Endothelium-Dependent and -Independent Perfusion Reserve and the Effect of L-arginine on Myocardial Perfusion in Patients With Syndrome X

Morten Bøttcher, MD; Hans Erik Bøtker, MD, PhD; Helle Sonne, MD; Torsten Toftegaard Nielsen, MD; Johannes Czernin, MD

Background—Impaired vasodilatation capacity in patients with angina pectoris and a normal coronary arteriogram (syndrome X [SX]) has been reported. Most studies report on the response in epicardial vessels. This does not necessarily reflect compromised myocardial microcirculation. Lack of the NO precursor L-arginine has been suggested as a possible cause.

Methods and Results—Myocardial blood flow (MBF) was measured, using PET, at rest (MBF-rest) and during intravenous dipyridamole (MBF-DIP) in 25 women (mean age 53±7 years) with SX. Thirty healthy volunteers served as controls. One group (A) consisted of 15 age-matched female volunteers (54±10 years). The other control group consisted of 15 young healthy women (B; 24±5 years). In 12 SX patients, MBF-rest and MBF during cold pressor testing were also measured after infusion of L-arginine (6.7 g/min for 45 minutes). The increase in MBF after cold pressor testing was similar in the SX group compared with controls. L-arginine did not affect MBF-rest (0.83±0.14 versus 0.89±0.13 mL·g⁻¹·min⁻¹) or MBF after cold pressor test (0.95±0.10 versus 1.03±0.17 mL·g⁻¹·min⁻¹). In contrast, the hyperemic response to DIP was blunted compared with the group A controls (1.68±0.49 versus 2.34±0.45 mL·g⁻¹·min⁻¹, P<0.05); this resulted in a significant reduction of the coronary flow reserve in SX patients relative to controls (2.03±0.53 versus 2.96±0.63 mL·g⁻¹·min⁻¹, P<0.01).

Conclusions—In patients with SX, the microcirculatory response to cold, reflecting the endothelium function, is normal and unaltered by intravenous L-arginine. This suggests preserved microcirculatory endothelial function. However, a markedly attenuated hyperemic flow and flow reserve after DIP suggest a dysfunction of the adenosine-mediated endothelium-independent vasodilatation at the microcirculatory level in these patients. (Circulation. 1999;99:1795-1801.)

Key Words: blood flow • L-arginine • angina • syndrome X • tomography

Approximately 15% to 20% of all patients undergoing coronary arteriography because of chest pain reveal no significant stenosis. A subgroup of these patients have ST-segment depression during exercise testing and are diagnosed as having syndrome X (SX). A reduced coronary flow reserve (CFR) can be seen in a significant proportion of patients with SX.¹ The reduced CFR in combination with angina pectoris and ST-segment depression during exercise have suggested an ischemic nature of SX and generated the term microvascular angina.² However, a reduced CFR is not a consistent finding in patients with SX. Furthermore, metabolic and functional evidence of myocardial ischemia is uncommon even in SX patients with a reduced CFR.³⁻⁵ Because an impairment of CFR does not per se explain the occurrence of angina, these findings challenge the reduced CFR as the underlying mechanism of angina pectoris in patients with SX.

On the other hand, myocardial perfusion studies have demonstrated an impaired flow response to both pace stress and pharmacological vasodilation in patients with SX.⁶ The fact that these abnormalities have been demonstrated by several methods, including PET,⁵,⁷,⁸ intracoronary Doppler guide wire,⁵,⁹ and thermodilution measurement of coronary sinus blood flow⁶,¹⁰ strengthens the conclusion that an abnormal flow reserve does exist. The mechanisms, which may impair CFR, are not known, when specific vascular or cardiac disorders have been excluded. Several investigations have suggested that not only endothelium-independent vasodilation but also dependent vasodilation is impaired in patients with SX.¹¹ Whether this causes the myocardial perfusion to decrease is not clear, and in the absence of myocardial ischemia, the mechanism for the generation of angina pectoris still lacks an explanation.
The aim of the present study was to investigate whether microcirculatory endothelium–dependent and –independent vasodilatation is impaired in patients with SX and whether L-arginine supplementation enhances endothelium-dependent vasodilatation at the level of microcirculation in these patients.

Methods

Study Population

The study population consisted of 25 women (mean age 53 ± 7 years) diagnosed with SX on the basis of a positive bicycle test (angina pectoris and simultaneous ST-segment depression ≥ 0.1 mV in at least 2 precordial leads), completely normal coronary arteriography, and a negative hyperventilation test. All patients had normal left ventricular function at ventriculography and normal echocardiographic examination. None of the patients had hypertension (systolic blood pressure > 180 mm Hg, diastolic blood pressure > 95 mm Hg), dyslipidemia (plasma cholesterol > 8 mmol/L, serum triglyceride > 3.5 mmol/L), history of diabetes mellitus, plasma glucose > 7.5 mmol/L or glucosuria, obesity (defined as body mass index > 30 kg/m²), or bundle branch block on the ECG. Ten of the SX patients were postmenopausal and 4 received hormone replacement therapy; 7 in control group A were postmenopausal and 3 were on hormone replacement therapy. Myocardial lactate exchange at rest and in response to pace stress at 150 bpm for 10 minutes was assessed with measurement of coronary sinus blood flow by thermodilution technique with a 7-F Wilton-Webster catheter (Webster Labs, Inc). Analysis of lactate in blood samples was drawn simultaneously from the coronary sinus and the femoral artery. Myocardial lactate production was not observed in any of the patients at rest, during pace stress, or in the recovery period after pacing.

Fifteen healthy age- and sex-matched volunteers served as controls (mean age 54 ± 10 years; group A). A group of 15 young female volunteers (group B; mean age 24 ± 5 years) served as a second control group. All volunteers had low likelihood of coronary artery disease by Baysian analysis; this was evidenced by a normal physical examination, resting ECG, and absence of any significant risk factors. None of the participants had a history of elevated serum cholesterol levels, hypertension, or diabetes. None of the participants received any medication. Individuals with bronchial asthma were excluded from the study because of the risk of untoward side effects of diprydiamole (DIP).

All study participants refrained from intake of caffeine-containing food or beverages for at least 24 hours before each study. All participants gave written informed consent as approved by the county ethics committee.

Study Protocol

The study protocol consisted of serial N-13 ammonia PET blood flow measurements. For 12 of the 25 SX patients on day 1, myocardial blood flow was studied at rest, during cold pressor testing (CPT) after infusion of L-arginine, and finally during DIP-induced hyperemia. On a different day, MBF was measured during CPT and at rest after infusion of L-arginine. The study sequence was performed in random order with a mean difference of 7 ± 2 days between the 2 studies. The remaining 13 SX patients underwent PET scanning for determination of rest- and DIP-induced hyperemic blood flow on the same day. Group B volunteers underwent rest and DIP studies on day 1 and rest and CPT measurements on day 2 (random order). Group A volunteers underwent measurements of myocardial blood flow at rest and after administration of DIP. None of the healthy controls received any medication. All SX patients stopped intake of medication before the study (nitroglycerin 2 days, beta blockers 1 week, calcium antagonists 3 weeks, and all other types of medication stopped 1 week before the study). L-arginine (300 g) was suspended in 500 mL saline to yield a concentration of 600 g/L. Infusion was performed with an automated infusion pump set at 11 mL/min. The amount given was therefore 6.7 g/min for a period of 45 minutes. The systolic and diastolic blood pressure measurements were taken with an automated arm cuff–based device; measurements were taken twice during the last 2 minutes of the infusion, average values are reported. Myocardial perfusion was corrected (MBF-RC) for changes in rate pressure product (RPP) by the formula (MBF-RC = 10.000 × (Rest-MBF/RPP).

PET

The Siemens/CTI 961/08–12 positron tomograph, which simultaneously acquires 47 transaxial images, was used. This scanner has an axial field of view of 15 cm, an intrinsic in-plane spatial resolution of 5.5 mm full width at half-maximum (FWHM), and an inter-plane spacing of 3.125 mm. The acquired images were reconstructed using a Shepp filter with a cut-off frequency of 0.3 Nyquist, resulting in an effective in-plane resolution of 7 mm FWHM.

After performing a 15-minute transmission image to be able to correct for photon attenuation, a 20 mL bolus of N-13 ammonia (20 mCi/740 MBq) was injected in a cubital vein over 30 s. Acquisition of a dynamic imaging sequence was started simultaneously. Fifty minutes (5 half-lives) were allowed for decay of the radiotracer activity before the next image sequence was acquired. CPT was performed using a container with ice water (≈1°C). The subject’s hand and distal part of the forearm were submerged in the container. After 30 s, the imaging sequence was started. The hand was kept in ice water for 90 s. Finally, after allowing another 50 minutes for decay of the tracer, the third imaging sequence was acquired; this was done after pharmacological vasodilatation was induced by intravenous infusion of DIP for 4 minutes (0.56 mg · kg⁻¹ · min⁻¹). Eight minutes after the DIP infusion was begun, the 20 mL N-13 ammonia (20 mCi/740 MBq) bolus was injected and an identical imaging sequence acquired. The dynamic imaging protocol consisted of twelve 10-s, two 30-s, one 60-s, and one 15-minute image (static image).

Throughout, the flow studies heart rate and blood pressure were measured approximately every 60 s. Continuous 12 lead ECG was monitored throughout the study. The rate pressure product was calculated as the average value of 2 measurements of heart rate and systolic blood pressure during the first 120 s of the image acquisition.

Quantification of Blood Flow

The sets of 47 transaxial images from the 16 frames were reoriented into 12 short axis planes as described previously. On the basis of the reoriented short axis images, we generated polar maps of the N-13 ammonia activity distribution and compared them to a database of normals. This was done to ascertain that all participant were actually without any significant coronary artery disease.

MBF was quantified in the vascular territories most commonly supplied by the left anterior descending artery, left circumflex, and right coronary artery. Regions of interest were assigned to tissue areas representing the 3 vascular territories on 3 short axis images (one basal, one mid ventricular, and one apical image). The right ventricle insertion onto the septum was used as a guide to assure identical regions of interest in all studies. For each of the 3 image sequences, a region of interest was centered in the left ventricular blood pool, one in each plane, to obtain 3 arterial input functions. The regions were then copied to the first 120 s of the dynamic imaging sequence to obtain tissue time activity curves for each region.

For each of the vascular territories the 3 tissue curves (basal, mid ventricular, and apical) were averaged and corrected for partial volume effects by assuming a uniform myocardial wall thickness of 1 cm. Both the blood pool and myocardial time activity curves were corrected for physical decay. Tissue time activity curves were then fitted with a previously validated 2 compartment model which corrects for spillover of activity from blood pool into left ventricular myocardium.
Statistical Analysis
Mean values are given with their standard deviations. The paired $t$ test was used to determine differences within each group. Unpaired $t$ test with Bonferroni correction was used to compare the 3 groups. $P<0.05$ was considered significant.

Results
Hemodynamic Findings
The table summarizes hemodynamics and flow measurements obtained during the PET studies. At rest, RPP was slightly higher in the SX group compared with the control group B (8364±1421 versus 7140±709, $P<0.05$). During CPT, the RPP increased in both SX and group B controls ($P<0.01$). The RPP remained significantly higher in the SX group compared with the group B controls during CPT. No changes in RPP occurred after infusion of the total amount of $\text{L-arginine}$ solution. During DIP-induced vasodilation, significant increases in RPP ($P<0.001$) were observed in all study groups.

Myocardial Blood Flow and Myocardial Flow Reserve
Myocardial perfusion values are listed with the RPP corrected values in the Table and are depicted in Figures 1 and 2. Resting flow values were increased in the SX group compared with the control group B (0.83±0.14 versus 0.66±0.14 mL·g$^{-1}$·min$^{-1}$, $P<0.01$) but similar to the group A controls. MBF during CPT was similar in SX patients and group B controls. Because MBF values at rest and during CPT depend on cardiac work flow, rates were also normalized to the RPP, a commonly used index of cardiac work. This correction yielded similar flow results in both SX patients and controls at rest and during CPT. However, DIP-induced hyperemic flow was significantly lower in the SX group compared with both control groups (1.68±0.49 versus 2.31±0.50 [A] and 2.16±0.55 [B] mL·g$^{-1}$·min$^{-1}$, $P<0.01$). Consequently, the coronary flow reserve (ratio between resting and hyperemic MBF) was significantly decreased compared with the control group A (2.03±0.53 [SX] versus 2.96±0.63 [A] mL·g$^{-1}$·min$^{-1}$, $P<0.01$). During DIP, the reduced MBF remained lower after correction for RPP in the SX group compared with both control groups.

Effect of $\text{L-arginine}$ Supplementation
The concentration of $\text{L-arginine}$ increased from 33±8 before infusion to 4278±1370 µmol/L during the last 2 minutes of the infusion. Myocardial perfusion was un-
changed at rest after infusion of L-arginine. Also, during CPT, L-arginine did not significantly affect MBF (Figure 1). Five patients complained of light nausea during L-arginine infusion. Seven patients complained of headache before the study, probably because of withdrawal symptoms from beta blocker treatment. Six of these patients spontaneously reported fewer symptoms after L-arginine infusion.

**Coronary Vascular Resistance Index**

To correct for potential differences in mean arterial blood pressure, an index of coronary vascular resistance (CVR) can be derived from the ratio between mean arterial blood pressure (the driving pressure) and myocardial blood flow and expressed in units of mm Hg \( \cdot \) mL\(^{-1} \cdot \) g\(^{-1} \cdot \) min\(^{-1}\). The CVR index at rest did not differ between SX patients and controls (107\( \pm \)19 [B] versus 104\( \pm \)18 [SX] mm Hg \( \cdot \) mL\(^{-1} \cdot \) g\(^{-1} \cdot \) min\(^{-1}\); \( P=NS \)). However, during DIP-induced hyperemia, the drop in CVR observed in the control group was significantly attenuated in the SX group (60\( \pm \)20 versus 38\( \pm \)10 [B] mm Hg \( \cdot \) mL\(^{-1} \cdot \) g\(^{-1} \cdot \) min\(^{-1}\), \( P<0.01 \)).

**Discussion**

The present study demonstrates that patients with SX have preserved perfusion response to CPT, an index of myocardial microcirculatory endothelial function. In contrast, the hyperemic blood flow response to DIP, which relaxes the resistance vessel smooth muscle cells independent of endothelial function, was substantially reduced. This resulted in a 44\% decrease of the myocardial flow reserve. Infusion of the NO precursor L-arginine had no influence on either the resting myocardial perfusion or the CPT-induced hyperemia.

**Myocardial Blood Flow During DIP-Induced Hyperemia**

We chose to achieve coronary vasodilation with DIP because pharmacologically induced hyperemia is reduced specifically with DIP, whereas adenosine and papaverine may yield preserved CFR in patients with SX.\(^{19} \) In normal healthy controls, DIP decreases coronary vascular resistance to about 30\% of normal resting values.\(^{20} \) The reduction induces reflex tachycardia but has little effect on blood pressure. The SX patients in this study had a normal hemodynamic response to DIP infusion. However, in accordance with previous studies,\(^{5,7,8,21} \) the flow increase was substantially decreased and the resistance only declined to approximately 55\% of resting values.

DIP-induced coronary vasodilation is a result of an adenosine effect on resistance vessels mediated by inhibition of the reuptake of adenosine released by cardiac myocytes. Adenosine binds to A\(_2\) purinoceptors on the vascular smooth muscle cells, stimulating adenylate cyclase and increasing intracellular cAMP, which mediates smooth muscle relaxation. Papaverine is a nonspecific smooth muscle relaxant which acts predominantly by inhibiting cAMP phosphodiesterase.\(^{8} \) Because DIP, adenosine, and papaverine act differently on the cell, the impaired response to DIP could result from an abnormality of adenosine metabolism. It has been proposed that adenosine production is impaired in these patients.\(^{19} \) This mechanism would explain not only why DIP is a less effective vasodilator than exogenously administered adenosine but also why exercise and atrial pacing, dependent on adenosine mediated vasodilation, cause submaximal increases in MBF.

Several findings in the present study support the presence of a microvascular dysfunction involving abnormalities in adenosine metabolism in patients with SX: (1) basal MBF was elevated and unstimulated presence of adenosine was abnormally high, (2) CFR was reduced with DIP as the vasodilating agent, stimulated production of adenosine was abnormally low, and (3) none of the patients revealed metabolic evidence of myocardial ischemia during atrial pacing.

Our data therefore lend support to the hypothesis that an inappropriate, patchily distributed prearteriolar constriction or inadequate dilation may induce compensatory release and accumulation of adenosine, which reaches concentrations sufficient to stimulate cardiac afferent nerves.\(^{22,23} \) Even in the absence of ischemia, this mechanism can explain the heterogeneous findings in patients with SX. At one end of the spectrum, involvement of a considerable number of arterioles can explain the reduced CFR in some but not all patients with SX and the presence of myocardial ischemia in a few patients. At the other end, the involvement of a very limited number of arterioles can explain the occurrence of pain in the absence of detectable signs of ischemia, because adenosine in addition to its vasodilatory effect also is a well-known pain messenger.\(^{24–26} \) Finally, accumulation of adenosine in the extra-cellular compartment of the myocardium alters electrical conduction and causes electrocardiographic changes.\(^{41} \)

The mechanisms responsible for the purported increase of the prearteriolar vasomotor tone cannot be identified with the results of the present study. Although an endothelium-dependent mechanism seems less likely, an increased sympathetic tone\(^{27,28} \) cannot be excluded and may gain support from the finding of increased RPP and increased resting myocardial blood flow in the patient group. In addition, abnormal responses to vasconstrictor stimuli such as neuropeptide Y\(^{29} \) and endothelin\(^{30,31} \) have been demonstrated.

**Effect of CPT and Influence of L-arginine Supplementation**

A selective impairment of the endothelium-dependent microvascular vasodilation has been proposed as a possible cause of reduced CFR.\(^{31} \) On the basis of a documented close correlation between coronary sinus blood flow and the response to acetylcholine and atrial pacing, this study suggests that microvascular endothelial dysfunction could play a central role in the reduction of the CFR.\(^{6} \)

Until now, impairment of endothelial-dependent microcirculatory vasodilation has been demonstrated using acetylcholine. Acetylcholine relaxes blood vessels by means of muscarinic receptors that stimulate the synthesis and release of endothelium-derived relaxing factor identical to
NO. Acetylcholine has a dual effect on the vascular smooth muscle; it causes relaxation, which is strictly dependent on the presence of intact endothelium, and it also causes vasoconstriction which results from stimulation of specific muscarinic receptors located on the smooth muscle cells. The net resulting coronary vasomotor response depends on the balance between the 2 opposing effects. Therefore, an impaired response to acetylcholine may not only be attributable to impaired endothelium-dependent vasodilation but also to enhanced smooth muscle cell muscarinic receptor response, altered signal transduction properties, or reduced production, release, or diffusion of NO.

In the present study, we sought to stimulate a physiological endothelium-dependent vasodilation specifically of the microvasculature using CPT. The rationale behind this approach was that the degree of microcirculatory vasoconstriction determines myocardial perfusion in the absence of epicardial stenoses. During CPT, the ensuing discomfort increases cardiac contractility and heart rate. Subsequent increases of shear stress stimulate the coronary endothelial cells to release NO, which mediates microcirculatory smooth muscle relaxation and increased perfusion, as also observed in the present study. The cold-induced vasodilation of normal coronary arteries is abolished by blocking NO with NG-monomethyl-L-arginine (L-NMMA) indicating that endothelial-derived NO accounts for vasodilation in response to CPT. In accordance with our findings, a preserved coronary vasodilator response to CPT has recently been demonstrated in patients with SX.

The finding of a similar myocardial perfusion response to CPT in patients and controls indicates that SX-patients react adequately to the approximately 20% increase in RPP.

A defect in the enzymes catalyzing production of NO has been proposed as a possible pathological mechanism for the endothelium dependent dysfunction. NO is believed to play a central role in the regulation of coronary tone. NO is produced primarily in the endothelial cells, and release of NO is stimulated by several substances and shear stress. The substrate for NO production is L-arginine and the production is controlled primarily by 2 NO synthetase enzymes I-NOS and E-NOS. We found no alterations of resting perfusion following L-arginine supplementation. Therefore, decreased substrate delivery cannot constitute a pathophysiological mechanism in SX patients. Furthermore, L-arginine supplementation seems to be unable to increase the microcirculatory reactivity to endothelium stimulation. This is in contrast to the findings of Egashira et al, who have demonstrated an increased epicardial flow response to acetylcholine after treatment with L-arginine. Several explanations can be offered for these discrepancies. First of all, a different mode of stimulation has been used. Secondly, an increased flow rate in the epicardial vessels does not necessarily represent an increase in perfusion. The activation of epicardial vessel by stimulation with acetylcholine may also activate collateral circulation, which does not reflect true perfusion. Finally, differences in patients characteristics may account for the different results.

Methodological Considerations
Alterations of CFR must be interpreted with caution because CFR can be reduced either by an elevation of basal coronary flow or by a reduction of maximal hyperemic flow. Therefore, we measured MBF by PET and present actual values of flow per unit mass at rest, during CPT, and after DIP. Patients with SX had elevated MBF at rest, but the reduced CFR was mainly caused by a reduction of the actual increase in blood flow after DIP.

The coronary perfusion pressure influences maximal vasodilation. To correct for potential differences in blood pressure, we derived the CVR, which is useful for comparison of the study groups, when the blood pressure and RPP are different.

Limitations of the Study
The current study investigated only one regimen of L-arginine administration, namely a 1-hour infusion of 30 g. Consequently, no dose-relationships could be established. However, the dose is greater than or equal to the doses used in invasive studies of endothelial dysfunction. The pharmacological regimen caused a documented approximately 100-fold increase in the L-arginine plasma concentration. Thus, it is unlikely that an insufficient dose is responsible for the impaired increment of perfusion after L-arginine in the patient group. A possible study limitation is the fact that the L-arginine could have affected the general ammonia handling to such an extent that the tracer uptake used for quantification of flow had been altered. However, the 2 compartment ammonia flow model used has proven very robust to metabolic changes in general and insensitive to changes in insulin and glucose concentrations and pH.

For ethical reasons our control group A did not undergo coronary arteriography. This procedure would enhance the probability that none of these controls had any significant coronary artery disease. If, however, any of the normal controls had coronary artery disease, it would tend to reduce the hyperemic response and, consequently, lead to less pronounced differences between the groups. In order to compensate for this, we included the control group B. This group has an extremely low risk of endothelial dysfunction and early signs of coronary artery disease.

Although our results are in accordance with a microvascular dysfunction involving adenosine metabolism, it must be noted that papaverine-induced vasodilation, which is independent on adenosine production, may be attenuated at least in a proportion of patients with SX. Furthermore, some patients with SX have evidence of coronary endothelial dysfunction together with myocardial ischemia. The extensive conflicting data in the literature reflect the heterogeneous nature of the disorder. We believe that SX encompasses several pathophysiological disease entities; these may even include disturbances without any evidence of vascular dysfunction, such as abnormal pain perception and disturbed electrolyte handling.

We did not look at the effect of L-arginine on the DIP-induced flow increase. It could be argued that DIP response is a combined response of endothelium-dependent and -independent flow increase because the relaxation of the smooth muscle cells and the resultant flow increase would also stimulate the endothelium. However, the endothelium-dependent and -independent responses are not only of differ-
ent mechanisms but also of very different magnitudes. Typical data would yield an increase in perfusion of approximately 15% to 25% after endothelium stimulation, whereas adenosine-mediated vasodilatation increases flow by 200% to 350%. In the most optimistic case, the full effect of the endothelium shear stress stimulation is activated during adenosine stimulation. In another very optimistic approach, the l-arginine increases the endothelium-dependent fraction of the flow increase by 50%. This would mean that flow increase would go up from 200% to 210%, a 5% increase. Given the individual variation with standard deviations in the 15% range, a simple sample size calculation (power 80%, alpha 0.05) yields a minimal sample size of 73; in light of these very optimistic presumptions, we found this to be unrealistic.

Clinical Implications

Our study supports the hypothesis that the microcirculation is abnormal. The abnormality is independent of the endothelial function in patients with SX. The fundamental underlying mechanism is not clear but does not involve myocardial ischemia in most cases. The abnormality seem to involve adenosine metabolism, providing a rationale for treatment with antagonists of adenosine receptors, eg, aminophylline.

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