Urinary proteins induce lysosomal membrane permeabilization and lysosomal dysfunction in renal tubular epithelial cells

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Running title: Urinary proteins impair lysosome in TECs

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ABSTRACT

Lysosomal membrane permeabilization (LMP) has been shown to cause the release of cathepsins and other hydrolases from the lysosomal lumen to the cytosol and initiate a cell death pathway. Whether proteinuria triggers LMP in renal tubular epithelial cells (TECs) to accelerate the progression of renal tubulointerstitial injury remains unclear.

In this study, we evaluated the TEC injury, as well as the changes of lysosomal number, volume, activity and membrane integrity after urinary protein overload in vivo and in vitro. Our results revealed that neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) levels were significantly increased in the urine of patients with minimal change nephrotic syndrome (MCNS) and the culture supernatant of HK-2 cells treated by urinary proteins extracted from MCNS patients. Urinary protein overload also induced apoptotic cell death in HK-2 cells. Importantly, we found that lysosomal volume and number were markedly increased in TECs of patients with MCNS and HK-2 cells overloaded with urinary proteins. However, lysosome function as assessed by proteolytic degradation of DQ-ovalbumin and cathepsin-B and cathepsin-L activities was decreased in HK-2 cells overloaded with urinary proteins. Furthermore, urinary protein overload led to a diffuse cytoplasmic immunostaining pattern of cathepsin-B and irregular immunostaining of lysosome associated membrane protein 1 (LAMP1), accompanying a reduction in intracellular acidic components which could be improved by pre-treatment with antioxidant. Taken together, our results indicate that overloading of urinary proteins caused LMP and lysosomal dysfunction at least partly via oxidative stress in TECs.
INTRODUCTION

Proteinuria, the most common manifestation in patients with glomerulopathy, is regarded as not only a marker of glomerular injury, but also a mediator that delivers renal injury from glomerulus to tubulointerstitium (15, 24). Accumulating evidence suggests that proteinuria is an important driver for the development of interstitial fibrosis and chronic renal failure in patients with glomerulopathy (8). One of the important mechanisms underlying proteinuria-induced injury is tubular epithelial cell (TEC) atrophy/apoptosis triggered by endoplasmic reticulum stress, oxidative stress and inflammation after activation of multiple intracellular signaling pathways (5, 26, 30).

The lysosome is known as a cytoplasmic membrane-enclosed organelle, which contains various hydrolytic enzymes and finishes the degradation process of cellular components and macromolecules (20). Several degradation pathways, including endocytosis, phagocytosis and autophagy, end up in lysosomes to be degraded (3). Normal degradation function is crucial to maintain cellular homeostasis and enable cell survival in physiological state. However, given its high content of hydrolytic enzymes, lysosomes are potentially harmful to the cell when damage occurs to the lysosomal membrane under pathological conditions, so called lysosomal membrane permeabilization (LMP) (4, 9). LMP might result in indiscriminate degradation of cellular organelles since lysosomes release hydrolytic enzymes into the cytoplasm. In addition, lysosomal destabilization has a close relationship with various types of cell death in association with oxidative stress and inflammation (11). In certain conditions,
the extent of lysosomal membrane damage is linked with different outcomes of cell injury. For example, partial lysosomal rupture induces apoptosis while massive lysosomal leakage leads to necrosis (4).

Lysosomal degradation and transport for re-use are important in dealing with reabsorbed urinary proteins (16, 23). However, whether LMP occurs in TECs and whether LMP leads to TEC apoptosis after urinary protein overload remain unclear. It has been implicated that in the case of glomerular injury, the increased filtration of urinary proteins may overload the lysosomal pathways and cause lysosomal rupture in tubular cells, leading to renal tubular injury (15, 25). This idea is attractive but lacks direct evidences. In this study, we assessed the morphological and functional changes of lysosomes after urinary protein overload in TEC in vivo and vitro.
MATERIALS AND METHODS

Patients. This study was approved by the Institutional Review Board of the Affiliated Hospital of Guangdong Medical College. Kidney specimens were obtained from biopsy-proven untreated minimal change nephrotic syndrome (MCNS, n =11), focal segmental glomerulosclerosis (FSGS, n =11) or membranous nephropathy (MN, n =11) patients. The patients with urinary protein excretion more than 3.5 g/24 h and age ranged from 16 to 40 were enrolled. The control kidney specimens (n = 4), were obtained from patients with hematuria only and biopsy-proven minimal change disease.

Cell culture and treatments. Human proximal tubular HK-2 cells (ATCC, Virginia, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) under standard conditions. HK-2 cells were exposed to 1, 2, 4 and 8 mg/ml urinary proteins for 0, 2, 4, 8 and 16 h before analysis. To suppress oxidative stress, HK-2 cells were pre-treated with 2000 U/ml catalase (Millipore, Billerica, MA, USA) or 1 mM N-acetyl cysteine (NAC, Sigma, St. Louis, MO, USA) before exposure to 8 mg/ml urinary proteins for 16 h. Urinary proteins were extracted from urine of patients with untreated, biopsy-proven and uncomplicated idiopathic MCNS using an ammonium sulfate precipitation method as described previously (22).

Immunofluorescence study. Immunostaining analysis for tissues or cells was conducted as described previously (14). Antibodies of rabbit anti-LAMP1, mouse anti-Cathepsin-D (Abcam, Cambridge, MA, USA) and mouse anti-Cathepsin-B
(Santa Cruz, CA, USA) were used in the staining. Immunoreactivity was visualized with Alexa Fluor® 488 donkey anti-rabbit IgG or Alexa Fluor® 594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA). Microscopic images were taken using a TCS SP5 II confocal microscope (Leica Microsystems, Wetzlar, Germany) or an Olympus BX43 microscope (Olympus, Tokyo, Japan). The average fluorescent intensity value was calculated from at least 50 LAMP1-positive cells for each test. LMP in HK-2 cells were graded as the percentage of cells displaying diffuse staining of Cathepsin-B immunostaining from 6 fields in each experiment.

Transmission electron microscopy. Kidney tissue specimens from patients were fixed, embedded and stained as described previously (14). Ultrathin sections were examined using a Philips CM100 electron microscope (Eindhoven, Netherlands) with a magnification of 8000×. Lysosomal diameters in TECs were measured in at least 10 fields.

Ovalbumin dequenching assay and Lyso-Tracker Red uptake test. After exposure to urinary proteins, HK-2 cells were incubated with 10 μg/ml of DQ-ovalbumin (Invitrogen) for 1 h or 50 nM Lyso-Tracker Red (Invitrogen) for 30 min at 37 °C. For ovalbumin dequenching assay, cells were washed with PBS and fixed in 4% paraformaldehyde. Green fluorescent dots of DQ-ovalbumin in individual HK-2 cells were counted and the average numbers of dots in at least 30 cells was presented in the figures. For Lyso-Tracker Red uptake test, cells were washed with PBS and fluorescent intensity was measured.

Flow cytometry analysis. After incubated with Lyso-Tracker Red dye, HK-2 cells
were trypsinized and re-suspended in PBS for FACS analysis of ‘pale’ cells with diminished and punctuated red fluorescence (BD, FACSCanto II, San Jose, CA, USA). Also, after loading with urinary proteins, cells were harvested and rinsed with PBS for apoptosis assay. Apoptosis was determined by the Annexin V-FITC Apoptosis Detection Kit (Dojindo, Kumamoto, Japan) following the manufacturer’s protocol.

Biochemical and enzymatic assays. The levels of neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) in patient’s urine and cell culture supernatant were measured with the Quantikine™ kits (R&D Systems, Minneapolis, Minnesota, USA). The activity of Cathepsin-B, Cathepsin-D or Cathepsin-L was measured with the fluorescence-based assay kit (BioVision).

Western blot analysis. HK-2 cells were homogenized and protein concentration in extracts was determined using BCA reagent. Equal amounts of proteins were loaded and separated by a 12 % SDS-PAGE gel and the samples were then transferred to a polyvinylidene difluoride membrane (Millipore). After blocking with 5% fat-free milk for 2 h, the blots were incubated overnight at 4℃ with mouse antibodies. After washing for 3 times, the membranes were probed with HRP-conjugated secondary antibodies (Beyotime Institute of Biotechnology, Jiangsu, China) for 1 hour at room temperature. The bands were detected using an enhanced chemiluminescence (ECATHEPSIN-L) solution and followed by exposure to X-ray film.

Statistical analysis. All statistical tests were performed with SPSS 16.0. All data are expressed as the means ± standard error of the mean (S.E.M.). Two group comparisons were carried out using the independent-sample t-test. Multiple group
comparison was carried out using ANOVA, followed by Bonferroni or Dunnett post-hoc tests. $P$ value was considered as statistically significant if it is less than 0.05.
RESULTS

Urinary proteins induced TEC injury. To explore the action of urinary protein overload in vivo, we first evaluated renal TEC lesion in patients with nephrotic syndrome. Considering that other mechanisms (e.g. leakage of the glomerular ultrafiltrate into the peritubular interstitial space, autoantibodies, and so on) in addition to urinary protein overload are involved in TEC lesion in FSGS or MN patients (10, 12), we predominantly focused on MCNS patients in this study. The clinical characteristics of MCNS and control patients are presented in Table 1. There was no statistically significant differences in age, serum creatinine, blood urea nitrogen or serum uric acid between the two groups. The 24-h urinary proteins, total cholesterol and triglyceride increased, whereas the plasma albumin decreased in MCNS patients compared to the controls. Morphologically, some TECs exhibited swelling, vacuolar and granular degeneration in patients with MCNS (Fig. 1A). Also, two renal tubular injury markers, urinary NGAL and KIM-1, were significantly increased in these patients compared to the control (Fig. 1B). In agreement with the study in vivo, we also found that exposure of HK-2 cells to urinary proteins for 16 h enhanced the NGAL and KIM-1 secretion in a dose-dependent manner. Also, exposure to 8 mg/ml urinary proteins significantly elevated NGAL and KIM-1 levels in the culture supernatant from 2 h to 16 h (Fig. 1C and 1D).

It is well known that FITC Annexin V can detect the exposure of phosphatidylserine on the outer leaflet of the plasma membrane, which occurs in the early stages of apoptosis, while only the membranes of late apoptotic cells are permeable to
propidium iodide (PI) (7). So early and late apoptosis were subsequently evaluated after coupled staining with FITC Annexin V and PI. When studying the relationship between urinary protein dose and cell injury, we found that both early (Annexin V+/PI⁻) and late (Annexin V+/PI⁺) apoptosis was enhanced at 8 mg/ml in HK-2 cells. Obvious early and late apoptosis was observed at the prolonged treatment time of 16 h (Fig. 2A). Also, urinary protein-induced apoptosis was confirmed by immunoblot of cleaved caspases-3 at 8 mg/ml and 16 h (Fig. 2B).

Urinary proteins increased the number and enlarged the volume of lysosomes in TECs.

To assess the impact of urinary proteins on lysosomes in vivo, we examined the changes of lysosomal number and volume in renal TECs of MCNS patients. The lysosomal number per cell was counted on TEM images. The number of lysosomes was significantly higher in renal proximal TECs of patients with MCNS than that in the controls. The lysosomes were also enlarged in these patients compared to the controls (Fig. 3A). This phenomenon was also seen in HK-2 cells following exposure to urinary proteins, of which exposure to 8 mg/ml urinary proteins significantly increased the lysosomal number and volume, as assessed by lysosome-specific fluorescence intensity and LAMP1-positive granules (Fig. 3B).

Urinary proteins suppressed the degradation of DQ-ovalbumin. To evaluate the efficiency of lysosome-mediated proteolytic degradation, a self-quenched substrate for proteases, DQ-ovalbumin, was utilized. Bright dots of DQ-ovalbumin were seen in HK-2 cells treated with the solvent as control. However, these dots were markedly reduced in HK-2 cells loaded with 8 mg/ml urinary proteins (Fig. 4). These results
suggested that degradability of lysosome was compromised in spite that the lysosomal volume was larger and the amount was higher after exposure to urinary proteins.

*Urinary proteins increased the protein level but decreased the enzymatic activity of cathepsins.* To investigate whether the suppressed proteolytic degradation was attributed to the variation of lysosomal enzymatic activity, we examined the activity and the protein level of lysosomal proteolytic enzymes with fluorescence-based enzymatic assays in HK-2 cells. Exposure to urinary proteins induced a significant decrease in Cathepsin-B and Cathepsin-L activities (Fig. 5A and 5B), but not in Cathepsin-D activity (Fig. 5C). Interestingly, the protein level of Cathepsin-B was increased while Cathepsin-D was not changed following exposure to urinary proteins compared to the control (Fig. 5D and 5E).

*Urinary proteins triggered defective lysosomal acidification and LMP in TECs.* Since the activity of enzymatic activity is closely related to the acid milieu of lysosomes (3), we next tested whether urinary proteins attenuated acidification of lysosomes in renal TECs. We used Lyso-Tracker Red to label the acidic intracellular compartments (lysosomes) in living cells. The punctuated red fluorescence (lysosomes) was clearly seen under control condition. However, exposure to urinary proteins abolished Lyso-Tracker Red labeling in a dose-dependent manner (Fig. 6A and 6C). In parallel, the number of ‘pale’ cells as examined by flow cytometry was also significantly increased after exposure to urinary proteins (Fig. 6B and 6D). These data suggest that acidification of lysosomes was attenuated, resulting from lysosomal destabilization, namely LMP (4). To test if LMP actually occurred, we examined if exposure to
urinary proteins elicited lysosomal rupture. In the control, Cathepsin-B immunoactivity displayed punctate pattern and co-localized to a large extent with LAMP1-positive dots in renal TECs. In contrast, in TECs from patients with MCNS, both Cathepsin-B and LAMP1 exerted as a diffuse immunostaining pattern, suggesting a release from the lysosome to the cytosol. Also, the patients with FSGS and MN, another two common pathologic types in the nephrotic syndrome, were studied in this experiment. Also, similar patterns of Cathepsin-B and LAMP1 staining were obtained in TECs of patients with heavy proteinuria (Fig. 7A). In HK-2 cells, Cathepsin-B release was also observed after exposure to 8 mg/ml urinary proteins extracted from MCNS patients, accompanied by larger and irregular LAMP1 immunostaining at 16 h (Fig. 7B and 7C).

Oxidative stress was involved in urinary protein-triggered LMP. We recently reported that some signaling pathways are activated by oxidative stress after exposure of TECs to urinary proteins (14). Thus, we subsequently tested whether oxidative stress was also an important mechanism underlying LMP. In our study, we found that the production of reactive oxygen species (ROS) was significantly enhanced after exposure of HK-2 cells to urinary proteins, which was suppressed by pre-treatment with antioxidant catalase or NAC (data not shown). Similar to the results shown in Fig. 6, exposure to urinary proteins abolished Lyso-Tracker Red labeling but elevated the proportion of ‘pale’ cells. However, pre-treatment with catalase or NAC markedly increased the mean fluorescence intensity of Lyso-Tracker Red (Fig. 8A and 8C) and reduced the proportion of ‘pale’ cells (Fig. 8B and 8D), indicating that oxidative stress
was involved in the lysosomal dysfunction and LMP induced by urinary proteins.
DISCUSSION

The aim of this study was to investigate the effect of urinary proteins on renal tubules with respect to TEC injury and lysosomal dysfunction. It is well known that, in addition to urinary proteins, tubulointerstitial injury has a relationship with some other factors, such as the clinical stages, pathological types of nephrotic syndrome, as well as the ages of patients. Therefore, to minimize the influence of these factors, untreated young patients with MCNS and normal serum creatinine were enrolled in this study. We found that exposure to urinary proteins induces renal proximal tubule injury and apoptotic death of TECs. It has been widely accepted that TEC apoptotic death, which could be triggered by LMP, is the driver for the development of fibrotic renal lesions, so it is important to explore whether urinary protein overload induces lysosome destabilization and LMP. In this study, our results revealed that cathepsin-B was accumulated in parallel with increased amount and volume of lysosomes both in vivo and in vitro following urinary protein overload. These data indicate an increased lysosomal mass in response to increased amounts of internalized proteins. However, it is not clear whether an increase in lysosomal content is sufficient to complete the clearance of overloaded proteins due to endocytosis. Notably, a previous study suggested that larger lysosomes were a result of cellular injury due to increased requirement for hydrolysis (6). In addition, other studies also demonstrated that larger lysosomes were more susceptible to breakage and that an increase in lysosomal volume was a common event in cell death induced by various factors (17, 18, 29). In our study, a significant increase in lysosome size and volume was observed in TECs
of proteinuria patients and HK-2 cells after urinary protein overload, indicating a potential damage of lysosomes.

Next, we investigated if the proteolytic power of the lysosomes changed following urinary protein overloading. Our data revealed that lysosomal proteolysis capacity of cathepsin-B and cathepsin-L but not cathepsin-D decreased as characterized by the DQ-ovalbumin assay. Most enzymes of lysosomes exert maximum activity at low pH, and the elevation of lysosomal pH could impair the activity of hydrolytic enzymes and elicit declined degradability of lysosomes (1, 3, 21). Also, our results showed the defective acidification of lysosomes after exposure to urinary proteins. These data suggest that the attenuated lysosomal milieu resulted in the reduction in enzymatic activity of cathepsin-B/L but not cathepsin-D that was reported to remain active at neutral pH (4). This is consistent with a previous report that after BSA overloading cathepsin-L was accumulated in parallel to a decreased capacity of lysosomal degradation (13). It indicates that the increased lysosomal contents might not be sufficient to maintain normal clearance of overloaded proteins.

The alteration of the intralysosomal pH and the release of lysosomal enzymes were used as an indicator of LMP (4, 9, 28). Therefore, the decreased labeling of Lyso-Tracker Red and redistribution of cathepsins from lysosomes to the cytosol might indicate a LMP after urinary protein overload in TECs. We have also shown that oxidative stress is one of the crucial factors to trigger LMP, which agrees with a previous study in neurons (27). Our recent study revealed that exposure to urinary proteins for 8 h activates autophagy at least partly via oxidative stress, which mounts
an adaptive response in TECs (14). It suggests that short-term treatment with urinary proteins (e.g. 8 h) does not trigger LMP, since the lysosome-mediated degradation system plays a key role in autophagy activation. With long-term exposure (e.g. 16 h), LMP occurs, and the reno-protective role of autophagy can not continue. It is well known that LMP has emerged as a trigger of cell death (3, 11). Therefore, the translocation of cathepsin-D to the cytoplasm observed in our study might play an important role in triggering apoptosis as reported previously (2, 19).

In summary, urinary protein overload results in TEC injury and apoptosis, accompanied by an increase in the amount and mass of lysosomes, a decrease in the lysosomal enzymatic activity and degradation ability. LMP triggered by oxidative stress is likely an important mechanism underlying TEC injury and apoptosis after urinary protein overload (Fig. 9). Stabilizing lysosomal membrane might be an intriguing strategy to alleviate renal tubular damage induced by urinary proteins.
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FIGURE LEGENDS

Fig. 1. TEC injury occurred in patients with MCNS. (A) Representative photomicrographs of Periodic Acid Schiff (PAS)-stained renal tubuli of patients with MCNS and controls. Scale bar, 50 μm. (B) The urinary NGAL and KIM-1 levels were measured by ELISA in patients with MCNS and controls. (C and D) The levels of KIM-1 and NGAL in culture supernatant were measured by ELISA after exposure to urinary proteins at different concentrations for 16 h or at 8 mg/ml for different times. **P < 0.01.

Fig. 2. Effect of urinary proteins on HK-2 cell apoptosis. (A) Early and late apoptosis of HK-2 cell was detected by Annexin V/PI flow cytometry after exposure to urinary proteins at different concentrations for 16 h or at 8 mg/ml for different times. (B) Western blot analysis of cleaved caspase-3 after exposure of HK-2 cells to urinary proteins at different concentrations for 16 h or at 8 mg/ml for different times. *P < 0.05.

Fig. 3. Effects of urinary proteins on lysosomal amount and volume. (A) Lysosomal numbers and diameters under transmission electron microscope (TEM) in TECs of patients with MCNS and controls. Scale bar, 2μm. (B) Immunofluorescence intensity of LAMP1 (green) after exposure to different concentrations of urinary proteins for 16 h. The nucleus was counter-stained by DAPI (blue). Scale bar, 10μm. *P < 0.05.

Fig. 4. Effect of urinary proteins on the degradation of DQ-Ovalbumin. (A) HK-2 cells were treated with different concentrations of urinary proteins or vehicle for 16 h
and then incubated with DQ-Ovalbumin (10μg/ml) for 1 h. (B) The degraded products presented as green puncta were quantified. Scale bar, 10μm. ***P < 0.001.

**Fig. 5. Effects of urinary proteins on enzymatic activity and protein level of cathepsin.** (A-C) Proteolytic activities of cathepsin-B (CB), cathepsin-L (CL) and cathepsin-D (CD) in HK-2 cells after exposure to urinary proteins at different concentrations for 16 h or at 8 mg/ml for different times. Urinary protein overload induced a significant decrease in proteolytic activity of CB and CL. (D and E) Western blot analysis of CB and CD protein level after exposure to different concentrations of urinary proteins for 16 h. The ratio of CB or CD to tubulin were expressed after quantifying the densitometry. The protein level of CB was increased at 16 h time point after exposure to 8 mg/ml urinary proteins. *P < 0.05 and **P < 0.01.

**Fig. 6. Effects of urinary proteins on Lyso-Tracker Red fluorescence and proportion of ‘pale’ cells.** (A and C) HK-2 cells were treated with urinary proteins at different concentrations for 16 h. After incubating with Lyso-Tracker Red for 1h, the intensity of cell fluorescence was analyzed. Scale bar, 10 μm. (B and D) Alternatively, the cells were trypsinized, centrifuged, and resuspended for flow cytometry analysis. Cells with decreased red fluorescence (‘pale’ cells) were gated, and their percentages were indicated. *P < 0.05 and **P < 0.01.

**Fig. 7. Effect of urinary proteins on the distribution of cathepsin-B and LAMP1.** (A) Immunofluorescent staining of LAMP1 and cathepsin-B (CB) in TECs of patients with MCNS, FSGS, MN or controls. Red immunofluorescence in lower panel illustrated the leakage of CB from lysosomes into the cytoplasm. Scale bar, 10 μm. (B)
Immunofluorescent staining of LAMP1 and CB in HK-2 cells after exposure to urinary proteins at different concentrations for 16 h. The percentage of cells displaying diffuse staining of CB was counted in at least 10 random fields. Scale bar, 10μm. ***P < 0.001.

**Fig. 8. Effects of antioxidant on Lyso-Tracker Red fluorescence and proportion of ‘pale’ cells in HK-2 cells overloaded by urinary proteins.** (A and C) HK-2 cells were pre-treated with vehicle, catalase (CAT, 2000 U/ml) or NAC (1 mM) before exposure to 8 mg/ml urinary proteins for 16 h. After incubating with Lyso-Tracker Red for 1h, the intensity of cell fluorescence was analyzed. Scale bar, 10 μm. (B and D) Alternatively, the cells were trypsinized, centrifuged, and resuspended for flow cytometry analysis. Cells with decreased red fluorescence (“pale” cells) were gated, and their percentages were indicated. *P < 0.05, **P < 0.01 and ***P < 0.001.

**Fig. 9. Schematic representation of lysosomal membrane permeabilization (LMP) and cell injury in urinary protein-overloaded TECs.** Urinary proteins trigger LMP and lysosomal dysfunction at least partly via oxidative stress. LMP is likely an important mechanism underlying TEC injury after urinary protein overload.
### Table 1. Clinical characteristics of the enrolled patients

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<th>Control</th>
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<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Male/female</td>
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<td>9/2</td>
<td>-</td>
</tr>
<tr>
<td>Age (year)</td>
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<td>23.3±3.2</td>
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<td>24-h urinary proteins (g)</td>
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<td>4.6±0.5</td>
<td>&lt;0.001</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
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<td>10.5±1.0</td>
<td>&lt;0.01</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
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<td>2.7±0.3</td>
<td>&lt;0.05</td>
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<tr>
<td>Plasma albumin (g/L)</td>
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<td>18.0±2.4</td>
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<td>Serum creatinine (μmol/L)</td>
<td>83.8±7.9</td>
<td>80.3±7.3</td>
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<td>Blood urea nitrogen (mmol/L)</td>
<td>5.6±0.5</td>
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<td>0.11</td>
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<td>Serum uric acid (μmol/L)</td>
<td>324.0±20.8</td>
<td>376.6±22.7</td>
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The data are expressed as counts or means ± S.E.M.
Urinary proteins → Oxidative stress → Lysosomal membrane permeabilization → TEC injury

- Lysosomal volume/amount
- Lysosomal enzymatic activity

- Lysosomal acidification
- Lysosomal degradation ability

Other mechanisms