Size and charge selectivity of the glomerular filter in early experimental diabetes in rats

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ABSTRACT

Microalbuminuria is an early sign of diabetic nephropathy. The aim of the present study was to investigate whether the changes of the glomerular filtration barrier in early experimental diabetes are due to size- or charge selective alterations. Wistar rats, made diabetic by streptozotocin (STZ) and having their blood glucose maintained at ~20mM for 3 weeks or 9 weeks, were compared with age matched controls. Glomerular clearances of native albumin (Cl-HSA) and neutralized albumin (Cl-nHSA) were assessed using a renal uptake technique. Glomerular filtration rate and renal plasma flow were assessed by ^{51}Cr-EDTA and ^{125}iodohippurate, respectively. In a separate set of animals, diabetic for 9 weeks, and in controls, glomerular sieving coefficients (θ) for neutral FITC-Ficoll (mol. radius 15-90 Å) were assessed using size exclusion chromatography. At 3 weeks of diabetes Cl-HSA and Cl-nHSA remained unchanged, indicating no alteration in either size or charge selectivity. By contrast, at 9 weeks of diabetes duration there was a 2-fold increase of Cl-HSA, while Cl-nHSA remained largely unchanged, at first suggesting a glomerular charge defect. However, according to a two-pore model, the number of large pores, assessed from both Ficoll and Cl-HSA, increased two-fold. In addition a small reduction in proximal tubular reabsorption was observed at 3 weeks, which was further reduced at 9 weeks. In conclusion, no functional changes were observed in the glomerular filtration barrier at 3 weeks of STZ-induced diabetes, whereas at 9 weeks there was a decrease in size selectivity owing to an increased number of large glomerular pores.

Key words: sieving coefficient, proteinuria, capillary permeability, fractional clearance, macromolecules.

INTRODUCTION

Diabetic nephropathy (DNP) is currently the leading cause of end-stage renal disease in the western world. In the early course of insulin-dependent diabetes several functional and structural alterations occur in the kidney. Some of these alterations include glomerular hyperperfusion/hyperfiltration (34), hypertrophy of the nephrons and gross renal enlargement (3, 25) and also changes in the glomerular extracellular matrix mass and composition. Microalbuminuria, i.e moderately increased levels of albumin excretion (20-200μg/min), is an early sign of DNP, reflecting either alterations in the glomerular barrier to (neg. charged) albumin or a reduction in the protein reabsorbing properties of the proximal tubules, the latter supported by a recent study (29). According to the "Steno hypothesis" loss of negative charges in the filtration barrier, conceivably due to altered activity of the enzymes involved in the metabolism of extracellular matrix components, may be the cause of the microalbuminuria in early DNP (6). The "Steno hypothesis" is supported by the preferential urinary excretion of albumin (neg. charged), and not that of other larger proteins, in early DNP (4, 7). There has been little evidence of impaired barrier size-selectivity in early DNP, i.e alterations primarily in the "large pore system" of the glomerular filtration barrier.

By contrast, late DNP is characterized by a prominent unselective proteinuria which develops due to gradual deterioration of the glomerular barrier. The structural changes occurring during the development of early to late DNP involve thickening of the glomerular and tubular basement membranes, and later, a decreased filtration surface area combined with loss of podocytes, which is furthermore accompanied by a clearly reduced size-selectivity of the glomerular filter (15, 16, 38). Hitherto the permselective properties of glomerular barrier in diabetic nephropathy have mainly been assessed using dextran as a probe for glomerular permeability. It is now well established, however, that dextran is hyperpermeable across the

glomerular filter. Therefore it is insensitive to small changes in the glomerular size-selectivity. Only in advanced DNP, i.e during the macroalbuminuric phase, increases in the large pore/shunt pathway have been detected using dextran (7, 14, 24, 31). Recently Ficoll, a polysaccharide being less hyperpermeable across the glomerular filter than dextran, was employed as a marker for glomerular permeability in patients with early DNP and microalbuminuria (1). Using this less hyperpermeable macromolecular probe, an increase in the large pore pathway was indeed detected (1), implying that a decreased size selectivity is responsible for the increased permeability to albumin in early DNP. Furthermore, in a recent careful micropuncture study albuminuria in early streptozotozin (STZ)-induced diabetes was found to be due to a reduced proximal tubular reabsorption of albumin (29).

The present study was performed in order to investigate the functional alterations of the glomerular barrier occurring in early DNP. The aim was to clarify whether the major early injury could be ascribed to size- or to charge-selective changes in the filter, or perhaps to both. This was accomplished by studying rats exposed to hyperglycemia in poorly treated STZ-induced diabetes for either 3 or 9 weeks, respectively. Glomerular size and charge selectivity were assessed *in vivo* using two different approaches. The glomerular clearances of native and neutralized albumin (HSA and nHSA, respectively) were measured using a renal tissue uptake technique. Furthermore, after nine weeks of diabetes, when a perturbation in the albumin transport was detected, glomerular sieving coefficients (θ) for Ficoll molecules with a broad size distribution (radius of 15-90 Å) was measured. This approach enabled us to provide, for the first time, a precise evaluation of both charge and size selectivity in the STZ-diabetic model *in vivo*.

METHODS

Experiments were performed in male Wistar rats (Møllegaard, Lille Stensved, Denmark). The rats had free access to standard chow and water until the day of the experiment. The studies were approved by the Animal Ethics Committee at Lund University.

Diabetic animals

49 animals, diabetic for either 3 or 9 weeks, were purchased from Møllegaard, Lille Stensved, Denmark, arriving a few days prior to the experiments. Briefly, rats weighing between 140-160g were made diabetic by a single i.v injection of streptozotozin (N-(Methylnitroscocarbamolyl)-α-D-glucosamine), 90mg/kg (Biochimika, Sigma-Aldrich, Denmark). Blood glucose levels were measured every morning. When glucose was detected in the urine and the plasma concentration of glucose was >25mmol/L the animals were considered diabetic. The rats received daily subcutaneous injections using 0.5 IU Insulin (Porcine insulin 40UI/mL, Caninsulin, Intervet, Skovlunde, Denmark). Rats having a plasma glucose concentration higher than 17mM were given 0.2 IU more insulin than the day before, while rats having a glucose concentration lower than 13 mM were given 0.2 IU less than the day before. The aim was to keep the animals at a blood glucose level between 18-25 mmol/L. After either 3 weeks or 9 weeks of diabetes glomerular filtration rate (GFR), plasma flow (RPF) and glomerular permselectivity were determined.

Surgery

The rats were anaesthetized intraperitoneally using 60 mg/kg sodium pentobarbital and placed on a heating pad to maintain body temperature at 37°C. The tail artery was cannulated (PE-50

cannula) for arterial pressure recordings on a polygraph (Model 7B, Grass Instruments, Quincy, MA, USA), and for the administration of drugs. A tracheotomy was performed using a PE-240 tube. The left carotid artery and left jugular vein were cannulated (PE-50) for blood sampling and infusions respectively. After surgery the animal was allowed to recover for at least 30 min.

Glomerular filtration rate (GFR) and renal plasma flow (RPF)

Glomerular filtration rate and renal plasma flow were determined at 3 weeks (n=10) and 9 weeks (n=8) of diabetes (D-3w and D-9w) and in their respective control groups (C-3w (n=4) and C-9w (n=6)). A catheter was placed in the urinary bladder via an abdominal incision for urine collection. ^{51}Cr-EDTA (0.37 MBq, Amersham, Biosciences, Buckinghamshire, UK) and ^{125}I-iodohippurate (0.08 MBq, Amersham, Biosciences, Buckinghamshire, UK) were given together as a bolus dose, immediately followed by a constant infusion (3ml/h) of the respective tracer (0.37MBq/ml of ^{51}Cr-EDTA and 0.08 MBq/ml of ^{125}I-iodohippurate in 0.9% NaCl). Five blood samples were collected from the carotid artery during a 20 min period. During the same period urine was collected. After volume loading the rats with 2 ml of horse serum (SVA, Uppsala, Sweden) GFR and RPF were assessed for another 20 min period. At the end of the experiment a sample was collected from the renal vein and the extraction-fraction of ^{125}I-iodohippurate was calculated and used for determination of its clearance.

Clearance of neutralized albumin (nHSA) and native albumin (HSA)

Clearance of neutralized human serum albumin (nHSA; Stokes-Einstein (SE)-radius 35.0 Å) and native (neg. charged) human serum albumin (HSA; SE-radius 35.5 Å) was measured at 3 weeks (n=8) and 9 weeks of diabetes (n=10) and in their respective control groups (n=6 and

n=8) using a tissue uptake technique as described in detail elsewhere (13). Briefly, ^{125}I-HSA (0.2 MBq, Institute for Energy Technique, Kjeller, Horten, Norway) was administered as a bolus dose together with ^{131}I-nHSA (0.15 MBq) in the tail artery. nHSA was prepared by Dr. Olav Tenstad (University of Bergen, Norway) by a graded modification of the COOH-groups and was labelled with ^{131}I, using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Iodo-Gen) as described at some length earlier (13). Six blood samples (25 μl) and one urine sample were collected during an 8 min period. To eliminate the tracer from the renal vasculature, a whole body washout (using a 1:2 mixture of 0.9% saline and heparinized horse serum, SVA, Uppsala, Sweden) was performed via the carotid artery (20 ml/min) for 8 minutes, after the inferior vena cava had been freed and cut open for collection of the rinse fluid. The kidneys were dissected free and the cortex and the urine sample were assessed with respect to radioactivity. Urine samples were precipitated using trichloro acetic acid (TCA) and the amount of free iodine was calculated. Clearance of albumin (nHSA or HSA) was calculated as the cortical tracer mass plus the precipitable urine mass of tracer divided by the average plasma tracer concentration and time. The fractional tubular albumin excretion was obtained from the precipitable urine mass divided by the total mass of albumin recovered in the urine and the renal cortex, and the fractional (proximal) tubular albumin reabsorption was obtained from 1 minus this entity.

Sieving of FITC-Ficoll

A separate set of diabetic rats (n=13) and controls (n=8) were used for assessing θ for Ficoll at 9-weeks. A mixture of fluorescein isothiocyanate (FITC) labeled Ficoll-400 (1 mg) and Ficoll-70 (42 μg) (TdB Consultancy, Uppsala, Sweden) was administered as a bolus dose together with FITC-Inulin (0.5 μg) and ^{51}Cr-EDTA (0.37 MBq). The bolus was followed by a constant infusion of 3ml/h (Ficoll-70: 94.5 μg/min, Ficoll-400: 3 mg/min, Inulin: 1.5 μg/min,

^{51}Cr-EDTA: 0.019 MBq/min). 20 minutes after Ficoll administration a laparotomy was performed and catheters (PE10 coupled to PE50) were placed in the left and the right ureter and used for urine collection. Urine was collected during a five minute period during which one midpoint (2.5 min) blood sample was taken. Plasma and urine were assessed on a size exclusion high-performance chromatography system (Waters) using an ultrahydrogel-500 column (Waters) and was calibrated as described in detail previously (2).

Urinary albumin/creatinine concentration ratio (ACR)

The albumin/creatinine concentration ratios (ACR) were obtained by sampling urine in metabolic cages for four hours two days prior to the experiments. The albumin concentration in urine was assessed using a simplified (one step incubation) enzyme linked immunosorbent assay (ELISA), as described at some length earlier (19, 33). Briefly, the plates were coated with rat albumin (Sigma) and then incubated with the urine sample, a clonal rabbit anti-rat antiserum (diluted 1:2,000, from Nordic Immunolog Laboratories, Tillberg, Netherlands) and an anti-rabbit IgG conjugated with alkaline phosphatase. The detection limit was 16μg/L and the intra- *vs*. inter-assay variations were 11.8% and 12.8%, respectively. Urinary creatinine was analyzed using the Jaffé reaction.

RESULTS

General

Both groups of diabetic rats, those with 3 weeks of diabetes duration (D-3w) and those with 9 weeks of diabetes duration (D-9w), had lower body weights compared to their age-matched controls (Table 1). The mean blood glucose concentrations for the diabetic rats 10 days prior to the start of the experiment were 23.2±2.2 for the D-3w group and 20.2±2.9 for the D-9w group.

Albumin excretion and Renal hemodynamics

After a diabetes duration of 3 weeks the urinary albumin to creatinine clearance ratio (ACR) was increased 7-fold compared to that in the control rats (9.16±1.63 mg/mole vs.1.26±0.20 mg/mole) (Figure 1). The ACR was further increased at nine weeks of diabetes (13.6±3.8 mg/mole compared to 1.04±0.17 mg/mole in the control group). Glomerular filtration rate (GFR) and renal plasma flow (RPF), measured simultaneously (see methods), were assessed in a parallel set of rats at 3 and 9 weeks. After 3 weeks of diabetes duration GFR was elevated, i.e 1.21±0.10 ml/g kidney compared to 0.82±0.12 ml/g (control) ($p<0.05$). After 9 weeks of diabetes duration GFR, if expressed per g kidney weight, had returned to control (Table 1). However, if GFR data for the two control groups (3 and 9 weeks) were pooled, a small increase was still observed for GFR, when expressed per 100g of body weight (BW), at 9 weeks of diabetes. This is consistent with the increased GFR in the 9 week diabetic group, compared to control, after plasma volume expansion. The "renal reserve" i.e the increment in GFR after (a large) plasma volume expansion was not significantly different between the groups. No significant increment in renal blood flow was observed (per g of kidney) in the diabetic vs. non-diabetic animals at any time point. However, the filtration fraction (FF) was elevated at 3 weeks of diabetes compared to control both during basal conditions and after plasma volume expansion (Table 1).

Clearance and θ for HSA and nHSA

The clearance of native albumin (HSA) and neutralized albumin (nHSA) did not increase at all after 3 weeks of diabetes compared to the control group. However, after 9 weeks of diabetes duration the clearance of HSA was significantly increased (1.8-fold) compared to control ($7.21\pm0.315 \times 10^{-4}$ ml/min vs. $4.06\pm0.714 \times 10^{-4}$ ml/min ($p<0.01$)) indicating an alteration in glomerular barrier characteristics. By contrast, the clearance of nHSA was not significantly different between any of the groups (Table 1). Thus the clearance ratio of nHSA/HSA showed a significant decrease at 9 weeks of diabetes ($p<0.01$) (Fig 1). Calculating the tubular reabsorption of HSA after 3 weeks of diabetes indicates a significant (6.7%) decrease from 95.2±1.1 per cent in the control to 88.8±2.3 per cent ($p<0.05$) and a 14% reduction at 9 weeks of diabetes (to 82±2.2 per cent $p<0.05$). The tubular reabsorption of nHSA was 99.2±0.1 per cent in the control groups and decreased in the diabetic situation to 97.7±0.4 ($p<0.05$) and 95.5±0.5 ($p<0.05$) per cent after 3 weeks and 9 weeks, respectively. θ for HSA was calculated by dividing the HSA clearance by the corresponding average GFR assessed in the parallel rats used for this purpose (see data above). θ for HSA was significantly increased only after 9 weeks of diabetes (Table 1), while θ for nHSA was slightly, but significantly, reduced at both 3 and 9 weeks of diabetes.

θ for FITC-Ficoll

To evaluate whether the increase in clearance of native albumin after 9 weeks of diabetes was due to a reduced charge selectivity or to an altered number of large pores, we investigated the sieving coefficients for FITC-Ficoll in the molecular range of 15-90Å. Our data show that after 9 weeks of diabetes there was indeed a significant increase in θ for molecules larger than 55Å in radius (Fig 2).

Two-pore parameters

The fractional ultrafiltration coefficient accounted for by the large pores (α_L) was calculated for HSA using the two-pore model assuming a filtration pressure of 9 mm Hg (to assess the LpS), a $\Delta\pi$ of 28 mmHg and a large pore radius of 112 Å (120Å minus a Debye length of 8Å for neg. charged HSA) . The "apparent" α_L, calculated from the θ for HSA, was significantly increased only after 9 weeks of diabetes (Fig 3). The parameters generated from the best fit of the measured Ficoll sieving curves to the two-pore model are shown in Table 2. After 9 weeks of STZ-induced diabetes α_L had increased two-fold compared to that in the control group (Fig 3), indicating an increased fraction of large pores in the glomerular filter. Further, in the diabetic animals the capillary pore area ($A_0/\Delta x$) remained unchanged, while the small pore radius (r_s) decreased very slightly (but significantly) from 46.1±0.06 Å to 45.3±0.14 Å compared to control ($p<0.05$).

Statistics

Statistical significance between groups was tested using a one-way ANOVA with the significance level set to $p<0.05$. Comparison between means was performed by Bonferroni´s correction.

DISCUSSION

This is the first comprehensive study investigating the early functional changes of the glomerular filtration barrier in streptozotozin (STZ)-diabetic in rats *in vivo*. The results demonstrate that in the very early stage of DNP (3 weeks) there was no change in glomerular barrier function whatsoever. However, after 9 weeks of poorly controlled diabetes (blood glucose ~20mM) a decreased clearance ratio of neutralized albumin (nHSA) to native albumin (HSA) was noticed, at first suggesting a reduction in the amount of negative charges in the glomerular barrier. Further, concomitantly with the decreased nHSA/HSA ratio, we observed an increased clearance of large Ficoll molecules (>55Å), exactly matching the increased excretion of HSA, suggesting that the main alteration in the glomerular barrier was an increase in the large pore number.

In humans overt proteinuria due to DNP seldom appears until a decade or so has elapsed from the onset of type-1-diabetes mellitus. The first sign of glomerular dysfunction is microalbuminuria. This early excretion of (neg.) albumin has been ascribed mainly to loss of anionic sites of the glomerular filtration barrier, according to the so called "Steno hypothesis". Indeed, a decreased selectivity index, i.e. a reduced ratio between (neutral) IgG or (neutral) α_2-macroglobulin and albumin (negatively charged), has been observed in several studies in microalbuminuric patients (4, 12, 32), and seems to support the concept of an alteration in the charge barrier. Furthermore, favoring the "Steno hypothesis" is the finding of a decreased amount of heparan sulphate (HS) side chains of the glomerular basement membrane (GBM) proteoglycans in patients with DNP (36), a decrease that is correlated to the degree of albuminuria (27). It should be noted, however, that the selectivity index is assessed in urine modified by tubular reabsorption, and does not represent the ratio of protein concentrations in the primary urine. Furthermore, even though the enzyme heparanase was found to be

increased in STZ-induced diabetic rats, this was actually not found to imply a significant reduction in the GBM HS content (37). Further, no change in HS sulfation or charge density has been detected in early STZ-induced diabetes (35). Hence, the contribution of a decreased charge selectivity in the urinary hyperexcretion of albumin in early DNP is controversial.

The early changes occurring in the kidney after the onset of diabetes is nephron hypertrophy and an increase in GFR and RPF. In accordance with this we found that GFR was increased compared to control in the very early development of STZ-induced DNP (3 weeks), both in the basal situation and after volume expansion At nine weeks of diabetes duration, however, the increment in GFR, both before and after volume expansion, was markedly blunted, in agreement with previous data (17). At 3 weeks of diabetes we did not find any increase whatsoever in the glomerular clearance of either native or neutralized albumin compared to control. This indicates that no deterioration of the glomerular barrier function had occurred at this point. However, we found a reduction in tubular reabsorption of both native and neutralized albumin ($p<0.05$) after three weeks of diabetes that was further enhanced at 9 weeks of diabetes. Note, that a reduction in fractional tubular albumin reabsorption from 95-96 per cent to 89 per cent, as found in the present study, at an unchanged GFR, would imply a near three-fold increment in albumin excretion, adding to the increases in albumin excretion that would follow upon increments in GFR. Actually, we found a sevenfold increment in rat albuminuria at 3 weeks of diabetes duration which, at least partly, may be explained by a combination of reduced proximal tubular albumin reabsorption and an increased GFR. These results are in essential agreement with the findings of Tojo *et al*. who showed, using a careful micropuncture technique, that the reabsorption of albumin in the proximal tubule was reduced, suggesting a dysfunction in the proximal tubular endocytotic process at early stages of diabetes (29). After 3 weeks of diabetes the reduction in proximal tubular reabsorption of

HSA vs nHSA was 6.7 and 1.6 per cent, respectively, and after 9 weeks 13.9 vs 3.7 per cent. Hence, we found that the reabsorption of HSA, compared to its neutral counterpart, was reduced. Thus microalbuminuria may be chiefly secondary to alterations in the proximal tubular protein reabsorption, affecting negatively charged albumin more (18) than neutral and larger macromolecules (IgG or α_2-macroglobulin). In fact, the renoprotective effets of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) seem not only confined to reductions in glomerular hyperfiltration but have recently also been shown to restore albumin reabsorption in the proximal tubules (30).

The glomerular barrier size-selectivity is bimodal, in that the glomerular filter can be described as a membrane having two different size selective pathways (21), "small pores" (r_s ~37.5 Å in radius) and a low number of "large pores" (r_L~120Å in radius, comprising one part per million of the "small pores")(13, 28). According to the two-pore concept native albumin, due to its net negative charge, is excluded from the small pores and normally passes to the urine exclusively through the large pore pathway. If the negative charges in the small pore pathway were to decrease, this would lead to an increased glomerular passage of native albumin. Neutralized albumin (nHSA), on the other hand, due to its lack of net negative charge, passes mainly through the small pores, and should be only marginally affected by a change in pore charge. This is because pore charge critically influences the effective pore radius to negatively charged species in the small pores but not to a significant extent in the large pores according to the Debye-Hückel theory of ion-ion interaction (21). In the present study the sieving coefficient (θ) of nHSA was approximately 10 fold higher than that of HSA. After nine weeks of diabetes duration the clearance of HSA increased 2-fold, whereas that of neutralized albumin was unchanged, yielding a reduced nHSA/HSA ratio. This could, in theory, be interpreted as a decrease in the negative charge of the small pores. However, the

sieving coefficients (θ) for neutral FITC-Ficoll molecules in the SE- radius range of >55Å was significantly increased (Fig 2) at this point, suggesting an increased macromolecular flux through the large-pore pathway, while the r_L was not significantly different from control. In line with this, the fractional ultrafiltration coefficient (LpS) accounted for by the large pores (α_L) was 2 fold higher in 9 weeks of DNP compared to control rats. Calculating an "apparent" α_L from the HSA clearance data yielded an almost identical increase in α_L as obtained with Ficoll (Fig 3), implying that the main structural change of the filtration barrier in early STZ-induced diabetes must have been an increase in the number of large pores and not primarily a decrease in the negative charge of the glomerular filter.

Among diabetic patients with overt proteinuria a reduced size selectivity of the glomerular barrier has been commonly noted using dextran as a macromolecular marker (7, 14, 24, 31). However in patients with microalbuminuria, clear evidence for a decreased size selectivity comes from one previous study only, where the more sensitive glomerular sieving probe, Ficoll, was employed (1). Dextran has been shown to be hyperpermeable through the glomerular filter compared to either proteins or Ficoll (5, 20). Furthermore, we have shown that also Ficoll molecules appear hyperpermeable through the (small) pores of the glomerular filter when they approach the pore radius in size (a_e>60% of r_s). However, we also found, somewhat surprisingly, that the permeability of Ficoll in the size range of 55-75Å (when a_e<60% of r_L) reflects the sieving of large proteins (of equivalent size) across the large pores (23). Actually, to be able to accurately measure the glomerular passage of very large Ficoll molecules we have introduced a mixture containing high concentrations of high-MW Ficoll (Ficoll-400kDa) and a lower amount of Ficoll-70kDa molecules. Thus, by enhancing the concentrations of very large Ficoll molecules in both plasma and urine, we have produced a

very sensitive method for assessing glomerular sieving of Ficoll molecules larger than 55Å in radius.

The results from the present study are partly in contrast to recent long-term experiments, in which the insulin-dependent diabetic NOD-mice were investigated with regard to glomerular permselectivity, also utilising Ficoll as a glomerular size probe. In these mice the sieving coefficients for $Ficoll_{12\text{-}70Å,}$ measured in the cooled isolated perfused kidney (cIPK), did not increase in the diabetic mice, neither after 10, nor after 40 weeks of diabetes duration (9). At the same time the clearance of albumin was increased 3 fold at 40 weeks of diabetes, indicating a defect in the charge barrier. However, in the cIPK the number of large pores is much higher than measured *in vivo* (10), making this model less sensitive to changes in the large pore number compared to the model used in the present study. As a result changes in size selectivity to very large macromolecules may have been overlooked in the cIPK.

Podocyte integrity is very important for the glomerular barrier function because podocytes "embrace" the capillaries and stabilize the whole glomerular barrier. Since the discovery of nephrin in 1998 and the loss of barrier function associated with the mutation in the nephrin gene (11), the podocyte slit diaphragm (PSD) has been considered to play a major role in the size selective properties of the glomerular barrier. However, a recent study indicates that the major hindrance to macromolecules is most probably located more proximally to the PSD, i.e in the endothelium or the GBM (8, 22). Although injury to any of the three layers of the glomerular filter (the endothelial cells, the GBM or the podocytes) results in loss of glomerular size selectivity, it is important to stress the integrity of all layers acting in concert for the barrier to function properly. In a recent study Siu *et al* (26) showed that STZ-diabetic rats with no insulin substitution had a decreased podocyte number already after two weeks.

Treating the rats with insulin for 6 weeks resulted in a much milder phenotype, where the podocyte number was not significantly reduced compared to control. It is reasonable to speculate that, after 9 weeks of poorly controlled diabetes, the podocyte density would be somewhat reduced, which in part could be responsible for a disintegration of the glomerular barrier, reducing its size selectivity.

In conclusion, the present study indicates that in the very early stage of STZ-diabetes albuminuria is due to a reduced proximal tubule reabsorption and not to functional alterations in the glomerular filtration barrier. However, this stage is followed by an increase in glomerular albumin clearance, primarily resulting from a reduced size selectivity of the glomerular filter.

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Table 1

Parameter	Control 3w	Diabetes 3w	Control 9w	Diabetes 9w
Body weight (g)	394±9g	245±5g*	367±9	266±7g*†
Kidney weight (g)	2.65±0.10	2.28±0.07	2.14±0.08	2.47±0.10
GFR (ml/min/g kidney)	0.818±0.069	1.21±0.101*	0.719±0.172	0.595±0.088†
GFR (ml/min/g kidney) Volume expansion	0.965±0.151	1.71±0.086*	1.20±0.071	1.24±0.074†
GFR (ml/min/100g BW)	0.474±0.077	1.07±0.082*	0.448±0.089	0.536±0.079†
GFR (ml/min/100g BW) volume expansion	0.559±0.100	1.52±0.070*	0.721±0.050	1.12±0.078*†
RPF (ml/min/g kidney)	3.57±0.157	3.13±0.286	2.83±0.551	2.43±0.217
RPF (ml/min/g kidney) vol. expansion	5.34±0.314	4.83±0.314	4.97±0.361	4.34±0.277
Filtration fraction (FF)	0.247±0.035	0.341±0.047*	0.279±0.0098	0.237±0.039
Filtration fraction (FF) vol. expansion	0.227±0.017	0.352±0.013*	0.269±0.0089	0.268±0.034
ACR	1.26±0.20	9.16±1.63	1.04±0.17	13.6±3.79
Clearance HSA (ml/min/g kidney) $\times 10^4$	3.32±0.96	3.46±0.149	4.60±0.714	7.21±0.315*†
Clearance HSA (ml/min/100g BW) $\times 10^4$	2.34±0.725	3.43±0.183	2.69±0.435	6.26±0.367
Clearance nHSA (ml/min/g kidney) $\times 10^4$	39.8±9.84	43.3±1.51	49.1±7.62	38.4±1.48
Clearance nHSA (ml/min/100g BW) $\times 10^4$	27.7±6.95	42.9±1.62	28.3±3.69	33.3±1.88
θ HSA $\times 10^4$	5.08±1.49	2.86±0.143	4.88±0.701	12.2±0.810*†

θ nHSA $\times 10^4$	76.0±2.85	35.7±1.21*	76.5±7.58	64.0±2.34†

* indicates statistical difference ($p<0.05$) between the diabetic and the control group at each time point and † indicates statistical difference between the two diabetic groups.

Table 2

Two-pore parameters	Control 9 weeks (n=8)	Diabetes 9 weeks (n=13)
Small pore radius (Å)	46.1±0.06	45.3±0.14*
Large pore radius (Å)	116±5.4	126±3.5
α_L ($\times 10^5$)	3.63±0.95	7.52±1.1*
$A_0/\Delta x$ (cm/g) ($\times 10^{-5}$)	5.13±0.34	4.96±0.43
Jv_L/GFR ($\times 10^5$)	7.77± 1.4	20.6±2.7*
GFR (ml/min/g kidney)	0.56±0.024	0.64±0.060

* indicates statistical difference ($p<0.05$) between the diabetic and the control group

FIGURE LEGENDS

Figure 1.

After 3 and 9 weeks of poorly controlled diabetes, the clearance of nHSA and HSA were determined simultaneously, using a tissue uptake technique, and compared to age matched controls. The nHSA/HSA ratio was significantly reduced after 9 weeks of diabetes.

Figure 2.

Sieving coefficients (θ) v.s SE-radius (a_e), plotted in a semi logarithmic diagram for Ficoll (black line -control; dashed line-diabetes) and HSA (open square-control; filled triangle-diabetes) after nine weeks of poorly controlled diabetes compared to age matched controls. Ficoll θ are given for 425 data points between 15 and 90 Å and θ for HSA is given at 36 Å.

Figure 3.

Relative fractional ultrafiltration coefficient (UF) accounted for by the large pores (α_L) in % for Ficoll and native albumin (HSA). α_L for Ficoll was obtained from the two pore model. α_L for HSA was calculated assuming a ΔP of 9 mm Hg, a $\Delta\pi$ of 28 mmHg and a large pore radius of 112Å (120 minus 8Å, due to the negative charge of albumin) as obtained from the two-pore fit of the Ficoll data (see Table 2).