Insulin-Resistant Glucose Metabolism in Patients With Microvascular Angina—Syndrome X

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Studies in patients with microvascular angina (MA) or the cardiologic syndrome X have shown a hyperinsulinemic response to an oral glucose challenge, suggesting insulin resistance and a role for increased serum insulin in coronary microvascular dysfunction. The aim of the present study was to examine whether patients with MA are insulin-resistant. Nine patients with MA and seven control subjects were studied. All were sedentary and glucose-tolerant. Coronary arteriography was normal in all participants, and exercise-induced coronary ischemia was demonstrated in all MA patients. A euglycemic, hyperinsulinemic clamp was performed in combination with indirect calorimetry. Biopsy of vastus lateralis muscle was taken in the basal state and after 4 hours of euglycemia and hyperinsulinemia (2 mU · kg^{-1} · min^{-1}). The fasting level of "true" serum insulin was significantly higher (43 ± 6 *v* 22 ± 3 pmol/L, $P < .02$) and the rate of insulin-stimulated glucose disposal to peripheral tissues was lower in patients with MA (13.4 ± 1.0 *v* 18.2 ± 1.4 mg · kg fat-free mass [FFM]$^{-1}$ · min^{-1}, $P < .02$) due to a decrease in nonoxidative glucose metabolism (8.4 ± 0.9 *v* 12.5 ± 1.3 mg · kg FFM^{-1} · min^{-1}, $P < .02$). No difference was found in glucose or lipid oxidation rates between the two groups. In patients with MA, as well as in the pooled group of all participants, a positive correlation was demonstrated between the insulin-stimulated increment in fractional activity of skeletal muscle glycogen synthase (GS) and the increment in nonoxidative glucose metabolism after in vivo insulin exposure (MA: $r = .73$, $P = .03$; all participants: $r = .73$, $P = .005$), indicating a reduced in vivo activation of GS in MA patients. In conclusion, (1) MA is part of the insulin resistance syndrome, and (2) the insulin resistance is predominantly localized to the glycogen synthesis pathway in skeletal muscle tissue.

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PATIENTS WITH ANGINA-LIKE chest pain and normal coronary arteries may comprise 10% to 30% of patients undergoing diagnostic cardiac catheterizations for evaluation of chest pain.[1] Previous studies have shown that a subgroup of these patients with exercise-induced ischemia—microvascular angina (MA) or the cardiologic syndrome X—have limited coronary-flow responses to rapid atrial pacing.[2] Administration of ergonovine results in an increase in coronary vascular resistance during pacing,[2] and furthermore, infusion of the potent coronary vasodilator dipyridamole may precipitate myocardial ischemic pain, suggesting that the increased coronary resistance in MA patients resides in the intramural prearterioles (MA).[3] Patients with MA also exhibit an impaired peripheral vasodilator response to local ischemia.[4] These findings suggest a generalized dysregulation of the microcirculation in these patients. Moreover, demonstration of abnormalities of esophageal motility and bronchoconstrictor responses to methacholine inhalation supports the hypothesis that patients with coronary microvascular dysfunction may suffer from a generalized disorder of smooth muscle function.[5] The heterogeneous clinical picture may reflect yet-unknown mechanisms responsible for the increased smooth muscle tone, or that inadequate dilation of prearterioles differs in patients with MA.

Recently, glucose-stimulated hyperinsulinemia but normal fasting serum insulin levels were demonstrated in glucose-tolerant patients with MA, indicating peripheral insulin resistance and a role for increased levels of serum insulin in coronary microvascular dysfunction.[6] In the present study, we have applied the euglycemic, hyperinsulinemic clamp technique to determine if patients with MA are insulin-resistant, and if so, to identify the tissue and the metabolic pathway responsible and to characterize some of the associated biochemical alterations.

SUBJECTS AND METHODS

Subjects

Due to angina-like chest pain, all patients were referred to the Department of Cardiology at National University Hospital in Copenhagen for cardiac catheterization. Coronary angiography demonstrated normal epicardial coronary arteries in all patients. Next, all cardiac medication was terminated for at least 48 hours, whereafter all patients underwent graded bicycle exercise testing, ie, they exercised maximally until they were limited by symptoms. According to the result of the exercise test, they were divided into two groups: group 1, nine patients with ≥1-mm horizontal or downsloping ST segment depression during exercise (MA patients), and group 2, seven patients with normal ECG during exercise (control group; Table 1).

The resting ECG was normal in all subjects, and bundle-branch block was not demonstrated in any subjects during exercise. All patients had a normal hyperventilation test, two-dimensional echocardiography, esophagogastroscopy, gastric acid production, esophageal motility, and no patients had chest-wall pain. All patients were normotensive, and all had a normal response to a 75-g oral glucose tolerance test (2-hour plasma glucose concentration, 6.1 ± 0.4 *v* 5.4 ± 0.3 mmol/L [NS], MA patients *v* control subjects, respectively), and no family history of diabetes or other known insulin-resistant disorders were present. None of the participants were taking any medication during the investigation.

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Table 1. Clinical Characteristics of Study Participants

	MA Patients	Control Subjects
Sex (F:M)	7:2	6:1
Age (yr)	48 ± 4	48 ± 3
Weight (kg)	73.9 ± 4.7	71.6 ± 4.5
Body mass index (kg/m^2)	25.6 ± 1.2	25.4 ± 1.6
FFM (kg)	48.8 ± 3.0	45.5 ± 4.3
Hemoglobin A_{1c} (%)	5.1 ± 0.2	5.1 ± 0.2
Blood pressure (mm Hg)	121/79 ± 6/5	121/80 ± 7/5
Fasting		
Plasma glucose (mmol/L)	5.3 ± 0.1	5.1 ± 0.1
Serum insulin (pmol/L)	43 ± 6	22 ± 3*
Serum C-peptide (mmol/L)	455 ± 46	423 ± 34
Plasma NEFA (mmol/L)	0.96 ± 0.11	0.66 ± 0.08
Plasma total cholesterol (mmol/L)	6.56 ± 0.5	5.77 ± 0.4
Plasma HDL cholesterol (mmol/L)	1.59 ± 0.12	1.34 ± 0.13
Plasma LDL cholesterol (mmol/L)	4.39 ± 0.43	3.85 ± 0.33
Plasma triglycerides (mmol/L)	0.99 ± 0.16	0.87 ± 0.10

NOTE. Results are the mean ± SE.

Abbreviations: NEFA, nonesterified fatty acids; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFM, fat-free mass.

*$P < .02$.

They refrained from physical exercise training and any major physical activity for 3 days before the examination. Before entry into the study, the purpose and risks of the experimental protocol were carefully explained to all the volunteers, and informed consent was obtained. The protocol was approved by the local Ethics Committee and was in accordance with the Helsinki II Declaration.

Study Protocol

Euglycemic, hyperinsulinemic clamp. All participants underwent a euglycemic, hyperinsulinemic clamp after a 10-hour overnight fast. Each clamp lasted for 6 hours: there was a 2-hour basal period (−120 to 0 minutes) followed by a 4-hour (0 to 240 minutes) hyperinsulinemic glucose clamp, with a muscle biopsy at the end of each study period. Details of the clamp technique have been described previously.[7] Briefly, on the morning of each clamp study, an intravenous catheter was inserted into an antecubital vein for infusion of 3-[^{3}H]glucose, insulin, and glucose. Another catheter was inserted in the contralateral arm for blood sampling, and the arm was placed in a heated box throughout the study to ensure arterialization of the venous blood. To assess total peripheral glucose uptake, 3-[^{3}H]glucose was administered throughout the study period as a primed infusion (25 μCi) followed by a continuous infusion (0.25 μCi · min^{-1}). The clamp was performed by continuous infusion of insulin 2 mU · kg^{-1} · min^{-1} (Actrapid; Novo Nordisk, Bagsvaerd, Denmark), and euglycemia was maintained by a variable infusion of 20% glucose at a rate determined by measurement of plasma glucose concentrations at 5- to 10-minute intervals using an automated glucose-oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Steady-state periods were defined as the last 30 minutes during basal measurements (−30 to 0 minutes) and as the last 30 minutes during insulin-stimulated measurements (210 to 240 minutes).

Glucose disposal. Total glucose disposal rate was calculated from plasma concentrations of tritiated glucose and plasma glucose using Steele's non–steady-state equations.[8] In these calculations, the distribution volume of glucose was defined as 200 mL/kg body weight and the pool fraction as 0.65. Negative rates of hepatic glucose output were calculated in all participants during insulin-stimulated steady-state periods, largely as a consequence of a model error emerging at high rates of glucose metabolism.[9] We used the negative numbers to indicate a nil hepatic glucose output. Because hepatic glucose output was fully suppressed during insulin infusion, glucose infusion rates were used to calculate total glucose disposal rate.

Glucose and lipid oxidation. Indirect calorimetry was performed using a flow-through canopy gas-analyzer system (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland). Briefly, air was suctioned at a rate of 40 L · min^{-1} through a canopy placed over the head of the subject. Samples of inspired and expired air were analyzed for oxygen concentration using a paramagnetic differential oxygen sensor, and for carbon dioxide, using an infrared carbon dioxide sensor. Signals from the gas analyzers were processed by the computer, and oxygen consumption and carbon dioxide production were calculated and recorded once per minute. After an equilibration period of 10 minutes, the average gas-exchange rates recorded over the two 30-minute steady-state periods were used to calculate rates of glucose oxidation and lipid oxidation. Protein oxidation rate was estimated from urea nitrogen excretion (1 g nitrogen = 6.25 g protein). Rates of oxidation were calculated from Frayn's equation.[10] Non-oxidative glucose metabolism was calculated as the difference between total glucose disposal rate and glucose oxidation, as determined by indirect calorimetry.

Muscle biopsies. Percutaneous muscle biopsies (~400 mg) were obtained under local anesthesia (1% lidocaine without epinephrine) from the vastus lateralis approximately 20 cm above the knee, using a modified Bergström needle (Stille-Werner; Copenhagen, Denmark). Muscle samples were blotted to remove blood, frozen within 30 seconds in liquid nitrogen, and stored at −80°C until assayed. Before biochemical analysis, muscle samples were freeze-dried and dissected free of visible connective tissue, fat, and blood.

Determination of muscle glycogen synthase activity. Extraction of muscle samples, assays for glycogen synthase (GS), and analysis of protein were performed as described previously.[11] GS activity was assayed in duplicate in the absence or presence of seven different concentrations (0 to 6.7 mmol/L) of glucose-6-phosphate (G6P). Total activity refers to GS activity in the presence of a saturating concentration of G6P (6.7 mmol/L). The final concentration of uridine diphosphate (UDP) glucose (unlabeled UDP-glucose + UDP-[U-^{14}C]glucose; New England Nuclear, Boston, MA) in the reaction mixture was 0.13 mmol/L. GS activity was expressed as nanomoles UDP-glucose incorporated into glycogen per minute per milligram soluble protein in the homogenate. Fractional velocity (Fv) values were calculated as GS activity in the presence of subsaturating levels of G6P divided by GS activity in the presence of 6.7 mmol/L G6P. The concentration of G6P producing half-maximal stimulation ($A_{0.5}$) of GS was calculated using a Hill plot. Interassay coefficients of variation were 0.12 for $A_{0.5}$ and 0.13 for total GS, respectively, when experiments were performed on the same muscle biopsy.

Preparation of GS antipeptide antibody and GS immunoblotting. These procedures were performed as described in detail previously.[12] GS antibody was produced against nine amino acids [(NH_2)-TSSLGEERN] in the carboxy terminus of GS. The homogenates used for GS activity measurements were also used for immunoblotting. Connective tissue and insoluble cell constituents with no measurable GS immunoreactivity were removed from homogenate by centrifugation at 6,000g_{max} (5 minutes at 4°C). Samples of 100 μg total protein per lane were separated in triplicate on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis with a 4% stacking gel. A human muscle protein standard preparation was assayed on each gel in triplicate and used

to correct for intergel variations in the immunoblots. Prestained molecular-weight markers were obtained from BioRad (Richmond, CA). The proteins were electrophoretically transferred to a 0.45-μm nitrocellulose membrane. After blocking for 12 to 24 hours, the membranes were incubated with GS (1:10,000) antiserum for 12 to 24 hours at 4°C. After washing, blots were incubated with [^{35}S]-protein A (Amersham, Arlington Heights, IL) 0.2 μCi/mL buffer for 1 hour at 20°C. Quantitative scanning of autoradiograms was performed within the linear response range, as determined by a standard curve of total GS protein using a Shimadzu CS 9000 flying-spot densitometer (Tokyo, Japan). When six different samples from the same muscle biopsy were analyzed for GS protein, the interassay coefficient of variation was 0.11.

Specificity of the GS antiserum was tested against purified GS (Sigma G 2259; Sigma, St Louis, MO) isolated from rabbit skeletal muscle. The antiserum produced a single band with a molecular weight of 84 kd. Absorption of the antiserum with peptide used for immunization of rabbits resulted in complete disappearance of the band at 84 kd.

Other analytical procedures. Glucose levels in plasma and in urine were measured by a hexokinase method.[13] Levels of intact insulin in serum were measured applying an enzyme-linked, two-site immunoassay.[14] The method uses two murine monoclonal antibodies that bind to two different epitopes on the insulin molecule. The immunoassay is specific, and no proinsulin is bound by the antibodies. C-peptide was analyzed by radioimmunoassay.[15] Hemoglobin A_{1C} level was measured by high-performance liquid chromatography (normal range, 4.1% to 6.1%). Tritiated glucose in plasma was analyzed as described previously.[16] Free fatty acids (nonesterified fatty acids) in plasma were determined using the method reported by Itaya and Michio.[17] Fat-free mass (FFM) was measured using the bioelectrical impedance technique.[18] Plasma levels of fasting total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined using routine methods.

Statistical Analysis

Statistical analysis was performed with the SPSS package (SPSS, Chicago, IL). Nonparametric statistics were used: Mann-Whitney *U* test for unpaired data, Wilcoxon test for paired data, and Spearman's test for correlation analysis. *P* values less than .05 were considered significant. All data are the mean ± SE.

RESULTS

Clinical Characteristics

Patients with MA were well matched to control subjects in terms of anthropometric variables and parameters of fasting glycemia and lipidemia (Table 1).

Glucose and Lipid Metabolism In Vivo

During the basal period of the clamp study, no difference was found in oxidative or nonoxidative glucose metabolism between MA patients and control subjects (oxidative, 1.5 ± 0.1 *v* 1.9 ± 0.3 mg · kg $FFM^{-1} \cdot min^{-1}$, NS; nonoxidative, 1.2 ± 0.3 *v* 1.0 ± 0.3, NS; Fig 1). Fasting plasma glucose concentration (5.3 ± 0.1 *v* 5.1 ± 0.1 mmol/L, NS) was comparable in the two groups, whereas fasting serum insulin level was significantly increased in patients with MA (43 ± 6 *v* 22 ± 3 pmol/L, $P < .02$). The increase in fasting serum insulin was not caused by increased levels of serum proinsulin in patients with MA, since the two-site immunoassay used does not measure human proinsulin levels.

Total glucose disposal rate and oxidative and nonoxidative glucose metabolism increased significantly ($P < .02$) from the basal state to the hyperinsulinemic state in both groups (Fig 1). However, after 4 hours of insulin infusion, total glucose disposal rate and nonoxidative glucose metabolism were significantly lower in patients with MA as compared with control subjects (disposal, 13.4 ± 1.0 *v* 18.2 ± 1.4 mg · kg $FFM^{-1} \cdot min^{-1}$, $P < .02$; nonoxidative, 8.4 ± 0.9 *v* 12.5 ± 1.3, $P < .02$; MA patients *v* controls, respectively), whereas no difference was seen for the glucose oxidation rate between the two groups. During the hyperinsulinemic period, serum insulin levels were similar for MA patients and control subjects (1,077 ± 101 *v* 1,021 ± 81 pmol/L, NS), and likewise, no difference was seen in plasma glucose concentrations (5.4 ± 0.1 *v* 5.4 ± 0.1 mmol/L, NS). Lipid oxidation in the basal state was comparable in MA patients and controls (1.3 ± 0.1 *v* 1.1 ± 0.2 mg · kg $FFM^{-1} \cdot min^{-1}$, NS). After 4 hours of hyperinsulinemia, lipid oxidation was significantly suppressed ($P < .03$) in both groups, and no statistical difference was found between the groups (0.1 ± 0.1 *v* −0.1 ± 0.1 mg · kg $FFM^{-1} \cdot min^{-1}$ [NS], MA patients *v* controls, respectively).

Muscle GS Activity

At basal circulatory insulin levels, total GS activity, ie, in the presence of a maximal concentration of the allosteric activator G6P, was not different between MA patients and controls (Table 2). At the highest steady-state serum insulin level, similar results were obtained. Insulin caused a similar increase in the Fv (ΔFv0.1) of GS at a physiologic G6P concentration of 0.1 mmol/L in MA patients and controls.

The G6P concentration that half-maximally stimulates GS decreased significantly ($\Delta A_{0.5}$) from the basal to highest serum insulin concentration in MA patients ($P < .01$), as

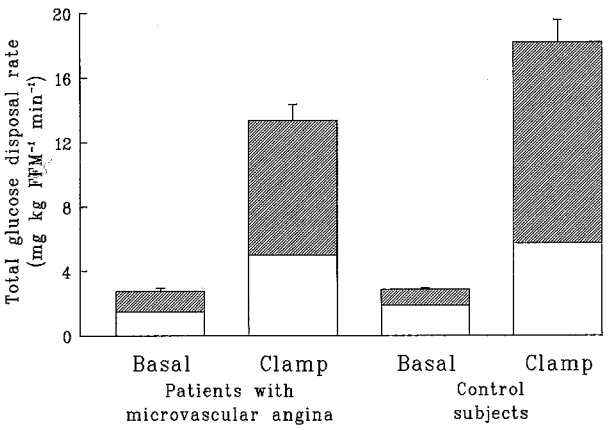

Fig 1. Total glucose disposal rate and oxidative (□) and nonoxidative (▨) glucose metabolism in 9 patients with MA and 7 control subjects during the basal period and after 4 hours of hyperinsulinemia (2 mU · $kg^{-1} \cdot min^{-1}$). During the basal period, no difference in oxidative and nonoxidative glucose metabolism was seen between the groups. After 4 hours of hyperinsulinemia, total glucose disposal rate and nonoxidative glucose metabolism were significantly lower in MA patients *v* control subjects ($P < .02$).

Table 2. GS Activity in Biopsies of Vastus Lateralis Muscle in the Basal State and After 4 Hours of Hyperinsulinemia

	MA Patients			Control Subjects		
	Basal	Clamp	Change	Basal	Clamp	Change
Total GS activity	28.3 ± 2.4	29.8 ± 1.6		27.7 ± 2.2	31.9 ± 3.9	
Fv0.1 (%)	19.5 ± 1.8	46.3 ± 5.0	26.8 ± 4.2	18.4 ± 3.7	50.4 ± 3.9	31.9 ± 1.9
$A_{0.5}$ (mmol/L)	0.35 ± 0.02	0.14 ± 0.02	0.21 ± 0.02	0.38 ± 0.09	0.11 ± 0.02	0.26 ± 0.08

NOTE. Results are the mean ± SE. Total activity refers to GS activity in the presence of a saturating concentration of G6P (6.7 mmol/L) and is shown as nanomoles UDP-glucose incorporated into glycogen per milligram protein per minute. Fv0.1 was calculated as GS activity in the presence of the physiologic concentration of G6P (0.1 mmol/L) divided by GS activity in the presence of 6.7 mmol/L G6P. $A_{0.5}$ is the G6P concentration that half-maximally stimulates GS.

well as in controls ($P < .03$). However, no difference was seen between the groups (Table 2). A significantly positive correlation was found between the increment in fractional activity of GS at the physiologic G6P concentration of 0.1 mmol/L (ΔFv0.1) and the increment in nonoxidative glucose metabolism in patients with MA ($r = .73, P < .03$) and in the pooled group of all participants ($r = .73$, $P = .005$), but not in control subjects ($r = .54, P > .1$; Fig 2). The lack of correlation in the control group probably reflects the small number of subjects in the group and the presence of one outlier point.

GS Immunoreactive Protein

The amount of GS immunoreactivity in homogenates from human skeletal muscle was quantified by immunoblotting using antipeptide antiserum specific for GS. In all participants, a single band of 84 kd was identified for GS immunoreactive protein (Fig 3). Densitometric scanning of autoradiograms showed no difference between the groups in the relative level of GS protein in the basal state when results were normalized for equal amounts of protein: 111 ± 9 (n = 9 patients with MA) versus 110 ± 7 (n = 6 controls) OD unit/100 μg protein (% of internal standard, NS). After 4 hours of euglycemia and hyperinsulinemia, still no significant difference was found between the groups: 115 ± 9 (n = 9 patients with MA) versus 122 ± 9 (n = 6 controls) OD unit/100 μg protein (% of internal standard, NS).

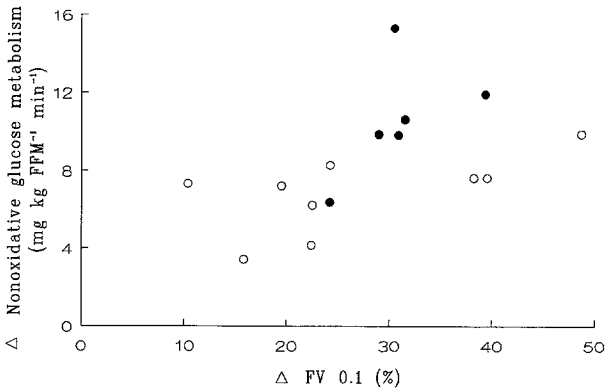

Fig 2. Relationship between the increase above basal values of muscle GS fractional activities (ΔFV0.1) and whole-body nonoxidative glucose metabolism during insulin infusion (2 mU · kg⁻¹ · min⁻¹), respectively, in 9 patients with MA (○: $r = .73, P < .03$) and 6 control subjects (●: $r = .54, P > .1$). Total group: $r = .73, P = .005$.

DISCUSSION

Although patients with MA or the cardiovascular syndrome X have been described for more than 20 years, the pathogenesis behind this condition is still unknown.[1,19] Among endocrinologists, the term syndrome X, or the insulin resistance syndrome, has recently been used to describe a constellation of metabolic changes associated with obesity, non–insulin-dependent diabetes mellitus

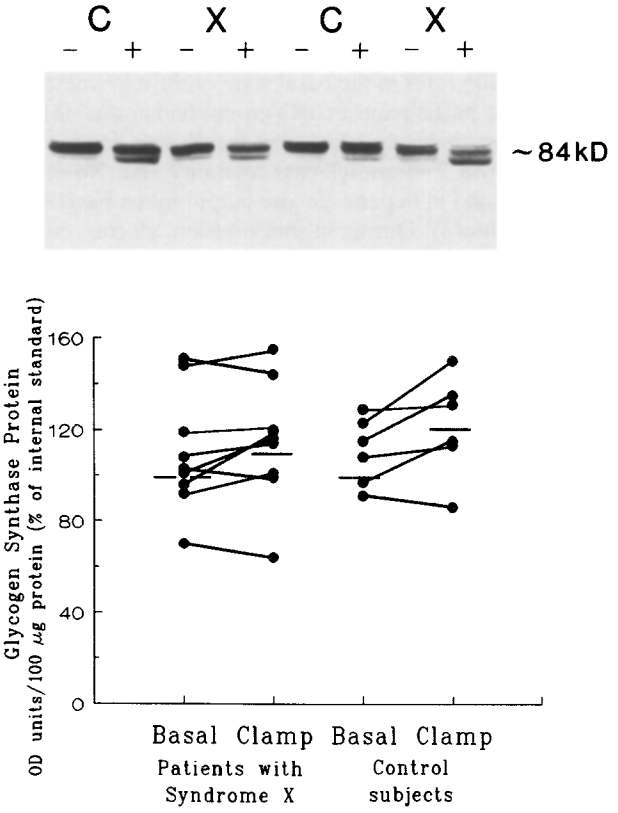

Fig 3. (A) Autoradiogram of immunoblotting analysis of GS protein levels in vastus lateralis muscle in the basal state (−) and after hyperinsulinemia (+) for patients with MA (X) and control subjects (C). Blot is representative of 15 subjects (9 MA patients and 6 sedentary controls). (B) Quantitation with densitometry showed no differences between the groups.

(NIDDM), dyslipidemia, essential hypertension, and premature coronary heart disease, which is thought to be secondary to insulin-resistant glucose metabolism and hyperinsulinemia.[20] In the cardiologic literature, the terms MA and syndrome X refer to angina-like chest pain and normal coronary arteries. Thus, although the two forms of syndrome X appeared to be related to coronary heart disease, no other characteristics were known to be shared between the two forms. However, since insulin resistance could be the inducer of the recently described hyperinsulinemia after a glucose load in MA patients,[6] the major objective of the present study was to examine whether patients with MA and no family history of known insulin-resistant disorders (eg, diabetes mellitus, hypertension, or obesity) were characterized by impaired insulin-stimulated whole-body glucose metabolism. Patients with MA had significantly increased circulating levels of true insulin in the fasting state, indicating insulin resistance of peripheral tissues. Applying the euglycemic, hyperinsulinemic clamp technique in combination with indirect calorimetry, we demonstrated that patients with MA are insulin-resistant with a significantly decreased rate of insulin-stimulated glucose disposal to peripheral tissues (predominantly skeletal muscle). This insulin resistance is due to a decrease in insulin-stimulated nonoxidative glucose metabolism as compared with that in matched control subjects, thus confirming previous studies.[21,22] No differences were found in whole-body glucose or lipid oxidation rates in the basal state or during hyperinsulinemia. The participants in both groups had normal fasting plasma glucose levels, and all were glucose-tolerant as estimated by a 75-g oral glucose tolerance test. No difference was found in hepatic glucose output in the basal state (data not shown). During insulin infusion, glucose output was equally supressed in both groups, indicating normal hepatic insulin responsiveness.

Under experimental conditions of euglycemia and hyperinsulinemia, nonoxidative glucose metabolism primarily reflects glycogen synthesis in skeletal muscle, with only minor amounts of glucose being converted to lipids or lactate, and in all insulin-resistant states examined thus far, reduced glucose storage in muscle represents the major intracellular abnormality responsible for the defect in insulin action.[23] GS, a key enzyme in the muscle glycogen synthesis pathway, exists in an active dephosphorylated form and a less active phosphorylated form.[24,25] The two forms of the enzyme are interconverted by protein kinase and phosphatase reactions,[24-26] with G6P allosterically activating the phosphorylated form of GS, whereas insulin acts covalently on GS by reducing and increasing the activities of specific kinases and phosphatases, respectively.[27,28] However, in vitro determinations of enzyme activity can only be regarded as approximations of in vivo enzyme activity. GS activity is subject to multiple regulatory factors,[25] and only factors that change the phosphorylation state (eg, insulin and G6P) of the enzyme are measurable in vitro, whereas rapid modulation of GS activity by noncovalent regulators cannot be assayed. In the present study, analysis of vastus lateralis muscle showed no differences in total and fractional GS activity either in the basal state or during hyperinsulinemia. However, in patients with MA and in the pooled group of all participants, we found a significantly positive correlation between the in vitro increment in fractional activity of GS at the physiologic G6P concentration of 0.1 mmol/L (ΔFv0.1) and the in vivo increment in nonoxidative glucose metabolism after 4 hours of hyperinsulinemia. This finding suggest a reduced in vivo activation of GS in skeletal muscle of patients with MA as compared with control subjects.

Probably due to the low number of control subjects in the present study, the positive correlation between changes in nonoxidative glucose metabolism and changes in Fv of GS failed to reach statistical significance ($r = .54$, $P = .27$). There is one outlier in the control group who causes the lack of correlation for the group (Fig 2). By eliminating this individual, the correlation in this group would have been significant ($r = .90, P < .05$).

The rate-limiting defect for glucose disposal in skeletal muscle of MA patients could obviously be located at the level of glucose transport. However, nothing is known about muscle glucose transport in patients with MA, but several studies of NIDDM patients may be used for comparison. Muscle glucose transport and glycogen synthesis are both insulin-stimulated pathways, and each has separately been suggested to be responsible for the reduced rate of insulin-stimulated glucose clearance in NIDDM patients. Thus, impaired insulin-stimulated 3-*O*-methylglucose transport in muscle strips isolated from NIDDM patients has been reported.[29,30] Moreover, studies in healthy volunteers and insulin-dependent diabetics show that glucose transport is rate-limiting for overall glucose disposal over a wide range of plasma insulin and glucose levels.[31,32] In patients with NIDDM, measurement of muscle G6P content by ^{31}P nuclear magnetic resonance during a hyperglycemic, hyperinsulinemic clamp showed a decrease both in G6P and in nonoxidative glucose metabolism as compared with healthy controls. These findings are compatible with a defect in glucose transport or phosphorylation reducing the rate of muscle glycogen synthesis.[33]

However, the latter suggestion is challenged by results from two other groups of investigators.[34,35] When NIDDM patients and matched control subjects were examined during similar glucose utilization rates and circulating insulin levels, NIDDM patients had an increased intracellular concentration of free glucose and G6P in muscle tissue, indicating that the rate-limiting step in muscle glucose metabolism in patients with NIDDM may be located after G6P. Based on these considerations, we cannot determine whether MA patients express additional impairments of glucose transport and phosphorylation.

The syndrome of angina pectoris with normal coronary arteriograms and exercise-induced ischemia is clearly heterogeneous. From several previous studies, it is evident that the most consistent finding in patients with MA seems to be a reduced vasodilatory capacity of the coronary microcirculation—thus, the condition is called MA.[19] However, the mechanisms that cause the abnormal coronary blood-flow

pattern are unknown. Patients with MA not only appear to have an impaired vasodilator reserve that affects the coronary circulation, but also show evidence of a reduced peripheral vasodilator response to the stress induced by local ischemia.[4] These findings suggest a more generalized disorder localized to the regulation of the microcirculation in patients with MA. A comparable impaired vasodilation of resistance vessels has also been shown in patients with arterial hypertension and diabetes mellitus,[36,37] diseases characterized by insulin resistance.[38] Hence, it appears that MA is part of the insulin resistance syndrome, which can be expressed in several ways depending on the genetics of the particular individual.

In conclusion, we have demonstrated that patients with angina-like chest pain and normal findings at coronary arteriography but with an ischemic response on ECG during exercise (MA) are characterized by fasting hyperinsulinemia and insulin-resistant whole-body nonoxidative glucose metabolism in peripheral tissues.

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