Early intensive insulin therapy attenuates the p38 pathway in the renal cortex and indices of nephropathy in diabetic rats

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Abstract. In this rodent study, we compared the effects of early versus late intensive insulin therapy on diabetic nephropathy and potential causal mechanisms. Diabetes was induced in rats by high-fat diet and low-dose streptozotocin. Intensive insulin therapy was initiated in the early intensive insulin therapy groups as soon as diabetes was confirmed and lasted for 8 (8wEI group) and 16 weeks (16wEI group). In the late insulin therapy group (LI group), intensive insulin treatment was initiated 8 weeks later and lasted for 8 weeks. Age-matched diabetic rats (8wDM group and 16wDM group) and non-diabetic rats (8wNC group and 16wNC group) served as controls. Histological analysis, real-time PCR, and western blot were performed in renal cortex specimens. Glomerular hypertrophy and mesangial matrix expansion were prominent in the 16wDM and LI groups while the EI groups remained normal and similar to the 16wNC group. Western blots revealed that p38 MAPK activities in the EI groups decreased significantly, whereas the level in the LI group was markedly higher than the 16wEI group, and not different from the DM groups. Activities of MKK3/6, CREB and MKP-1 proteins as well as CREB and MKP-1 mRNA showed a similar pattern. Therefore, we concluded that early intensive insulin treatment and attainment of good glycemic control counteracted some renal molecular pathways associated with epigenetic metabolic memory to minimize risk of diabetic nephropathy. However, late insulin therapy did not abrogate the increased renal cortical p38 MAPK pathway activation in diabetic rats and led to glomerular hypertrophy and extracellular matrix expansion.

Key words: Early intensive insulin treatment, Diabetic nephropathy, p38 mitogen-activated protein kinase (MAPK), Metabolic memory

IN TYPE 2 DIABETES, insulin therapy is usually initiated late when near-normal glycemia can no longer be maintained with oral hypoglycemic agents. However, interestingly, our previous clinical studies revealed that compared with oral hypoglycemic agents, early intensive insulin treatment had favourable outcome in inducing long-term euglycemic control (remission without drug therapy for more than 12 months) in patients with newly diagnosed type 2 diabetes with severe hyperglycemia [1-2]. However, it is not known whether this will work in patients with more advanced disease including signs of diabetic nephropathy. Furthermore, because such mechanistic studies are not possible in clinical trials, we studied rodents to assess the effects of early or late intensive insulin therapy on signs of diabetic nephropathy and potential causal mechanisms.

P38, a member of the mitogen-activated protein kinase (MAPK) family, appears to contribute to the pathogenesis of diabetic nephropathy [3]. P38 MAPK was activated in vivo in glomeruli from diabetic rats and in vitro in mesangial cells exposed to high glucose [3-4]. Moreover, increased renal cortical p38 MAPK activity in diabetic rats could be attenuated by intensive (blood glucose=172 ± 10 mg/dL) but not poor or moderate metabolic control [5]. Therefore, attenuation of the p38 MAPK pathway in the renal cortex could be one beneficial effect of intensive insulin therapy. However, no previous study has investigated whether initiation of intensive insulin treatment in different stages of diabetes has different effects on the renal cortical p38 MAPK pathway.

Therefore, the goal of this study was compare the effects of early versus late intensive insulin therapy on glycemic control and contributors to diabetic nephrop-
Diabetic rats were induced by high-fat diet and low dose streptozotocin (STZ). Intensive insulin treatment was initiated in different phases of diabetes and lasted for different periods to test the effects of intensive insulin therapy on pathological abnormalities, the p38 MAPK pathway and other potential contributors to diabetic nephropathy.

**Materials and Methods**

**Animal model**

Male Sprague–Dawley (SD) rats (150-180 g) aged 7-8 weeks were purchased from the Experimental Animal Center of Guangdong Medical Sciences and raised in animal center of Sun Yat-sen University. The study was approved by Ethics Committee and followed the Guidelines for Animal Care of the First Affiliated Hospital of Sun Yat-sen University.

The rats were allocated into normal diet or high-fat diet (HFD) (58.3% fat, 7.9% protein and 33.8% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for 5 weeks. Diabetes was induced in HFD rats by intraperitoneal injection of STZ (Sigma, Saint Louis, MI), 40 mg/kg body weight. The normal-diet rats were injected an equal volume of vehicle citrate buffer. Three days after STZ injection, rats with the random blood glucose values ≥16.7 mmol/L were considered diabetic and selected for further studies.

**Experimental protocol**

Diabetic rats were randomly divided into six groups:

- **Diabetic groups (DM groups):** NPH insulin (Humulin N, Eli Lilly, Indianapolis, IN), 1-2 units per day was used only when the glucose level went higher than 33.3mmol/L.
  - 8wDM group, n=6, diabetes was induced and lasted for 8 weeks.
  - 16wDM group, n=8, diabetes was induced and lasted for 16 weeks.

- **Early intensive insulin therapy groups (EI groups):** NPH insulin, 6-10 units per day was injected. The doses of NPH insulin were titrated to maintain non-fasting blood glucose≤8 mmol/L.
  - 8wEI group, n=6, maintain near-normal glycemic control for 8 weeks as soon as diabetes was confirmed.
  - 16wEI group, n=9, maintain near-normal glycemic control for the entire 16 weeks as soon as diabetes was confirmed.

- **Late insulin therapy group:** LI group, n=7, NPH insulin, 1-2 units, three times per week were injected for 8 weeks as soon as diabetes was confirmed. Then 6-10 units NPH insulin per day was injected for the following 8 weeks.

- **The age-matched normal-diet non-diabetic SD rats served as controls (8wNC, n=6, 16wNC group, n=8).**

  Blood samples were obtained from the tail veins. Blood glucose was measured by a glucometer (Roche, Basel, Switzerland). During insulin treatment, non-fasting blood glucose was measured once every day. Before and after completing insulin treatment, fasting blood glucose was measured 10 hours after food was removed.

After completing the treatment, all the rats were maintained in metabolic cages to collect 24-hour urine. The urine volume was measured. Urine protein was tested by chemiluminescence analysis. Urinary protein excretion (mg/24 h) was assessed as: urine protein (mg/mL) × urine volume (mL) /24h.

In the end of study, the rats were fasted and anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3mL/ 100g body weight). The right kidneys were removed for histological analysis. The left kidneys were removed, decapsulated, weighed, and then divided into cortical and medullary portions. Kidney cortices were snap-frozen in liquid nitrogen and stored at -80°C for further analysis.

**Histological analysis**

The right kidneys were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 um. These specimens were then stained with hematoxylin and eosin (HE) and periodic acid-silver metheramine (PASM). In PASM-stained sections, the glomerular cross-sectional area (Ag), tuft area (At) and mesangial matrix area (Am) were measured in 30 glomerular profiles per rat by using Image Pro Express 6.0 software. Mesangial matrix area was defined as the PASM-positive area. Quantitative measurement of mesangial matrix expansion (mesangial matrix index) was expressed as PASM-positive area per total glomerular tuft cross-sectional area [7]. The glomerular volume (Vg) was determined as: $Vg = \beta / \kappa \cdot \frac{Ag}{3/2}$, where $\beta$ is 1.38 as a shape factor and $\kappa$ is 1.1 as a distribution factor [8].

**RNA extraction and real-time RT-PCR**

Total RNA was extracted from the kidney cortices...
using Trizol Reagent (Invitrogen, Carlsbad, CA). Two microgramma total RNA was reverse transcribed at a final volume of 20 μl by using ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). One μl of the RT products was amplified and quantified in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) by using the SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan). The results were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression. The sequences of the primers were as follows: transcription factor cyclic AMP response element binding protein (CREB), Forward, 5′- GGGAAATCCTTTCAAGGAGGC -3′, Reverse, 5′- CGACATTCTTGTGCTGCTTCC -3′; mitogen-activated protein phosphatase-1 (MKP-1), Forward: 5′- GATCAACGTCTCGCCAATT -3′, Reverse: 5′- GCACAAAACACCTTC ACCTCA -3′; transforming growth factor β1 (TGF-β1), Forward, 5′- CTCCCGTGGCTTCTAGTGC -3′, Reverse, 5′- GGGTGACTTCTTGTGCCGTAG -3′; GAPDH, Forward, 5′- AGCCAGAACATCATCCTCCCTG -3′, Reverse, 5′- CACACCTTCTTGATGTAC -3′.

**Western blot analysis**

Total proteins were extracted from the kidney cortices with a commercially available kit (Kangchen, Shanghai, China). The protein concentration was determined by BCA analysis (Kangchen, Shanghai, China). Thirty microgramma protein samples were separated on a 10.5-12% SDS–polyacrylamide gel electrophoresis (PAGE) medium and transferred to poly-vinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked with 5% non-fat milk in Tris-buffered saline Tween-20 (TBST) for 1 h at room temperature and incubated at 4°C overnight with primary antibody. The total p38, phospho-p38 (Thr180/Tyr182), MAPK kinase 3 (MKK 3), MAPK kinase 6 (MKK 6), phospho-MKK3/6 (Ser189/207), CREB, phospho-CREB (Ser 133) and β-actin (Cell Signalling, Beverly, MA) primary antibodies were at dilutions of 1:1000, and MKP-1 (SantaCruz Biotech, Santa Cruz, CA) primary antibody was at 1:200. After washed three times with TBST, the membranes were further incubated for 1 h at room temperature with horseradish peroxidase conjugated anti-rabbit secondary antibody at 1:2000 dilutions (Thermo, Rockford, IL) and washed three times with TBST. Proteins were detected with the enhanced chemiluminescence (ECL) system (Millipore, Bedford, MA).

**Statistical analysis**

Results are expressed as means ± S.D. Statistical analysis was performed by the SPSS 11.0 statistical package. One-way-analysis of variance (one-way-ANOVA) was used for comparison of more than two groups followed by LSD test for multiple comparisons. Kruskal-Wallis test was used when the data departed substantially from a normal distribution. Significance was defined as p<0.05.

**Results**

**Metabolic and weight data**

Diabetic rats developed severe hyperglycemia 3 days after injected with STZ. In the 8wEI and 16wEI groups, good glycemic control was maintained from the first day of initiation of insulin therapy until end of study with nonfasting blood glucose values being similar to the 8wNC and 16wNC groups, respectively. In the LI group, the nonfasting blood glucose values before attainment of good glycemic control were comparable to those in the DM groups. However, the values were significantly decreased immediately after initiation of intensive insulin therapy and were maintained at nearly normal levels for the next 8 weeks (Fig. 1A). The body weight showed a reverse pattern, the rats gained weight significantly during insulin treatment. Rats appear to be in a growth phase that is attenuated by diabetes but restored by insulin and good glycemic control. Therefore, insulin therapy also has beneficial effect on growth (Fig. 1B).

Induction of 16-week diabetes significantly increased the kidney weight associated with glomerular hypertrophy as well as the relative kidney weight compared with the increase found in the 16wNC group (p<0.05). The increase associated with diabetes was significantly counteracted in the EI groups, but in the LI group the kidney weight and the relative kidney weight were notably higher than the 16wEI group. The kidney weights in the 16wDM and LI groups were not different. When normalized for body weight, relative kidney weight in the LI group was markedly lower than the 16wDM group, with the most likely reason being that the LI group gained weight over the insulin-treated period. The kidney weight in the LI group was higher than that in the 8wDM group, while the relative kidney weight was much lower. The potential mechanism may also be that the LI group gained weight during insulin treatment (Table 1).
Moreover, the 16wEI was lower than the LI group and reached borderline statistical significance (p=0.054) (Fig. 2). The potential reason is that increasing urinary protein excretion is a symptom developed in a relative late period of diabetic nephropathy and the interval of early and late insulin treatment is too short to observe the differences in the 16wEI and LI groups. Besides, the variance of urinary protein excretion rate in the LI group was relatively large that the difference between early and late insulin treatment was diminished. Accordingly, if the poor glycemic-control period is extended or the case number is increased in the LI group, the significant difference

**Table 1** Kidney weight and relative kidney weight

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Fasting body weight (g) (final)</th>
<th>Kidney weight (g)</th>
<th>Relative kidney weight (mg/g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8wNC</td>
<td>6</td>
<td>461.00 ± 35.68*#</td>
<td>1.44 ± 0.07*#</td>
<td>3.13 ± 0.17*#</td>
</tr>
<tr>
<td>16wNC</td>
<td>8</td>
<td>547.75 ± 34.50</td>
<td>1.79 ± 0.10</td>
<td>3.28 ± 0.15</td>
</tr>
<tr>
<td>8wDM</td>
<td>6</td>
<td>304.50 ± 19.92*#</td>
<td>1.52 ± 0.15*#</td>
<td>4.98 ± 0.22*</td>
</tr>
<tr>
<td>16wDM</td>
<td>8</td>
<td>389.88 ± 25.06*</td>
<td>2.05 ± 0.15*</td>
<td>5.27 ± 0.56*</td>
</tr>
<tr>
<td>8wEI</td>
<td>6</td>
<td>445.67 ± 19.01*#</td>
<td>1.45 ± 0.09*#</td>
<td>3.24 ± 0.13*</td>
</tr>
<tr>
<td>16wEI</td>
<td>9</td>
<td>539.56 ± 46.91*#</td>
<td>1.90 ± 0.28</td>
<td>3.53 ± 0.42*</td>
</tr>
<tr>
<td>LI</td>
<td>7</td>
<td>505.71 ± 41.53*#</td>
<td>2.09 ± 0.35*</td>
<td>4.21 ± 1.03*</td>
</tr>
</tbody>
</table>

The increased kidney weight and relative kidney weight associated with diabetes were significantly counteracted in the EI groups, but in the LI group were notably higher than the 16wEI group.* p<0.05 vs. NC group; # p<0.05 vs. DM group.

**Urinary protein excretion**

Urinary protein excretion was already significantly elevated after 8-week diabetes and aggravated in the next 8 weeks. Both 8-week and 16-week intensive insulin treatment markedly reduced the urinary protein excretion rate to near normal levels. The LI group was markedly higher than the 16wNC and similar to the 8wDM group. Moreover, the 16wEI was lower than the LI group and reached borderline statistical significance (p=0.054) (Fig. 2). The potential reason is that increasing urinary protein excretion is a symptom developed in a relative late period of diabetic nephropathy and the interval of early and late insulin treatment is too short to observe the differences in the 16wEI and LI groups. Besides, the variance of urinary protein excretion rate in the LI group was relatively large that the difference between early and late insulin treatment was diminished. Accordingly, if the poor glycemic-control period is extended or the case number is increased in the LI group, the significant difference.
Early insulin therapy attenuates DN

Histological analysis

PASM-stained glomeruli are representatively shown in Fig. 3Aa-g. As compared with the 16wNC group, the glomerular volume and mesangial matrix index were increased significantly in the 16wDM and LI groups, and remained normal in the 16wEI group. These data suggested that glomerular hypertrophy and mesangial matrix expansion were developed in diabetic rats. These morphological changes were reversed to near-normal in the EI groups after 8-week and 16-week good glycemic control, but not in the LI group even though intensive insulin treatment was initiated 8 weeks after induction of diabetes. However, that the mesangial matrix index in the LI group was notably less than the 16wDM group and was not different from the 8wDM group revealed that intensive insulin therapy although initiated late could partly delay the progression of diabetic nephropathy. Diabetic glomerulosclerosis was not observed in any specimen (Fig. 3B-C).

Early but not late insulin treatment down-regulated the activations of renal cortical p38 MAPK and MKK3/6 proteins

The finding that early but not late insulin treatment ameliorated glomerular hypertrophy and mesangial matrix expansion in diabetic rats prompted us to investigate the underlying mechanisms. We tested the activation of p38 MAPK pathway using western blot analysis. After 8 or 16 weeks of severe hyperglycemia, the levels of renal cortical p-p38 protein and p-p38/total p38 protein increased in the DM groups compared with the NC groups. In the EI groups, early intensive insulin treatment for 8 and 16 weeks resulted in a significant attenuation in the activation of p38 MAPK, whereas activity in the LI group was similar to the DM groups (Fig. 4A).

Next, the activity of MKK 3/6, a typical upstream activator of p38 MAPK was assessed [9]. Similarly, the levels of renal cortical p-MKK3/6 protein, p-MKK3/6/ total MKK3 protein and p- MKK3/6/ total MKK6 protein were elevated in the DM groups and LI group, whereas the levels in the EI groups were not different from the NC groups (Fig. 4B).

Early but not late insulin treatment decreased the activations of renal cortical CREB protein and mRNA

We further examined the activation of CREB, an immediate downstream of p38 MAPK [10]. Similar to the activation of p38 MAPK and MKK3/6, p-CREB protein and p-CREB/CREB protein remained elevated in renal cortex of the DM groups and the LI group, but did not differ between the NC and EI groups (Fig. 5A).

CREB activation was also evident at the mRNA level. Real-time PCR revealed when compared with the NC groups, level of CREB mRNA in renal cortex of the 8wDM group was elevated and further increased in the 16wDM group, while in the 8wEI and 16wEI groups, remained unchanged (p>0.05). As compared with the 16wNC group, mRNA level of CREB in the LI group was 2.63 ± 0.56 fold higher (p<0.001). Therefore late insulin treatment could not suppress the aggravation (Fig. 5C).

Early but not late insulin treatment declined the levels of renal cortical MKP-1 protein and mRNA

We also measured the activity of MKP-1, which mediated the inactivation of p38 MAPK signaling through dephosphorylation [10]. The activations of MKP-1 protein and mRNA showed similar trends as CREB (Fig. 5B and D). In comparison to the 16wNC group, the mRNA levels of MKP-1 were 1.75 ± 0.20, 3.10 ± 0.75 and 3.33 ± 0.93 fold higher in the 8wDM, 16wDM and LI groups respectively (p<0.05), and 1.47 ± 0.35 fold higher in the 16wEI group (p>0.05). The level in the LI group was markedly higher than that in the 8wDM group (p<0.05) (Fig. 5D). These data, taken together, indicated that the increase of renal cortical p38 activity was paralleled with the activities of MKK3/6, CREB and MKP-1. Therefore, the potential reason of the abrogation of pathological abnormalities in the EI groups is that the increased activation of p38 MAPK pathway was eliminated by early intensive insulin treatment. The high activation of renal cortical p38 MAPK pathway, which was not attenuated by late insulin therapy, may be responsible for glomerular hypertrophy and mesangial matrix expansion in the LI group. Since previous study has recognized that p38 MAPK involves in signaling related to mitogenesis, growth, and differentiation, which is the leading cause of glomerular hypertrophy and extracellular matrix accumulation [11]. Moreover, the above changes have been occurred in the 8-week diabetic rats and could not be reversed, even aggravated after late insulin treatment. However, histological abnormalities and urinary protein excretion rate did not progressed after intensive insulin treatment in the LI group, it meant that intensive insulin therapy although initiated late still could
Fig. 3  PASM staining sections of the kidneys, the glomerular volume and mesangial matrix index. Aa, the 8wNC group; Ab, the 16wNC group; Ac, the 8wDM group; Ad, the 16wDM group; Ae, the 8wEI group; Af, the 16wEI group; Ag, the LI group. Original magnification for Aa–g was ×400. B-C, Glomerular hypertrophy and mesangial matrix expansion were prominent in the 16wDM group. These histological changes were attenuated in the EI groups but not the LI group, and were similar in the 8wDM and LI groups. However, significant diabetic glomerulosclerosis was not observed in any specimen. *p<0.05 vs. 16wNC group; †p<0.05 vs. 16wDM group.
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Fig. 4  Early but not late insulin treatment down-regulated the activations of renal cortical p38 MAPK and MKK3/6 proteins. A, P-p38/total p38 protein, B, p-MKK3/6/total MKK3 protein and p-MKK3/6/total MKK6 protein were found to be increased in the DM groups and LI group as compared with the 16wNC group. In the EI groups early intensive insulin treatment for 8 and 16 weeks resulted in a significant decline in the activations of the above proteins.

Fig. 5  Early but not late insulin treatment decreased the activities of renal cortical CREB and MKP-1. A, P-CREB/CREB protein remained elevated in the DM groups and LI group, but did not differ between the NC groups and EI groups. C, The mRNA levels of CREB were consistent with the activation of CREB protein. B, The activations of MKP-1 protein and D, MKP-1 mRNA showed similar results as CREB. * p<0.05 vs. 16wNC group; # p<0.05 vs. 16wDM group.

Fig. 6  Both early and late insulin treatment suppressed the high mRNA level of renal cortical TGF-β1 in diabetic rats. The mRNA levels of TGF-β1 in the 8wDM and 16wDM groups were increased. However, the levels in the 8wEI, 16wEI and LI groups were similar. * p<0.05 vs. 16wNC group; # p<0.05 vs. 16wDM group.
partly delay the development of diabetic nephropathy, the change of p38 pathway activation may not be the only mechanism involved in the abnormalities developed in the LI group and further studies involved in other signaling are needed.

Both early and late insulin treatment suppressed the high mRNA level of renal cortical TGF-β1 in diabetic rats

We last tested the expression of TGF-β1, which could stimulate p38 MAPK expression [12-13]. The mRNA levels of renal cortical TGF-β1 in the 8wDM and 16wDM groups were 1.50 ± 0.47 and 1.81 ± 0.83 fold higher respectively, in comparison to the 16wNC group (p<0.05). However, despite the different stages of intensive insulin therapy, the levels in the 8wEI, 16wEI and LI groups were similar (Fig. 6). Diabetic glomerulosclerosis is characteristics of late diabetes and usually found in renal tissue of long-term diabetic rats and TGF-β1 was a key mediator of diabetic glomerulosclerosis [12-14]. In contrast, in our study, diabetic rats were induced only for 8 and 16 weeks, thereby no diabetic glomerulosclerosis was observed and intensive insulin treatment may be induced too early to observe the difference in the early and late treatment groups. The above results showed that insulin may have direct effect in blocking the increased renal cortical TGF-β1 level in diabetic rats and intensive insulin treatment even though initiated late also could antagonize the pathogenesis of diabetic glomerulosclerosis.

Discussion

The key findings of the present study are that treatment with intensive insulin therapy immediately after confirmation of diabetes in rats is able to prevent histological changes associated with diabetic nephropathy and the beneficial effects of early insulin therapy may be mediated by p38 MAPK pathway. In addition, the present study found for the first time that the elevated activation of renal cortical p38 MAPK pathway, which was not attenuated by late insulin therapy, might be one of the potential molecular mechanisms leading to glomerular hypertrophy and mesangial matrix expansion. Whereas, late intensive insulin treatment only had beneficial effect on blocking the development of proteinuria.

One potential process that may contribute to the improvements in proteinuria, the morphological changes and the p38 MAPK pathway activity after early intensive insulin treatment is that the glucotoxicity generated by hyperglycemia was eliminated. As previously reported, long term hyperglycemia was the critical reason that led to proteinuria, glomerular hypertrophy, extracellular matrix accumulation and an increase in the activation of p38 MAPK pathway [11]. As our results show here, the decline in the urinary protein excretion, glomerular volume, mesangial matrix index and p38 MAPK pathway activity coincided with the decline of the blood glucose levels. Therefore, the attenuated p38 MAPK pathway activity induced by early intensive insulin therapy in this study was dependent on the attenuation of hyperglycemia. Another potential mechanism of the decline of p38 MAPK pathway activity is that the direct effects of insulin on the renal cortical cells, which occur independently of glycemic control. Insulin has direct signaling effects on endothelial cells and podocytes in vitro [15-16]. Insulin affects on a complex, highly integrated signaling network in the endothelial cells. One of the most critical signaling branches downstream from the insulin receptor is the MAPK pathway [15]. Secondly, insulin is able to signal to the podocyte also through the MAPK pathway and inhibits the increased of matrix and thickness of glomerular basement membrane [16]. In summary, the evidences mentioned above suggest that interactions between hyperglycemia, insulin and the p38 pathway activity may be more complex. Insulin treatment attenuated renal cortical p38 activity through simply elimination of glucotoxicity and the direct effects in glomerulus. Use of a model with non-insulin therapy to achieve glycemic control is required to discriminate between these potential causal mechanisms.

Clinical trials have been shown the beneficial effects of intensive insulin therapy on diabetic microvascular complications [17-18]. Recent rodent study indicated that renal cortical p38 activity increased in diabetic rats but was attenuated by improved glycemic control achieved by insulin therapy [5]. However, that study was performed in type 1 diabetes rodent model and did not investigate the effects of late insulin therapy. Our study was performed in a model for type 2 diabetes, which was induced by high-fat diet and low-dose STZ [6] and we found that high-fat diet feeding had no impact on the relative kidney weight, renal cortical morphology and p38 MAPK activation in kidney (data were not shown). Moreover, our results indicated that proteinuria and renal cortical p38 activity could not be easily reversed if restoration of good glycemic control
Early insulin therapy attenuates DN thereby to induce sustained euglycemia as well as delay or reduce the risk of diabetic complication.

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No potential conflicts of interest relevant to this article were reported.

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Conflict of Interest

All authors declare no conflict of interest.

References


