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Urinary biomarkers track the progression of nephropathy in hypertensive and obese rats

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Abstract

Aims—To determine whether urinary biomarkers of acute kidney injury can be used to monitor the progression of chronic kidney injury in a rat model of hypertension and obesity.

Materials & methods—A suite of novel urinary biomarkers were used to track the progression of kidney damage in SHROB and SHR-lean rats.

Results—Urinary albumin, NAG, clusterin, osteopontin, RPA-1 and fibrinogen levels were significantly elevated over time and were closely associated with the severity of histopathologically determined nephropathy in both SHROB and SHR-lean rats.

Conclusion—Urinary biomarkers, such as albumin, fibrinogen, NAG, clusterin, RPA-1 and osteopontin, may serve as useful tools to track the progression of chronic kidney disease associated with hypertension and obesity.

Keywords

chronic kidney disease; hypertension; obesity; urine biomarker

Hypertension and obesity are highly prevalent chronic diseases throughout the world. Data from the National Health and Nutrition Examination Survey indicate that hyper tension affects approximately 30% of adults in the USA [1]. Furthermore, the prevalence of obesity

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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in the USA has increased in recent years; more than 33% of adults and 17% of youth were obese in 2009–2010 [2]. Hypertension and obesity have been identified as major risk factors for the development of chronic kidney disease (CKD). Independently, hypertension or obesity can increase the risk of renal dysfunction. For example, hypertension plays a role in the development of one quarter of the reported cases of renal dysfunction [3]. Overweight and obesity are associated with 24.2 and 33.9% of CKD among US men and women, respectively [4]. Hypertension and obesity are inter-related conditions that strongly predispose patients to CKD. When hypertension is combined with obesity, epidemiological studies have demonstrated that renal injury is more severe compared with renal damage induced by each risk factor alone [5,6]. From a public health perspective, early detection of kidney damage is key for preventing the progression of nephropathy in hypertensive and obese patients.

Kidney function is traditionally evaluated by blood and urine tests in preclinical and clinical studies, as well as in routine clinical care. Blood urea nitrogen (BUN) and serum creatinine (used to estimate glomerular filtration rate) are the gold standard tests that are typically conducted to assess kidney function. Other tests may include urinary volume, specific gravity, protein and fractional electrolytes; however, these routinely used indicators of renal function often do not identify renal injury until a significant degree of kidney function is lost [7]. This lack of sensitivity severely limits the ability to detect kidney disease at the earliest stages. Therefore, there is an urgent need to identify more sensitive, specific and reliable biomarkers for the early diagnosis of kidney injury in preclinical and clinical studies, and for routine patient monitoring. Consistent with this need, the US FDA Critical Path Initiative calls for the development of improved methods for a ‘better product safety toolkit’, including more sensitive and predictive biomarkers of toxicity [101]. As a result, seven urinary renal safety biomarkers (Kim-1, albumin, clusterin, TFF3, total protein, cystatin C and β_2-microglobulin) submitted by the Predictive Safety Testing Consortium’s Nephrotoxicity Working Group were approved by the FDA and the EMA for monitoring nephrotoxicity during preclinical testing of drugs [8]. Subsequently, the EMA/FDA working group approved for use an eighth urinary biomarker, RPA-1, to detect acute drug-induced nephrotoxicity for safety assessment studies [102]. In 2011, the FDA published a strategic plan for new or enhanced engagement in regulatory science, defined as the science of developing new tools, standards and approaches to assess the safety, efficacy, quality and performance of FDA-regulated products. One of the eight priority areas – modernizing toxicology to enhance product safety – highlights a need to identify and evaluate better biomarkers for monitoring toxicities, side effects and abnormalities in preclinical and clinical studies [103]. Therefore, efforts are ongoing to identify improved biomarkers of nephrotoxicity in order to improve the preclinical and clinical safety evaluation of drugs and medical device materials.

In recent years, considerable progress has been made in identifying more sensitive and specific biomarkers for early detection of kidney injury. Novel biomarkers have been developed to detect acute kidney injury (AKI) associated with nephrotoxic drug exposure, cardiopulmonary bypass and acute tubular necrosis [9–14]. These biomarkers include urinary Kim-1, NGAL, RPA-1, α-GST, GST-Yb1, cystatin C, clusterin, osteopontin and fibrinogen [9,13,15–20]. In our previous studies, we have demonstrated that a number of novel biomarkers (Kim-1, NGAL, RPA-1, α-GST, GST-Yb1) can detect AKI at earlier stages following exposure of rats to nephrotoxicants, such as gentamicin, mercury and chromium, than the traditionally used markers, BUN and serum creatinine [21]. However, it is unknown whether these biomarkers of AKI can be used to track the progression of CKD. Given their potential to detect AKI in the earliest stages, it is important to determine if these biomarkers can also be used to track the progression of nephropathy associated with chronic conditions such as hypertension and obesity. Therefore, the goal of this study was to

determine if a suite of recently identified biomarkers of AKI can be used to track the progression of nephropathy associated with CKD in an animal model of hypertension and obesity.

Materials & methods

Animals

All procedures requiring the use of animals were approved by the FDA Institutional Animal Care and Use Committee. Male SHROB/ KolGmiCrl-*Lepr*^{cp}/Crl rats, strain code 375, and SHR-lean rats, strain code 376, were purchased from Charles River Laboratories (MA, USA). Animals (ten per group) at 12 weeks of age, for acclimation, were housed individually in standard plastic cages and maintained on a 12-h light/ dark cycle at room temperature with free access to food and water. Rats were fed with commercial chow 5K20 containing 10% fat (LabDiet, MD, USA). Bodyweight was recorded at 3-week intervals. Rats were placed individually in plastic metabolism cages for 24-h urine collections at 3-week intervals. The volume of the urine was recorded and the urine samples were centrifuged at 3000×g for 10 min at 4°C. The supernatants were collected and stored at −80°C until analysis. For practical reasons, the study was divided into 2-week segments for each interim urine collection and blood pressure measurement. Therefore, half the animals in each group (five SHROB and five SHR-Lean) were evaluated within the first week of each interim assessment, and the remaining animals in each group were evaluated during the following week. Based on this design, at the end of the study, half of the SHROB and SHR-lean rats were euthanized under isofluorane anesthesia at week 38 and the other half at week 39. Blood was collected by cardiac puncture for biochemical analyses. Kidneys were removed immediately and sections were preserved for histological analyses.

Blood pressure measurement

Blood pressure was monitored at 18 and 21 weeks of age, and then at 6-week intervals from weeks 21 to 38 in both SHROB and SHR-lean rats. Conscious rats were placed in restrainers with a darkened nose cone to limit the animal's view and reduce the level of animal stress. Blood pressure was noninvasively measured by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, CT, USA). In each rat, at least five successful measurements were taken in order to obtain an average value in a given recording session.

Blood chemistry

Heparinized blood was analyzed for BUN, creatinine and glucose using an *i-STAT*® Blood Analysis System (Abbott Point of Care, NJ, USA). Plasma levels of cholesterol and triglyceride were measured using a Reflotron Clinical Chemistry Analyzer (Boehringer Mannheim, CT, USA).

Urinary biomarkers analysis for renal injury

Urine NAG was measured using the NAG assay kit (Bio-quant, CA, USA). Urine creatinine was determined using a creatinine colorimetric detection kit (Enzo Life Sciences, NY, USA). Fibrinogen was measured using a Luminex-based assay kit (Millipore, MA, USA). Urinary protein biomarkers of Kim-1, NGAL, albumin, osteopontin, α-GST, GST-Yb1, RPA-1 and clusterin were measured using the rat multiplex assay kits and SECTOR™ Imager 2400 electrochemiluminescence detection platform (Meso Scale Discovery, MD, USA).

Histopathology

Kidneys were fixed in 10% zinc-formalin (Anatech Ltd, MI, USA) for 48 h and then stored in 70% ethanol at room temperature prior to processing. Kidneys were routinely processed, embedded in paraffin, sectioned at approximately 5 μm, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy. The severity of chronic nephropathy was graded on a scale of 0–5 as follows: 0 = normal histology, 1 = <1%, 2 = 1–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100% of the renal cortex showing various combinations of tubulointerstital and glomerular changes, as described in Table 1.

Statistical analysis

Data analysis was conducted using Graphpad Prism 5.0 statistical software. For urinary protein biomarkers, one-way ANOVA followed by a Bonferroni *post hoc* test was used to compare differences between time points for each group of rats. For blood chemistries, a t-test was used to compare differences between SHROB and SHR-lean groups. Data were expressed as mean ± standard deviation (n = 9 SHROB rats; n = 10 SHR-lean rats). A p-value <0.05 was considered statistically significant.

Results

Animal morbidity

All animals were observed daily for general health as per the Institutional Animal Care and Use Committee study protocol. Animals that lost more than 20% bodyweight or became moribund were euthanized. During the study period, a single SHROB rat was removed at 36 weeks of age due to significant bodyweight loss and signs of morbidity. All other animals were clinically normal and completed the study.

Bodyweight & blood pressure

Bodyweight in both SHROB and SHR-lean rats was recorded at 3-week intervals from 18 to 38 weeks of age. As shown in Table 2, SHROB rats at the beginning of the study (18 weeks of age) weighed approximately 150 g more than SHR-lean rats. Moreover, SHROB rats had a moderately higher rate of bodyweight gain compared with the SHR-lean rats throughout the study. At the end of study, the average weight of rats in the SHROB and SHR-lean groups was 692 ± 72 g and 466 ± 18 g, respectively. SHROB rats had a moderately higher rate of bodyweight gain compared with the SHR-lean rats throughout the study; the average weight gain at successive 3-week intervals was 31, 50, 50, 30, 6, 0 and 7 g, respectively. In SHR-lean rats, the average weight gain at successive 3-week intervals was 10, 23, 16, 9, 10, 24 and 19 g, respectively. Blood pressure was measured at 18 and 21 weeks of age, and thereafter at 6-week intervals from 21 to 38 weeks of age in both SHROB and SHR-lean rats (Table 3). Compared with the normal WKY rats with mean systolic blood pressure of 135 ± 2 mmHg at week 18 [22], both groups of rats in our study developed higher systolic and diastolic blood pressure at the beginning of the study, and remained elevated at similar levels for the entire study period. No significance differences were observed in systolic and diastolic blood pressure between the two groups of animals.

Clinical biochemistry parameters

Blood levels of BUN and creatinine were measured at the end of the study to assess kidney function. As shown in Table 4, 3.6- and 4.6-fold higher levels of BUN and serum creatinine, respectively, were observed in SHROB rats compared with SHR-lean rats ($p < 0.05$), and were statistically significant. Serum levels of cholesterol and triglyceride were analyzed at the end of the study. As presented in Table 4, 2.8- and 2.5-fold higher levels of serum total cholesterol and triglyceride, respectively, were observed in SHROB rats compared with

SHR-lean rats ($p < 0.05$), and were statistically significant. The levels of blood glucose showed no significant difference between SHROB rats and SHR-lean rats.

Renal histopathological changes

Representative photomicrographs of SHROB and SHR-lean rat kidneys are presented in Figure 1. The incidence and severity of kidney lesions consistent with chronic nephropathy in SHROB and SHR-lean rats are shown in Table 5. At the end of the study, all kidneys examined in SHROB rats at necropsy appeared to be enlarged with a mottled surface. By contrast, kidneys from all SHR-lean rats revealed a normal morphology with a smooth surface.

Histopathology results showed that SHROB rats had an increased severity of kidney damage compared with SHR-lean rats. The average score of chronic nephropathy lesions in SHROB rats (4.9) was higher than that for SHR-lean rats (3.0) (Table 5). Figure 1A & B demonstrate chronic nephropathy in SHROB rats characterized by numerous dilated tubules that are empty or contain proteinaceous casts, numerous atretic tubules, interstitial fibrosis and infiltrates of lymphocytes. Furthermore, severe glomerular damage was observed in SHROB rats, characterized by basement membrane thickening around Bowman's capsule, enlarged glomeruli with sclerosis and adhesions to Bowman's capsule. In Figure 1C & D, there are similar glomerular, tubular and interstitial changes present in SHR-lean rats, but the severity of lesions is decreased and has a multifocal rather than a diffuse pattern compared with that observed in the SHROB rats.

Analysis of urinary biomarkers

Meso Scale Discovery's multiple array technology enables the detection of urinary biomarkers in multiplex formats using electrochemiluminescence detection [23]. In this study, we used this multiplex immunoassay system to analyze a number of urinary biomarkers (albumin, Kim-1, NGAL, osteopontin, fibrinogen, α-GST, GST-Yb1, RPA-1 and clusterin) (Figure 2A–J). Absolute values for the basal levels of urinary biomarkers at 18 weeks of age are listed in Table 6. Overall, urinary biomarker levels demonstrated differences between SHROB and SHR-lean rats for the entire study duration. Higher biomarker levels were observed in SHROB rats compared with SHR-lean rats at the beginning of the study (week 18) as described above, although the differences were not always statistically significant. To track the progression of chronic nephropathy, we compared levels of urinary biomarkers over time to those measured at the beginning of study (18 weeks of age) in each group of rats. The results show that urinary albumin was the earliest urinary biomarker elevated, with increases seen at week 30 compared with week 18, and these levels remained elevated through week 36 in SHROB rats. However, an increase in albumin was not observed until week 36 in SHR-lean rats. Urinary NAG, clusterin, osteopontin and fibrinogen levels were elevated either at week 33 or 36 in SHROB rats only, but not elevated at any time point in SHR-lean rats, compared with levels measured at week 18. Interestingly, RPA-1 levels were increased at week 24 and remained elevated through week 36 in SHR-lean rats, but no time-dependent changes were observed in SHROB rats. There were no increases observed at any point in the study in urinary levels of Kim-1, α-GST, GST-Yb1 or NGAL for either strain of rat. For all urinary biomarkers analyzed, large standard deviations were observed in both groups of rats. In this chronic disease animal model of hypertension and obesity, many factors (e.g., high-fat diet and stress) could affect the animal responses, including blood pressure and progression of nephropathy. The large variation around the data means might result from higher than typical variability in individual animal responses.

Discussion

It is becoming increasingly recognized that tests routinely used to evaluate renal dysfunction, notably BUN and serum creatinine, have poor sensitivity for the detection of renal disease since these biochemical indicators are often elevated only after a significant amount of kidney function is lost. Reliance on these biomarkers can result in delayed diagnosis of renal disease. As a result, there is an urgent need to identify and develop novel biomarkers to diagnose renal injury at the earliest stages. Recently, a number of candidate markers have been widely investigated to detect AKI at the earliest stages in patients and experimental animals. However, it is not clear if these new biomarkers of AKI can be used to track the progression of CKD that occurs in certain disease states. Therefore, the goal of the current study was to determine if a suite of novel AKI biomarkers have the ability to track the progression of CKD in an animal model of hypertension and obesity.

Epidemiological studies have shown that obesity combined with hypertension results in an increased risk for the development of CKD in patients compared with when only one of the conditions is present [3]. In this study, we used genetic animal models of hypertension and obesity to evaluate the value of a suite of novel biomarkers for tracking the progression of nephropathy in chronic disease. The SHROB rat is a unique animal model with genetic obesity superimposed on a background of genetic hypertension [24]. The obese phenotype results from a mutation in the leptin receptor gene [25], which normally regulates appetite. The SHROB strain rats are not only hypertensive, similar to the SHR-lean rats, but also show additional characteristics including obesity, hypertriglyceridemia, hypercholesterolemia, insulin resistance and metabolic syndrome [24,26,27]. In our study, the blood chemistry data confirmed the clinical characteristics described above. For example, rats developed hyperlipidemia with higher levels of plasma cholesterol and triglyceride in the SHROB group. Glucose levels in SHROB rats were lower than those of SHR-lean rats, and this may be associated with hyperinsulinism. We observed that both SHROB and SHR-lean rats had higher blood pressure through the study, compared with historical values in normotensive Wistar rats [104], with no significant difference observed between the two groups. Spontaneous hypertension usually occurs at approximately 12–13 weeks of age in SHROB rats [27]. In SHR-lean rats, systolic blood pressure measured using telemetry monitoring increased until 15 weeks of age, and the pressure then plateaued during 16–28 weeks of age [104]. In the current study, we started with 18-week-old rats, which may explain the lack of change in systolic and diastolic blood pressure throughout the entire study. Some studies have demonstrated that both SHROB and SHR-lean animals develop accelerated kidney damage that is similar to chronic nephropathy, as seen in patients with CKD [24,26–28]. Therefore, these animals can serve as valuable models for the study of CKD associated with hypertension, hyperlipidemia, hyperinsulinemia and obesity. Male rats were chosen for evaluation in this study for the following reasons. First, based on an SHR rat growth chart and technical data obtained from Charles River Laboratories, male rats gain more weight compared with female rats from 7 weeks until 15 weeks. Second, historical data showed that higher elevation of blood pressure with increasing age is observed in male compared with female SHR-lean rats using direct and indirect blood pressure measurements [104]. Therefore, in order to maximize the progression of nephropathy over the time course used in our study, we chose to use male rats because male rats would be expected to gain more weight and develop more severe high blood pressure, two critical risk factors for nephropathy.

A number of studies have demonstrated how a suite of novel urinary biomarkers can be used to detect AKI. For example, Kim-1, α-GST, GST-Yb1, RPA-1, NAG, clusterin, fibrinogen and osteopontin have been shown to detect early AKI following exposure to nephrotoxic chemicals and drugs, and following a period of kidney ischemia and reperfusion. Notably,

these responses were anchored to and correlated with histopathologic evidence of AKI [8,12,16,21]. However, there are limited reports on the value of these biomarkers for tracking the progression of nephropathy in CKD. For example, urinary NGAL has been reported to be an independent renal predictor of CKD, which is correlated significantly with serum creatinine, glomerular filtration rate and proteinuria in patients with CKD [29]. A prospective cohort study demonstrated that combining urinary cystatin C, creatinine and albumin:creatinine ratio could improve the accuracy to predict CKD progression [30]. In the present study, we evaluated the ability of a number of urinary biomarkers to track the development and progression of nephropathy in rat models of CKD. Among the biomarkers evaluated in our study, urinary albumin was the earliest elevated biomarker. A significant increase was observed at week 30 in SHROB rats; however, an increase in albumin did not occur until week 36 in SHR-lean rats. Large amounts of albumin excreted into urine might reflect changes in glomerular permeability, which occurs earlier in SHROB rats compared with SHR-lean rats [31,32]. The renal histopathologic changes, including numerous dilated tubules containing proteinaceous casts, were correlated with large amounts of urinary albumin levels in SHROB rats. Several other urinary biomarkers, such as NAG, clusterin, fibrinogen and osteopontin, were increased at week 33 or 36 in SHROB rats, compared with week 18, but not in SHR-lean rats. Elevations of these biomarkers may be related to the development of severe chronic nephropathy in SHROB rats consistent with the histopathological changes (e.g., glomerular, tubular and interstitial changes). As shown in Table 5, SHROB rats showed a higher overall chronic nephropathy score (4.9) than SHR-lean rats (3.0). Significantly higher levels of BUN and serum creatinine in SHROB rats at the end of the study also provided evidence for higher severity of kidney damage compared with SHR-lean rats. By contrast, several promising candidate biomarkers for proximal and distal tubular injury, such as Kim-1, α-GST and GST-Yb1, exhibited no changes in both SHROB and SHR-lean rats through the entire study period. In addition, there was no increase in NGAL levels observed from 18 to 36 weeks of age in both rats. These results may be explained by the relatively more severe glomerular damage compared with tubular injury in SHROB rats. The histopathological analysis showed severe glomerular damage with basement membrane thickening around Bowman's capsule and enlarged glomeruli with sclerosis in SHROB rats. Such changes in the glomerular basement membrane could significantly alter the glomerular filtration function. Therefore, several biomarkers that are filtered into urine (e.g., albumin, NAG, clusterin, osteopontin and fibrinogen) were significantly elevated over time with the progression of disease. By contrast, there was less histopathologic damage in proximal and distal tubules, which might explain why some proximal (e.g., Kim-1, α-GST and NGAL) and distal tubular injury biomarkers (e.g., GST-Yb1 and NGAL) were not elevated. Interestingly, we also observed that urinary biomarker levels were different between SHROB and SHR-lean rats for the entire study duration; higher levels of biomarkers were observed in SHROB rats compared with SHR-lean rats. It is not clear why SHROB rats showed elevations of urinary biomarkers throughout the study period. One possible explanation is that urinary biomarkers may start to increase prior to week 18 in SHROB rats compared with SHR-lean rats, although significant increases were observed until 30 weeks of age when compared to rats at 18 weeks of age within each group. Another explanation is that differences in responses to kidney damage might be related to strain-specific parameters, such as the genetic differences between these two strains of animals.

Conclusion

In summary, a suite of novel urinary biomarkers of AKI was examined in this study to determine their ability to track the progression of CKD in an animal model of hypertension and obesity. The results demonstrated that urinary biomarkers such as albumin, NAG, clusterin, RPA-1, fibrinogen and osteopontin are significantly elevated over time with the

progression of nephropathy. The data suggested that these biomarkers may serve as useful tools to track the progression of CKD associated with hypertension and obesity. The development of sensitive and specific urinary biomarkers would be helpful for diagnosing early kidney injury, monitoring disease progression and establishing effective therapies in patients. From a regulatory perspective, the results of this study establish a baseline of renal injury that may be useful for evaluating the pre- and post-market safety of FDA-regulated products (drugs or devices) for patients with risk factors such as hypertension and obesity.

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Executive summary

Background

- Development of more sensitive, specific and reliable urinary biomarkers is needed for early diagnosis of kidney injury in preclinical and clinical studies, and in routine patient monitoring.
- Early detection of kidney damage is key for preventing the progression of nephropathy in chronic kidney disease patients, including those with the risk factors of obesity and hypertension.

Results

- We have demonstrated that several urinary biomarkers of acute kidney injury are significantly elevated over time in a rat model of hypertension and obesity, and these levels correlate well with the severity of nephropathy.

Conclusion

- As a result, these biomarkers show early promise as useful tools to track the progression of chronic kidney disease.

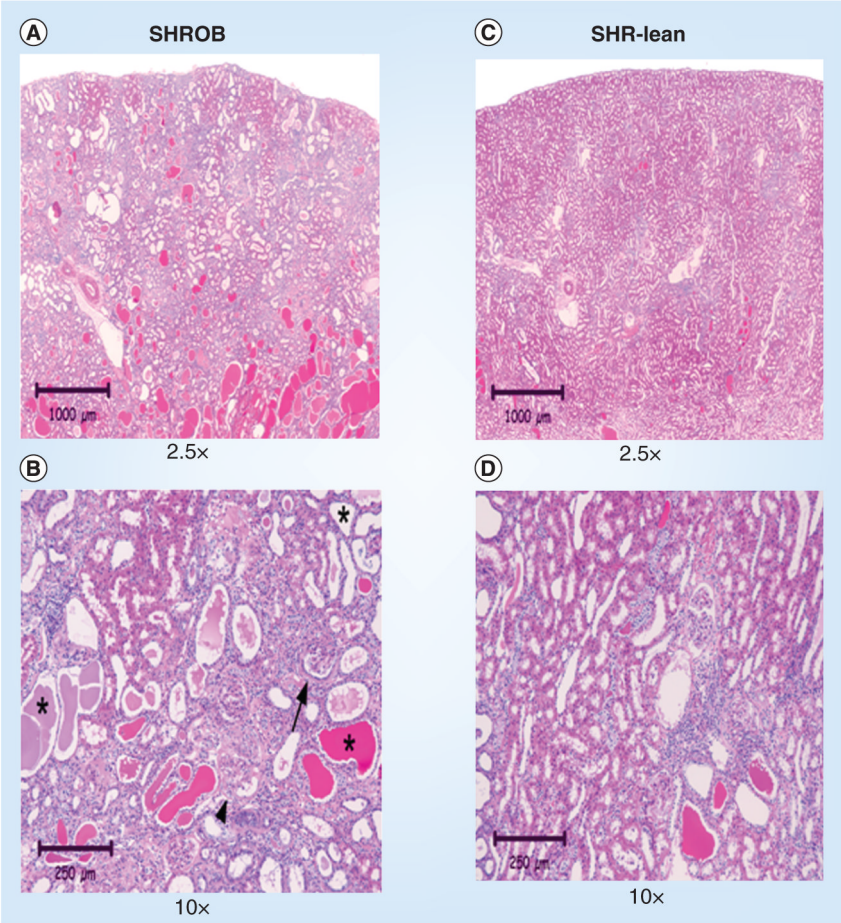
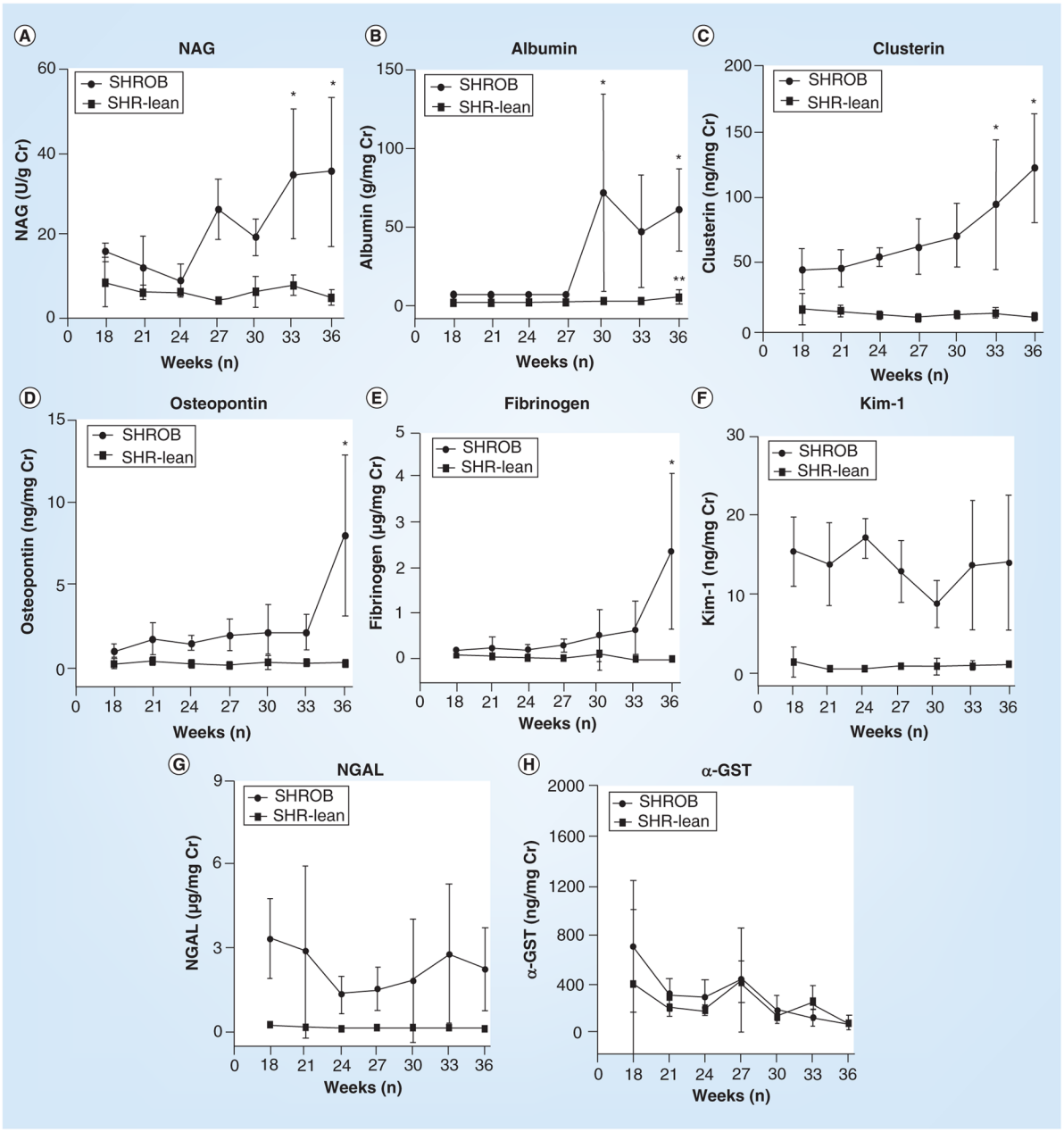


Figure 1. Representative photomicrographs of chronic nephropathy in SHROB and SHR-lean rats at the end of study (week 39)

(A) (×2.5) and **(B)** (×10): photomicrographs of hematoxylin and eosin-stained kidneys of SHROB rats; **(C)** (×2.5) and **(D)** (×10): photomicrographs of SHR-lean rats. Arrow indicates basement membrane thickening around Bowman's capsule. Arrowhead indicates enlarged glomerulus with sclerosis. Asterisks indicate dilated tubules that are empty or contain pale to brightly eosinophilic protein casts.



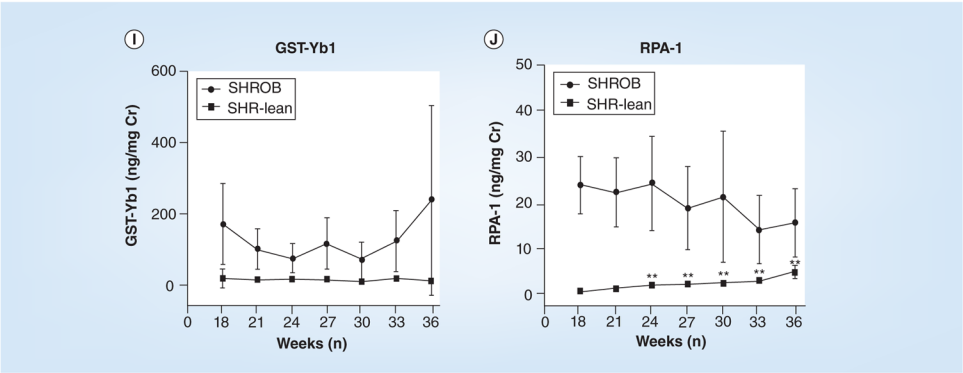


Figure 2. Expression of urinary biomarkers in SHROB and SHR-lean rats from 18 to 36 weeks of age

(A) NAG, **(B)** albumin, **(C)** clusterin, **(D)** osteopontin, **(E)** fibrinogen, **(F)** Kim-1, **(G)** NGAL, **(H)** α-GST, **(I)** GST-Yb1 and **(J)** RPA-1.

All the above urinary biomarkers are normalized by urinary Cr concentration.

Data are expressed as mean ± standard deviation for each group (n = 9 SHROB rats; n = 10 SHR-lean rats).

*Statistically significant difference ($p < 0.05$) compared with rats at 18 weeks of age in SHROB group.

**Statistically significant difference ($p < 0.05$) compared with rats at 18 weeks of age in SHR-lean group.

Cr: Creatinine.

Table 1

Summary of observed kidney histopathology changes.

Nephron segment	Histopathological lesions
Glomerulus	Thickened glomerular basement membranes, glomerular hypertrophy or atrophy, thickened Bowman's capsule, glomerular mesangial proliferation, increased glomerular mesangial matrix, glomerular adhesions, increased size and number of parietal cells, segmental or global glomerular sclerosis, small sclerotic glomerular tufts
Tubule	Necrosis/loss, degeneration, regeneration, dilatation, atrophy and protein casts, epithelial cell pigmentation, thickening of tubular basement membranes
Interstitial	Lymphocytic infiltration, interstitial fibrosis

The severity of chronic nephropathy was graded on a scale as described in the 'Materials & methods' section. The renal cortex was scored based on the various combinations of glomerular changes and tubulointerstital changes described in this table.

Table 2

Summary of bodyweight changes in SHROB and SHR-lean rats.

Rat	Age (weeks)							
	18	***21***	***24***	***27***	***30***	***33***	***36***	***38***
SHROB	516 ± 21 g	547 ± 25 g*	597 ± 33 g*	647 ± 39 g*	679 ± 32 g*	685 ± 29 g*	685 ± 70 g*	692 ± 72 g*
SHR-lean	373 ± 10 g	383 ± 14 g*	406 ± 13 g*	422 ± 14 g*	431 ± 15 g*	441 ± 16 g*	465 ± 17 g*	466 ± 18 g*

Data are expressed as mean ± standard deviation for nine SHROB and ten SHR-lean rats. Bodyweight was obtained in each rat from 18 to 38 weeks of age.

* $p < 0.05$ indicates the statistically significant difference compared with rats at 18 weeks of age in the SHROB and SHR-lean groups, respectively.

Table 3

Summary of blood pressure changes in SHROB and SHR-lean rats.

Rat	Blood pressure (mmHg)	Age (weeks)				
		18	***21***	***27***	***33***	***38***
SHROB	Systolic	209 ± 3.7	218 ± 7.0	226 ± 5.5	235 ± 4.3	228 ± 6.3
	Diastolic	158 ± 7.5	166 ± 10.5	179 ± 9.6	190 ± 10	183 ± 14
SHR-lean	Systolic	227 ± 6.1	225 ± 4.9	225 ± 5.2	223 ± 4.7	226 ± 3.2
	Diastolic	175 ± 9.4	178 ± 9.5	180 ± 9.4	166 ± 7.4	180 ± 4.4

Data are expressed as mean ± standard deviation for nine SHROB and ten SHR-lean rats. Blood pressure was obtained in each rat from 18 to 38 weeks of age by a tail-cuff monitoring method. There are no statistically significant differences in systolic and diastolic pressures for each group of rats from 18 to 38 weeks, or between the two groups.

Table 4

Changes in plasma levels of blood urea nitrogen, creatinine, glucose, cholesterol and triglyceride at the end of study (age 38 weeks) in SHROB and SHR-lean rats.

Rat	BUN (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)
SHROB	73.9 ± 35*	1.48 ± 1.14*	671 ± 65*	1218 ± 435*	150 ± 23
SHR-lean	20.2 ± 1.8	0.32 ± 0.11	240 ± 29	484 ± 182	181 ± 26

Data are expressed as mean ± standard deviation for nine SHROB and ten SHR-lean rats.

* $p < 0.05$ indicates the significant difference in SHROB rats compared with SHR-lean rats.

BUN: Blood urea nitrogen.

Table 5

Incidence and severity of chronic nephropathy lesions based on histopathology evaluation at conclusion of the study (38–39 weeks of age).

Data	SHROB	SHR-lean
Rats examined (n)	9	10
Rats with chronic nephropathy (n)	9	10
Individual rat severity scores†	5, 5, 4, 5, 5, 5, 5, 5, 5	3, 3, 3, 3, 3, 3, 3, 3, 3, 3
Average severity score	4.9	3.0

†Chronic nephropathy grading score: 0 = normal histology, 1 = <1%, 2 = 1–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100% of the renal cortex showing tubular, interstitial and glomerular changes. One SHROB rat was removed early (week 36 of age) due to significant bodyweight loss and signs of morbidity.

Table 6

Summary of the basal levels of urinary biomarkers at 18 weeks of age.

Biomarker	SHROB	SHR-lean
NAG (U/g Cr)	16.1 ± 2.3	8.6 ± 6.0
Albumin (g/mg Cr)	5.53 ± 2.3	0.52 ± 0.97
Clusterin (ng/mg Cr)	44.3 ± 15.6	13.9 ± 12
Osteopontin (ng/mg Cr)	1.13 ± 0.4	0.39 ± 0.26
Fibrinogen (ng/mg Cr)	186 ± 64	126.8 ± 57.1
Kim-1 (ng/mg Cr)	15.7 ± 4.4	1.9 ± 1.9
NGAL (μg/mg Cr)	3.38 ± 1.44	0.31 ± 0.07
α-GST (ng/mg Cr)	716 ± 539	408 ± 602
GST-Yb1 (ng/mg Cr)	176 ± 113	25.1 ± 26.2
RPA-1 (μg/mg Cr)	24.2 ± 6.2	1.2 ± 0.4

Cr: Creatinine.