Regulation of Matrix Metalloproteinases 2 and 9 Activities by Peroxynitrites in Term Placentas From Type 2 Diabetic Patients

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Regulation of Matrix Metalloproteinases 2 and 9 Activities by Peroxynitrites in Term Placentas From Type 2 Diabetic Patients

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Abstract

Matrix metalloproteinases (MMPs) are proteolytic enzymes related to a proinflammatory environment in several diseases, including diabetes, which can be activated by reactive nitrogen species. This work aimed to determine MMP-2 and MMP-9 activities and nitration in term placentas from type 2 diabetic patients and verify the hypothesis that peroxynitrites are positive regulators of placental MMP-2 and MMP-9 activities. For this purpose, term placentas from healthy and type 2 diabetic patients were analyzed for MMP-2 and MMP-9 levels and activities, protein nitration, and nitration of MMP-2 and MMP-9. Villous explants were cultured in the presence of peroxynitrites for further evaluation of MMP-2 and MMP-9 activities. We found that MMP-2 and MMP-9 activities were increased in term placentas from diabetic patients. These changes were found even when MMP-2 protein concentrations were diminished and MMP-9 protein concentrations were not changed in the diabetic group. Increased protein nitration and specific nitration of MMP-2 and MMP-9 were found in term placentas from diabetic patients. Peroxynitrites were able to increase the activity of placental MMP-2 and MMP-9. Taken together, this study has shown for first time that peroxynitrites can nitrate and activate MMP-2 and MMP-9 in the placenta, a nitrative pathway possibly related to MMPs overactivity in the placentas from type 2 diabetic patients.

Keywords

diabetes, pregnancy, placenta, matrix metalloproteinases (MMPs), peroxynitrites

Introduction

The increasing incidence of type 2 diabetes, the earlier age at onset of this pathology, and the increase in maternal age during the last decades, have made type 2 diabetic pregnant women an increasing population in most countries. Similar to type 1 diabetes, type 2 diabetes increases the risks of miscarriage and malformations.[1,2] Besides, type 2 diabetes shares with gestational and type 1 diabetes increased risks of perinatal morbidity and mortality and of programming of metabolic and cardiovascular diseases in the offspring's life.[3,4] Despite these common features, many specific changes may be present in type 2 diabetic gestations and their identification may help in the design of specific therapeutic approaches.

The placenta is a unique fetal and maternal organ. Its proper development and function is crucial for fetal development and programming of diseases in the neonatal life.[5,6] Studies performed in type 2 diabetic patients have identified increased oxidative stress in the placentas from type 2 diabetic patients.[7] Excessive reactive oxygen species can combine with nitric oxide (NO) leading to the formation of the potent oxidant peroxynitrite.[8] Although aberrant NO and peroxynitrite metabolism has been identified in gestational and type 1 diabetes, it has not been previously addressed in pregnant type 2 diabetic patients.[9–12] Previous studies performed in human term placentas and experimental models of diabetes have shown the capacity of oxidative stress and NO to regulate matrix metalloproteinases (MMPs) activity.[13,14] Matrix metalloproteinases are proteolytic enzymes capable of cleaving all components of the extracellular matrix, needed during fetal and placental

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development.[15] Both MMP-2 and MMP-9 are relevant for trophoblast invasion and differentiation, and for implantation, vascularization, angiogenesis, and parturition processes.[16–19] Both NO and peroxynitrite can regulate MMPs activities, at least in part through posttranscriptional modifications that s-nitrosylate and nitrate MMPs.[20,21] Although these posttranscriptional modifications are physiological in processes such as implantation and trophoblast invasion,[22] they can turn into pathological processes when MMPs are overactivated. Overactivity of MMPs is clearly related to many pathologies such as cardiovascular disease, nephropathy, and cancer.[16,23] Increased MMPs are markers of a proinflammatory state and are increased in sera from both type 1 and type 2 diabetic patients.[24,25] Increased MMP-2 and MMP-9 activities are found in the placenta from animal models of diabetes throughout gestation.[26,27] Although MMP-9 activity has been previously found increased in term placentas from type 1 diabetic women, placentas from diet-treated gestational diabetic women show reduced MMP-9 activity.[14] In this work, we aimed to analyze MMP-2 and MMP-9 activities in term placentas from type 2 diabetic and healthy patients and to evaluate the relationship between placental MMPs activity and peroxynitrite-induced MMPs nitration.

Materials and Methods

Participants and Tissue Collection

Women with no pregnancy complications (controls) were recruited at the time of delivery in term pregnancy. Based on clinical history, women with type 2 diabetes and without other comorbidities were recruited in the second trimester of pregnancy. The placentas ($n = 12$ in both the control and the diabetic group) were obtained at the term of cesarean section delivery, carried out due to obstetric reasons. Medical and obstetrical criteria for exclusion were vascular/renal complications, preeclampsia, anemia with total hemoglobin $\leq 8\%$, preterm labor, premature rupture of membranes, chorioamnionitis, placental abruption, and acute foetal distress. The protocol was approved by the institutional review boards of Hospital Materno-Infantil "Ramón Sardá." Written consent was given by volunteers in accordance with the Institution's guidelines.

Placental Preparations

Placental villous tissues from control and type 2 diabetic patients were obtained after the basal and the chorial plates were dissected out. Villous tissues (100 mg) were immediately frozen at $-80^{\circ}C$ for further determination of MMP-2 and MMP-9 protein content, lipid peroxidation, NO production, peroxynitrite-induced protein nitration, specific MMP-2 and MMP-9 protein nitration, and total protein content (evaluated by the Bradford method[28]). Placental villous tissues from control and type 2 diabetic patients were incubated (100 mg) in a metabolic shaker under an atmosphere of 5% CO_2 in air at $37^{\circ}C$, with or without the addition of peroxynitrites (10 and 100 μmol/L), as previously described,[14,29] for further determination of MMP-2 and MMP-9 activities by zymography.

Zymography Analysis of MMP-2 and MMP-9 Activities

Zymography was performed to evaluate the presence of MMP-2 and MMP-9 activities as previously described.[26] Both MMPs and pro-MMPs were analyzed by zymography, since the exposure to sodium dodecyl sulfate (SDS) induces changes in pro-MMPs conformation which are associated with their activation.[30] Protein derived from placental explants culture media (75 μg) was subjected to a 10% SDS–polyacrylamide gel electrophoresis (SDS-PAGE), in which 1 mg/mL gelatin (type A from porcine skin) had been incorporated. Following electrophoresis, gels were washed in 30% Triton X-100 for 60 minutes to remove SDS. Then, the gels were incubated in 50 mmol/L Tris Buffer pH 7.4, containing 0.15 mmol/L NaCl and 30 mmol/L $CaCl_2$, for 18 hours at $37^{\circ}C$. Gels were stained with Coomassie blue and then destained with 10% acetic acid and 30% methanol in water. The areas of proteolytic activity appeared as negative-stained bands in the dark background.

The identities of MMPs were based on their molecular weights and a positive internal control (HT-1080 conditioned medium). The enzymatic activity was quantified using the Image J analysis program (Bethesda, Maryland) and expressed as arbitrary densitometric units, which were normalized to the internal control.

Zymography was also performed in culture media from placentas exposed to peroxynitrites (10 and 100 μmol/L); the placental explant cultures were performed as described in the placental preparation section.

Analysis of NO Production

Nitric oxide production was evaluated by the determination of the concentration of its stable metabolites nitrates/nitrites, as previously described,[26] by using a commercial assay kit (Cayman Chemical Co, Michigan, USA). Briefly, 100 mg of placental tissue was homogenized in Tris-HCl solution (0.1 mmol/L, pH 7.4) and an aliquot was separated to determine the protein content. Nitrates in the supernatant were reduced to nitrites using nitrate reductase, and total nitrites were then quantified by the Griess reaction. Different amounts of sodium nitrate were used as standards. Resulting optical densities were measured at 540 nm in a microliter plate. Results are expressed as nmol/mg of protein.

Analysis of Lipid Peroxidation

Lipid peroxidation was evaluated as previously described,[13] by measuring the concentrations of thiobarbituric acid reactive substances (TBARS), a method widely used to assess peroxidation of fatty acids.[31] Briefly, 100 mg of placental tissue was homogenized in 100 mmol/L Tris-HCl buffer, pH 7.6. The homogenate was added with trichloroacetic acid (40%) and centrifuged at 900 g for 10 minutes. The supernatant was added with an equal volume of thiobarbituric acid (46 mmol/L), and

the solution was heated at 95°C for 15 minutes. Then, the samples were cooled and quantified spectrophotometrically at 530 nm. Different concentrations of malondialdehyde (Sigma-Aldrich, St Louis, Missouri) subjected to the same conditions as the tissue homogenates were used as standards. Thiobarbituric acid reactive substances are expressed as nmol/mg protein.

Determination of Peroxynitrite-Induced Damage

Evaluation of protein nitration, an index of peroxynitrite-induced damage[32] was evaluated as previously described,[29] by using a commercial assay kit (Nitrotyrosine Assay Kit, Millipore, California). Briefly, 100 mg of placental tissue was homogenized in Tris-HCl 0.1 mol/L buffer and then subjected to the assay kit. Samples or standards together with an antibody against nitrotyrosine were added to nitrated Bovine Serum Albumin (BSA)-coated plates to carry out a competitive enzyme-linked immunosorbent assay (ELISA). After incubation and washing, a secondary horseradish peroxidase-conjugated antibody was added to the wells. The excess of antibody was washed and LumiGLO Chemiluminiscent Substrate added that allow to develop luminiscence for 10 minutes. Resulting relative units of light were measured in a Luminoskan Ascent Luminescence Microplate Reader (Lab System, Israel). The relative units of light values were compared with those from the standard curve. Data are reported as μg nitro-BSA equivalents/mg of protein.

Western Blot Analysis of MMP-2 and MMP-9 Protein Concentrations

Placental tissues were homogenized in 500 μL of ice-cold lysis buffer (20 mmol/L HCl pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, and 5 μL protease inhibitor cocktail) incubated for 2 hours on ice and then centrifuged at 9600*g* for 10 minutes. Proteins on the supernatant were separated using 10% SDS-PAGE and transferred to nitrocellulose membranes, as previously described.[33] The membranes were blocked and incubated overnight at 4°C with one of the following primary antibodies: mouse anti-MMP-2 antibody (1:200; Santa Cruz Biotechnologies, California), goat anti-MMP-9 antibody (1:600; Calbiochem, La Jolla, California), or rabbit anti-actin antibody (1:500; Sigma-Aldrich) as an internal control. Then the membranes were washed and incubated for 1 hour at room temperature with the appropriate horseradish peroxidase-labeled secondary antibody: anti-mouse (1:15000), anti-goat (1:12000), or anti-rabbit (1:10000; Jackson ImmunoResearch Laboratories, Inc, Baltimore, Maryland). The signal was revealed using the enhanced chemiluminescence (ECL) system (GE Healthcare, Piscataway, New Jersey). The bands corresponding to MMPs and actin were identified with 2 molecular weight standards: pre-stained full-range rainbow (12-225 kDa; GE Healthcare, Buckinghamshire, UK) and Magic Mark XP (20-120 kDa; Invitrogen, California). The relative intensity of protein signals was quantified by densitometric analysis using the Image J software (Bethesda, Maryland). Results showing the MMP protein/actin protein ratio are expressed as relative units.

Analysis of MMP-2 and MMP-9 Protein Nitration

Placental tissues from healthy and type 2 diabetic patients were homogenized in immunoprecipitation buffer (Tris-HCl 50 mmol/L, EDTA 1 mmol/L, Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) 1 mmol/L, NaCl 150 mmol/L, $MgCl_2$ 1 mmol/L, octylphenoxypolyethoxyethanol (IGEPAL) 1%, SDS 0.1%, glycerol 10%, and protease inhibitor cocktail (Sigma-Aldrich). The immunodetection of nitrated proteins was performed as previously described.[29,34] An equivalent volume of 1 mg protein from each of the placental homogenate was incubated with 3 μg of anti-nitrotyrosine antibody (Calbiochem) overnight at 4°C. The nitrotyrosine–antibody complexes were incubated with 30 μL of sepharose bound protein A/G (Santa Cruz Biotechnologies) for 1 hour at 4°C. The immunocomplexes were centrifuged at 9600*g* for 10 minutes at 4°C, saving the supernatant to be used as a control of the experiment (Negative control: equal volumes of the immunoprecipitate supernatants were loaded into an SDS gel and incubated with an anti-nitrotyrosine antibody. No signal was detected, data not shown). The precipitated beads containing the nitrated proteins were washed with buffer plus protease inhibitors. Then the sample loading buffer (62.5 mmol/L Tris, 2% SDS, 0.7 mol/L 2-mercaptoethanol, 10% glycerol, and 0.12% bromophenol blue) was added to the beads and heated for 5 minutes at 95°C. After centrifugation at 9600*g* for 1 minute, the supernatants containing the nitrated proteins were loaded to an SDS-PAGE for detection of nitrated MMPs by Western blot, performed as described above using mouse anti-MMP-2 antibody (1:200) and goat anti-MMP-9 (1:600) as primary antibodies. Secondary antibodies (anti-mouse and anti-goat) were used at a concentration of 1:6000. Relative intensity of protein signals was quantified by densitometric analysis using the Image J software and expressed as relative units.

Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM). Groups were compared by Student *t* test or one-way analysis of variance (ANOVA) in conjunction with Tukey test where appropriate. Statistical analyses were carried out using Prism 4.0 software (GraphPad). Differences between groups were considered significant when P values $< .05$.

Results

Participants

Demographic data of all participants involved in this study are shown in Table 1. There were no significant differences in maternal age, parity, fasting glucose, body mass index, and gestational age when the patients with type 2 diabetes and the healthy pregnant women (controls) were compared. Multiparity was observed in most of the control and diabetic patients

Table 1. Anthropometric and Metabolic Data of the Study Population[a]

	Control Patients (n = 12)	T2DM Patients (n = 12)
Maternal age (years)	27.7 ± 1.7	32.4 ± 1.7
Parity		
Primiparous	1	1
Multiparous	11	11
Fasting glucose (mg/dL)[b,c]	< 99	75-100
Maternal BMI (kg/m^2)[b]	26 ± 1.8	29 ± 1.6
Treatments[c]		
Diet (kcal)	—	1800-2000
Insulin (Units per day)	—	24-138
Gestational age (wk)[d]	38.5 ± 0.2	38.6 ± 0.3
Placental weight (g)[d]	508 ± 12	581 ± 28[e]
Weight at birth (g)[d]	3217 ± 96	3795 ± 113[f]
Neonatal complications[d]	None	RDS (n = 1) Macrosomia (n = 2)

Abbreviations: T2DM, type 2 diabetes mellitus; BMI, body mass index; RDS, respiratory distress syndrome.
[a] Data are given as means ± SEM. Student *t* test.
[b] First trimester of pregnancy.
[c] Third trimester of pregnancy.
[d] Delivery.
[e] $P < .05$.versus control patients (healthy participants).
[f] $P < .001$ versus control patients (healthy participants).

(75% had had 2-4 previous pregnancies). Three of the evaluated type 2 diabetic women were obese, although no significant differences were observed in the evaluated parameters when compared to the nonobese diabetic women. All women with type 2 diabetes received insulin therapy and dietary treatment for metabolic control, as shown in Table 1. Placental weight and neonatal weight were increased in the type 2 diabetic patients when compared to controls ($P < .05$). Neonatal complications were observed in 3 of the type 2 diabetic patients evaluated (2 cases of macrosomia and 1 case of respiratory distress syndrome).

Placental MMP-2 and MMP-9 Activities and Protein Concentrations

Zymography was performed to analyze the activity of MMP-2 and MMP-9 in their active and proenzyme forms. The MMP-2 in its proenzyme form and MMP-9 in its active and proenzyme forms were found overactivated in term placentas of diabetic patients when compared with those of healthy patients ($P < .01$; Figure 1A). When protein concentrations were analyzed by Western blot, concentrations of MMP-2 in their active and proenzyme forms were reduced ($P < .05$), and concentrations of MMP-9 in their active and proenzyme forms were not changed in the diabetic group when compared to controls (Figure 1B).

Placental Lipid Peroxidation, NO, and Peroxynitrites

The TBARS, measured as an index of lipid peroxidation, were found increased in term placentas of type 2 diabetic patients when compared to controls ($P < .01$; Figure 2A). Nitric oxide concentrations, evaluated by the measurement of its stable metabolites nitrates/nitrites, were also increased in the placentas of type 2 diabetic patients ($P < .05$; Figure 2B). In addition, protein nitration, which indicates peroxynitrite-induced damage, was increased in the placentas of type 2 diabetic patients when compared to controls ($P < .001$; Figure 2C).

Nitration of MMP-2 and MMP-9

As overactivity of MMP-2 and MMP-9 was not related to increased protein levels, and as oxidative and nitrative stress was detected in the placentas of type 2 diabetic women, we analyzed whether these MMPs were nitrated in the placentas. For this, nitrated proteins were immunoprecipitated with anti-nitrotyrosine antibody, and MMP-2 and MMP-9 were identified by Western blot with specific antibodies in the immunoprecipitate. Figure 3 shows that MMP-2 in its proenzyme form and MMP-9 in its active and proenzyme forms were nitrated, and that the concentrations of nitrated pro-MMP-2, pro-MMP-9, and active MMP-9 were increased in term placentas of type 2 diabetic patients ($P < .05$) when compared to controls.

Effect of Peroxynitrites on MMP-2 and MMP-9 Activities

In order to determine whether peroxynitrites can modulate MMP-2 and MMP-9 activities, zymography was performed in the culture media of villous explants from placentas of type 2 and healthy women cultured for 1 hour in the presence of peroxynitrites (10 and 100 μmol/L). Figure 4 shows that the activity of MMP-2 and MMP-9 in their proenzyme forms are enhanced in the presence of peroxynitrites in placental explants from healthy patients ($P < .01$), while only proMMP-9 was further activated in the presence of the higher dose of peroxynitrite evaluated in placental explants from diabetic patients ($P < .01$). The MMP-2 in its active form was not detected and there was no further activation of the active form of MMP-9 in the placentas from both control and type 2 diabetic patients with the 2 doses of peroxynitrites evaluated.

Discussion

In this work, oxidative stress, nitrative stress, and MMP-2 and MMP-9 overactivation were identified in term placentas of type 2 diabetic patients, indicating a proinflammatory environment similar to that previously found in type 1 diabetic patients and in several animal models of pregestational diabetes.[35,36] An important finding was that MMP-2 and MMP-9 overactivities are associated with an increase in peroxynitrites, as peroxynitrites were capable of activating MMP-2 and MMP-9 in the placenta.

Although relevant in physiological developmental processes including implantation and placentation, MMPs overactivation is clearly associated with several diseases, including gestational diseases such as preeclampsia and premature rupture of

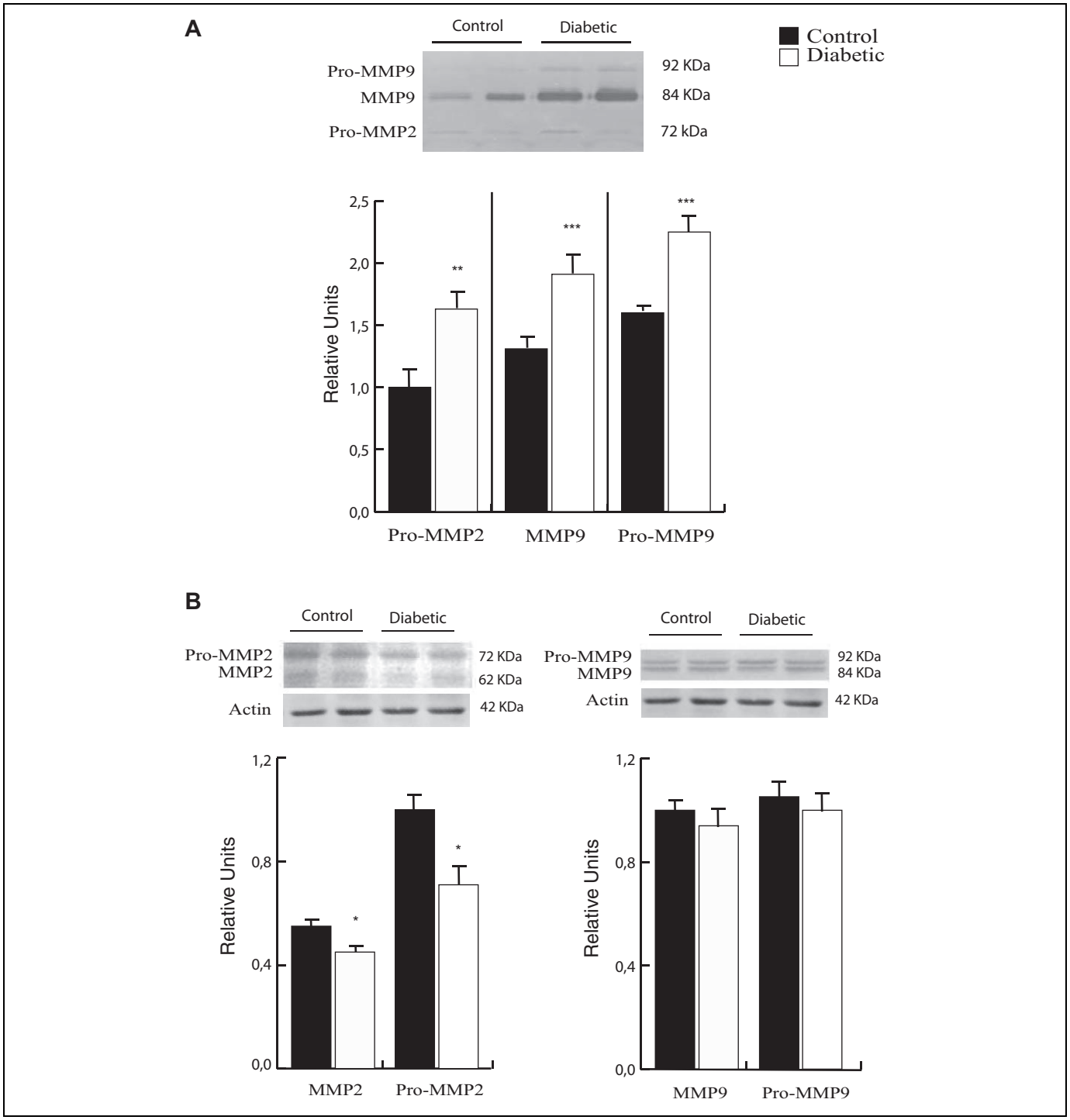

Figure 1. Effect of type 2 diabetes on placental MMP-2 and MMP-9 activities and protein expression. A, Gelatinolytic activity of MMP-2 and MMP-9 evaluated in placentas from type 2 diabetic and healthy patients by zymography. Densitometric analysis and representative zymography bands are shown. B, MMP-2 and MMP-9 protein expression evaluated in placentas from type 2 diabetic and healthy patients by Western blot. Densitometric analysis and representative Western blot bands are shown. All data are displayed as the mean $\pm$ SEM. ANOVA and post hoc test of Tukey, n = 12 in each group, $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$ versus placentas of healthy participants (controls). MMP indicates matrix metalloproteinase; ANOVA, analysis of variance; SEM, standard error of the mean.

membranes.[16–18] Both MMP-2 and MMP-9 can cleave many components of the extracellular matrix, promote the release of factors with proinflammatory properties encrypted in the extracellular matrix, and change the structure and function of many proteins, growth factors, and receptors, both in the extracellular matrix and in the cell membrane.[37,38] This indicates the

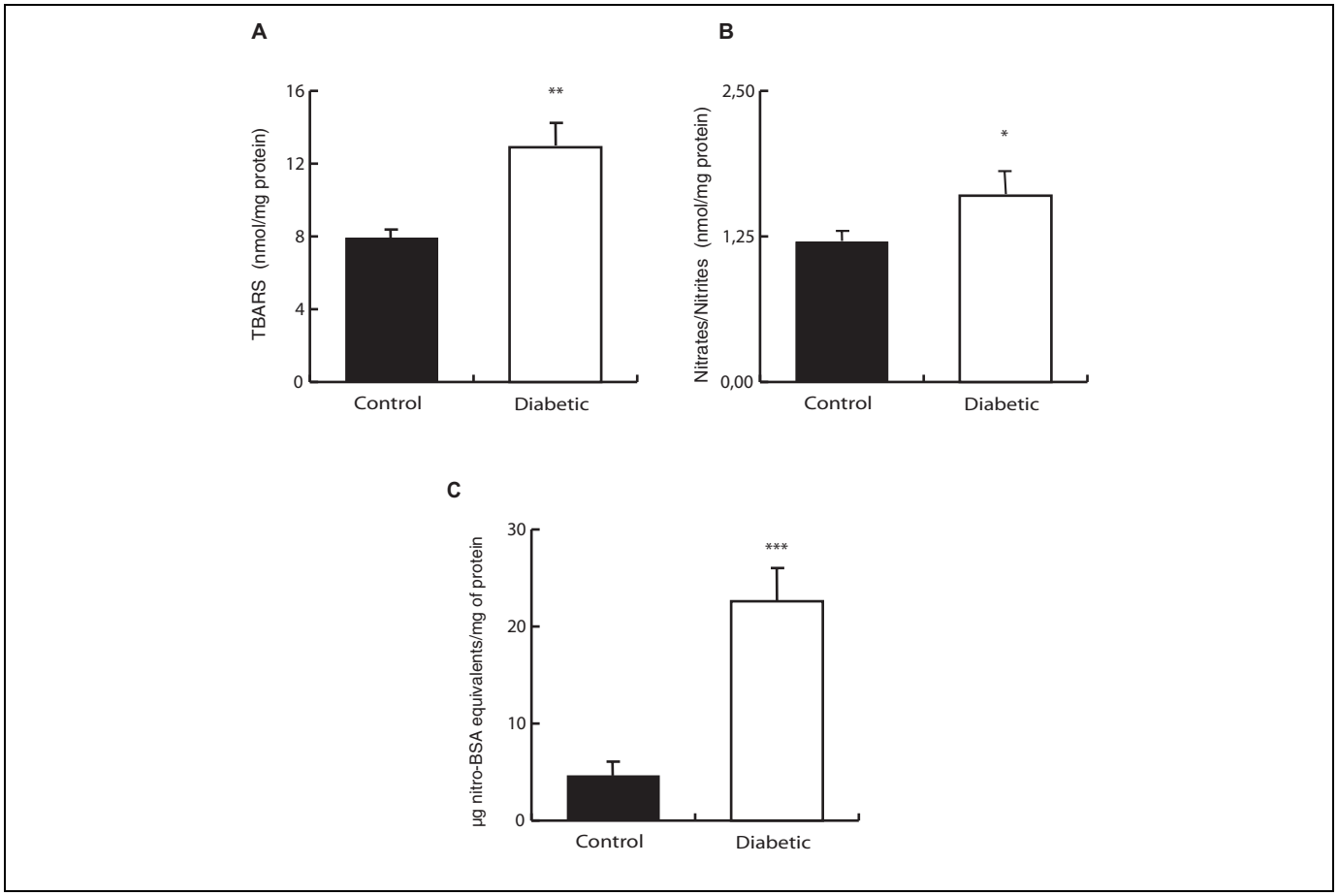

Figure 2. Effect of type 2 diabetes on placental lipid peroxidation, nitric oxide production, and peroxynitrite-induced damage. A, TBARS, a marker of lipid peroxidation, (B) nitrates-nitrites, nitric oxide stable metabolites, and (C) protein nitration, an index of peroxynitrite-induced damage evaluated in placental tissues from type 2 diabetic and healthy patients. All data are displayed as the mean ± SEM. Student *t* test, n = 12 in each group, **P* < .05, ***P* < .01; ****P* < .001 versus placentas of healthy participants (controls). TBARS indicates thiobarbituric acid reactive substances; SEM, standard error of the mean.

relevance of an appropriate control of MMPs activity to prevent adverse changes in the cell-to-cell and cell-to-extracellular matrix interactions. Active MMP-9 is abundant in term placentas, and active MMP-2 is relevant in implantation and the initial steps of placentation.[18,39,40] Pro-MMPs are relevant for further processing but can also exert enzymatic activity in the presence of certain substrates which allow a conformational change, as the prodomain does not need to be removed from the zymogen to acquire activity.[30,41] Besides, certain extracellular matrix components can sequester MMP-2 and prevent its activation.[42] As we here found that the active form of MMP-2 is not detected by zymography, it may be highly retained by extracellular matrix components or by endogenous tissue inhibitors of matrix metalloproteinases (TIMPs) in the placenta at term gestation.[30,43] On the other hand, it is known that reactive oxygen and nitrogen species can activate MMPs, a pathway that can be accompanied by further enzymatic autocatalysis leading to the cleavage of the propeptide.[44] Indeed, the association of MMPs overactivity with a proinflammatory environment is clearly defined in several pathologies, including neurodegenerative diseases, cardiovascular diseases, and cancer.[23,45] Overactivity of MMPs promotes the infiltration of macrophages, capable of secreting MMPs and also reactive oxygen and nitrogen species, positive regulators of MMPs activity.[44,46] Whether the observed overactivity of MMPs is related to an increased recruitment of immune cells requires further studies. We here found that overactivity of MMPs is not the result of increased MMPs protein expression, but rather a result of the increase in oxidative and nitrogen species. We previously found that NO and reactive oxygen species can stimulate MMP-9 activity in human term placentas and in placentas from animal models.[13,14,33] In this work, we found that increased oxidative stress and NO production are increased in term placentas from type 2 diabetic patients, alterations that result in an increase in the formation of peroxynitrites, as demonstrated by the observed increased protein nitration.

In addition, we demonstrated that peroxynitrites can upregulate MMP-2 and MMP-9 activities in the placenta. There are reports of a localized increase in MMP-9 S-nitrosylation in trophoblasts at the migration edge, related to their invasiveness

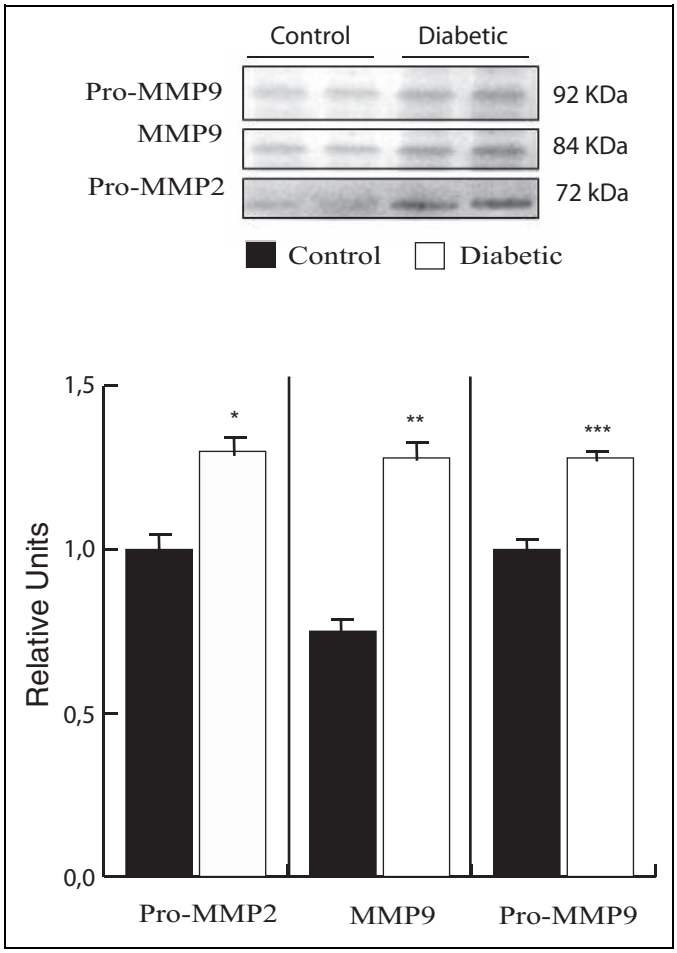

Figure 3. Effect of type 2 diabetes on placental nitration of MMP-2 and MMP-9. Nitrated MMP-2 and MMP-9 evaluated in placentas from type 2 diabetic and healthy patients by immunoprecipitation with an anti-nitrotyrosine antibody, followed by Western blot analysis of the nitrated MMP-2 and MMP-9 performed using specific antisera. Densitometric analysis and representative Western blot bands are shown. All data are displayed as the mean ± SEM. ANOVA and post hoc test of Tukey, n = 12 in each group, *$P < .05$, **$P < .01$; ***$P < .001$ versus placentas of healthy participants (controls). MMP indicates matrix metalloproteinase; ANOVA, analysis of variance; SEM, standard error of the mean.

capacity.[22] Although related to physiological processes such as implantation, peroxynitrite-induced nitration in tyrosine residues is considered a marker of peroxynitrite-induced damage.[32] Indeed, it reflects changes in the functions of multiple enzymes frequently associated with pathological processes such as the inactivation of prostacyclin, which leads to the loss of a potent vasodilator agent; the activation of cyclooxygenase, which leads to the formation of proinflammatory prostaglandins; and the activation of Poly ADP Ribose Polimerase (PARP), which leads to the induction of apoptosis.[32,47] Nitrated proteins change the function of relevant proteins in the placenta. Indeed, nitrated superoxide dismutase is increased in placentas from diabetic rats, and p38 MAP kinase nitration is enhanced in placentas from patients with preeclampsia, alterations associated with a loss of enzymatic activity.[29,48] In this work, we identified that pro-MMP-2 and MMP-9 in its active and proenzyme forms are nitrated in the placentas from type 2 diabetic women, showing that the MMPs that present increased enzyme activity by zymography are those that are highly nitrated. Moreover, peroxynitrites can increase MMP-2 and MMP-9 activities mainly in the placentas from healthy patients, suggesting that these MMPs were already activated by peroxynitrites in the diabetic placentas. The fact that both MMP-2 and MMP-9 proenzymes can become activated in the presence of peroxynitrites suggests that this is a relevant posttransductional mechanism involved in the activation of these enzymes. This suggests that a complete different pattern of activation and thus function may occur in the placenta from type 2 diabetic patients, as a result of increased oxidative and nitrative stress.

In conclusion, our findings identified increased MMP-2 and MMP-9 activities in term placentas from type 2 diabetic patients, together with an increase in placental oxidative and nitrative stress. Furthermore, peroxynitrite-induced nitration of MMPs seems to be the mechanism by which these MMPs are overactivated.

Our findings have potential clinical implications due to the identification of the placenta as a target for type 2 maternal diabetes-induced oxidative and nitrative stress, with a particular impact on the activities of MMP-2 and MMP-9. Besides their relevant role as markers of a proinflammatory state, these changes may have adverse consequences in the fetuses, which will require further studies.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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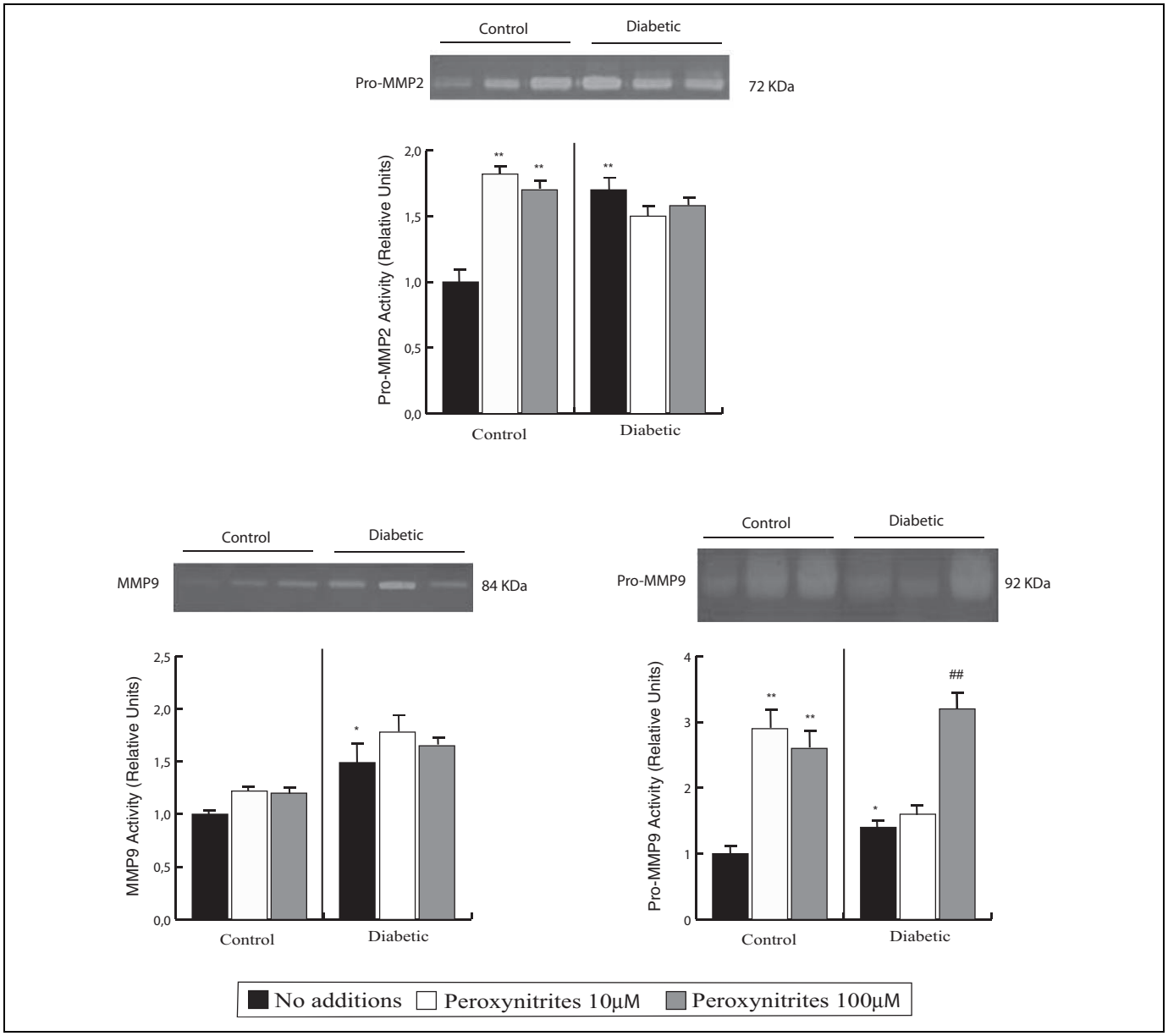

Figure 4. Effects of peroxynitrite on MMP-2 and MMP-9 activities in the placenta from type 2 diabetic and healthy patients. Placental explants from type 2 diabetic and healthy patients cultured in the presence of peroxynitrites 10 and 100 μmol/L for 1 hour, for further determination of MMP-2 and MMP-9 activities by zymography. Densitometric analysis and representative zymography bands are shown. All data are displayed as the mean ± SEM. ANOVA and post hoc test of Tukey, n = 12 in each group, *$P < .05$, **$P < .01$ versus placentas from healthy participants (controls), $^{\#\#}P < .01$ versus placentas from type 2 diabetic participants.

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