Electronic supplementary material

Methods

Serum analyses BUN was measured using a commercial kit from Jiancheng Bioengineering Institute (Nanjing, China). Serum creatinine was measured as described by Park et al. [1] with the Quantichrom Creatinine Kit (BioAssay Systems, Hayward, CA, USA). Serum triacylglycerol was assayed with a commercial kit from Dongou Tianjinma Biotech (Wenzhou, China). Total cholesterol, and HDL- and LDL-cholesterol levels were measured using commercial kits from Beihuakangtai (Beijing, China).

Urine analyses Urine albumin was measured with an ELISA kit from ADL (Beijing, China). Urinary creatinine concentrations were measured as described above and used to normalise the urine albumin. The GFR was calculated according to the formula:

GFR (l/24 h) = [urine creatinine (μmol/l) × urine volume (l/24 h)]/serum creatinine (μmol/l).

Histological analyses Kidney samples were collected and then fixed in 4% paraformaldehyde overnight. Fixed tissues were embedded in paraffin. Sections at 5 μm thickness were stained with PAS and examined under a light microscope. For each genotype, the images of at least 60 glomeruli (20 glomeruli/mouse, n=3) were recorded with an Olympus camera (Tokyo, Japan). The glomerular areas and the mesangial matrix areas were digitally quantitated with the Image-Pro Plus software (Media Cybernetics, Shanghai, China) for each glomerulus. The ECM accumulation indices were calculated by dividing the mesangial matrix area with the glomerular area.

Western blots for TGF-β1 and collagen IV Western blots were performed with a rabbit anti-TGF-β1 antibody (1:1,000) (Santa Cruz, CA, USA) and anti-collagen antibody (1:1000) (Abcam, Cambridge, MA, USA) and anti-β-actin (1:8,000) (Sigma, Saint Louis, MO, USA). The detection was achieved using the Immobilon Western chemiluminescent substrate kit (Millipore, Billerica, MA, USA).

Immunohistochemistry for renal expression of collagen IV Renal cortical sections (5 μm) were incubated overnight with the anti-collagen IV antibody (1:200 in blocking solution) in a humidified chamber at 4ºC. Biotinylated anti-rabbit IgG was then applied as a secondary antibody, followed by the addition of horseradish peroxidase conjugated with streptavidin and incubated in 3,3-diaminobenzidine substrate solution with nickel chloride enhancement. The immunostaining was examined and photographed under light microscopy (Olympus, Tokyo, Japan).

Reference

1. Park CW, Zhang Y, Zhang X et al (2006) PPARalpha agonist fenofibrate improves diabetic nephropathy in db/db mice. *Kidney Int* 69: 1511–1517