DOI: 10.1111/j.1365-2362.2009.02200.x

ORIGINAL ARTICLE

MMP/TIMP balance is modulated *in vitro* by $15dPGJ_2$ in fetuses and placentas from diabetic rats

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ABSTRACT

Background Maternal diabetes is associated with morphological placental abnormalities and foeto-placental impairments. These alterations are linked with a dysregulation of the activity of matrix metalloproteinases (MMPs). We investigated the action of 15deoxy$\Delta^{12,14}$ prostaglandin J_2 ($15dPGJ_2$), a natural ligand of the peroxisome proliferator activated receptor (PPAR) γ, on MMP-2 and MMP-9 activities and tissue inhibitors of matrix metalloproteinases (TIMP) levels in foetuses and placentas from diabetic rats.

Materials and methods Diabetes was induced in rat neonates by a single streptozotocin administration (90 mg kg^{-1} s.c.). At 13·5 days of gestation, foetal and placental homogenates were prepared for the determination of PPARγ levels (western blot) and $15dPGJ_2$ concentration (enzyme-immunoassay), whereas the *in vitro* effect of $15dPGJ_2$ (2 μM) was evaluated on placental and foetal MMPs and TIMP activities (zymography and reverse zymography), nitrate/nitrite concentrations (Griess method) and thiobarbituric acid reactive substances (TBARS).

Results PPARγ was increased while $15dPGJ_2$ was decreased in placentas and foetuses from diabetic rats. $15dPGJ_2$ additions were able to reduce the high activities of MMP-2 and MMP-9 present in diabetic placental tissues. $15dPGJ_2$ additions reduced MMP-2 activity in control and diabetic foetuses. TIMP-3 levels were decreased in diabetic placentas and $15dPGJ_2$ was able to enhance them to control values. Nitrates/nitrites and TBARS, metabolites of MMPs activators, were increased in the diabetic placenta and reduced by $15dPGJ_2$.

Conclusions This study demonstrates that $15dPGJ_2$ is a potent modulator of the balance between MMP activities and TIMP levels, which is needed in the correct formation and function of the placenta and foetal organs.

Keywords $15dPGJ_2$, diabetes, fetus, MMPs, placenta, PPARγ.

Eur J Clin Invest 2009; 39 (12): 1082–1090

Introduction

Diabetes mellitus is a pathology associated with morphological placental abnormalities, embryo malformations and embryo-placental metabolic disorders [1–4]. In particular, disturbances in extracelullar matrix (ECM) components as laminin, fibronectin and type IV collagen have been found in placentas from diabetic rats [5,6]. These alterations in the ECM have also been described in embryonic tissues during diabetic gestations [7]. Although the direct consequence of the alterations of the ECM in the diabetic foeto-placental unit is not fully understood, it is clear that these changes can affect placental and embryo development and alter gas exchange and nutrient transfer from the mother to the foetus [8]. These alterations in the components of the ECM are associated with a dysregulation of the activity of matrix metalloproteinases (MMPs). MMPs are a family of zinc-dependent proteinases, which have the capacity to break down all the components of the ECM and are inhibited by specific endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs). Most MMPs are secreted as inactive proenzymes and converted into active forms by the proteolytic removal of a pro-domain. Due to their potential for tissue damage, MMPs are strictly regulated at multiple levels, including transcription, activation of the zymogen form, and inhibition of the active form [9]. In addition, nitric oxide (NO) and reactive oxygen species (ROS) are involved in the MMP pro-domain chemical disruption, leading to the formation of the biologically active MMPs [10]. We have previously found that MMP-2 and MMP-9 are increased in human diabetic placenta at term and in foetuses and placentas from experimental diabetic models [11,12]. Besides, the increased levels of both NO and ROS present in the diabetic tissues have the capacity to produce MMP-2 and MMP-9 over-activation [11,13–15].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that directly control several genes involved in lipid metabolism, glucose homeostasis, cellular proliferation and differentiation, by binding to specific DNA sequences called PPAR-response elements (PPREs) located in the promoter regions of the target genes to initiate gene transcription [16,17]. Three PPAR isotypes have been identified: α, β (also called δ) and γ and they all conform heterodimers with the receptor for 9-cis retinoic acid α (RXRα). PPARs are expressed in a variety of organs including the liver, kidney, heart, skeletal muscle and adipose tissue [18]. Furthermore, PPARs have recently been described to have important functions in the reproductive system [19]. The presence of PPAR isoforms has been determined throughout the development of rodent placenta and in human placenta at term [20–23]. In the foetuses, PPARγ is expressed after placentation, and genetic studies performed in mice have established that PPARγ$^{-/-}$ is lethal for the foetus due to failures in both the terminal differentiation of the trophoblast and placental vascularization [24,25]. Among the endogenous agonists, 15deoxyΔ [12,14] prostaglandin J$_2$ (15dPGJ$_2$) is the most potent natural ligand of PPARγ [26]. In addition, there are several synthetic PPARγ agonists such as the thiazolidinediones frequently used in the treatment of type 2 diabetes [27].

We have previously found reduced 15dPGJ$_2$ concentrations in term placental tissues obtained from diabetic rats and pregestational and gestational diabetic patients [21,28], being 15dPGJ$_2$ a prostaglandin capable of regulating NO production in rat foetuses and placentas [28] and in human term placental tissues [21]. On the other hand, several authors have reported the ability of 15dPGJ$_2$ and thiazolidinediones to inhibit MMP-2 and MMP-9 expression and gelatinolytic activity in cancer cell lines and monocyte/macrophage lineages *in vitro* [29–31].

The aim of this study was to evaluate whether the PPARγ agonist 15dPGJ$_2$ modulates the MMPs/TIMPs system in foetuses and placentas from control and diabetic rats at midgestation.

Materials and methods

Animals

Albino Wistar rats were bred in the animal facility of the Institution with free access to Purina rat chow and water, under a 14 h light : 10 h dark cycle. At 2 days of age, they received a single injection of either streptozotocin (90 mg kg^{-1} s.c.) (Sigma-Aldrich, St Louis, MO, USA) dissolved in citrate buffer (0·05 M, pH 4·5) or buffer alone (controls). Four days after birth, neonates exhibiting glycosuria higher than 500 mg dL^{-1} were considered diabetic [32]. Adult diabetic rats had hyperglycaemias ranging between 180 and 230 mg dL^{-1}, while control rat glycaemia levels were below 100 mg dL^{-1}. Besides, 2 h after a glucose challenge (1·5 g kg^{-1} glucose i.p.) blood glucose concentrations of diabetic rats were greater than 250 mg dL^{-1}, whereas blood glucose concentrations of control rats were lower than 110 mg dL^{-1}. This experimental model is compatible with the pregnant state, and the reproductive characteristics of this model have been reported previously [33]. In the evening of pro-oestrus, control and diabetic females weighing between 200 and 300 g were caged overnight with control males. The following day was designated as day 0·5 of pregnancy if the sperm cells were found in the vaginal smear. The guidelines for the care and use of animals approved by the local institution were followed, according to 'Principles of laboratory animal care' (NIH publication No. 85–23, revised 1985); http://grants1.nih.gov/grants/olaw/references/phspol.htm). Glycaemias on day 13·5 of gestation were 102 ± 10 mg dL^{-1} in the control group and 218 ± 19 mg dL^{-1} in the diabetic group ($P < 0·001$).

Tissue preparation

On day 13·5 of gestation, animals were killed by cervical dislocation and their placentas and foetuses were removed and placed in Petri dishes containing Krebs-Ringer-Bicarbonate solution (KRB composition: 11 mM glucose, electrolyte concentrations: 145 mM Na^+, 5·9 mM K^+, 2·2 mM Ca^{2+}, 1·2 mM Mg^{2+}, 127 mM Cl^-, 25 mM $HCO3^-$, 1·2 mM SO_4^{2-}, 1·2 mM PO_4^{3-}). Some placentas and foetuses selected at random were incubated for 1 h in a metabolic shaker under an atmosphere of 5% CO_2 and 95% O_2 at 37 °C, either with or without 15dPGJ$_2$ (2 μM) (Cayman Chemical Company, Ann Arbor, MI, USA). After incubations, aliquots of the incubation medium were frozen at −70 °C for further determination of MMP and TIMP activities, whereas tissues were frozen at −70 °C for further determination of nitrates/nitrites and thiobarbituric acid reactive substances (TBARS). The other placentas and foetuses were directly frozen at −70 °C for the rest of determinations.

Measurement of 15dPGJ$_2$

The 15dPGJ$_2$ concentration was determined with a commercial enzyme immunoassay kit (Assay Designs Inc., Ann Arbor, MI, USA). For that, placentas and foetuses from control and diabetic rats were homogenized in phosphate-buffer saline and an aliquot reserved for protein determination. The homogenate was extracted twice in absolute ethanol. The extracts were dried in a Speed-Vac concentrator and reconstituted with 200 μL of the assay buffer provided by the commercial kit. The kit was used following the manufacturer's instructions, as previously described [21].

Western blot to detect protein expression of PPARγ

Tissues were homogenized in 500 μL of ice-cold lysis buffer (20 mM HCl pH 7·4, 150 mM NaCl, 1% Triton X-100 and 5 μL

protease inhibitor cocktail) and incubated on ice for 2 h. Tissue homogenates were centrifuged at 9600 g for 10 min and the supernatant removed. Total proteins (100 μg) were separated using 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto a nitrocellulose membrane. The membrane was then blocked with 10% of non-fat milk and incubated overnight at 4 °C, with rabbit anti PPARγ polyclonal antibody (1 : 200) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). As an internal control, actin was detected with mouse monoclonal antibody (Sigma-Aldrich, St Louis, MO, USA). The membrane was then probed for 1 h at room temperature with horseradish peroxidase-labelled second antibody. The signal was revealed using the enhanced chemiluminescence system (Amersham Biosciences; Piscataway, NJ, USA), as previously described [21]. The relative intensity of protein signals was quantified by densitometric analysis using the Sigma Gel Program.

Zymography

Zymography was performed to evaluate the presence of gelatinase activity as previously described [11,34]. Both MMPs and pro-MMPs were also analysed using zymography, as the exposure to SDS induces changes in pro-MMPs conformation which are associated with their activation. Briefly, 25 μg of protein derived from foetal and placental tissues was subjected to a 7·5% SDS–PAGE, in which 1 mg mL^{-1} gelatin (type A from porcine skin) had been incorporated. Following electrophoresis, gels were washed in 30% Triton X-100 for 60 min to remove SDS. Then, the gels were incubated in 50 mM Tris Buffer pH 7·4, containing 0·15 mM NaCl and 30 mM $CaCl_2$, for 18 h at 37 °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) that was run in each gel to allow the standardization of the results obtained in the different zymograms.

The enzymatic activity was quantified using an image analysis program (Sigmagel; Sigma-Aldrich) and expressed as arbitrary densitometric units, which were normalized to the internal control. Data are shown as relative to the value 1 assigned to the mean values for MMP-9 in control placentas and those for MMP-2 in control foetuses.

Reverse zymography

To analyse the activity of TIMP proteins in placenta and foetuses from control and diabetic rats, reverse zymographic analysis was performed. Briefly, 50 μg of protein derived from placental or foetal tissues was mixed with loading buffer [2% (w/v) SDS, 10% (v/v) glycerol, 0·1% (w/v) bromophenol blue, 50 mM Tris–HCl, pH 6·8] and applied to 15% (w/v) polyacrylamide gels containing 0·1% (w/v) SDS and 1 mg mL^{-1} gelatin plus 25% (v/v) conditioned medium of human fibrosarcoma HT-1080 cells, which is a rich source of various MMPs. After electrophoresis, gels were rinsed twice with 2·5% (v/v) Triton X-100 and then incubated at 37 °C for 72 h in Tris buffer (50 mM Tris–HCl, 0·2 M NaCl, 5 mM $CaCl_2$, pH 8·0). Subsequently, gels were stained with Coomassie blue and then destained with 10% acetic acid and 30% methanol in water. MMPs provided by the HT-1080 conditioned medium digest the gelatin within the gel, whereas the TIMPs in the samples inhibit MMP action, allowing the identification of TIMPs as dark bands on a clear background. The identities of TIMPs were based on their molecular weights, determined through prestained SDS–PAGE protein standards (Bio-Rad Laboratories, Hercules, CA, USA) that were run in the same gel. TIMP activity was quantified using an image analysis program (Sigmagel; Sigma-Aldrich) and expressed as arbitrary densitometric units.

Measurement of NO production

NO production was determined by measuring the concentration of its stable metabolites nitrates/nitrites with a commercial assay kit (Cayman Chemical Company). For that, placental tissues were homogenized in 1 mL Tris–HCl buffer pH 7·6, and an aliquot was separated for protein determination. Nitrates in the supernatant were reduced to nitrites using nitrate reductase and total nitrites were measured using the Griess method [35]. Optical densities were measured at 540 nm in a microlitre plate using $NaNO_3$ and $NaNO_2$ as standard. Results are expressed as nmol mg^{-1} of protein.

Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the levels of TBARS, as previously described [14]. For that, placental tissues were homogenized in 100 mM Tris–HCl buffer pH 7·6. The homogenate was added with trichloroacetic acid (40% w/v) and centrifuged at 900 g for 10 min. The supernatant was added with an equal volume of thiobarbituric acid (46 mM) and the solution was heated at 95 °C for 15 min. Then, the samples were cooled and quantified spectrophotometrically at 530 nm. Malondialdehyde (MDA), subjected to the same conditions as the tissue homogenates, was used as a standard. TBARS are expressed as nmol mg^{-1} protein.

Statistical analysis

Results are expressed as means ± SEM. Comparisons between groups were performed using either one-way analysis of variance in conjunction with Turkey's test or Student's *t*-test, where appropriate. The statistical level of significance was defined as $P < 0·05$.

Results

PPARγ protein expression and 15dPGJ$_2$ concentrations in the placenta and the foetus

Western blot analysis showed that PPARγ protein was expressed in both placentas and foetuses from the control and diabetic rats, as a specific band corresponding to PPARγ protein at a molecular size of about 55 kDa. Placentas and foetuses from diabetic rats expressed more PPARγ protein than their respective controls, $P < 0{\cdot}05$ (Fig. 1a–c).

The concentration of the endogenous agonist of PPARγ 15dPGJ$_2$ was evaluated in both placentas and foetuses from control and diabetic rats. 15dPGJ$_2$ concentrations were reduced in both the placentas and foetuses ($P < 0{\cdot}001$) from diabetic animals when compared with the controls (Fig. 1d).

15dPGJ$_2$ effects on MMP-2 and MMP-9 activities in the placenta and the foetus

To analyse the influence of 15dPGJ$_2$ on placental MMP activation, placental tissues were incubated for 1 h either in the presence or absence of 15dPGJ$_2$ (2 μM). The analysis of gelatinolytic activity showed that both MMP-2 and MMP-9 activities ($P < 0{\cdot}01$ and $P < 0{\cdot}05$), as well as proMMP-2 and proMMP-9 activities ($P < 0{\cdot}005$ and $P < 0{\cdot}01$), were increased in the placentas from diabetic rats when compared with the controls (Fig. 2). The addition of 15dPGJ$_2$ reduced the activity of proMMP-2 ($P < 0{\cdot}01$) in control placental tissues when compared with those without additions. Interestingly, in the diabetic group the inhibitory effect of 15dPGJ$_2$ was observed in all the MMPs evaluated: proMMP-9 ($P < 0{\cdot}05$), MMP-9 ($P < 0{\cdot}005$), proMMP-2 ($P < 0{\cdot}05$) and MMP-2 ($P < 0{\cdot}005$) when compared with diabetic placentas incubated without additions (Fig. 2).

On the other hand, MMP-9 activity could not be detected in the foetuses by zymography at this developmental stage. ProMMP-2 and MMP-2 activities were enhanced in foetuses from diabetic rats when compared with the controls ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively) (Fig. 3). The addition of 15dPGJ$_2$ reduced both proMMP-2 and MMP-2 activities in foetuses from both control ($P < 0{\cdot}05$ and $P < 0{\cdot}01$) and diabetic ($P < 0{\cdot}005$ and $P < 0{\cdot}05$) animals when compared with foetuses incubated without additions (Fig. 3).

15dPGJ$_2$ effects on TIMPs activities in the placenta

With the purpose of analysing the effect of 15dPGJ$_2$ on the activity of the TIMPs, placentas and foetuses from control and diabetic rats were incubated for 1 h in either the presence or absence of 15dPGJ$_2$ (2 μM). The TIMPs present in the incubated medium were analysed by reverse zymography because of their ability to inhibit MMP hydrolysis of protein substrates present in the gel.

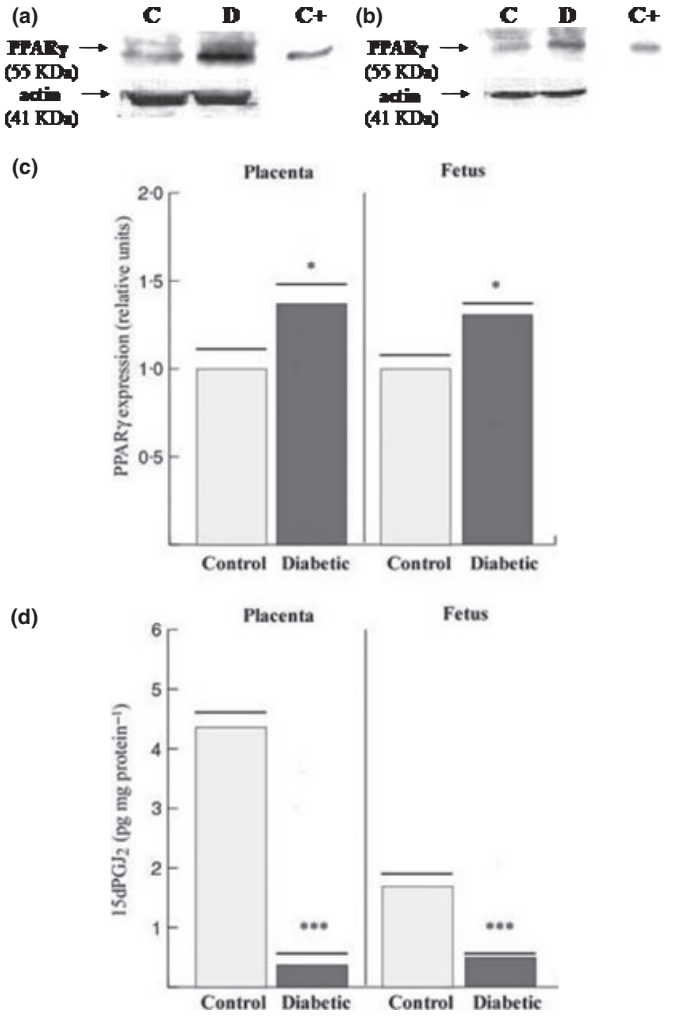

Figure 1 Protein expression of peroxisome proliferator activated receptor gamma (PPARγ) in placentas and foetuses from control and diabetic rats. (a) Representative western blot of PPARγ in placenta from control (C) and diabetic (D) rats. (b) Representative western blot of PPARγ in foetuses from control (C) and diabetic (D) rats. A cell lysate from rat lung was used as a positive control (C+). Actin was used as internal control. (c) Relative densitometric analysis of PPARγ expression in placentas and foetuses from control and diabetic rats on day 13·5 of gestation. Student's *t*-test was performed. Data are the means ± SEM ($n = 6$ in each group). *$P < 0{\cdot}05$ vs. their respective controls. (d) 15dPGJ$_2$ concentrations in placentas and foetuses from control and diabetic rats on day 13·5 of gestation. Values are means ± SEM; $n = 7$ rats per group. Student's *t*-test was performed. ***$P < 0{\cdot}001$ denotes differences with the control groups.

The analysis of TIMP activities detected only TIMP-3 in the placentas of control and diabetic rats, whereas no TIMPs were detected in the foetuses at the evaluated developmental stage.

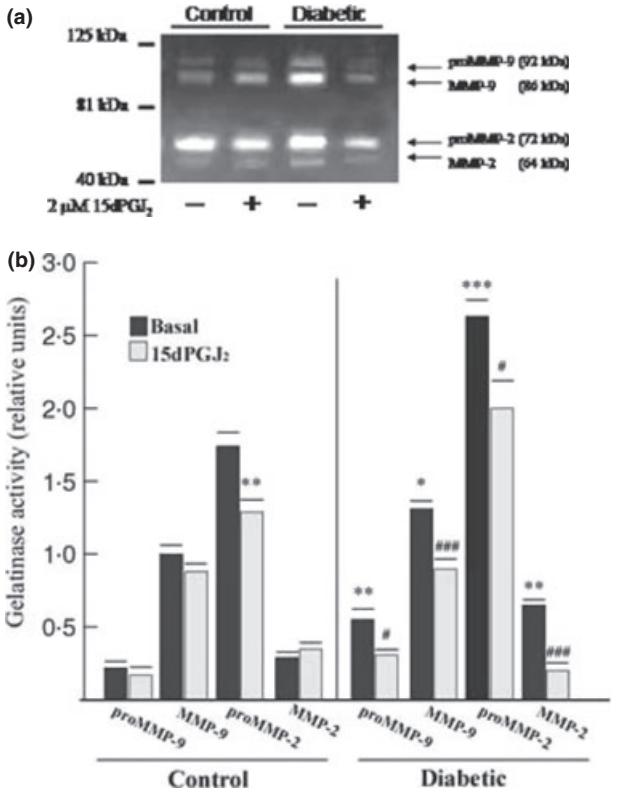

Figure 2 15dPGJ$_2$ effects on MMP-2 and MMP-9 activities in the placenta. (a) Representative zymogram of MMP-2 and MMP-9 activities in placentas from control and diabetic rats on day 13·5 of gestation, with or without the addition of 15dPGJ$_2$ (2 μM). (b) Relative densitometric analysis of the activities of MMP-2 and MMP-9 and their proenzymes in placentas from control and diabetic rats on day 13·5 of gestation. Data are the means ± SEM; n = 7 in each group. ANOVA followed by Tukey's test was performed. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}005$ vs. control without additions; $^{\#}P < 0{\cdot}05$, $^{\#\#\#}P < 0{\cdot}005$ vs. diabetic without additions.

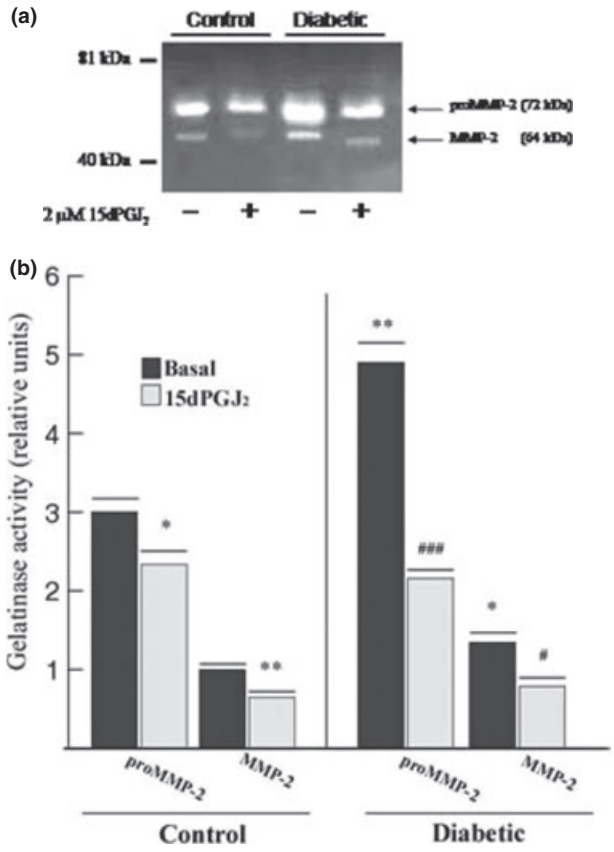

Figure 3 15dPGJ$_2$ effects on MMP-2 activity in the foetus. (a) Representative zymogram of MMP-2 activity in foetuses from control and diabetic rats on 13·5 of gestation, with or without the addition of 15dPGJ$_2$ (2 μM). (b) Relative densitometric analysis of the activities of MMP-2 and its proenzyme in foetuses from control and diabetic rats on day 13·5 of gestation. Data are the means ± SEM, n = 7 in each group. ANOVA followed by Tukey's test was performed. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ vs. control without additions; $^{\#}P < 0{\cdot}05$, $^{\#\#\#}P < 0{\cdot}005$ vs. diabetic without additions.

TIMP-3 was decreased in the diabetic placenta ($P < 0{\cdot}01$) when compared with the controls (Fig. 4). Moreover, the addition of 15dPGJ$_2$ was able to enhance TIMP-3 activity to control values in placental tissues from diabetic rats ($P < 0{\cdot}01$), but did not modify TIMP-3 activity in placentas from control rats (Fig. 4). The identity of TIMP-3 was confirmed using western blot analysis (data not shown).

15dPGJ$_2$ effects on NO production and TBARS concentrations in the placenta

To determine the effect of 15dPGJ$_2$ on the production of NO and the levels of TBARS, placentas from control and diabetic rats were incubated in either the presence or absence of 15dPGJ$_2$ (2 μM). The concentration of nitrates/nitrites, stable NO metabolites, was increased in placental tissues from diabetic rats when compared with the control ($P < 0{\cdot}001$). The addition of 15dPGJ$_2$ reduced nitrates/nitrite concentration in the control placentas ($P < 0{\cdot}01$) but failed to decrease the levels of nitrates/nitrites in diabetic placentas (Fig. 5a). Furthermore, the levels of TBARS (an index of lipid peroxidation) were increased in placentas from diabetic rats when compared with the control ($P < 0{\cdot}01$) and the addition of 15dPGJ$_2$ was able to prevent this increase ($P < 0{\cdot}05$) (Fig. 5b).

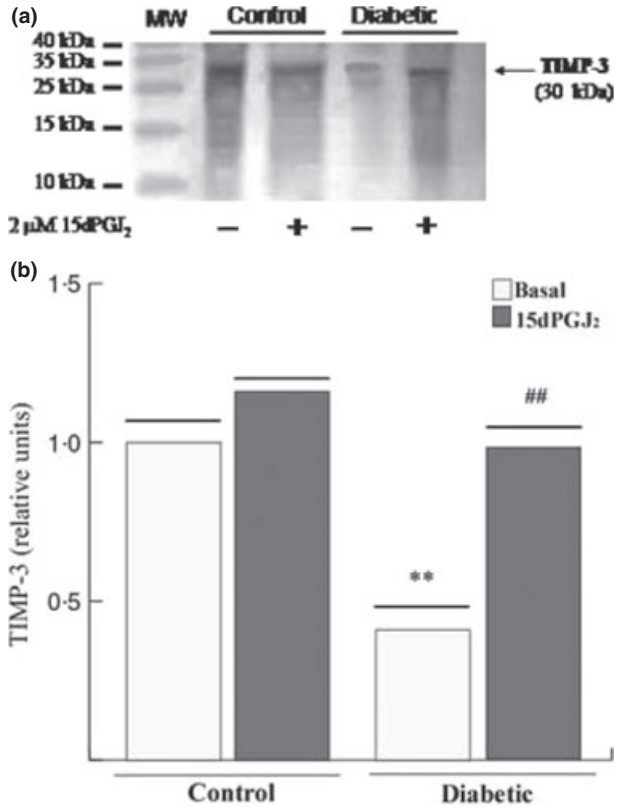

Figure 4 15dPGJ$_2$ effects on TIMP-3 activity in the placenta. (a) Representative reverse zymogram of TIMP-3 activity in placentas from control and diabetic rats on day 13·5 of gestation, with or without the addition of 15dPGJ$_2$ (2 μM). MW: Molecular weights derived from the markers in the first lane are shown on the left. (b) Relative densitometric analysis of the activity of TIMP-3 in placentas from control and diabetic rats on day 13·5 of gestation. Data are the means ± SEM, n = 7 in each group. ANOVA followed by Tukey's test was performed. $^{**}P < 0{\cdot}01$ vs. control without additions; $^{\#\#}P < 0{\cdot}01$ vs. diabetic without additions.

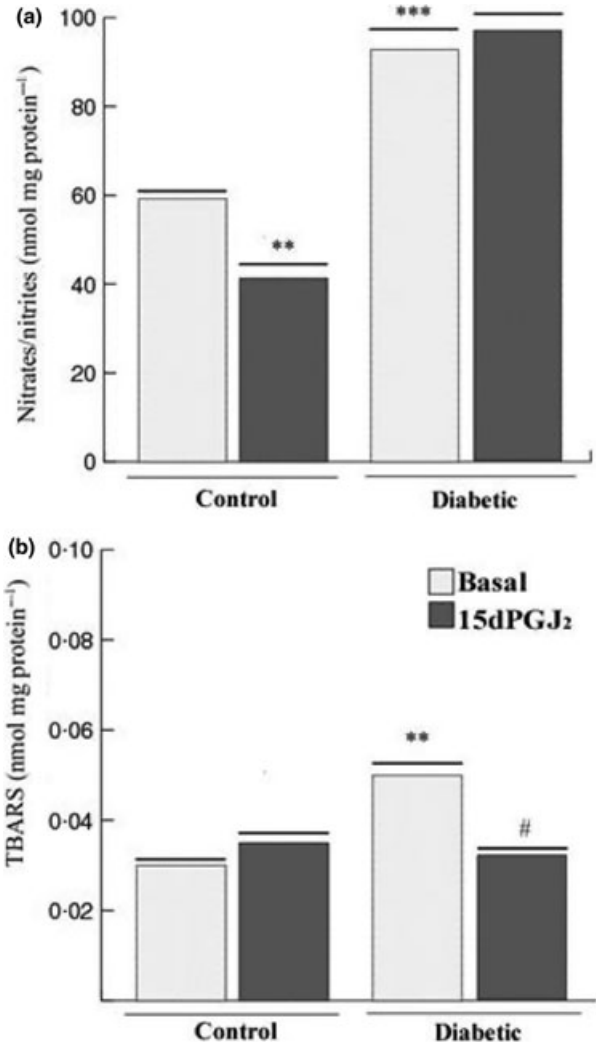

Figure 5 15dPGJ$_2$ effects on NO production and TBARS levels in the placenta. (a) 15dPGJ$_2$ effect on nitrates/nitrite concentrations in placentas from control and diabetic rats on day 13·5 of gestation. Values are means ± SEM; n = 7 rats per group. ANOVA followed by Tukey's test was performed. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ vs. control without additions. (b) 15dPGJ$_2$ effect on TBARS levels in placentas from control and diabetic rats on day 13·5 of gestation. Values are means ± SEM; n = 7 rats per group. ANOVA followed by Tukey's test was performed. $^{**}P < 0{\cdot}01$ vs. control without additions; $^{\#}P < 0{\cdot}05$ vs. diabetic without additions.

Discussion

PPARs are involved in cellular metabolic and inflammatory processes [17]. PPARγ has been initially described in adipose tissue, but later detected in a wide range of tissues including placental and developing foetal tissues [18,20–22]. PPARγ has a significant anti-inflammatory role and is involved in the down-regulation of pro-inflammatory enzymes like nitric oxide synthase inducible form (iNOS) and cyclooxygenase 2 and of pro-inflammatory cytokines like tumour necrosis factor alpha, interleukin-1 and interleukin-6 [36,37]. Besides, PPARγ is an important regulator of proliferation, differentiation and cellular death [29,30,38].

In this work, we found that PPARγ was increased in placentas and foetuses from diabetic rats when compared with the controls. PPARγ over-expression has been shown in diabetic murine placenta and in the choriocarcinoma cell line BeWo under severe hyperglycaemic conditions [23]. How-

ever, under moderate diabetic conditions, PPARγ increases seem to be specific to this developmental stage, as PPARγ decrease has been reported in term placenta from both diabetic patients and diabetic rat experimental models [21,28]. On the other hand, we found that the endogenous ligand of PPARγ 15dPGJ$_2$, was significantly decreased in placentas and foetuses from diabetic rats compared with the controls. This effect of diabetes on 15dPGJ$_2$ levels has been previously reported by our group [21,28,39–41]. Altogether, these results suggest the existence of an early compensatory response that induces the increase in PPARγ levels tending to prevent the effects of dramatic 15dPGJ$_2$ decreases.

The ECM remodelling is a key feature in reproductive processes such as placental establishment and growth, as well as embryo implantation and development. However, when this ECM remodelling is imbalanced, this physiological phenomenon turns into a pathological one. Diabetes affects ECM degradation through the modification of the activities of several MMPs, central regulators of matrix turnover. MMP-2 and MMP-9 have been studied extensively in various diabetic tissues [42–45]. We have previously described a significant increase in both MMP-2 and MMP-9 in the placenta of diabetic rats at midgestation and of diabetic women at term pregnancies [11,12]. This increase in MMP-2 activity is also found in diabetic rat foetuses [12,14,15].

Several studies conducted mainly in cellular lines have revealed that activation of PPARγ is involved in decreasing the expression and activity of MMP-2 and MMP-9 [29,31]. Moreover, PPARγ agonists are also able to reduce MMP-9 activity in amnion tissues, although not in placental tissues obtained from healthy women at term [46]. The present results show that 15dPGJ$_2$ was able to down-regulate the activity of proMMPs, MMP-2 and MMP-9 in diabetic placental tissue, but to decrease only proMMP-2 in placentas from healthy rats. These results suggest that the action of this prostaglandin is greater in diabetic tissues, where its production is poor and PPARγ is overexpressed.

In foetuses from control and diabetic rats, the addition of 15dPGJ$_2$ results in a decrease in the activity of both proMMP-2 and MMP-2, a regulation that is more marked in foetuses from diabetic mothers.

The TIMPs regulate MMPs activity during the formation of the rat placenta, being TIMP-3 a prominent and relevant TIMP in this process [47]. It is interesting and novel that not only MMPs but also the concentration of TIMP-3, the only TIMP detected by reverse zymography in the placenta at the evaluated developmental stage, is dysregulated in maternal diabetes. Indeed, TIMP-3 levels were found decreased in diabetic placentas compared with the controls, an alteration that probably results in a greater MMP activity. Moreover, 15dPGJ$_2$ was able to increase the level of this MMP inhibitor in the placenta from diabetic rats, but this increase was not observed in control placental tissue. Similar results have been found in human monocyte cells line THP-1, in which the addition of 15dPGJ$_2$ promotes an increase in TIMP-1 when MMPs are over-expressed [48]. No TIMP was found in foetal samples from control and diabetic rats using the technique of reverse zymography. Other authors have reported the presence of TIMP-2 and TIMP-3 in foetal tissues at midgestation, although these were assessed using different techniques such as ELISA and RT-PCR [49].

The present results demonstrate that 15dPGJ$_2$ has the ability to modulate MMPs and TIMP-3 in the foeto-placental unit. The actual mechanisms by which 15dPGJ$_2$ may produce MMP down-regulation and TIMP-3 over-expression is not yet fully understood. Throughout the activation of PPARγ, transrepression of transcription factors such as AP-1 and NF-kB results in the inhibition of MMP expression [50]. On the other hand, the function of 15dPGJ$_2$ may also be regulated by a PPARγ-independent pathway, leading to a decrease in cytokine production, iNOS expression and reactive oxygen species and anti-inflammatory effects [36,37,51,52]. Both reactive oxygen species and nitric oxide are able to activate MMPs in the placenta, and are increased in the diabetic placenta [14,15]. In this work, we found that 15dPGJ$_2$ was able to down-regulate NO production in control placentas and reduce ROS in diabetic tissues. As the influence of 15dPGJ$_2$ on MMP activity is more marked in diabetic tissues, the effects of 15dPGJ$_2$ in controlling ROS generation is likely to be involved in the regulation of placental MMP activity in maternal diabetes.

In this study, we demonstrated that the action of 15dPGJ$_2$ on the MMPs/TIMPs system on diabetic tissues leads to a restoration of the correct balance between MMP activities and TIMP-3 levels. Although further investigations are needed to completely elucidate the pathway that 15dPGJ$_2$ follows to regulate MMPs/TIMPs levels, this prostaglandin becomes a potential agent to modulate the ECM disorders present in diabetic gestations.

Acknowledgements

This work was supported by grants from Agencia de Promoción Científica y Tecnológica de Argentina (PICT 05-32268 and PICT 06-00084). In memoriam of Elida González (1953–2007). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 30 March 2009; accepted 3 July 2009

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