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Endothelial and Metabolic Characteristics of Patients With Angina and Angiographically Normal Coronary Arteries

Comparison With Subjects With Insulin Resistance Syndrome and Normal Controls

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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 (ΔAUCs [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 ΔAUCs of NOx ($p < 0.05$) were lower in CSX group than in MSX and normal controls, and the ΔAUCs 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 ΔAUCs 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

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Abbreviations and Acronyms

AUC = area under the curve
BMI = body mass index
CSX = "cardiac" syndrome X
ECG = electrocardiograph
ET-1 = endothelin-1
FSIGT = frequently sampled intravenous glucose tolerance test
FGOx = forearm glucose oxidation
FGSt = forearm glucose storage
FGU = forearm glucose uptake
GIR = glucose infusion rate
HDL = high-density lipoprotein
MSX = "metabolic" syndrome X
NOx = nitrite/nitrate
RIA = radioimmunoassay

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 vasoactive 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 been also 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.

METHODS

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/m^2) 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/m^2). 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/m^2), 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,

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

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

Data are means ± 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.

78.2 ± 26.1 and 61.6 ± 14.8 μmol/liter) and alanine levels (242 ± 26.3, 246.5 ± 21.5 and 195.4 ± 12.2 μmol/l) were similar in the three groups.

Experimental protocol. All subjects were admitted to the metabolic unit in the morning after an overnight fast, and samples were withdrawn after at least 30 min of rest in the supine position. On the morning of each test, a 20-gauge plastic cannula (Abbocath T; Abbot, Ireland Ltd., Sligo, Ireland) was inserted in a dorsal hand vein of one hand, in retrograde position, and the hand was placed in a plexiglass box and maintained at 55°C for intermittent sampling of arterialized blood. In order to monitor the arterial blood glucose and accurately infuse glucose to maintain blood glucose at baseline levels, it is necessary to arterialize venous blood by heating the hand. This is the method of choice because obtaining arterial samples is difficult and is associated with some risks related to arterial cannulation. Many investigators 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 antecubital vein of the same arm for infusions. Another 18-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 counterregulatory 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 subjects (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; $p <$

0.11). 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) × 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 O_2 and CO_2 as previously published in non–steady-state conditions (19,20). In addition, in order to evaluate the degree of blood CO_2 variability in the same subject in the absence of external stimulation, eight subjects were studied in the fasting state, and arterialized and deep venous CO_2 content was measured every 15 min for 1 h. The coefficients of variation (CV) of the arterialized and deep venous CO_2 were 2.0 ± 0.3% and 3.34 ± 0.26%, respectively.

Three sets of arteriovenous measurements were performed at each time point. Forearm O_2 consumption (VO_2) and CO_2 production (VCO_2) 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 spectrofluorimetric 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 antiserum was a rabbit-anti-ET-1 antibody, and the tracer was I^{125}-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 CO_2 content was calculated from measured CO_2 tension and pH, and adjusted to whole CO_2 blood content using an empirically derived regression equation (25). O_2 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 CO_2, 1.3% for forearm venous CO_2 and 1.5% for arterialized O_2, while mean intrasubject CV was 4.6% for venous O_2 content.

Statistical analysis. All values are expressed as mean ± standard error at each time interval. Areas under the curves (ΔAUCs) 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.

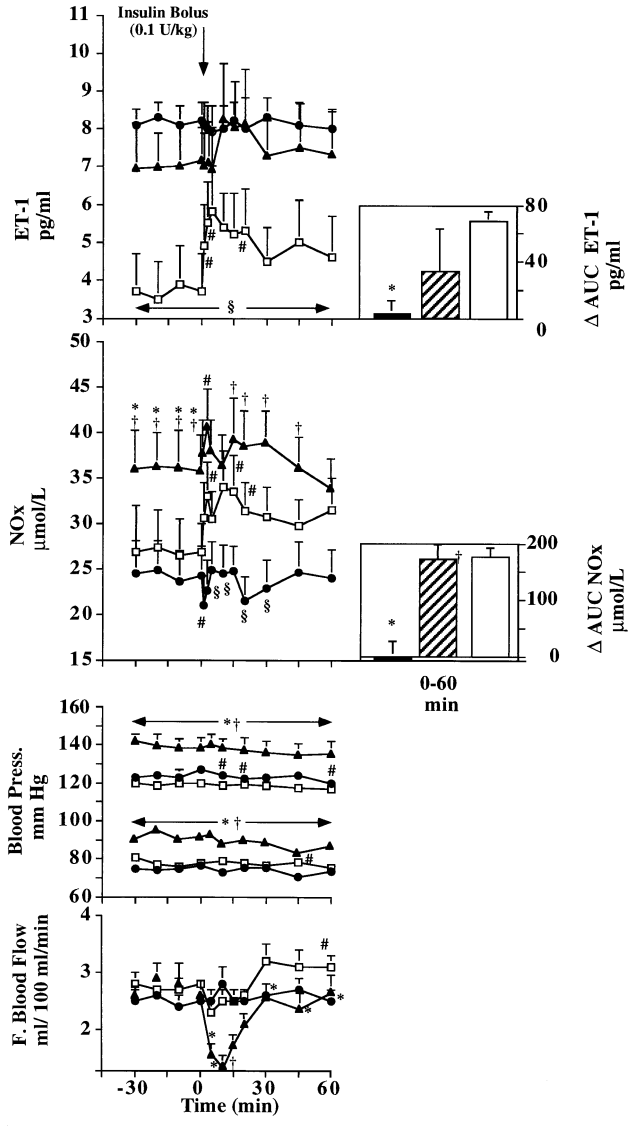

Figure 1. ET-1, NOx, systolic and diastolic blood pressure and forearm blood flow levels after an intravenous administration of 0.1 U/kg of insulin in 13 CSX patients **(circles)**, in 9 MSX subjects **(triangles)** and in 13 normal controls **(squares)**. Histograms represent ΔAUCs for the different parameters in CSX patients **(filled bars)**, in MSX subjects **(hatched bars)** and in normal controls **(open bars)**. Data are means ± SE. Blood Press. = blood pressure; ET-1 = endothelin-1; F. Blood Flow = forearm blood flow; NOx = nitrite/nitrate. #$p < 0.05$ vs. basal; *$p < 0.05$ vs. normal controls; §$p < 0.01$ vs. normal controls; †$p < 0.05$ vs. CSX patients.

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

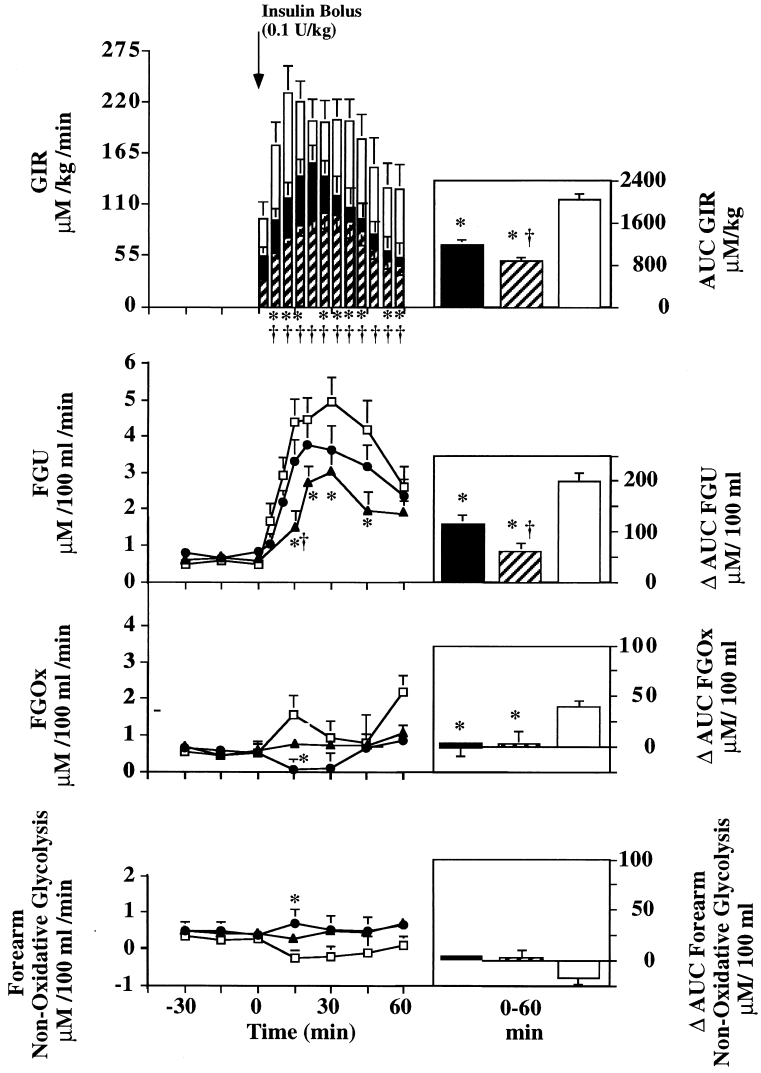

Figure 2. GIR FGU and forearm glucose metabolism after an intravenous administration of 0.1 U/kg of insulin in 13 CSX patients **(circles)**, in 9 MSX subjects **(squares)** and in 13 normal controls **(triangles)**. Histograms represent ΔAUCs for the different parameters in CSX patients **(filled bars)**, in MSX subjects **(hatched bars)** and in normal controls **(open bars)**. Date are means ± SE. GIR = glucose infusion rate; FGU = forearm glucose uptake; FGOx = forearm glucose oxidation. *$p < 0.05$ vs. normal controls; †$p < 0.05$ vs. CSX patients.

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

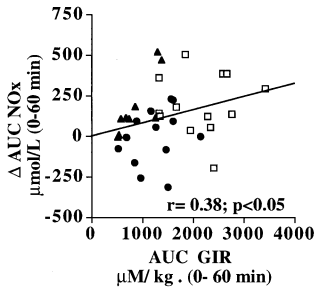

Figure 3. Relationship between the AUCs of GIR and the ΔAUCs of NOx levels in 13 CSX patients **(circles)**, in 9 MSX subjects **(triangles)** and in 13 normal controls **(squares)**. Nox = nitrite/nitrate; GIR = glucose infusion rate.

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 Steimberg 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).

Insulin-mediated glucose metabolism. In our study, similar to MSX subjects, the presence of supraphysiological insulin levels did not overcome the defect in insulin sensitivity in CSX patients. However, even if CSX patients and MSX subjects are less sensitive to insulin than normal controls, the degree of insulin resistance is different in the two groups, and, in particular, MSX subjects are significantly more insulin-resistant than CSX patients.

The presence of insulin resistance in CSX patients has already been described by 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 glucose storage measured during the test, which was correctly efficient in CSX patients while severely impaired in MSX subjects. These findings suggest that MSX subjects show a more severe impairment in the intracellular partitioning of muscle glucose metabolism.

Table 2. Similarities and Differences Between "Cardiac" and "Metabolic" Syndrome X

	"Cardiac" Syndrome X	"Metabolic" Syndrome X
Endothelial factors		
Basal NOx	Normal	High
Basal ET-1	High	High
Insulin-mediated NOx	Blunted	Normal
Insulin-mediated ET-1	Blunted	Normal
Insulin sensitivity		
Insulin-stimulated glucose infusion rate	Decreased	Highly decreased
Insulin-stimulated forearm glucose intake	Decreased	Highly decreased
Insulin-stimulated forearm glucose oxidation	Highly decreased	Highly decreased
Insulin-stimulated forearm glucose storage	Normal	Highly decreased
Insulin-stimulated non-oxidative glycolysis	Slightly increased	Slightly increased

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

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.

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REFERENCES

1. Kemp HG. Left ventricular function in patients with anginal syndrome and normal coronary arteriograms. Am J Cardiol 1973;32: 375–6.
2. Cannon RO, Camici PG, Epstein SE. Pathophysiological dilemma of syndrome X. Circulation 1992;85:883–92.
3. Sax FL, Cannon RO, Hanson C, Epstein SE. Impaired forearm vasodilator reserve in patients with microvascular angina. Evidence of a generalized disorder of vascular function? N Engl J Med 1987;317: 1366–70.
4. Vrints C, Bult H, Hitter E, et al. Impaired endothelium dependent coronary vasodilatation in patients with angina pectoris and normal coronary arteriograms. J Am Coll Cardiol 1992;19:21–31.
5. Motz W, Vogt M, Rabenay O, et al. Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiograms. Am J Cardiol 1991;68:996–1003.
6. Egashira K, Inou T, Hirooka Y, et al. Evidence of impaired endothelium-dependent coronary vasodilatation in patients with angina pectoris and normal coronary angiograms. N Engl J Med 1993;328:1659–64.
7. Tweddel AC, Martin W, Hutton I. Thallium scans in syndrome X. Br Heart J 1992;68:48–50.
8. Fragasso G, Rossetti E, Dosio F, et al. High prevalence of the thallium-201 reverse redistribution phenomenon in patients with syndrome X. Eur Heart J 1966;74:1482–7.
9. Kaski JC, Elliott PM, Salomone O, et al. Concentration of circulating plasma endothelin in patients with angina and normal coronary angiograms. Br Heart J 1995;74:620–4.
10. Yanagisawa M, Kurihara M, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cell. Nature 1988;332:411–5.
11. Botker HE, Moller N, Ovesen P, et al. Insulin resistance in microvascular angina (syndrome X). Lancet 1993;342:136–40.
12. Godsland IF, Crook D, Stevenson JC, et al. Insulin resistance syndrome in postmenopausal women with cardiological syndrome X. Br Heart J 1995;74:47–52.
13. Dean JD, Jones CJH, Hutchison SJ, et al. Hyperinsulinaemia and microvascular angina ("syndrome X"). Lancet 1991;337:456–7.
14. Piatti PM, Monti LD, Conti M, et al. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes 1996;45:316–21.
15. De Fronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–23.
16. Alford FP, Martin FIR, Pearson MJ. The significance and interpretation of mildly abnormal oral glucose tolerance. Diabetologia 1971; 7:173–80.
17. Bonora E, Moghetti P, Zancanaro C, et al. Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp. J Clin Endocrinol Metab 1989;68: 374–8.
18. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factor controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 1981;68:1456–67.
19. Piatti PM, Monti LD, Davis SN, et al. Effects of an acute decrease in non-esterified fatty acid levels on muscle glucose utilization and forearm indirect calorimetry in lean NIDDM patients. Diabetologia 1996;39:103–12.
20. Kelley DE, Mitrakau A, Marsh H, et al. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. J Clin Invest 1988;81: 1563–71.
21. Natali A, Buzzigoli G, Taddei S, et al. Effects of insulin on hemodynamics and metabolism in human forearm. Diabetes 1990;39:490–500.
22. Kelley DE, Reilly J, Veneman T, Mandarino LJ. The influence of physiologic hyperinsulinemia on skeletal muscle glucose storage oxidation, and glycolysis in man. Am J Physiol 1990;258:E923–9.
23. Monti LD, Sandoli E, Costa S, et al. Fluorimetric method for the measurement of intermediate metabolites (lactate, pyruvate, alanine, β-hydroxybutyrate, glycerol) using a Cobas Fara centrifugal analyzer. J Autom Chem 1993;15:177–81.
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 griess reaction is used to assay for nitrite. Anal Biochem 1995;224:502–8.
25. Douglas AR, Jones NL, Reed JW. Calculation of whole blood CO2 content. J Appl Physiol 1988;65:473–7.
26. Baron AD. The coupling of glucose metabolism and perfusion in human skeletal muscle. The potential role of endothelium-derived nitric oxide. Diabetes 1996;45:S105–9.
27. Steimberg HO, Brechtel G, Johnson A, et al. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 1994;94:1172–9.
28. Laakso M, Edelman SV, Brechtel G, Baron AD. Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. Diabetes 1992;41:1076–83.
29. Laine H, Yki-Jarvinen H, Kirvela O, et al. Insulin resistance of glucose uptake in skeletal muscle cannot be ameliorated by enhancing endothelium-dependent blood flow in obesity. J Clin Invest 1998;101: 1156–62.
30. McVeigh GE, Brennan GM, Johnston GD, et al. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non–insulin-dependent) diabetes mellitus. Diabetologia 1992; 35:771–6.
31. Young ME and Leighton B. Evidence for alterated sensitivity of the nitric oxide/cGMP signalling cascade in insulin-resistant skeletal muscle. Biochem J 1998;329:73–9.
32. Camici PG, Marraccini P, Lorenzoni R, et al. Coronary hemodynamics and myocardial metabolism in patients with Syndrome X: response to pacing stress. J Am Coll Cardiol 1991;17:1461–70.
33. Utriainen T, Takala T, Luotolahti M, et al. Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM. Diabetologia 1998;41:555–9.
34. Saito Y, Nakao K, Mukoyama M, Imura H. Increased plasma endothelin-1 in patients with essential hypertension. N Eng J Med 1990;322:205.
35. Miyauchi T, Yanagishawa M, Tomizawa T, et al. Increased plasma concentration of endothelin-1 and big endothelin-1 in acute myocardial infarction. Lancet 1989;ii:53–4.
36. Lerman A, Edwards BS, Hallet JW, et al. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. N Engl J Med 1991;325:997–1001.
37. Lerman A, Burnett JC Jr, Higano ST, et al. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. Circulation 1998;97:2123–8.
38. Rabinowitz D, Zierler K. Forearm metabolism in obesity and its response to intra-arterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. J Clin Invest 1962;41: 2173–81.
39. Jackson RA, Perry G, Rogers J, et al. Relationship between the basal glucose concentration, glucose tolerance and forearm glucose uptake in maturity-onset diabetes. Diabetes 1973;22:751–61.
40. Jackson RA, Advani U, Perry G, et al. Dietary diabetes. The influence of low carbohydrate diet on forearm metabolism in man. Diabetes 1973;22:145–59.
41. Radziuk J, Inculet R. The effects of ingested and intravenous glucose on forearm uptake of glucose and glucogenic substrate in normal man. Diabetes 1983;32:977–81.