Published in final edited form as:

Diabetes Metab Res Rev. 2011 May ; 27(4): 341–347. doi:10.1002/dmrr.1192.

Glomerulopathy in Spontaneously Obese Rhesus Monkeys with Type 2 Diabetes: A Stereological Study

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Abstract

Background—Animal models provide insights into the diabetic nephropathy pathogenesis, however, available rodent models do not mirror the heterogeneity of lesions in type 2 diabetic (T2DM) patients, and do not progress to end stage renal disease. Previous studies showed that spontaneously obese T2DM rhesus monkeys develop many of the features of human diabetic glomerulopathy, and may progress to ESRD. Here, in order to further characterize diabetic glomerulopathy in this model, we used electron microscopic stereology..

Methods—Renal biopsies from 17 diabetic, 17 pre-diabetes/metabolic syndrome (preDM/MS) and 11 non-diabetic monkeys were studied. Fractional volumes of mesangium [Vv(Mes/glom)], mesangial matrix [Vv(MM/glom)] and mesangial cells [Vv(MC/glom)], glomerular basement membrane (GBM) width and peripheral GBM surface density per glomerulus [Sv(PGBM/glom)] were estimated. Glomerular filtration (GFR) and albumin excretion rates (AER) were measured in a limited number of animals. Glomerular structural and biochemical/metabolic data were compared among the groups.

Results—Diabetic monkeys showed classical diabetic nephropathy changes, including GBM thickening ($p=0.001$), increased Vv(Mes/glom) ($p=0.02$), and reduced Sv(PGBM/glom) ($p=0.03$) compared to non-diabetic monkeys. Increased Vv(Mes/glom) was primarily due to increased Vv(MM/glom) ($p=0.03$). Glomerular structural parameters inter-relationships in diabetic monkeys mirrored those of human diabetic glomerulopathy. AER was greater ($p=0.03$) in diabetic vs. non-diabetic monkeys. There was evidence for a positive relationship between AER and Vv(Mes/glom).

Conclusions—These studies indicate that this primate model shares many features of human diabetic glomerulopathy. Mesangial expansion in this model, similar to human diabetic nephropathy and different from available rodent models of the disease, is primarily due to increased mesangial matrix.

Keywords

Diabetic Nephropathy; Rhesus Monkeys; Stereology; Diabetes Mellitus; Metabolic Syndrome; Type 2 Diabetes

Introduction

Diabetic nephropathy causes more than 45% of end-stage renal disease (ESRD) in the US [1] with over 80% of diabetic nephropathy ESRD cases being secondary to type 2 diabetes (T2DM) [1]. Further, the incidence of T2DM-induced ESRD has markedly increased [1], despite improved glycemic and blood pressure control, and the emergence of promising drugs, such as renin-angiotensin system blockers. Therefore, new insights into the diabetic nephropathy pathogenesis and treatment are needed. Animal models could provide crucial insights concerning mechanisms and potential therapeutic targets. Currently available rodent models do not reliably reflect the complexity and heterogeneity of human T2DM nephropathy [2]. Herein, we argue that the spontaneously obese and naturally T2DM rhesus monkey represents a major advance as an animal model of diabetic nephropathy. These primates, when protected from their natural predators while having free access to food, frequently become obese [3] and may subsequently develop metabolic syndrome and overt T2DM which is metabolically indistinguishable from human obesity-associated T2DM [4–6]. Our previous studies have documented glomerular hyperfiltration, glomerular hypertrophy and glomerular basement membrane (GBM) thickening in these T2DM monkeys [7]. The present work extends these initial studies and demonstrates significant increases in mesangial matrix in diabetic animals. Moreover, we found glomerular diabetic nephropathy changes in pre-diabetic/metabolic syndrome (preDM/MS) monkeys.

Subjects and Methods

Animals

Kidney biopsy specimens from 17 diabetic, 17 preDM/MS and 11 non-diabetic obese animals from a colony of outbred rhesus monkeys (*Macaca mulatta*) were studied. The monkeys were housed in individual stainless steel primate cages in accordance with the NIH recommendations for the Care and Use of Laboratory Animals. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Maryland and the University of South Florida.

Laboratory Studies

All metabolic and biochemical studies and blood pressure measurements were performed after a 16 hour overnight fast. Blood pressure was in supine position under light ketamine anesthesia, using a Dynamap blood pressure monitor (Critikon, Johnson & Johnson, Tampa, FL) placed on the right upper arm. Intravenous glucose tolerance testing (IVGTT) was performed as detailed elsewhere [8]. PreDM/MS status was defined as the presence of all of the following: hyperinsulinemia or insulin resistance, normoglycemia or impaired or reduced glucose tolerance, obesity [(body weight >15 kg; body fat >22% by Lunar dual X-ray energy absorptiometry (DXA)], and dyslipidemia (elevated triglycerides and low HDL cholesterol). Diabetes was defined as at least two fasting plasma glucose (FPG) ≥ 126 mg/dL, in accordance with the ADA guidelines for human diagnosis. Acute insulin response (AIR) was calculated as the mean change in plasma insulin per minute from basal levels for the period 0–10 min during the IVGTT [9]. FPG and insulin were measured every 6 months in the non-diabetic monkeys and glucose was tested 1–2 times daily in the diabetic monkeys. Diabetic monkeys received daily subcutaneous insulin injections to maintain plasma glucose

levels at approximately 140 to 240 mg/dL. Plasma glucose, serum cholesterol, triglyceride and creatinine were measured quarterly using standard laboratory methods. Plasma insulin was measured by double-antibody radioimmunoassay. Glomerular filtration rate (GFR) was measured in 4 diabetic, 6 preDM/MS and 6 non-diabetic obese monkeys using the Iohexol method. Albumin excretion rate (AER) was measured using an immunoassay [10] in 6 diabetic, 10 preDM/MS and 5 non-diabetic obese animals.

Quantitative Renal Structural Studies

Renal tissues were fixed in 2% glutaraldehyde, and embedded in Epon. Semi-thin sections were prepared to provide three random glomeruli per animal as previously described [11]. Ultrathin sections were studied using a JEOL C100 electron microscope. Overlapping digital images at 3,900 $\times$ were obtained to make montage images of the entire glomerular profiles. Poorly fixed biopsies and glomeruli with biopsy-related trauma were excluded by a masked observer. Mesangial fractional volume per glomerulus [Vv(Mes/glom)] was estimated by point counting and surface density of peripheral GBM per glomerulus [Sv(PGBM/glom)] by line intercept counting as previously described [11,12]. Systematic uniform random images of glomeruli at 11,700 $\times$ were used to estimate mesangial matrix [Vv(MM/glom)] and mesangial cell [Vv(MC/glom)] fractional volumes per glomerulus by point counting, and GBM width using the orthogonal intercept method [11,13]. All measurements were performed by a masked observer.

Statistics

Data are presented as mean $\pm$ SD, except for age, serum triglyceride and cholesterol, which are presented as median (range). Group comparisons were performed by ANOVA and least squared distance method for normally distributed variables, and otherwise by the Kruskal-Wallis test. Pearson and Spearman correlations were used where indicated. Values with $p \leq 0.05$ were considered statistically significant.

Results

Demographic and Clinical Parameters

Age was greater in the diabetic monkeys [median: 23 (15–38 years)] *vs.* non-diabetic animals 14 (10–30)] ($p=0.003$), but was not different between diabetic and preDM/MS [23 (12–29)] monkeys (Table 1). Diabetic monkeys had 3.9 ± 1.9 (range 0.3 to 8.0) years of T2DM. The duration of prediabetes, if defined as the period of slight elevation of glucose (100 to 126 mg/dL) and impaired glucose tolerance was one to two years; and if included the insulin resistant/dyslipidemic state was 5 to 8 years. Body weight and mean systolic and diastolic blood pressures were not different among the groups (Table 1). FPG levels were higher in diabetic *vs.* non-diabetic and preDM/MS monkeys ($p<0.00001$), but were similar between non-diabetic and preDM/MS monkeys (Table 1). FPG correlated directly with body weight in non-diabetic monkeys ($r=0.62$, $p=0.04$), and with serum cholesterol ($r=0.49$, $p=0.048$) and triglyceride ($r=0.56$, $p=0.02$) levels in diabetic monkeys. Plasma insulin was significantly higher in preDM/MS monkeys compared to non-diabetic ($p=0.0002$) and diabetic animals ($p=0.0001$) (Table 1). Plasma insulin levels were inversely correlated with serum HDL in diabetic monkeys ($r=-0.84$, $p=0.008$). Acute insulin response was directly correlated with serum VLDL in non-diabetic monkeys ($r=0.91$, $p=0.002$). Acute insulin response (AIR) was significantly reduced ($p=0.0004$) in diabetic monkeys compared to preDM/MS animals (Table 1). Serum triglyceride levels were greater in diabetic compared to both non-diabetic ($p=0.001$) and preDM/MS ($p=0.001$) monkeys (Table 1). Serum cholesterol and triglyceride levels were tightly correlated in diabetic monkeys ($r=0.94$, $p<0.00001$). Serum LDL was not statistically different among the groups. Serum HDL was lower in diabetic ($p=0.0003$) and preDM/MS ($p=0.02$) compared to non-diabetic monkeys

(Table 1). Conversely, serum VLDL levels were higher in diabetic compared to non-diabetic ($p=0.005$) and preDM/MS ($p=0.006$) monkeys. HbA_{1C} was higher in diabetic ($N=8$) compared to non-diabetic ($N=7$) ($p<0.00001$) or preDM/MS ($N=15$) ($p<0.00001$), but not different between non-diabetic and preDM/MS monkeys. GFR values in 4 DM, 6 PreDM/MS and 6 non-diabetic monkeys were not significantly different due to the wide ranges in these small groups. On a human scale for AER classification (normoalbuminuria: AER <20 $\mu\text{g}/\text{min}$; microalbuminuria: AER $20\text{--}200$ $\mu\text{g}/\text{min}$; macroalbuminuria: AER >200 $\mu\text{g}/\text{min}$), two out of the six T2DM monkeys with AER measurements were macroalbuminuric and one was microalbuminuric; three out of ten preDM/MS monkeys with AER measurements had mild macroalbuminuria (maximum AER = 255 $\mu\text{g}/\text{min}$) and one had microalbuminuria; while none of the non-diabetic monkeys had micro- or macroalbuminuria (Figure 1). AER values were greater ($p=0.03$) in DM ($N=6$) [$67.3(1.6\text{--}539.3)$ $\mu\text{g}/\text{min}$] vs. non-diabetic obese ($N=5$) [$1.6(0.3\text{--}4.5)$ $\mu\text{g}/\text{min}$] monkeys. AER values were directly correlated with serum triglyceride ($r=0.73$, $p<0.05$) and VLDL ($r=0.69$, $p<0.05$) and inversely correlated with serum HDL ($r=-0.56$, $p<0.05$) values.

Renal Structural Parameters

Electron micrographs revealed changes similar to those seen in human diabetic glomerulopathy, including mesangial expansion and GBM thickening (Figure 2). No changes suggestive of diseases other than diabetic nephropathy were identified. Glomeruli of non-diabetic monkeys appeared qualitatively normal.

GBM width was increased in diabetic vs. non-diabetic ($p=0.001$) and in diabetic vs. preDM/MS monkeys ($p=0.005$) (Table 1). There was no significant difference in GBM width between non-diabetic and preDM/MS monkeys. $V_v(\text{Mes}/\text{glom})$ was increased in diabetic compared to non-diabetic monkeys ($p=0.02$). This was primarily due to increased $V_v(\text{MM}/\text{glom})$ ($p=0.03$) rather than $V_v(\text{MC}/\text{glom})$ which was not significantly different among groups (Table 2). There was also a trend toward greater $V_v(\text{Mes}/\text{glom})$ in preDM/MS monkeys compared to non-diabetic animals ($p=0.06$). $S_v(\text{PGBM}/\text{glom})$ was significantly reduced in diabetic compared to non-diabetic monkeys ($p=0.03$).

GBM width correlated directly with $V_v(\text{Mes}/\text{glom})$ ($r=0.62$, $p=0.008$) and $V_v(\text{MM}/\text{glom})$ ($r=0.51$, $p=0.04$) and was inversely correlated with $S_v(\text{PGBM}/\text{glom})$ ($r=-0.71$, $p=0.02$) in diabetic monkeys. $V_v(\text{Mes}/\text{glom})$ was inversely correlated with $S_v(\text{PGBM}/\text{glom})$ ($r=-0.70$, $p=0.002$) in diabetic monkeys.

Relationships between Clinical and Renal Structural Parameters

Body weight was correlated with $V_v(\text{Mes}/\text{glom})$ ($r=0.64$, $p=0.03$) and $V_v(\text{MM}/\text{glom})$ ($r=0.69$, $p=0.02$) in non-diabetic monkeys. Fasting plasma glucose was directly correlated with GBM width ($r=0.60$, $p=0.01$), $V_v(\text{Mes}/\text{glom})$ ($r=0.65$, $p=0.005$) and $V_v(\text{MM}/\text{glom})$ ($r=0.80$, $p<0.00001$) and inversely correlated with $S_v(\text{PGBM}/\text{glom})$ ($r=-0.71$, $p=0.001$) in diabetic monkeys, but not in the other groups. Plasma insulin was correlated with GBM width ($r=0.69$, $p=0.03$) in non-diabetic obese monkeys. Acute insulin response was directly correlated with $V_v(\text{Mes}/\text{glom})$ in non-diabetic ($r=0.76$, $p=0.03$) and inversely correlated with this parameter in diabetic ($r=-0.56$, $p=0.02$) monkeys. Acute insulin response was also inversely correlated with $V_v(\text{MM}/\text{glom})$ ($r=-0.51$, $p=0.04$) in diabetic monkeys. Serum triglyceride levels were directly correlated with $V_v(\text{MM}/\text{glom})$ in non-diabetic ($r=0.64$, $p=0.03$) and diabetic ($r=0.57$, $p=0.02$) monkeys. When preDM/MS and diabetic monkeys were combined, serum triglyceride levels were also directly correlated with GBM width ($r=0.46$, $p=0.006$) and $V_v(\text{MM}/\text{glom})$ ($r=0.49$, $p=0.004$), and inversely correlated with $S_v(\text{PGBM}/\text{glom})$ ($r=-0.35$, $p=0.045$). Serum VLDL was directly correlated with GBM

width in non-diabetic ($r=0.62$, $p=0.04$) and with $V_v(\text{MC}/\text{glom})$ in diabetic ($r=0.68$, $p=0.003$) monkeys.

AER showed a trend towards correlation with $V_v(\text{Mes}/\text{glom})$ ($r=0.43$, $p=0.06$) and towards an inverse correlation with $S_v(\text{PGBM}/\text{glom})$ ($r=-0.41$, $p=0.07$) in the entire cohort. There was significant direct correlation between $V_v(\text{MM}/\text{glom})$ ($r=0.45$, $p=0.04$) and AER in this cohort. There was no correlation between GFR (measured in a small number of monkeys: 4 DM, 6 PreDM/MS and 6 non-diabetic monkeys) and any of the glomerular structural parameters measured in this study.

Discussion

Diabetic nephropathy natural history, especially in T2DM patients, is incompletely understood. The most careful longitudinal studies have been carried out with the Pima Indians, where patients were repeatedly evaluated over time, and thus the onset of T2DM was known [14,15], while in ordinary practice, T2DM may be undetected for months to years, and renal lesions may be well established at the time of DM diagnosis. Moreover, renal biopsies are not routinely obtained during the early stages of the disease or in patients with metabolic syndrome. Even once clinical signs of renal disease are detectable, biopsies are generally limited to clinically atypical cases, which may lead to observations not extendable to the typical T2DM patient [17]. Therefore, an animal model with close resemblance to human T2DM nephropathy could be of great advantage to determine the natural history and to understand the mechanisms of this disease, as well as for evaluation of potential therapeutic interventions. While murine models have suggested possible mechanisms underlying diabetic nephropathy, the characteristics of the disease in mice and rats differ from the human disorder. Aside from marked differences in size and life span, other limitations of rodent models, including absence of progressive renal insufficiency impose difficulties in extrapolating the findings to humans [21]. Newer rodent models of DN are under development, but to date, do not adequately mimic human diabetic nephropathy.

Non-human primate models, although expensive and therefore limited in the numbers of animals that can be studied, carry significant genetic and phenotypic similarity to humans, and are not inbred. The progression from obesity to insulin resistance and T2DM has been well characterized in longitudinal studies in aging rhesus monkeys, which shares many of the features of human T2DM [5]. Renal failure and moderate-to-heavy proteinuria has been reported in non-insulin-dependent diabetic chimpanzees [23]. Although T2DM duration in monkeys in the present study was too short to observe ESRD and the available GFR data was limited to draw any meaningful statistical comparison among the groups, we have observed ESRD in other rhesus monkeys in our colony with naturally occurring diabetes (unpublished data). Hypertriglyceridemia, increased VLDL and reduced HDL in diabetic monkeys showed that these animals share similar lipid profile abnormalities with human T2DM patients.

We confirmed increased GBM width in diabetic monkeys. Previous studies of rhesus monkeys and baboons made diabetic with streptozotocin injections or total pancreatectomy showed GBM thickening and mesangial expansion after 2 to 12.5 years of diabetes [24–26]. Poorly controlled diabetic female baboons showed GBM thickening and increased $V_v(\text{Mes}/\text{glom})$ 9–12 years following pancreatectomy and developed lesions similar to human diabetic nephropathy [27]. Our study, for the first time, presents the results of unbiased electron microscopic stereological measurements of diabetic glomerulopathy structural parameters in spontaneously T2DM monkeys. The limited available data from research human T2DM renal biopsies makes exact comparison of glomerular structural changes in

the present T2DM monkeys with those of human challenging. Nevertheless, we documented lesions characteristic of diabetic nephropathy in this non-human primate model. The glomerular structural parameters of non-diabetic monkeys were remarkably close to those seen in normal adult humans [28]. The increased Vv(Mes/glom) and GBM thickening and the reduced Sv(PGBM/glom) documented in the diabetic monkeys in the present study are similar in magnitude to changes that we have previously described in human diabetic nephropathy of type 1 and 2 diabetic patients [11,29–31]. GBM thickening in spontaneously obese T2DM rhesus monkeys with diabetes duration comparable to the present study was previously shown by Cusumano *et al* [7]. However, these authors found only a non-statistically significant trend toward mesangial expansion by subjective light microscopy assessment in diabetic monkeys [7], while we identified a clear increase in Vv(Mes/glom) in diabetic animals. This probably reflects the value of electron microscopy stereological methodologies to detect the early structural changes of diabetic nephropathy. Perhaps the most important finding of our study was that increased Vv(Mes/glom) in diabetic monkeys was primarily due to increased Vv(MM/glom), while Vv(MC/glom) was not statistically different among the groups. This is similar to human diabetic glomerulopathy and different from most rodent models of this disease, where mesangial cells and matrix increase in parallel [32,33]. Although precise renal functional studies (AER and GFR) were performed in a limited number of monkeys in this study, direct correlation between AER and Vv(MM/glom) and the trend of relationship between AER and Vv(Mes/glom) were similar to human DN. These relationships need to be confirmed in a larger group of animals. In addition, studies to explore if biochemical changes associated with human diabetic nephropathy (i.e., increased protein kinase C, oxidative stress, etc.) are also present in this model will be important to complement these structural observations.

We also observed a trend toward increased Vv(Mes/glom) in preDM/MS compared to non-diabetic monkeys, which suggests that glomerular structural changes may begin before the onset of overt diabetes as defined by current guidelines. On the other hand, the mechanisms of renal injury by the different components of metabolic syndrome, such as abnormal glucose metabolism, hypertension, and dyslipidemia may be different from one another and not a continuum of pre-diabetic to diabetic status. The relationship between these traits and changes in glomerular structure is, however, beyond the scope of the present study, and would require prospective longitudinal observations and repeated kidney biopsies in a larger number of animals beginning with pre-diabetes or earlier.

The present study showed that increased body weight was related to increased Vv(Mes/glom) and Vv(MM/glom) in non-diabetic monkeys. These relationships were not observed in preDM/MS or diabetic monkeys, probably due to the body weight loss that frequently occurs in overtly diabetic monkeys prior to initiation of insulin treatment, and/or emergence of other pathogenetic mechanisms related to diabetic nephropathy in preDM/MS and diabetic monkeys. Body weight loss may also be accounted for possible masking of blood pressure elevations in T2DM monkeys compared to non-diabetic animals (Table 1).

Epidemiologic studies have shown that insulin resistance and hyperinsulinemia are strong predictors of chronic kidney disease in non-diabetic individuals [36]. High insulin milieu, *per se*, was associated with glomerular hypertrophy and increased extracellular matrix accumulation in both non-diabetic and streptozotocin-induced diabetic rats [37]. The present study showed direct relationships between plasma insulin levels and GBM width, and between acute insulin response and Vv(Mes/glom) in non-diabetic obese monkeys, further supporting these previous light microscopy observations.

Dyslipidemia is also a known risk factor for chronic kidney disease [29,38], and is a part of both the metabolic syndrome/preDM and T2DM states in monkeys. However, the

mechanisms of dyslipidemia-induced renal injury are not clearly understood. The present study demonstrated multiple associations between dyslipidemia and glomerular structural parameters related to diabetic nephropathy. Additional metabolic-structural-functional studies of these monkeys, progressing from lean to obese to preDM/MS, and finally to T2DM are needed, which may unravel the mechanisms linking dyslipidemia to renal injury. These additional studies will also allow for comparisons of these structural-functional relationships with those of human T2DM.

Although the authors acknowledge that this is an expensive diabetic nephropathy model as compared to rodent models, multiple studies can be performed on the same colony to share the cost. In fact, the present model which includes many living diabetic and prediabetic monkeys and necropsy material is made available for multiple investigators.

In summary, this environmentally controlled outbred colony of rhesus monkeys, providing genetic heterogeneity similar to that seen in humans, could allow for a more realistic assessment of the interaction between genes and environment in the risk of disease. Moreover, the relatively large size of these animals permits frequent blood sampling and serial renal biopsies that would be more difficult in rodents and likely precluded in humans. In addition, non-human primate models could provide an excellent intermediate experimental platform to validate and extend pre-clinical studies of efficacy and toxicity before undertaking large human clinical trials aiming to prevent or halt the progression of diabetic nephropathy.

Acknowledgments

This study was partially supported by NIH grant NO1-AG3-1012 and HHSN263200800022C (to BC Hansen). We wish to thank Drs. Noni Bodkin, and Xenia Tigno for their assistance with the renal samples, Theresa Alexander for excellence in overseeing primate care, Thomas Groppoli for electron microscopy studies, and John M. Basgen for performing the stereological measurements. Dr. M. Luiza Caramori is supported by a Career Development Award from the Juvenile Diabetes Research Foundation.

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Figure 1. Albumin excretion rate (AER) in obese non-diabetic (ND), pre-diabetic-metabolic syndrome (preDM/MS) and diabetic (T2DM) monkeys. AER was greater in T2DM vs. ND monkeys ($p=0.03$).

Figure 2.

Electron micrographs (11,000 $\times$) of glomeruli from a non-diabetic (A) and a diabetic (B and C) monkeys. Increased mesangial (Mes) matrix (asterisk) and glomerular basement membrane (GBM) thickening is evident in B and C. Abbreviations: Cap = capillary; E = endothelial cell; PC = podocyte.

Table 1

Clinical data of non-diabetic, preDM/MS and diabetic rhesus monkeys.

	Non-diabetic n=11	PreDM/MS n=17	Diabetic n=17	p-value
Age (years)	14 (10–30)	23 (12–29)	23 (15–38)	0.003 *
BW (kg)	13±3	14±5	12±4	NS
FPG (mg/dL)	64±10	66±14	203±67	<0.00001 * [†]
HbA_{1C} (%)[‡]	4.2±0.1	5.0±1.4	9.1±1.4	<0.00001 * [†]
Insulin (μU/dL)	43±31	136±34	45±60	0.0002 [‡] , 0.0001 [†]
AIR (mU/L)	194±166	306±322	18±24	0.0004 [†]
SBP (mmHg)	136±27	125±23	128±20	NS
DBP (mmHg)	70±15	63±11	68±12	NS
Chol (mg/dL)	145 (128–245)	134 (93–184)	154 (104–598)	NS
LDL (mg/dL)	72±15	64±18	76±29	NS
HDL (mg/dL)	81±22	60±20	49±19	0.02 [‡] , 0.0003 *
TG (mg/dL)	55 (19–96)	179 (72–302)	242 (34–2858)	0.001 * [‡]
VLDL (mg/dL)	12±3	17±7	55±58	0.005 *, 0.006 [†]
Cr (mg/dL)	1.0±0.2	1.1±1.7	1.3±1.5	NS

Abbreviations: BW=body weight; FPG=fasting plasma glucose; AIR=acute insulin response; SBP=systolic blood pressure; DBP=diastolic blood pressure; TG=triglyceride; Chol=cholesterol; Cr=serum creatinine; * different by definition. Data are expressed as mean ± SD, except for TG which is median (range).

* diabetic vs. non-diabetic;

[†] diabetic vs. PreDM/MS;

[‡] PreDM/MS vs. non-diabetic.

[‡] measured in 8 diabetic, 15 preDM/MS and 7 non-diabetic monkeys.

Table 2

Electron microscopy glomerular structural parameters in non-diabetic, PreDM/MS and diabetic rhesus monkeys

	Non-diabetic n=11	PreDM/MS N=17	Diabetic n=17	p-value
GBM width (nm)	361±50	383±57	450±83	0.001 [*] , 0.005 ^{**}
Vv(Mes/glom)	0.18±0.05	0.21±0.05	0.22±0.06	0.02 [*] , 0.06 [†]
Vv(MM/glom)	0.09±0.02	0.11±0.03	0.12±0.04	0.03 [*]
Vv(MC/glom)	0.07±0.03	0.08±0.02	0.09±0.04	NS
Sv(PGBM/glom), (μm²/μm³)	0.15±0.02	0.14±0.03	0.12±0.02	0.03 [*]

Abbreviations: PreDM/MS=prediabetic/metabolic syndrome; GBM=glomerular basement membrane; Vv(Mes/glom)=glomerular mesangial fractional volume; Vv(MM/glom)=glomerular mesangial matrix fractional volume; Vv(MC/glom)=glomerular mesangial cell fractional volume; Sv(PGBM/glom)=surface density of peripheral GBM. Data are expressed as mean±SD;

*
diabetic vs. non-diabetic;

[†]
PreDM/MS vs. non-diabetic;

**
diabetic vs. PredM/MS