Endothelial Activation in Patients With Cardiac Syndrome X

Giovambattista Desideri, MD; Achille Gaspardone, MD; Marco Gentile, MD; Anna Santucci, MD; Pier Agostino Gioffrè, MD; Claudio Ferri, MD

Background—The presence of endothelial dysfunction with increased endothelin-1 plasma concentrations in patients with cardiac syndrome X is still under debate. The aim of the present study was to evaluate the presence of endothelial dysfunction in patients with cardiac syndrome X.

Methods and Results—Endothelin-1 levels were evaluated with a sensitive radioimmunoassay with previous purification through reverse phase HPLC in 24 patients (3 men and 21 women, mean age 54\pm7 years) with typical angina, instrumental evidence of ischemia, and normal coronary angiograms both under baseline conditions and after oral glucose load (75 g d-glucose). We also measured plasma nitrite-plus-nitrate levels, a sharp index of endothelial nitric oxide production, and circulating concentrations of the soluble fraction of the endothelial adhesion molecule vascular cell adhesion molecule-1, a well-recognized marker of early endothelial dysfunction. Fourteen healthy subjects (1 man and 13 women, mean age 47\pm15 years) served as controls. There were no significant differences in baseline plasma endothelin-1 concentrations between patients and control subjects (0.55\pm0.34 versus 0.48\pm0.22 pg/mL, \(P=0.503\)). Plasma nitrite-plus-nitrate and soluble vascular cell adhesion molecule-1 concentrations were also similar between the 2 groups. After glucose ingestion, circulating endothelin-1 concentrations were significantly higher in patients with cardiac syndrome X than in control subjects (\(P<0.03\) at 60, 90, and 120 minutes).

Conclusions—Our findings show that no basal endothelial damage is present in patients with cardiac syndrome X. Nevertheless, increased responsiveness of endothelin-1 to glucose loading suggests that patients with cardiac syndrome X present an increased susceptibility to releasing endothelin-1 under stressful circumstances. (Circulation. 2000;102:2359-2364.)

Key Words: endothelin • cell adhesion molecules • endothelium-derived factors • angina

A number of patients manifest angina-like chest pain despite the presence of myocardial ischemia but normal coronary angiograms.\(^1,2\) Because a definite nosography of such condition is still under debate, the term “cardiac syndrome X” has become popular to identify some of these patients with typical anginal pain, noninvasive tests indicative of myocardial ischemia, angiographically normal epicardial vessels with no inducible coronary artery spasm, and no known associated cardiovascular diseases.\(^3,6,7\) Coronary microcirculation dysfunction has been proposed as the cause of angina.\(^3,6,7\) Indeed, a sizeable proportion of patients with cardiac syndrome X exhibit an alteration of coronary microcirculation as suggested by an abnormally small increase in coronary blood flow in response to dipyridamole or pacing\(^3,8,9\) and increased heterogeneity of myocardial perfusion in small myocardial regions both at rest and during dipyridamole infusion.\(^10,11\)

Elevated levels of the potent endothelium-derived vasoconstrictive peptide endothelin-1 (ET-1)\(^12\) have been found in patients with cardiac syndrome X.\(^13,14\) Because ET-1 is a potent vasoconstrictor of coronary microcirculation\(^15\) and a marker of endothelial damage,\(^16–18\) it was hypothesized that elevated ET-1 levels might be involved in the pathogenesis of microvascular dysfunction in cardiac syndrome X.\(^13\) Accord- ing to this, elevated ET-1 activity was recently found to be associated with reduced coronary vasomotor responses in patients with chest pain and normal coronary arteriograms.\(^19\) Although a report by Hoffman et al\(^20\) confirmed this hypothesis, normal levels of circulating ET-1 and big ET-1 were recently observed in patients with cardiac syndrome X.\(^21\) Furthermore, peripheral resistance vessel sensitivity to ET-1 was found to be reduced rather than increased due to ET-1 type A receptor downregulation.\(^21\) Thus, the existence of abnormal production of ET-1 in patients with cardiac syndrome X is still under debate.

To elucidate this aspect, we evaluated circulating ET-1 concentrations in patients with cardiac syndrome X through...
the use of a newly developed sensitive radioimmunoassay with previous reverse phase HPLC extraction.\textsuperscript{22,23} Moreover, circulating concentrations of nitrite plus nitrate, a sharp index of vascular nitric oxide production,\textsuperscript{14,23,24} and soluble vascular cell adhesion molecule (VCAM)-1, a sensitive marker of early endothelial dysfunction,\textsuperscript{25–27} were evaluated. Finally, because oral glucose loading affects circulating levels of ET-1\textsuperscript{28} and because patients with cardiac syndrome X present with abnormal glucose tolerance,\textsuperscript{29,30} we also measured ET-1 during an oral glucose tolerance test.

**Methods**

**Patients With Cardiac Syndrome X**

The present study included 24 consecutive patients (3 men and 21 women, mean age 54±7 years) who were referred to our institution for suspected coronary artery disease. Some of the study cohort had participated in previous studies.\textsuperscript{29,30} The entry criteria were recurrent typical chest pain at rest and on effort, a normal 12-lead ECG at rest, repeatedly positive exercise tests for ischemia-like ECG changes (horizontal or downsloping ST-segment depression of >1.5 mm at 60 ms after the J point in ≥2 contiguous leads that lasts for >1 minute), normal left and right ventricular function at rest, the absence of valvular heart disease and myocardial hypertrophy on M- and B-mode echocardiography, the absence of clinical and ultrasonographic evidence of atherosclerotic lesions of the neck and limb vessels, sitting systolic/diastolic blood pressure levels of <140/90 mm Hg in the absence of antihypertensive medications, no heredity for essential hypertension and/or diabetes, and no concomitant acute and chronic diseases.

All patients had a recent normal coronary angiogram without evidence of focal or diffuse coronary spasm after intracoronary infusion of ergonovine maleate.\textsuperscript{20} TI scintigraphy was positive for adenosine-induced regional uptake abnormalities in 12 patients. All patients discontinued cardiovascular treatment 10 days before they entered the study. Fourteen women were in menopause; of these women, 5 had undergone surgical hysterectomy in the past (>2 years). None was taking substitutive medications.

**Control Group**

As previously reported,\textsuperscript{29,30} 14 age-, sex-, and weight-matched (1 man and 13 women, mean age 47±15 years) healthy volunteers who had been recruited among the physicians and nurses of our institution served as controls. None had a genetic predisposition for hypertension or diabetes. Further, their clinical examinations were normal, they had no concomitant acute or chronic diseases, and they did not take any kind of drugs or dietary supplements, including vitamins, antioxidants, and so on. Their chest radiography, resting ECG, M- and B-mode echocardiography, exercise stress test, and clinical and ultrasound evaluations of the neck and limb vessels were also normal. Menopause was present in 6 women, and none were taking substitutive medications.

**Glucose Tolerance Test**

As previously described,\textsuperscript{29} patients and control subjects followed their usual diet until the morning of the study day. After an overnight fast, they were given a glucose solution to drink (75 mg d-glucose in 200 mL tap water). Venous blood samples were taken during fasting and at 30, 60, 90, 120, and 180 minutes after the oral glucose load.

This study was approved by our institutional review committee. All subjects gave their informed consent.

**Laboratory Measurements**

Venous blood samples were obtained from a large antecubital vein 60 minutes after venous catheterization to avoid interference due to blood stasis.\textsuperscript{31} Patients and control subjects remained seated and at rest during this time interval. The venous catheter was kept patent with saline infusion (0.2 mL/h) until blood was drawn into chilled EDTA tubes on ice. Plasma was separated by centrifugation (2000g for 15 minutes at 4°C) and stored at −80°C until assayed.

Plasma ET-1 levels were assessed with reverse phase HPLC followed by radioimmunoassay (Peninsula Laboratories) according to a previously described method.\textsuperscript{22,23} Intra-assay and interassay variations were <10%. Purification through HPLC allowed us to recognize a single peak that corresponded perfectly to ET-1; neither ET-2, ET-3, nor big ET-1 was present in the HPLC eluates.\textsuperscript{22,23} The sensitivity of the assay was 0.2 pg/mL. Circulating soluble VCAM-1 concentrations were assessed with an enzyme-linked immunosorbent assay (R & D Systems).\textsuperscript{26,27} Nitric oxide activity in plasma was evaluated as nitrite-plus-nitrate concentration according to the well-recognized method based on the use of Griess reagents.\textsuperscript{23,24}

Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed with enzymatic methods (Boehringer-Mannheim; in the case of HDL cholesterol, after precipitation of LDL cholesterol with phosphotungstate). Serum LDL cholesterol levels were assessed according to the Friedewald method.\textsuperscript{52} Plasma glucose levels were assessed at baseline and 30, 60, 90, 120, and 180 minutes after the oral glucose load according to the glucose oxidation method and with a glucose analyzer II (Beckman Instruments).

**Statistical Analysis**

Continuous normally distributed data are expressed as mean±1 SD and were analyzed by 2-way unpaired Students’ t test. Proportions were compared by χ² test. Two-factor ANOVA for repeated measures and 1 factor was used to compare ET-1 data during glucose tolerance test in patients with cardiac syndrome X or control subjects. When significant differences were detected, pairwise comparisons were made by Scheffe’s F test. Differences were considered to be statistically significant at P<0.05.

**Results**

The general characteristics of the study populations are shown in Tables 1 and 2. Total cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride concentrations were higher in patients with cardiac syndrome X than in control subjects (Table 1). Although plasma glucose levels were in the range of normality, they were higher in patients with cardiac syndrome X than in control subjects both under baseline conditions and at 120 and 180 minutes during the glucose tolerance test (Table 2).

Baseline circulating ET-1 concentrations did not differ between patients and control subjects (Figure, A). After glucose loading, ET-1 levels increased in both groups and reached statistical significance compared with baseline values at 60 and 90 minutes (Figure, B). However, the increase in ET-1 levels was higher in patients than in control subjects (Figure, B). Both under baseline conditions and at the time of each oral glucose tolerance test, plasma ET-1 levels did not correlate with heart rate×pressure product at 1 mm ST-segment depression in patients with cardiac syndrome X.

Nitric oxide production and plasma soluble VCAM-1 concentrations were similar for the patients and control subjects (Table 1). No significant changes in the 2 variables were observed with glucose loading in either group.

**Discussion**

In patients with cardiac syndrome X, we observed marked fasting dyslipidemia and higher plasma glucose levels (although in the normal range) both under baseline conditions and after glucose loading. These data confirm the previously described link between metabolic and cardiac syndrome X.\textsuperscript{14,29,30,33,34} Moreover, the present results indicate that base-
line levels of the potent endothelium-derived vasoconstrictor ET-1 are not elevated in patients with cardiac syndrome X compared with control subjects. Furthermore, plasma concentrations of nitrite plus nitrate, a sharp index of nitric oxide production, and soluble VCAM-1, an adhesion molecule released by damaged vascular endothelial cells that is increased in patients with endothelial dysfunction even without overt atherosclerotic lesions or early ultrasonographically detectable intimal changes, are not increased in patients with cardiac syndrome X compared with control subjects. These findings suggest that under baseline conditions, no endothelial damage was detected in these patients with endothelium-derived markers.

The results of the present study contrast with those of previous reports, in which a significant increase in baseline ET-1 concentrations was observed in patients with cardiac syndrome X compared with control subjects. In those studies, patients were carefully selected, and discrepancies with our results cannot reflect differences in the study populations. However, in the previous studies, commercial radioimmunoassays for ET-1 measurements with a sensitivity of 2 pg/mL were used, and the mean difference between patients and control subjects was close to 1.0 pg/mL. Thus, it seems that such a difference, although significant, might be affected by assay sensitivity. Indeed, monoclonal antibodies against ET-1 markedly cross-react with ET-2, ET-3, and big ET-1 (50%, 90%, and 10%, respectively), leading to

### TABLE 1. Characteristics of the Study Populations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Syndrome X (n=24)</th>
<th>Control Subjects (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>3/21</td>
<td>1/13</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>54±7</td>
<td>47±15</td>
<td>0.06</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±3</td>
<td>25±4</td>
<td>0.8</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>9</td>
<td>6</td>
<td>0.09</td>
</tr>
<tr>
<td>Menopause, n</td>
<td>14</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>Symptom duration, mo</td>
<td>48±19</td>
<td>...</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>132±12</td>
<td>128±9</td>
<td>0.1</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79±11</td>
<td>76±10</td>
<td>0.1</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>76±10</td>
<td>72±8</td>
<td>0.9</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>73±10</td>
<td>70±9</td>
<td>0.69</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>244±27</td>
<td>187±33</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>173±24</td>
<td>132±25</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>40±8</td>
<td>42±6</td>
<td>0.262</td>
</tr>
<tr>
<td>VLDL cholesterol, mg/dL</td>
<td>32±10</td>
<td>18±6</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>157±51</td>
<td>84±27</td>
<td>0.0001</td>
</tr>
<tr>
<td>NO, µg/L</td>
<td>25±8</td>
<td>26±7</td>
<td>0.134</td>
</tr>
<tr>
<td>VCAM-1, µg/L</td>
<td>1186±475</td>
<td>998±532</td>
<td>0.394</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Values are mean±SD.

### TABLE 2. Plasma Glucose After Oral Glucose Ingestion

<table>
<thead>
<tr>
<th>Glucose, mg/dL</th>
<th>Syndrome X (n=24)</th>
<th>Control Subjects (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>87±10</td>
<td>79±8</td>
<td>0.01</td>
</tr>
<tr>
<td>30 min</td>
<td>134±26</td>
<td>120±44</td>
<td>0.11</td>
</tr>
<tr>
<td>60 min</td>
<td>138±33</td>
<td>116±47</td>
<td>0.09</td>
</tr>
<tr>
<td>90 min</td>
<td>125±38</td>
<td>102±44</td>
<td>0.09</td>
</tr>
<tr>
<td>120 min</td>
<td>121±35</td>
<td>98±34</td>
<td>0.046</td>
</tr>
<tr>
<td>180 min</td>
<td>101±31</td>
<td>78±34</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Values are mean±SD.
overestimation of true ET-1 concentrations in biologic fluids. To avoid this cross reaction, we used the radioimmunoassay after HPLC separation of plasma samples. This procedure increased ET-1 sensitivity to 0.2 pg/mL, which allowed us to identify the sample peak that corresponded to ET-1 alone and to exclude interference with other ETs and big ET-1. Therefore, discrepancies with the mentioned studies might reflect the different methodologies used for ET-1 evaluation.

Of note, our findings are in agreement with those obtained by Newby et al., who found in patients with cardiac syndrome X normal baseline levels of ET-1 and stimulated nitric oxide activity. Furthermore, in this study, the vasoconstriction caused by ET-1 correlated inversely with plasma ET-1 concentrations, thus suggesting a reduced responsiveness to exogenous ET-1 that possibly reflected overactivity of the ET-1 system and ET-1 type A receptor downregulation. Interestingly, these authors also found normal baseline plasma levels of von Willebrand factor, a well-recognized marker of endothelial dysfunction, further supporting the lack of endothelial alterations in patients with cardiac syndrome X.

Although in the present study we found normal baseline ET-1 plasma levels in patients with cardiac syndrome X, it is worth noting that glucose loading, which determines an elevation of ET-1 plasma levels in both insulin-resistant patients and healthy subjects, caused a significantly higher release of ET-1 in patients with cardiac syndrome X compared with control subjects at 60, 90, and 120 minutes. This finding might be due to a synergistic stimulatory effect of insulin and glucose on ET-1 secretion. In fact, although insulin is a powerful regulator of ET-1 secretion in vitro, insulin alone was not able to stimulate ET-1 secretion when infused intravenously in patients with cardiac syndrome X. By contrast, simultaneous infusion of both insulin and glucose significantly increased plasma ET-1 concentration.

Thus, higher glucose and insulin levels manifested by patients with cardiac syndrome X compared with control subjects after glucose ingestion are probably responsible for the enhanced ET-1 response to glucose load observed in patients with cardiac syndrome X. In addition, patients with cardiac syndrome X showed a dyslipidemic pattern, which could have influenced ET-1 release. Indeed, both LDL cholesterol and triglycerides are known to stimulate ET-1 secretion in vitro and in vivo. In contrast to this possible explanation of our results, patients with cardiac syndrome X had normal circulating ET-1 concentrations at baseline. However, the fact that the presence of dyslipidemia may represent a sort of metabolic background that favors the enhanced ET-1 response to glucose ingestion cannot be excluded. Further, our patients are not necessarily comparable to those of other series. Although dyslipidemia has often been reported in patients with cardiac syndrome X, our patients showed a particularly evident degree of association between metabolic and cardiac syndrome X. Therefore, the potential role of dyslipidemia in modulation of ET-1 secretion should be restricted to the subset of patients with cardiac syndrome X who simultaneously manifest a dyslipidemic pattern. In any case, the correlation we found in the present study as well as in previous studies between metabolic disturbances and cardiac syndrome X cannot reflect selection bias. Indeed, our patients were not previously seen in either the metabolic unit or diabetes department. On the contrary, patients were recruited among consecutive patients who were directly referred to the department of cardiac surgery due to chest pain suspected to be of cardiac origin.

Of note, ET-1 response to glucose loading in cardiac syndrome X is similar to that observed in other insulin-resistant conditions either after glucose ingestion or during insulin infusion. Despite this, the lack of any additional evidence of endothelial damage both under baseline conditions and after glucose loading in cardiac syndrome X makes ET-1 response to glucose loading a specific pattern of endothelial activation that might contribute to an explanation of the tendency to develop microvascular dysfunction rather than atherosclerotic lesions.

Our data suggest that patients with cardiac syndrome X might have an enhanced tendency to release ET-1 under stress circumstances. According to this, previous data showed an increased release in ET-1 at the peak of exercise testing in patients with syndrome X compared with control subjects. Thus, it seems that patients with cardiac syndrome X present an increased susceptibility to different stimuli in the release of ET-1. This latter in turn, by causing vasoconstriction of the coronary microcirculation, may play a role in the pathogenesis of myocardial ischemia and angina in patients with cardiac syndrome X. In addition, exaggerated increments of ET-1 plasma levels after glucose loading could impair insulin-mediated vasodilation and the consequent increase in peripheral glucose uptake, thereby contributing to the hyperinsulinemic response to glucose ingestion known to be manifested by patients with cardiac syndrome X.

However, ET-1 represents only 1 of the neurohumoral mediators that affect vascular response in patients with syndrome X. According to this, we did not find any correlation between plasma ET-1 levels and heart rate x pressure product at 1 mm ST-segment depression, an accepted index of coronary vascular response. Although our data seem to contrast with those of the recent report by Cox et al., who described higher ET-1 levels associated with reduced coronary vasoconstrictor responses in patients with chest pain and normal coronary arteriograms, we agree with Cox et al. in their emphasis that ET-1 is primarily a paracrine mediator, with up to 75% secreted into the basolateral side (ie, toward the smooth vascular muscle cells), with different release capabilities in different vascular districts. Thus, it is extremely interesting to note that Cox et al. found a correlation between plasma ET-1 levels and vasodilator responses during atrial pacing by assaying arterial ET-1 but not venous ET-1. Because we assayed venous ET-1 and used a different method for ET-1 measurements, which is able to eliminate overestimation of true ET-1 levels, a discrepancy between their study might reflect the different methodology used.

Study Limitations

The failure to detect an endothelial activation under baseline conditions in patients with cardiac syndrome X might reflect the use of a limited number of circulating molecules as
markers of endothelial damage. However, a number of studies indicate that circulating concentrations of ET-1,16–18 and soluble VCAM-125–27 are well-recognized markers of endothelial damage. In particular, endothelial cells poorly express VCAM-1 in resting conditions,19 and an increased release of its soluble form with marked elevation of its plasma level occurs immediately after generalization26,27 as well as local25 endothelial damage. Further, an elevated plasma-soluble VCAM-1 concentration is associated not only with the extension of advanced atherosclerosis but also with early intimal changes in both high- and low-risk subjects.35,46

Another potential limitation of the present study is that patients showed a trend toward an older age. This could have influenced both insulin sensitivity47 and circulating ET-1 levels.48,49 However, age-related increase in insulin resistance is determined by increments in body fat.49 Because the body mass index was similar for patients with cardiac syndrome X and control subjects, it is unlikely that the small difference in age between the 2 groups influenced peripheral insulin-mediated glucose extraction. Similarly, small increments in circulating ET-1 levels have been described in healthy subjects with a mean age of >65 years.48,49 Because none of our patients were >65 years old and only 3 were 61 to 64 years old, it seems extremely unlikely that the small difference in age between the patient and control groups, which was also nonsignificant, influenced our findings. In accordance to our interpretation, which is at variance with previous studies in elderly subjects,48,49 baseline ET-1 concentrations were at similar levels in the 2 groups. Thus, the small, nonsignificant difference in age between patients and control subjects does not represent a study bias.

In conclusion, our findings indicate the absence of endothelial activation in patients with cardiac syndrome X under baseline conditions. However, after glucose loading, patients with cardiac syndrome X appear to have an enhanced tendency to release ET-1, thus suggesting an increased susceptibility of vascular endothelium to be activated. Because ET-1 has the potential to affect microvascular tone,12,15 its increased release under stress circumstances might play a role, together with other neurohumoral mediators, in the pathogenesis of myocardial ischemia in patients with cardiac syndrome X.

**References**


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