Assessment of iris vasculature abnormalities in dogs with diabetes mellitus

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OBJECTIVE
To identify and characterize abnormalities of iris vasculature in dogs with diabetes mellitus, compared to clinically normal, age-matched control dogs, by means of anterior segment angiography.

ANIMALS
10 dogs with naturally occurring diabetes mellitus and 10 age-matched control dogs with no ocular or systemic disease.

PROCEDURES
The day before iris vasculature abnormality (IVA) assessment, all dogs underwent complete physical and ophthalmic examinations and baseline clinicopathologic analyses. For diabetic dogs, serum fructosamine concentration and a 12-hour blood glucose concentration curve were generated. The next day, all dogs were sedated and anterior segment angiography (following IV injection of indocyanine green [1 mg/kg] and subsequently sodium fluorescein [20 mg/kg]) was performed with a full-spectrum camera and camera adapter system. Group findings were compared, and multiple linear regression analysis was performed to identify potential factor associations with IVAs.

RESULTS
During anterior segment angiography, the arterial, capillary, and venous phases were identified in all dogs. Times to onset of all phases in diabetic dogs were significantly less than those in control dogs. Vascular disruptions within the peripupillary region (evident following sodium fluorescein administration) were common in diabetic dogs. Severity of dye leakage into the iris stroma and aqueous humor was significantly greater in diabetic dogs than in control dogs. Duration of disease, mean blood glucose concentration, and serum fructosamine concentration were significantly associated with IVAs.

CONCLUSIONS AND CLINICAL RELEVANCE
In diabetic dogs, anterior segment angiography revealed IVAs that were not evident in control dogs. The severity of those changes appeared to be associated with disease duration and blood glucose regulation.

Diabetes mellitus is one of the most common endocrinopathies affecting dogs. It is estimated that 1 in 100 dogs to 1 in 500 dogs will develop DM.[1] Diabetes mellitus–associated ocular changes such as cataracts, decreased corneal sensitivity, and retinopathy have been reported.[2–5] The latter has been considered to be of low prevalence or clinical importance.[6] Detection of retinal vascular abnormalities by posterior segment evaluation is hindered by the rapid onset of cataracts in diabetic dogs, thereby preventing longitudinal assessment of vascular changes.[7,8]

ABBREVIATIONS

ASICGA	Anterior segment indocyanine green angiography
ASSFA	Anterior segment sodium fluorescein angiography
DM	Diabetes mellitus
ICG	Indocyanine green
IVA	Iris vasculature abnormality
MBGC	Mean blood glucose concentration
SABP	Systolic arterial blood pressure
SF	Sodium fluorescein

Alternative approaches to better evaluate and characterize these ocular vascular abnormalities over time are needed. Microangiopathies that develop secondary to DM involve the entire vascular system of the eye.[9] In humans, both diabetic retinopathy and iridopathy have been well characterized.[10–14] The latter is considered to be one of the most serious ocular complications associated with DM, and ≤ 90% of diabetic patients are affected.[15] Early detection of IVAs is paramount and relies on the use of anterior segment angiographic techniques because routine ophthalmic evaluation is not sufficiently sensitive for detection of these vascular changes.[16,17] As such, anterior segment angiography would likely be a viable alternative for assessment of ocular microangiopathies in dogs.

Vascular abnormalities indicative of diabetic iridopathy include capillary leakage from the peripupillary border and leakage into the iris stroma.[15,18] These changes are thought to reflect direct vas-

cular endothelial damage and loss of iris vascular system integrity (ie, the blood-aqueous barrier).[19,20] Additionally, perfusion abnormalities in the iris have also been described.[10,11] The pathophysiologic development and progression of DM-related ocular microangiopathy is complex, and hyperglycemia is considered to be a major contributing factor.[21,22] Ocular inflammation and specific inflammatory mediators (eg, vascular endothelial growth factor) have been proposed to have roles as well.[23,24] Additionally, arterial hypertension, high serum creatinine concentration, and proteinuria have been associated with more severe vascular changes.[11] The only factor shown to prevent or reduce progression of IVA in humans is good glycemic control,[21,22] and blood pressure monitoring is also important in preventive management.[25]

To our knowledge, there are no reports of studies documenting IVAs in diabetic dogs, and risk factors (eg, duration of disease and glycemic control) for development of such abnormalities in that species have not been determined. As such, the purpose of the study reported here was to identify and characterize IVAs in dogs with DM, compared to findings in clinically normal age-matched control dogs, by means of ASICGA and ASSFA. Additionally, we sought to correlate the prevalence and severity of IVAs with duration and control of DM.

Materials and Methods

Animals

Client-owned dogs with naturally occurring DM from the Michigan State University Veterinary Medical Center and surrounding primary care facilities were included in the study. Diabetic dogs, regardless of disease control status and degree of cataract formation, were considered eligible for study enrollment. However, dogs with any signs suggestive of concurrent systemic disease (eg, hyperadrenocorticism, cardiac or renal failure, or neoplasia) or ophthalmic abnormalities with the exception of cataract formation (eg, keratoconjunctivitis sicca, corneal ulceration, phacolytic uveitis, or glaucoma) were excluded from the study. Diabetic dogs receiving corticosteroids orally within the preceding 30 days or with a prior history of receiving any topical ocular anti-inflammatory medication were excluded. Diabetic dogs received a complete physical and ophthalmic examination prior to enrollment to ensure the exclusion criteria were not met, and owners were asked to complete a simple questionnaire documenting insulin type, insulin dosage, and daily water consumption for their dog. After diabetic dogs were enrolled in the study, a matching number of client-owned, age-matched control dogs that had no systemic or ophthalmic disease were subsequently enrolled. The sample size for the study was determined on the basis of recent research[26] by assuming a difference of at least 2 seconds in perfusion times between groups (diabetic dogs vs control dogs) with a power of 0.80, α value of 0.05, and SD of 1.5. The sample size determined was 10 dogs with DM and 10 control dogs.

Written informed consent was obtained from all owners of dogs included in the study. The study protocol was approved by the Institutional Animal Care and Use Committee at Michigan State University and conformed to the statement of the Association for Research in Vision and Ophthalmology regarding use of animals in vision research.

Clinical and laboratory assessments

All dogs underwent complete ophthalmic and physical examinations on the day prior to the anterior segment angiography procedures. The ophthalmic examination included evaluation of menace, dazzle, and pupillary light reflexes and fluorescein[a] staining of the ocular surface; slit-lamp biomicroscopy[b]; rebound tonometry[c]; and indirect ophthalmoscopy,[d] when possible, because of lens opacification.

For all dogs with DM, a blood sample (5 or 6 mL) was collected for a CBC, serum biochemical profile, and assessment of serum fructosamine concentration. A urine sample was collected for urinalysis, microbial culture, and urine protein-to-creatinine concentration ratio assessment. Systolic arterial blood pressure measurement (Doppler sphygmomanometry) was also performed; the mean of 5 readings was calculated for each dog, and the same personnel performed the testing for all dogs with DM. Thereafter, the diabetic dogs underwent measurement of blood glucose concentration over a 12-hour period (8 AM to 8 PM) to evaluate glycemic control. A blood sample (0.1 mL) was collected at 7 time points (at 2-hour intervals) during the 12-hour period of assessment (ie, at 8 AM, 10 AM, 12 PM, 2 PM, 4 PM, 6 PM, and 8 PM). Blood glucose concentration in the collected samples was measured with a glucometer[e] that was calibrated annually. Blood glucose monitoring was performed by the same personnel for each dog with DM.

For all age-matched control dogs, a blood sample (5 mL) was collected for a CBC and serum biochemical profile. A urine sample was collected for urinalysis. Systolic arterial blood pressure measurement (Doppler sphygmomanometry) was also performed in the manner described for dogs with DM. With the exception of the blood glucose concentration measurements, all clinicopathologic analyses were performed at the Michigan State University Veterinary Diagnostic Laboratory.

Anterior segment angiography

The day after clinical examinations and laboratory assessments were performed, ASICGA and ASSFA were performed in all dogs. Anterior segment angiography was performed with a digital single lens reflex camera adapter system that included a modified (full-spectrum) camera,[f] camera adapter, and camera lens,[g] as previously described.[26] Each dog was sedat-

ed once in accordance with a standardized protocol. Twenty minutes prior to sedation, all dogs received maropitant citrate[h] (1 mg/kg, SC) and diphenhydramine[i] (2 mg/kg, SC). These medications were used as a prophylactic measure to counteract potential emesis and anaphylaxis, respectively, that may be associated with IV dye administration. A cephalic catheter was placed (sterile technique used), and midazolam[j] (0.2 mg/kg, IV) and butorphanol tartrate[k] (0.2 mg/kg, IV) were administered. For each dog, the selection of the primary eye for the entire imaging sequence (ie, the eye in which the time of onset for each angiographic phase and the arterial and capillary phase durations would be determined) was made by means of sequential randomization (coin toss). Nevertheless, both eyes of each dog underwent imaging at various time points to identify and characterize the degree of dye extravasation or leakage.

Once each dog was adequately sedated, gentle manual restraint and retraction of the eyelids was used to ensure proper positioning of the head and exposure of the eyes. Standard color and near-infrared images were obtained. The same angiographic imaging protocol was used regardless of the angiographic dye injected. On completion of bolus dye administration, imaging of the primary eye occurred at a rate of 3 images/s for a total of 30 seconds. Thereafter, imaging was performed at 1, 2, 3, 4, and 5 minutes. Imaging of the secondary eye was performed at 35 seconds and 1.5, 2.5, 3.5, 4.5, and 5.5 minutes after completion of bolus dye administration. For the purpose of conducting ASICGA and ASSFA, IV administration of ICG[l] (1 mg/kg) was performed followed by IV administration of SF[m] (20 mg/kg), respectively. Ten minutes was allowed to elapse between dye administrations. When imaging was completed, all dogs were allowed to recover from sedation and were monitored for a minimum period of 2 hours, whereupon they were returned to their owners.

Angiographic evaluations

Angiographic measurements obtained included times to onset of the arterial, capillary, and venous phases and durations of the arterial and capillary phases, as described previously.[27] Times to onset of the arterial, capillary, and venous phases were identified by the initial filling of dye within the major arterial circle of the iris, pupillary capillaries, and iris veins, respectively **(Figure 1)**. Phase durations were defined as the time from the onset of one phase to onset of the next phase. All images were converted to black and white with the black and white adjustment tool (entire image) of graphic image editing software.[n] All measurements were performed in duplicate by one of the authors (CGP), and mean values for all dogs were calculated on completion of the study. Angiographic images were compared between groups, and any vascular changes were characterized and the degree of dye extravasation was assessed. The severity of dye leakage within the iris stroma was categorized as grade 0 to 4 (no leakage to severe leakage) in accordance with a previously published grading scheme[17] **(Appendix; Figure 2)**. The severity of dye leakage within the aqueous humor was subjectively characterized as grade 0 = none, grade 1 = mild, grade 2 = moderate, and grade 3 = severe.

Statistical analysis

For each variable involving more than 1 measurement (SABP, MBGC, times to onset of angiographic phases, and phase durations), means were first calculated for each dog and subsequently used to generate medians for each group (control dogs and dogs with DM). Data were assessed for normality by Shapiro-Wilk testing and inspection of probability plots and were reported as medians and ranges. Times to onset of the arterial, capillary, and venous phases; arterial and capillary phase durations; severity of vascular leakage; and severity of dye leakage into the aqueous humor for each dye injection for diabetic dogs and control dogs were compared with the Wilcoxon signed-rank test. For data from dogs with DM, multivariable linear regression analyses were performed to further evaluate potential associations of various clinical and laboratory factors with selected angiographic features. The angiographic features included as the primary response variable in each model were the time to onset of each angiographic phase and the arterial and capillary phase durations, as well as severity of dye leakage within the iris stroma and into the aqueous humor. The variables of cataract stage, duration of DM, MBGC, serum fructosamine concentration, and SABP were considered in each regression model. Nonsignificant variables were removed from

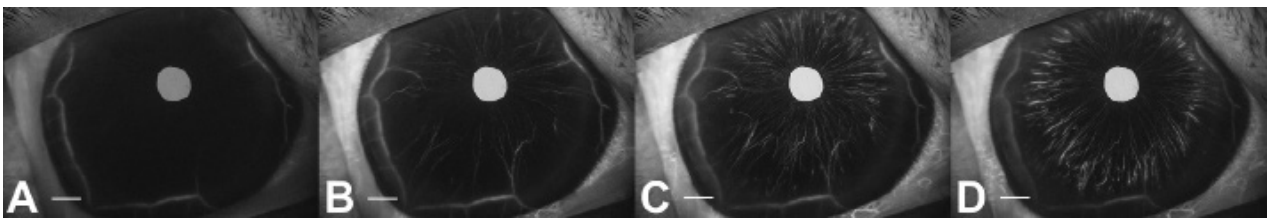

Figure 1—Representative ASICGA images of the right eye of an 8-year-old spayed female mixed-breed dog with a brown iris. After IV injection of ICG, notice the filling of the major arterial circle and early filling of radial iris arteries at 6 seconds (arterial phase; A), progressive filling of radial iris arteries and initial filling terminal capillary loops within the peripupillary region at 9 seconds (capillary phase; B), and progressive filling of radial iris veins at 11 seconds (venous phase; C). The image in panel D was obtained during the late period of ASICGA (at 5 minutes following ICG injection). In each panel, bar = 2 mm.

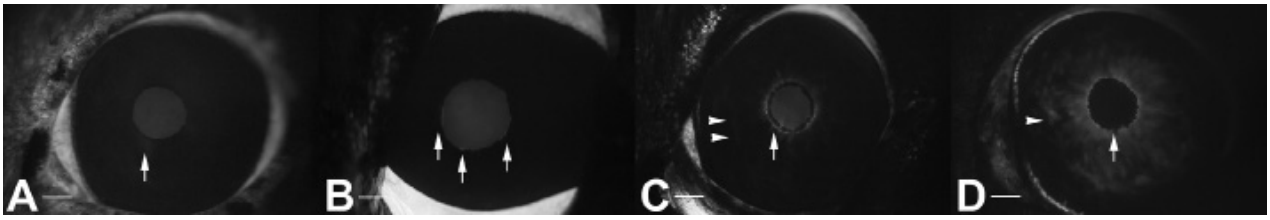

Figure 2—Representative ASSFA images of the left eyes of 4 dogs with DM to illustrate the grading scheme used to characterize the severity of dye leakage following IV injection of SF. All images were acquired at 30 seconds after SF injection; peripupillary SF leakage is denoted by arrows, and multifocal regions of stromal leakage are denoted by arrowheads. Grade 0 corresponds to no fluorescein leakage (not shown). A—In a 3-year-old neutered male mixed-breed dog, mild leakage is evident in 1 or 2 quadrants of the peripupillary border (grade 1). B—In a 5-year-old neutered male mixed-breed dog, mild leakage is evident in 3 or 4 quadrants of the peripupillary border (grade 2). C—In a 9-year-old spayed female mixed-breed dog, SF leakage is evident in 3 or 4 quadrants of the peripupillary border with leakage in 1 or 2 quadrants of the iris stroma (grade 3). D—In an 8-year-old spayed female Pug, SF leakage is evident in 3 or 4 quadrants of the peripupillary border with leakage in > 2 quadrants of the iris stroma (grade 4). In each panel, bar = 2 mm.

the model, but all deleted variables were individually added back to the final model to assess for significance. Analyses were performed with commercially available software.[o] For all analyses, a value of $P < 0.05$ was considered significant.

Results

Animals

The study included 10 dogs with DM and 10 clinically normal, age-matched control dogs. A total of 11 dogs with DM were considered eligible for the study; however, 1 dog was excluded from participation because of progressive retinal atrophy, as noted during the ophthalmic examination. The control dogs included 7 mixed-breed dogs, 2 Golden Retriever–Poodle mixed-breed dogs, and 1 Labrador Retriever. Median age and weight of the control dogs were 7.0 years (range, 3.0 to 10.0 years) and 24.6 kg (range, 4.9 to 31.8 kg), respectively. Four control dogs were < 7 years of age, and 6 dogs were ≥ 7 years of age. Eight control dogs were castrated males, and 2 were spayed females. The dogs with DM enrolled in the study included 5 mixed-breed dogs and 1 Labrador Retriever, 1 Chesapeake Bay Retriever, 1 Cairn Terrier, and 1 Miniature Poodle. Median age and weight of the diabetic dogs were 7.1 years (range, 3.0 to 10.0 years) and 20.8 kg (range, 7.0 to 44.3 kg), respectively. Four dogs with DM were < 7 years of age, and 6 dogs were ≥ 7 years of age. Of the diabetic dogs, 6 were castrated males and 4 were spayed females. Age, weight, sex distribution, and neuter status were not different between the 2 groups.

Ophthalmic examination findings

No notable ocular abnormalities were observed in the control dogs (n = 20 eyes). Iris pigmentation was considered heavy (dark brown) and moderate (light brown) in 7 and 2 control dogs, respectively. One control dog had heterochromia in the right eye and a poorly pigmented iris (blue) in the left eye. Five control dogs had signs of mild iris atrophy bilaterally, and nuclear sclerosis in both eyes of 7 control dogs was noted. Among the control dogs, median intraocular pressure was 13.5 mm Hg (range, 10.0 to 20.0 mm Hg) in the right eye and 14.0 mm Hg (range, 10.0 to 18.0 mm Hg) in the left eye.

Four dogs with DM (n = 8 eyes) lacked menace response in both eyes owing to the stage of cataract formation. Iris pigmentation was heavy (dark brown) in 8 diabetic dogs and moderate (light brown) in 2 diabetic dogs. Two diabetic dogs had mild iris atrophy bilaterally. Cataract formation was evident in 9 of the 10 diabetic dogs. For each of those 9 dogs, the stage of cataract progression in both eyes was comparable; 1 dog had incipient cataracts, 3 dogs had immature cataracts, 4 dogs had mature cataracts, and 1 dog had hypermature cataracts. No clinically detectable sign of aqueous flare was noted in any eye of the diabetic dogs. Among the diabetic dogs, median intraocular pressures in the right and left eyes were 15.0 mm Hg (range, 12.0 to 20.0 mm Hg) and 14.5 mm Hg (range, 12.0 to 19.0 mm Hg), respectively. With regard to median intraocular pressure of either the right or left eye, there was no significant difference between diabetic and control dogs. Detailed fundic examination was only possible in 2 dogs with DM; findings for both of those dogs were deemed to be normal.

Clinical and clinicopathologic findings

Control dogs did not have clinicopathologic abnormalities suggestive of systemic disease. Clinicopathologic abnormalities in diabetic dogs were consistent with DM and included hyperglycemia, hypercholesterolemia, and glucosuria. Median SABP in control and diabetic dogs was 127.5 mm Hg (range, 120 to 140 mm Hg) and 150 mm Hg (range, 80 to 170 mm Hg), respectively; median values were not significantly different.

Among the diabetic dogs, the median duration of DM was 5.0 months (range, 2.0 to 36.0 months). The diagnosis of DM had been made < 1 year prior to study enrollment for 8 of the 10 dogs and < 6 months prior to study enrollment for 6 of those 8 dogs. Seven dogs with DM were receiving NPH insulin,[p] and 3 dogs were receiving porcine insulin zinc suspension (Lente insulin[q]) that was approved for use in cats and dogs. The median insulin dose, based on body

weight, was 0.5 U/kg (range, 0.3 to 1.0 U/kg). For the 10 diabetic dogs, the median MBGC derived from the 12-hour blood glucose concentration curves was 366.8 mg/dL (range, 124.1 to 448.7 mg/dL). Two dogs had an MBGC < 200 mg/dL, 1 dog had an MBGC between 200 and 300 mg/dL, and 7 dogs had an MBGC > 300 mg/dL. Median serum fructosamine concentration (reference interval, 179 to 324 µmol/L) was 559.5 µmol/L (range, 328 to 872 µmol/L). One dog had a serum fructosamine concentration < 400 µmol/L,

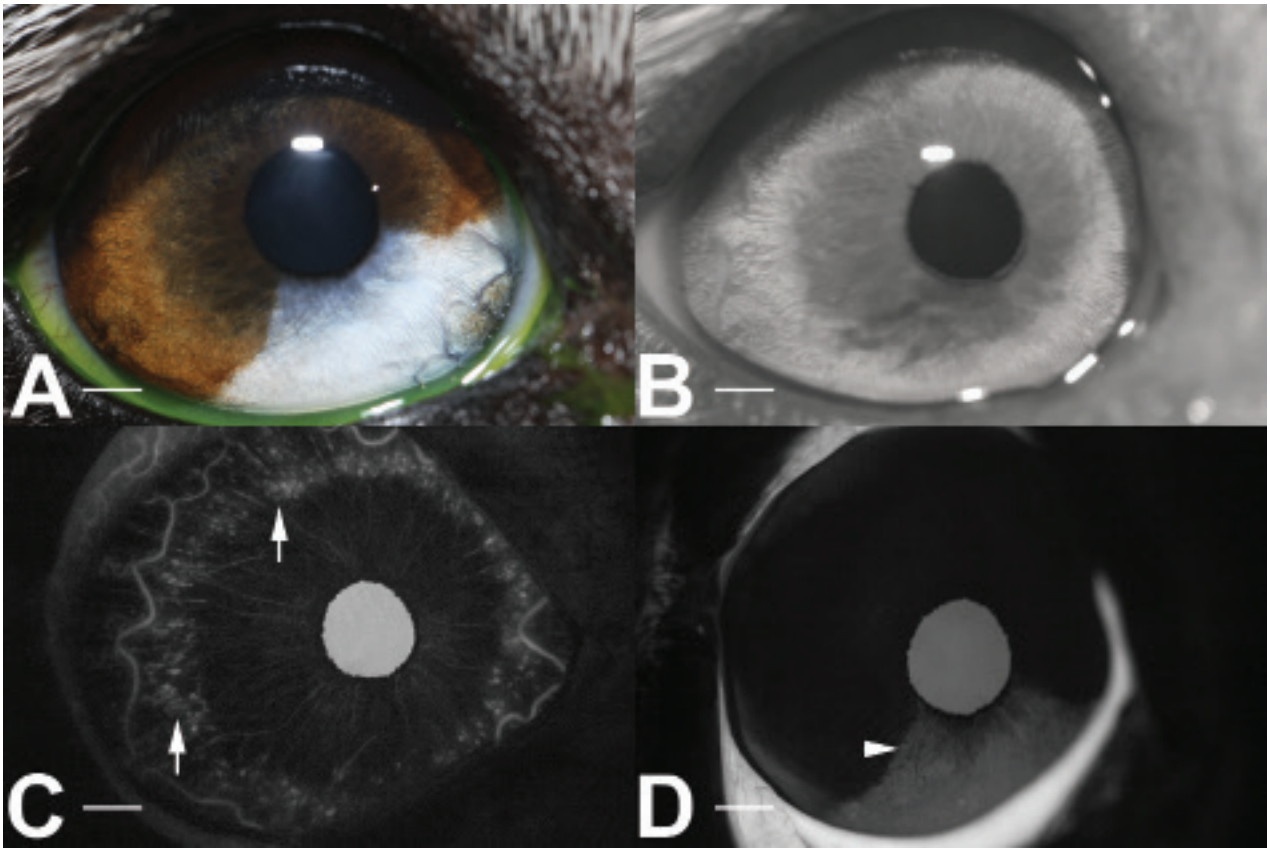

Figure 3—Representative standard color (A), near-infrared (B), ASICGA (C), and ASSFA (D) images of the right eye of a 7-year-old neutered male mixed-breed control dog. Angiographic images (C and D) were obtained at 30 seconds after IV dye injection. Numerous multifocal regions of venular dilations (arrows) are highlighted after ICG injection (C) but not after SF injection (D). No progression or stromal dye leakage associated with these dilations was observed following ICG or SF injection. However, following SF injection, diffuse staining of the stroma within the blue portion of this heterochromic eye (arrowhead) is visible. In each panel, bar = 2 mm.

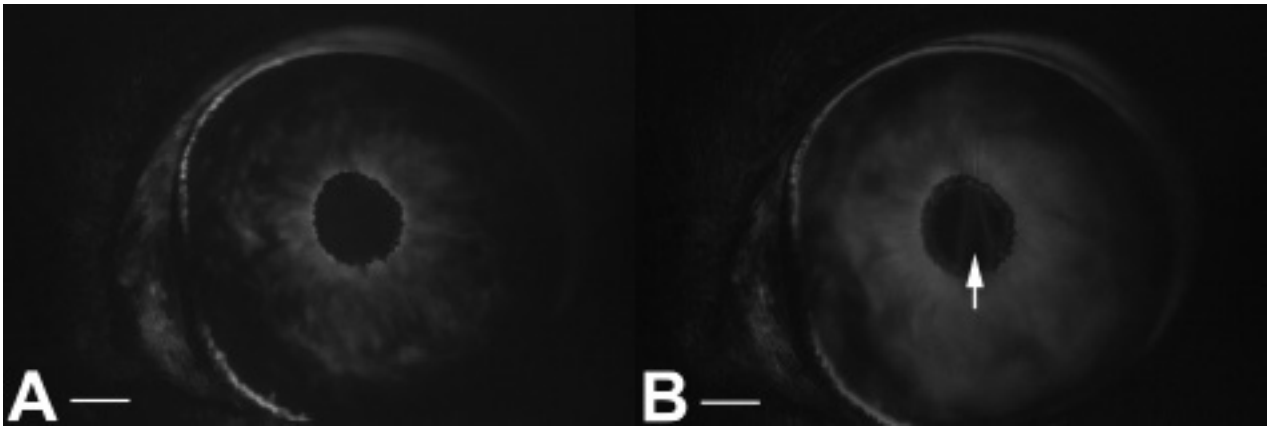

Figure 4—Representative ASSFA images obtained from the left eye of an 8-year-old neutered male Pug. A diagnosis of DM had been made for this dog 5 months prior to imaging. After SF injection, notice the prominent peripupillary and stromal dye leakage of SF obtained at 30 seconds (A; same image depicted in panel D of Figure 2) and at 5 minutes (B). Progressive leakage of SF is readily apparent (A vs B) with visible leakage occurring from the peripupillary region and into the aqueous humor (arrow). For this eye, the leakage severity grade is 4. In each panel, bar = 2 mm.

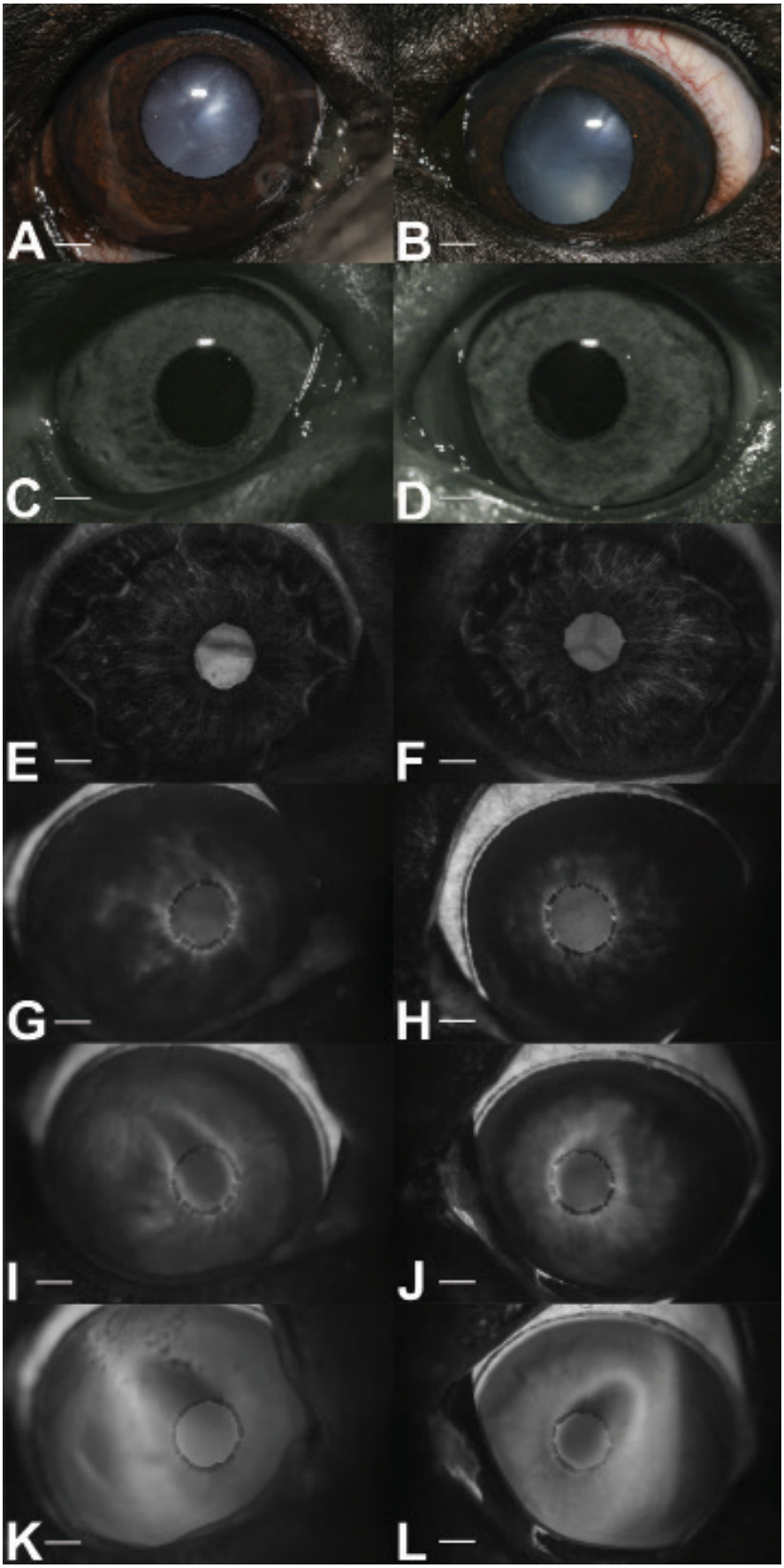

Figure 5—Representative standard color (A and B), near-infrared (C and D), ASICGA (E and F), and ASSFA (G through L) images of the right (A, C, E, G, I, and K) and left (B, D, F, H, J, and L) eyes of a 5-year-old neutered male mixed-breed dog. A diagnosis of DM had been made for this dog 4 months prior to imaging. After IV injection of ICG, there was evidence of dye leakage, capillary dilations, or regions of hypoperfusion at 5 minutes (E and F). After IV injection of SF, bilateral, progressive leakage of SF within the peripupillary region and stroma is apparent at 30 seconds (G and H), 1 minute (I and J), and 5 minutes (K and L). There is marked leakage of SF into the aqueous humor (K and L; arrows). For both eyes, the leakage severity grade is 4. In each panel, bar = 2 mm.

whereas 9 dogs had serum fructosamine concentrations > 400 µmol/L.

Angiographic imaging

In both the control and diabetic groups, the right eye of 5 dogs and the left eye of 5 dogs was randomized as the primary eye for ASICGA and ASSFA. Data collected for those eyes were subsequently used for temporal calculations. Anterior segment angiography with ICG allowed clear visualization of the iris vasculature, and temporal phase calculations were performed for all primary eyes, regardless of the degree of iris pigmentation present. After IV injection of ICG bolus in control dogs, the median times of onset of the arterial, capillary, and venous phases in the 10 primary eyes were at 6.2 seconds (range, 5.0 to 10.0 seconds), 9.0 seconds (range, 7.0 to 12.3 seconds), and 10.0 seconds (range, 8.0 to 15.0 seconds), respectively. After IV injection of ICG bolus in diabetic dogs, the median times of onset of the arterial, capillary, and venous phases in the 10 primary eyes were at 4.5 seconds (range, 2.0 to 12.0 seconds), 6.5 seconds (range, 4.0 to 15.0 seconds), and 8.0 seconds (range, 6.0 to 17.0 seconds), respectively. The time to onset of each phase was significantly ($P < 0.05$) faster in diabetic dogs than in control

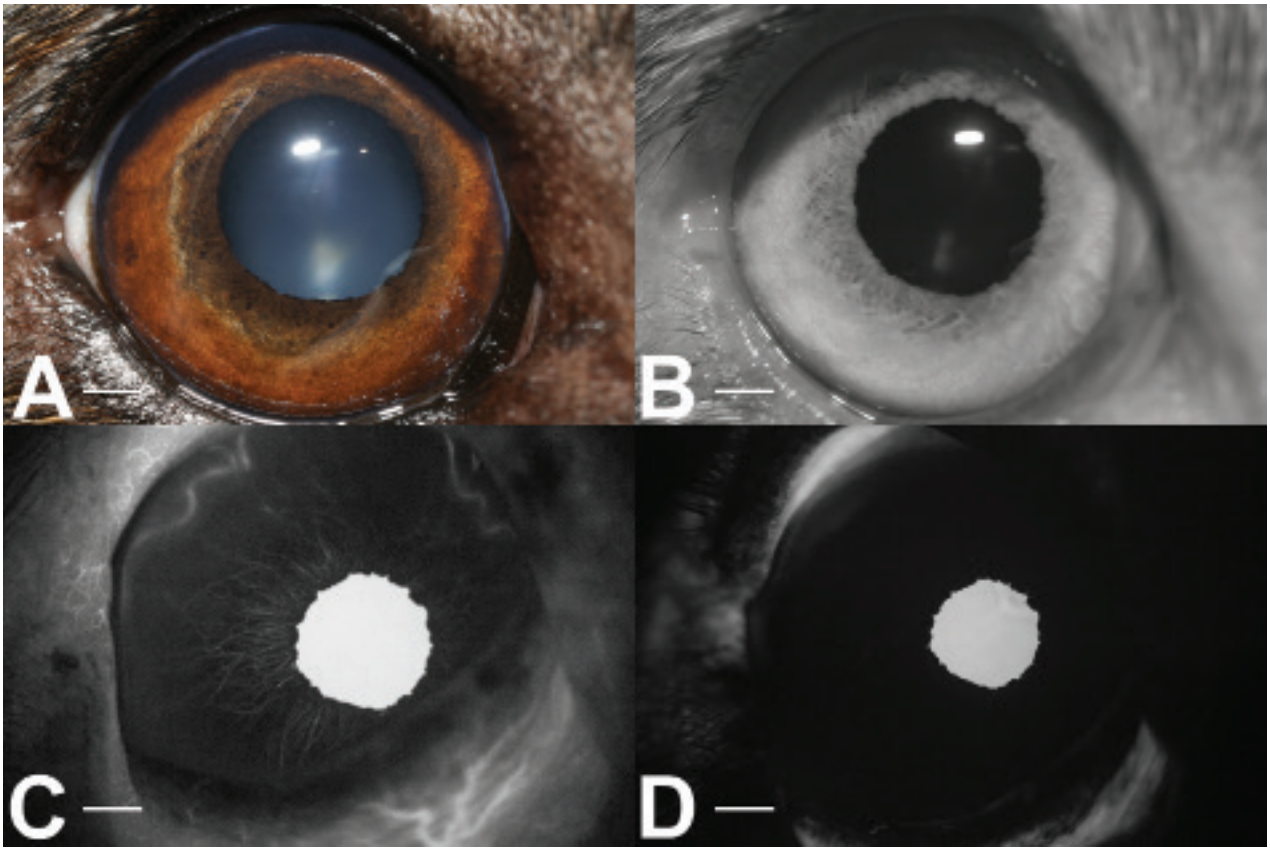

Figure 6—Representative standard color (A), near-infrared (B), ASICGA (C), and ASSFA (D) images of the right eye of a 9-year-old spayed female mixed-breed control dog. Angiographic images were obtained at 30 seconds (C and D) after IV injection of dye. Scalloping of the pupillary edge is readily apparent, indicative of iris atrophy. There is no evidence of leakage of ICG (C) or SF (D) despite age-related degenerative changes to the iris. In each panel, bar = 2 mm.

dogs. No evidence of dye extravasation or leakage of ICG was detected in any eye of the control (20 eyes) or diabetic dogs (20 eyes). However, 4 control dogs (8 eyes) and 4 diabetic dogs (8 eyes) had bilateral multifocal venous dilations, primarily within the ciliary zone of the iris **(Figure 3)**. The median interval between ICG injection and occurrence of these dilations in the primary eye was 25.0 seconds (range, 20.0 to 30.0 seconds) and 20.0 seconds (range, 10.0 to 20.0 seconds) for the control and diabetic dogs, respectively. The time to occurrence of these venular dilations was significantly (P = 0.03) shorter in diabetic dogs, compared to control dogs; however, the number of venular dilations was subjectively greater in those control dogs. No dye leakage was associated with these venular dilations at the time of occurrence or following their detection.

Anterior segment angiography with SF failed to allow clear visualization of the iris vasculature in 4 and 8 primary eyes of the control and diabetic dogs, respectively. After IV injection of the SF bolus, the median time to observation of fluorescence in the pupillary opening of the primary eyes (ie, onset of the arterial phase) was 6.0 seconds (range, 5.0 to 10.0 seconds) in control dogs and 6.0 seconds (range, 4.0 to 15.0 seconds) in diabetic dogs. Although clear observation of the iris vasculature was not readily achieved during ASSFA, SF leakage was more common and the severity of leakage was significantly (P < 0.001) greater in diabetic dogs, compared to control dogs. All 10 diabetic dogs had some degree of bilateral, slow, progressive leakage of SF within the peripupillary region of the iris **(Figure 4)**. The onset of leakage in the primary eyes of the diabetic dogs occurred following the capillary phase; the median time to leakage after injection of the SF bolus was 16 seconds (range, 10 to 30 seconds). Additionally, multiple foci of SF leakage throughout the iris stroma were observed in both eyes of 5 diabetic dogs. On the basis of the applied grading scheme,[17] iris leakage of SF in the eyes of the diabetic dogs was classified as grade 1 in 3 dogs (6 eyes), grade 2 in 2 dogs (4 eyes), grade 3 in 3 dogs (6 eyes), and grade 4 in 2 dogs (4 eyes; **Figure 5**). No peripupillary capillary or stromal leakage of SF was observed in any control dog (20 eyes), including the 5 dogs that had signs of iris atrophy **(Figure 6)**. However, leakage of SF within the anterior chamber of the primary and fellow eye was a common observation in both groups. The severity of leakage into the aqueous humor was significantly (P = 0.006) greater in diabetic dogs than in control dogs **(Figure 7)**. After injection of the SF bolus, the median time to observation of dye emanating into the aqueous humor was 1 minute (range, 30 seconds to 4 minutes) in diabetic dogs, which was significantly (P < 0.001)

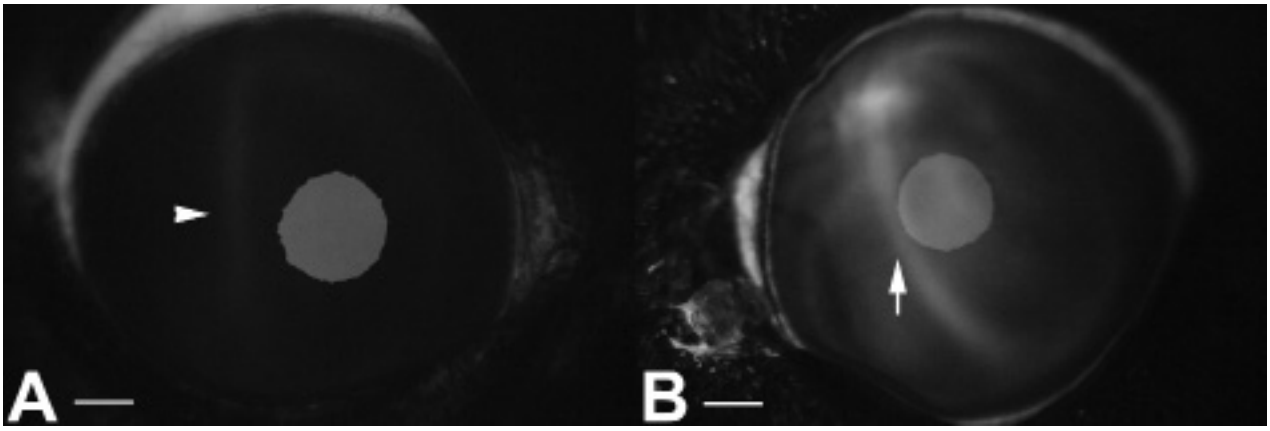

Figure 7—Representative ASSFA images obtained from the right eye of an 8-year-old spayed female mixed-breed control dog (A) and the left eye of a 7-year-old spayed female mixed-breed dog with DM (B). Angiographic images were obtained at 5 minutes after IV injection of SF. Notice the greater degree of SF leakage within the aqueous humor in the diabetic dog's eye (B; arrow), compared with that in the control dog's eye (A; arrowhead). In each panel, bar = 2 mm.

faster than the time of 1.5 minutes (range, 30 seconds to 5 minutes) in control dogs.

Multiple linear regression analysis was conducted to assess whether duration of DM, stage of cataract, MBGC, serum fructosamine concentration, or SABP was significantly predictive of the time to onset of the angiographic phase following IV injection of the ICG bolus and SF leakage severity grades following IV injection of SF. Regarding times to onset of the angiographic phases after ICG injection, results of the regression analysis indicated that the model explained 85.9% (adjusted R^2) of the variance. Duration of disease (β = 0.15 unit increase; P = 0.03), MBGC (β = 0.012 unit increase; P = 0.03), serum fructosamine concentration (β = 0.02 unit increase; P = 0.003), and SABP (β = 0.058 unit decrease; P = 0.01) contributed to the model. However, stage of cataract did not contribute to the model (β = 0.011 unit increase; P = 0.97). Regression analysis revealed that the model could explain 32.5% (adjusted R^2) and 79.9% (adjusted R^2) of the variance with regard to severity of stromal SF leakage and severity of SF leakage into the aqueous humor, respectively. Serum fructosamine concentration (β = 0.003 unit increase; P = 0.01) contributed to the model with regard to stromal SF leakage severity, whereas duration of DM (β = 0.010 unit increase; P = 0.002) and MBGC (β = 0.003 unit increase; P = 0.038) contributed to aqueous humor SF leakage severity.

Discussion

Results of the present study indicated that perfusion and structural abnormalities exist within the iris vasculature of dogs with DM. Similar abnormalities in humans with DM have been previously described (termed diabetic iridopathy) and are associated with alterations involving the iris vasculature.[12,13,16,28] The severity of vascular disruptions in the diabetic dogs of the present report appeared to be related to DM regulation status and duration of disease. Diabetic iridopathy is a microangiopathy with characteristic angiographic patterns, which include capillary dilations, regions of hypoperfusion, dye leakage within the peripupillary margin of the iris and iris stroma, and iris neovascularization.[29] These vascular alterations often precede posterior segment changes (ie, diabetic retinopathy) in human patients.[20] Routine diagnostic examination techniques (eg, slit lamp ophthalmoscopy) often do not adequately identify subtle vascular changes, warranting angiographic assessment.[16,17] Diabetic dogs in the present study had peripupillary and stromal leakage of SF with no evidence of capillary dilations or regions of hypoperfusion. Detection of capillary dilations and hypoperfusion within the iris stroma are typically observed with ASICGA, whereas observations of dye leakage and the presence neovascularization are often more readily observed with ASSFA.[28] Clinically, diabetic iridopathy is classified as nonproliferative or proliferative[16]; the latter is associated with neovascularization and may progress to the development of neovascular glaucoma. On the basis of clinical grading schemes used in human medicine, all diabetic dogs in the present study had signs consistent with nonproliferative diabetic iridopathy. The severity of diabetic iridopathy is generally evaluated on the basis of leakage characteristics, changes that may reflect either a breakdown in the blood-aqueous barrier or iris neovascularization.[30] Given that iris neovascularization was not observed in the diabetic dogs of the present study, we believe the angiographic changes primarily reflected alterations in the blood-aqueous barrier. By use of the previously published grading scheme,[17] the severity of iris dye leakage in 5 of 10 dogs was classified as a grade 3 or higher.

In the dogs of the present report, only ASICGA permitted clear visualization of the iris vasculature, thereby allowing temporal assessment of the arterial, capillary, and venous phases to be identified, regardless of the degree of iris pigmentation present. This finding was consistent with prior reports of ASICGA and ASSFA in healthy dogs, whereby observation of

SF was only possible in poorly pigmented eyes.[26,31] No evidence of capillary dilations or regions of hypoperfusion were observed in any eye of the dogs in the present study. However, the time to onset of each angiographic phase was significantly reduced in diabetic dogs, compared to findings for age-matched control dogs. Similar temporal phase differences have been observed between diabetic and nondiabetic humans.[10,11] The clinical importance of and physiologic reason for these temporal phase differences remain unknown. In the present study, results of multiple linear regression analysis indicated that disease duration, DM control status (MBGC and serum fructosamine concentration), and SABP were all associated with these temporal phase changes in diabetic dogs. Further work will be necessary to determine the role of each of these factors; however, it is interesting to note that control and monitoring of blood pressure in human diabetic patients are important aspects of prevention or progression of DM-related microangiopathies.[32]

In the present report, all 10 diabetic dogs (20 eyes) had no clinically detectable vascular changes on ophthalmic examination yet had dye leakage during ASSFA. With regard to the degree of SF leakage, the regression analysis revealed associations with both duration of DM and glycemic control. Onset of SF leakage first occurred within the peripupillary margin of the iris, followed by slow progressive leakage within various regions of the iris stroma. All 10 diabetic dogs had evidence of peripupillary SF leakage, and 5 of the 10 diabetic dogs (10 eyes) had evidence of stromal leakage as well. On the basis of the confluence and fluorescence pattern of SF within the iris, we believe these angiographic findings were reflections of structural alterations to the walls of the preexisting iris vasculature rather than neovascularization. This was supported by the time to onset of initial SF leakage and the rate of progression thereafter in addition to the absence of neovascularization observed during ASICGA.

The pathophysiologic processes associated with the development of ocular microangiopathies (eg, diabetic iridopathy and retinopathy) are complex. Ocular microangiopathies have been associated with various systemic changes including metabolic disturbances; alterations in blood flow characteristics; the induction of ischemia, changes in intravascular pressure, and inflammation; and the generation of inflammatory mediators. Hyperglycemia is considered to be a major factor contributing to the development and progression of ocular microangiopathies.[21,22] Tight glycemic regulation is considered to be the most effective measure to reduce the risk of developing ocular microangiopathies and minimizing their progression. For the diabetic dogs of the present study, significant associations of the severity of dye leakage to DM regulation status and duration of DM were identified. Most of the diabetic dogs had poor glycemic control, which supported the notion that hyperglycemia has an important role in the development of microangiopathy. Duration of DM was also associated with dye leakage, but it is important to note that the diagnosis of DM was made within the 6 months preceding study enrollment for most dogs. Some of the dogs for which diagnosis of DM had recently been made had high grades for dye leakage severity during ASSFA. Collectively, these findings suggested that major disruptions in ocular vasculature occur very early during the course of DM in dogs and that perhaps glycemic control is a more important risk factor than the duration of DM. Longitudinal assessment of diabetic dogs prior to, during, and following regulation of metabolic disease is needed to further clarify this speculation. Also, although findings for the dogs of the present study were consistent with diabetic iridopathy, other potential factors including age and cataract formation and the subsequent development of phacolytic uveitis need consideration.

With regard to age, peripupillary leakage and leakage of SF into the aqueous humor are normal findings in human patients > 50 years old.[27,33] However, similar leakage in younger patients is considered pathological. In the present study, 4 of the 10 diabetic dogs were < 7 years of age; these 4 dogs had a variable degree of peripupillary SF leakage, and 2 of them had marked stromal leakage (Figure 5). Conversely, 6 of 10 control dogs were > 7 years of age, yet no control dog had leakage within the peripupillary region or stroma, even in the presence of age-related degenerative changes (eg, iris atrophy).

Diabetes mellitus is known to induce several ocular complications in dogs. Cataract formation is a common sequela of DM, occurring in up to 75% of diabetic dogs within 1 year after diagnosis of DM.[8] Cataract formation is known to induce anterior uveitis (ie, phacolytic uveitis), which has been described in as many as 71% of dogs at the time of presentation, and there is evidence to suggest that the frequency of subclinical uveitis may be higher.[34,35] The presence of anterior uveitis is an indication that the blood-aqueous barrier has been disrupted, a change that could result in SF leakage. However, no diabetic dog in the present study had any clinical signs consistent with anterior uveitis (eg, flare). All intraocular pressures were > 12 mm Hg, and values did not differ between dogs with DM and control dogs. Additionally, 2 diabetic dogs had minimal to no lenticular involvement yet had mild to moderate degrees of dye extravasation. Although we cannot exclude the presence of subclinical anterior uveitis in any diabetic dog in the present study, we believe that explanation to be unlikely for the angiographic findings.

An interesting finding of the present report was the observation of numerous venular dilations. Similar observations have been noted in prior angiographic studies involving dogs and cats.[26,36]

Limitations of the present study included the relatively small sample of diabetic dogs evaluated and

their overall poor DM regulation status. To further evaluate the true effects of DM duration and regulation status and SABP, studies involving a larger number of dogs with broader ranges of disease duration and glycemic control will ultimately be necessary. Additionally, to more stringently control for direct effects of cataracts on disruption of the blood-aqueous barrier and to separate those effects from the metabolic alterations caused by DM, further studies could include a group of nondiabetic dogs with cataracts at various stages.

Results of the present study indicated that anterior segment vascular abnormalities consistent with diabetic iridopathy are present in diabetic dogs and that duration of DM and glycemic control are factors that affect the development and progression of those abnormalities. Disruption of the iris vasculature in and of itself is pathological in humans because alteration of the blood-aqueous barrier contributes to the development or persistence of uveitis. Iris vasculature alterations often precede the development of posterior segment alterations. Diabetic iridopathy is a known risk factor for the development of complications in humans who undergo routine cataract surgery or have glaucoma.[16,18] In diabetic dogs, anterior uveitis is a common finding that impacts their quality of life and is associated with poor long-term success rates following routine cataract surgery.[34] Development of anterior uveitis is often thought to be attributable to the leakage of soluble lens material following cataract formation (ie, phacolytic uveitis). However, disruption of the blood-aqueous barrier may reflect or be exacerbated by structural alterations of the iris vasculature as a direct result of DM and its regulation. Future work is needed to further explore the roles of factors such as DM regulation status, duration of DM, and SABP on the development of these vascular alterations and determine the extent to which such alterations affect ocular health in diabetic dogs.

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Footnotes

a. Ful-Glo, Akorn Inc, Lake Forest, Ill.
b. Kowa SL-17 portable slit-lamp biomicroscope, Kowa Co Ltd, Tokyo, Japan.
c. TonoVet, iCare, Vantaa, Finland.
d. Binocular indirect ophthalmoscope, Welch Allyn, Skaneateles Falls, NY.
e. Accu-Chek Performa, Accu-Chek, North Ryde, Australia.
f. Canon 7D, Canon, Tokyo, Japan.
g. Canon EF-S (60 mm f/2.8 macro lens), Canon, Tokyo, Japan.
h. Cerenia, Pfizer, New York, NY.
i. Diphenhydramine hydrochloride, Baxter Healthcare Corp, Deerfield, Ill.
j. Midazolam, Akorn Inc, Lake Forest, Ill.
k. Torbugesic-SA, Zoetis, Florham Park, NJ.
l. IC-Green, Akorn Inc, Lake Forest, Ill.
m. AK-Fluor, Akorn Inc, Lake Forest, Ill.
n. Creative Cloud, Adobe Systems Inc, San Jose, Calif.
o. Excel, Office 365, Microsoft Corp, Redmond, Wash.
p. Novulin N, Novo Nordisk, Plainsboro, NJ.
q. Vetsulin, Merck Animal Health, Madison, NJ.

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Appendix

Grading scheme used to characterize the severity of dye leakage detected during ASSFA in dogs with or without DM.

	Location and degree of SF leakage	
Grade	**Peripupillary region**	**Stroma**
0	None	None
1	Leakage in 1 or 2 quadrants of the peripupillary border	None
2	Leakage in 3 or 4 quadrants of the peripupillary border	None
3	Leakage in 3 or 4 quadrants of the peripupillary border	Leakage in 1 or 2 quadrants of the iris stroma
4	Leakage in 3 or 4 quadrants of the peripupillary border	Leakage in 3 or 4 quadrants of the iris stroma