Endothelial and Metabolic Characteristics of Patients With Angina and Angiographically Normal Coronary Arteries
Comparison With Subjects With Insulin Resistance Syndrome and Normal Controls

PierMarco Piatti, MD,* Gabriele Fragasso, MD,§ Lucilla D. Monti, MD,† Andrea Caumo,‡ Chuong Van Phan,† Giampietro Valsecchi, PhD,† Sabrina Costa,† Elena Fochesato, MD,* Guido Pozza, MD,† Antonio E. Pontiroli, MD,* Sergio Chierchia, MD, FESC, FACC§
Milano, Italy

OBJECTIVES
This study was performed to characterize the endothelial and metabolic alterations of patients with angina and angiographically normal coronary arteries (“cardiac” syndrome X [CSX]) compared with subjects with insulin resistance syndrome (“metabolic” syndrome X [MSX]) and normal controls.

BACKGROUND
Previous studies have found high endothelin-1 levels, impaired endothelium-dependent vasodilation and insulin resistance in patients with angina pectoris and angiographically normal coronary arteries. On the other hand, subjects with insulin resistance syndrome have shown high endothelin-1 levels.

METHODS
Thirty-five subjects were studied: 13 patients with angina pectoris and angiographically normal coronary arteries (CSX group); 9 subjects with insulin resistance syndrome (MSX group) and 13 normal controls. All subjects received an acute intravenous bolus of insulin (0.1 U/kg) combined with a euglycemic clamp and forearm indirect calorimetry. Endothelin-1 levels, nitrite/nitrate (NOx) levels, end products of nitric oxide metabolism, glucose infusion rates (index of insulin sensitivity) and their incremental areas (∆AUCs [area under curves]) were measured during this period.

RESULTS
Basal endothelin-1 levels were higher in CSX and MSX groups than in normal controls (8.19 ± 0.46 and 6.97 ± 0.88 vs. 3.67 ± 0.99 pg/ml; p < 0.01), while basal NOx levels were significantly higher in MSX group than in CSX and normal controls (36.5 ± 4.0 vs. 24.2 ± 3.3 and 26.8 ± 3.2 mol/liter, p < 0.05). After insulin administration, the ∆AUCs of NOx (p < 0.05) were lower in CSX group than in MSX and normal controls, and the ∆AUCs of endothelin-1 were lower in group CSX than in normal controls. Glucose infusion rate was significantly lower in CSX and MSX groups than in normal controls (p < 0.01), suggesting that in both CSX and MSX groups insulin resistance is present. A positive correlation was found between the ∆AUCs of nitric oxide and the AUCs of glucose infusion rate.

CONCLUSIONS
Blunted nitric oxide and endothelin responsiveness to intravenously infused insulin is a typical feature of patients with angina pectoris and angiographically normal coronary arteries and may contribute to the microvascular dysfunction observed in these subjects. (J Am Coll Cardiol 1999;34:1452–60) © 1999 by the American College of Cardiology

Patients with angina pectoris and angiographically normal coronary arteries (“cardiac” syndrome X [CSX] patients) show a relative frequent condition characterized by history of typical angina pectoris, presence of ischemic-like ST segment changes on exercise testing and neither obvious epicardial coronary disease nor inducible spasm on coronary arteriography (1). Several pathophysiological theories have been put forward to explain this syndrome, which has been denied, by some authors, the dignity of a real cardiac disease (2). Others, however, claim that these patients’ symptoms are, indeed, cardiac in origin and relate, at least in part, to a generalized microvascular dysfunction that also involves the coronary circulation and limits coronary flow reserve (3–6). In
addition, these patients exhibit a blunted hyperemic response to forearm ischemia (3) and often show regional perfusion abnormalities on myocardial scintigraphy (7,8).

Recently, increased levels of endothelin-1 (ET-1) have been found in these patients (9), suggesting that this powerful vasoreactive peptide (10) may play a role in this syndrome. Furthermore, other studies (11–13) suggested that CSX patients are insulin resistant and exhibit decreased insulin-induced glucose disposal, impaired total body glucose oxidation and reduced nonoxidative glucose metabolism. Conversely, their liver glucose output and lipid oxidation are similar to those of normal controls (11). On the other hand, increased ET-1 levels have also been found in insulin resistance syndrome ("metabolic" syndrome X [MSX]), characterized by the association in the same subject of insulin resistance, hyperinsulinemia, impaired glucose tolerance, hypertriglyceridemia, visceral obesity and hypertension (14). Until now, it has been impossible to define whether the endothelial and metabolic abnormalities previously shown in CSX patients have any association with coronary microvascular dysfunction.

The purpose of our study was to characterize the endothelial and metabolic alterations of CSX patients compared with MSX subjects and normal controls. In particular, our interest was extended in evaluating whether altered nitric oxide (nitrite/nitrate [NOx]) and ET-1 responsiveness to intravenously infused insulin is a typical feature of CSX patients or is a common feature when insulin resistance is present. Therefore, an intravenous insulin bolus combined with a euglycemic clamp (14) was performed in CSX patients, in MSX subjects without cardiovascular symptoms and in normal controls. This approach allowed us to evaluate simultaneously insulin sensitivity and dynamic effects of insulin on ET-1 and NOx release. In order to evaluate intracellular glucose metabolism, forearm muscle indirect calorimetry was also performed.

Patients and controls. All subjects gave informed consent to participate in the study that was approved by the local Ethics Committee. Thirty-five subjects were studied and divided in three groups. The first group (CSX group) consisted of 13 consecutive patients (eight women, five men; age 52 ± 2 years, body mass index [BMI] 24.1 ± 0.5 kg/m²) with rest and/or effort angina pectoris, a reproducible positive exercise test (>1-mm planar or downsloping ST-segment depression) and angiographically smooth epicardial coronary arteries. In all, prolonged hyperventilation and/or ergonovine administration, performed during coronary angiography, failed to induce epicardial coronary spasms. In these patients, all treatments were withdrawn 15 days before the study that was performed and only after an angina-free period of at least 3 days. The second group (MSX group) consisted of nine asymptomatic subjects with insulin resistance syndrome (five women, four men; age 49 ± 3 years, BMI 28.2 ± 1.4 kg/m²). Insulin resistance syndrome was defined by the association of impaired glucose tolerance and at least three of the following alterations: hyperinsulinemia (>96 pmol/liter), insulin resistance (HOMA [homeostasis model assessment] index >3.94), hypertriglyceridemia (>2.3 mmol/liter), low high-density lipoprotein (HDL) cholesterol levels (<1.19 mmol/liter), visceral obesity and hypertension (systolic blood pressure >160 mm Hg and diastolic blood pressure >95 mm Hg). The cutoff presented for each variable of the insulin resistance syndrome was derived considering >2 standard deviations (SD) of the mean values of group 3, which was considered our reference normal population. Diagnosis of impaired glucose tolerance was made after a standard OGTT (oral glucose tolerance test) (75 g) according to World Health Organization criteria. In all subjects, resting and exercise electrocardiograph (ECG) and two-dimensional echocardiographic and Doppler studies were normal. Five out of nine patients were affected by hypertension, and in these subjects, antihypertensive therapy was withdrawn 10 days before the study. The third group consisted of 13 normal controls (seven women, six men; age 50 ± 2 years, BMI 23.1 ± 0.8 kg/m²), undergoing routine cardiological evaluation before general surgery. In all, physical examination, chest roentgenogram, resting and exercise ECG and two-dimensional echocardiographic and Doppler studies were normal. They had no diabetes, hypertension, left ventricular hypertrophy, pericardial or valve disease or cardiomyopathy. All study subjects were nonsmokers.

In Table 1 clinical, hormonal and metabolic data of the three groups are represented. Body weight, BMI, waist/hip ratio, systolic and diastolic blood pressure, basal glucose, insulin, triglyceride, total cholesterol, HDL cholesterol and free fatty acid levels were similar in CSX group and in normal controls. In contrast, all these variables were higher in MSX group, as expected. Lactate (557.7 ± 68.8, 643.3 ± 124.6 and 553.5 ± 153.7 μmol/liter), pyruvate (61.7 ± 8.9,
clamp technique (15) is a widely used method by which tory hormones on endothelial factor release. The euglycemic euglycemic clamp to avoid the influence of counterregula-
tion carried out in the present study was the addition of a revised by Bonora et al. (17), is a well-known and simple technique (15). The intravenous bolus of insulin, or insulin 1 ml saline (14) combined with the euglycemic clamp received an intravenous bolus of 0.1 U/kg insulin diluted in antecubital vein of the controlateral arm for intermittent 18-gauge plastic cannula was inserted into a large, deep antecubital vein of the same arm for infusions. Another clamp. A 20-gauge plastic cannula was inserted into a large blood glucose levels during a euglycemic hyperinsulinemic investigators have, therefore, used the arterialized venous blood by heating the hand. This is the method of choice because obtaining arterial samples is difficult and is associ-
ated with some risks related to arterial cannulation. Many patients have, therefore, used the arterialized venous blood samples obtained from the heated hand to measure blood glucose levels during a euglycemic hyperinsulinemic clamp. A 20-gauge plastic cannula was inserted into a large, deep antecubital vein of the controlateral arm for intermittent sampling of deep venous forearm blood.

After a 30-min period of equilibration, all subjects received an intravenous bolus of 0.1 U/kg insulin diluted in 1 ml saline (14) combined with the euglycemic clamp technique (15). The intravenous bolus of insulin, or insulin tolerance test (ITT), used since 1971 (16) and more recently revised by Bonora et al. (17), is a well-known and simple method to evaluate insulin sensitivity. The only modification carried out in the present study was the addition of a euglycemic clamp to avoid the influence of counterregula-
tory hormones on endothelial factor release. The euglycemic clamp technique (15) is a widely used method by which blood glucose levels are maintained at baseline values by means of a variable 20% glucose infusion according to the blood glucose measurements obtained every 5 min. By using this method, the amount of glucose infused during the test corresponds to the degree of insulin sensitivity of the subjects.

To validate our method, we assessed the relationship between insulin sensitivity measured with our insulin bolus test and the insulin sensitivity index measured with the frequently sampled intravenous glucose tolerance test (FSIGT), according to Bergman et al. (18)—another test that is considered a reference test for the measurement of insulin sensitivity. We found that there was a highly significant correlation between the two insulin sensitivity indices ($r = 0.80$, $p < 0.01$). The choice to use the FSIGT as a reference method for our test was related to the fact that in both cases, during the first 60 min of the test, insulin levels are not in steady state.

Because, during the euglycemic clamp studies, arterialized instead of arterial samples were performed, in a previous study, we evaluated whether arterialized ET-1 levels are a reliable index of forearm arterial levels (14). In the preparatory study, we found that there was a highly significant correlation between arterial and arterialized ET-1 levels in 30 subjects ($r = 0.96$; $p < 0.001$) with a slope not different from 1 (0.93 ± 0.51; $p < 0.5$) and an intercept not different from 0 (0.140 ± 0.05; $p < 0.6$). In the present study, we evaluated whether arterialized NOx levels are a reliable index of forearm arterial levels. Arterial and arterialized samplings were obtained simultaneously in 20 sub-
jects (5 CSX patients, 7 MSX subjects and 8 normal controls). There was a highly significant correlation between arterial and arterialized NOx levels ($r = 0.90$; $p < 0.001$), with a slope not different from 1 (0.93 ± 0.11; $p < 0.16$) and an intercept not different from 0 (1.32 ± 2.86; $p < 0.01$).

**Table 1. Clinical, Hormonal and Metabolic Details of the Subjects in the Study (Mean ± SE)**

<table>
<thead>
<tr>
<th>Group</th>
<th>CSX</th>
<th>MSX</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>5/8</td>
<td>4/5</td>
<td>6/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 ± 2</td>
<td>49 ± 3</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.4 ± 2.4</td>
<td>75.3 ± 5.2</td>
<td>65.5 ± 3.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 0.5</td>
<td>28.3 ± 2.3$^*$</td>
<td>23.1 ± 0.8</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.89 ± 0.01</td>
<td>0.98 ± 0.03$^*$</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>129 ± 4</td>
<td>146.0 ± 3$^*$</td>
<td>120.0 ± 3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74 ± 2</td>
<td>90 ± 2$^*$</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>B Glucose (mmol/liter)</td>
<td>5.11 ± 0.17</td>
<td>5.50 ± 0.63</td>
<td>4.86 ± 0.14</td>
</tr>
<tr>
<td>S Insulin (pmol/liter)</td>
<td>55.6 ± 6.9</td>
<td>129.0 ± 12.2$^*$</td>
<td>40.0 ± 6.6</td>
</tr>
<tr>
<td>S Triglycerides (mmol/liter)</td>
<td>1.27 ± 0.11</td>
<td>3.08 ± 0.35$^*$</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>P Free fatty acids (mmol/liter)</td>
<td>0.75 ± 0.16</td>
<td>0.83 ± 0.10</td>
<td>0.63 ± 0.06</td>
</tr>
<tr>
<td>S Cholesterol (mmol/liter)</td>
<td>5.72 ± 0.20</td>
<td>5.98 ± 0.20</td>
<td>5.84 ± 0.45</td>
</tr>
<tr>
<td>S HDL cholesterol (mmol/liter)</td>
<td>1.35 ± 0.34</td>
<td>1.05 ± 0.07$^*$</td>
<td>1.52 ± 0.33</td>
</tr>
</tbody>
</table>

Data are mean ± SE. $^*$p < 0.05 vs. CSX and normal controls; $^†$p < 0.01 vs. CSX and normal controls.

B = blood; CSX = "cardiac" syndrome X; MSX = "metabolic" syndrome X; P = plasma; S = syndrome.
0.11). Therefore, arterialized ET-1 and NOx levels are a reliable index of forearm arterial levels.

Arterialized samples for ET-1, NOx and insulin were withdrawn at time -30, -20, -10, 0, 1, 3, 5, 10, 15, 20, 30, 45 and 60 min after the insulin bolus. Arterialized and deep venous samples for glucose and intermediate metabolite (lactate, pyruvate, alanine) measurements were withdrawn at -30, -15, 0, 5, 10, 15, 20, 30, 45 and 60 min. Arterialized samples for triglyceride and free fatty acid measurements were drawn at -5 and 0 min. This test thus permits simultaneous evaluation of insulin sensitivity, forearm indirect calorimetry, ET-1 and NOx response to insulin.

Blood flow and blood pressure measurements. Blood flow of the proximal forearm was measured immediately after each blood sample by venous occlusion plethysmography at time -30, -15, 0, 5, 10, 15, 20, 30, 45 and 60 min. Two cuffs were inflated simultaneously to obtain a collecting pressure of 60 mm Hg and a wrist occlusion pressure of 220 mm Hg. Changes in forearm volume were measured by means of a temperature-compensated mercury rubber strain gauge placed distally to the tip of the cannula, as previously reported (19). Blood flow was expressed in ml/min/100 ml forearm tissue volume. In addition, at least three determinations of arterial blood pressure were performed at 10-min intervals after the start of the test.

Forearm metabolite balance and forearm indirect calorimetry studies. Forearm balances of glucose, lactate, pyruvate and alanine were calculated by using the Fick principle: (arterialized blood concentration) - (deep venous blood concentration) x forearm blood flow. Forearm glucose oxidation (FGOx) rates were estimated by forearm indirect calorimetry using arterialized and deep venous blood samples obtained at -30, -15, 0 min and every 15 min after the start of the test, for the measurements of O2 and CO2 as previously published in non–steady-state conditions (19,20). In addition, in order to evaluate the degree of blood CO2 variability in the same subject in the absence of external stimulation, eight subjects were studied in the fasting state, and arterialized and deep venous blood CO2 content was measured every 15 min for 1 h. The coefficients of variation (CV) of the arterialized and deep venous CO2 were 20 ± 0.3% and 3.34 ± 0.26%, respectively.

Three sets of arteriovenous measurements were performed at each time point. Forearm O2 consumption (VO2) and CO2 production (VCO2) were calculated as the product of the arterialized-venous difference and forearm blood flow. FGOx was derived according to Natali et al. (21), and nonoxidative glycolysis was derived as the net balance of lactate, pyruvate and alanine, in glucose equivalents. In addition, forearm glucose storage (glycogen formation [FGSt]) was calculated as the difference between glucose uptake and the sum of glucose oxidation and nonoxidative glycolysis (22).

Assays. Blood glucose was measured with a glucose-oxidase based analyzer (YSI, Yellow Springs, Ohio). Samples for intermediate metabolite measurements were collected into weighted tubes containing chilled 0.5M perchloric acid. All samples were assayed for metabolites and insulin in a single assay. Alanine (intraassay CV 3%, interassay CV 3%), lactate (intraassay CV 4.0%, interassay CV 7.5%) and pyruvate (intraassay CV 8.0%, interassay CV 9.5%) were assayed using automated enzymatic spectrophotometric methods adapted to COBAS FARA II (Roche, Basel, Switzerland) (23). Plasma-free fatty acid (intraassay CV 3%, interassay CV 3%) and serum triglyceride levels were measured using automated enzymatic spectrophotometric techniques adapted to COBAS FARA II (Roche, Basel, Switzerland). Serum insulin levels (intraassay CV 3.0%, interassay CV 5.0%) were measured by radioimmunoassay using commercial kits (Insulin I125 Ria kit; Incstar Corporation, Stillwater, Minnesota).

ET-1 samples were measured with a commercial radioimmunoassay (RIA) kit (Biomedica Gruppe, Wien, Germany). In particular, in order to enrich the peptide from the plasma sample to measurable values, ET-1 was extracted on SepPack C18, and the eluate was evaporated in a Speedvac concentrator (Speed Vac SC110, Savant, Roma, Italy). The samples were then reconstituted in 250 μl of RIA buffer and assayed. In the RIA kit, the antisera were a rabbit-anti-ET-1 antibody, and the tracer was 125I-labeled ET-1. An intraassay CV of 3.0%, and an interassay CV of 11.9% were reported.

Nitrite/nitrate levels were evaluated through the measurement of metabolic end products, that is, nitrite and nitrate, using enzymatic catalysis coupled with Griess reaction, as previously reported (24).

Forearm arterialized and venous blood gas samples were analyzed at the patient’s bedside (Corning Medical and Scientific, Medfield, Massachusetts). Plasma CO2 content was calculated from measured CO2 tension and pH, and adjusted to whole CO2 blood content using an empirically derived regression equation (25). O2 content was calculated from the hemoglobin content and percent of saturation, using a constant of 1.34. The CV was calculated for each individual, and the mean of intrasubject CVs were 0.1% for pH, 0.6% for arterialized CO2, 1.3% for forearm venous CO2 and 1.5% for arterialized O2, while mean intrasubject CV was 4.6% for venous O2 content.

Statistical analysis. All values are expressed as mean ± standard error at each time interval. Areas under the curves (AAUCs) were calculated for each parameter by the trapezoidal rule. Comparisons within groups were performed by means of Student t test for paired data. Comparisons among groups were performed by means of analysis of variance followed by the Scheffe F test when indicated. Linear regression analyses or Spearman test were used as appropriate. A two-tailed probability level of less than 0.05 was considered statistically significant.
RESULTS

Basal levels. Basal ET-1 levels were significantly higher in CSX and MSX groups than in normal controls (8.19 ± 0.46 and 6.97 ± 0.88 vs. 3.67 ± 0.99 pg/ml, p < 0.01; Fig. 1), while no differences were found between CSX and MSX groups. Nitrite/nitrate levels were higher in MSX group than in CSX group and in normal controls (36.5 ± 4.0 vs. 24.2 ± 3.3 and 26.8 ± 3.2 μmol/liter, p < 0.05; Fig. 1), while no differences were found between CSX group and normal controls.

Arterial systolic and diastolic blood pressures were significantly higher in MSX group than in CSX group and in normal controls (p < 0.01, Fig. 1), while forearm blood flow was similar in the three groups.

Forearm glucose uptake (FGU), FGOx, nonoxidative glycolysis, FGSt and lipid oxidation (data not shown) were similar in the three groups (Fig. 2).

Insulin effects on endothelium activity and blood pressure. One minute after the insulin bolus, insulin levels peaked at about 5,000 pmol/liter in the three groups and rapidly declined to basal levels; blood glucose was successfully clamped to the baseline with a CV below 6%.

In Figure 1, ET-1, NOx, blood pressure and forearm blood flow levels after insulin bolus are reported. Endothelin-1 response was flat in CSX group, while a significant increase in ET-1 levels was elicited in MSX group, similar to that observed in normal controls. This determined a significantly lower ΔAUC of ET-1 in CSX group than in normal controls, while there were no significant differences between MSX group and normal controls. Nitrite/nitrate levels did not significantly increase in CSX group, while they increased significantly, with a similar pattern, in MSX group and in normal controls. Nitrite/nitrate levels remained significantly higher in MSX group than in CSX group, while no differences were found between MSX group and normal controls. The ΔAUCs of NOx were significantly lower in CSX group than in MSX group and in normal controls (p < 0.01), while no differences were found between MSX group and normal controls.

After insulin bolus, arterial systolic and diastolic blood pressure slightly decreased in all groups, however, blood pressure remained significantly higher in MSX group than in CSX group and in normal controls.

Forearm blood flow remained unchanged in CSX group, while it significantly decreased during the first 15 min in MSX group, returning at basal levels in the second half of the test. On the contrary, forearm blood flow was significantly higher during the last 30 min of the test in normal controls.

Insulin effects on glucose utilization and forearm indirect calorimetry. In Figure 2, the patterns of glucose utilization and forearm glucose metabolism after insulin bolus are reported.

Glucose infusion rates (GIRs) were significantly lower in CSX and MSX groups than in normal controls throughout the test. The AUCs for GIR were significantly lower in CSX and MSX groups than in normal controls (1,242.2 ± 126.7 and 651.4 ± 66.1 vs. 2,143.6 ± 178.7 μM/kg/min; p < 0.05).

It is interesting that the ΔAUC of GIR was significantly higher in CSX than MSX group (p < 0.05). A similar pattern was observed in FGU. ΔAUCs of FGOx were
significantly lower in CSX and MSX groups than in normal controls without differences between the two groups.

Nonoxidative glycolysis tended to be greater in CSX group, while it remained similar to baseline in MSX group and slightly decreased in normal controls. The ΔAUC of FGSt was lower in MSX group than in normal controls (44.1 ± 20.3 vs. 116.3 ± 23.3 μmol/100 ml forearm/min; p < 0.05). In CSX group, the ΔAUC of FGSt was 106.4 ± 48.7 μmol/100 ml forearm/min (NS vs. normal controls). The profiles and the ΔAUC of lipid oxidation were similar in all groups (data not shown).

By pooling all the subjects of the three groups, a negative correlation was found between basal ET-1 levels and the ΔAUC of NOx levels (r = −0.34, p < 0.05; data not shown). In addition, a negative correlation between basal ET-1 levels and FGU was observed (r = −0.47; p < 0.01; data not shown) and a positive correlation was found between the AUCs of GIR and NOx levels (r = 0.38; p < 0.05; Fig. 3).

**DISCUSSION**

The aim of the study was to evaluate whether an unbalance between ET-1 and NOx release exists in patients with angina pectoris and angiographically normal coronary arteries and whether it is typical of this disease or is a common feature of subjects with insulin resistance syndrome. An attempt was also made to investigate whether the observed alterations in insulin action on glucose metabolism might be
indicating an impairment in the intracellular NO/cGMP could be due to a defect in the second messenger activity, et al. (6), while in MSX subjects, the defect in vasodilation could be related primarily to a defect in nitric oxide synthesis, as previously suggested by Egashira et al. (6), Camici et al. (32), and Botker et al. (11), who found a reduction in total body glucose disposal and oxidation at physiological insulin levels (about 770 pM). Taking the results of both studies together, it is tempting to speculate that in CSX patients the metabolic action of insulin is defective and involves aerobic glycolysis and that such a defect might involve the skeletal muscle as well as the heart (32). In Figure 2, an uncoupling of glycolysis and glucose oxidation in response to insulin in CSX patients can be observed. Similar data, but relative to myocardial metabolism, have been reported in these patients by Egashira et al. (6) and Camici et al. (32). In the first study, an increase in myocardial lactate production was found after papaverine infusion (6), while in the second study, carbohydrate oxidation was not stimulated by pacing, and net pyruvate release was observed at maximal pacing and during recovery (32). In the present study, an increase in skeletal muscle nonoxidative glycolysis was also found in MSX subjects. From our data, it is impossible to draw any conclusion about the effect of insulin on myocardial metabolism in these subjects, although a normal insulin-induced myocardial glucose uptake has been found in patients with non-insulin-dependent diabetes mellitus despite a severe decrease in insulin-induced skeletal glucose uptake (33). Although evaluation of myocardial lactate production in subjects with insulin resistance syndrome was beyond the scope of the present study, we believe that this issue requires further investigation.

Another important difference between “cardiac” and “metabolic” syndrome X was related to an impairment in the insulin-induced release of endothelial factors. In the present study, we found that there are some similarities between “cardiac” and “metabolic” syndrome X concerning the presence of high basal ET-1 levels and insulin resistance in both groups. On the other hand, the evaluation of the dynamic response after insulin stimulation demonstrated that there are some important differences between the two syndromes, such as the presence of different basal NOx levels and insulin-stimulated ET-1 and NOx releases. In addition, MSX subjects were more insulin resistant, and insulin-mediated FGSt was compromised only in these subjects. Similarities and differences between “cardiac” and “metabolic” syndrome X related to endothelial-factor release and insulin sensitivity are reported in Table 2.

Insulin-stimulated endothelial release. The finding that in CSX patients insulin is not effective in stimulating NOx release is new. In normal controls, Baron (26) and Steinberg et al. (27) demonstrated that insulin-mediated vasodilation is largely dependent on the action of insulin on nitric oxide activity. To our knowledge, our data are the first to provide direct evidence that insulin-induced release of endothelium-derived relaxing factors is impaired in CSX patients and also to confirm the hypothesis that in subjects with insulin resistance, vasodilation is impaired (28–30). Indeed, after insulin injection, forearm blood flow significantly increased by 11% only in normal controls. However, different results were found when measuring the effect of insulin on NOx release in CSX and MSX patients. In CSX patients, basal NOx levels were normal, and the insulin-induced NOx release was severely impaired, while in MSX subjects, basal NOx levels were high, and the insulin-induced NOx release was normal. To explain these apparent discordant data, we postulate that in CSX patients the defect in vasodilation could be related primarily to a defect in nitric oxide synthesis, as previously suggested by Egashira et al. (6), while in MSX subjects, the defect in vasodilation could be due to a defect in the second messenger activity, indicating an impairment in the intracellular NO/cGMP signaling cascade. Further support to this hypothesis comes from previous in vitro studies, showing that rat skeletal muscle of insulin-resistant (obese Zucker; fa/fa) rats possesses multiple defects in the nitric oxide/cyclic GMP pathway (31). Moreover, a selective cyclic-GMP phosphodiesterase inhibitor, zaprinast, was able to increase cyclic-GMP levels and glucose utilisation in incubated soleus muscle isolated from lean, but not obese, insulin-resistant Zucker rats (31).
Relationship between endothelial factors and glucose metabolism. One interesting finding of the present study was the existence of a relationship linking endothelial factors and insulin action to glucose metabolism, as demonstrated by the positive correlations between the AUCs of GIR and the ΔAUCs of NOx levels. However, our data do not allow us to clarify whether insulin resistance in these subjects was mediated by a blunted response of NOx to insulin or by ET-1 overproduction, or both. Further studies are required to answer this question, although a negative correlation between basal ET-1 levels and FGU could suggest that ET-1 might directly influence glucose metabolism.

Clinical implications. Whereas previous studies have shown a strict correlation between the increment in triglyceride and insulin levels and the presence of high ET-1 levels in MSX subjects without myocardial complications (14), the mechanism responsible for the increase in basal ET-1 levels in CSX patients remains unknown. In fact, the latter group of subjects did not show hypertension (34), hyperinsulinemia, hypertriglyceridemia and diabetes (14) or other diseases, such as ischemic heart disease (35) and atherosclerosis (36), that could determine or be determined by a sustained stimulation of ET-1 release. In a previous study, the chronic administration of L-arginine decreased ET-1 levels and angina episodes in CSX patients (37), suggesting that there is a defect of nitric oxide activity (possibly synthesis) in these subjects. The findings of the present study may confirm this hypothesis—but only because of the supraphysiological insulin levels.

Study limitations. In the present study, we used the forearm balance technique to measure FGU before and after insulin administration. Although, from the existing literature, it frequently appears that the forearm technique is used to measure FGU during a perturbation (19,20,38–41), this approach does have limitations. In fact, the assessment of glucose uptake across the forearm hinges upon the Fick principle, which is valid only at steady-state conditions when blood flow is constant and arterial and venous glucose concentrations are stable. Under non–steady-state conditions, when blood flow or glucose concentrations change in time, the Fick principle does not hold, and systematic errors may affect the estimated fluxes. Therefore, the FGU results obtained in the present study during the insulin perturbation provide only qualitative insights into insulin-stimulated FGU in the two groups. On the other hand, even though there were systematic errors, they were likely to affect all groups to a similar extent. As a result, the time course of the difference among the FGUs in CSX patients, MSX subjects and normal controls was probably more reliable than the individual FGU profiles, suggesting an impairment of FGU in CSX patients and MSX subjects. As a matter of fact, the difference between the profiles of FGU at the regional level parallels the difference between the profiles of GIRs measuring glucose metabolism (glucose uptake, plus production) at the whole-body level. This is confirmed by a direct, significant correlation between the ΔAUCs of FGU and the AUCs of GIR (r = 0.43; p < 0.01). All in all, we are confident that our results suggesting a defect of insulin activity on glucose uptake in CSX patients are correct.

Conclusions. In summary, similar to MSX subjects, CSX patients show high basal ET-1 levels and insulin resistance. On the other hand, CSX patients exhibit a decrease of NOx and ET-1 release after insulin stimulation, while MSX subjects show high basal NOx levels, normal NOx release after insulin stimulation and a severe impairment of glucose storage. In conclusion, blunted nitric oxide and endothelin responsiveness to intravenously infused insulin is a typical feature of CSX patients and may contribute to the microvascular dysfunction observed in these subjects.

Acknowledgment
We are indebted to Dr. Fabio Pellegatta for providing the method of nitrite and nitrate assay.

Table 2. Similarities and Differences Between “Cardiac” and “Metabolic” Syndrome X

<table>
<thead>
<tr>
<th></th>
<th>“Cardiac” Syndrome X</th>
<th>“Metabolic” Syndrome X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal NOx</td>
<td>Normal</td>
<td>High</td>
</tr>
<tr>
<td>Basal ET-1</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Insulin-mediated NOx</td>
<td>Blunted</td>
<td>Normal</td>
</tr>
<tr>
<td>Insulin-mediated ET-1</td>
<td>Blunted</td>
<td>Normal</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-stimulated glucose infusion rate</td>
<td>Decreased</td>
<td>Highly decreased</td>
</tr>
<tr>
<td>Insulin-stimulated forearm glucose intake</td>
<td>Decreased</td>
<td>Highly decreased</td>
</tr>
<tr>
<td>Insulin-stimulated forearm glucose oxidation</td>
<td>Highly decreased</td>
<td>Highly decreased</td>
</tr>
<tr>
<td>Insulin-stimulated forearm glucose storage</td>
<td>Normal</td>
<td>Highly decreased</td>
</tr>
<tr>
<td>Insulin-stimulated non-oxidative glycolysis</td>
<td>Slightly increased</td>
<td>Slightly increased</td>
</tr>
</tbody>
</table>

ET-1 = endothelin-1; NOx = nitrite/nitrate.
REFERENCES

24. Verdon CP, Burto BA, Prior RL. Sample pretreatment with nitrate reductase and glucose 6-phosphate dehydrogenase quantitatively reduces nitrate while avoiding interference by NADP+ when the griss reaction is used to assay for nitrite. Anal Biochem 1995;224:502–8.