Role of 12-lipoxygenase in decreasing P-cadherin and increasing angiotensin II type 1 receptor expression according to glomerular size in type 2 diabetic rats

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Guo QY, Miao LN, Li B, Ma FZ, Liu N, Cai L, Xu ZG. Role of 12-lipoxygenase in decreasing P-cadherin and increasing angiotensin II type 1 receptor expression according to glomerular size in type 2 diabetic rats. Am J Physiol Endocrinol Metab 300: E708–E716, 2011. First published February 1, 2011; doi:10.1152/ajpendo.00624.2010.—12-lipoxygenase (12-LO) was implicated in the development of diabetic nephropathy (DN), in which the proteinuria was thought to be associated with a decreased expression of glomerular P-cadherin. Therefore, we investigated the role of 12-LO in the glomerular P-cadherin expression in type 2 diabetic rats according to the glomerular sizes. Rats fed with high-fat diet for 6 wk were treated with low-dose streptozotocin. Once diabetes onset, diabetic rats were treated with 12-LO inhibitor cinnamyl-3,4-dihydroxy-cyanocinnamate (CDC) for 8 wk. Then glomeruli were isolated from diabetic and control rats with a sieving method. RT-PCR, Western blotting, and immunofluorescent staining were used for mRNA and protein expressions of P-cadherin and angiotensin II (Ang II) type 1 receptor (AT1). We found that CDC did not affect the glucose levels but completely attenuated diabetic increases in glomerular volume and proteinuria. Diabetes significantly decreased the P-cadherin mRNA and protein expressions and increased the AT1 mRNA and protein expressions in the glomeruli. These changes were significantly prevented by CDC and recaputered by direct infusion of 12-LO product [12(S)-HETE] to normal rats for 7 days. The decreased P-cadherin expression was similar between large and small glomeruli, but the increased AT1 expression was significantly higher in the large than in the small glomeruli from diabetic and 12(S)-HETE-treated rats. Direct infusion of normal rats with Ang II for 14 days also significantly decreased the glomerular P-cadherin expression. These results suggest that diabetic proteinuria is mediated by the activation of 12-LO pathway that is partially attributed to the decreased glomerular P-cadherin expression.

GLOMERULAR HYPERTROPHY AND PROTEINURIA are hallmarks of the early diabetic nephropathy (DN) and can be induced by hyperglycemia and several growth factors, such as angiotensin II (Ang II), via their specific receptors (19). There are two Ang II plasma receptors, referred to as Ang II type I receptor (AT1) and type 2 receptor subtypes (6). Most of the known effects of Ang II in the adult tissues are attributable to AT1 intracellular signaling (6, 20, 25). Thus the expression of AT1 is important for the biological effect of Ang II. A number of clinical and experimental studies have demonstrated that AT1 antagonists reduce the damage of renal tissue and decrease proteinuria (3, 5, 26). One of the renal hemodynamic effects of Ang II was assumed to be the increase in the products of the 12-lipoxygenase (12-LO) pathway (2). To support this early study (2), we demonstrated recently that the upregulation of AT1 is associated with the activated 12-LO pathway in the diabetic kidneys and the mesangial cells exposed to high levels of glucose (27, 29).

The 12-LO pathway derives several products to exert numerous physiological and pathological effects in the kidney. One of the 12-LO products is the formation of oxidized lipids such as 12(S)-hydroxyicosatetraenoic acid [12(S)-HETE]. Both 12-LO and 12(S)-HETE were implicated in DN (10, 22, 27, 29). However, how 12-LO induces renal dysfunction remains under investigation.

The pathological change responsible for proteinuria in various glomerular diseases, including DN, is due predominantly to the loss of size-selective and/or charge-selective properties of the glomerular filtration barrier. The urinary side of the capillary loop in the glomerulus is covered by highly branched glomerular visceral epithelial cells called podocytes (21). Evidence indicates that the podocyte slit diaphragm that provides a structural framework for the filtration barrier is also essential as a signaling platform (12). In the past, the slit diaphragm was considered to be a modified tight junction due to the presence of the tight junction protein zonula occludens-1 at the cytoplasmic side of the slit diaphragm (14). However, recent studies using immunofluorescent and immunoelectron microscopy revealed that the slit diaphragm is a modified adherens junction rather than a modified tight junction and that, in addition to nephrin, NEPH1, and FAT, the P-cadherin was also localized at the slit diaphragm (7, 23).

The contribution of changes in nephrin expression to proteinuria in DN has been investigated. Bonnet et al. (4) found a reduction in nephrin mRNA and protein expression in streptozotocin (STZ)-induced diabetic spontaneously hypertensive rats at 32 wk after diabetes onset. Aaltonen et al. (1) observed an increase in nephrin mRNA levels in STZ-induced diabetic rats and in nonobese diabetic mice before the development of significant albuminuria. Studies showed that the prevention of the decreased nephrin expression with AT1 antagonist can significantly ameliorate proteinuria levels (24). All of these studies suggest an important role of slit diaphragm in the maintenance of the normal glomerular filtration. Furthermore, Kim et al. (13) demonstrated that nephrin mRNA and protein expression were decreased in hypertrophied glomeruli, whereas nephrin expression is increased in the relatively smaller glomeruli at the early stage of diabetes. This study...
suggests that the decreased expression of nephrin in the hyper-
trophied glomeruli may be responsible for the albuminuria at
the early stage of diabetes.

Besides nephrin, P-cadherin as one of the key components of
the slit diaphragm was also found to play critical role in the
maintenance of the normal glomerular filtration. In the nondi-
babetic condition, proteinuria was found to be related to the
depression of P-cadherin expression (16). It is also reported
that the detrimental effect of adriamycin on podocyte perme-
ability is associated with the disruption of the tight junctions by
decreasing P-cadherin expression (15). We have demonstrated
the decrease of P-cadherin expression in the diabetic kidney
and in the podocytes exposed to high levels of glucose in vitro
(28). However, whether the decrease of P-cadherin expression
varies according to the sizes of glomeruli under diabetic
conditions and whether the decreased expression of P-cadherin
in different sizes of glomeruli is related to diabetic activation of
the 12-LO pathway needs to be investigated.

Thus, the present study was to test a hypothesis that 12-LO
inhibition may ameliorate proteinuria by preventing the reduc-
tion of glomerular P-cadherin expression caused by diabetes.
To this end, we used a type 2 diabetic rat model that was
induced by a high-fat diet (HFD) for 6 wk, followed by
treatment with low-dose STZ. Eight weeks after hyperglycemia,
glomeruli were isolated from both diabetic and control rats and
separated into hypertrophied [large-sized (LG)] and relatively
small-sized (SG) groups. The P-cadherin expression according to
the sizes of isolated glomeruli was examined at mRNA and
protein levels. To define the role of 12-LO in diabetic
proteinuria and P-cadherin expression, 12-LO inhibitor cin-
namyl-3,4-dihydroxy-cyanocinnamate (CDC) was used to
treat the type 2 diabetic rats for 8 wk. In addition, to define
the direct roles of the 12-LO metabolic pathway and Ang II
in the decrease of glomerular P-cadherin expression, 12-LO
products 12(S)-HETE and Ang II, respectively, were in-
fused into normal rats.

MATERIALS AND METHODS

Materials

The 12(S)-HETE and CDC were purchased from Biomol (Plym-
outh Meeting, PA). We purchased the STZ, Ang II, and β-actin
antibody from Sigma (St. Louis, MO), P-cadherin and AT1 antibody
and goat anti-rabbit IgG-horseradish peroxidase from Santa Cruz
Biotechnology (Santa Cruz, CA), and supersignal chemiluminescent
reagent from Pierce (Rockford, IL). The relative multiplex reverse
transcription-polymerase chain reaction (RT-PCR) kits were pur-
bought from Ambion (Austin, TX), the RNA STAT-60 reagents were
bought from Tel-Test (Friendswood, TX), the 12(S)-HETE EIA kit
was bought from Assay Designs (Ann Arbor, MI), and the urinary
albumin ELISA Kit was bought from Exocell (Philadelphia, PA). We
purchased the osmotic minipump (Alzet Model 1002) from Direcot
(Cupertino, CA), the TINA image software from Raytest (Straub-
hardt, Germany), the digital image analysis (MetaMorph version
4.6r5) from Universal Imaging (Downingtown, PA), and the Prism
software from GraphPad (San Diego, CA).

Animal Models

All animal studies were conducted by following the protocol approved
by the Committee for the Care and Use of Laboratory Animals of Jilin
University. The animals were housed in a temperature-controlled room
and given free access to water. Male Wistar rats with 200–220 g body wt
were used for the following three animal models.

Type 2 diabetes model. Rats were randomly assigned to either
regular rat chow (control, n = 10) or 60% HFD (n = 24) for 6 wk
based on published studies (8, 18). After 6-wk HFD, rats received
either a low-dose STZ (35 mg/kg) to induce hyperglycemia or citrate
buffer (as control). STZ was dissolved in citrate buffer (0.01 mol/l, pH
4.5) and injected intraperitoneally. Blood glucose levels were mea-
sured 3 days after the STZ injection to confirm the development of
diabetes. Hyperglycemic rats were given with and without CDC
treatment at 4 mg/kg subcutaneously in the hind leg every other day
for 8 wk (n = 10). CDC was dissolved in sesame oil, and its dose used
in this study was selected on the basis of our previous studies (29). For
the CDC study, diabetic rats were injected with CDC mix in sesame
oil as diabetes/CDC group (CDC) or only with diluent sesame oil as
diabetic group (DN). At euthanization time, glomeruli were isolated
from part of the kidney and stored at −70°C for further study. The Rest
of the kidney specimens were fixed in 10% formalin for histological
evaluation or snap-frozen in liquid nitrogen for biochemical studies.
Blood glucose was measured by glucometer, and 24-h urinary albu-
min excretion was determined by ELISA kit.

12(S)-HETE infusion model. Sixteen male Wistar rats were ran-
domly assigned to receive either 12(S)-HETE infusion at a rate of 1
mg/kg daily or vehicle (normal saline) for 7 days by osmotic minic-
pumps (n = 8). For implantation of the osmotic minipumps the rats
were anesthetized with isoflurane, and incisions were made in the rats' backs to subcutaneously insert the osmotic minipumps under sterile
conditions. After a 7-day infusion of 12(S)-HETE the rats were
euthanized for isolation of glomeruli, as described in the above study.

Ang II infusion model. Sixteen male Wistar rats were randomly
assigned to receive either Ang II infusion at a rate of 400 ng·kg−1·min−1
or vehicle (ethanolamine) by osmotic minipumps for 14 days (n = 8).
Osmotic minipumps were implanted as described above, and the glomer-
ular isolation was the same as the above two studies.

Glomerular Isolation

Glomeruli were isolated with a sieving method, using sieves with pore
sizes of 250, 150, 125, and 75 μm, based on our previous study (13). The
glomeruli were collected under an inverted microscope to minimize
saline contamination. We classified glomeruli into small (on the 75-μm
sieve; SG) and large glomeruli (on the 125-μm sieve; LG) groups. Then,
glomeruli were frozen in liquid nitrogen and stored at −70°C.

Morphometric Measurement of Glomerular Volume

Glomerular volume (VG) was calculated as described previously
(10). Briefly, photographs of 50 glomeruli were taken using a digital

<table>
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<th>Gene</th>
<th>Sense</th>
<th>Antisense</th>
<th>Size, bp</th>
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<td>5'-ATCAGGACAGAGGCTGG-3'</td>
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<tr>
<td>AT1</td>
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<tr>
<td>GAPDH</td>
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<td>5'-GTCAAGAAAGACAGGAGG-3'</td>
<td>538</td>
</tr>
</tbody>
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AT1, angiotensin II type 1 receptor.
camera at the time of sieving, and the surface areas were traced using a computer-assisted color image analyzer. VG was calculated using the equation: \[ VG = \frac{4}{3} \pi \left( \frac{area}{\pi} \right)^{\frac{3}{2}}. \]

**Measurement of 12(S)-HETE**

Glomeruli were lysed in sodium dodecyl sulfate sample buffer, and the supernatants from glomerular lysate were stored at −70°C. The levels of 12(S)-HETE in the SG and LG groups were measured using a commercial EIA kit according to the manufacturer’s instructions, and protein content was measured with the modified Lowry method.

**Relative and Competitive RT-PCR**

Total RNA was isolated from glomeruli using RNA-STAT 60 reagents. The preparation of cDNA, relative multiplex PCR, and quantitative competitive PCRs were performed as described previously (27). Briefly, cDNA was synthesized with 1 μg of RNA using murine leukemia virus reverse transcriptase and random hexamers. Relative multiplex PCRs were performed using gene-specific primers along with primers for the GAPDH RNA as an internal control. The primers used are summarized in Table 1. In some experiments, we used quantitative competitive RT-PCR to determine AT1 mRNA expression, using the AT1 competitor cDNA (212 bp) as internal standard. The AT1 competitor cDNA used as internal standard was designed to contain the same base pair sequence as the target cDNA that would allow efficient priming but had a portion deleted so that the competitor PCR-generated fragment could be easily distinguished electrophoretically by size (Fig. 1). The RT-PCR products were separated by electrophoresis, and competitive PCR measurements were expressed with a ratio of the wild-type divided by the competitor band densities.

**Western Blot Analysis**

Glomeruli on 75- and 125-μm sieves were lysed in sodium dodecyl sulfate sample buffer. Lysate was centrifuged at 12,000 rpm for 30 min at 4°C, and the supernatants were stored at −70°C. Fifty micrograms of protein were loaded per lane, separated by 8% SDS-PAGE, transferred onto a nitrocellulose membrane, and immunoblotted with antibody to AT1 (1:1,000), P-cadherin (1:1,000), or α-actin (1:3,000). After being washed, membranes were incubated for 1 h with horseradish peroxidase-linked secondary antibody (1:2,000) and immunoreactive proteins detected by Supersignal chemiluminescent reagent. Immunoblots were scanned using densitometer, and the protein bands were quantified with TINA image software.

**Kidney Histology**

Renal cortical slices were snap-frozen in OCT compound and sectioned immediately for immunofluorescent staining. Briefly, for P-cadherin staining, monoclonal anti-P-cadherin antibody was diluted 1:50 with 2% casein in bovine serum albumin and applied for a 1-h incubation at 4°C. After washing, a secondary goat anti-rabbit IgG-FITC was added for 1 h.

**Statistical Analysis**

Data are expressed as means ± SD of multiple experiments. Paired Student’s t-tests were used to compare two groups or ANOVA for multiple groups, followed by Dunnett’s posttest using Prism software. Statistical significance was detected at the 0.05 level.

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**Fig. 1.** The nucleotide sequence of rat angiotensin II (Ang II) type 1 receptor (AT1) competitor. As an internal standard, an AT1 deletion mutant (competitor) that is coamplified with the endogenous gene was used in this study. Using the same primers for the mutant and the endogenous genes ensures comparable amplification efficiencies. The nucleotides used as primers for RT-PCR are indicated by gray boxes.

**Fig. 2.** Clinical characteristics of rats at the end of the study. Blood glucose levels (A), body weight (B), kidney weight/body weight ratio (C), glomerular volume (D), and urinary albumin excretion (E) were measured at end of the study. The data shown are means ± SD (n = 10 in each group). *P < 0.01 vs. control (Ctrl); †P < 0.05 vs. Ctrl; ‡P < 0.05 vs. diabetic nephropathy (DN); ‡‡P < 0.05 vs. diabetic nephropathy (DN); ††P < 0.01 vs. DN. CDC, cinnamyl-3,4-dihydroxy-cyanocinnamate.
RESULTS

Effect of 12-LO Inhibitor on the Renal Function of Type 2 Diabetic Rats

Rats with 6-wk HFD were given one injection of low-dose STZ, which induced hyperglycemia on day 3 (fasting blood glucose levels >300 mg/dl). Diabetic rats had elevated blood glucose levels throughout the study period compared with control rats (Fig. 2A). Rats with 6-wk HFD feeding had significantly higher body weight before STZ treatment than those with normal diet. After STZ treatment, diabetic rats did not gain significant body weight, whereas the rats with normal diet continually gained body weight so that the body weights in control and diabetic groups were not significantly different at

![Graph showing glomerular P-cadherin expression](image1)

Fig. 3. Glomerular P-cadherin expression. P-cadherin mRNA (A) and protein (B) expression in the small-sized (SG) and large-sized (LG) groups were detected by semiquantitative RT-PCR and Western blot, respectively. C: immunofluorescent staining for the P-cadherin was performed with the kidney tissue. A significant decrease in glomerular P-cadherin expression was exhibited with linear/punctuate distribution along the glomerular capillary loops in DN rats (×400). The data shown are means ± SD (n = 10 in each group). a P < 0.01 vs. corresponding Ctrls; b P < 0.05 vs. corresponding DNs.
the termination of experiment (Fig. 2B). The untreated diabetic rats had significant increases in the kidney weight/body weight ratio (Fig. 2C), the VG (Fig. 2D), and the proteinuria (Fig. 2E) compared with the control rats.

CDC treatment significantly prevented diabetic increases in the kidney weight/body weight ratio, the VG, and the proteinuria without influence of the body weight or blood glucose levels (Fig. 2).

**Effect of 12-LO Inhibitor on the Glomerular P-cadherin Expression of Type 2 Diabetic Rats**

A significant decrease in glomerular P-cadherin mRNA expression was observed in diabetic rats compared with controls (P < 0.01) without significant difference between LG and SG groups (Fig. 3A). Similarly, Western blotting also showed a significant decrease in the P-cadherin protein expression without significant difference between the two size groups of glomeruli (Fig. 3B). Immunofluorescent staining for whole kidney confirmed the glomerular-specific expression of P-cadherin and the decreased expression of P-cadherin in diabetic glomeruli (Fig. 3C). CDC treatment partially but significantly prevented diabetic decrease in the P-cadherin expression.

**Effect of 12-LO Inhibitor on the Glomerular AT1 Expression of Type 2 Diabetic Rats**

A significant increase in the glomerular AT1 mRNA expression detected by RT-PCR (Fig. 4A) was observed in diabetic rats compared with the controls (P < 0.01). The AT1 expression was significantly higher in the LG group than that in the SG group (P < 0.05; Fig. 4A). To ensure the accurately reflected difference for AT1 mRNA expression between the LG and the SG groups, the competitive RT-PCR with AT1-specific primers was performed. As an internal standard, an AT1 deletion mutant (competitor) that is coamplified with the endogenous gene was used. Using the same primers for the mutant and the endogenous genes ensures comparable amplification efficiencies. Each reaction was performed with a fixed amount of AT1 wild-type cDNA and a fixed amount of AT1 competitor cDNA. Data are expressed as the ratio of wild-type to competitor optical densities measured by densitometer. Competitive RT-PCR data also showed a significant increase in AT1 mRNA expression in the LG group compared with the SG group (P < 0.05; Fig. 4B). Consistent with the mRNA finding, Western blotting showed a significant increase in AT1 protein expression, with a higher expression in the LG than in the SG group of diabetic rats (Fig. 4C). CDC treatment significantly prevented a diabetic increase in the expression of AT1 at both mRNA and protein levels (Fig. 4).

**12(S)-HETE Levels in Type 2 Diabetic Glomeruli**

We have shown previously that 12-LO product 12(S)-HETE levels were increased significantly in whole kidney under diabetic conditions (27). Here, we have thus tested 12(S)-HETE levels in the SG and LG groups under diabetic conditions by EIA. As expected, the levels of 12(S)-HETE in glomeruli were increased significantly in diabetic rats compared with controls. The level of 12(S)-HETE is significantly higher in the LG than in the SG group (P < 0.05; Fig. 5). CDC could significantly attenuate diabetic increases in the levels of 12(S)-HETE.

**Effect of 12(S)-HETE Infusion on the Glomerular Expression of P-cadherin and AT1**

To define the causative role of 12(S)-HETE in the diabetic decrease in the glomerular P-cadherin expression, normal rats
were infused with 12(S)-HETE for 7 days. Direct infusion of 12(S)-HETE caused a significant decrease in glomerular P-cadherin mRNA (Fig. 6A) and protein (Fig. 6B) expression without a significant difference between the LG and SG groups.

Furthermore, we examined whether infusion of 12(S)-HETE also induces the glomerular AT1 expression, as observed in diabetic glomeruli (Fig. 4). Similarly, subcutaneous infusion of 12(S)-HETE caused a significant increase in the glomerular AT1 mRNA (Fig. 7A) and protein (Fig. 7B) expressions. The expression of AT1 at both mRNA and protein levels was higher in the LG than in the SG group (Fig. 7).

Effect of Ang II Infusion on the Glomerular P-cadherin Expression

Next, we examined the change of glomerular P-cadherin mRNA and protein expression in the normal rats that were directly infused with Ang II for 14 days. Subcutaneous infusion of Ang II significantly decreased the glomerular expression of P-cadherin mRNA (Fig. 8A) and protein (Fig. 8B) without significant difference between the LG and the SG groups.

DISCUSSION

P-cadherin is one of the classical cadherins, a superfamily of glycoproteins involved in cell-cell adhesion (9, 23). Based on the location of P-cadherin at the slit diaphragm, it has been proposed to function as a basic scaffold for the slit diaphragm, whereas the permselectivity is provided by the slit diaphragm complex composed of P-cadherin and other proteins, such as nephrin (23). Previous studies have indicated the potential role of decreased P-cadherin in the development of proteinuria in nondiabetic conditions (15, 16). Furthermore, intravenous injection of anti-P-cadherin antibody reportedly resulted in a 49% increase in urinary protein excretion independent of nephrin or NEPH1 (17), confirming the important role of P-cadherin in maintaining the glomerular filtration barrier. We have demonstrated previously the decreased expression of P-cadherin in the diabetic kidneys and the podocytes exposed to high levels of glucose in vitro (28), which was further supported by the present study to show the decreased expression of P-cadherin mRNA and protein specific in the glomeruli (Fig. 3C). Furthermore, we also provide the following new findings.

First, we demonstrated that diabetic decrease in the glomerular expression of P-cadherin is partially mediated by the activation of 12-LO pathways. In a type 2 diabetic rat model, we found that inhibition of 12-LO pathways with its inhibitor can partially, but significantly, attenuate diabetic decrease in the glomerular expression of P-cadherin (Fig. 3) along with an almost complete improvement of renal dysfunction (Fig. 2). Direct infusion of 12-LO product 12(S)-HETE to normal rats also significantly decreased the glomerular expression of P-cadherin at both mRNA and protein levels (Fig. 6). These
results suggest that activation of the 12-LO pathway plays a critical role in the development of DN by multiple pathogenic mechanisms (11, 22), one of which is the decreased expression of glomerular P-cadherin.

The second new finding is that diabetic decrease in the glomerular expression of P-cadherin is independent of glomerular sizes. We demonstrated for the first time that no statistical difference between the LG and the SG group was found for the decreased P-cadherin expression whether it was induced by diabetes (Fig. 3), 12(S)-HETE (Fig. 6), or Ang II (Fig. 8). Several studies demonstrated that diabetic glomerular sizes were different by examination with microdissection or a sieving technique. However, most of the previous studies that have focused on changes in glomerular nephrin showed inconsistent results. Bonnet et al. (4) found a reduction of the glomerular expression of nephrin mRNA and protein in STZ-induced diabetic and spontaneously hypertensive rats, whereas another study in STZ-induced diabetic rats and nonobese diabetic mice revealed an increase in nephrin mRNA levels (1). Kim et al. (13) found an increased nephrin expression in the SG group and a decreased nephrin expression in the LG group under the diabetic group compared with controls. Based on the finding that nephrin expression is diminished in the LG, they assumed that albuminuria may arise first in the hypertrophied glomeruli, i.e., the LG group at the early stage of DN. In line with this

Fig. 7. Effects of 12(S)-HETE infusion on the glomerular AT1 expression. AT1 mRNA (A) and protein (B) expression in the SG and LG groups were detected by semiquantitative RT-PCR and Western blot, respectively. The data shown are means ± SD (n = 8 in each group). aP < 0.05 vs. corresponding Ctrls; bP < 0.05 vs. HETE-SG.

Fig. 8. Effects of Ang II infusion on the glomerular P-cadherin expression. P-cadherin mRNA (A) and protein (B) expression in the SG and LG groups were detected by semiquantitative RT-PCR and Western blot, respectively. The data shown are means ± SD (n = 8 in each group). *P < 0.01 vs. corresponding Ctrls.
finding, we found that P-cadherin expression is significantly decreased in type 2 diabetic glomeruli, but we did not find a significant difference for the decreased P-cadherin expression between the LG and the SG groups under type 2 diabetic conditions. This suggests that the profiles of P-cadherin and nephrin expressions in diabetic glomeruli may be different; nephrin expression, but not P-cadherin expression, varies among the different sizes of glomeruli under diabetic conditions. Therefore, we proposed that protein excretion is partially dependent on the decreased expression of nephrin that changes according to the sizes of glomeruli and also partially dependent on the decreased P-cadherin expression that does not change according to sizes of glomeruli. This will explain why in the previous studies the changes of glomerular nephrin expression did not consistently parallel proteinuria results (1, 4, 13).

The third new finding of the present study is the direct role of 12-LO in the diabetic upregulation of AT1 expression in both the LG and SG groups. As shown in Fig. 4, the levels of AT1 mRNA and protein are significantly higher in the LG than in the SG group, which could be almost completely prevented by CDC. Interestingly, 12(S)-HETE level was also higher in the LG than in the SG group (Fig. 5). Direct infusion of 12(S)-HETE to normal rats increased the glomerular AT1 expression, which is also significantly higher in the LG than the SG group (Fig. 7). These direct correlation findings suggest that 12(S)-HETE may be the direct cause of glomerular AT1 upregulation.

Finally, we demonstrated that 12(S)-HETE-mediated diabetic suppression of glomerular P-cadherin expression is accompanied by upregulation of AT1 expression. We found that diabetic renal dysfunction paralleled the upregulated AT1 expression, particularly in the LG group. Inhibition of the 12-LO pathways almost completely prevented renal dysfunction and upregulated glomerular AT1 expression. The fact that, like diabetes, direct infusion of 12(S)-HETE to normal rats induced a significant decrease in glomerular P-cadherin expression (Fig. 6), along with a significant increase in AT1 upregulation (Fig. 7), suggests the possible involvement of Ang II in the diabetic suppression of glomerular P-cadherin. This was confirmed by the finding that glomerular P-cadherin expression was significantly decreased in rats with Ang II infusion (Fig. 8). However, the pattern of increased AT1 expression in different sizes of glomeruli was not directly correlated with the decreased expression of P-cadherin according to the different sizes of glomeruli. For instance, diabetes- and 12(S)-HETE-treated AT1 expression is higher in the LG group than in the SG group (Figs. 4 and 7), but the P-cadherin expression in the glomeruli was decreased without significant difference according to the sizes of glomeruli in diabetic (Fig. 3), 12(S)-HETE-treated (Fig. 6), and even Ang II-treated rats (Fig. 8). Therefore, diabetes-induced P-cadherin downregulation may be partially associated with Ang II/AT1 signaling pathway.

In summary, the present study has investigated the effect of 12-LO on the glomeruli P-cadherin expression with a focus on the profile in different sizes of glomeruli under type 2 diabetic conditions. Using the HFD/STZ-induced diabetic rat model, we demonstrated for the first time that the glomerular expression of P-cadherin at both mRNA and protein levels was significantly decreased in diabetic groups, which was significantly prevented by inhibition of 12-LO. Like diabetes, direct infusion of 12-LO product to normal rats for 7 days also significantly decreased the glomerular expression of P-cadherin. Direct infusion of Ang II can also decrease glomerular P-cadherin expression. However, there was no significant difference for the P-cadherin expression between different sizes of glomeruli under diabetic conditions. All of these results suggest that diabetic proteinuria is mediated by the activation of the 12-LO pathway. The decreased glomerular P-cadherin expression probably contributes partially to the 12-LO pathogenic effect on DN.

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

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