

Original Article

The ZSD rat: a novel model of diabetic nephropathy

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Received April 5, 2017; Accepted July 24, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: The ZSD rat is a new obese-diabetic rat model that expresses type 2 diabetes in the presence of an intact leptin pathway. During a long pre-diabetic state, the animals exhibit most of the features of metabolic syndrome including obesity, hyperlipidemia, hypertension, insulin resistance and decreased glucose disposal. The animals used in these studies were either allowed to become spontaneously diabetic at 16-30 weeks of age, or diabetes was induced with a diabetogenic diet. In the presence of either spontaneous or diet-induced diabetes, they develop progressive albuminuria as well as increases in other urinary markers of impaired renal function (kidney injury molecule-1 (KIM-1), β 2-microglobulin, clusterin and cystatin C). Typical morphological changes of nephropathy, such as glomerular capillary basement membrane thickening and podocyte effacement, accompany these marker increases. Lisinopril (ACEi) treatment (30 mg/kg/day via the diet) dramatically reduced diabetes-induced albuminuria by 85%, independent of the duration of diabetes or the initial albumin excretion. These results position the ZSD rat as a relevant model of diabetic nephropathy that can be treated with clinically effective compounds.

Keywords: Diabetes, kidney, nephropathy, renal, rat

Introduction

The physiologic environment of chronic hyperglycemia with obesity is associated with a variety of co-morbidities including cardiovascular disease, atherosclerosis, retinopathy, neuropathy and nephropathy. Prolonged exposure to hyperglycemia is now recognized as a major factor in the pathogenesis of diabetic complications in type 1 and type 2 diabetic patients [1, 2]. Chronic hyperglycemia results in accumulation of reactive oxygen species, pro-inflammatory cytokines and advanced glycation end-products (AGE) that ultimately induce end organ damage in vasculature, heart and kidneys [3]. Diabetic nephropathy (DN) is one of the most serious complications of diabetic microvascular changes [4], and is the most common cause of chronic kidney injury leading to end-stage renal disease (ESRD). Chronic hyperglycemia induces hemodynamic as well as metabolic changes in the diabetic kidney [5]. Oxidative stress resulting from hyperglycemia is associated with endothelial dysfunction and the development of hypertension [6]. Hypertension is identified in approximately 50% of patients with type 2 diabetes and metabolic

syndrome; the addition of hypertension accelerates the development of DN and ESRD [7]. The prevalence of chronic kidney disease is much higher in patients identified as pre-diabetic or diabetic compared with normoglycemic patients [8]. Insulin resistance, as a precursor to hyperglycemia, is also causative in DN due to the induction of vasoconstriction, sodium retention and arterial hypertension [9].

DN is characterized by albuminuria, increases in serum creatinine and a decreased glomerular filtration rate (GFR). Histological changes include glomerular mesangial expansion, glomerular basement membrane (GBM) thickening, excessive extracellular matrix proteins and fibrosis [7-9]. Severe diabetes is not necessary for the development of DN. Indeed, it has been shown that pre-diabetic patients with impaired glucose tolerance and insulin resistance, before onset of severe hyperglycemia, exhibit the same prevalence for development of chronic kidney disease as overtly diabetic patients [8]. Treatment options for diabetic nephropathy are directed toward lowering blood pressure and improving glycemic control [10]. Angiotensin II receptor blockers (ARB) and angiotensin II

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converting enzyme inhibitors (ACEi) have been the mainstay to stop or slow progression to ESRD [10-13].

Pre-clinical studies in DN are often carried out in diabetic rodents. The most common model is the STZ rat which employs a normoglycemic rat induced to become diabetic through injection of streptozotocin. This model has been used extensively to evaluate the mechanisms and potential interventions for DN [7, 14-16]. The rapid induction of type 1 diabetes in rats after a single dose of STZ (>45 mg/kg) increases blood glucose levels to more than 500 mg/dL within 48 hours [17], and is associated with rapidly developing renal damage. Streptozotocin can also be described as a direct renal toxin; it causes tubular and glomerular hypertrophy with mesangial expansion and thus exerts renal damage in addition to that caused by its induction of beta cell apoptosis and hyperglycemia [17]. The development of renal damage following STZ is likely dramatically different from the development of DN in patients with pre-diabetes/metabolic syndrome who slowly progress to type 2 diabetes.

Rat models with defective leptin receptors, such as the ZDF and ZSF1, have been characterized and used to evaluate compounds to treat DN [18-29]. However, although the ZDF rat does have kidney changes that appear to be specifically associated with the diabetic condition [23], it also has kidney hydronephrosis [30] that makes it less than an ideal model for DN. The ZSF1 rat has been used effectively for the study of DN, but it has the leptin receptor defects from both the ZDF and the SHHF rats [24] which, when combined, cause the obesity in this model. Although these established models have, and likely will continue, to be used in DN studies, the defects in the morphology of the kidney shown by the ZDF, and the leptin receptor defects present in both models are problematic for a translational model.

Mouse models have also been diligently sought as models featuring all of the characteristics of human DN. A recent review by Betz and Conway states that “no model exhibits with all of the features of human DN” [31]. As a consequence, the quest for ideal DN models continues.

The ZDSD/Pco (ZDSD) rat is a new model of obesity, metabolic syndrome and diabetes [32]. The model was developed by crossing a homo-

zygous lean Zucker diabetic fatty (ZDF) male rat with a sub strain of the CrI:CD (SD) rat, selectively bred for high fat diet induced obesity [33, 34]. The standard CrI:CD (SD) rat is a sub strain of SD rats that is significantly heavier and more obese than other lines of SD rats; a percentage of these rats is very susceptible to developing obesity, when fed high fat diets [33, 34]. The original design was to combine the defect in β -cell gene transcription that is found in lean and obese ZDF rats [35] with the obesity of the CrI:CD (SD) model, to produce an obese diabetic model that preserves the critical leptin pathway. The animals were fed regular rodent chow (Purina 5008) during the 12 years of the model development process. The offspring from the initial crosses were screened and selected for obesity, the propensity to become diabetic and the expression of the other characteristics of metabolic syndrome. This model has been selectively inbred for >30 generations. The ZDSD rat has been shown to develop microvascular and macrovascular complications of diabetes in a fashion similar to that occurring in human diabetes [36]. Spontaneous development of diabetic complications such as impaired wound healing [37], osteoporosis [38, 39], decreased nerve conduction velocity [36] and hypertension [40] have also been identified in the model.

Obesity and metabolic syndrome are clear predictors of chronic kidney disease largely due to the potentiation of chronic inflammation by insulin resistance [41]. In addition, the lipoprotein abnormalities and increased hemodynamics and vascular dysfunction associated with metabolic syndrome have all been implicated as causative for the development of renal disease [42, 43]. This cluster of metabolic dysregulations is exhibited by the ZDSD rat, positioning the model as a unique tool for the study of DN.

In validation of the ZDSD rat as a novel tool for the study of DN, we have characterized the spontaneous development of DN in the ZDSD rat and determined the efficacy of the ACEi, Lisinopril, in reducing albuminuria.

Materials and methods

Spontaneous development of renal dysfunction in untreated ZDSD rats

Male ZDSD/Pco rats ($n=16$) were obtained from the PreClinOmics, A Crown Bioscience compa-

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ny (now Crown Bioscience-Indiana), colony. They were maintained on Purina 5008 regular rodent chow from weaning and throughout the study duration. Beginning at 10 weeks of age, blood samples (fed) were collected from the tail vein for assessment of blood glucose, blood urea nitrogen (BUN) and creatinine. Twenty-four-hour urine samples were collected at room temperature and without additives. Blood and urine samples were obtained in animals every 2-4 weeks until 30 weeks of age. Animals were considered diabetic when morning blood glucose (fed) reached ≥ 250 mg/dL. Urine volume and body weight were recorded. Urine was assayed for cystatin C, clusterin, urinary kidney injury molecule-1 (KIM-1) and $\beta 2$ -microglobulin using Luminex multiplex kits RKT1-37K (clusterin, KIM-1) and RKT2-37K (albumin, $\beta 2$ -microglobulin, cystatin C). Kidneys were fixed in 10% buffered formalin and stained with periodic acid Schiff (PAS) and H&E for pathological assessment of untreated 33 week old animals. Age-matched SD rats ($n=10$) were included for comparison.

Kidneys from selected diabetic animals at 33 weeks of age, as described above, were also prepared for light microscopy. Representative sections were selected for microscopy.

Electron microscopy

Control Crl:CD (SD) ($n=2$) and ZSD male rats that were spontaneously diabetic for 12 ($n=2$) and 16.5 ($n=2$) weeks were perfused with 4% paraformaldehyde then fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate buffer. After fixation the specimens were rinsed with phosphate buffered saline (PBS) followed by post fixation with 1% osmium tetroxide in phosphate buffer for one hour. After rinsing with PBS, the tissue specimens were dehydrated through a series of graded ethyl alcohols from 70 to 100%. After dehydration, the specimens were infiltrated with 2 changes of 100% propylene oxide and a 50:50 mixture of propylene oxide and the embedding resin (Embed 812, Electron Microscopy Sciences, Hatfield, PA) overnight. The next day the specimens were transferred to fresh 100% embedding media for a minimum of 2 hours, then embedded in a fresh change of 100% embedding media. Following polymerization overnight at 60°C, the blocks were ready to be sectioned. Thin sections were cut (70-80 nm), stained with uranyl

acetate and lead citrate, then viewed on a Tecnai BioTwin (FEI, Hillsboro, OR) with digital images taken with an AMT (Advanced Microscope Techniques, Danvers, MA) CCD camera. Multiple measurements were taken (144 to 162 per group) using the software on the Tecnai of three peripheral capillary loops in each of three glomeruli from each animal. Thickness values that were more than 2.5 SD from the mean were considered outliers and not used leaving 141 to 158 measurements per group.

Effect of Lisinopril on renal function

Male ZSD rats, were maintained on Purina 5008 chow *ad lib* until 18 weeks of age. A diabetogenic diet (Purina 5SCA) was initiated and continued for 3 weeks to accelerate the development of hyperglycemia. Animals were then maintained for the duration of the study on Purina 5008 regular rodent chow. In this study, treatment was started 5, 9 and 13 weeks after animals became hyperglycemic (ages 29, 33 and 37 weeks; respectively). In each age group, diabetic rats were sorted into untreated (Purina 5008 chow, $n=12$) and treated (5008 chow admixed with Lisinopril 250 ppm, $n=13$) based on body weight and fed glucose level. Treatment was continued for 4 weeks. Blood and 24-hour urine samples were collected before and after 4 weeks of treatment. Urine was collected at room temperature and without preservatives. Fed glucose was measured in whole blood by StatStrip (Xpress II, Novo Biomedical); HbA1c (whole blood), creatinine (serum) and BUN (serum) were assayed in serum using a AU480 clinical analyzer (Beckman Coulter, Brea, CA); albumin and creatinine were measured in urine by MSD (MesoScale Discovery, Rockville, MD) and AU480 analyzer, respectively. Estimated glomerular filtration (eGFR) was calculated using the following formula: Urine creatinine (mg/dL) \times urine volume (mls/min)/serum creatinine (mg/dL).

Statistical analysis

All data are presented as Mean \pm SEM. Statistical analysis was performed using Prism for Windows (version 6.07 GraphPad, San Diego, CA). With regard to data presented on the spontaneous development of DN in ZSD rats, a two-way ANOVA was conducted to compare the effect of strain on body weight, blood glucose, urine volume, urine albumin, urine KIM-1, urine

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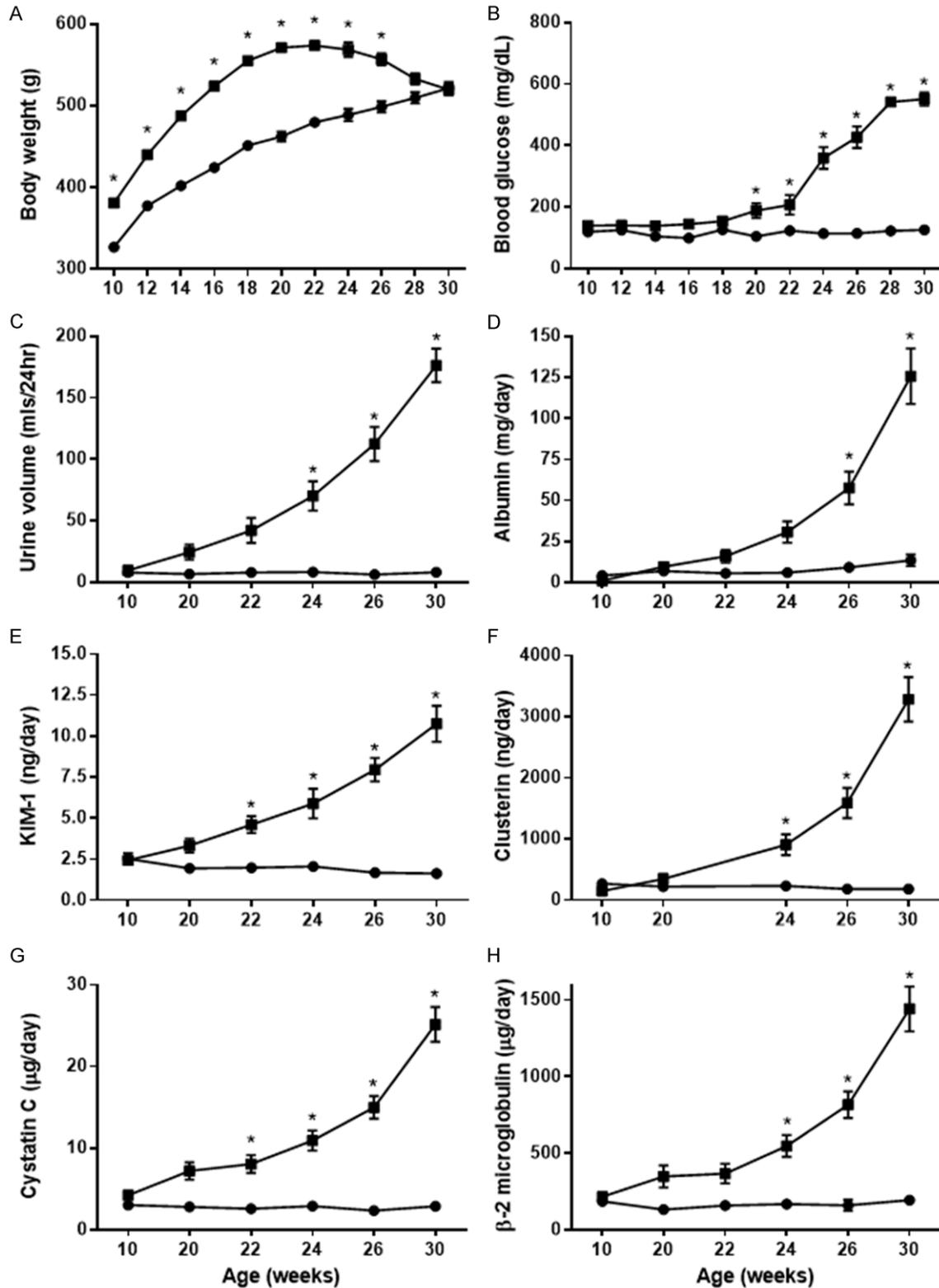


Figure 1. Weight (A), blood glucose (B) and urinary markers (C-H) of renal injury in ZSD rats compared to age-matched SD rats. ZSD rats (■, 10-30 weeks of age) are significantly heavier (A) and have significantly higher blood glucose levels (B) compared to age-matched SD rats (●). Renal injury is evident in ZSD rats compared to SD rats as evidenced by significantly higher urine volume, albumin excretion and excretion of renal injury biomarkers (C-H) (two-way ANOVA, Sidak's; *, $P < 0.05$).

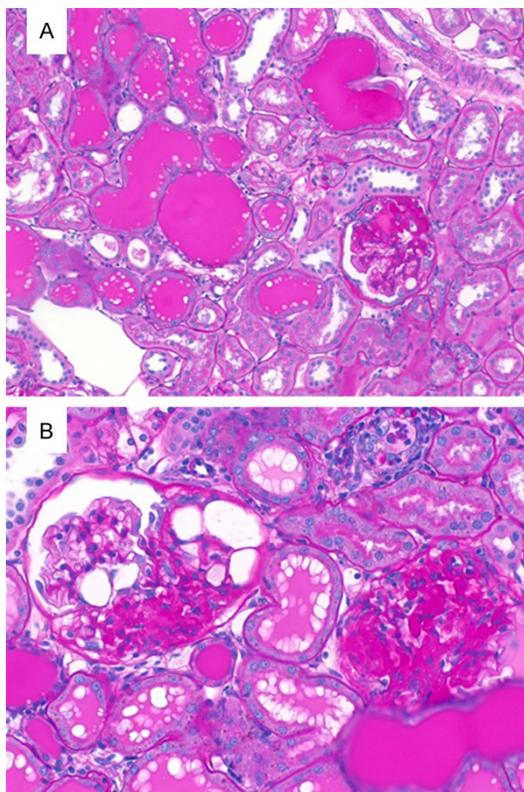


Figure 2. Light microscopic pictures of diabetic kidney pathology. The upper panel (A) shows a glomerulus with a nodule in the lower right quadrant of the picture. This glomerulus also demonstrates mesangial expansion (A). The lower panel illustrates sclerosis in two glomeruli and other pathological changes (B).

clusterin, urine cystatin C and urine β 2-microglobulin. Post-hoc comparisons between strains were made using Sidak's multiple comparison tests and are indicated on graphs. With regard to the effects of Lisinopril, means were compared using one-way ANOVA/pooled t-test or paired t-tests. Significant effects indicated on graphs (* $P < 0.05$).

Results

Spontaneous development of renal dysfunction in untreated ZSD

ZSD rats were significantly heavier when compared to age-matched SD rats at 10 weeks of age (380.5 ± 5.0 vs. 326.4 ± 3.6 g) through 26 weeks of age (556.8 ± 7.6 vs. 498.5 ± 6.9 g). ZSD weight gain leveled at about 20 weeks and subsequently decreased. Body weight in 28-30 week old animals was not significantly different when compared to control SD animals (Figure 1A).

Hyperglycemia developed spontaneously in ZSD rats compared to SD rats and although glucose levels tended to run higher in animals as young as 10 weeks of age (118.8 ± 2.9 vs. 139.4 ± 2.1 mg/dL) they do not become statistically significant until 20 weeks of age. Glucose levels remained quite steady in SD animals as they age, while there was a progressive increase in glucose in aging ZSD rats. Glucose levels were significantly higher compared to SD rats from 20 weeks of age, and reached 550.9 ± 21.2 mg/dL at 30 weeks (Figure 1B).

Urine volume (mls/day) was not significantly different in ZSD rats compared to age-matched SD rats at 10 weeks of age. However, as blood glucose increased, urine volume became significantly different starting at 24 weeks when compared to age-matched SD rats (Figure 1C).

Albumin excretion was comparable to that of SD rats in animals up to 20 weeks of age (6.9 ± 1.4 vs. 9.5 ± 2.7 mg/day for SD and ZSD rats, respectively). Urinary albumin excretion became significantly different, compared to SD rats, at 26 weeks reaching 125.7 ± 16.9 mg/day in 30 week old animals (Figure 1D).

In addition to increased albumin excretion, age-related increases in the excretion of early urinary markers of renal injury (β 2-microglobulin, KIM-1, clusterin and cystatin C) were noted in ZSD animals (Figure 1E-H). Figure 1G demonstrates significantly higher excretion of cystatin C in the urine of ZSD animals compared to SD animals starting at 22 weeks of age (8.1 ± 0.1 vs. 2.6 ± 0.3 μ g/day, respectively). Figure 1G illustrates that cystatin C levels in urine of SD animals remained steady throughout the study period; however, levels in ZSD rats increased rapidly compared to baseline values over the 20-week observation period and were significantly higher compared to SD rats at each of 22 to 30 weeks. In 30 weeks old animals, cystatin C was increased by 9 fold over that of SD rats (25.1 ± 2.1 vs. 2.9 ± 0.2 μ g/day, respectively). A similar pattern was apparent for KIM-1, clusterin and β 2-microglobulin; significant differences in these markers first appeared in 22 to 24 week old animals (Figure 1E, 1F and 1H). A seven-fold increase in KIM-1 (10.8 ± 1.1 vs. 1.6 ± 0.1 ng/day) and a seven-fold increase in β 2-microglobulin (1441.9 ± 146.5 vs. 194.1 ± 22.5 ng/day) were prominent in 30 week old rats.

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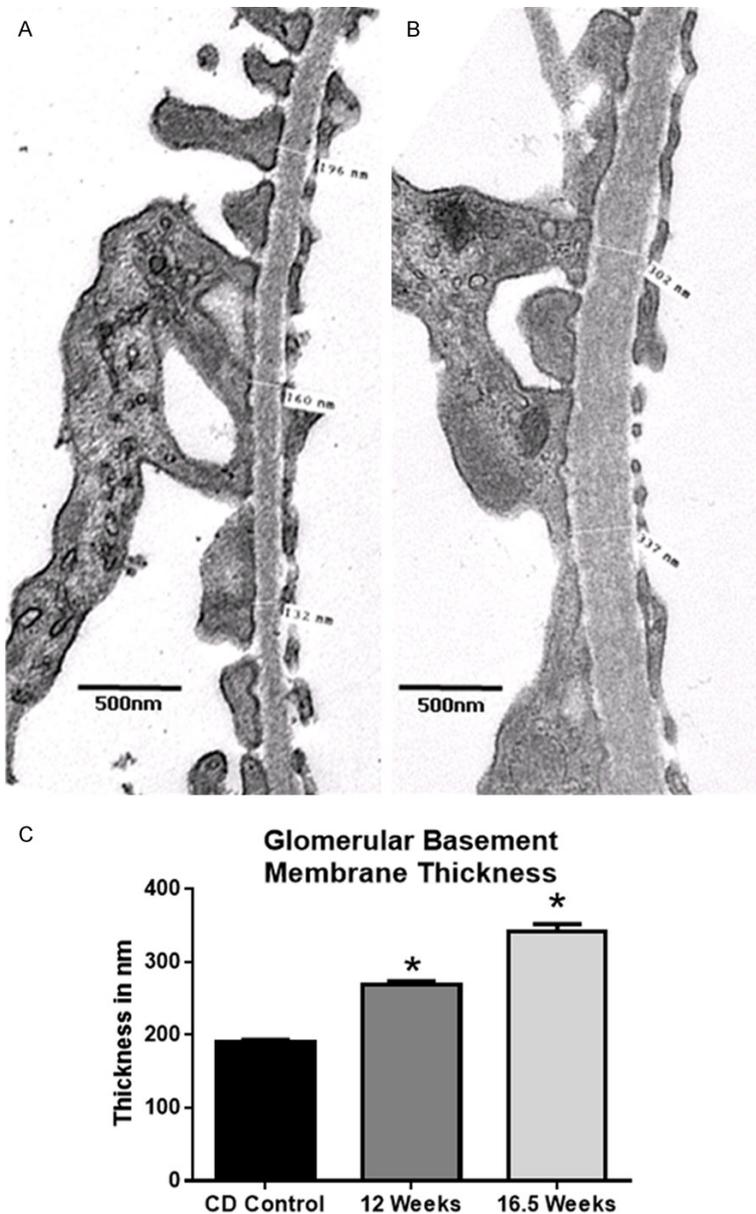


Figure 3. Electron micrographs of glomerular capillary walls. The picture on the left (A) is from a control kidney while the one on the right (B) is from a ZSD rat that was diabetic for 12 weeks. The micrographs are aligned with podocytes on the left, and capillary endothelium on the right of the basement membrane. There is a clear thickening of the basement membrane in the diabetic ZSD when compared to control capillary of age-matched SD rat. (C) represents the quantification of the basement membrane thickness between CD control and ZSD at 12 weeks and 16.5 weeks of diabetes (155, 158 and 141 measurements, respectively; 6 glomeruli from 2 animals, one-way ANOVA followed by Dunnett's multiple comparisons test; * $P < 0.05$).

Fixed kidneys from 33 weeks old ZSD rats were examined for morphological evidence of renal disease. **Figure 2** represents a typical pattern of tubule, glomerular and interstitial changes that developed in ZSD rats as a

result of long-standing diabetes. Histopathology scores (0-5) were assigned for glomerulopathy with mesangial expansion and capsule thickening, tubular dilation and degeneration, protein casts and inflammation. Scores averaged 2.5-3.0 for each assessment for ZSD kidneys (**Figure 2A** and **2B**).

Basement membrane thickening was apparent in electron micrographs of glomeruli in ZSD rats when compared to control rats (≈ 320 nm vs. 163 nm, respectively) (**Figure 3A** and **3B**). Podocyte effacement was also evident on the convex surface (left side) of the diabetic glomerular capillary (**Figure 3B**) when compared to regular distribution of podocyte foot processes in **Figure 3A**. **Figure 3C** demonstrated significant increases in thickness of the glomerular capillary basement membranes at 12 and 16.5 weeks of diabetes.

Effect of Lisinopril on renal function in diabetic ZSD

Body weight: Based on average feed intake and body weight, Lisinopril was delivered at approximately 30 mg/kg/day over the 4-week period. Lisinopril had no significant effect on body weight in diabetic ZSD rats following 4 weeks of treatment (**Table 1**).

Glucose: Baseline blood glucose in diabetic ZSD rats was not different between the two groups (**Table 1**). Following vehicle treatment,

blood glucose did not change significantly, but administration of Lisinopril elicited a significant increase compared to baseline and vehicle treatment (**Table 1**). Although hyperglycemia was accompanied by elevated HbA1c values

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Table 1. Data before and after vehicle and Lisinopril treatment

Measurement	Vehicle		Lisinopril	
	Baseline	Termination	Baseline	Termination
Body Weight (g)	496.0 ± 6.7	468.2 ± 6.9 [†]	500.1 ± 6.4	452.6 ± 6.9 [†]
Blood Glucose (mg/dL)	563.2 ± 15.2	572.0 ± 15.7	563.2 ± 17.7	718.3 ± 16.6 ^{*,†}
HbA1c (%)	10.2 ± 0.21	10.9 ± 0.20 [†]	10.4 ± 0.2	12.3 ± 0.3 [†]
Urinary Volume (ml/day)	171.4 ± 18.4	224.3 ± 9.3 [†]	139.5 ± 18.9	222.5 ± 17.9 ^{*,†}
Urinary Albumin (mg/day)	47.3 ± 16.8	145.8 ± 39.4 [†]	43.7 ± 12.2	4.6 ± 1.1 ^{*,†}
eGFR (ml/min)	5.07 ± 0.20	5.72 ± 0.24 [†]	4.63 ± 0.33	3.93 ± 0.19 ^{*,†}
Serum Creatinine (mg/dL)	0.44 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.49 ± 0.02 ^{*,†}
Serum BUN (mg/dL)	18.8 ± 0.6	22.3 ± 0.7 [†]	18.9 ± 0.6	37.6 ± 1.2 ^{*,†}

*Significantly different from vehicle at termination, t-test P<0.05. [†]Significantly different from corresponding baseline data, paired t-test P<0.05.

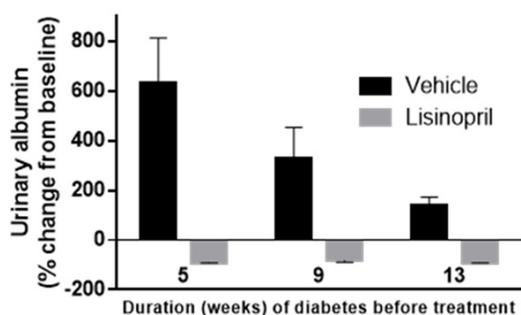


Figure 4. Effect of Lisinopril on urinary albumin excretion in animals with the duration of diabetes. Lisinopril treatment elicited a significant decrease in urinary albumin excretion when compared to vehicle treatment. When stratified according to the duration of diabetes before initiation of treatment, the normalization of albumin excretion by Lisinopril treatment is consistent. All paired groups of animals are significantly different (paired t-test; *P<0.05).

from baseline with both vehicle and Lisinopril (4 weeks of treatment), Lisinopril treatment resulted in a significant increase in HbA1c compared to vehicle treated animals at 4 weeks (Table 1).

Urine volume: Baseline urine volume in diabetic ZSD rats was not significantly different between the groups at the beginning and the end of treatment; however, urine volume increased significantly in both groups as diabetes progressed. No significant effect of Lisinopril compared to vehicle was noted (Table 1).

Albumin excretion: Baseline albumin excretion averaged 45.5 ± 10.0 mg/day in diabetic ZSD rats and there were no significant differences between treatment groups at baseline (Table 1). Albuminuria increased significantly after ad-

ministration of vehicle for 4 weeks while administration of Lisinopril significantly reduced the albuminuria compared to vehicle treatment at 4 weeks and reduced albumin compared to its baseline value by 89% (Table 1).

Lisinopril prevented the progressive rise in albumin excretion as diabetes developed, independent of the duration of diabetes at the start of treatment (-89.2 ± 3.3, -81.8 ± 7.5 and -90.9 ± 1.9%), and lowered the levels compared to baseline values in animals that were diabetic for 5, 9 and 13 weeks, respectively (Figure 4).

Glomerular filtration: The estimated glomerular filtration rate (eGFR) was calculated from urine and serum creatinine values. At baseline, eGFR averaged 4.8 ± 0.2 ml/min in diabetic ZSD rats and was not different among treatment groups. Following 4 weeks of treatment, eGFR increased significantly in the vehicle treated animals; in contrast, eGFR in Lisinopril treated animals decreased significantly and was significantly lower following treatment with Lisinopril compared to vehicle (Table 1).

Serum creatinine: Baseline serum creatinine averaged 0.43 ± 0.004 mg/dL in diabetic ZSD rats and there were no significant differences among treatment groups. Serum creatinine did not change with the progression of more severe diabetes in the vehicle group, but serum creatinine in Lisinopril treated rats increased significantly compared to baseline values and to vehicle treated animals after 4 weeks of treatment (Table 1).

Serum BUN: Baseline serum BUN averaged 18.9 ± 0.41 mg/dL and there were no differ-

ences among treatment groups. Compared to vehicle, administration of Lisinopril elicited a significant increase in BUN following the 4-week treatment (**Table 1**).

Discussion

Diabetic nephropathy (DN) is regarded as the leading cause of ESRD and is estimated to occur in 20-40% of diabetic patients [44]. The cluster of conditions known as metabolic syndrome carries with it a number of risk factors for the development of DN including obesity, hypertension and insulin resistance. Obesity has been shown to be a predictor for development of DN, and high fat feeding has been shown to increase body fat and induce renal injury in obese mice [45] and in humans, where reduction in obesity (via gastric bypass) produced remission in DN [46]. Hypertension occurs in 50% of patients with type 2 diabetes and contributes to the development of DN through a number of mechanisms associated with arterial damage and hemodynamics [47]. In addition, increased levels of inflammatory mediators and over production of reactive oxygen species (ROS) have been identified in diabetic animals and patients. Inflammation and ROS have been shown to be major contributors to the initiation and progression of DN [48-50]. Indeed, current therapeutic approaches for DN are centered on the control of blood pressure, reduction of hyperglycemia and life-style changes to reduce or eliminate obesity [51, 52]. Albuminuria represents a biomarker of generalized endothelial dysfunction within the kidney and is predictive of the existence of endothelial dysfunction within the cardiovascular system [53]. Albuminuria has been identified as a risk factor for both renal and cardiovascular morbidity and mortality in diabetic [54, 55] and in non-diabetic patients [56]. These risk factors have all been identified as contributory to the DN that spontaneously develops in the ZSD rat.

In current clinical practice, DN presents in two stages: microalbuminuria (30-300 mg/day) and macroalbuminuria (>300 mg/day). Early DN is defined as the presence of microalbuminuria with a normal or mildly decreased eGFR (>60 ml/min/1.73 m²) [57]. While the diagnosis of nephropathy in diabetic patients is focused on the presence of albuminuria, it has become clear that significant changes in renal architec-

ture and function have been shown in patients with normal albuminuria [58, 59]. Declining renal function in the absence of albuminuria has been shown to occur in 25% of diabetic patients [60, 61]. Histological examination of renal biopsies from diabetic patients with impaired renal function in the absence of albuminuria indicated vascular and tubulo-interstitial damage [62]. Thus, while concurrent monitoring of albuminuria and eGFR are the mainstay of diagnoses and evaluation of treatment for DN, earlier detection is paramount to preventing or slowing the progression to ESRD in diabetic patients. Early tubular injury has been identified as a major contributor to early DN and is predictive of progression to ESRD [63-65]. Indeed, tubular damage markers are elevated in the urine of diabetic patients [66]. Tubular damage markers such as KIM-1 and β 2-microglobulin were elevated in the urine of diabetic patients before the onset of microalbuminuria and, as such, represent sensitive markers for the early detection of DN [64, 67-72]. High urinary KIM-1, like albumin, was associated with increased long term risk. Clusterin is upregulated in renal tissues following insult and is associated with the process of cellular repair. Increases in urinary clusterin have been observed in a wide variety of renal diseases including glomerulonephritis and renal tubular injury [73, 74]. Clusterin was found to be elevated by 2 fold in the urine of diabetic patients compared to normal volunteers [75]. Increased urinary [76, 77] and serum [78] cystatin C are recognized as markers of renal tubular dysfunction. Although not yet routinely used in clinical practice, measurements of serum cystatin C may be preferred over serum creatinine in estimation of GFR especially when renal function is not stable, as occurs in early DN [79]. Cystatin C was elevated by 4 fold in the urine of diabetic patients compared to normal volunteers [65]. It is evident from the biomarker profile in human diabetic patients that glomerular and tubular injury occurs in the setting of hyperglycemia. In a similar fashion, biomarkers for glomerular (albumin) and tubular injury (KIM-1, clusterin, cystatin C and β 2-microglobulin) in the setting of hyperglycemia are reflected in the spontaneous development of DN in the ZSD rat shown in this investigation.

The interruption of an upregulated renin-angiotensin-aldosterone system (RAAS) remains the cornerstone of renoprotective strategies for

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diabetic patients. This is accomplished with monotherapy using angiotensin converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARB), direct renin inhibitors (aliskiren), or dual therapy with ACEi/ARB. The ACEi Lisinopril is a commonly used first line anti-hypertensive agent and has been shown to reduce blood pressure and reduce albuminuria in hypertensive and normotensive diabetic patients [80-82].

It has been suggested that in early DN, chronic hyperglycemia enhances glucose transport to the proximal tubule which elicits hyperfiltration, increased glomerular pressure and subsequent mesangial expansion with proteinuria [83, 84]. This hyperfiltration has been documented in the early stages of DN in humans [85] and in hypertensive rats made diabetic with STZ [86]. A reversal of the hyperfiltration is a predominant benefit with ACEi therapy and is thought to be the result of preferential dilation of the efferent arteriole, and a decrease in glomerular capillary pressure, with improvement in renal blood flow. Concomitant with this reduction in GFR, increases in BUN and serum creatinine have been noted in patients with renal insufficiency [87]. Similar to human DN, and as illustrated by high eGFR in untreated ZSDS rats, hyperfiltration is also a key feature of DN in this model.

Large increases in creatinine are seen clinically in some patients [88], but these are relatively rare and even these large increases are not typically considered problematic unless other clinical signs of worsening nephropathy are present. In a clinical study [88] of 13,166 cases, 31 had increases of serum creatinine from <1.2 to >2.5 mg/dL. Although smaller increases are more common, they are also not typically considered to be an indication of worsening nephropathy but rather considered to be indications of drug efficacy [87]. Nevertheless, elevated creatinine levels are issues with ACEi and ARB treatments [89] and require monitoring to identify potentially worsening nephropathy.

The ZSDS rat presents with a progressive hyperglycemia and albuminuria consistent with DN in human disease. Also, similar to DN in patients, urinary biomarkers used clinically to assess glomerular and tubular injury were prominently elevated in the model prior to the development of overt diabetes or albuminuria.

Histologic lesions that are also identified in human DN including basement membrane thickening and mesangial expansion were evident with chronic hyperglycemia in ZSDS rats. In addition, this model responded to ACEi treatment in a similar fashion to that of diabetic patients with nephropathy. Similar to clinical experience, Lisinopril reduced hyperfiltration, increased serum BUN and creatinine and prevented albuminuria. The changes in intra-renal hemodynamics elicited by ACEi therapy could also explain the rises in blood glucose and associated increase in HbA_{1c} seen in the paper since a decrease in glomerular filtration will likely also result in a decrease in glucose excretion in these untreated rats. This may not be seen clinically since this phenomenon would not be observed with reasonably well treated patients.

Conclusion

Obesity and metabolic syndrome are clear predictors of chronic kidney disease, largely due to the potentiation of chronic inflammation by insulin resistance. In addition, the lipoprotein abnormalities, increased hemodynamics and vascular dysfunction associated with metabolic syndrome have all been implicated as causative for renal disease. The ZSDS rat spontaneously develops significant renal disease corresponding to hyperglycemia and hypertension [40], the severity of which correlates with the level of hyperglycemia. Elevations in biomarkers for renal dysfunction (i.e., cystatin C, KIM-1, clusterin, and β 2-microglobulin) as well as significant albuminuria and histological analysis have proven the ZSDS rat to exhibit nephropathy that closely mimics that observed in obese, insulin resistant patients.

ACEi treatment, which is the mainstay of clinical treatment for DN, was also effective in significantly reducing albuminuria in the diabetic ZSDS rat. Due to the demonstration of hypertension in this model, it should be considered that this reduction is likely to be at least partially due to a reduction in the hypertension-induced glomerular hyperfiltration [19], although it is known that ACEi can reduce albuminuria at doses that do not effect blood pressure [90]. The increased creatinine and BUN levels resulting from ACEi therapy may increase acceptance of the model, as these are indications of clinical efficacy of ACEi and

ARB therapy according to Schoolwerth [87]. Interestingly, while reductions in hyperfiltration were noted in the STZ rat following Lisinopril, the elevations in BUN and creatinine seen both clinically and in the ZDSD rat were not apparent in the STZ model.

This study strongly supports the ZDSD rat as a translational model of diabetic nephropathy with the potential to add value to mechanistic as well as drug discovery efforts.

Acknowledgements

The project was partially funded by a grant from the NIH R44 DK074242 and by internal funds at PreClinOmics, Indianapolis, IN 46268. The authors would like to acknowledge our deceased colleague and co-author, Vincent H Gattone, 2nd from the Department of Anatomy at Indiana University School of Medicine who encouraged the pursuit of the nephropathy in this model and, with the assistance of Caroline Miller, contributed the light and electron microscopy to this paper. The authors would also like to acknowledge the multiple staff at PreClinOmics, now Crown Bioscience Indiana, who made contributions to the development, breeding and monitoring of the ZDSD rat and their valuable assistance in the experiments presented in this paper. Of these, the longest term contributors are Leah Shanahan, Kathryn Coy, Melissa Bass and Julius Mongu. A special acknowledgement goes to Joseph Pesek, past President of PreClinOmics, who encouraged and contributed to the development of the ZDSD model and to the review of the research performed with the model. RGP and KMZ contributed to the design, analysis of data, drafting and review. CVJ reviewed, edited and approved the final document.

Disclosure of conflict of interest

The ZDSD rat was developed, in part, from the ZDF rat and as such RGP receives a small portion of license fees that are paid to Indiana University for sales of the ZDSD rat, he is also a consultant with Crown Bioscience, the distributor of the ZDSD rat. CVJ is an employee and KMZ is a consultant with Crown Bioscience.

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