

L-Arginine Normalizes Coronary Vasomotion in Long-Term Smokers

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Background—Noninvasive measurements of myocardial blood flow (MBF) with PET revealed an abnormal coronary vasomotor response to cold pressor test in healthy long-term smokers. If coronary endothelial dysfunction accounted for this abnormality, we hypothesized that it could be reversed by L-arginine as the substrate for NO synthase.

Methods and Results—MBF was quantified with ^{13}N -labeled ammonia and PET in 11 healthy smokers (age, 45 ± 10 years; 27 ± 10 years of smoking) and in 12 age-matched nonsmokers on 2 separate days. On day 1, MBF was measured at rest and, after intravenous L-arginine, during cold pressor test. On day 2, MBF was measured during cold pressor test and then at rest during L-arginine. Baseline rate-pressure product (RPP) (6559 ± 1590 versus 7144 ± 1157 bpm \times mm Hg) and MBF (0.65 ± 0.14 versus 0.73 ± 0.13 mL \cdot g $^{-1}$ \cdot min $^{-1}$) were similar in nonsmokers and smokers. Cold pressor test increased RPP similarly in both groups ($53 \pm 26\%$ versus $46 \pm 26\%$), whereas MBF increased in nonsmokers (to 0.93 ± 0.25 mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P < 0.05$) but not in smokers (0.80 ± 0.16 mL \cdot g $^{-1}$ \cdot min $^{-1}$). The percent MBF increase differed between nonsmokers and smokers ($44 \pm 25\%$ versus $11 \pm 14\%$; $P = 0.0017$). However, after L-arginine, the magnitude of MBF response to cold pressor test no longer differed between groups ($48 \pm 36\%$ versus $48 \pm 28\%$), whereas RPP again increased similarly in the 2 groups ($59 \pm 30\%$ versus $44 \pm 16\%$). L-Arginine had no effect on resting MBF in smokers or nonsmokers.

Conclusions—Our findings implicate the coronary endothelium as the major site of the abnormal vasomotor response in long-term smokers. Cold pressor test combined with PET imaging may allow the noninvasive identification of coronary endothelial dysfunction in humans. (*Circulation*. 1999;99:491-497.)

Key Words: blood flow ■ smoking ■ cold pressor test ■ endothelium ■ tomography

Cigarette smoking is a well-recognized coronary risk factor that induces endothelial dysfunction.^{1,2} We previously observed an abnormal myocardial blood flow (MBF) response to cold pressor test in chronic but healthy smokers.³ The cold pressor test evokes a mixed, adrenergically mediated vasoconstrictor and vasorelaxant response; the vasorelaxant effects are thought to be mediated through β -adrenoreceptor stimulation and, importantly, through NO release from endothelium. The cold pressor test has therefore been considered a noninvasive probe of endothelium-dependent coronary vasomotion.^{4,5} In the normal coronary circulation, the complex interplay between vasoconstrictor and vasorelaxant effects produces a net increase in coronary flow. However, in patients with coronary artery disease or with coronary risk factors, the cold pressor test can cause a paradoxical constriction of the epicardial vessels and an attenuated flow response.⁴ The reported correlation between abnormal responses to cold pressor test and to intracoronary acetylcholine in the large epicardial conduit and the resistance vessels of the coronary circulation implicate the endothelium as the primary site for the abnormal response to cold pressor test.⁶

If the abnormal response to cold pressor test in smokers reflects endothelial dysfunction, we postulated that intrave-

nous L-arginine should improve or restore the flow response. L-Arginine is the substrate for NO synthase (NOS)⁷ and has been found to improve abnormal flow responses to intracoronary acetylcholine in patients with coronary risk factors.^{8,9} L-Arginine-mediated restoration of the flow response to cold pressor test would then support the hypothesis that an abnormal cold pressor test signifies endothelial dysfunction. If true, the combination of this test with PET-based measurements of MBF could offer a means for probing endothelial dysfunction noninvasively.

Therefore, it was the aim of this study to determine with ^{13}N -labeled ammonia and PET imaging whether L-arginine modifies the MBF response to cold pressor test in healthy long-term smokers.

Methods

Study Population

Twenty-three normal volunteers (11 long-term smokers and 12 nonsmokers) were studied. The group of smokers consisted of 8 men and 3 women (age, 45 ± 10 years; range, 39 to 60 years) who had been smoking cigarettes for 27 ± 10 years (range, 14 to 50 years;

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26±17 pack-years). The group of 12 age-matched, healthy, lifelong nonsmokers (9 men, 3 women; age, 46±11 years; range, 28 to 59 years) served as controls. Four smokers and 6 nonsmokers had participated in a previous study³ and participated again a year later in the current study. Each participant was studied on 2 different days (mean interval of 8±5 days). None had hypertension, diabetes, familial hyperlipidemia, a history of coronary artery disease, or evidence of atherosclerosis (determined by absence of angina, intermittent claudication, or cerebrovascular ischemia) or was taking any medication. Long-term smoking was defined as ≥2 pack-years of cigarette smoking before the PET study.¹ ECGs were normal at rest, during cold pressor test, and during L-arginine infusion in all subjects. Smokers and nonsmokers >50 years old had either a normal treadmill or normal pharmacological stress test. All participants refrained from consuming caffeine-containing food or beverages for ≥24 hours, and smokers refrained from smoking for ≥4 hours before study.^{3,10} Each participant signed an informed consent form approved by the University of California at Los Angeles Human Subject Protection Committee.

Positron Emission Tomography

MBF was measured with ¹³N-labeled ammonia, PET, and a previously validated tracer kinetic model.¹¹ The CTI/Siemens ECAT EXACT HR⁺ device used in the study acquires 63 transaxial planes with an in-plane resolution of 4.3 mm full-width half-maximum (FWHM) and a 15.5-cm axial field of view (FOV).¹² After adequate positioning of the heart in the FOV, aided by a rectilinear transmission scan, a 20-minute transaxial transmission scan was acquired. The images were reconstructed with a Hann filter with a transaxial cutoff frequency of 0.093 mm⁻¹ cycles per pixel, and an axial cutoff frequency of 0.082 mm⁻¹, resulting in an effective isotropic resolution of 11 mm FWHM.

The study protocol consisted of 2 study sessions, both performed in the afternoon. On day 1, MBF was measured at rest and, after L-arginine infusion, during cold pressor test. Beginning with intravenous ¹³N-labeled ammonia administration (15 to 20 mCi), serial transaxial emission images were acquired (12 image frames of 10 seconds each, 2 frames of 30 seconds each, and 1 frame of 900 seconds). This was followed by intravenous infusion of L-arginine (30 g as 10% arginine hydrochloride) for 45 minutes at a rate of 6.67 mL/min. The cold pressor test was performed during the last 2 minutes of the L-arginine infusion. The patient immersed the left hand in ice water for 45 seconds before a second dose of ¹³N-labeled ammonia (15 to 20 mCi) was injected. The image-acquisition sequence used for the baseline study was repeated while the cold pressor test was maintained for another minute to permit trapping of ¹³N-labeled ammonia in the myocardium. On day 2, MBF was measured first during cold pressor test and then during the last 2 minutes of L-arginine infusion. Cold pressor test, administration of L-arginine and of ¹³N-labeled ammonia, and image acquisition were identical to those performed on day 1.

Heart rate, arterial blood pressure, and 12-lead ECG were recorded continuously. Heart rates and arterial blood pressures during the first 2 minutes of each image acquisition sequence were averaged and used to calculate the rate-pressure product (RPP).

Quantification of MBF

Regional MBF was quantified in the territories of the left anterior descending, left circumflex, and right coronary arteries.¹¹ Thus, 69 coronary territories were analyzed in the 23 participants (33 territories in smokers and 36 in nonsmokers). Sectorial regions of interest (ROIs; 70° to 90° each) were placed in each coronary territory on a basal, mid, and apical short-axis image. The same anatomic landmark (insertion of right ventricle into the intraventricular septum) ensured identical assignment of ROIs for the 4 study conditions. A small ROI (25 mm²) was centered in the left ventricular blood pool for determination of the arterial input function.¹³ The ROIs were copied to the first 120 seconds of the serially acquired images for generation of blood pool and myocardial time-activity curves. A single time-activity curve was obtained for each coronary vascular territory by averaging the time-activity data from the 3 short-axis

cross sections. Because MBF did not differ between the vascular territories, a single value of MBF was obtained by averaging the time-activity data in the 3 coronary territories.^{11,14} Effects of partial volume were corrected for with a recovery coefficient that assumed a uniform 1-cm-thick left ventricular wall.¹⁵ Time-activity curves were corrected for physical decay and fitted with a 2-compartment tracer kinetic model that corrects for spillover of activity from the blood pool into the left ventricular myocardium.¹⁶

Blood Chemistry

On day 1, total serum cholesterol and HDL cholesterol were determined at baseline by enzymatic methods.¹⁷ LDL cholesterol was calculated mathematically.¹⁸ Serum concentrations of L-arginine, citrulline, and ornithine (by fluorescent high-performance liquid chromatography),^{19,20} insulin (by radioimmunoassay), and glucose (by standard enzymatic methods) were measured at baseline and at 35 minutes of L-arginine infusion on day 1 and before cold pressor test and at 35 minutes of L-arginine infusion on day 2.

Statistical Analysis

Descriptive statistics are expressed as mean±SD. Hemodynamic parameters and MBFs at baseline, during cold pressor test after L-arginine, and during cold pressor test and L-arginine infusion were analyzed by repeated-measures ANOVA. Post hoc comparisons were made with the Bonferroni test. Changes in serum measurements before and after L-arginine as well as the interobserver reproducibility of the MBF measurements were assessed by paired *t* testing.

Differences in hemodynamic parameters, MBF, and serum measurements between smokers and nonsmokers were assessed with the unpaired *t* test. Differences between groups in the magnitude of the MBF response to cold pressor test at baseline and after L-arginine were evaluated by 2-sample Wilcoxon rank sum (Mann-Whitney) test. All probability values are 2-tailed; *P*<0.05 was considered statistically significant.

Results

Hemodynamic Findings

Heart rate and blood pressure measurements in smokers and nonsmokers are summarized in Table 1. At baseline, systolic, diastolic, and mean arterial blood pressures (82±7 versus 84±13 mm Hg) and RPPs (6559±1590 versus 7144±1157 bpm×mm Hg) were similar in both groups. Smokers and nonsmokers responded to cold pressor testing after L-arginine infusion with significant increases in heart rate, mean arterial blood pressure (to 102±14 and 100±16 mm Hg, respectively), and RPP (to 10 365±2943 and 10 208±1557 bpm×mm Hg, respectively). Also, during baseline cold pressor testing, mean arterial blood pressure increased similarly in both groups (to 101±11 and 109±14 mm Hg, respectively), whereas heart rate failed to significantly increase in nonsmokers. However, RPPs did not differ between both groups (10 059±2911 versus 10 361±2079 bpm×mm Hg, respectively; *P*=NS), indicating that cold pressor testing induced comparable increases in cardiac work in smokers and nonsmokers. L-Arginine infusion had no effect on heart rate or mean arterial blood pressure (84±13 versus 84±15 bpm and 82±7 versus 80±6 mm Hg, respectively; *P*=NS) at baseline in smokers and nonsmokers, respectively. Furthermore, the increase in cardiac work induced by cold pressor test was independent of L-arginine in both groups (10 365±2943 bpm×mm Hg before versus 10 059±2911 bpm×mm Hg after L-arginine for smokers; 10 208±2911 bpm×mm Hg before versus 10 361±2079 bpm×mm Hg after L-arginine for nonsmokers; *P*=NS). RPP during L-arginine administration

TABLE 1. Hemodynamic and Blood Flow Responses to Cold Pressor Test and L-Arginine

	Rest				L-Arginine+CPT				CPT				L-Arginine			
	HR	SBP	DBP	MBF	HR	SBP	DBP	MBF	HR	SBP	DBP	MBF	HR	SBP	DBP	MBF
Smokers																
1	70	109	73	0.82	77	114	77	0.92	77	126	82	0.83	83	104	76	1.20
2	69	129	75	0.76	72	160	95	0.94	84	162	97	0.87	66	110	61	0.76
3	69	113	75	1.01	88	150	85	1.34	73	198	105	1.07	69	140	84	1.04
4	60	114	68	0.63	62	160	79	1.27	71	156	104	0.86	55	127	79	0.70
5	54	131	73	0.56	69	151	78	0.93	58	141	71	0.69	46	117	70	0.46
6	62	102	60	0.66	81	126	81	0.92	68	140	85	0.66	61	107	60	0.74
7	61	102	55	0.85	72	125	80	1.09	83	132	81	1.10	56	98	53	0.49
8	63	111	74	0.72	70	127	77	1.14	67	131	83	0.68	62	104	71	0.70
9	60	100	64	0.60	72	131	87	1.11	70	145	95	0.61	59	101	66	0.58
10	61	151	92	0.65	70	177	109	0.86	60	161	105	0.75	61	145	96	1.02
11	65	86	55	0.72	82	104	66	1.11	68	116	81	0.72	66	83	48	0.69
Mean	63	113	69	0.73	74*	139*	83*	1.06*	71	146*	90*	0.80	62	112	69	0.76
SD	5	18	11	0.13	7	22	11	0.16	8	23	12	0.16	9	19	14	0.23
Nonsmokers																
1	81	122	75	0.89	75	121	70	0.83	78	138	79	1.06	78	102	63	0.86
2	56	127	80	0.89	91	150	81	1.19	78	147	91	1.48	79	123	65	1.22
3	52	102	69	0.57	64	132	75	0.68	62	128	78	0.63	58	115	74	0.75
4	80	103	62	0.74	113	138	76	1.24	109	146	91	1.19	98	97	53	1.03
5	48	109	70	0.51	61	135	80	0.90	61	152	84	0.91	52	114	66	0.72
6	53	104	73	0.63	59	150	95	0.76	58	125	87	0.68	52	104	70	0.67
7	47	100	56	0.52	52	125	56	0.61	49	130	80	0.65	54	108	61	0.67
8	64	105	61	0.60	105	130	75	1.26	95	132	80	1.08	69	107	62	0.67
9	68	116	73	0.73	82	145	87	1.03	75	156	92	1.08	72	119	75	0.76
10	46	109	66	0.55	60	111	69	1.13	55	113	63	0.86	55	110	59	0.69
11	63	118	71	0.59	82	147	85	0.90	75	164	97	0.84	64	112	67	0.60
12	54	105	65	0.52	74	132	75	0.75	73	123	75	0.73	65	105	62	0.71
Mean	59	110	68	0.65	77*	135*	77*	0.94*	72*	138*	83*	0.93*	66	110	65	0.78
SD	12	9	7	0.14	19	12	10	0.23	17	15	9	0.25	14	7	6	0.18

CPT indicates cold pressor test; HR, heart rate (bpm); SBP, systolic blood pressure (mm Hg); DBP, diastolic blood pressure (mm Hg). MBF is mL · g⁻¹ · min⁻¹. **P*<0.05 by ANOVA (repeated measures).

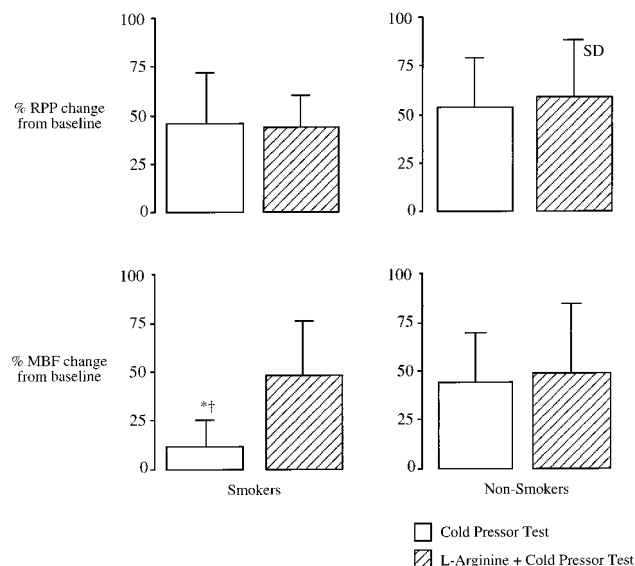
was similar in smokers and nonsmokers (7251±1432 versus 6969±1484 bpm×mm Hg; *P*=NS). Last, the resting RPP at baseline on day 1 was similar to that at baseline before cold pressor testing on day 2 in smokers (7144±1157 versus 7465±1698 bpm×mm Hg; *P*=NS) but not in nonsmokers (6559±1590 versus 7227±1726 bpm×mm Hg; *P*=0.042). This finding was related to a higher heart rate on day 2 during transmission scan than that on day 1 obtained during measurement of MBF at baseline (63±11 versus 59±12 bpm; *P*=0.032) despite similar systolic blood pressures (110±9 versus 114±3 mm Hg; *P*=NS). However, when heart rates during transmission imaging for both studies were compared, they no longer differed between days (65±12 versus 63±11 bpm; *P*=NS). Therefore, baseline hemodynamic conditions were comparable for the 2 sessions in smokers and nonsmokers.

PET Image Analysis

There were 69 coronary territories (3 per participant) in the 23 participants. On visual examination, all 69 vascular territories exhibited homogeneously distributed perfusion at rest, during cold pressor test with and without L-arginine infusion, and during L-arginine administration. There were no rest or stress perfusion defects in any of the smokers or nonsmokers.

MBF Measurements

MBF at rest was similar for the 2 groups (0.73±0.13 versus 0.65±0.14 mL · g⁻¹ · min⁻¹). L-Arginine infusion did not affect resting MBF in smokers or nonsmokers (0.73±0.13 versus 0.76±0.23 mL · g⁻¹ · min⁻¹ before versus after infusion in smokers and 0.65±0.14 versus 0.78±0.18 mL · g⁻¹ · min⁻¹ before versus after infusion in nonsmokers; *P*=NS; Table 1). Whereas cold pressor testing increased



Cold pressor test-induced changes in RPP and MBF before and after L-arginine administration in smokers (left) and nonsmokers (right). Percent MBF increase during cold pressor test differed between smokers and nonsmokers ($11 \pm 14\%$ versus $44 \pm 25\%$; $*P=0.0017$) despite comparable percent increases in RPP in both groups. However, after L-arginine infusion, MBF during cold pressor test increased similarly in smokers and nonsmokers ($48 \pm 28\%$ versus $48 \pm 36\%$), whereas similar increases in RPP occurred in the 2 groups. $\dagger P=0.0044$ cold pressor test vs L-arginine plus cold pressor test in smokers.

RPPs similarly in smokers and nonsmokers (from 7144 ± 1157 to 10361 ± 2079 and from 6559 ± 1590 to 10059 ± 2911 bpm \times mm Hg, respectively; $P<0.05$), MBF increased only in nonsmokers (from 0.65 ± 0.14 to 0.93 ± 0.25 mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P<0.05$) and not in smokers (from 0.73 ± 0.13 to 0.80 ± 0.16 mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P=NS$; Table 1). The percent MBF increase during cold pressor testing differed between nonsmokers and smokers ($44 \pm 25\%$ versus $11 \pm 14\%$, respectively; $P=0.0017$; Figure) despite comparable percent increases in RPP ($53 \pm 26\%$ versus $46 \pm 26\%$). However, after L-arginine infusion, the magnitude of the MBF response to cold pressor testing no longer differed between the 2 groups ($48 \pm 36\%$ versus $48 \pm 28\%$), whereas RPPs again increased similarly in both groups ($59 \pm 30\%$ versus $44 \pm 16\%$; Figure). During cold pressor test after L-arginine administration, MBF increased similarly in smokers and nonsmokers (1.06 ± 0.16 versus 0.94 ± 0.23 mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P=NS$). To remove any observer bias, MBF data were analyzed by 2 independent observers (Dr Campisi and Dr Schöder). Average MBF values in 6 participants did not differ between observers (0.75 ± 0.19 versus 0.72 ± 0.12 mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P=0.51$).

Coronary Vascular Resistance

An index of coronary vascular resistance was calculated from the ratio of mean arterial blood pressure (mm Hg) to MBF (mL \cdot g $^{-1}$ \cdot min $^{-1}$).²¹ At rest, this index was similar for smokers and nonsmokers (120 ± 30 versus 131 ± 20 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P=NS$). In smokers, coronary vascular resistance was lower during cold pressor test after L-arginine than during baseline cold pressor test (97 ± 25

TABLE 2. Serum Chemistry at Baseline and in Response to L-Arginine Infusion

	Smokers (n=11)		Nonsmokers (n=12)	
	Rest	L-Arginine	Rest	L-Arginine
Glucose, mmol/L	2.49 ± 0.40	2.57 ± 0.38	2.54 ± 0.53	2.69 ± 0.39
Insulin, μ U/mL	10 ± 7	$33 \pm 22^*$	14 ± 12	$47 \pm 32^*$
Arginine, μ mol/L	105 ± 35	$5613 \pm 1661\dagger$	94 ± 29	$4721 \pm 1928\dagger$
Citrulline, μ mol/L	36 ± 12	$46 \pm 12\dagger$	35 ± 11	$44 \pm 10\dagger$
Ornithine, μ mol/L	73 ± 16	$412 \pm 120\dagger$	65 ± 21	$421 \pm 71\dagger$

* $P<0.01$ vs baseline; $\dagger P<0.001$ vs baseline; $\ddagger P<0.0001$ vs baseline.

versus 139 ± 25 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P<0.05$) and remained unchanged during L-arginine infusion (117 ± 31 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P=NS$). In nonsmokers, this index averaged at baseline (rest) 131 ± 20 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$ and was lower at rest with L-arginine (106 ± 21 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$) and during cold pressor testing with and without L-arginine (99 ± 24 and 115 ± 27 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P<0.05$ for all versus baseline). Of note, coronary resistance during cold pressor test was higher in smokers than in nonsmokers (139 ± 25 versus 115 ± 27 mm Hg/mL \cdot g $^{-1}$ \cdot min $^{-1}$; $P<0.05$).

Blood Chemistry

Total serum cholesterol was similar in smokers and nonsmokers (188 ± 40 mg/dL [range, 131 to 235 mg/dL] versus 167 ± 45 mg/dL [range, 93 to 253 mg/dL]; $P=NS$). In the smoking group, 4 participants had borderline elevated cholesterol levels (200 to 239 mg/dL). In the nonsmoking group, 1 participant had borderline and 1 had elevated cholesterol levels (>240 mg/dL).²² There were no differences in HDL and LDL cholesterol levels between groups (46 ± 19 versus 45 ± 13 mg/dL [HDL] and 118 ± 43 versus 99 ± 30 mg/dL [LDL], for smokers and nonsmokers, respectively; $P=NS$). HDL/LDL ratios were similar in smokers and nonsmokers (0.47 ± 0.34 versus 0.49 ± 0.20 ; $P=NS$). Serum arginine levels were similar in smokers and nonsmokers at baseline and during L-arginine infusion (Table 2). During L-arginine administration, levels of citrulline increased by $32 \pm 23\%$ and $33 \pm 31\%$, respectively, and ornithine levels increased by $468 \pm 143\%$ and $595 \pm 277\%$ in smokers and nonsmokers, respectively. Insulin levels at baseline were similar in smokers and nonsmokers and increased similarly in both groups during L-arginine infusion (by $349 \pm 440\%$ and $361 \pm 416\%$, respectively). Glucose levels remained unchanged during L-arginine infusion in smokers and nonsmokers (Table 2). There were no differences in serum measurements between the 2 days of the study protocol.

Discussion

The major finding of the current study is that acute administration of L-arginine as the substrate of NOS reversed the abnormal MBF response to cold pressor testing in healthy long-term smokers. This finding supports the hypothesis that the abnormal response to cold pressor test depends on endothelial dysfunction.

Cold Pressor Test and Endothelial Function

The previously reported correlation between the epicardial vasomotor response to intracoronary acetylcholine and that to cold pressor test in patients with mild atherosclerosis⁴ indicated that the cold pressor test could be used as a probe of endothelium-dependent coronary vasomotion.⁵ By combining cold pressor testing with PET-based MBF measurements, we had shown that endothelium-dependent dilation of the coronary microcirculation could be demonstrated noninvasively. The MBF response to cold pressor test was attenuated in healthy long-term smokers; the degree of this impairment correlated with the number of years of smoking.³ The current findings confirm and expand on the previous observations. Again, the flow responses to cold pressor test were diminished in long-term smokers. Importantly, L-arginine reversed the abnormal response, which suggests that the cold pressor test acted through an endothelium-dependent mechanism. Increases in serum citrulline during L-arginine infusion support the possibility that the NOS pathway was stimulated. Support for this possibility comes from Gellman et al,²³ who reported that L-arginine normalized the paradoxical coronary resistance response to cold pressor test in patients with coronary artery disease, suggesting again that coronary responses to the cold pressor test involve the L-arginine–NO pathway. Furthermore, Tousoulis et al²⁴ showed that the cold pressor test caused dilation of proximal and distal artery segments in patients with normal coronary arteriograms and that this dilation was abolished by *N*^G-monomethyl-L-arginine, a competitive antagonist of NO. Our findings together with these earlier observations support the role of the cold pressor test as a tool to evaluate coronary endothelial function. Cold pressor testing combined with PET imaging may thus allow the noninvasive identification of coronary endothelial dysfunction in humans.

L-Arginine and Endothelial Dysfunction in Cigarette Smokers

L-Arginine is the substrate of the stereospecific enzyme NOS.⁷ Several studies report a modulating effect of L-arginine supplementation on NO production in vivo under conditions known to be associated with endothelial dysfunction, such as hypercholesterolemia,^{8,25} hypertension,²⁶ aging,⁹ and diabetes.²⁷ In healthy young cigarette smokers, oral administration of L-arginine reversed the increased monocyte–endothelial cell adhesion to endothelial cells, an early event in atherogenesis.²⁸

In the current study, cold pressor test failed to increase MBF in long-term smokers despite appropriate increases in RPP and thus in cardiac work. Accordingly, coronary vascular resistance was higher in smokers than in nonsmokers but became normal after L-arginine administration. Because it continued to have no effect on the flow response to cold pressor test in nonsmokers, L-arginine appeared to act selectively in long-term smokers with endothelial dysfunction. We tested the possibility that our findings might have been attributed to a lack of reproducibility of the MBF measurements. However, mean flow values obtained in 6 participants were similar for both

observers. Furthermore, Nagamachi et al²⁹ reported no significant differences between estimates of MBF at rest either on the same day or after 26.5 ± 18.9 days. In the present study, each participant was studied twice at an interval of 8 ± 5 days. Thus, it is unlikely that the observations can be attributed to systematic measurement error.

It remains uncertain how L-arginine administration increases NO production. One explanation is that L-arginine supplementation enhances the availability of intracellular substrate for endothelial NOS. However, because intracellular levels of L-arginine exceed the K_m of NOS, administration of the substrate is unlikely to affect NO production.³⁰ Nevertheless, total cellular L-arginine concentration might not necessarily reflect the concentration in microdomains of the cell (for example, in the plasmalemmal caveolae as the site of endothelial production of NO).^{30,31} L-Arginine levels in endothelial cells can be modulated by the enzyme arginase, which degrades L-arginine to ornithine and urea.³² This reaction has also been reported in hepatocytes,³³ macrophages,³⁴ and neurons.³⁵ According to Wu and Meininger,³⁶ arginase and not NOS is the major pathway of arginine metabolism in normal endothelial cells. Therefore, it is possible that catabolism of arginine independent of NOS (ie, arginase) may play a role in regulating intracellular arginine levels. In the current study, serum ornithine levels increased more than citrulline levels during L-arginine infusion in both smokers and nonsmokers. The increases were similar for both groups. Therefore, arginine appears to degrade mainly by the arginase pathway.

Another possibility is that vascular effects of L-arginine could be mediated by release of endogenous substances. L-Arginine stimulates insulin release from pancreatic β -cells. The hormone possesses vasoactive properties possibly mediated by endogenous NO release.³⁷ The increase in serum insulin during L-arginine raises the possibility of an insulinotropic effect of L-arginine for normalizing the MBF response to cold pressor test. Creager et al²⁵ demonstrated that infusion of L-arginine in hypercholesteremic individuals induced an increase in insulin levels and in brachial blood flow. In contrast, the enantiomer D-arginine (which is not a substrate for NOS) also induced insulin release, although without affecting brachial artery blood flow. Thus, despite enhanced insulin release during L- or D-arginine infusions, the reversal of endothelial dysfunction in hypercholesterolemia or long-term cigarette smokers, as observed in the present study, may be related to a specific action of L-arginine. However, Giugliano et al³⁷ reported that vascular responses to L-arginine are mediated in part by endogenous insulin secretion in healthy individuals. In particular, octreotide inhibition of basal insulin secretion diminished the vasodilating effect of L-arginine. Studies are needed to clarify this important issue. Alternatively, L-arginine may enhance NO release by reversing the inhibitory effect of L-glutamine in receptor-mediated NO release³⁸ or might overcome the effects of endogenous NOS antagonists such as asymmetric dimethyl arginine.³⁹ Finally, L-arginine might exert its beneficial effect on the vascular system through antioxidative properties.⁴⁰ Cigarette smoke contains oxidants, and recent reports described

a role of oxygen-derived free radicals in mediating endothelial dysfunction.⁴¹ Thus, L-arginine may decrease NO catabolism by smoke-enhanced oxygen-derived free radicals.⁴²

Study Limitations

The blood flow measurements in long-term smokers could have been affected by acute nicotine effects. Nicotine evokes the release of catecholamines, with subsequent adrenergically mediated increases in cardiac work and coronary blood flow. However, smokers abstained from smoking before the PET study for ≥ 4 hours, which is sufficient to reduce serum nicotine to nearly unmeasurable levels.^{3,10}

Abnormalities in total cholesterol levels might have affected the MBF response to cold pressor test. However, only 5 of the 23 participants had borderline values, and 1 had elevated cholesterol levels. Of note, average total cholesterol levels did not differ between smokers and nonsmokers. Furthermore, HDL/LDL ratio, not total cholesterol, is a predictor of the blood flow response to cold pressor test.³ In our study, HDL/LDL ratios were similar for both groups.

Flow-limiting coronary stenoses might have affected MBF responses to cold pressor testing in long-term smokers. Coronary artery disease could have been ruled out with certainty only by use of coronary arteriography, which seemed unjustified in these asymptomatic individuals. However, none of the 23 volunteers had a history of coronary artery disease or of atherosclerosis (determined by absence of angina, intermittent claudication, and cerebrovascular ischemia). In addition, smokers and nonsmokers >50 years old had either a previous normal treadmill or normal pharmacological stress test.

Corrections for partial volume effects, which assumed a uniform myocardial wall thickness of 1 cm, might have introduced a systematic error in the MBF measurements.¹⁵ On the basis of phantom studies, partial volume effects were corrected with a recovery coefficient of 0.73. Assuming a constant wall thickness between studies, a systematic error in correcting for partial volume would have equally affected all 4 MBF estimates.

Conclusions

Acute administration of L-arginine as the precursor of NO reversed the abnormal MBF response to cold pressor test in healthy long-term smokers. This finding supports the hypothesis that the abnormal response to cold pressor test depended on endothelial dysfunction in long-term smokers. Cold pressor test combined with PET imaging may allow the noninvasive identification of coronary endothelial dysfunction in humans.

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