Long-term renal changes in the Goto-Kakizaki rat, a model of lean type 2 diabetes

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ABSTRACT

Background. Type 2 diabetes has become the single most frequent cause of end-stage renal disease. The Goto-Kakizaki rat is currently used as a model for lean type 2 diabetes, but its renal changes have not been fully characterized. We investigated long-term functional and structural renal changes in the Goto-Kakizaki rat to evaluate if this animal model resembles the changes observed in human diabetic kidney disease.

Methods. Urinary albumin excretion, creatinine clearance and blood pressure were measured at the age of 2, 8 and 14 months in 12 female Goto-Kakizaki rats and 10 female, non-diabetic Wistar rats. To study kidney morphology, kidney weight, glomerular volume, basement membrane thickness, mesangial fraction and total mesangial volume were determined at 14 months.

Results. Urinary albumin excretion rose progressively over time in both groups, but was significantly higher in Goto-Kakizaki rats than in Wistar rats. Creatinine clearance decreased over time in Goto-Kakizaki rats but not in Wistar rats. Blood pressure was in the normotensive range in all animals throughout the study. Kidney weight, glomerular volume, basement membrane thickness, mesangial fraction and total mesangial volume were significantly higher in Goto-Kakizaki rats than in Wistar rats. Body weight and blood glucose levels were higher whereas serum insulin levels were non-different or lower in Goto-Kakizaki rats compared to Wistar rats.

Conclusion. The Goto-Kakizaki rat is a lean, hyperglycaemic, euinsulinaemic, normotensive experimental model of type 2 diabetes with robust functional and structural renal changes.

KEY WORDS

Albuminuria, basement membrane thickness, glomerular volume, Goto-Kakizaki rat, kidney, mesangium

ADDITIONAL KEY WORDS

Creatinine clearance, fructosamine, glucose tolerance test, insulin, 8-isoprostane, type 2 diabetes mellitus

RUNNING TITLE

Long-term renal changes in the Goto-Kakizaki rat

INTRODUCTION

The prevalence of type 2 diabetes is increasing worldwide. Type 2 diabetes has become the single most frequent cause of end-stage renal disease in western countries. Nevertheless, the pathophysiology of diabetic nephropathy has mainly been studied in experimental models of type 1 diabetes. In view of the reported differences in the pathophysiology of nephropathy between type 1 and type 2 diabetes [1], it is important to develop good experimental models for the study of nephropathy in type 2 diabetes.

The Goto-Kakizaki rat has been presented as a model for type 2 diabetes with hyperglycaemia in the absence of obesity or hypertension. The Goto-Kakizaki rat has been bred from non-diabetic Wistar rats selected from a normal population with a glucose tolerance test slightly deviating from the normal range [2]. Although this animal model has been widely used for metabolic studies, the renal changes have not yet been fully characterized. The aim of this study was to describe the long-term renal morphological and some functional changes in the Goto-Kakizaki rat, in order to evaluate its suitability as a model for renal disease in human type 2 diabetes.

ANIMALS AND METHODS

Experimental design

Twelve female Goto-Kakizaki rats and 10 female non-diabetic Wistar rats (M&B, Li. Skensved, Eiby, Denmark) of 2 months old with body weights approximately 200 g were studied. Animals were housed 2 per cage in a room with a 12:12 hour artificial light cycle, a temperature of $21 \pm 1°C$, and a humidity of 55 ± 5 %. The animals had free access to standard chow (Altromin #1324; Lage, Germany) and tap water throughout the experiment. The study complied with Danish regulations for care and use of laboratory animals.

At 2, 8 and 14 months, body weight (BW) and food consumption (FC) were measured in all rats. Blood glucose was determined on tail-vein blood. Under light ether anaesthesia, blood samples were drawn from the retro-orbital venous plexus using heparinized capillary tubes. After centrifugation, serum samples were stored at -80°C for later determination of serum insulin, fructosamine and 8-isoprostane levels. An intraperitoneal glucose tolerance test (IPGTT) was performed in 6 rats of each group. The animals were placed in metabolic cages to collect 24-hour urine samples, which were stored at -80°C for later analysis of urinary albumin excretion (UAE).

As previously described, systolic blood pressure (BP) was recorded in the early afternoon by the tail-cuff method on awake rats after they were accustomed to rest quietly in warmed plexi-glass restrainers [3]. For each animal the blood pressure level was determined as the mean value of 10 consecutive measurements. At 14 months, the rats were anaesthetized with pentobarbital (50 mg/kg intraperitoneally), and non-fasting blood samples were taken from the retro-orbital venous plexus.

Right and left kidney were quickly removed, carefully cleaned and weighed. The middle piece of the right kidney (including the papilla) was fixed in a 0.1 M cacodylatebuffer with 2 % paraformaldehyde and 1 % glutaraldehyde, for electron microscopic determination of basement

membrane thickness (BMT) and mesangial fraction, and the middle piece of the left kidney (including the papilla) was fixed in 4 % paraformaldehyde for light microscopic measurement of glomerular volume. Liver and heart were removed and weighed. In each group, one animal was excluded from the study due to unexplained massive body weight loss.

Metabolic measurements

Blood and urinary glucose concentration

Blood glucose was measured in tail-vein blood by Haemo-Glucotest 1-44 (Roche Diagnostics Scandinavia AB, Copenhagen, Denmark) and Reflolux II reflectance meter (Boehringer-Mannheim, Mannheim, Germany), and urine was tested for ketone bodies by Neostix-4 (Ames, Stoke Poges, Slough, UK).

IPGTT

An IPGTT was performed by intraperitoneal injection of a 20% glucose-solution in a dose of 2 g/kg BW. Animals were tested at 1.00 p.m. after a 6 hours fast. Blood glucose was measured by Haemo-Glucotest 1-44 in tail-vein blood prior to, and 30, 60, 90, 120 and 150 minutes after the glucose administration.

Serum insulin levels

Serum insulin levels were determined using an ultrasensitive Rat Insulin ELISA Kit (DRG Diagnostics, Marburg, Germany). The intra- and inter-assay coefficients of variance were <5 % and <10 %, respectively.

Serum fructosamine levels

The fructosamine assay (Fructosamine Test Plus, Hoffman-La Roche, Basle, Switzerland) was performed as previously described [4]. The principle of the assay relies on the reducing potential of ketoamines in alkaline medium. The serum fructosamine assay measures all

glycated serum proteins by forming the corresponding eneaminols, which in turn reduce nitroblue tetrazolium to the colored formazan derivative. The rate of formazane color development correlates with the fructosamine level. The intra- and inter-assay coefficients of variance were <5% and <10%, respectively.

Serum 8-isoprostane (8-epi PGF$_{2\alpha}$) levels

Total serum 8-*epi* PGF$_{2\alpha}$ levels were determined using a 8-Isoprostane EIA Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. For sample purification by a solid phase extraction method, a 250 µl serum sample was diluted with 500 µl ethanol. After centrifugation, and incubation of the supernatant with 15% KOH, the sample was diluted with Ultra Pure H$_2$O to a total volume of 2.5 ml. In the final step of the purification protocol, 8-*epi* PGF$_{2\alpha}$ was eluated with 1.25 ml ethyl acetate with 1% methanol. All samples were run in the same assay. The intra-assay coefficient of variance was below 5%.

Renal measurements

UAE and creatinine clearance (C$_{Cr}$).

As previously described, urinary albumin concentration in 24-hour urine collections was determined by an in house rat albumin radioimmunoassay, using rabbit anti-rat albumin antibody RARa/Alb (Nordic Pharmaceuticals and Diagnostics, Tilburg, The Netherlands), and globulin-free rat albumin for standard and iodination (Sigma Chemical Co., St. Louis, Missouri, USA) [5]. Serum and urinary creatinine concentrations were measured by an automated technique adapted from the method of Jaffé and corrected for the prevailing glucose contents interfering in the Jaffé reaction. The C$_{Cr}$ was expressed in ml/min. The intra- and inter-assay coefficients of variance were <5 % and <10 %, respectively, for both assays.

Estimation of glomerular volume.

The middle part of the left kidney (containing the papilla) was embedded in paraffin for light microscopic examination. Two micron-thick sections were cut on a rotation microtome (Leica Rotation Microtome RM 2165, Leica, Vienna, Austria) and stained with periodic acid schiff and haematoxylin. The thickness of the sections was controlled routinely by a Digital Microcator ND 221 (Heidenhain, Traunreut, Germany) attached to the microscope. In each animal, the mean glomerular tuft volume (V_G) was determined from the mean glomerular cross-sectional area (A_G) in an Olympos light microscope (BX51TF, Tokyo, Japan) at a magnification of 420x as previously described [6;7]. The areas were determined with a 2D-version of the nucleator (CAST, Olympus, Denmark) by light microscopy as the average area of a total of 80 to 100 glomerular profiles (that is, capillary tuft omitting the proximal tubular tissue and the Bowmann capsule) [6]. V_G was calculated as: $V_G = \boldsymbol{b}/k \times (A_G)^{3/2}$ where $\beta = 1.38$, which is the shape coefficient for spheres (the idealised shape of glomeruli), and $k = 1.1$, which is a size distribution coefficient [7].

Estimation of mesangial fraction, total mesangial volume and basement membrane thickness (BMT).

For electron microscopy, small blocks (2x2x4mm) were cut perpendicular to the cortex and embedded in Epon, cut on a Ultramicrotome (Reichert Ultracut S, Leica, Vienna, Austria) and stained with uranyle acetate and lead citrate. In each animal 2 blocks were cut for electron microscopy and 3 glomeruli were examined. Images were recorded with a video camera (Proscan, Münster, Germany) mounted on a Tecnai 12 electron microscope (Phillips, Enthoven, Holland). The mesangial fraction was determined by point counting the image on a monitor at a final magnification of 4,400x as previously described [8]. The total mesangial volume was calculated by multiplying the mesangial fraction by the total glomerular volume. For

determination of BMT, images at a final magnification of 42,000x were used. The BMT was measured by AnalySIS (Proscan, Münster, Germany) applying orthogonal intercept as previously described and the BMT given as a harmonic mean [9].

Statistical analysis

All results are given as mean values ± SEM with *N* indicating the number of rats studied. Differences between groups were analyzed by one-way ANOVA in combination with the unpaired or paired Student's *t*-test when appropriate. When data did not follow a normal distribution or failed equal variance test, the Mann-Whitney Rank Sum test was used to analyse differences between groups. Statistics were performed using GraphPad Prism version 3.00 for Windows 95 (GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS

Body weight and food consumption

At the age of 2 months, Goto-Kakizaki rats had a significant higher body weight (17 %) than Wistar rats *(Table 1)*. At 8 months and 14 months, this difference in body weight was only 11 and 10 %, respectively. Food consumption was not different between the two groups except at the end of the study period *(Table 1)*.

Parameters of metabolic control

Non-fasted and fasted blood glucose levels were significantly higher in Goto-Kakizaki rats than in Wistar rats at all time points *(Table 1)*. Fasted blood glucose levels were in general lower than the non-fasted. Goto-Kakizaki rats showed non-fasted and fasted serum insulin levels that were not significantly different from Wistar rats, except for the non-fasted serum insulin levels at 2 months and the fasted serum insulin levels at 8 months *(Table 1)*. The IPGTT displayed an obvious diabetic curve for Goto-Kakizaki rats at 2, 8 and 14 months (data not shown). Serum fructosamine levels were not different between the Goto-Kakizaki rats and Wistar rats (253 ± 5 µmol/L vs. 266 ± 6 µmol/L, P=0.1). None of the animals showed glucosuria or ketone bodies measured on morning urine by Neostix-4 at any of the time points studied.

Serum 8-epi PGF$_{2\alpha}$ levels

No significant differences were observed in the serum levels of 8-*epi* PGF$_{2\alpha}$ in Goto-Kakizaki rats and Wistar rats (973 ± 243 ng/L vs. 2053 ± 533 ng/L, respectively, P=0.2).

BP, UAE and C_{Cr}

BP was not different between the groups at 2 months and 8 months of age *(Table 1)*. At 14 months, there was a significant difference in BP between Goto-Kakizaki and Wistar rats due to a reduction in BP in the Wistar rats of 12 % compared to the BP at 8 months (*P*<0.05). In both groups, systolic BP remained within the normotensive range at all times, ranging from 91 to 142 mmHg. UAE rose progressively over the study period in Goto-Kakizaki rats and the rise in UAE was much more pronounced and significantly higher than in Wistar rats *(Figure 1)*. C_{Cr} was similar in both groups at the age of 2 months. In Wistar rats, C_{Cr} did not change over time (*Figure 2*). In Goto-Kakizaki rats, C_{Cr} was significantly decreased at the age of 8 months with no further decrease at 14 months. C_{Cr} was significantly lower in Goto-Kakizaki rats than in Wistar rats at 8 months and at 14 months.

Kidney weight and morphology

Kidney weight and glomerular volume were significantly higher in Goto-Kakizaki than in Wistar rats (*Figure 3 and 4*). Kidney weight to body weight ratio (KW/BW) was not significantly different between both groups although there was a tendency for an increased ratio in Goto-Kakizaki rats (3.14±0.07 vs. 2.93±0.08 mg/g BW, *P*=0.07). BMT, total mesangial volume (*Figure 5*) and fractional mesangial volume (35±2 % vs. 30±1 %, *P*=0.04) were significantly higher in Goto-Kakizaki rats than in Wistar rats.

Liver and heart weight

Liver weight was not different between Goto-Kakizaki and Wistar rats (9910±384 mg vs. 9056±548 mg, respectively). Heart weight was significantly higher in Goto-Kakizaki than in

Wistar rats (856±20 mg vs. 707±22 mg, respectively, *P*<0.001). Macroscopic inspection at sacrifice showed slightly dilated hearts in Goto-Kakizaki rats.

DISCUSSION

The present study is, to our knowledge, the first to provide a detailed description of the long-term renal structural and functional changes in the Goto-Kakizaki rat. Goto-Kakizaki and non-diabetic Wistar rats were studied up to the age of 14 months. Goto-Kakizaki rats exhibited renal and glomerular hypertrophy with increased basement membrane thickness and mesangial expansion compared to Wistar rats. In the long-run, urinary albumin excretion rose progressively and creatinine clearance decreased over time in Goto-Kakizaki rats.

Goto-Kakizaki rats showed an impressive glomerular hypertrophy of 47 % versus Wistar rats at 14 months. This glomerular hypertrophy is partly due to expansion of the mesangial region, as both the total and the fractional mesangial volume were higher in the Goto-Kakizaki rats than in the Wistar rats.

The progressive increase in urinary albumin excretion was much more prominent in Goto-Kakizaki rats than in non-diabetic Wistar rats. We studied female rats, because male rats are known to spontaneously develop proteinuria of glomerular origin and glomerulosclerosis [10]. In addition, proteinuria in male rats is characterized by elevated excretion of the sex-dependent α_{2u}-globulin and by increasing albumin excretion with increasing age, resulting in alterations in the composition of urinary proteins with age [11;12]. It is therefore important to include only female animals when the influence of diabetes on renal functional changes is investigated. Creatinine clearance was significantly lower in Goto-Kakizaki rats than in Wistar rats at the age of 8 and 14 months. Due to the design of the study and our primary interest in glomerular morphology we chose to determine the creatinine clearance as an estimate of glomerular filtration rate instead of the gold-standard procedure, inulin clearance.

Goto-Kakizaki rats had a slightly higher body weight than Wistar rats although food consumption was similar in both groups. Whether this difference in body weight is due to a difference in body composition is unknown.

Goto-Kakizaki rats are characterized by hyperglycaemia and glucose intolerance [2;13]. In contrast to other animal models of type 2 diabetes such as the obese Zucker diabetic fatty rat, the Otsuka-Long-Evans-Tokushima fatty rat, the ob/ob mouse and the db/db mouse [14], the Goto-Kakizaki rat lacks obesity, hypertension and dyslipidaemia, factors which may result in renal damage independent of diabetes. The Goto-Kakizaki rat may be a useful model to study the pathogenesis of diabetic kidney disease in the absence of these confounding variables. In addition, mice models have the disadvantage that information on blood pressure is often not available.

It is interesting to note that the Goto-Kakizaki rat develops severe renal functional and structural changes in spite of rather mild diabetic metabolic changes. This suggests that, although hyperglycaemia is the most important risk factor for the development of microvascular complications in diabetes [15], other factors are also involved. We measured serum 8-isoprostane $F_{2\alpha}$ levels as a parameter of oxidative stress but serum 8-isoprostane $F_{2\alpha}$ levels were not different between Wistar and Goto-Kakizaki rats suggesting no important increase in oxidative stress in the circulation of the Goto-Kakizaki rat. Transient hyperglycaemia may stimulate other factors such as growth factors and cytokines which activate further downstream molecules and pathways that lead to pathological renal events even when glycaemia is normalised again.

In Europe and America, the majority of patients with type 2 diabetes (> 80 %) are obese using body mass index (BMI) criteria of > 25 kg/m^2 for women and > 27 kg/m^2 for men. In developing countries such as India, however, non-obese type 2 diabetic patients constitute a common category (> 60 %) and many are actually lean with a BMI of < 18.5 kg/m^2 [16]. Lean type

2 diabetes probably represents a more severe form of diabetes with an increased risk of microvascular complications [16].

In conclusion, the results demonstrate that the Goto-Kakizaki rat is a lean, hyperglycemic, non-hyperinsulinaemic, normotensive experimental model of type 2 diabetes with robust renal structural and functional changes i.e. glomerular hypertrophy, basement membrane thickening, mesangial expansion, progressive increase in albuminuria and decline in glomerular filtration rate, that resemble the changes found in long-term type 2 diabetic patients. The Goto-Kakizaki rat may be a useful model to study the pathogenesis of diabetic nephropathy in lean type 2 diabetes, a condition which is common in developing countries.

ACKNOWLEDGMENTS

Conflict of interest statement. None declared.

REFERENCES

1. Ruggenenti P, Remuzzi G. Nephropathy of type 1 and type 2 diabetes: diverse pathophysiology, same treatment? *Nephrol Dial Transplant* 2000; 15: 1900-1902

2. Goto Y, Kakizaki M, Masaki N. Spontaneous diabetes produced by selective breeding of normal wistar rats. *Proc Japan Acad* 1975; 51: 80-85

3. Pfeffer JM, Pfeffer MA, Frohlich ED. Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. *J Lab Clin Med* 1971; 78: 957-962

4. Boye N, Ingerslev J. Rapid and inexpensive microdetermination of serum fructosamine results in diabetics, uraemics, diabetics with uraemia and healthy subjects. *Scand J Clin Lab Invest* 1988; 48: 779-783

5. Christensen C, Ørskov H. Rapid screening PEG radioimmunoassay for quantification of pathological microalbuminuria. *Diabetic Nephrology* 1984; 3: 92-94

6. Gundersen HJ, Bagger P, Bendtsen TF *et al*. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 1988; 96: 857-881

7. Weibel ER. *Stereological methods, Vol.1. Practical methods for biological morphometry*. Academic Press, London: 1979; 51-57

8. Østerby R, Gundersen HJ, Nyberg G, Aurell M. Advanced diabetic glomerulopathy. Quantitative structural characterization of nonoccluded glomeruli. *Diabetes* 1987; 36: 612-619

9. Jensen EB, Gundersen HJ, Østerby R. Determination of membrane thickness distribution from orthogonal intercepts. *J Microsc* 1979; 115: 19-33

10. Remuzzi A, Puntorieri S, Alfano M *et al*. Pathophysiologic implications of proteinuria in a rat model of progressive glomerular injury. *Lab Invest* 1992; 67: 572-579

11. Harvey AM, Malvin RL. Comparison of creatinine and inulin clearances in male and female rats. *Am J Physiol* 1965; 209: 849-852

12. Roy AK, Chatterjee B, Demyan WF *et al*. Hormone and age-dependent regulation of alpha 2u-globulin gene expression. *Recent Prog Horm .Res* 1983; 39: 425-461

13. Yagihashi S, Goto Y, Kakizaki M, Kaseda N. Thickening of glomerular basement membrane in spontaneously diabetic rats. *Diabetologia* 1978; 15: 309-312

14. Janssen U, Phillips AO, Floege J. Rodent models of nephropathy associated with type II diabetes. *J Nephrol* 1999; 12: 159-172

15. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352: 837-853

16. Mohan V, Vijayaprabha R, Rema M *et al*. Clinical profile of lean NIDDM in South India. *Diabetes Res Clin Pract* 1997; 38: 101-108

Table 1. Clinical and metabolic parameters in Wistar (W) and Goto-Kakizaki rats (GK).

Age	BW (g)	FC (g/24 h)	NF BG (mmol/L)	F BG (mmol/L)	NF I (µg/L)	F I (µg/L)	BP (mmHg)
2 months							
W (N = 6)	178±5	16.7±0.2	5.9±0.4	4.7±0.3	3.00±0.83	0.72±0.13	114±0.7
GK (N = 6)	209±6*	16.1±0.4	14.9±0.8*	8.0±0.4*,‡	1.49±0.09*,‡	0.47±0.07	114±0.9
8 months							
W (N = 9)	253±4	15.9±0.4	4.9±0.3	4.7±0.1	0.64±0.29	0.09±0.04	118±4.9
GK (N = 11)	280±6*	15.7±0.3	17.3±0.8*,‡	9.6±0.6*,‡	0.42±0.09	0.64±0.04*,‡	124±3.0
14 months							
W (N = 9)	268±6	21.8±0.8	5.2±0.3	7.7±0.3	0.50±0.24	0.27±0.08	104±1.8§
GK (N = 11)	292±6†,‡	17.2±0.4*,‡	16.1±0.6*,‡	12.7±0.5*	0.20±0.04	0.34±0.06	116±1.7*

Values are mean ± SEM. BW: body weight, FC: food consumption, NF: non-fasted, BG: blood glucose, F: fasted, I: insulin,

BP: blood pressure. $N = 6\text{-}11$, *$P<0.01$ vs. W, †$P<0.05$ vs. W, ‡Mann Whitney Rank Sum Test, §$P<0.05$ vs. W at 8 months

FIGURE LEGENDS

Figure 1. Urinary albumin excretion (UAE) in Wistar (open bars) and Goto-Kakizaki rats (closed bars) at 2, 8 and 14 months of age. Results are presented as mean + SEM. N = 6-11, *P < 0.05, **P < 0.0001 vs. age-matched Wistar rats.

Figure 2. Creatinine clearance in Wistar (open bars) and Goto-Kakizaki rats (closed bars) at 2, 8 and 14 months of age. Results are presented as mean + SEM. N = 6-11, *P < 0.01 vs. age-matched Wistar rats.

Figure 3. Right kidney weight and glomerular volume in Wistar (open bars) and GK-rats (closed bars) at 14 months of age. Results are presented as mean + SEM. N = 9-11, *P < 0.01, **P < 0.001 vs. Wistar rats.

Figure 4. Light micrographs of renal cortices stained with periodic acid shiff and haematoxylin of a Wistar rat (A) and a Goto-Kakizaki rat (B) at 14 months of age. (Original magnification, 420x)

Figure 5. Basement membrane thickness (BMT) and total mesangium volume in Wistar (open bars) and Goto-Kakizaki rats (closed bars) at 14 months of age. Results are presented as mean + SEM. N = 8, *P < 0.05, **P < 0.001 vs. Wistar rats.
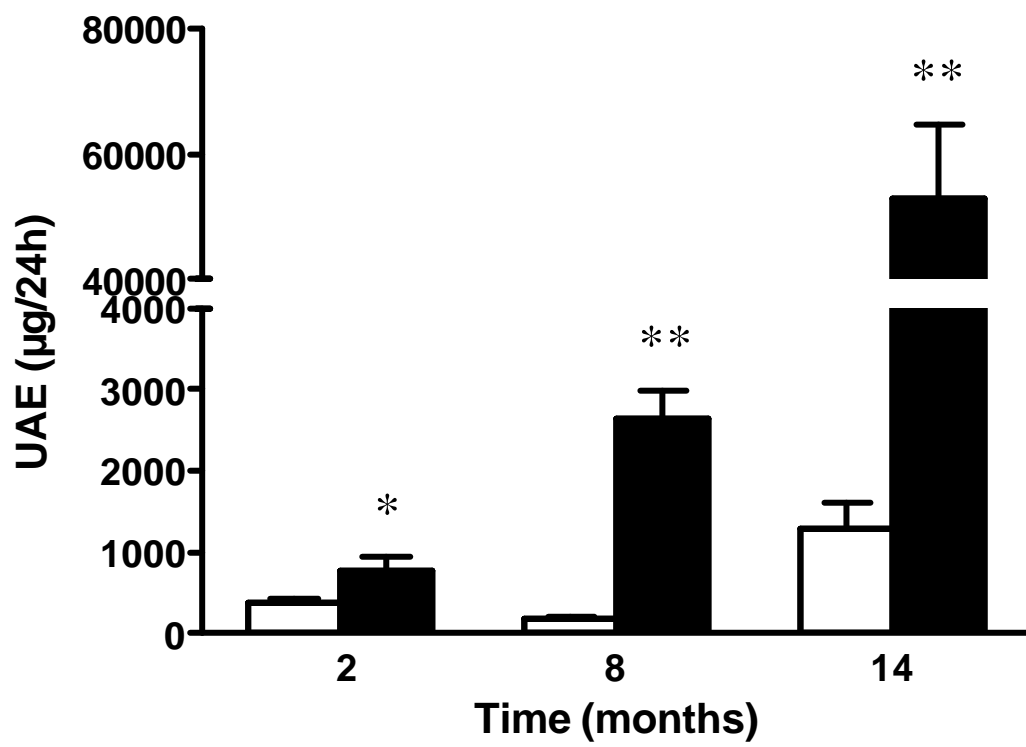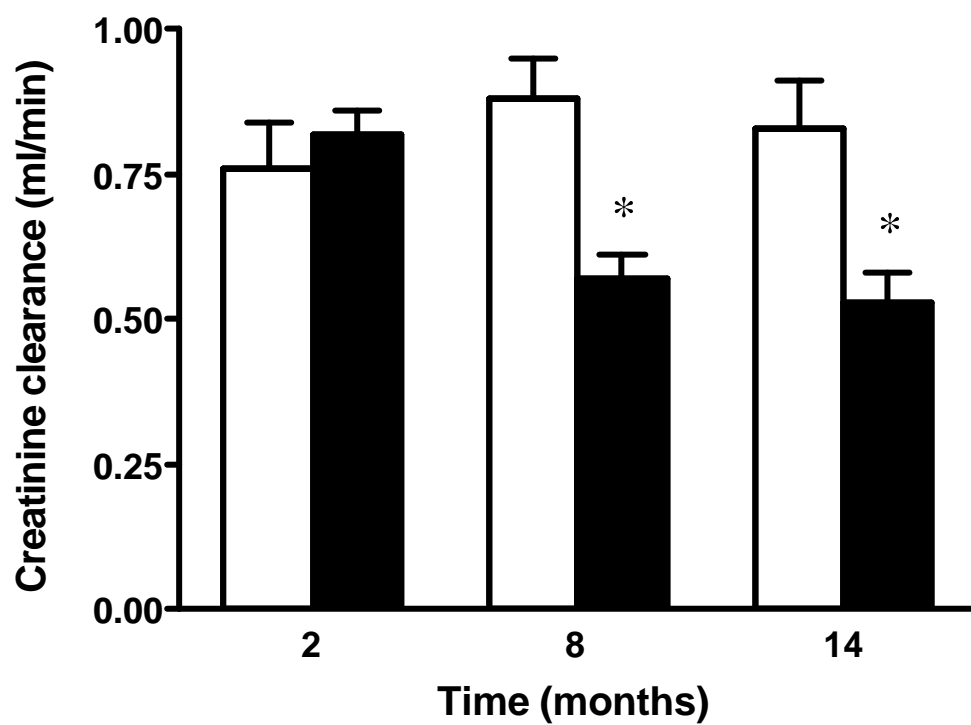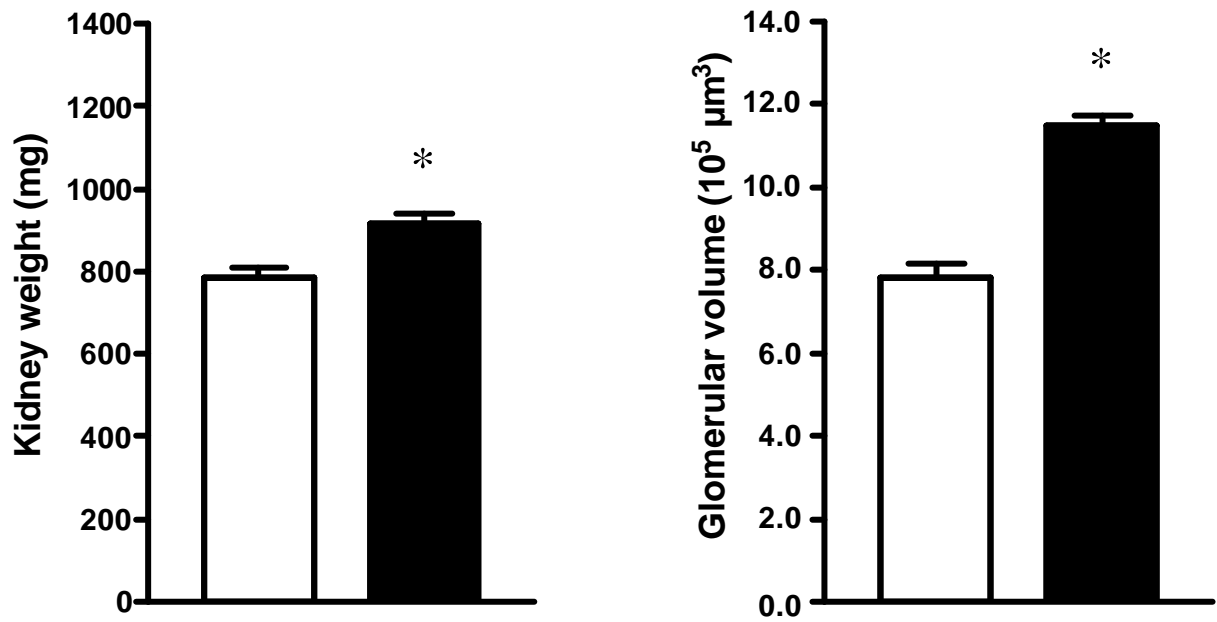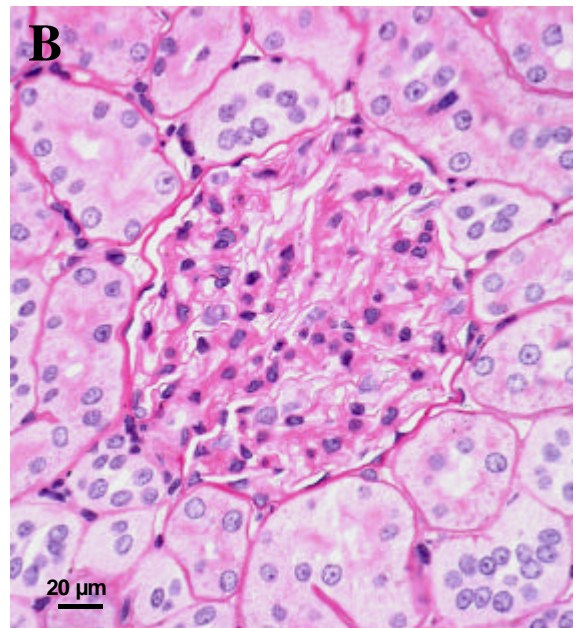
Figure 1.

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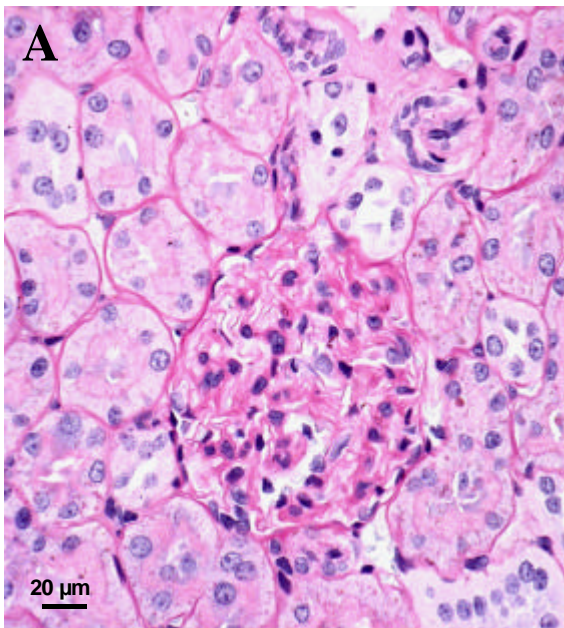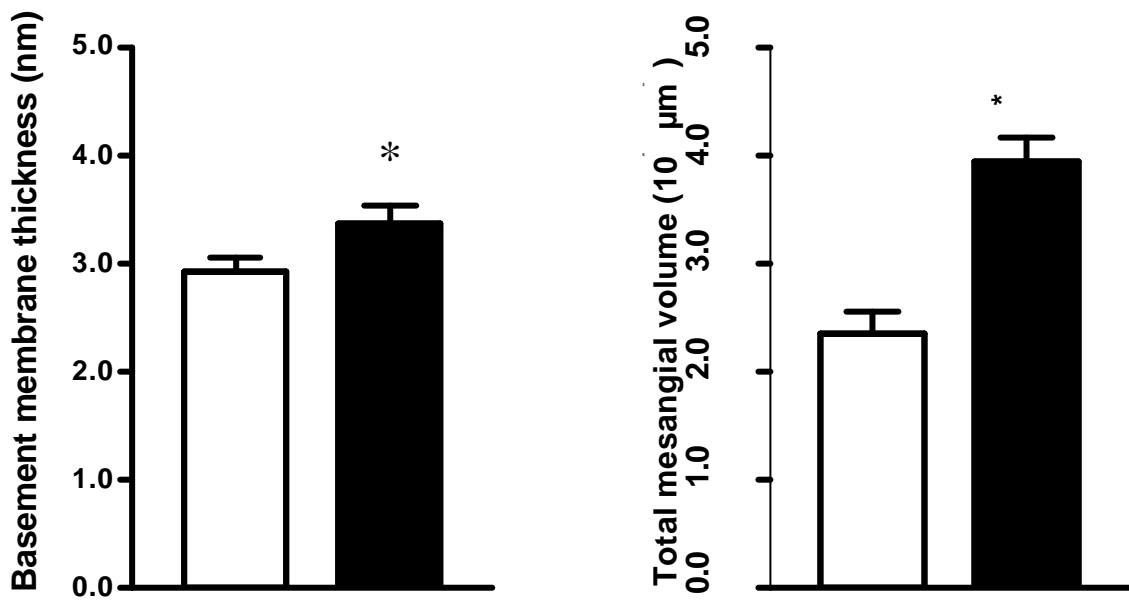Figure 4.

Figure 5.

APPENDIX

ANOVA: Analysis of variance

A_G: Mean glomerular cross-sectional area

β: Shape coefficient for spheres

BP: Blood pressure

BMT: Basement membrane thickness

BW: Body weight

EIA: Enzyme immunoassay

ELISA: Enzym-linked immuno sorbent assay

F: Fasted

FC: Food consumption

GFR: Glomerular filtration rate

H_2O: Water

I: Insulin

IPGTT: Intraperitoneal glucose tolerance test

κ: Size distribution coefficient

KOH: Potassium hydrochloride

NF: Non-fasted

SEM: Standard error of the mean

UAE: Urinary albumin excretion

V_G: Mean glomerular tuft volume

8-*epi* $PGF_{2\alpha}$:   8-*epi* prostaglandin $F_{2\alpha}$ = 8-isoprostane