L-Arginine Normalizes Coronary Vasomotion in Long-Term Smokers

Roxana Campisi, MD; Johannes Czernin, MD; Heiko Schöder, MD; James W. Sayre, PhD; Heinrich R. Schelbert, MD

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×mm Hg) and MBF (0.65±0.14 versus 0.73±0.13 mL·g$^{-1}$·min$^{-1}$) were similar in nonsmokers and smokers. Cold pressor test increased RPP similarly in both groups (53±26% versus 46±26%), whereas MBF increased in nonsmokers (to 0.93±0.25 mL·g$^{-1}$·min$^{-1}$; P<0.05) but not in smokers (0.80±0.16 mL·g$^{-1}$·min$^{-1}$). The percent MBF increase differed between nonsmokers and smokers (44±25% versus 11±14%; P=0.0017). However, after L-arginine, the magnitude of MBF response to cold pressor test no longer differed between groups (48±36% versus 48±28%), whereas RPP again increased similarly in the 2 groups (59±30% versus 44±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 $\beta$-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 intravenous 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;...
Positron Emission Tomography

MBF was measured with $^{18}$N-labeled ammonia, PET, and a previously validated tracer kinetic model. The CTI/Siemens ECAT EXACT HR$^2$ 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). 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 0.082 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 $^{18}$N-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 $^{18}$N-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 the administered intravenous $^{18}$N-labeled ammonia. The heart rate and blood pressure measurements in smokers and nonsmokers were evaluated by 2-sample Wilcoxon rank sum (Mann-Whitney) test. All probability values are 2-tailed; P<0.05 was considered statistically significant.

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
PET Image Analysis

Campisi et al February 2, 1999

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 ± 14% versus 44 ± 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 ± 28% versus 48 ± 36%), whereas similar increases in RPP occurred in the 2 groups. †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 10 361 ± 2079 and from 6559 ± 1590 to 10 059 ± 2911 bpm × mm Hg, respectively; P<0.05), MBF increased only in nonsmokers (from 0.65 ± 0.14 to 0.93 ± 0.25 mL · g⁻¹ · min⁻¹; P<0.05) and not in smokers (from 0.73 ± 0.13 to 0.80 ± 0.16 mL · g⁻¹ · min⁻¹; P=NS; Table 1). The percent MBF increase during cold pressor testing differed between nonsmokers and smokers (44 ± 25% versus 11 ± 14%, respectively; P=0.0017; Figure) despite comparable percent increases in RPP (53 ± 26% versus 46 ± 26%). However, after L-arginine infusion, the magnitude of the MBF response to cold pressor testing no longer differed between the 2 groups (48 ± 36% versus 48 ± 28%), whereas RPPs again increased similarly in both groups (59 ± 30% versus 44 ± 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 · g⁻¹ · min⁻¹; P=NS). To remove any observer bias, MBF data were analyzed by 2 independent observers (Dr Campisi and Dr Schöneder). Average MBF values in 6 participants did not differ between observers (0.75 ± 0.19 versus 0.72 ± 0.12 mL · g⁻¹ · 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 · g⁻¹ · min⁻¹).²¹ At rest, this index was similar for smokers and nonsmokers (120 ± 30 versus 131 ± 20 mm Hg/mL · g⁻¹ · 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 versus 139 ± 25 mm Hg/mL · g⁻¹ · min⁻¹; P<0.05) and remained unchanged during L-arginine infusion (117 ± 31 mm Hg/mL · g⁻¹ · min⁻¹; P=NS). In nonsmokers, this index averaged at baseline (rest) 131 ± 20 mm Hg/mL · g⁻¹ · min⁻¹ and was lower at rest with L-arginine (106 ± 21 mm Hg/mL · g⁻¹ · min⁻¹) and during cold pressor testing with and without L-arginine (99 ± 24 and 115 ± 27 mm Hg/mL · g⁻¹ · 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 · g⁻¹ · 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 ± 23% and 33 ± 31%, respectively, and ornithine levels increased by 468 ± 143% and 595 ± 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 ± 440% and 361 ± 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Smokers (n=11)</th>
<th>Nonsmokers (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>L-Arginine</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>2.49±0.40</td>
<td>2.57±0.38</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>10±7</td>
<td>33±22*</td>
</tr>
<tr>
<td>Arginine, μmol/L</td>
<td>105±35</td>
<td>5613±1661†</td>
</tr>
<tr>
<td>Citrulline, μmol/L</td>
<td>36±12</td>
<td>46±12†</td>
</tr>
<tr>
<td>Ornithine, μmol/L</td>
<td>73±16</td>
<td>412±120†</td>
</tr>
</tbody>
</table>

*P<0.01 vs baseline; †P<0.001 vs baseline; ‡P<0.0001 vs baseline.
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 atherosclerosis indicated that the cold pressor test could be used as a probe of endothelium-dependent coronary vasomotion. 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. 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, who reported that the 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, Toussoulis et al 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 Nω-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. 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, hypertension, aging, and diabetes. 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. 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 al 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 Km of NOS, administration of the substrate is unlikely to affect NO production. 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). L-Arginine levels in endothelial cells can be modulated by the enzyme arginase, which degrades L-arginine to ornithine and urea. This reaction has also been reported in hepatocytes, macrophages, and neurons. According to Wu and Meininger, 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. 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 al 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 dysfunc- 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 al 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 release or might overcome the effects of endogenous NOS antagonists such as asymmetric dimethyl arginine. Finally, L-arginine might exert its beneficial effect on the vascular system through antioxidative properties.

Cigarette smoke contains oxidants, and recent reports described
a role of oxygen-derived free radicals in mediating endothelial dysfunction. Thus, \textit{l}-arginine may decrease NO catabolism by smoke-enhanced oxygen-derived free radicals.\textsuperscript{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 \(\geq 4\) hours, which is sufficient to reduce serum nicotine to nearly unmeasurable levels.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 \textit{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.

**Acknowledgments**

The Laboratory of Structural Biology and Molecular Medicine, University of California, Los Angeles, is operated for the US Department of Energy by the University of California under contract No. DE-FC03-87ER66015. This study was supported in part by the Director of the Office of Energy Research, Office of Health and Environmental Research, Washington, DC; by research grant HL-33177, National Institutes of Health, Bethesda, Md; and by an Investigative Group Award, American Heart Association, Los Angeles (Calif) Affiliate. The authors thank Ron Sumida, Larry Pang, Francine Aguilar, Der-Jenn Liu, Priscilla Contreras, and Suman Wongpiya for their excellent assistance in performing the PET studies and N. Satyamurthy and his cyclotron staff for production of the \textsuperscript{15}N-labeled ammonia. We also thank Deborah Dorsey, RN, for her assistance; Diane Martin and David Twomey for preparing the artwork; and Eileen Rosenfeld for preparing this manuscript.

**References**


l-Arginine Normalizes Coronary Vasomotion in Long-Term Smokers
Roxana Campisi, Johannes Czernin, Heiko Schöder, James W. Sayre and Heinrich R. Schelbert

Circulation. 1999;99:491-497
doi: 10.1161/01.CIR.99.4.491
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/4/491

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/