Approximately 10–20% of patients with typical angina pectoris have normal coronary arteries, and most of these patients are concluded to have syndrome X. However, syndrome X is a heterogeneous group that includes slow coronary flow (SCF), which is characterized by late opacification of the epicardial coronary arteries without occlusive disease. SCF was first described by Tambe et al in 1972 and the exact etiopathogenesis is still unknown. Endothelial and vasomotor dysfunction, microvascular dysfunction, and occlusive disease of small coronary arteries have been suggested.

Endothelin-1 (ET-1) is the strongest known vasoconstrictor peptide and has significant effects on the cardiovascular system. It increases coronary vascular resistance and has positive inotropic effects on cardiomyocytes and is mitogenic for smooth muscle cells. Because of these properties, it contributes to the progression of atherosclerosis. Recent studies have indicated that peripheral immune ET-1 concentrations are also elevated in patients with chest pain and angiographically patent coronary arteries (syndrome X patients). Furthermore, Lubov et al found that ET-1 is indeed released during exercise and is related to the severity of the ischemia as reflected by perfusion defects on single photon emission computerized tomography (SPECT) sestamibi. It has also been suggested that abnormalities in nitric oxide (NO)-mediated endothelium-dependent dilation of small coronary arteries may play a role in the pathogenesis of syndrome X.

In this study, the endothelin-1 (ET-1) and nitric oxide (NO) concentrations in slow coronary flow (SCF) patients were assessed before and at the peak of the exercise stress test and compared with the values from healthy controls. The study population was 25 patients who underwent coronary angiography and were diagnosed as SCF (11 females (44%), aged 56.7±9.8 years) and 20 normal subjects (9 females (45%), aged 54.3±9.2 years). Mean TIMI frame count in the patients was 54.1±13.4. Blood samples were drawn at rest and immediately at the end of exercise testing. The baseline ET-1 concentrations of the control subjects were lower than those of the patients (7.0±4.5 pg/ml vs 11.1±5.9 pg/ml, p<0.0001) and this difference increased after exercise (6.2±4.3 pg/ml vs 20.1±10.4 pg/ml, p=0.0001). Post-exercise ET-1 concentrations were significantly higher than baseline in patients with SCF (p<0.0001) and a reduction in the ET-1 concentrations was observed in control subjects (p<0.05). Baseline NO concentrations of the patients were lower than those of the control subjects (27±5.1 pmol/L vs 31.2±4.9 pmol/L, p=0.0001). Although the NO concentrations in both groups were significantly increased after exercise (29.4±5.9 pmol/L vs 33.3±5.6 pmol/L, p<0.05 for both), the difference was not significant. A significant negative correlation among post-exercise ET-1 concentrations and maximal heart rate, exercise duration and exercise rate–pressure product, and a significant positive correlation among post-exercise NO concentrations and maximal heart rate and exercise duration were observed in both groups. The results of this study show that endothelial function (assessed by ET-1 and NO concentrations) and its response to exercise were abnormal in SCF patients compared with healthy subjects, and this may play some pathophysiologic role.
ventricular hypertrophy and contraindication for exercise testing. After signed informed consent was obtained, all concomitant medication was stopped for at least 5 half lives before the test. As a control group, we studied 20 normal subjects (9 females (45%); aged 54.3±9.2 years), none of whom had evidence of structural cardiac or systemic diseases, and all had normal exercise and echocardiographic studies; only 3 were current smokers.

The study was carried out according to the principles of the Declaration of Helsinki and approved by the Investigational Review Board of Mersin University, School of Medicine.

Coronary Angiography and TIMI (Thrombolysis in Myocardial Infarction) Frame Count

Coronary angiography used the femoral approach and standard Judkins technique to demonstrate the coronary arteries in the left and right oblique planes, and cranial and caudal angles. Left ventricular and aortic pressures were obtained. Lopromide (Ultravist-370, Schering AG) was used as the contrast agent and was manually injected (6–8 ml at each position). The proximal coronary lumen diameter was measured by a quantitative computer-assisted (QCA) facility and those with a caliber of 3 mm or more were enrolled for further SCF measurements. For the quantitative measurement of coronary blood flow, the time elapsed from the appearance of contrast agent until it reached the distal end of the coronary arteries in terms of cineframe count was considered to be the TIMI frame count.10,19 Thereafter, the final count was subtracted from the initial and the exact TIMI frame was calculated for the given artery. The cut-off values for the TIMI frame counts were taken from the study of Gibson et al19 in which 78 normal coronary arteries were evaluated. Their cut-off values because of the length of time needed for normal visualization of the coronary arteries were 36.2±2.6 frames for the left anterior descending coronary artery (LAD), 22.2±4.1 frames for the left circumflex coronary artery (LCX) and 20.4±3 frames for the right coronary artery (RCA). They proposed that the TIMI frame count for the LAD was 1.7-fold higher than those of the LCX and RCA. In their study, the reason given for this differences was that the LAD is 1.5-fold longer than RCA and 1.6-fold longer than LCX anatomically. Therefore, the TIMI frame count was divided by 1.7 when the LAD was the case, for adjusted correction. The corrected cut-off value for the LAD was 21.1±1.5 frames. Any values obtained above these thresholds were considered to be SCF in our study. The TIMI frame counting was undertaken separately by 2 cardiologists and in the case of conflict, the frames were referred to a third one. All TIMI frame counts were measured in matched projections using Medcon Telemedicine Technology (version 1.900, Israel).

Exercise Treadmill Testing

The 12-lead maximal effort exercise tests were performed using standard graduated treadmill protocols consistent with American Heart Association guidelines20 with all medications discontinued for at least 5 half lives before evaluation. Patients were encouraged to give their maximal effort, but not to allow their angina to reach levels higher than previously experienced. The results were analyzed and reported using a computerized database (EXTRA; Mosby Publishers; Chicago, IL, USA).21 The ST-segment response considered was the most horizontal or downsloping ST-segment depression in any lead, except aVR, during exercise or recovery. An abnormal response was defined as ≥0.1 mV at 0.08 s after the J point of horizontal or downsloping ST-segment depression and chest pain onset during exercise.

Blood samples were drawn from the forearm vein at rest and immediately at the end of exercise testing.

Analysis of ET-1

Plasma samples were drawn into chilled EDTA tubes (1 mg/ml blood) containing aprotinin (500 KIU/ml of blood). The whole blood samples were centrifuged at 1,600 G for 15 min at 0 °C. The plasma fractions were transferred to a plastic tube and stored at –70°C for long-term storage. After a short incubation the excess sample was washed out and a polyclonal antibody to ET-1 labeled with the enzyme horseradish peroxidase was added. This labeled

### Table 1 Baseline and Post-Exercise Endothelin-1 and Nitric Oxide Concentrations in Patients With Slow Coronary Flow (SCF) and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>SCF (n=25)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1 (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>11.1±5.9*</td>
<td>7.0±4.5</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>20.1±10.4*</td>
<td>6.2±4.3†</td>
</tr>
<tr>
<td>Nitric oxide (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>27±5.1#¶</td>
<td>31.2±4.9</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>29.4±5.9&lt;£</td>
<td>33.3±5.6</td>
</tr>
</tbody>
</table>

*p<0.0001 (comparing baseline concentrations between SCF and controls); †p<0.0001 (comparing baseline and post-exercise concentrations in SCF group); #p<0.0001 (comparing post-exercise concentrations between SCF and controls); §p<0.05 (comparing baseline and post-exercise concentrations in SCF group); ¶p=0.0001 (comparing baseline concentrations between SCF and controls); £p<0.05 (comparing baseline and post-exercise concentrations between SCF and controls); ¥p=0.0001 (comparing post-exercise concentrations between SCF and controls).

### Table 2 Exercise Parameters of the Slow Coronary Flow (SCF) Patient and Control Groups

<table>
<thead>
<tr>
<th></th>
<th>SCF (n=25)</th>
<th>Controls (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>84±15</td>
<td>79±19</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline systolic blood pressure (mmHg)</td>
<td>129±22</td>
<td>124±27</td>
<td>NS</td>
</tr>
<tr>
<td>Peak exercise heart rate (beats/min)</td>
<td>152±16</td>
<td>168±13</td>
<td>0.0001</td>
</tr>
<tr>
<td>Peak exercise systolic blood pressure (mmHg)</td>
<td>150±14</td>
<td>165±14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Exercise duration (s)</td>
<td>440±124</td>
<td>522±131</td>
<td>0.05</td>
</tr>
<tr>
<td>Exercise time to angina pectoris (s)</td>
<td>395±124</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Exercise time to &gt;0.1 mV ST segment depression (s)</td>
<td>412±156</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maximal ST-segment depression (mV)</td>
<td>0.13±0.11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rate–pressure product [(mmHg×beats/min)]×10²</td>
<td>227±57</td>
<td>279±76</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
antibody bound to the ET-1 captured on the plate. After a short incubation the excess labeled antibody was washed out and substrate was added. The substrate reacted with the labeled antibody bound to the ET-1 captured on the plate. The color generated by the substrate was read at 450 nm, and was directly proportional to the concentration of ET-1 in the sample (Human Endothelin-1, catalog no EIA-3111, DRG International Inc. USA).

Analysis of Serum Nitrite and Nitrate Concentrations

The concentrations of nitrite and nitrate were determined using a procedure based on the Griess reaction. Blood samples were centrifuged at 4,000 rpm for 10 min. Serum samples were then separated and stored at −70°C until assay. Equal volumes of serum and potassium phosphate buffer were placed in an ultrafilter and centrifuged at 4,000 rpm for 45 min. The ultrafiltrate was collected and used in the test. Nitrates were quantitatively converted to nitrites for analysis. Enzymatic reduction of nitrate to nitrite was carried out using coenzymes (NADPH, FAD) in the presence of nitrate reductase in a stepwise incubation assay. N-1-(naphthyl) ethylenediamine dihydrochloride, sulfanilamide and incubation solutions were mixed at a ratio of 1:1:2 (v/v). These mixtures were incubated for 5 min at room temperature and measured at 540 nm. Sodium nitrite of 1.00 mmol/L was used as standard for determination of nitrite and potassium nitrate of 80 mmol/L was used as standard for determination of nitrate (Nitric oxide colorimetric assay, 1-756-281, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS software package (Version 10.0, SPSS Inc, Chicago, IL, USA). Paired t-test was used to compare the baseline and post-exercise values of NO and ET-1. Comparison of changes in these variables in both groups were analyzed with an independent-t test. Correlations were examined by Pearson’s correlation. Continuous variables were expressed as means±SD. All hypothesis testing was 2-tailed. A p-value of <0.05 was considered significant.

Results

Baseline and post exercise ET-1 and NO concentrations of the patients with SCF and the control group are given in Table 1. The 2 groups did not differ in age, sex, blood cholesterol, or baseline heart rate and blood pressure. Exercise was stopped in 9 patients because of chest pain (heart rate 128–156 beats/min) and in 10 patients because of ≥0.1 mV ST segment depression with a heart rate of

Fig 1. Endothelin-1 and nitric oxide concentrations in slow coronary flow patients with and without ≥0.1 mV ST depression and angina pectoris during an exercise test.
134–170 beats/min. Control subjects completed the whole protocol and none showed ST-segment changes, tachyarrhythmias or chest pain throughout the procedure. Peak exercise heart rate, peak exercise duration, peak exercise systolic blood pressure and rate–pressure product were significantly higher in the control group than in the patients. The exercise parameters of both groups are summarized in Table 2.

Baseline ET-1 concentrations of the control subjects were lower than those of the patients (7.0±4.5 pg/ml vs 11.1±5.9 pg/ml, p<0.0001) and this difference was significantly increased after exercise (6.2±4.3 pg/ml vs 20.1±10.4 pg/ml, p<0.0001), (Table 1). The post-exercise ET-1 concentrations were significantly higher than baseline in the patients with SCF (p<0.0001) and a slight reduction was observed in the control subjects (p<0.05).

Baseline NO concentrations of the patients were lower than those of the control subjects (27±5.1 μmol/L vs 31.2±
Although the difference was not significant, the NO concentrations in both groups were significantly increased (29.4±5.9 μmol/L vs 33.3±5.6 μmol/L, p<0.05 for both) after exercise; patients still had lower NO values than controls (p=0.0001) (Table 1).

SCF patients with ST depression of ≥0.1 mV had significantly increased peak exercise ET-1 (29.02±6.47 pg/ml vs 15.12±8.62 pg/ml) and less increased NO (26.06±6.52 μmol/L vs 31.55±4.35 μmol/L) concentrations than the patients without ST changes (Fig 1). Patients who had angina during exercise also had higher ET-1 (26.1±8.71 pg/ml vs 16.62±9.1 pg/ml) and lower NO (26.1±6.68 μmol/L vs 31.12±4.51 μmol/L) concentrations than patients without angina (Fig 1).

A significant negative correlation among post-exercise ET-1 concentrations and maximal heart rate, exercise duration and exercise rate – pressure product (Fig 2), and a significant positive correlation among post-exercise NO concentrations and maximal heart rate and exercise duration were observed in both groups (Fig 3). However, peak exercise systolic blood pressure was not associated with either ET-1 or NO concentrations.

The mean TIMI frame count was 54.1±13.4 (computed in 16 patients from LAD, 7 patients from LCX, and 2 patients from RCA) in the SCF patients. TIMI frame count positively correlated with baseline ET-1 concentrations and negatively correlated with NO concentrations (Fig 4). Mean vessel diameter was 3.6±0.4 mm. No correlation existed among diameter and ET-1, NO and TIMI frame count.

Neither age nor gender was related to ET-1 and NO concentrations.

**Discussion**

The results of this study show that baseline ET-1 plasma concentrations were higher and NO plasma concentrations were lower in patients with SCF than in a matched group of control subjects. Furthermore, the differences in the ET-1 concentrations between the 2 groups became greater after the exercise treadmill test, as a result of a significant increase in ET-1 in patients with SCF. Although the NO concentrations of both groups increased with exercise, the difference between 2 groups did not change and was still lower in the SCF group (Table 1).

Some biopsy studies of patients with SCF have shown that SCF could be the result of increased resistance in arterioles\(^6,8,9\). Mangieri et al\(^9\) and Kurtoglu et al\(^10\) have observed remarkable progress in restoring coronary flow when they studied the effect of dipyridamole therapy in this group of patients. All these studies support the theory that the pathophysiology underlying this disorder is closely related to the microvasculature and has a dynamic character. On the other hand, Von Lider et al have shown that coronary flow reserve confirmed the extremely slow blood flow velocity in a patient with SCF, but that coronary flow reserve and coronary blood flow were within the normal range\(^5\); and these findings suggest that SCF may not always be a microvascular disease. They speculated that SCF may be caused by epicardial artery disease such as coronary ectasia.

The findings to date do not exactly and clearly delineate the borders of this disorder nor do they imply any interaction between the micro and macrovasculature of the heart. Accordingly, some postmortem studies have revealed a co-incidence of epicardial and small vessel disease\(^22,23\). According to previous reports, in the early phase of atherosclerosis or with strong risk factors for coronary artery disease, the vasodilation capacity of coronary resistive arterioles by pharmacologic and physical stress was disturbed before the development of angiographic atherosclerotic disease\(^24,25\). Additionally, intravascular ultrasound (IVUS) can detect intimal thickening and therefore indicate early atherosclerosis, which cannot be detected by conventional angiography\(^26-28\). Wiedermann et al used IVUS to demonstrate that most patients with syndrome X have abnormal coronary arteries, and they identified 3 distinct morphologic subgroups: normal coronary arteries, atheromatous plaque and intimal thickening\(^29\). Exercise-vasomotion is normal in patients with syndrome X who have normal coronary arteries on IVUS; patients with abnormal arteries (plaque or intimal thickening) have an abnormal (constrictive) response to exercise. Accordingly, as the present results support, the abnormal rest and exercise ET-1 and NO responses because of diseased epicardial coronaries (such as plaque or intimal thickening) would explain SCF.

ET-1 and NO are key molecules in the normal autoregulatory mechanisms; that is, modulating the vasodilator response to tachycardia and exercise\(^30,31\). Endothelial injury causes an increase in the plasma concentration of ET-1\(^32\). Endothelial dysfunction in patients with syndrome X is
also associated with an increased release of ET-1 after rapid atrial pacing.\textsuperscript{33} On the other hand, the epicardial coronary artery dilatation induced by pacing depends on the release of endogenous NO.\textsuperscript{34} Tousoulis et al reported that endothelin in the region of atheromatous stenosis can produce NO even in a normal amount.\textsuperscript{\textit{28}} In the present study, the increase in NO concentrations post exercise may be explained by that finding. Dysfunction and resistance of diseased microvasculature to NO may be another cause of angina and ST depression despite the increased concentrations of NO.\textsuperscript{\textit{20}} However, in the present study SCF patients with ST depression and angina had lower peak exercise NO and higher peak exercise ET-1 concentrations than patients who had not.

In a previous study, Sezgin et al concluded that there was probable endothelial dysfunction in SCF patients? The endothelial dysfunction in the small coronary arteries (increased ET-1 concentrations) in patients with angina pectoris and normal coronary angiography may increase coronary resistance and so cause myocardial ischemia.\textsuperscript{\textit{35,36}} The effects of adrenergic stimulation, such as exercise, on the coronary microvascular tone complex are controversial. Sympathetic activation increases coronary flow indirectly through metabolic vasodilatation secondary to an increase in heart rate and myocardial contractility and, in part, to endothelium-mediated vasodilation.\textsuperscript{\textit{35}} On the other hand, it may have both direct vasodilator and vasoconstrictor effects through $\beta$- and $\alpha$-receptor stimulation, respectively.\textsuperscript{\textit{37}} The net effect likely depending on the pathophysiological state of the small coronary arteries. Indeed, increased sympathetic stimulation may cause abnormal microvascular constriction in endothelial dysfunction.\textsuperscript{\textit{39}} Additionally, ET-1 can modulate central and peripheral sympathetic outflow.\textsuperscript{\textit{40}} In the present study, we observed that patients with angina and ST depression >0.1 mV had higher peak exercise ET-1 and lower NO concentrations. As well, a significant negative correlation among post-exercise ET-1 concentrations and maximal heart rate, exercise duration and exercise rate pressure product, and a significant positive correlation among post-exercise NO concentrations and maximal heart rate and exercise duration were observed in all subjects. Therefore, increased sympathetic activity during exercise may contribute to decreased coronary flow by the previously discussed mechanisms.

Angiographic evaluation of SCF was first defined by Tambe et al\textsuperscript{a} and Gibson et al\textsuperscript{\textit{19}} developed the TIMI frame count system, using it particularly to evaluate coronary artery patency and flow velocity after thrombolytic treatment in patients with acute myocardial infarction. Later, this system was used to quantitatively evaluate flow velocity in patients with SCF.\textsuperscript{\textit{41}} The TIMI frame count system is an important method of giving an objective numeric value of anterograde blood flow in patients with SCF.\textsuperscript{\textit{10}} In another previous study, Goel et al\textsuperscript{\textit{4}} found that patients with SCF had more frequent typical angina pectoris and positive exercise test results than patients with normal coronary flow. They conclude that SCF patients constitute a definite subset within the wide spectrum of syndrome X and that the phenomenon of SCF could be used as a marker for myocardial ischemia. Accordingly, in the present study, the TIMI frame count positively correlated with ET-1 and negatively correlated with NO in the SCF patients, which supports the theory of the development of myocardial ischemia in these patients.

In conclusion, baseline and peak exercise ET-1 and NO concentrations are impaired in patients with SCF, particularly in patients with angina and ST depression >0.1 mV. These findings suggest that endothelial dysfunction may play an active role in the pathophysiology of SCF and myocardial ischemia in these patients.

**References**


