Podocytes are key cells in the glomerular filtration barrier, and there is considerable evidence demonstrating their major role in the development of diabetic nephropathy (DN) (29, 34). Previous studies have shown the presence of all the renin-angiotensin system (RAS) components required for angiotensin (ANG) II production, the main effector of this system, within the podocyte (18, 32). Central elements of DN pathophysiology, such as hyperglycemia, change the expression of intrapodocyte RAS to a “pro-ANG II” profile (9, 37). One element of high interest in the RAS is angiotensin-converting enzyme 2 (ACE2), an enzyme whose main role is to cleave angiotensin-II into angiotensin-(1–7), have been demonstrated in podocytes. Conditionally immortalized mouse podocytes were cultured with insulin in the presence and absence of albumin. We found that insulin increases ACE2 gene and protein expression, by real-time PCR and Western blotting, respectively, and enzymatic activity within the podocyte and these increases were maintained over time. Furthermore, insulin favored an “anti-ANG II” regarding ACE/ACE2 gene expression balance and decreased fibronectin gene expression as a marker of fibrosis in the podocytes, all studied by real-time PCR. Similarly, insulin incubation seemed to protect podocytes from cell death, studied by a terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay. However, all these effects disappeared in the presence of albumin, which may mimic albuminuria, a main feature of DN pathophysiology. Our results suggest that modulation of renin-angiotensin system balance, fibrosis, and apoptosis by insulin in the podocyte may be an important factor in preventing the development and progression of diabetic kidney disease, but the presence of albuminuria seems to block these beneficial effects.

PODOCYTES ARE KEY CELLS IN the glomerular filtration barrier, with a major role in the development of diabetic nephropathy. Podocytes are insulin-sensitive cells and have a functionally active local renin-angiotensin system. The presence and activity of angiotensin-converting enzyme 2 (ACE2), the main role of which is to cleaving profibrotic and proinflammatory angiotensin-II into angiotensin-(1–7), have been demonstrated in podocytes. Conditionally immortalized mouse podocytes were cultured with insulin in the presence and absence of albumin. We found that insulin increases ACE2 gene and protein expression, by real-time PCR and Western blotting, respectively, and enzymatic activity within the podocyte and these increases were maintained over time. Furthermore, insulin favored an “anti-ANG II” regarding ACE/ACE2 gene expression balance and decreased fibronectin gene expression as a marker of fibrosis in the podocytes, all studied by real-time PCR. Similarly, insulin incubation seemed to protect podocytes from cell death, studied by a terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay. However, all these effects disappeared in the presence of albumin, which may mimic albuminuria, a main feature of DN pathophysiology. Our results suggest that modulation of renin-angiotensin system balance, fibrosis, and apoptosis by insulin in the podocyte may be an important factor in preventing the development and progression of diabetic kidney disease, but the presence of albuminuria seems to block these beneficial effects.

Podocytes; insulin; renin-angiotensin system; ACE2; albumin

Podocytes are key cells in the glomerular filtration barrier, and there is considerable evidence demonstrating their major role in the development of diabetic nephropathy (DN) (29, 34). Previous studies have shown the presence of all the renin-angiotensin system (RAS) components required for angiotensin (ANG) II production, the main effector of this system, within the podocyte (18, 32). Central elements of DN pathophysiology, such as hyperglycemia, change the expression of intrapodocyte RAS to a “pro-ANG II” profile (9, 37). One element of high interest in the RAS is angiotensin-converting enzyme 2 (ACE2), an enzyme whose main role is to cleave ANG II into ANG-(1–7) (30). Deletion of the ACE2 gene in mice leads to worsening of ANG II-induced hypertension and diabetic kidney injury (11, 36). Although ACE2 expression and activity have been demonstrated in cultured podocytes (32), there are no studies in the literature focused on its variations in a diabetic state. Podocyte-specific overexpression of ACE2 transiently attenuates the development of DN in transgenic mice with streptozotocin-induced diabetes (23). Podocytes in culture are insulin-sensitive cells and are the only cells of the glomerular filtration barrier that present this feature (5). Mice with specific deletion of the insulin receptor from their podocytes present kidney damage as demonstrated by albuminuria and podocyte loss at 5 wk of age. In later stages, 8 wk of age, there are histological changes in the kidney comparable to DN but in an environment of normoglycemia (35). It is known that RAS modulation is essential for delaying the progression of DN; therefore, studies focused on the effect of insulin on the RAS within the podocytes are needed. Albuminuria is not only a clinical feature in DN, but it has a major role in DN pathophysiology. Albumin overload favors podocyte injury by different mechanisms: redistribution of cytoskeleton actin fibers; a decrease in specific cell markers such as synaptopodin; increased cell apoptosis; or the release of cytokines, proinflammatory, and vasoactive molecules by the podocytes (1, 20, 24). Our hypothesis is that insulin can exert a direct role on RAS modulation within the podocyte. As albuminuria is a main feature of DN and has effects on these cells, the introduction of this variable seems to be of high interest in evaluating podocyte RAS. In the in vitro studies that we present, the cells were co-incubated with albumin with the hypothesis that its presence could modify podocyte response to insulin. Our results show that insulin increases ACE2, favors an “anti-ANG II” profile regarding ACE/ACE2 gene expression balance, and decreases fibrosis and apoptosis in the podocyte. Furthermore, these effects are inhibited by co-incubation with albumin.

METHODS

Cell culture. Experiments were performed in a growth-restricted, conditionally immortalized mouse podocyte cell line cultured as previously described (21). Briefly, cells were grown under permissive conditions with mouse γ-interferon (Life Technologies) at 32°C in RPMI media containing 10% fetal bovine serum (FBS Gold), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (all from PAA Laboratories). When cells reached 80–90% of confluence, they were trypsinized and induced to differentiate in restrictive conditions (absence of mouse gamma-interferon at 37°C), when proliferation is reduced and cells gradually resembled the phenotype of differentiated podocytes in vivo. During this process, cells were cultured in DMEM with standard glucose (5 mM) containing 1% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine, and 0.1 mM sodium pyruvate (all from PAA Laboratories). Cells were grown and differentiated in culture dishes coated with 50 μg/ml type I collagen (BD Bioscience). On day 12 of differentiation, serum was withdrawn; after 24 h, cells were exposed to complete DMEM with high glucose concentration (25 mM). For insulin time-dependent study, cells were incubated in a concentration of 200 nM insulin from bovine pancreas, I6634, Sigma-Aldrich) (5, 15) for 1, 24, or 48 h (POD1, POD24, and POD48, respectively). There was a control group for each time point (PODc1, PODc24, and PODc48, respectively). To study how albumin may influence insulin-induced expression of ACE2 at 48 h (as a prolonged exposure), four study groups were established: control (without intervention; PODc); insulin incubation (200 nM concentration; PODins); albumin incubation...
Table 1. Primer sequences used in quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agt</td>
<td>Angiotensinogen (Agt)</td>
<td>CGTGGCCCTAGTGAGAGAGAC</td>
<td>TCTAAATGCAGACTGTCCCT</td>
</tr>
<tr>
<td>Ace2</td>
<td>Angiotensin-converting enzyme 2 (ACE2)</td>
<td>TACCAACGGGAAGGATGTTCTT</td>
<td>GCCGTCTTGGGACAATAAT</td>
</tr>
<tr>
<td>Ace</td>
<td>Angiotensin-converting enzyme (ACE)</td>
<td>TGGCTCTGGACACAAATGTTTCTT</td>
<td>AGGAAAGGGGATGGATGTTTCCCT</td>
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<tr>
<td>Agtr1a</td>
<td>Angiotensin receptor type 1 (AT1-R)</td>
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<td>GTAGGACTGCGAAAAGGATGTTTCCCT</td>
</tr>
<tr>
<td>Agtr2</td>
<td>Angiotensin receptor type 2 (AT2-R)</td>
<td>TGGAGGCTCCGGAATTTCAAC</td>
<td>GTAGGACTGCGAAAAGGATGTTTCCCT</td>
</tr>
<tr>
<td>Fn1</td>
<td>Fibronectin</td>
<td>GCGACGACGATCGGGTTTCTT</td>
<td>GCCACAGACGACGATCGGGTTTCTT</td>
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<tr>
<td>Tgf1</td>
<td>Transforming growth factor-β (TGF-β)</td>
<td>TCTGGAGGCTCCGGAATTTCAAC</td>
<td>GTAGGACTGCGAAAAGGATGTTTCCCT</td>
</tr>
<tr>
<td>Actb</td>
<td>β-Actin (β-Actin)</td>
<td>GCCACGACGACGATCGGGTTTCTT</td>
<td>GCCACAGACGACGATCGGGTTTCTT</td>
</tr>
</tbody>
</table>

Table 1. Primers sequences used in quantitative PCR.
Briefly, cells were fixed in a 4% paraformaldehyde solution and permeabilized with a 0.1% Triton X-100/0.1% sodium citrate solution. Cells were labeled with the TUNEL reaction mixture. Cell death was measured by fluorescence intensity with Image J software.

Statistical analysis. Statistical analyses were performed using SPSS 16.0 statistical software. Because the sample size was small, nonparametric tests were conducted. A Mann-Whitney U-test was used for comparison between two groups. In addition, a Kruskal-Wallis test was performed for multiple comparisons in the study. Significance was defined as \( P < 0.05 \), and data are expressed as means \( \pm \) SE.

RESULTS

Insulin increases ACE2 in podocytes early and is maintained in long-term incubation. ACE2 gene expression was significantly increased in podocytes in insulin-exposed group at all time points (1, 24, and 48 h) compared with its own control group (POD1 1.19 \( \pm \) 0.06 vs. PODc1 as 1; POD24 1.55 \( \pm \) 0.16 vs. PODc24 as 1; POD48 1.29 \( \pm \) 0.07 vs. PODc48 as 1; all \( P < 0.05 \)). There was an increase in gene expression in POD24 compared with POD1 (\( P < 0.05 \)), but there was no difference from POD48 (Fig. 1A). ACE2 protein expression was higher in podocytes after 1- and 24-h incubation than in its appropriate controls (POD1 1.81 \( \pm \) 0.1 vs. PODc1 as 1; POD24 2.10 \( \pm \) 0.3 vs. PODc24 as 1; all \( P < 0.05 \)). After 48 h of insulin incubation, ACE2 expression was significantly higher compared with its control group as well (POD48 1.42 \( \pm \) 0.06 vs. PODc48 as 1, \( P < 0.01 \)). However, there were no differences in ACE2 protein expression between each time point (Fig. 1, B and C).

The immunofluorescence study also showed an increase in ACE2 staining in all insulin-incubated groups compared with their own control groups (Fig. 1D).

There was an increase in ACE2 enzymatic activity, expressed as RFU per microgram per hour, in each group with respect to its own control group: POD1 22%, POD24 64%, and POD48 27% increase. Therefore, enzymatic activity was concordant with the protein expression, but it did not reach statistical significance.

Albumin modifies insulin-induced ACE2 expression in podocytes. In a prolonged exposure, 48 h, the presence of albumin did not influence ACE2 gene expression in the podocytes compared with controls [PODalb 1.02 \( \pm \) 0.14 vs. PODc as 1, \( P = \) not significant (NS)]. In the presence of albumin, the increase in ACE2 gene expression observed with insulin incubation alone was maintained (PODai 1.33 \( \pm \) 0.13 vs. PODins 1.38 \( \pm \) 0.06, \( P = \) NS) (Fig. 2A). In concordance with gene expression studies, the incubation with albumin did not modify ACE2 protein expression within the podocyte compared with control group (PODalb 0.89 \( \pm \) 0.13 vs. PODc as 1, \( P = \) NS) (Fig. 2, B and C). Notably, the incubation with insulin in the presence of albumin resulted in a significant decrease in ACE2

![Figure 1](http://ajprenal.physiology.org/)

**Fig. 1.** Insulin incubation time-dependent studies. Podocytes were incubated with insulin (concentration of 200 nM) for 1, 24, or 48 h. Each time point had its own control group (PODc). There is an increase at all time points compared with their specific control group in angiotensin-converting enzyme 2 (ACE2) gene expression by qPCR (A) and ACE2 protein expression by Western blotting (WB, B). *\( P < 0.05 \) vs. appropriate PODc. **\( P < 0.01 \) vs. corresponding PODc. ACE2 staining in all insulin-incubated groups compared with their own control groups (Fig. 1D).
protein expression (PODai 0.38 ± 0.05 vs. PODins 1.27 ± 0.09, P < 0.05). There was a statistically significant increase in ACE2 enzymatic activity (30%) in the insulin group compared with the control group (P < 0.05). The presence of albumin did not modify the enzymatic activity (Fig. 2D). In concordance with protein expression, insulin incubation in the presence of albumin resulted in a 35% decrease in ACE2 enzymatic activity that was statistically significant compared with insulin alone (PODins vs. PODai, P = 0.001) (Fig. 2D).

Localization of ACE2 in the endoplasmic reticulum explains the differences between gene expression and protein expression. To find an explanation for the discordance among ACE2 gene expression, protein expression, and enzymatic activity in PODai group at 48 h, a colocalization study was performed. As the endoplasmic reticulum (ER) plays a critical role in the processing, folding, and transporting to the membrane of newly synthesized proteins, its relationship with ACE2 was studied.

As shown in Fig. 3, ACE2 and calnexin colocalized in the three intervention groups, but this colocalization was clearly stronger in the group incubated with albumin plus insulin (PODai).

Insulin changes gene expression of key components of the RAS in podocytes, but its effect disappeared in the presence of albumin. In addition to ACE2 evaluation at 48 h, gene expression of other key elements of the RAS was studied. Results are shown in Table 2. The ACE/ACE2 index was calculated for evaluating the balance between ACE and ACE2 gene expression. There was a significant decrease in the ACE/ACE2 index in PODins compared with PODc (index 0.43 ± 0.06, P < 0.05 vs. PODc). In the presence of albumin, this effect disappeared and there was an increase in the ACE/ACE2 index compared with the control group and PODins (PODai 1.63 ± 0.23, P < 0.05 vs. PODc and PODins) (Fig. 4).

Albumin increases transforming growth factor-β and fibronectin gene expression, and the presence of insulin cannot reverse this effect. To study a possible influence of insulin over inflammation and fibrosis in the podocytes, we studied two elements of these processes, fibronectin and transforming growth factor (TGF)-β, in the presence of insulin alone or combined with albumin for 48 h. There were no statistically significant differences in TGF-β gene expression between PODc and PODins (PODins 1.39 ± 0.15, P = NS vs. PODc as 1). There was a significant increase in TGF-β gene expression in PODalb compared with PODc and PODins, and the addition of insulin did not reverse this effect (PODalb 2.54 ± 0.31, PODai 2.43 ± 0.20, P < 0.01 vs. PODc and PODins). (Fig. 5A).

The presence of insulin decreased fibronectin gene expression compared with the control group (PODins 0.89 ± 0.20 vs. PODc as 1, P < 0.05). Albumin increased fibronectin gene expression compared with the control group (PODalb 1.66 ± 0.17 vs. PODc as 1, P < 0.05). This effect was not avoided when insulin was added to albumin, with the observation of an increase in the same way as the albumin group (PODai 1.67 ± 0.15 vs. PODc as 1, P < 0.05) (Fig. 5B).

Influence of insulin in podocyte cell death in the presence of albumin. We observed a significant decrease in cell number in the PODalb group at 48-h incubation compared with baseline
In the groups incubated with insulin, there was an increase in cell number between point 0 and the end point without reaching statistical significance. There was 22% less podocyte apoptosis in PODins compared with PODc, but with no statistical significance. There was an increase in TUNEL staining, consistent with apoptosis, in the cells incubated with albumin (PODalb 19%, PODai 47%), but it was statistically significant only in the PODai group (P < 0.05 vs. PODc) (Fig. 6B).

**DISCUSSION**

Podocytes have an important role in the development of DN (29, 34). These cells express all the RAS components (18, 32) and are modulated in a diabetic milieu (8, 9, 37). ACE2, the RAS enzyme that cleaves ANG II into ANG(1–7), is of high interest in our studies. Podocytes in culture are insulin-sensitive cells (11), and knocking down the insulin receptor specifically in podocytes in a mice model leads to kidney damage as demonstrated by albuminuria, podocyte loss, and other kidney histological changes comparable to DN in an environment of normoglycemia (12).

In this study, we demonstrated for the first time that insulin increases ACE2 gene transcription, protein expression, and enzymatic activity in podocytes. The effects of insulin appeared early (1-h incubation) and were maintained in long-term incubation (48 h). These effects may have great significance in

### Table 2. Gene expression of RAS components in insulin and albumin incubation studies

<table>
<thead>
<tr>
<th></th>
<th>Agt</th>
<th>ACE</th>
<th>ACE/ACE2</th>
<th>AT1-R</th>
<th>AT2-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>PODc</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PODins</td>
<td>1.44 ± 0.14*</td>
<td>0.80 ± 0.08*</td>
<td>0.43 ± 0.06*</td>
<td>0.99 ± 0.08</td>
<td>0.63 ± 0.06*</td>
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<tr>
<td>PODalb</td>
<td>2.53 ± 0.2*S</td>
<td>1.96 ± 0.36*S</td>
<td>2.47 ± 0.81</td>
<td>1.84 ± 0.24*S</td>
<td>1.57 ± 0.24*S</td>
</tr>
<tr>
<td>PODai</td>
<td>2.34 ± 0.31*S</td>
<td>2.06 ± 0.21*S</td>
<td>1.63 ± 0.23*S</td>
<td>2.14 ± 0.16*S</td>
<td>1.83 ± 0.21*S</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as gene mRNA fold-increase. RAS, renin-angiotensin system. Data were normalized by setting the untreated control group as 1. Podocytes were incubated for 48 h with insulin in the presence or not of albumin (PODc, control group; PODins, incubation with insulin with a concentration of 200 nM; PODalb, incubation with albumin in a concentration of 10 mg/ml; PODai, incubation with insulin and albumin with the previous concentrations).

*P < 0.05 vs. PODc. $P < 0.05$ vs. PODins.
glomerular and podocyte function in DN. Nadarajah et al. (23) described the importance of ACE2 in podocytes in an animal model of streptozotocin-induced diabetes with podocyte-specific overexpression of ACE2. In the earliest points of follow-up (4 and 8 wk), there were no differences in albuminuria between the control group and the transgenic mice, but the nontransgenic diabetic mice had higher levels of albuminuria than the other two groups. In the histological analysis, at 8 wk of follow-up, mesangial expansion decreased in nephrin and synaptopodin and podocyte loss was significantly ameliorated in the transgenic group compared with the nontransgenic diabetic group. The same pattern was shown in TGF-β1 cortical expression. In the later follow-up (16 wk), there were no differences between both diabetic mice in all these parameters. If targeted overexpression of ACE2 in these mice had a protective role, we hypothesized that the effect of insulin over ACE2 expression in podocytes shown in our in vitro model could reflect the observed in vivo, and it could be associated with renoprotective effects.

Albuminuria is a major finding in DN, and it is known to exert direct damage in podocytes, causing loss of differentiation and increase in mesenchymal markers (1), rearrangement of their cytoskeleton (20), and an increase in cell apoptosis (24). Recently, Peired et al. (26) published an article showing that albuminuria increases cell death and blocks the potential differentiation of renal progenitor cells within Bowman’s capsule in podocytes, remarking on the effect of albumin over the podocyte capital from a glomerulus. We did not find any effect on ACE2 expression with the incubation with albumin, but its presence blocked insulin effects. Interestingly, in the group coincubated with insulin and albumin we found an evident discordance between gene expression (same elevation as insulin alone) and protein expression and activity (decreased compared with insulin alone). There are several ER stress inducers in a diabetic environment such as an excess of reactive oxygen species or cytokines (6). Exposure of podocytes to stimuli like albumin overload leads to ER stress, which causes an accumulation of unfolded proteins in the ER that leads to cell injury (4). In this regard, we found a marked colocalization of ACE2 and ER in the group coincubated with insulin and albumin. This finding seems to reflect the increase in the synthesized proteins that need to be processed (ACE2 in the presence of insulin), along with a decrease in ER capacity of managing this overcharge in the presence of albumin.

We also showed that there was a change in the balance between ACE and ACE2 gene expression. In the presence of insulin, ACE/ACE2 index shows a significant decrease compared with both groups incubated with albumin as well as with the control group. Regarding other elements of RAS studied, there was a pro-ANG II gene profile in the groups incubated with albumin compared with the one incubated with insulin alone. Although the existence of other enzymes involved in ANG II generation [chymases as non-ACE pathways or nephrin with a role in generation of ANG-(1–7) from ANG I] and cleavage (aminopeptidase) (18, 32) should be taken into account. Our results are in agreement with other studies in podocytes in a diabetic milieu. The exposure to mechanical strain causes an increase in ANG II levels in primary cultured podocytes, and an animal model of glomerular capillary hypertension shows an increase in ANG II type 1 receptor (AT1-R) expression in podocytes (8). In addition, a high-glucose medium increases ANG II peptide by ACE-dependent and -independent pathways, along with an increase in Agt and AT1-R in cultured podocytes (9, 37). In our study, all the cells, including the control group, were incubated with high glucose levels in the media; then the increase in Agt, AT1-R, and ACE could be ascribed to the presence of albumin, not to high glucose exposure. It is interesting to remark that insulin was not able to reverse the negative effect of albumin over the
podocytes. One might surmise that the lack of the beneficial effect of insulin on the podocytes in the presence of albumin may be in part explained by albumin overload as previously mentioned.

Previous publications were focused on the relationship between insulin signaling pathways and RAS (22, 33), but not specifically on the podocytes. A recent study evidenced crosstalk between AT1-R and insulin receptor signaling pathways to competitively regulate megalin expression in proximal tubular cells in culture (14). Despite that kidney tubular cells and podocytes are epithelial cells, they are not comparable, but that study demonstrated the existence of an interaction between RAS and the signaling pathways of insulin in the kidney. These results and ours emphasize the need to explore this relationship in greater depth, since both RAS and insulin are central to the pathophysiology and treatment of DN.

TGF-β has a central role in the pathophysiology of DN podocytopathy (16, 38). An increase in TGF-β in podocytes leads to dedifferentiation of these cells, rearrangement of the cytoskeleton, and an increase in albumin permeability in a monolayer of podocytes (12, 17). Fibronectin is a glycoprotein that is part of the mesangial matrix, and it is produced by the podocyte (10). Its increment is related to mesangial matrix expansion, sclerosis, and glomerular fibrosis. In our study, we noted that albumin increased fibronectin and TGF-β gene expression and that insulin decreased fibronectin gene expression but could not reverse albumin effects. It is interesting to remark on the relationship between TGF-β and fibronectin. In cultured podocytes, there is an increase in fibronectin gene expression secondary to exogenous TGF-β (12, 17), caused by the presence of oxidized-LDL cholesterol (7) or high glucose levels (31).

Finally, we studied podocyte cell death in the presence of insulin, as podocyte loss is one critical feature in DN (29, 34). Podocytes incubated with insulin showed an evident decrease in TUNEL staining, consistent with a decrease in apoptosis, compared with the control group and both albumin groups, revealing a possible protective antia apoptotic effect of insulin dependent on the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (13, 19). Despite that there are no specific studies in the literature about insulin and podocyte survival, we hypothesized that insulin may have the same protective effects on podocytes. Supporting this hypothesis, exposure to statins or insulin growth factor-I has been shown to decrease podocyte apoptosis via PI3K/Akt (2, 3). Surprisingly, we could not find any increase in phosphorylated-AKT in the insulin treated podocytes. These data suggest that in our experiment the decrease in apoptosis might not be ascribed to a direct effect of insulin via PI3K/Akt (data not shown).

In summary, our results suggest that insulin may exert a renoprotective effect within the podocyte by different pathways: 1) increasing ACE2 protein expression, 2) decreasing the ACE/ACE2 gene expression index, 3) decreasing fibronectin gene expression as a maker of fibrosis, and 4) decreasing podocyte apoptosis. All these protective effects of insulin disappear in the presence of albumin, leading us to suggest that early administration of insulin in diabetes mellitus may have a protective effect at this cellular level before DN is already established.

ACKNOWLEDGMENTS

We thank Sergi Mojal (Statistician, Scientific and Technical Department, IMIM-Hospital del Mar, PRBB, Barcelona, Spain) for generous help with the statistical analyses.

GRANTS

This work was partially supported by research grants from the projects ISCIII-FEDER, PI081688 and PI 11/01549, Ajut Jordi Gras, FIMIM, 2010–2012; and ISCIII-RETICS REDinREN RD12/0021/0024.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


