Longitudinal Prevalence of Hypertension, Proteinuria, and Retinopathy in Dogs with Spontaneous Diabetes Mellitus

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Background: The prevalence and progression of vascular complications of spontaneous diabetes mellitus (DM) in dogs have not been described.

Objectives: To investigate the effects of duration of disease, as estimated by time since DM diagnosis, and glycemic control on prevalence of systemic hypertension, proteinuria, and diabetic retinopathy in dogs with spontaneous DM.

Animals: Seventeen client-owned dogs with spontaneous DM.

Methods: Prospective, longitudinal observational study. Dogs with DM of less than 1 year's duration were recruited and evaluated once every 6 months for 24 months. Recorded measures included indirect BP, urine albumin, protein and creatinine concentrations, serial blood glucose and serum fructosamine concentrations, ophthalmic examination, and a standardized behavioral questionnaire.

Results: Eleven dogs completed the 2-year follow-up period, during which the highest recorded prevalence of systolic and diastolic hypertension was 55 and 64%, respectively. Prevalence of microalbuminuria and elevated urine protein:creatinine ratio (UPC) ranged up to 73 and 55%, respectively. Prevalence of retinopathy ranged up to 20%. No significant effect of time since DM diagnosis or glycemic control was detected for any of the measures examined. Additionally, no significant associations between BP, urine albumin concentration, UPC and retinopathy were detected.

Conclusions and Clinical Relevance: With the exception of proteinuria, which was substantial in some cases, clinically deleterious diabetic vascular complications were not identified in dogs in this study.

Key words: Blood pressure; Canine; Kidney; Ophthalmology; Renal/urinary tract; Retina.

D iabetes mellitus (DM) can result in vascular complications such as nephropathy, systemic hypertension, and retinopathy. These comorbidities are well documented in dogs with experimentally induced DM and were found to develop after several months to 2.5 years of disease, depending on the model.[1–3] While systemic arterial hypertension,[4] nephropathy,[4,5] and retinopathy[6–8] are recognized in dogs with spontaneous DM, little is known about the prevalence of these conditions in dogs, their onset after diagnosis of DM, nor etiopathogenic factors leading to their development and progression.

Vascular complications of DM are very important causes of morbidity and mortality in humans. Nephropathy associated with DM is the most common cause of end-stage renal disease in humans.[9] Renal injury manifest by microalbuminuria occurs in 20–40% and overt proteinuria because of diabetic nephropathy is present in approximately 10–20% of humans with type 1 DM within 7–15 years of diagnosis with conventional treatment.[10–12] The presence of microalbuminuria is a predictor of the development of end-stage renal disease in diabetic humans, and hypertension is associated with its progression.[6] Although diabetic retinopathy is an important cause of vision

Abbreviations:

BP	blood pressure
DM	diabetes mellitus
MBG_{8h}	mean blood glucose from 8-hour curve
UPC	urine protein:creatinine ratio

loss in humans, being more prevalent than nephropathy,[10–12] it appears to be of little clinical significance in the dog. However, its association with duration of DM and presence of hypertension has not been examined.

The purpose of this study was to document longitudinal changes in prevalence of systemic hypertension, proteinuria, and retinopathy in a group of dogs with spontaneously occurring DM and to evaluate the impact of glycemic control and duration of DM, as estimated by time since DM diagnosis, on the development and progression of these conditions. We hypothesized that vascular complications in dogs with DM, including systemic hypertension, retinopathy, and proteinuria, are progressive and that they are associated with duration and control of disease.

Materials and Methods

Case Selection

Dogs with naturally occurring DM diagnosed less than 1 year before evaluation were used in this study. Initial evaluations at the VA-MD Regional College of Veterinary Medicine occurred between March 2005 and March 2009. DM was diagnosed by finding persistent marked hyperglycemia (plasma glucose >250 mg/dL) and glucosuria in dogs with clinical signs consistent with the disease. Duration of disease was approximated based upon time since definitive diagnosis and institution of treatment, which were concurrent events in all cases. Dogs were excluded if

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a concurrent endocrinopathy or primary chronic kidney disease was diagnosed at the time of initial evaluation or if significant illness unrelated to DM developed during the study period. Specific screening for concurrent endocrinopathies (eg, hypothyroidism, hyperadrenocorticism) was performed only in cases where concurrent clinical signs or laboratory abnormalities were suggestive of their presence. Primary chronic kidney disease was diagnosed by the presence of plasma creatinine and urea above the reference range and concurrent isosthenuria. Dogs with elevated urine protein:creatinine ratio (UPC), but without other evidence of renal dysfunction, were included.

Clinical Examination and Clinicopathologic Evaluation

Dogs were evaluated as described below at initial enrollment and every 6 months thereafter for 2 years. At each evaluation, dogs were hospitalized for 8–10 hours. A physical examination by a single investigator (DLP) and complete ophthalmologic examination by a single investigator (IPH), including slit lamp biomicroscopy and indirect ophthalmoscopy, were performed. In addition, owners completed a standardized questionnaire (see Supporting Information) to document changes in water and food consumption, urination frequency, and activity, relative to normal for their pet.

Systolic, diastolic, and mean BPs were measured in triplicate at 3 separate time points (2–3 hours apart) during each visit, utilizing an oscillometric method.[a] All measurements were performed with the dog in sternal recumbency.[13] Measurements were performed by 1 of 2 experienced licensed veterinary technicians and all measurements on a given day were performed by the same individual. For data analysis, the daily mean value for each parameter was utilized for analysis.

At each evaluation, blood samples were collected by venipuncture and urine samples by cystocentesis for the following laboratory assessments: complete blood count, serum biochemistry, serum fructosamine, routine urinalysis, urine microalbumin, UPC, and urine aerobic bacterial culture. Blood glucose concentration was measured every 2 hours for 8 hours using a hand held glucometer.[b] Dogs were fed and administered insulin by their owners before presentation to the VTH. Initial blood glucose measurements were obtained within 2 hours of insulin administration in all dogs.

Data Recording and Analysis

Glycemic Control Classification. Serum fructosamine concentrations were designated as indicating good, fair, or poor control based upon the guidelines provided by the laboratory.[c] Before July 2007, fructosamine concentrations of <450, 450–550, and >500 µmol/L were designated good, fair, and poor glycemic control, respectively. Thereafter, a change in reagents resulted in adjustment of the interpretation of serum fructosamine concentrations with <500, 500–614, and >614 µmol/L designating good, fair, and poor glycemic control, respectively.

Mean blood glucose concentration during the 8-hour glucose curve (MBG_{8h}) was utilized as a gauge of good (<250 mg/dL), fair (250–300 mg/dL), or poor (>300 mg/dL) glycemic control.[14]

A glycemic control score was derived based upon owner perceptions regarding the presence of polyuria, polyphagia, and polydipsia. For each of these 3 parameters, a score of 0 or 1 was assigned to indicate that the condition was not present or was present, respectively. The mean of these 3 scores was utilized to designate the glycemic control score as good (mean score value <0.34), fair (mean score value 0.34–0.67), or poor (mean score value >0.67).

Blood Pressure. Systolic hypertension was defined as systolic blood pressure (BP) >150 mmHg and diastolic hypertension was defined as diastolic BP >95 mmHg.[15]

Proteinuria. Urine albumin concentration and UPC were utilized to assess proteinuria. Urine creatinine was measured using a modified Jaffe procedure on an automated chemistry analyzer.[d] Urine protein was measured using a colorimetric method on an automated chemistry analyzer.[d] Urine albumin concentration was measured using a quantitative immunoturbidometric assay utilizing monoclonal antibody to canine albumin.[c] Results were normalized to a urine specific gravity of 1.010. Microalbuminuria was defined as urine albumin concentrations ≥2.5 mg/dL.[c] Urine albumin concentrations exceeding the assay limit of 30 mg/dL were arbitrarily assigned a concentration of 31 mg/dL. Overt proteinuria was defined as UPC ≥0.5.[16]

Diabetic Retinopathy. Diabetic retinopathy was diagnosed when petechial retinal hemorrhages or microaneurysms were detected upon funduscopic examination, as previously described.[7,8] Dogs were classified as affected if retinopathy was detected in either eye. Dogs were excluded from analysis if at least 1 fundus could not be visualized because of the presence of cataract or other cause.

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Virginia Tech and all pet owners provided signed consent.

Statistical Analysis

Normal probability plots showed that mean systolic BP, mean diastolic BP, and urine albumin concentrations were normally distributed, while the UPC was skewed. Accordingly, a log (base e) transformation was applied to UPC before analysis. Prevalence of hypertension, proteinuria, and retinopathy at baseline were estimated as binomial proportions.

A mixed-model ANOVA followed by Tukey's procedure for multiple comparisons was applied to assess the effect of time since DM diagnosis and glycemic control measures (including MBG_{8h}, serum fructosamine and client assessment scores) on systolic and diastolic BP, urine albumin and log UPC. Effect of time since DM diagnosis and glycemic control on each of systolic hypertension, diastolic hypertension, significant microalbuminuria, elevated UPC and retinopathy was assessed using the Cochran–Armitage test. Agreement between MBG_{8h}, serum fructosamine and client-derived glycemic control scores was assessed using a kappa statistic.

Effect of UTI on log UPC was assessed using mixed-model ANOVA and effect of UTI on overt proteinuria was assessed using a Mantel–Haenszel chi-square test.

Association between retinopathy and each of systolic BP, diastolic BP, urine albumin concentration and log UPC was assessed using-mixed model ANOVA. Associations between retinopathy and systolic hypertension, diastolic hypertension were assessed using a Mantel–Haenszel chi-square test. Mixed-model linear regression was used to assess associations between BP and urine albumin concentration, between systolic and diastolic BP and UPC and between urine albumin concentration and UPC. Statistical significance was set at $P < .05$. All analyses were performed using SAS version 9.3.[e]

Results

Study Population

Seventeen dogs met the study inclusion criteria during the enrollment period. Insulin treatment was initiated at the time of DM diagnosis and before study

enrollment in all cases. Included in this group were 9 neutered male, 5 spayed female, and 3 intact male dogs (all of which were neutered during the course of the study). Breeds include mixed (5), Miniature Schnauzer (4), Labrador Retriever (2), Yorkshire Terrier (2) and one each of 4 different breeds (Pomeranian, Basset Hound, Pug, Samoyed). At initial presentation, the mean (±SD) age was 6.8 ± 2.6 years and the mean (±SD) time since DM diagnosis was 4.3 ± 2.9 months. This group is utilized to report prevalence data for the various comorbidities at initial presentation. Of these 17 dogs, 6 did not complete the study. Four dogs withdrew from the study because of owner's unwillingness to continue to participate. One dog died of unknown cause after the 12-month visit and one was euthanized because of neurologic signs after the 12-month visit. Two dogs were excluded from the study at initial evaluation, one for concurrent hypothyroidism, and one for primary chronic kidney disease.

Eleven dogs returned for evaluation every 6 months for the prescribed 2-year time frame and are included in the full statistical analysis of data. Included in this group were 4 neutered male, 4 spayed female, and 3 intact male dogs (all of which were neutered during the course of the study). Breeds include mixed (3), Miniature Schnauzer (2), Labrador Retriever (2), and one each of 4 different breeds (Basset Hound, Pug, Samoyed, Yorkshire Terrier). The mean (±SD) age and time since DM diagnosis at initial presentation were 6.6 ± 2.8 years and 5.5 ± 2.8 months, respectively. The mean (±SD) administered insulin dosage over the course of the study was 0.8 (±0.451) U/kg. There was no attempt to control diet or insulin choice in the study participants. Over the course of the study, 6 dogs were receiving porcine lente insulin[f] at all visits, 4 dogs were receiving recombinant human NPH[g] at all visits and 1 dog was switched from porcine lente insulin[e] to recombinant human NPH[h] after initial evaluation.

Glycemic Control

Table 1 depicts glycemic control scores for all 11 dogs over the course of the study. In general, clients tended to assign better control scores to their pets than the other measures, with poor control being assigned in only 2/55 instances, compared with designations by serum fructosamine and MBG_{8h}, which each resulted in designations of poor control in 8/55 instances.

Evaluation of the 3 measures of glycemic control revealed fair agreement between MBG_{8h} and serum fructosamine concentrations ($\kappa = 0.2766$), but poor agreement between MBG_{8h} and client score ($\kappa = 0.0796$) and between serum fructosamine concentration and client score ($\kappa = 0.0776$).

Blood Pressure

At initial evaluation of all 17 dogs, mean (±SD) BP was 144.5 (±11.8) mmHg and the prevalence of systolic hypertension (systolic BP >150 mmHg) was 35.3% (6/17 cases). Systolic BP exceeded 160 mmHg in 2 dogs, but did not exceed 165 mmHg in any dog.

Systolic hypertension was present initially in 36% (4/11) of dogs with complete follow-up. Of these 4, 2 were hypertensive at all subsequent time points, one was hypertensive at 3 of 4 subsequent time points and one was hypertensive at 1 of 4 subsequent time points. At the 6-, 12-, 18-, and 24-month evaluations, systolic hypertension was detected in 6/11 (55%), 5/11 (46%), 5/11 (46%), and 5/11 (46%) cases, respectively. No significant change in systolic BP (Table 2) or prevalence of systolic hypertension was associated with time since DM diagnosis or with glycemic control ($P > .05$). The highest recorded systolic BP was 180 mmHg in 1 dog at a single time.

At initial evaluation, the prevalence of diastolic hypertension (diastolic BP >95 mmHg) was 59% (10/17). Diastolic BP exceeded 100 mmHg in 8 dogs, with the highest measurement being 124 mmHg. Both systolic and diastolic hypertensions were present in 4 dogs at initial evaluation.

At initial evaluation in the 11 dogs with complete follow-up, diastolic hypertension was present in 64% (7/11). Of these 7 dogs, 5 had diastolic hypertension at all subsequent time points, 1 had diastolic hypertension at 3 of 4 subsequent time points and 1 had diastolic hypertension at 1 of 4 subsequent time points. At the 6-, 12-, 18-, and 24-month evaluations, diastolic hypertension was detected in 6/11 (55%), 7/11 (64%), 5/11 (456%), and 7/11 (64%) cases, respectively. No significant change in diastolic BP (Table 2) or preva-

Table 1. Number of dogs with good, fair, or poor glycemic control score categorizations, based upon MBG_{8h}, serum fructosamine, and owner behavioral scores for 11 dogs with diabetes mellitus over a 2-year period.

Visit Time (months)	MBG_{8h} Score			Fructosamine Score			Owner Score		
	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor
0	8	3	0	4	5	2	7	3	1
6	8	0	3	6	4	1	10	1	0
12	8	2	1	6	3	2	9	1	1
18	7	1	3	7	2	2	10	1	0
24	5	5	1	8	2	1	9	2	0
Total	36	11	8	31	16	8	45	8	2

MBG_{8h}, mean 8 hours blood glucose.

Table 2. Mean (± SD) systolic BP, diastolic BP, and urine albumin concentration and median (range) UPC in 17 diabetic dogs at initial evaluation and 11 diabetic dogs over a 2-year period.

	0 months (n = 17)	0 months (n = 11)	6 months (n = 11)	12 months (n = 11)	18 months (n = 11)	24 months (n = 11)
Systolic BP (mmHg)	145 ± 11.8	146 ± 12.4	149 ± 15.9	146 ± 15.1	144 ± 18.2	151 ± 16.4
Diastolic BP (mmHg)	97 ± 16	99 ± 15.6	98 ± 10.1	101 ± 18.1	95 ± 18.1	100 ± 18
Urine Alb (mg/dL)	8.8 ± 8.9	7.5 ± 8.4	11.6 ± 9.4	9.3 ± 9.0	9.5 ± 8.7	8.4 ± 9.9
UPC	0.41 (0.10–5.06)	0.53 (0.10–4.11)	0.53 (0.10–3.42)	0.41 (0.05–7.20)	0.56 (0.08–3.75)	0.71 (0.05–6.32)

BP, blood pressure; UPC, urine protein:creatinine ratio.

lence of diastolic hypertension was associated with time since DM diagnosis or glycemic control ($P > .05$). The highest recorded diastolic BP in dogs with long-term evaluation was 129 mmHg and was recorded in 2 dogs, each at a single time point. When dogs administered enalapril were excluded from the analysis, significant changes in systolic and diastolic BPs and prevalence of systolic and diastolic hypertension over time were still absent.

Renal Parameters

At initial evaluation of all dogs, mean (±SD) urine albumin concentration was 8.83 (±8.93) mg/dL and prevalence of microalbuminuria was 10/16 (62.5%) dogs (the urine sample for this assay was misplaced for 1 case at initial evaluation). Three dogs (all with complete follow-up) had microalbuminuria without an elevation of UPC at the initial evaluation.

In dogs with complete follow-up, mean (±SD) urine albumin concentration was 7.5 (±8.4) mg/dL and microalbuminuria was detected in 7/11 (64%) dogs at initial evaluation. Serial mean (±SD) urine albumin concentrations are depicted in Table 2. At the 6-, 12-, 18-, and 24-month evaluations, significant elevation of urine albumin was detected in 6/9 (67%), 6/10 (60%), 8/11 (73%), and 8/11 (73%) of cases, respectively. Urine samples for this assay were misplaced for 2 dogs at the 6-month visit and 1 dog at the 12-month visit. Two of the 3 dogs with microalbuminuria but with a normal UPC at initial evaluation developed an elevated UPC ratio at a subsequent evaluation, but the

UPC did not exceed 1.0 in either case. Two dogs had a urine albumin concentration >30 mg/dL at 1 and 2 time points, respectively. No significant change in mean urine albumin was associated with time since DM diagnosis or with glycemic control ($P > .05$).

At initial evaluation of all dogs, the median (range) UPC was 0.41 (0.10–5.06) and an overt proteinuria was detected in 7/16 (44%) dogs. In dogs with complete follow-up, the median (range) UPC at initial evaluation was 0.39 (0.10–4.11) and overt proteinuria was present in 4/11 (36%) dogs. All 4 of these dogs exhibited overt proteinuria at all subsequent time points. At the 6-, 12-, 18-, and 24-month evaluations, UPC >0.5 was present in 6/11 (55%), 5/11 (46%), 6/11 (55%), and 6/11 (55%) cases, respectively (Fig 1). No significant change in prevalence of elevated UPC was associated with time since DM diagnosis or glycemic control ($P > .05$). No significant association between positive urine culture and proteinuria was detected ($P > .05$).

The 4 dogs with the highest UPCs were administered enalapril for management of proteinuria at various times during the course of the study (Fig 1). Once enalapril was started in a dog, the treatment was continued for the remainder of the study, resulting in a total of 8 instances of enalapril treatment during the study. When these 8 instances were excluded from analysis, statistical significance was unchanged. In these 4 dogs, the median (range) UPC before enalapril treatment was 2.75 (0.57–7.20) (n = 12 instances) and median (range) UPC during enalapril treatment was 3.87 (2.53–6.32) (n = 8 instances). There was a decline
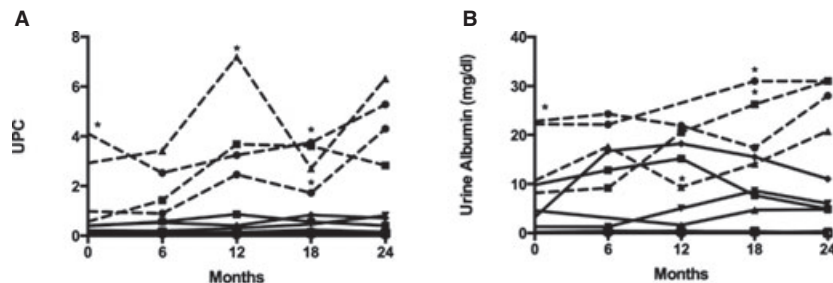

Fig 1. Serial (A) urine protein:creatinine ratio and (B) urine albumin concentrations in 11 diabetic dogs over a 2-year period. —Dogs treated with enalapril, *Initiation of enalapril treatment.

in median UPC in only 1 of these 4 dogs during ena-lapril treatment and all 4 dogs had a higher UPC at 24 months compared with that at initial enrollment. Of the 4 dogs that received enalapril, only one experienced a urinary tract infection (a single instance). In that case, the infection was diagnosed at the same visit that the dog was initially started on enalapril, at which time the UPC was 1.72. At the subsequent visit, urine culture was negative and the UPC was 4.3.

Serum creatinine and BUN remained within reference intervals for all dogs in the longitudinal evaluation and did not change significantly over the course of the study ($P > .05$).

Bacteria were cultured in urine samples on 15 instances in 6 study dogs. Of the 6 affected dogs, positive culture was found once in 1 dog, twice in 1 dog and on 3 occasions in 4 dogs. Median (range) UPC for all instances of positive and negative urine culture were 0.2 (0.05–2.46) (n = 15) and 0.56 (0.05–4.3) (n = 40), respectively. Mean (±SD) urine albumin concentrations for all instances of positive and negative urine culture were 8.2 (±10.60) mg/dL (n = 13; 2 missing data points) and 11.2 (±9.72) mg/dL (n = 39; 1 missing data point), respectively. When the 15 instances of urinary tract infection were excluded from analysis, statistical significance was unchanged.

Retinopathy

At initial evaluation, fundic examination was precluded by cataract in 7/17 (41%) dogs. Retinopathy was documented in 1/10 dogs in which fundic examination was possible.

In dogs with complete follow-up, fundic examination was precluded by cataract in 6/11 dogs at initial evaluation. Retinopathy was not detected on initial examination in any of the 5 dogs whose fundus could be examined. At the 6-, 12-, 18-, and 24-month evaluations, retinopathy was documented ophthalmoscopically in 1/11 (9%), 1/10 (10%), 2/10 (20%), and 1/10 (10%) study dogs. Four of the 17 dogs had retinopathy at 1 or more time points during the study and only 1 dog had retinopathy on more than 1 examination. Cataract extraction surgery was performed to address clinically significant cataract in 8 dogs during the course of the study. Cataract formation was not substantial enough to impair fundus examination in 2 dogs over the course of the study. In 1 dog, complete cataract precluded fundus examination at the 12-, 18-, and 24-month visits, but cataract surgery was declined. No significant associations were detected between the presence of ophthalmoscopically evident retinopathy and time since DM diagnosis, glycemic control, systolic BP, systolic hypertension, diastolic BP, or diastolic hypertension ($P > .05$).

Discussion

Diabetic vascular complications, including hypertension, proteinuria, and retinopathy were documented in this longitudinal study, but there were no significant associations between these conditions and time since DM diagnosis or degree of glycemic control. In addition, no significant associations were found between these various complications in this group of dogs.

The prevalence of systolic and diastolic hypertension in the present study was similar to that previously reported in dogs with spontaneous DM.[4,17] The severity of systolic hypertension, when considered as risk of target organ damage, was mild in all but 2 dogs, which had a risk considered "moderate" at most measurements, and "severe" at 1 measurement for 1 dog.[15] Similar to a previous report, diastolic hypertension occurred more frequently than systolic hypertension.[4] Additionally, the diastolic pressure elevation was higher in magnitude than the systolic pressure, relative to proposed risk to target organs.[15] However, the 2 dogs with complete follow-up that reached a diastolic BP >120 mmHg did not have overt proteinuria or retinopathy.

Blood pressure did not significantly increase during the course of the 2-year study, leading to the conclusion that any effect of DM on BP may occur early in the course of the disease. This is not consistent with a previous study that found the risk of hypertension to increase with more prolonged DM.[4] However, dogs were evaluated once in that study, where the median time from diagnosis of DM to evaluation was 6 months, rather than longitudinally as in the present study. In addition, dogs with unilateral nephrectomy and induced DM had a peak increase in mean arterial pressure after 4 months that then decreased through the remainder of the 1-year study.[18] In humans, hypertension occurs in approximately 30% of those with type 1 DM and 50–80% of those with type 2 DM.[19] Hypertension is commonly present at the time of diagnosis of diabetes in humans with type 2 disease, while in type 1 diabetes, it occurs later in the course of disease and is most commonly believed to be a consequence of nephropathy.[20]

Microalbuminuria develops in 30–60% of humans with type I diabetes,[21,22] and commonly precedes the development of overt glomerular disease and proteinuria.[23] However, there is a paucity of information regarding urine albumin concentration in dogs with spontaneous DM. Mazzi et al reported mean ± SD urine albumin concentrations of 20 ± 43.3 mg/dL in a group of 20 diabetic dogs.[17] Elevated urine albumin was found in 55% of the dogs, with over half of them having concurrent elevation in UPC. The prevalence of microalbuminuria of 59% in the present study, with 7/17 dogs also having elevated UPC, is similar. Although 2 of the 3 dogs with microalbuminuria and normal UPC at initial evaluation developed an elevated UPC during the longitudinal study, the degree of proteinuria remained modest (ie, UPC <1.0).

In dogs, a UPC >0.5 is considered abnormal.[16] Based upon this definition, the prevalence of abnormal UPC in our study population ranged from 48.3 to 60% over the course of this investigation. Urine samples with positive bacterial culture were included in the analysis, as statistical findings were unchanged after removing

instances of positive urine culture. Although inflammation associated with lower urinary tract infection is a commonly cited source of proteinuria, the majority of pyuric samples in a recent study were not albuminuric nor did they have elevated UPC.[24] Microalbuminuria commonly precedes the development of overt proteinuria in humans, but we were unable to document a similar course of disease in our study population.[23] This may be a reflection of the fact that the vast majority of dogs demonstrating increased urine albumin concentration already had elevated UPC at the time of albuminuria diagnosis. This does not preclude the possibility that screening for microalbuminuria may prove a useful tool to predict the onset of diabetic nephropathy in dogs, but earlier testing may be necessary. Alternatively, the overall lack of significant progression of proteinuria for the 2-year duration of the study may be an indication that the proteinuria is not a result of diabetic nephropathy. Based on the tests utilized, diabetic dogs in this study did not have progression of renal dysfunction during the 2-year study period. Lack of progression of renal dysfunction is consistent with the described lack of clinically important nephropathy in dogs with spontaneous DM,[25] despite development of lesions typical of diabetic nephropathy in dogs with spontaneous and experimentally induced DM.[2,5] In addition, no significant effect of time since DM diagnosis, glycemic control or BP on urine albumin or UPC could be demonstrated.

Despite the lack of significant effect of duration of DM on proteinuria, the 4 dogs with a UPC >0.5 at initial evaluation had increases in UPC throughout the study period despite treatment with enalapril (Fig 1). At the end of the 24-month study, all 4 dogs had a UPC >2, and would be considered candidates for intervention as was done in the present study. Further investigation is warranted to determine if a subset of dogs with DM have progressive glomerulopathy as in humans.[26]

Elevated BP is associated with increasing risk of diabetic nephropathy in humans and dogs with experimentally induced DM.[27] While angiotensin-converting enzyme inhibitors attenuate progression of diabetic nephropathy in alloxan-induced diabetic dogs,[1] results of the present study did not discern an association between BP or hypertension status and indicators of nephropathy (ie, microalbuminuria or overt proteinuria). Enalapril was administered to 4 dogs to address persistent and substantially elevated UPC; in no dog was it administered for the treatment of hypertension. The number of dogs being treated with enalapril at 6, 12, 18, and 24 months were 1/11, 1/11, 2/11, and 4/11, respectively. A consistent reduction in proteinuria was not noted in any dog receiving enalapril. While it is possible that this treatment may have influenced both BP and proteinuria, removing instances of enalapril treatment from analysis did not change the statistical outcomes for either condition.

In dogs whose fundus could be examined, the prevalence of ophthalmoscopically detectable retinopathy ranged from 9.1 to 20.0% over the 2-year course of the study. The lesions detected in this study were similar to those previously described and consisted of single to multiple retinal hemorrhages/microaneurysms visible in the tapetal fundus.[7] Prevalence of retinopathy in humans with diabetes in the United States was recently reported as 28.5%, with a strong correlation with duration of diabetes.[28] Previous studies describing retinopathy in dogs with naturally occurring diabetes have reported similar prevalence values as this study, ranging from 3 to 21%.[5,7,29,30] Although hypertension is also a well-known risk factor for development and progression of diabetic retinopathy in humans,[28,31] a similar correlation was not found in the present study. Time since DM diagnosis and glycemic control scores were also not correlated with presence of ophthalmoscopically visible retinopathy in this study. Studies comparing methodologies for diagnosis of diabetic retinopathy are lacking in dogs, but it is reasonable to assume that ophthalmoscopy alone may miss subtle lesions. Although ophthalmoscopy is accepted as the most commonly used screening tool for diabetic retinopathy in humans, fluorescein angiography and multiple field stereoscopic fundus photography are more sensitive than ophthalmoscopy alone in detecting the condition.[32,33] However, neither multiple field stereo fundus photography nor fluorescein angiography is practical to employ on a routine basis with clinical veterinary patients. Additionally, fluorescein angiography carries a risk of severe adverse reaction,[34] which is not justifiable in context of what is regarded as an essentially benign condition in the dog.

Because a primary objective of this study was to assess the relationship of glycemic control with the development of vascular complications, multiple means of evaluating this factor were employed, including serial blood glucose measurement, serum fructosamine concentration, and owner observations. Although a previous study reported that owner perceptions of polyphagia, polyuria, and polydipsia correlated well with other measures of glycemic control, this was not evident in the present study.[14] Agreement between MBG_{8h} and serum fructosamine concentration was better, but only fair. In general, owners tended to assess their pets control as better than was suggested by MBG_{8h} or serum fructosamine concentration. While the effect of variable glycemic control on progression of vascular complications would best have been evaluated by prospectively assigning dogs to more or less intensive treatment, use of client-owned dogs with spontaneous DM made this ethically impossible.

The spectrum of vascular complications associated with DM in the dog is similar to that recognized in humans and includes systemic hypertension, proteinuria, and retinopathy. Failure to document progression of these complications in the present study despite less than optimal glycemic control may have resulted from the small sample size or insufficient time for progression to manifest. Some investigators report that morphologic nephropathy develops consistently only after experimentally induced DM of >2 years duration,[3] whereas others report glomerular changes within 6–23 months.[35] In addition, dogs with unilateral nephrec-

tomy developed increased mean arterial pressure and proteinuria within 2–8 months induction of DM and had increased GFR, renal blood flow, glomerular capillary pressure and histopathologic changes of diabetic nephropathy after 1 year.[1,2,18] Yet another factor that necessitates caution in interpretation of the results of the present study is the considerable variability in proteinuria that is noted between samples from the same dog, particularly at the modest levels present in most dogs of this study. The variability could mask statistical elucidation of progression of the proteinuria. Subsequent studies of spontaneous DM should be continued for a longer duration, particularly in dogs with overt proteinuria at diagnosis, to determine if clinically important complications occur.

Footnotes

[a] MEMOPRINT, S + B medVET, Babenhausen, Germany
[b] ACCU-CHEK Advantage, Roche Diagnostics Corp, Indianapolis, IN
[c] ANTECH Diagnostics, Irvine, CA
[d] Olympus AU400 Chemistry Analyzer, Beckman Coulter Inc, Brea, CA
[e] SAS Inc, Cary, NC
[f] Vetsulin· Intervet Inc., Merck Animal Health, Summit, NJ
[g] Humulin N, Lilly USA, LLC, Indianapolis, IN
[h] Novolin N, Novo Nordisk Inc, Princeton, NJ

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Diabetes mellitus – recheck owner questionnaire.