Psammomys obesus, a particularly important animal model for the study of the human diabetic nephropathy

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Abstract: The *Psammomys obesus* lives in natural desert habitat on low energy (LE) diet, however when maintained in laboratory conditions with high energy (HE) diet it exhibits pathological metabolic changes resembling those of type 2 diabetes. We have evaluated and correlated the histopathology, metabolic and functional renal alterations occurring in the diabetic Psammomys. Renal function determined by measuring glomerular filtration rate (GFR), protein excretion, protein/creatinine ratio and morpho-immunocytochemical evaluations were performed on HE diet diabetic animals and compared to LE diet control animals. The diabetic animals present a 54% increase in GFR after one month of hyperglycemic condition and a decrease of 47% from baseline values after 4 months. Protein excretion in diabetic animals was 5 folds increased after 4 months. Light microscopy showed an increase in glomeruli size in the diabetic Psammomys, and electron microscopy and immunocytochemical quantitative evaluations revealed accumulation of basement membrane material as well as frequent splitting of the glomerular basement membrane. In addition, glycogen-filled Armanni-Ebstein clear cells were found in the distal tubules including the thick ascending limbs of the diabetic animals. These renal complications in the Psammomys, including changes in GFR with massive proteinuria sustained by physiological and histopathological changes, are very similar to the diabetic nephropathy in human. The *Psammommys obesus* represents therefore a reliable animal model of diabetic nephropathy.

Key words: Diabetic nephropathies, Psammomys, Glycogen nephrosis

Introduction

Diabetic nephropathy is a clinical syndrome characterized by persistent microalbuminuria in concomitance with insulin- or non-insulin-dependent diabetes [1, 2]. The human nephropathy proceeds through several distinct patho-physiological stages, including an early stage of glomerular hyperfiltration, followed by the silent phase in which glomerular filtration rate (GFR) returns to normal [3]. The last stage is characterized by sequential development of microalbuminuria, and progressive decline in GFR leading to renal failure [4]. Different animal models of rats and mice are used to study the diabetic nephropathy [5-9]. These models have focused on the earlier harbingers of nephropathy, including the development of albuminuria and histological changes, but not on renal insufficiency as an end point [10]. In a previous study on long-term streptozotocin-induced diabetes in rats, we showed a continued increase

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in GFR up to 40 weeks and then a decline, but never below the normal levels [8]. Our findings question reliability of the streptozotocin diabetic model for the investigation of human nephropathy, as renal failure is not achieved. This stresses out the need for new animal models that mimic the human diabetic nephropathy.

Psammomys obesus (often referred to as the "sand rat") is prone to develop obesity, hyperglycemia and hyperinsulinemia when fed a high energy (HE) diet [11]. The Psammomys is genetically predisposed to non-insulin-dependent diabetes mellitus, whereas environmental and nutritional factors contribute to the expression of diabetes. The progression of the nutritionally induced diabetes in Psammomys resembles in many respects that occurring in human, with hyperglycemic-hyperinsulinemic disorders [12]. The prolonged state of hyperglycemia leads to several complications in the Psammomys model, such as cataracts [13], neuropathy [13], nephropathy and renal failure [14].

A previous study from our laboratory [14] showed that after feeding a HE diet, the Psammomys becomes hyperglycemic and hyperinsulinemic and develops diabetes. According to their renal function, we herein show that animals in an early diabetes state have a high GFR, whereas in advanced stages the animals display low GFR, similar to diabetic chronic renal disease. The diabetic Psammomys were also characterized by an increase in urinary protein excretion, and a decrease in the Na-K-ATPase activity in the kidney medulla. As the Psammomys developed signs of diabetic nephropathy only at 4 weeks, we suggested that they may offer a more appropriate animal model for human diabetic nephropathy. In order to verify our hypothesis, we evaluated whether diabetic Psamommys will develop nephropathy and renal failure after relatively longer periods of time (16 weeks).

Diabetic albuminuria in human is associated with the development of characteristic renal-histopathologic features which include: glomeruli, tubules, interstitium, and the blood vessels. There may be tubular changes secondary to alterations in the perm-selectivity of the glomerulus, including extensive glycogen deposition in the tubular cells, i.e., the Armanni-Ebstein lesions [15]. In diabetic patients, the presence of glycogen in nephron was first demonstrated by Armanni and Ebstein in 1877 [15] and named Armanni-Ebstein lesion (AE). In experimental diabetes, AE has been established in the thick ascending limb of Henle's loop (TAL) and distal tubules [16]. The introduction of insulin led to a considerably reduction of these lesion in human [15], although some insulin-treated

patients may still present it [17].

In the present study, Psammomys in uncontrolled glycemic state developed type 2 diabetes and, similar to the findings in human, their GFR changed from early to advanced stages accompanied by increasing proteinuria, tubular damage and renal failure.

A certain correlation between the physiological and the structural changes was demonstrated.

Materials and Methods

Animals and tissue sampling

All animal experiments were approved by the Institutional Animal Welfare Committee.

Thirty-six male *Psammomys obesus*, aged 6 weeks and weighing 170 g, of the Hebrew University strain, were used. Each animal was housed in an individual cage with water and food intake ad libitum. At the beginning of the experiments, the animals were anaesthetized with ketamine/xylazine (85%/15%) at 0.1 ml/100 g body weight, and blood was drawn for baseline determinations of glucose, insulin and creatinine levels. For kidney function, the animals were kept in metabolic cages. Urine creatinine and protein excretion were determined in four hr urine collection. The animals were divided randomly into 2 groups: 1) control group, consisting of 12 normoglycemic animals fed a low energy laboratory diet (LE) (2.38 kcal/g), (Koffolk, Tel-Aviv, Israel) (Table 1), and 2) experimental group consisting of 24 animals fed with commercial HE diet (2.93 kcal/g) (Table 1). In the HE group all the animals gained weight and developed diabetes. The experimental group was further divided into 2 experimental periods 8 and 16 weeks. Blood glucose levels and urine collections were monitored weekly for a period of 16 weeks. At the end of the experimental period, blood was drawn from the aorta under ketamine/xylazine anaesthesia, for the

Table 1. Composition of the low energy (LE) and high energy (HE) diets

Diets	LE	HE
Protein (%)	16.2	23.6
Fat (%)	3.1	2.4
Carbohydrate (%)	70	68
NaCl (%)	1.1	1.1
Ash (%)	9.6	5.0
Digestible energy (kcal/g)	2.38	2.93

determinations of creatinine, glucose and insulin levels. The animals were killed, the abdominal cavity was opened and the kidneys were cut into small fragments for morphological evaluation.

Metabolic studies

Renal function: Renal functions were performed by measuring creatinine clearance, using the endogenous creatinine production.

Creatinine clearance: Plasma creatinine was determined in blood samples obtained upon termination of the experiments. Urinary creatinine was determined in 4 hr urine collections in metabolic cages at weekly intervals. Creatinine levels in plasma and urine were measured using automated picric acid methods (Cobas Mira Analyzer, Hoffman-La Roche & Co., Basel, Switzerland). Creatinine clearance was calculated using the standard clearance formula.

Protein excretion: Urine was collected for 4 hr in metabolic cages at weekly intervals. Urinary protein excretion was detected by a Pyrogallol Red direct colorimetric method in urine (Cobas-Mira analyzer).

Plasma glucose: Plasma glucose levels were measured using Glucometer Elite (Bayer Corporation, Elkhart, IN, USA).

Plasma insulin: Plasma insulin levels were measured by radioimmunoassay utilizing antibodies against human insulin (Medgenix, Brussels, Belgium) and human insulin as standard.

Histology

Kidneys were removed at 8 and 16 weeks of the experiment for evaluation of the histopathological changes by light and electron microscopy.

Light microscopy: Kidneys fragments were fixed by immersion for 24 hr in 4% paraformaldehyde and embedded in paraffin. Histopathology was carried out on sections stained with hematoxyline and eosin (H&E), periodic acid Schiff (PAS) and Masson trichrome.

Electron microscopy: Samples of renal cortex were fixed by immersion in 1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) and processed for embedding in Lowicryl K4M at -20°C as previously described [18, 19]. Tissue sections were mounted on nickel grids stained with uranyl acetate and lead citrate and examined at the electron microscope (Philips 410LS, Phillips Ltd., Montreal, QC, Canada).

Immunocytochemistry: Other tissue sections were pro-

cessed for immunogold using an anti-mouse serum albumin antibody (Sigma, Oakville, ON, Canada) at 1 : 50 dilution for overnight at 40°C. The sections were then rinsed with 0.01 M phosphate buffer saline (PBS) and incubated with the protein A-gold (10 nm) complex for 30 minutes at room temperature. The protein A-gold was prepared with 10 nm gold particles according to techniques described previously [18]. Upon thorough rinsing with PBS and water and counterstaining, the sections were examined by electron microscopy. The distribution of the immunogold labeling, revealing circulating albumin was assessed through morphometrical evaluations as reported previously [18, 19].

Determination of glomerular size by image analysis: The glomerular size was measured using the segmentation tool of Image-Pro Plus 4.5 (Media Cybernetic, Silverspring, MD, USA). Digital images were taken from 40 randomly selected glomeruli in H&E-stained sections derived from each animal. All images were taken using a 63× objective and computer assisted-image analysis was used to measure the area of the glomerulus.

The circumference of each glomerulus was outlined using the polygonal tracing tool of Image-Pro Plus (Media Cybernetics, Inc., Bethesda, MD, USA). Glomerular area was computed utilizing the spatial measurement tool calibrated to a stage micrometer.

Statistical evaluation

Results of the different groups were compared by non-paired Student's t-test with modified levels of significance according to the Godfrey method [20].

Results

Metabolic data

Animals' body weight: Body weights of the diabetic and non-diabetic Psammomys at time 0, 8 and 16 weeks are presented in Table 2. The diabetic Psammomys, fed on HE diet, increased in weight by 20% during the experimental period, compared with the LE diet animals who served as a control, non diabetic group.

Plasma glucose levels: Table 2 shows at 8 and 16 weeks that HE-fed Psammomys have high blood glucose levels during the experimental period compared to the LE-fed group ($P<0.001$).

Blood insulin levels: During feeding with the HE diet,

Table 2. Body weight, blood glucose and insulin levels, urine protein excretion and urine protein/urine creatinine ratio in the control and diabetic Psammomys at the beginning of the experiment (time 0) and at 8 and 16 wk of the experimental period

	Time 0		8 wk		16 wk	
	Control	Diabetes	Control	Diabetes	Control	Diabetes
Weight (g)	187.2±4.8 (n=12)	193.9±5.9 (n=24)	204.8±7.4 (n=12)	238.1±7.3* (n=24)	199.2±5.4 (n=6)	242.3±10[†] (n=12)
Blood glucose (mg%)	84.1±5.1 (n=12)	73.6±3.1 (n=24)	82.7±2.2 (n=12)	306.2±43.2[†§] (n=24)	78.8±5.9 (n=6)	293.4±20.5[†§] (n=12)
Blood insulin (μU/ml)	45.3±15 (n=12)	48.5±10 (n=24)	50.5±8.2 (n=12)	304[†‡§]±7.5 (n=24)	35 ±10 (n=6)	550[†§]±14 (n=12)
Urine protein (mg Prot/L)	0.37±0.06 (n=12)	0.58±0.1 (n=24)	0.86[‡§]±0.08 (n=12)	2.27[†‡§]±0.26 (n=12)	1.28[§]±0.07 (n=6)	3.53[†§]±0.39 (n=12)
U-Protein/U-Creatinine (mg/μMole)	2.31±0.27 (n=12)	2.43±0.16 (n=24)	2.56±0.2 (n=12)	5.94[†§]±0.51 (n=24)	3.41[§]±0.33 (n=6)	6.72[†§]±0.63 (n=12)

*$P<0.005$ vs. control, [†]$P<0.001$ vs. control, [‡]$P<0.001$ vs. time 16 wk, [§]$P<0.005$ vs. time 0.
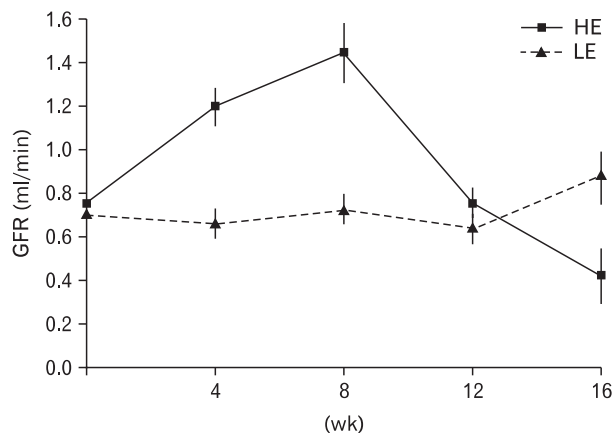

Fig. 1. Glomerular filtration rate (GFR) of the control low energy (LE) diet and the diabetic high energy (HE) diet Psammomys after 4, 8, 12, and 16 weeks. Data are presented as means±SE. $P<0.005$ vs. controls animals.

Table 3. Glomerular areas (μm^2) in control and diabetic Psamommys

Time 0	8 wk	16 wk
9,340±450 (n=40)	13,358±880*[‡] (n=40)	22,635±985[†] (n=40)

*$P<0.005$ vs. control, [†]$P<0.001$ vs. control, [‡]$P<0.001$ vs. 16 wk.

proteinuria, about 6 times higher than that in the control group ($P<0.001$). The normoglycemic animals, fed on the LE diet, showed a slight increase in protein excretion during the experimental period ($P<0.01$).

Protein/creatinine ratio: The urine protein/creatinine ratio of the experimental groups is shown in Table 2. In the diabetic animals this ratio progressively increased up to 176%, while in the control Psammomys the increase was only by 47% from baseline.

Light microscopy

Glomeruli: Light microscopy revealed an increase in glomeruli size in the diabetes Psammomys (Fig. 2) compared to control animals. There was a 2.4-fold increase in the glomerular area of the diabetic Psammomys ($P<0.001$) (Table 3). No difference in the fibrosis was detected using the Masson Trichrome staining (data not shown).

Fig. 3 shows the particular appearance of special cells, the C-cells that are stained neither by PAS nor by H&E.

Electron microscopy

Electron microscopy (Fig. 4) revealed that these C-cells not stained by H&E or by PAS, are in fact tubular epithelial cells containing large amounts of glycogen (Fig. 4B). These glycogen loaded cells are tubular epithelial cells of the TAL and distal tubules. Their clear appearance is due to the extraction of the glycogen deposits during tissue preparation. The cells appeared clear with a thin rim of cytoplasm containing mitochondria and other cellular organelles. Microvilli and
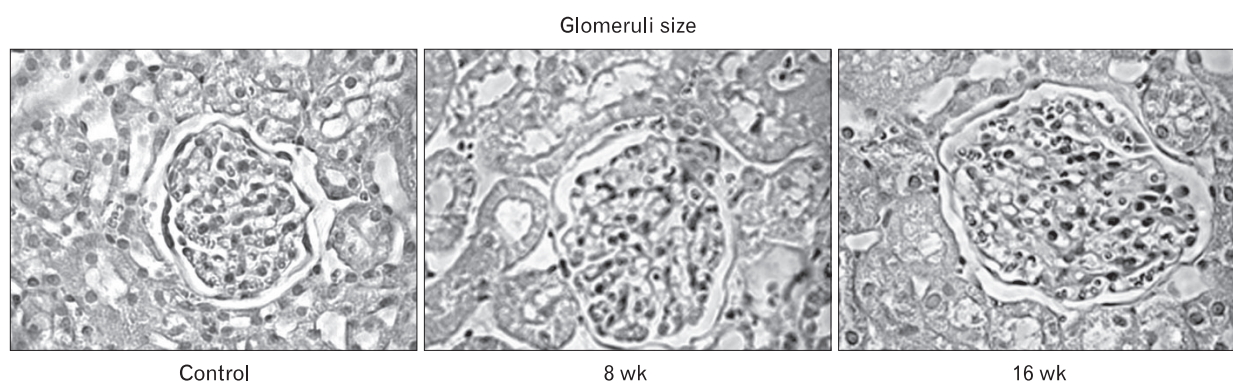
the Psammomys that developed diabetes showed increases in insulin levels up to 304±7.5 μU/ml after 8 weeks ($P<0.001$). This increase reached 550±14 μU/ml after 16 weeks, while it remained at low levels in the control group, 35±10 μU/ml ($P<0.001$) (Table 2).

GFR: Fig. 1 shows the changes in the GFR of the Psammomys during 16 weeks of the HE diet. There were no changes in the GFR of the control group, while in the diabetic Psammomys the GFR rose and doubled within 8 weeks (range, 0.74±0.19 to 1.45±0.12 ml/min, $P<0.005$). The diabetic animals showed a decreased GFR towards normal values at 12 weeks (0.78±0.01) and continued to drop reaching the lowest levels (lower than the baseline) at the end of the study at 16 weeks (0.47±0.05).

Protein excretion: Total protein excretion is depicted in Table 2. There was a gradual increase in protein excretion of the hyperglycemic Psammomys. Sixteen weeks after the HE diet was started the Psammomys developed massive

Glomeruli size

Control   8 wk   16 wk

Fig. 2. Histology of representative glomeruli from the diabetic (high energy diet) Psammomys at the beginning of the diet (time 0) and at 8 and 16 wk. Glomeruli area gradually increased in 8 and 16 weeks compared to time 0 (×300). Quantitative data appear in Table 3.

Periodic acid shiff (PAS)

Control (LE)   Diabetes (HE)
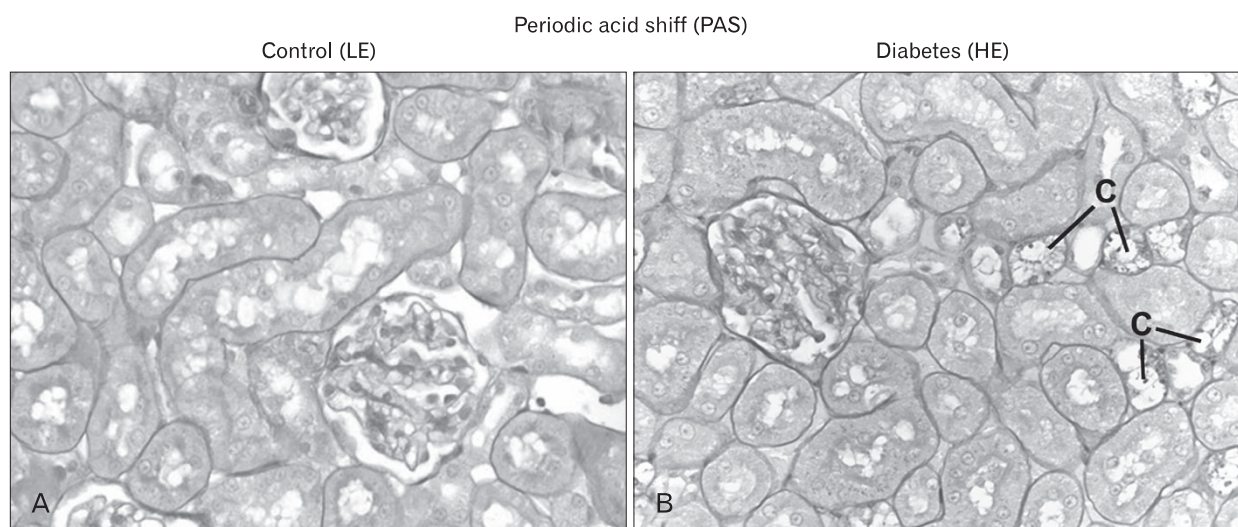

Fig. 3. (A, B) PAS staining of sections of kidneys from control (low energy [LE] diet) and the diabetic (high energy [HE] diet) Psammomys after 16 wk of feeding with the appropriate diet (×200). C, clear cells.
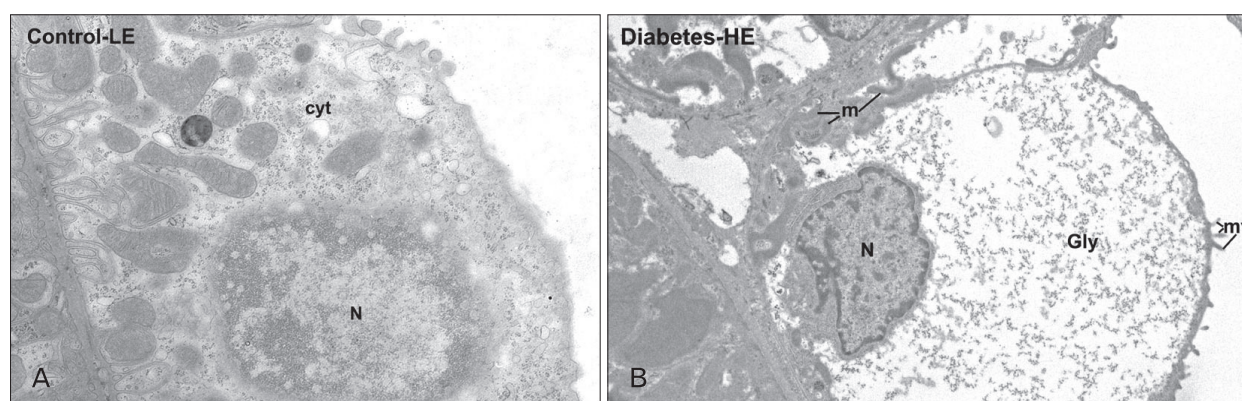

Fig. 4. Renal distal tubules. Epithelial cells of the control (low energy [LE] diet) (A) and a diabetic (high energy [HE] diet) (B) animals. In (A) the cytoplasm (cyt) appears normal with its different components, while in (B), the clear cell is filled by glycogen (gly) which was extracted during tissue preparation (×15,000). The cell cytoplasm appears clear; m, mitochondria; mv, microvilli; N, nucleus.

basal infoldings tended to disappear. The nucleus remained more centrally located. These changes were not seen in the non-diabetic animals (Fig. 4A).

The glomerular tuft in normoglycemic animals displayed the usual structures with a thin basement membrane between the fenestrated endothelial cells and the podocytes (Fig. 5A). The hyperglycaemic animals preserved the general normal structure of the glomerular tuft (Fig. 5B). The basement membrane, however, showed sites of enlargements as well as splitting into two layers (Fig. 5B). Despite these focal differences the average thickness of the basement membrane in the hyperglycaemic animals was not statistically different from that of normoglycemic animals (164.75±9.90 nm for the HE diet *vs.* 159.25±9.10 nm for the LE diet animals). Podocytes also displayed normal structures with no significant fusion of the foot processes.

Immunocytochemistry

We applied the immunogold approach for the detection of circulating serum albumin. The gold particles revealed albumin over the capillary lumina, in association with the endothelial cells and in the glomerular basement membrane (Fig. 6). In the normoglycemic animals no labeling was detected in the urinary space (Fig. 6A). In the hyperglycaemic animals the labeling over the basement membrane appeared to be distributed throughout the thickness of the basement membrane (Fig. 6B). The gold particles were associated with the membranes of the endothelial cells as well as those of the podocytes. Some labeling was also detected over dense material present in the urinary space (Fig. 6B). These results indicate that in the diabetic animals, circulating albumin, besides being present in the capillary lumen, is also present throughout the glomerular basement membrane and in the urinary space.

Morphometrical evaluation of the distribution of labeling through the glomerular basement membrane showed that in tissues of normoglycemic animals this distribution was asymmetrical with most of the gold particles located towards the endothelial side of the basement membrane (Fig. 6C). Such a distribution reflects retention of albumin by the endothelial side of the basement membrane. In the hyperglycaemic animals, on the other hand, the distribution appears to be more uniform, with densities of labeling being almost as high on the podocyte side as on the endothelial side (Fig. 6C). These results confirm the qualitative observations and demonstrate that in hyperglycaemic condition, serum albumin crosses the glomerular wall to reach the urinary space.

Discussion

The present study is the first to provide a detailed description of long-term renal structural changes and their correlation with the functional disturbances in a particular animal model with diabetic renal injuries. We have shown that the changes in renal functions and structures that occur in human diabetes are also present in the diabetic Psammomys: there is a decrease in the GFR with a gradual increase in protein excretion and presence of glycogen ne-
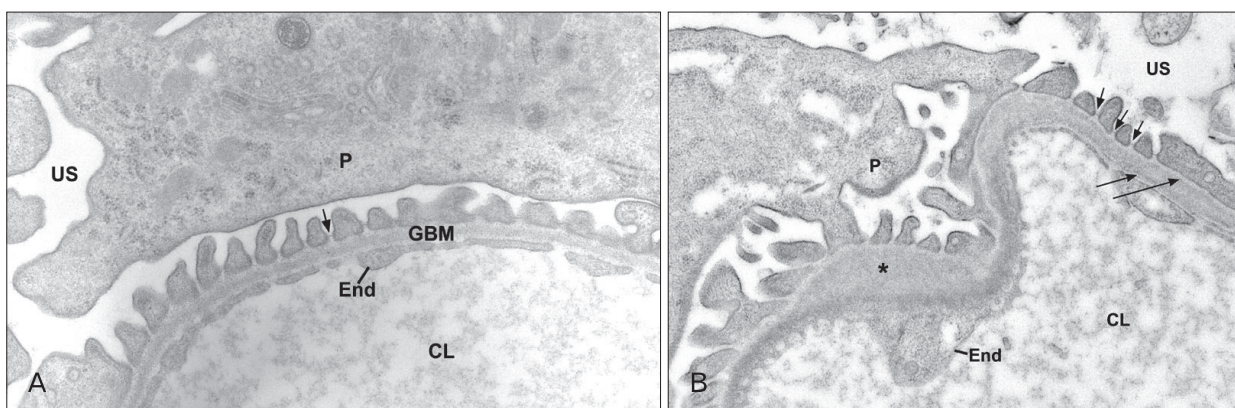

Fig. 5. Electron micrographs of the glomerular wall of a control (low energy diet) (A) and of a diabetic (high energy diet) (B) animals. For the control animal (A) the glomerular wall appears normal with a thin glomerular basement membrane (GBM) between the endothelial cell (End) and the podocyte (P). On the other hand, for the diabetic animal (B), the GBM, shows sites of increase thickness and material deposition (*) as well as sites where the GBM splits in two (long arrows) (×30,000). Small arrows, slit diaphragm. CL, capillary lumen; US, urinary space.
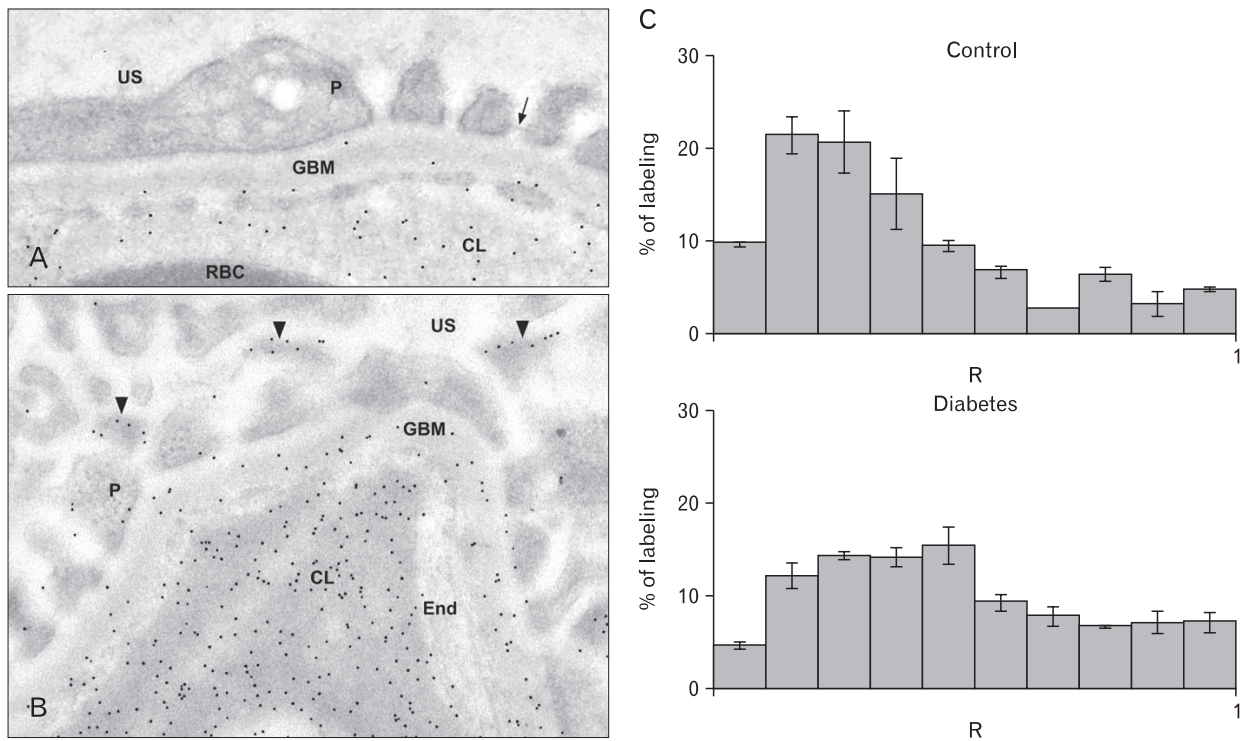
Fig. 6. (A, B) Immunocytochemical detection of endogenous serum albumin by the protein A- gold technique. Glomerular walls of a control (A) and a diabetic animal (B). The gold particles reveal that serum albumin antigenic sites are present in large amounts over the capillary lumen (CL) and over the glomerular basement membrane (GBM). In the control animals (A), the labeling appears to be mainly on the endothelial side of the basement membrane, and no gold particles are seen in the urinary space (US). Arrow: slit diaphragm. For the diabetic animal (B), the labeling over the GBM appears to be distributed throughout the GBM and we can detect dense labeled areas in the urinary space (arrowheads) (×40,000). P, podocyte; End, endothelial cell. (C) Distribution of serum albumin immunolabeling across the glomerular wall as expressed over ratio values (R). In the control animals the distribution is asymmetrical, demonstrating that most of the albumin is retained on the endothelial side of the glomerular basement membrane. The distribution for the diabetic animals is more uniform across the basement membrane indicating alteration in the passage of albumin across the glomerular wall.

phrosis. The physiological changes at the beginning of the diabetic condition consisted in a doubling of the GFR, followed by a gradual decline in GFR with the increase in urine protein excretion. The above pathopysiological changes were accompanied by glomeruli hypertrophy with leakage of albumin from the glomerular wall to the urinary space and glycogen nephrosis at the TAL and distal tubules.

Human diabetic nephropathy is characterized by changes in GFR with increasing proteinuria which leads to renal failure [2, 3]. In contrast to streptozotocin animal models used by us [8] as well as other models [5, 21-23] in which renal failure is difficult to achieve even after a 40 week follow-up, the diabetic Psammomys showed significant reductions in renal function already at 8 weeks. Increases in glomerular filtration rate are the earliest factional abnormality of the Psammomys diabetic kidney. Glomerular hyperfiltration in the early phase of diabetes has been widely documented in other animal

models of diabetes and in humans [24, 25]. Others [26, 27] as well as our group [28-30] have hypothesized that glomerular hyperfiltration observed in rats with streptozotocin-induced diabetes mellitus is the consequence of resting tubuloglomerular feedback [26-30].

As in humans, the diabetic Psammomys showed the same stages of proteinuria; during the experimental period the diabetic animals developed microalbuminuria that progressively reached the degree of massive proteinuria. The increment in protein excretion was followed by impaired glomerular filtration rates (Fig. 1).

In different rat models of type 2 diabetes, proteinuria was also observed. However, those investigations were not able to correlate the proteinuria with the changes in GFR [22, 23, 31, 32]. This lack of correlation displayed by different models of diabetes in rats and mice, as opposed to the one present in the diabetic Psammomys, emphasizes the closeness of this model

with the human nephropathy and its potential as a better tool for the investigation and understanding diabetes.

The initially basic proteinuria of our control animals slightly increased during the experimental period, confirming that proteinuria is normally present in rodents [33, 34], and the slight increase may be due to aging.

Anatomical-functional correlation

The important results of this communication are the histological changes observed in the glomeruli and tubules of the Psammomys diabetic kidney.

The histological changes of the glomeruli: In the glomeruli we found progressive hypertrophy, with leakage of albumin from the glomerular capillary into the urinary space. Renal hypertrophy is common in diabetes, in humans as well as in animal models. Although is not well understood this finding has been generally attributed to glomerular hyperfiltration. Our present study raises certain questions regarding the correlation between glomerular hyperfiltration and glomerular hypertrophy in the diabetic Psammomys. In the very early phase of diabetes in the Psammomys, glomerular hyperfitration is associated with glomerular hypertrophy. Interestingly, as the hyperglycemic state continues, the glomerular filtration returns to normal levels at 12 weeks, and then falls far below those of control levels at 16 weeks. Despite this significant drop in glomerular filtration rate (two-fold below control) there is a dramatic increase in glomerular volume. This paradoxical inverse morphological - functional correlation in the present study is not yet understood.

The marked drop in the glomerular filtration rate at 16 weeks is most likely due to alteration in the filtering barrier of the glomerular tuft, which consists of endothelial cells, basement membrane and visceral epithelial cells (the podocytes). In fact, experimental studies by Myers et al. [35] have shown a decrease in glomerular filtration coefficient (Kf) in this setting. These investigators have demonstrated in the diabetic kidney a reduction in the number of restrictive pores that leads to loss of ultrafiltration capacity. Parallel to the fall in filtration capacity, there is an impairment of glomerular barrier size selectivity leading to progressive albuminuria [36, 37].

Histopathological changes of the tubule: The present study has shown that the earliest morphological change noticeable by light microscopy (Fig. 3) in the diabetic Psammomys kidney is the appearance of glycogen deposits in the tubular epithelial cells of the distal nephron (Fig. 4). Similar findings

were reported in rat kidney with streptozotocin-induced diabetes mellitus [38]. This pathological abnormality is known in diabetic patients as glycogen nephrosis or Armanni-Ebstein. With insulin therapy in humans, the incidence and severity of the lesion became less pronounced [39]. The pathophysiological importance of glycogen accumulation in epithelial cells is not yet fully understood. Theoretically, if localized to the macula densa glycogen-induced injury could potentially interfere with the tubuloglomerular feedback. Consequent inhibition of vasoconstrictor signalling to afferent arteriole would contribute to afferent arteriolar dilatation leading to an increased glomerular capillary hydraulic pressure and hyperfiltration. Meyer et al. [40], by investigating the renal and hepatic glucose metabolism in type 2 diabetes in humans, indicated that renal glucose uptake is markedly increased in type 2 diabetes; which could explain the accumulation of glycogen found in diabetic kidneys where it triggers cell apoptosis leading to renal failure.

Increased renal deposits of glycogen in diabetic humans and rats have been the object of extensive investigations [16, 41, 42]. Various studies agree that the predominant location of the pathologic glycogen accumulation is in the TAL, whereas less extensive deposition occurs in the distal tubules and in other portions of the nephron [16, 38, 41, 43-45]. Whether glycogen accumulation interferes with the physiological functions of the affected segments is uncertain. Normally, in physiological states, a major function of the TAL is sodium chloride reabsorption. The highest activity of the Na-K-ATPase activity is in the medulla of the kidney and especially in the TAL because of its role in controlling the hyperosmolarity of the medulla. Structurally, the Na-K-ATPase is localized in the cell membrane of the large and numerous basal infoldings. In our previous report on diabetic Psammomys [14] we clearly demonstrated that Na-K-ATPase activity was determined by glomerular filtration. We also showed a decrease in Na-K-ATPase activity in the medulla of the Psammomys with GFR values below those in the control group. Here we show that in long-term diabetes there are glycogen-containing cells located in the TAL, with reduced basolateral infoldings.

Taking together these findings we can propose that the decrease in renal function of the diabetic Psammomys may be caused by the structural changes that happened in the glycogen containing cells in the TAL that led to reduced activity of the Na-K-ATPase enzyme. It is possible that this reduction in enzyme activity in the medulla of the dia-

betic Psammomys [14] is part of the progression of renal disease. Tubular apoptosis found associated with glycogen accumulation [41] must contribute significantly to the diabetic nephropathy. Support for this possibility is provided by the finding that patients with type I glycogen storage disease frequently develop renal disease [46]. Moreover, striking similarities between diabetes mellitus and inherited glycogen storage disease with regard to renal pathology have been well documented [46]. Glycogen deposits in the kidney are the first shared morphological features early in both diseases [47]. Interestingly, the pathophysiology in both conditions is almost identical [46-48]. Furthermore, the analogy encompasses the natural history of both diseases that follow an inexorable detrimental course of progression to glomerular sclerosis with proteinuria and hypertension, culminating in end-stage renal disease [46-48].

In summary, the results of the present study demonstrate parallel physiological and histological changes in the diabetic Psammomys kidney and human diabetes. We have shown: 1) close relationships between structural changes such as increasing glomerular size with leakage of albumin from the glomerular wall to the urinary space; 2) the existence of glycogen-containing cells with the physiological changes, e.g. reduction of the GFR and massive proteinuria. Accordingly, the diabetic the Psammomys appears to be an excellent model for the study of the human diabetic renal failure.

Acknowledgements

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