Disorder of fatty acid metabolism in the kidney of PAN-induced nephrotic rats

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PROTEINURIA is considered to play an essential role in the progression of tubulointerstitial damage, which is a common pathway to end-stage renal disease (28, 30). Fatty acids (FAs) bound to serum albumin (7) are filtered through glomeruli and reabsorbed into proximal tubular epithelial cells (PTECs) via endocytosis. FAs were markedly reabsorbed and accumulated in PTECs of patients and experimental animals with nephrosis (16, 32). FAs also play a role in the development of tubulointerstitial damage, which causes end-stage renal disease. Fatty acid-binding albumins are filtered through glomeruli and reabsorbed into proximal tubular epithelial cells (PTECs). However, the role of fatty acid metabolism associated with albuminuria in the development of tubulointerstitial damage remains unclear. Thus, the present study was designed to determine the changes of fatty acid metabolism in the nephrotic kidney. To induce nephrotic syndrome, Sprague-Dawley rats (SDRs) and Nagase analbuminemic rats (NARs) with inherited hypoalbuminemia were treated with a single injection of puromycin aminonucleoside (PAN). In SDRs, PAN treatment induced massive proteinuria and albuminuria and caused tubular damage, apoptosis, and lipid accumulation in PTECs. Among the enzymes of fatty acid metabolism, expressions of medium-chain acyl-CoA dehydrogenase (MCAD) and cytochrome P-450 (CYP)4A significantly decreased in PTECs of PAN-treated SDRs. Expressions of peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α and estrogen-related receptor (ERR) were also significantly decreased, without changes in the expression of PPAR-α. In NARs, PAN treatment induced proteinuria but not albuminuria and did not cause tubular damage, apoptosis, or lipid accumulation. Expressions of MCAD, PGC-1α, or ERRα did not change in the kidney cortex of PAN-treated NARs, but the expression of CYP4A4 significantly decreased. These results indicate that massive albuminuria causes tubular damage and lipid accumulation with the reduction of MCAD, CYP4A4, PGC-1α, and ERRα in PTECs.

albuminuria; lipid accumulation; lipotoxicity; fatty acid metabolism; proximal tubular epithelial cells; puromycin aminonucleoside

FA-binding protein (FABP) delivers FAs to intracellular sites of utilization. FA synthesis is catalyzed through FA synthase (FAS) and acetyl-CoA carboxylase (ACC). FAs undergo oxidation in mitochondria, the peroxisome, and the microsome. Medium-chain FAs (6–12 carbon chains) freely diffuse into mitochondria, and long-chain FAs are transported into mitochondria by carnitine palmitoyltransferase (CPT)-I. Acyl-CoA dehydrogenases are mitochondrial enzymes catalyzing the initial step in each cycle of FA β-oxidation and are categorized into distinct groups, such as very long-chain acyl-CoA dehydrogenase (VLCAD), long-chain acyl-CoA dehydrogenase (LCAD), medium-chain acyl-CoA dehydrogenase (MCAD), and short-chain acyl-CoA dehydrogenase (SCAD). Besides fatty acid β-oxidation in mitochondria, acyl-CoA oxidase (ACOX) is a peroxisomal enzyme breaking very long-chain FAs (20 or more carbon chains) down to a manageable size. Cytochrome P-450 (CYP)4A is a microsomal enzyme relating FA ω-oxidation of medium-chain and long-chain FAs.

Sterol regulatory element-binding proteins (SREBPs) serve as the master regulators of cellular lipid synthesis; SREBP-1 regulates fatty acid synthesis, and SREBP-2 regulates cholesterol synthesis (3). Peroxisome proliferator-activated receptor (PPAR)-α is essential for the maintenance of FA metabolism (1, 13, 44) as well as the function of PTECs (13). In addition to PPAR-α, PPAR-γ coactivator (PGC)-1α regulates FA metabolism and mitochondrial biogenesis (8, 33, 37). PGC-1α upregulates the expression and activity of the orphan nuclear receptor estrogen-related receptor (ERR)α (34), which is a transcriptional regulator of the MCAD gene (36).

Previous studies have reported disorders of FA metabolism in several kidney diseases. Lipid contents are increased in the kidney of chronic nephrotic rats (16). Lipid accumulation was also found in the remnant kidney of 5/6 nephrectomized rats accompanied by the upregulation of FAS and ACC expression and downregulation of FABP, CPT-I, ACOX, SREBP, and PPAR-α expression (4, 15). MCAD expression was downregulated in the mouse kidney with cisplatin-induced acute renal failure and ischemia-reperfusion injury (20, 21, 26). ACOX and CYP4A expressions were downregulated in the rat kidney with ischemia-reperfusion injury (27). Although the described animal models with kidney diseases have various disorders of FA metabolism, the role of FA metabolism associated with albuminuria in the development of tubulointerstitial damage remains unclear. Thus, the present study was designed to determine the changes of FA metabolism in the nephrotic kidney of Sprague-Dawley rats (SDRs) and to further compare them with those of Nagase analbuminemic rats (NARs) with inherited hypoalbuminemia.
MATERIALS AND METHODS

Animals. Six-week-old male SDRs and NARs weighing 160–180 g were obtained from SLC (Shizuoka, Japan). Rats were housed in an animal care facility at Tohoku University School of Medicine and had free access to standard laboratory chow and water while housed under controlled environmental conditions (12:12-h light-dark cycle and 24°C room temperature). All protocols involving rats were reviewed and received prior approval by the Animal Welfare Committee of Tohoku University.

Rats were randomly divided into four groups: the control SDR group (n = 24), the puromycin aminonucleoside (PAN)-treated SDR (PAN-SDR) group (n = 24), the control NAR group (n = 6), or the PAN-treated NAR (PAN-NAR) group (n = 6). Vehicle (0.9% NaCl) or PAN (Sigma-Aldrich) at a dose of 100 mg/kg (35) was injected intravenously in each group. Rats were placed in individual metabolic cages (TOYO-RIKO, Tokyo, Japan) for 24 h, and urine samples were collected. Blood samples were collected by decapitation under anesthesia (TOYO-RIKO) for 24 h, and urine samples were treated with 0.3% H2O2 in absolute ethanol for 30 min and then processed for immunostaining with antibodies against caspase 3, MCAD, SCAD, ACOX, PPAR-α, PGC-1α, and ERRα (Santa Cruz Biotechnology) or CYP4A11 (Becton Dickinson) for 1 h. After being washed in TBST, immunoblots were developed using an enhanced chemiluminescence kit (Super Signal, Thermo Fisher Scientific). Relative intensities were quantified using ImageJ software (version 1.44, National Institutes of Health). Band intensities for each protein were normalized to those for β-actin as an internal standard, and the band intensity in the control SDR group was assigned a value of 1. Immunoblot specificities of kidney homogenates were verified by blockade with corresponding control peptides for FAS, ACC, L-FABP, CPT-I, ACOX, PPAR-α, PGC-1α, and ERRα (Santa Cruz Biotechnology) or CYP4A11 (Becton Dickinson).

Tissue lipid contents. Total lipids were extracted from 100 mg of the kidney cortex by a previously described method (6). Briefly, samples were homogenized in 6 ml of chloroform-methanol (2:1). The mixture stood for 1 h, after which 1.5 ml water was added, and the mixture was centrifuged at 2,000 × g for 10 min. The organic phase was evaporated to dryness and taken up in chloroform; 100 μl of this organic phase were solubilized by adding 1 ml 2-propanol, and free FA and triglyceride contents were determined using lipid assay kits (NEFA C-Test Wako and Triglyceride E-Test Wako) from Wako Pure Chemical (Osaka, Japan). Data are expressed as the amount of the lipids per 1 g of the kidney cortex mass.

Histopathology (electron microscopy), immunohistochemistry, and oil red O stain. For electron microscopy, small blocks of the kidney cortex were fixed with 2% glutaraldehyde and postfixed in 1% OsO4. Samples were dehydrated in graded ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy (JEM-100SX, JEOL).

For immunohistochemical analysis, kidney tissues were fixed with 10% paraformaldehyde and embedded in paraffin. Paraffin sections (3 μm thick) were deparaffinized in xylene and ethanol and rinsed in PBS. To block endogenous peroxidase activity, rehydrated sections (6 μm thick) were deparaffinized in xylene and ethanol and rinsed in PBS. To block endogenous peroxidase activity, rehydrated sections were treated with 0.3% H2O2 in absolute ethanol for 30 min and then processed for immunostaining with antibodies against caspase 3, MCAD, PGC-1α, and ERRα (1:500, respectively, Santa Cruz Biotechnology) or CYP4A (1:500, Nosan, Tokyo, Japan) according to the manufacturer’s instructions. After being washed with PBS, a chromagen solution, consisting diaminobenzidine and H2O2, was applied to the sections. Slides were counterstained with hematoxylin for 1 min.

For oil red O staining of tissue lipids, kidney tissues were fixed with 10% paraformaldehyde and made into frozen sections, and a portion was then stained with oil red O. Sections (6 μm thick) were rinsed in distilled water, rinsed in 60% isopropanol for 1 min, stained for 15 min in the oil red O working solution (Muto Pure Chemicals, Tokyo, Japan), rinsed again for 1 min in 60% isopropanol, and then returned to distilled water. Slides were counterstained with hematoxylin for 1 min.

Statistical analysis. Data are presented as means ± SE. Data were analyzed by repeated-measures ANOVA followed by a Tukey test for multiple comparisons among the groups. Paired data were analyzed by a Student’s t-test. P values of <0.05 were considered to indicate statistical significance.

RESULTS

General data. A single intravenous injection of PAN induced massive proteinuria in SDRs. Time courses of urinary protein excretion in the control SDR and PAN-SDR groups are shown in Fig. 1A. Urinary protein excretion increased after the 3rd day after PAN treatment, continued to increase to a maximum level until the 14th day, and normalized on the 28th day. Therefore, the later experiments provided the data obtained on the 14th day after PAN treatment. Urinary protein
excretion and albumin excretion were significantly higher in the PAN-SDR group than in the control SDR group (Fig. 1, B and C). In contrast to the SDR group, urinary protein excretion was significantly higher in the PAN-NAR group than in the control SDR group (Fig. 1B), but urinary albumin excretion was barely detected and not significantly different between the control NAR group and PAN-NAR group (Fig. 1C).

The effects of PAN treatment on plasma parameters in SDRs and NARs are shown in Table 1. Plasma total protein and albumin were significantly lower in the control NAR group than in the control SDR group, and total cholesterol, triglyceride, and creatinine were significantly higher in the control NAR group than in the control SDR group. In SDRs, plasma total protein and albumin were significantly lower in the PAN-SDR group than in the control SDR group, and total cholesterol, triglyceride, and creatinine were significantly higher in the control SDR group than in the control SDR group. In NARs, plasma total protein and albumin were not significantly different between the control NAR group and PAN-NAR group. Plasma total cholesterol, triglyceride, and creatinine were significantly higher in the control NAR group than in the control NAR group, but the PAN-induced change of plasma creatinine was less in NARs than in SDRs.

Glomerular damage in nephrotic rats. Electron microscopy experiments were performed to determine the structural changes in glomerular morphology (Fig. 2A). The glomeruli in the control SDR and control NAR groups had tall and slender foot processes without foot process effacement (Fig. 2A, a and c). Compared with the control groups (Fig. 2A, a and c), the glomeruli in the PAN-SDR and PAN-NAR groups showed complete foot process effacement with increased dense materials (Fig. 2A, b and d). Lysosomes in the podocytes of the PAN-SDR group were prominent (Fig. 2A, b), whereas those of the PAN-NAR group were indistinctive (Fig. 2A, d). To confirm PAN-induced glomerular podocyte damage, levels of nephrin protein, a molecule of the slit diaphragm of the podocyte, which is known to be reduced by PAN treatment (14), and levels of dendrin protein, a novel component of the slit diaphragm with proapoptotic signaling properties (2), which is also known to be reduced by PAN treatment (5), were examined in isolated glomeruli of the four groups. Levels of nephrin protein were not significantly different between the control SDR group and control NAR group. Compared with the corresponding control group, levels of nephrin protein were significantly decreased to 46% and 38% in the PAN-SDR group and PAN-NAR group, respectively, and were not significantly different between the control SDR group and control NAR group. Levels of dendrin protein were not significantly different between the control SDR group and control NAR group. Compared with the corresponding control group, levels of dendrin protein were significantly decreased to 72% and 68% in the PAN-SDR group and PAN-NAR group, respectively, and were not significantly different between the PAN-SDR group and PAN-NAR group (Fig. 2C).

Table 1. Effect of PAN on plasma parameters in SDRs and NARs

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<th>SDRs</th>
<th>NARs</th>
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<td></td>
<td>Control</td>
<td>PAN</td>
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<tr>
<td>Total protein, g/dl</td>
<td>6.1 ± 0.4</td>
<td>5.7 ± 1.3*</td>
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<tr>
<td>Albumin, g/dl</td>
<td>3.7 ± 0.3</td>
<td>2.1 ± 0.3*</td>
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<tr>
<td>Total cholesterol, mg/dl</td>
<td>65.7 ± 17.1</td>
<td>203.5 ± 64.9*</td>
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<tr>
<td>Triglyceride, mg/dl</td>
<td>40.1 ± 24.1</td>
<td>86.3 ± 25.6*</td>
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<tr>
<td>Free fatty acid, meq/l</td>
<td>0.76 ± 0.29</td>
<td>0.69 ± 0.15</td>
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<tr>
<td>Urea nitrogen, mg/dl</td>
<td>16.0 ± 2.2</td>
<td>14.1 ± 1.9</td>
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<tr>
<td>Creatinine, mg/dl</td>
<td>0.13 ± 0.07</td>
<td>0.97 ± 0.66*</td>
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Values are expressed as means ± SE; n = 6 rats/group. PAN, puromycin aminonucleoside; SDRs, Sprague-Dawley rats; NARs, Nagase analbuminemic rats. *P < 0.05 compared with the corresponding control group; †P < 0.05 compared between the control groups.
Tubular damage, apoptosis, and lipid accumulation in nephrotic rats. Urinary NAG, a marker of acute PTEC injury (45), was significantly higher in the control NAR group than in the control SDR group and significantly higher in the PAN-SDR group than in the control SDR group (Fig. 3A). Urinary 8-OHdG, a marker of oxidative stress (24), was significantly higher in the control NAR group than in the control SDR group (Fig. 3B). In contrast to SDRs, urinary NAG or 8-OHdG was not significantly different between the control NAR group and PAN-NAR group (Fig. 3, A and B).

Apoptosis in the kidney cortex was examined by Western blot and immunohistochemical analyses for caspase 3, a family of cysteine proteases acting as molecular executioners for programmed cell death (22). Levels of caspase 3 protein were significantly higher in the PAN-SDR group than in the control SDR group but were not significantly different among the control SDR, control NAR, and PAN-NAR groups (Fig. 3C). Representative photomicrographs of immunohistochemical analysis for caspase 3 are shown in Fig. 3D. Strong stainings for caspase 3 protein were detected in several PTECs and distal tubular cells in the kidney cortex of the PAN-SDR group (Fig. 3D,b), but staining was barely detected in the kidney cortex of the control SDR, control NAR, and PAN-NAR groups (Fig. 3D,a, c, and d).

To clarify lipid accumulation, kidney sections were stained with oil red O, and representative photomicrographs are shown in Fig. 4A. Oil red O stain was barely detected in several PTECs of the control SDR group and control NAR group (Fig. 4A,a and c). Oil red O stain identified noteworthy amounts of lipid deposits in the kidneys of the PAN-SDR group (Fig. 4A,b), and little lipid stained in the kidneys of the PAN-NAR group (Fig. 4A,d). Free FA content and triglyceride content in the kidney cortex were significantly higher in the PAN-SDR group than in the control SDR group (Fig. 4B and C). In contrast to SDRs, free FA content and triglyceride content in the kidney cortex were not significantly different between the control NAR group and PAN-NAR group (Fig. 4, B and C).

Expressions of enzymes for FA metabolism in the kidneys of SDRs and NARs. To clarify the mechanism of lipid accumulation in the kidneys of the PAN-SDR group, expressions of enzymes for FA metabolism in the kidney cortex were com-
pared among the four groups by Western blot analysis. Levels of the enzymes for FA synthesis (FAS and ACC) were not significantly different among the four groups (Fig. 5, A and B). Levels of the enzymes for FA transport (L-FABP and CPT-I) were not significantly different among the four groups (Fig. 5, C and D).

Levels of the enzymes for FA oxidation (VLCD, LCAD, MCAD, SCAD, ACOX, and CYP4A) were not significantly different between the control SDR group and control NAR group (Fig. 6). Compared with the control SDR group, levels of MCAD protein were significantly decreased to 35% in the PAN-SDR group but were not significantly different between the control NAR group and PAN-NAR group (Fig. 6C). Compared with the corresponding control group, levels of CYP4A protein were significantly decreased to 49% and 68% in the PAN-SDR group and PAN-NAR group, respectively (Fig. 6F). Levels of the other enzymes for FA oxidation (VLCD, LCAD, SCAD, and ACOX) were not significantly different between the control group and the corresponding PAN-treated group (Fig. 6, A, B, D, and E).

Levels of the transcriptional regulators for MCAD and CYP4A (PPAR-α, PGC-1α, and ERRα) were not significantly different between the control SDR group and control NAR group (Fig. 7). Levels of PPAR-α protein were not significantly different between the control group and the corresponding PAN-treated group (Fig. 7A). Two distinct bands (~115 and ~130 kDa) for PGC-1α protein were detected by Western blot analysis (Fig. 7B). Three isoforms of PGC-1α (PGC-1α-a, PGC-1α-b, and PGC-1α-c) have been cloned from mouse skeletal muscles, but PGC-1α-a is only isoform expressed in the mouse liver (22). The PGC-1α isoforms that are expressed in the kidney are unknown; however, the protein size of the lower band for PGC-1α protein in rat kidney samples is identical with that of a single band for PGC-1α protein in rat liver samples (data not shown). Compared with the control SDR group, levels of the lower band for PGC-1α protein were significantly decreased to 37% in the PAN-SDR group but were not significantly different between the control NAR and PAN-NAR groups (Fig. 7B). Levels of the upper band for PGC-1α protein were not significantly different between the control group and the corresponding PAN-treated group. Compared with the control SDR group, levels of ERRα protein were significantly decreased to 31% in the PAN-SDR group but were not significantly different between the control NAR and PAN-NAR groups (Fig. 7C).

Expressions of MCAD, CYP4A, PGC-1α, and ERRα proteins were further examined by immunohistochemistry in the kidney cortex of the control SDR and PAN-SDR groups. The representative photomicrographs are shown in Fig. 8. Strong stainings for MCAD, CYP4A, PGC-1α, and ERRα proteins were detected in PTECs of the control SDR group, and weak stainings for these proteins were detected in the distal tubules, glomeruli, and renal vessels (Fig. 8, a–d). Immunohistochemical experiments confirmed marked decreases in the levels of MCAD, CYP4A, PGC-1α, and ERRα proteins in PTECs of the PAN-SDR group (Fig. 8, e–h).
DISCUSSION

The present study determined the tubular damage and disorder of FA metabolism in the nephrotic kidney. PAN treatment induced proteinuria and dyslipidemia in both SDRs and NARs through damage of glomerular podocytes, but the PTEC damage, apoptosis, and lipid accumulation concomitant with albuminuria were found only in SDRs, suggesting that albuminuria causes the disorder of FA metabolism in PTECs. To clarify mechanisms for the disorder of FA metabolism, the present study examined expressions of enzymes for FA synthesis, transport, and oxidation and disclosed a specific downregulation of MCAD and CYP4A expressions in the nephrotic kidney. Therefore, the lipid accumulation might be explained in part by the downregulation of MCAD and CYP4A.

MCAD is essential for complete FA oxidation, and MCAD-null mice develop severe hepatosteatosis and cardio-

Fig. 4. Effects of PAN treatment on lipid accumulation in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. A: representative photomicrographs of the oil red O-stained kidney cortex in the control SDR group (a), PAN-SDR group (b), control NAR group (c), and PAN-NAR group (d) (magnification: ×200). Bar = 100 μm. Noteworthy amounts of lipoid deposits were identified in proximal tubular epithelial cells (PTECs) of the PAN-SDR group (b), and little lipid staining was identified in PTECs of the PAN-NAR group (d). B and C: free fatty acid content (B) and triglyceride content (C) in the kidney cortex were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Data are presented as means ± SE; n = 6 in rats/group. *P < 0.05 compared with the corresponding control group.

Fig. 5. Effects of PAN treatment on expressions of enzymes for fatty acid catabolism in the kidney cortex of SDRs and NARs on the 14th day after PAN treatment. Protein levels of fatty acid synthase (FAS; A), acetyl-CoA carboxylase (ACC; B), liver-type fatty acid-binding protein (L-FABP; C), and carnitine palmitoyltransferase (CPT)-I (D) in the kidney cortex were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots are shown at the top, and each lane from the left to the right was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for each protein were normalized to those for β-actin (bottom), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; n = 6 rats/group.
Inhibits Na\(^{+}\), 20-HETE (31). 20-HETE constricts renal microvessels and long-chain FAs. CYP4A metabolizes arachidonic acid to 20-HETE analogs, which may attenuate renal injury (29). The PPAR-\(\alpha\)-null ligand and transgene prevented acute tubular necrosis (15). PPAR-\(\alpha\)-null mice (12). The constitutive expressions and activities of VLCAD and LCAD were significantly lower in the kidneys of PPAR-\(\alpha\)-null mice (1, 12), but expression and activity of MCAD were not different between PPAR-\(\alpha\)-null and wild-type mice (1, 21), suggesting that VLCAD and LCAD might serve as rate-limiting enzymes for FA oxidation. The PAN-induced nephrosis caused hypoalbuminemia with increased plasma cholesterol and triglyceride, and the albumin overload caused hyperalbuminemia with increased plasma free FAs (38). The discrepancy of expressions of FA metabolism in the two nephrotic models can be explained by differences in dyslipidemia and plasma albumin levels.

CYP4A is expressed in PTECs, thick ascending limbs of the loop of Henle’s loop (TALs), glomeruli, and renal microves-sels (10) and catalyzes the \(\omega\)-hydroxylation of medium-chain and long-chain FAs. CYP4A metabolizes arachidonic acid to 20-HETE (31). 20-HETE constricts renal microvessels and inhibits Na\(^{+}\) reabsorption in PTECs and TALs. It regulates kidney functions and activates a number of intracellular signal transduction pathways involved in cell growth and survival (23, 31). Reduction of 20-HETE may contribute to increasing Na\(^{+}\) reabsorption and \(O_2\) consumption after tubular hypoxia and damage in the nephrotic kidney. In agreement with this speculation, a CYP4A inhibitor exacerbated ischemia-reperfusion kidney injury, and 20-HETE analogs attenuated the injury (29).

The present study further examined the expressions of transcriptional regulators for MCAD and CYP4A and disclosed that PGC-1\(\alpha\) and ERR\(\alpha\) but not PPAR-\(\alpha\) expressions were downregulated in the nephrotic kidney. The PPAR-\(\alpha\) ligand clofibrate induced MCAD and CYP4A expressions in the rat kidney (9, 25). PPAR-\(\alpha\) expression was reduced in the remnant kidneys of 5/6 nephrectomized rats (4, 15) and in the kidneys of mice with cisplatin-induced acute renal failure (20). The PPAR-\(\alpha\) ligand and transgene prevented acute tubular necrosis in mice with cisplatin-induced acute renal failure and ischemia-reperfusion injury (20, 21, 27). Acute FA toxicity induced by albumin-overload proteinuria was exaggerated in the kidneys of PPAR-\(\alpha\)-null mice (12).

PGC-1\(\alpha\) has been recently pointed as an important regulator of FA metabolism. PGC-1\(\alpha\) upregulates FA oxidation and expressions of mitochondrial \(\beta\)-oxidation enzymes such as
MCAD, LCAD, and CPT-I, particularly when it coactivates PPAR-α, PPAR-γ coactivator (PGC)-1α, and estrogen-related receptor (ERR)α in tissues with high activity of FA oxidation, such as in the heart, brown fat, liver, and kidney. In the kidney, PGC-1 expression is localized in PTECs and TALs (26). PGC-1α mRNA was diminished in PTECs of mice with cisplatin-induced acute renal failure (26). PGC-1α, ERRα, and MCAD mRNA were reduced in the kidneys of mice treated with lipopolysaccharide (17). Furthermore, PGC-1α expression was proportionally suppressed with the degree of renal impairment, and the reduced PGC-1α expression involved O2 consumption in response to TNF-α (41). PGC-1α reduces ROS accumulation and apoptosis with upregulation of the mitochondrial antioxidant defense system in vascular endothelial cells under basal and oxidative stress conditions (42). Additionally, suppression of PGC-1α would be a critical event that influences...
the duration of functional impairment (41). Indeed, in our study, the reduced PGC-1α expression improved on the 28th day after PAN treatment when the urinary protein excretion normalized (data not shown).

Proteinuria is considered to play an essential role in the progression of tubulointerstitial damage and end-stage renal disease (28, 30). PPAR-α has been considered a therapeutic target for FA toxicity associated with proteinuria (12), as in tubular injury after ischemia-reperfusion or toxic insults (20, 21, 26, 27). The present study revealed unchanged PPAR-α expression in the nephrotic kidney and suggested a novel mechanism of proteinuria-induced tubular damage with the disorder of FA metabolism in PTECs may play key roles in the control of FA toxicity in animals with constitutive PPAR-α expression and in humans with lower PPAR-α activity.

Although MCAD, CYP4A, PGC-1α, and ERRα were downregulated in the nephrotic kidney, functional roles of these molecules in kidney damage remain unclear. To the best of our knowledge, there is no report of the effective activator of PGC-1α in the kidney, whereas a β2-adrenergic receptor agonist induced PGC-1α-b and PGC-1α-c expressions in skeletal muscle (22). Our preliminary study showed that the PPAR-α ligand clofibrate prevented kidney damage in the PAN-SDR group with upregulation of MCAD and CYP4A expressions in the kidneys (data not shown). These results suggest that activation of PPAR-α functions and maintenance of FA β- and ω-oxidations could protect the kidney from albuminuria-induced damage. To clarify the roles of PGC-1α in kidney damage, future studies using genetically modified animals for PGC-1α (3, 22) will be required.

In conclusion, the present study demonstrated that albuminuria had the specific effect of inducing tubular damage, apoptosis, and lipid accumulation with downregulation of MCAD, CYP4A, PGC-1α, and ERRα in PTECs of PAN-induced nephrotic rats. The disorder of FA metabolism in PTECs may contribute the development of albuminuria-induced tubulointerstitial damage.

GRANTS
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DISCLOSURES
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AUTHOR CONTRIBUTIONS
Author contributions: Y.M., O.I., and K.T. conception and design of research; Y.M., R.R., D.I., P.C., Y.N., and K.J. performed experiments; Y.M. analyzed data; Y.M. and K.J. interpreted results of experiments; Y.M. prepared figures; Y.M. drafted manuscript; Y.M. and O.I. edited and revised manuscript; Y.M., O.I., and M.K. approved final version of manuscript.

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