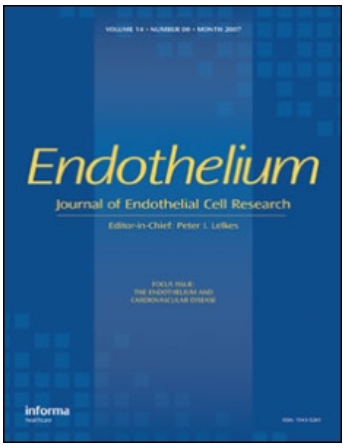


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# Metalloproteinases in Diabetics and Nondiabetics during Acute Coronary Syndromes and after 3 Months

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# Metalloproteinases in Diabetics and Nondiabetics during Acute Coronary Syndromes and after 3 Months

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**The authors hypothesized that matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1, and TIMP-2 would be abnormal in acute coronary syndromes (ACS). Forty-six diabetic and 78 nondiabetic patients during ACS and after 3 months were enrolled in this study. MMP-2, -9 and TIMP-1, -2 plasma levels were measured. Significant decrease of MMP-2, TIMP-1, and TIMP-2 plasma levels was observed in the nondiabetic group with ACS after 3 months compared to the baseline value. Significant decrease of MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma levels was observed in the diabetic group with ACS after 3 months compared to the baseline value. MMP-9, TIMP-1, and TIMP-2 plasma levels were higher in diabetic patients during ACS compared to nondiabetic patients during ACS. TIMP-1 and TIMP-2 increases were observed in diabetic patients with ACS at 3 months compared to nondiabetic patients after ACS. MMPs and TIMP-1 and -2 plasma levels were alterated in nondiabetic and diabetic patients during ACS and after 3 months, which may reflect abnormal extracellular matrix metabolism in diabetes during and after acute event.**

**Keywords** Extracellular Matrix, Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases, Acute Coronary Syndromes, Diabetes Mellitus

It is well-known that the main cause of acute coronary syndromes (ACS) consists of plaque disruption, with subsequent superimposed intracoronary thrombus, leading to prolonged coronary obstruction (Falk et al. 1995). Both matrix metalloproteinase (MMP)-2 or MMP-9 are synthesized and secreted locally in atherosclerotic lesions, predominantly by monocyte-derived macrophages and endothelial cells (Death et al. 2003). In addition, through their proteolytic activity, these MMPs are capable of degrading the fibrous cap of atherosclerotic plaques, thus contributing to plaque destabilization (Kai et al. 1998).

Earlier studies provided evidence that high MMP plasma values are associated with the presence of ACS (Jones et al. 2003). In particular, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been reported to be markedly increased in patients with ACS, both within the atherosclerotic plaque and into the peripheral circulation (Brown et al. 1995).

Type-2 diabetic patients are at high risk for acute coronary events due to an increased propensity of their atherosclerotic plaques to ulceration and overlying thrombosis (Cooper et al. 2001).

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The most common extracellular pathology in diabetes is the thickening of the basement membrane as a result of the deposition of extracellular matrix proteins (Vranes et al. 1999). Extracellular matrix is a dynamic structure that requires constant synthesis and degradation by MMPs (Nagase and Woessner 1999). This is tightly controlled by tissue inhibitors of metalloproteinase (TIMPs) (Vincenti 2001).

The expression and activity of MMPs in diabetes thus far have been reported predominantly in relation to microvascular complications (Noda 2003; Maxwell et al. 2001). Instead, there are very few data available on the role of MMPs and TIMPs in macrovascular and microvascular events in diabetic patients. To date, only two studies, which undertook the latter investigation, have reported significantly higher levels of MMP-2 and MMP-9 in diabetic patients with coronary artery disease (CAD) compared to nondiabetics with CAD (Marx et al. 2003; Derosa et al. 2007). Moreover, as far as it is known, there are no data on the association of MMP-2 and MMP-9 with acute coronary events amongst individuals with type-2 diabetes.

Therefore, in the present study we investigated whether plasma MMP-2 and MMP-9 concentrations are increased and linked with acute coronary events in nondiabetic patients and in type-2 diabetic patients when dosed during ACS and after 3 months; the levels of TIMP-1 and TIMP-2 are also investigated.

## MATERIALS AND METHODS

### Study Design

This multicenter case-control trial was conducted in the Department of Internal Medicine and Therapeutics at University of Pavia, in the "G. Descovich" Atherosclerosis Study Center, "D. Campanacci" Clinical Medicine and Applied Biotechnology Department at University of Bologna, and in the Catheterization Laboratory, Cardiovascular Department at Policlinico of Monza, Italy.

The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki and its amendments.

### Study Population

We recruited 124 patients of either sex admitted to the Coronary Care Unit (CCU) with a diagnosis of ACS associated with at least one of the following findings: (1) ischemic electrocardiographic changes consisting of new (or presumably new) ST-segment depression, persistent (>20 min) ST-segment elevation, T-wave inversion; (2) elevated cardiac markers, including cardiac troponin T (cTnT) and cardiac troponin I (cTnI).

The patients were normoweight or overweight (body mass index [BMI], 23.5 to 29.7 kg/m$^2$) (World Health Organization 1997).

Exclusion criteria are a history of impaired hepatic function (defined as plasma aminotransferase and/or $\gamma$-glutamyltransferase level higher than the upper limit of normal [ULN] for age and sex); impaired renal function (defined as serum creatinine level higher than the ULN for age and sex); severe anemia; and neoplastic, infectious, or autoimmune disease. Patients with cerebrovascular conditions within 6 months before study enrollment also were excluded.

Diagnosis of ST-segment elevation acute myocardial infarction (STEMI) was based on chest pain for 30 min and ST-segment elevation >1 mm in two or more contiguous leads on the 12-lead electrocardiogram (ECG). Patients with unstable angina (UA-NSTEMI) were included if they presented with recurrent chest pain at rest associated with ischemic ST-segment or T-wave changes. Most of these patients also had elevated values of creatine kinase (CK)-MB and cTnT.

Participants included 90 men (72.6%) and 34 women (27.4%) aged 62.8 to 75.3 years. There were no significant differences among centers in sex distribution, age, CAD, and diabetes duration, and in CAD and diabetes treatment. All patients were also evaluated after 3 months at the same centers. All patients provided written informed consent to participate.

### Cardiologic Procedures

All patients underwent coronary angiography in Judkins' technique (Judkins 1967). Two experienced, blinded observers visually assessed the coronary angiographies. When needed, quantitative assessment was performed by a third blinded observer. Hemodynamically relevant CAD was defined as $\geq$75% area reduction with respect to prestenotic segment area in at least one major epicardial coronary artery or major branch (>2.5 mm diameter). Patients were classified as having 1-, 2-, or 3-vessel disease. Coronary artery territories were defined from the angiogram in patients with 1-vessel disease by using the American Heart Association/American College of Cardiology guidelines (Fox 2004).

### Laboratory Methods

Before starting the study, and also after 3 months, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs, a 12-lead ECG, measurements of fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostasis model assessment (HOMA index), blood pressure, lipid profile, coagulation, fibrinolytic, and inflammation parameters, MMP-2, MMP-9, TIMP-1, and TIMP-2.

All plasmatic parameters were determined after a 12-h overnight fast, determined 2 h after lunch. Venous blood samples were taken for all patients between 08.00 and 09.00 and were drawn from an antecubital vein with a 19-gauge needle without venous stasis.

We used plasma obtained by addition of $Na_2$-EDTA, 1 mg/mL, and centrifuged at 3000 $\times g$ for 15 min at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory except biochemical

markers for the diagnosis of myocardial injury, which were determined within 24 h of admission. cTnT and cTnI levels exceeding the upper normal limit of each local laboratory were considered as increased. As elevated CK and/or CK-MB levels within 24 h of admission were considered the values exceeding twice the upper normal limit of each local laboratory.

BMI was calculated by the investigators as weight in kilograms divided by the square of height in meters. The estimate of insulin resistance was calculated by HOMA index with the formula: FPI ($\mu$U/mL) $\times$ FPG (mmol/L)/22.5, as described by Matthews and coworkers (Matthews et al. 1985). Blood pressure (BP) measurements were obtained from each patient (using the right arm) in the seated position, using a standard mercury sphygmomanometer (Erkameter 3000; ERKA, Bad Tolz, Germany) (Korotkoff I and V) with a cuff of appropriate size. BP was measured by the same investigator at each visit, in the morning and after the patient had rested for $\geq$10 min in a quiet room. Three successive BP readings were obtained at 1-min intervals, and the mean of the three readings was calculated.

Plasma glucose was assayed by glucose-oxidase method (GOD/PAP; Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV) of <2% (European Diabetes Policy Group 1999). Plasma insulin was assayed with Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound $^{125}$I-insulin (intra- and interassay CsV: 4.6% and 7.3%, respectively) (Heding 1972).

Total cholesterol (TC) and triglycerides (Tg) levels were determined using fully enzymatic techniques (Klose et al. 1978; Wahlefeld 1974) on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0 and 2.1 for TC measurement and 0.9 and 2.4 for Tg measurement, respectively. High-density lipoprotein cholesterol (HDL-C) level was measured after precipitation of plasma apo B–containing lipoproteins with phosphotungstic acid (Havel 1955), intra- and interassay CsV were 1.0 and 1.9, respectively; low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula (Friedewald et al. 1972).

Plasminogen activator inhibitor-1 (PAI-1) was assayed with a commercial two-stage indirect enzymatic assay (Spectrolyse; Biopool AB, Umea, Sweden), intra- and interassay CsV were 5.9% (Juhan-Vague and Collen 1992). Fibrinogen (Fg) was determined according to Clauss. The intra-assay CV for the Fg method was less than 5% (Clauss 1959).

Homocysteine (Hct) was measured by a modified procedure of Araki and Sako (1987), with high-performance liquid chromatography and fluorescence detection. The intra-assay CV of the method was 2.5%.

High-sensitivity C-reactive protein (Hs-CRP) was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE, USA). The intra- and interassay CsV were 5.7% and 1.3%, respectively (Rifai 1999).

Lipoprotein (a) [Lp(a)] was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) method, which is insensitive to the presence of plasminogen, using the commercial kit Macra-Lp(a) (SDI, Newark, DE, USA) (Uterman and Weber 1987; Scanu and Scandian 1991); the intra- and inter-assay CsV of this method were 5% and 9%, respectively.

MMP-2 and MMP-9 levels and activities and TIMP-1 and TIMP-2 levels were determined by a two-site ELISA using commercial reagents (Amersham Biosciences, Uppsala, Sweden). The intra- and interassay CsV for measuring MMP-2 levels were 5.4%, and 8.3%, respectively, whereas those for measuring MMP-2 activities were 5.4% and 17.9%, respectively (Fujimoto et al. 1993). The intra- and interassay CsV to evaluate MMP-9 levels were 4.9% and 8.6%, respectively, whereas those for measuring MMP-9 activities were 3.4% and 20.7%, respectively (Fujimoto et al. 1994). The intra- and interassay CsV for measuring TIMP-1 levels were 9.3% and 13.1%, respectively (Clark et al. 1991), whereas those for measuring TIMP-2 levels were 5.4% and 5.9%, respectively (Fujimoto et al. 1993b).

Quantitative cTnT was determined with the second-generation TnT ELISA (Enzymun-Test Troponin-T) on ES 300 system (Boehringer Mannheim, Mannheim, Germany). This assay uses the two cardiac-specific monoclonal antibodies M11.7 and M7 (Muller-Bardoff et al. 1997). The detection limit was 0.04 $\mu$g/L. The intra- and interassay CsV for measuring cTnT levels were 3.2% and 6.2%, respectively. Quantitative cTnI was measured with the Opus Troponin I assay (Behring Diagnostics, Westwood, MA, USA) performed on the Opus Plus Analyser, with a detection limit of 0.5 $\mu$g/L (Larue et al. 1993). The intra- and interassay CsV for measuring cTnI levels were 3.9% and 7.6%, respectively.

Concentrations of CK were measured with dry chemistry using Ektachem 950ICR System (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). The CV for measuring CK levels was 8.6% at 192 U/L (Toffaletti et al. 1983).

CK-MB mass was analyzed by microparticle enzyme immunoassay (MEIA) technology with AxSYM system (Abbott Diagnostics, Abbott Park, IL, USA). The CV for measuring CK-MB mass levels was 8.3% at 3.8 $\mu$g/L (Jockers-Wretou and Pfleiderer 1975).

### Statistical Analysis

Nonparametric tests were employed in the statistical analysis of the data because data were not normally distributed (Kolmogorov-Smirnov test). Mann-Whitney $U$ test was used to compare two independent groups. A $p$ value of less than .05 was considered statistically significant. All tests were two-sided. Statistica 6.0 (Statsoft, 2003, Tulsa, OK) was used for statistical computations.

## RESULTS

### Study Sample

A total of 124 patients were enrolled in the trial. The characteristics of the patient population at study entry are shown in Table 1.

TABLE 1
Baseline characteristics and parameter changes at the third month of the study in both groups

| | Nondiabetics with ACS | | Diabetics with ACS | |
|---|---|---|---|---|
| | Baseline | 3 months | Baseline | 3 months |
| BMI (kg/m$^2$) | 26.5 ± 3.0 | 25.7 ± 2.6$^{\cdot}$ | 27.0 ± 2.7 | 26.7 ± 2.7$^{\wedge}$ |
| $HbA_{1c}$ (%) | 5.3 ± 0.4 | 5.3 ± 0.4 | 7.4 ± 0.5$^{\S\S\S}$ | 6.8 ± 0.4$^{\wedge\wedge\wedge}$*** |
| FPG (mg/dL) | 92.4 ± 7.6 | 91.1 ± 5.7 | 146.2 ± 11.4$^{\S\S\S}$ | 131.7 ± 11.5$^{\wedge\wedge\wedge}$*** |
| FPI ($\mu$U/mL) | 8.3 ± 2.8 | 7.9 ± 2.5$^{\cdot\cdot}$ | 14.6 ± 3.8$^{\S\S\S}$ | 13.2 ± 3.3$^{\wedge\wedge}$*** |
| HOMA index | 1.9 ± 0.6 | 1.8 ± 0.6$^{\cdot\cdot\cdot}$ | 5.2 ± 1.5$^{\S\S\S}$ | 4.4 ± 1.2$^{\wedge\wedge}$*** |
| SBP (mm Hg) | 139.8 ± 12.3 | 134.1 ± 7.0$^{\cdot\cdot\cdot}$ | 140.0 ± 10.5 | 134.7 ± 6.1$^{\wedge\wedge}$ |
| DBP (mm Hg) | 81.7 ± 9.2 | 77.8 ± 6.5$^{\cdot\cdot}$ | 88.0 ± 8.1$^{\S\S\S}$ | 83.9 ± 5.5$^{\wedge\wedge}$*** |
| TC (mg/dL) | 208.5 ± 25.8 | 194.7 ± 15.2$^{\cdot\cdot\cdot}$ | 215.8 ± 25.2 | 188.5 ± 14.2$^{\wedge\wedge\wedge}$** |
| LDL-C (mg/dL) | 129.0 ± 24.1 | 123.8 ± 14.2$^{\cdot}$ | 127.5 ± 27.7 | 116.4 ± 16.7** |
| HDL-C (mg/dL) | 42.6 ± 6.5 | 41.5 ± 5.9$^{\cdot}$ | 42.5 ± 6.6 | 40.9 ± 5.2$^{\wedge}$ |
| Tg (mg/dL) | 183.2 ± 56.5 | 143.9 ± 32.4$^{\cdot\cdot\cdot}$ | 221.3 ± 28.0$^{\S\S\S}$ | 150.5 ± 21.9$^{\wedge\wedge\wedge}$ |
| Lp(a) (mg/dL) | 15.2 ± 15.0 | 16.1 ± 18.4$^{\cdot\cdot}$ | 18.8 ± 16.1 | 12.9 ± 11.3 $^{\wedge\wedge}$ |
| PAI-1 (ng/mL) | 73.9 ± 27.3 | 41.6 ± 14.2$^{\cdot\cdot\cdot}$ | 77.0 ± 30.3 | 63.0 ± 24.0$^{\wedge\wedge}$*** |
| Hct ($\mu$mol/L) | 10.2 ± 7.2 | 8.4 ± 5.8$^{\cdot\cdot\cdot}$ | 18.5 ± 23.0$^{\S}$ | 13.7 ± 15.8$^{\wedge\wedge}$* |
| Fg (mg/dL) | 329.0 ± 92.2 | 302.6 ± 56.2$^{\cdot\cdot}$ | 383.5 ± 98.3$^{\S\S}$ | 320.9 ± 45.9$^{\wedge\wedge}$ |
| Hs-CRP (mg/dL) | 1.5 ± 1.2 | 0.7 ± 0.4$^{\cdot\cdot\cdot}$ | 1.2 ± 1.2$^{\S}$ | 0.5 ± 0.3$^{\wedge\wedge}$* |

*Note*. Data are means ± *SD*. $^{\cdot}p < .05$ versus baseline; $^{\cdot\cdot}p < .01$ versus baseline; $^{\cdot\cdot\cdot}p < .0001$ versus baseline; $^{\S}p < .05$ versus baseline ACS; $^{\S\S}p < .01$ versus baseline ACS; $^{\S\S\S}p < .0001$ versus baseline ACS; $^{\wedge}p < .05$ versus baseline; $^{\wedge\wedge}p < .01$ versus baseline; $^{\wedge\wedge\wedge}p < .0001$ versus baseline; $^{*}p < .05$ versus ACS; $^{**}p < .01$ versus ACS; $^{***}p < .0001$ versus ACS.

ACS = acute coronary syndromes; BMI = body mass index; $HbA_{1c}$ = glycated hemoglobin; FPG = fasting plasma glucose; FPI = fasting plasma insulin; HOMA index = homeostasis model assessment index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; Tg = triglycerides; Lp(a) = lipoprotein(a); PAI-1 = plasminogen activator inhibitor-1; Hct = homocysteine; Fg = fibrinogen; Hs-CRP = high-sensitivity C-reactive protein.



## Body Mass Index

A significant BMI decrease ($p < .05$) was observed after 3 months in both groups compared to the baseline. There was no difference in BMI value between diabetic and nondiabetic patients with ACS during and after acute event, as reported in details in Table 1.

## Glycemic Control

No $HbA_{1c}$and FPG changes were present in the nondiabetic group with ACS after 3 months, whereas significant FPI and HOMA index decreases ($p < .01$ and $p < .0001$, respectively) were obtained in this group compared to baseline value. $HbA_{1c}$, FPG, FPI, and HOMA index variations ($p < .01$ and $p < .0001$, respectively) were observed in the diabetic group with ACS after 3 months compared to baseline values. Significant $HbA_{1c}$, FPG, FPI, and HOMA index increases ($p < .0001$) were present in the diabetic group with ACS during and after acute event compared to the nondiabetic with ACS (Table 1).

## Blood Pressure Control

Significant SBP and DBP decreases ($p < .01$, and $p < .0001$, respectively) were observed after 3 months in the nondiabetic group with ACS compared to baseline. SBP and DBP changes ($p < .01$) were present in the diabetic group with ACS after acute event respect to the baseline values. A significant DBP increase ($p < .0001$) was obtained in diabetic patients with ACS during and after acute event with respect to nondiabetic patients with ACS, whereas no SBP change was observed in both groups, as reported in Table 1.

## Lipid Profile and Lipoprotein Variables

Significant decreases of LDL-C and HDL-C ($p < .05$, respectively) and significant decreases of TC and Tg ($p < .0001$, respectively) were observed after 3 months in the nondiabetic group with ACS, whereas a significant Lp(a) increase ($p < .01$) was obtained in this group compared to the baseline. TC and Tg ($p < .0001$, respectively), HDL-C ($p < .05$), and Lp(a) ($p < .01$) changes were present in diabetic patients with ACS after acute event, whereas no LDL-C variation was observed with respect to the baseline values. A significant Tg increase ($p < .0001$) was obtained in the diabetic group with ACS during acute event, whereas no TC, LDL-C, HDL-C, and Lp(a) variations were present in this group with respect to nondiabetic patients with ACS. TC and LDL-C decreases ($p < .01$) were present after 3 months in the diabetic group with ACS, whereas no HDL-C, Tg, and Lp(a) changes were present

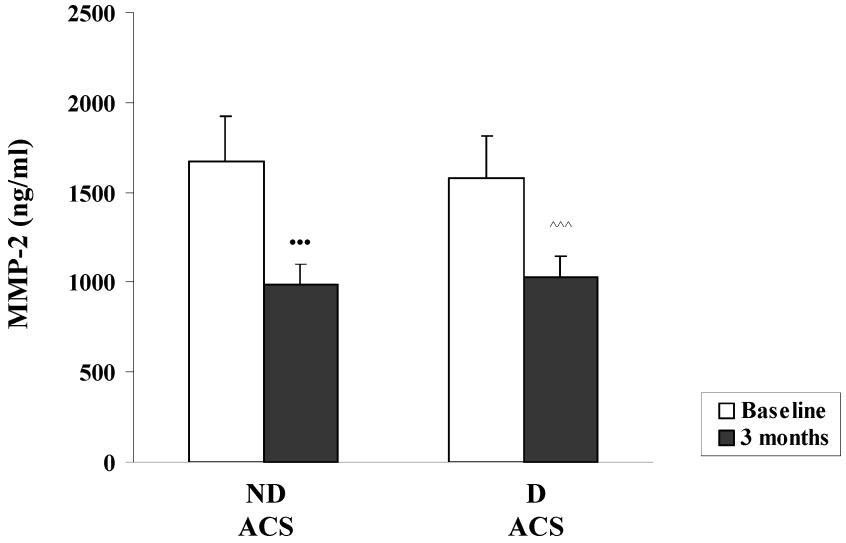


FIG. 1. MMP-2 levels at baseline and at the third month of the study in both groups. Values are mean ± *SD*. ··· $p < .0001$ versus baseline; ^^^ $p < .0001$ versus baseline. ACS = acute coronary syndromes; NDACS = nondiabetics with ACS; DACS = diabetics with ACS; MMP-2 = matrix metalloproteinase-2.

in this group with respect to nondiabetic patients with ACS (Table 1).

## Coagulation, Fibrinolytic, and Inflammation Parameters

Significant PAI-1, Hct, hs-CRP ($p < .0001$, respectively), and Fg ($p < .01$) decreases were observed after 3 months in respect to baseline values in the nondiabetic group, while significant PAI-1, Hct, Fg, and hs-CRP ($p < .01$, respectively) decreases were observed after 3 months compared to the baseline values in the diabetic group. Significant Hct and Fg increases ($p < .05$ and $p < .01$, respectively) were present in diabetic patients with ACS during acute event, whereas hs-CRP was lower ($p < .05$) in these patients compared to nondiabetic patients with ACS. No PAI-1 change was present in the diabetic group with ACS during acute event compared to nondiabetic patients with ACS. PAI-1 and Hct increases ($p < .05$ and $p < .0001$, respectively) were obtained after 3 months in diabetic patients with ACS, whereas hs-CRP was lower ($p < .05$) in these patients compared to the nondiabetic group with ACS. No Fg change was present in the diabetic group with ACS after acute event compared to nondiabetic patients with ACS (Table 1).

## Enzymatic Characterization

MMPs, TIMP-1, and TIMP-2 levels quantified in the diabetic group with ACS and the nondiabetic group with ACS are reported in Figures 1 to 4. Significant MMP-2, TIMP-1, and

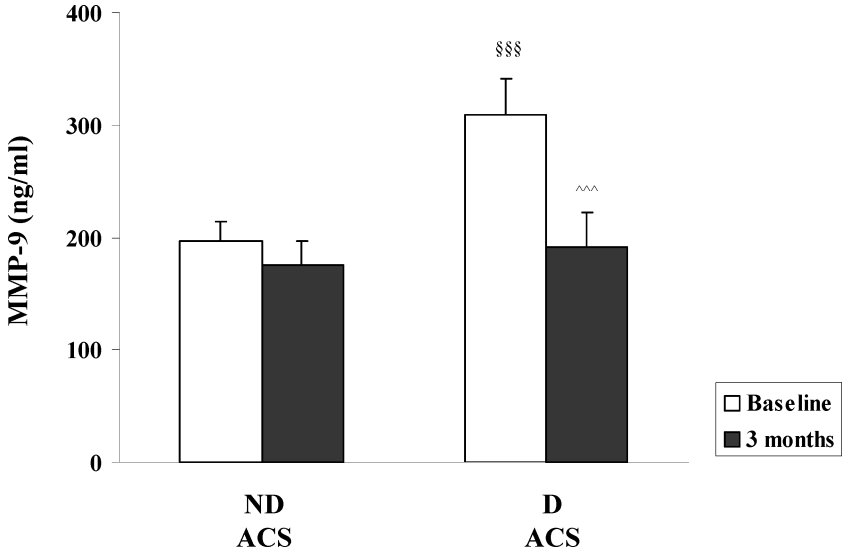


FIG. 2. MMP-9 levels at baseline and at the third month of the study in both groups. Values are mean ± *SD*. §§§ $p < .0001$ versus baseline ACS; ^^^ $p < .0001$ versus baseline. ACS = acute coronary syndromes; NDACS = nondiabetics with ACS; DACS = diabetics with ACS; MMP-9 = matrix metalloproteinase-9.

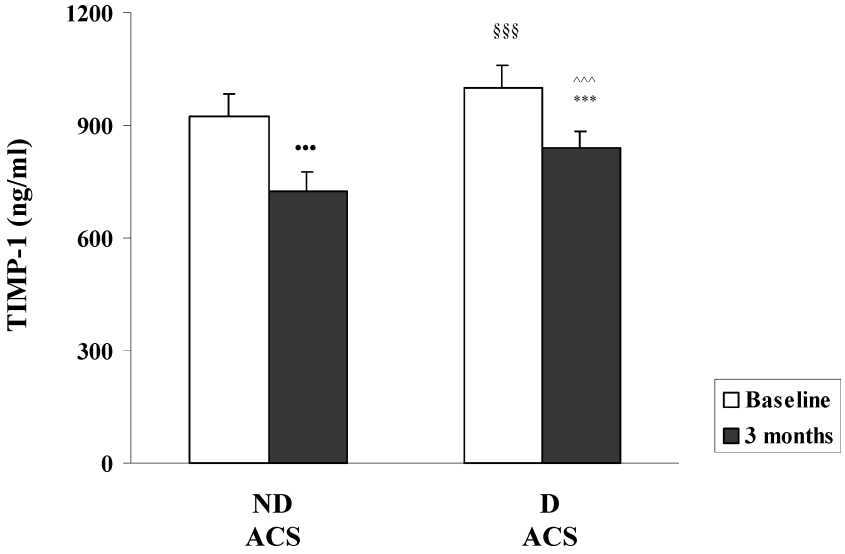


FIG. 3. TIMP-1 levels at baseline and at the third month of the study in both groups. Values are mean ± *SD*. $^{...}p < .0001$ versus baseline; $^{§§§}p < .0001$ versus baseline ACS; $^{\wedge\wedge\wedge}p < .0001$ versus baseline; $^{***}p < .0001$ versus ACS. ACS = acute coronary syndromes; NDACS = nondiabetics with ACS; DACS = diabetics with ACS; TIMP-1 = tissue inhibitor of metalloproteinase-1.

TIMP-2 decreases ($p < .0001$) were observed after 3 months in the nondiabetic group with ACS, whereas no MMP-9 variation was obtained in this group compared to the baseline. MMP-2, MMP-9, TIMP-1, and TIMP-2 changes ($p < .0001$) were present in diabetic patients with ACS after acute event respect to the baseline values. MMP-9, TIMP-1, and TIMP-2 levels were significantly higher ($p < .0001$) in diabetic patients with ACS during acute event with respect to nondiabetic patients with ACS, whereas no significant MMP-2 variation was present in both groups. Significant increase was observed for TIMP-1 and TIMP-2 levels ($p < .0001$) after 3 months in the diabetic group with ACS compared to the nondiabetic with ACS (Figures 1 to 4).

## Correlation Analyses

Correlation analyses did not indicate various patterns of associations in MMP-2, MMP-9, TIMP-1, and TIMP-2 with any other parameters in diabetic and nondiabetic patients with ACS before and after acute event.

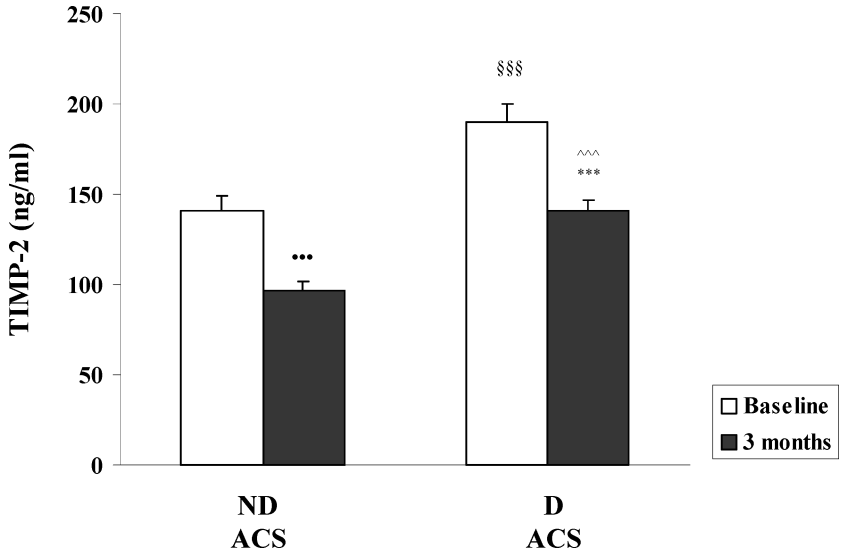


FIG. 4. TIMP-2 levels at baseline and at the third month of the study in both groups. Values are mean ± *SD*. $^{...}p < .0001$ versus baseline; $^{§§§}p < .0001$ versus baseline ACS; $^{\wedge\wedge\wedge}p < .0001$ versus baseline; $^{***}p < .0001$ versus ACS. ACS = acute coronary syndromes; NDACS = nondiabetics with ACS; DACS = diabetics with ACS; TIMP-2 = tissue inhibitors of metalloproteinase-2.

## DISCUSSION

The interest of the scientific community toward the MMPs is rapidly increasing during recent years. MMPs represent a marker of vascular disease (Maxwell et al. 2001), as was also demonstrated by our group in children and adolescents with type-1 diabetes (Derosa et al. 2004) and in patients with hypertension (Derosa et al. 2006) and pathological conditions associated with chronic endothelial dysfunction and unstable plaque formation (Nakazawa et al. 2005).

Furthermore, it has been postulated that they could be a relevant target for atherothrombotic cardiovascular disease treatment (Sierevogel et al. 2003). In fact, circulating MMPs levels are elevated in patients with acute myocardial infarction, unstable angina, and also after coronary angioplasty, which is related to late loss index after the procedure: these observations suggest that MMP expression may not only be related to the instability of the plaque but also to the formation of restenotic lesions (Ikeda et al. 2003).

In our study we observed that diabetic patients affected by ACS have MMP-9, TIMP-1, and TIMP-2 levels significantly higher than those of nondiabetic ACS patients, whereas MMP-2 levels are not significant.

It is possible that peripheral macrophages and leucocytes might be a source of elevated MMPs because monocytes can be activated in patients with ACS (Jude et al. 1994). In this sense, our findings are in agreement with the most part of the available ones, except for those very recently reported by Eckart et al. (2004), who observed an increased level of MMP-2 in myocardial infarction patients, whereas paradoxically MMP-9 decreased. On the other hand, Ferroni and colleagues reported that MMP-9 was markedly elevated in myocardial infarction patients, compared to healthy controls, whereas MMP-2 did not change (Ferroni et al. 2003).

Several lines of evidence have implicated MMPs and TIMPs in the rupture of atherosclerotic plaques and subsequent ACS (Dollery et al. 1995; Kai et al. 1998; Inokubo et al. 2001). Although convincing data exist demonstrating the association of MMPs with atheromatous plaques and colocalization of MMPs in the shoulder region of vulnerable lesions, a direct association with actual plaque rupture is less established.

Diabetes mellitus is a key player in cardiovascular morbidity and mortality, where it is closely linked to the genesis and progression of coronary atheroma, generalized vascular atherosclerosis, hypertension, and dyslipidemia (Lim et al. 2004).

Furthermore, diabetes and/or hyperglycemia per se are associated with significant change to the structure and function of cardiac and vascular tissue. These changes indicate alterations of MMPs that are a central factor in the control of extracellular matrix (ECM) turnover.

There is increasing evidence of the role of MMP-9 in atherogenesis (Uemura et al. 2001; Shah and Galis 2001). Recently, Galis and Khatri (2002) used an MMP-9 knockout mouse carotid artery model to demonstrated that a MMP-9 deficiency leads to a decrease in intimal hyperplasia and lumen loss, but an accumulation of interstitial collagen. This finding showed that the MMP-9 inhibition could increase the mechanical stability of arteries by increasing their collagen content and decreasing lumen loss, indicating the involvement of MMP-9 in the pathogenesis of atherosclerosis. This observation could explain the increase of MMP-9 levels in our diabetic patients with ACS with respect to nondiabetics.

We also showed significant MMP-2, TIMP-1, and TIMP-2 decreases in both the nondiabetic group and diabetic group with ACS after 3 months, whereas a reduction of MMP-9 was observed only in diabetics. However, a significant increase was observed for TIMP-1 and TIMP-2 levels after 3 months in diabetics ACS patients compared to nondiabetics with ACS, whereas no change of MMP-2 and MMP-9 was present in both groups.

Past clinical studies have demonstrated alterations in MMPs and TIMPs after myocardial infarction (MI) (Tyagi et al. 1996; Hirohata et al. 1997). The early post-MI period has been associated with induction of MMPs secondary to the acute inflammatory/injury response (Peterson et al. 2000; Creemers et al. 2001; Lindsey et al. 2001; Frangogiannis et al. 2002). Most notably, MMP-9 release from endogenous myocardial cells and neutrophils has been documented early after MI (Rohde et al. 1999; Lindsey et al. 2001). Conversely, MMP-2 is ubiquitously distributed in cardiac myocytes and fibroblasts (Cheung et al. 2000) and it has been shown to be persistently up-regulated after MI (Dixon et al. 1996; Carlyle et al. 1997; Podesser et al. 2001). Therefore, MMP-2 may play an important role in early myocardial healing and the late postinfarct remodeling process (Hayashidani et al. 2003).

Recently, Wilson et al. (2003) measured regional levels of myocardial MMPs and TIMPs in a post-MI sheep model at 2 months after myocardial infarction. MMP-2 level was increased, whereas MMP-9 level was reduced within the transition and MI regions at 8 weeks after MI. TIMP-1 and TIMP-2 were undectable within the MI region (Wilson et al. 2003). These results showed that there was a significant induction of MMPs within the post-MI myocardium, which was type and region specific. In particular, MMP-9 levels are reduced 2 months after MI, probably because this MMP is associated with early post-MI events (Creemers et al. 2001; Lindsey et al. 2001). Moreover TIMP-1 and TIMP-2 levels declined after an established MI, indicating a loss of MMP inhibitory control (Wilson et al. 2003). These observations could explain the reduction of TIMP-1 and TIMP-2 in both nondiabetic and diabetic ACS subjects after 3 months and why MMP-9 plasma level decreased only in diabetic subjects.

In addition, our data are partially in contrast with those of Portik-Dobos et al. (2002), which report reduced syntheses of both MMP-2 and MMP-9 in a small sample of diabetic patients undergoing coronary artery bypass grafting surgery versus nondiabetic controls (Portik-Dobos et al. 2002).

Taken together, our findings show divergent patterns of MMP and TIMP expression, suggesting their species-specific response post ACS. Moreover, these observations might give insight into

molecular events influencing ECM remodeling in nondiabetic and diabetic ACS patients and might contribute to clarifying the potential role of MMPs in the progression of atherosclerosis and plaque vulnerability.

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