Aldose reductase activity and glucose-related opacities in incubated lenses from dogs and cats

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Objective—To determine responses of canine and feline lenses to incubation in a medium with a high glucose concentration.

Sample Population—Lenses from 35 dogs and 26 cats.

Procedure—Glucose concentrations were measured in paired lenses from 25 dogs and 17 cats after incubation for 14 days in high-glucose (30 mmol of glucose/L) or control (6 mmol of glucose/L) medium. Aldose reductase activity was measured spectrophotometrically in the incubated lenses and in freshly frozen lenses from 10 dogs and 9 cats. Two lenses of each group were studied histologically.

Results—Canine and feline lenses in high-glucose medium developed glucose-specific opacities of variable localization and extent. Canine lenses developed equatorial vacuoles, but severity of the lesions was not associated with the age of the dog. Lenses from young cats (≤ 4 years old) developed extensive posterior cortical opacities, whereas those from older cats (> 4 years old) did not. Glucose concentrations were similar in all lenses incubated in high-glucose medium; however aldose reductase activity was significantly lower in lenses from older cats, compared with lenses from young cats and from dogs.

Conclusions and Clinical Relevance—High aldose reductase activity and glucose-related opacities suggest a central role for this enzyme in the pathogenesis of diabetic cataracts in dogs and cats. Because onset of diabetes mellitus usually occurs in cats > 7 years of age, low activity of aldose reductase in lenses of older cats may explain why diabetic cataracts are rare in this species despite hyperglycemia. (Am J Vet Res 2002;63:1591–1597)

Diabetes mellitus (DM) is 1 of the most common endocrinopathies in dogs and cats, with an incidence of 0.2 to 1.5% in small animal hospital populations.1–3 Onset of DM in dogs and cats usually does not occur before 7 years of age.1,3 An important complication of DM is the development of intumescent cataracts,4,5 which are common in diabetic dogs but rare in diabetic cats.5,6 In general, blood glucose concentration, duration of hyperglycemia, and activity of the enzyme aldose reductase are believed to be responsible for the development of diabetic cataracts.6–11 In the avascular lens, glucose is the primary source of energy and is mainly (80%) metabolized to pyruvate and lactate via the glycolytic pathway. About 15% of glucose is metabolized via the pentose phosphate pathway, thereby generating NADPH, which is used in several metabolic systems in the lens. In healthy eyes, only 5% of glucose passes through the sorbitol pathway, because the Michaelis constant of aldose reductase for glucose is exceptionally high (200 mmol/L).12,13 However, when the concentration of glucose exceeds the capacity of the glycolytic pathway enzyme hexokinase, glucose spills over into the sorbitol pathway to be metabolized via the enzyme aldose reductase.14,15 Furthermore, high glucose concentration increases the activity of aldose reductase, which catalyzes the reduction of glucose to sorbitol, using NADPH as a coenzyme.16–18 Sorbitol cannot readily diffuse through cell membranes because of its polar character.19 With increasing aldose reductase activity, sorbitol accumulates intracellularly, causing hyperosmotic effects and leading to an influx of water that results in swelling of lens fibers, altered membrane permeability, and loss of electrolytes and other solutes.19–22 The involvement of aldose reductase in hyperglycemic cataracts has been supported by the use of aldose reductase inhibitors, which interfere effectively with the production of sorbitol, thus preventing osmotic imbalance.20–22 Results of many studies23–25 indicate that the rate of diabetic cataract formation is proportional to the concentrations of plasma glucose and aldose reductase and sorbitol concentrations in the lens.

The high incidence of diabetic cataracts in dogs was previously considered to be attributable to insufficient response to insulin administration and resultant chronic hyperglycemia.2 However, even dogs with good adjustment to insulin and acceptable blood glucose concentrations still develop cataracts, whereas diabetic cats with insulin resistance and persistent high glucose concentrations do not. A retrospective study comparing blood glucose concentrations in diabetic dogs and cats revealed no significant difference between these species, either before or during insulin treatment.5 Similar blood glucose concentrations in dogs and cats are also detected by comparing glucose concentrations of diabetic animals of different studies.3–4 Metabolic studies in lenses have been carried out both in vivo and in vitro with comparable results.23 In vitro lens incubations, which simulate the physiologic environment of the lens, have several advantages over in vivo studies. Glucose load and medium additives are standardized, paired sets of lenses provide experimen-
nal and control tissues from the same animal, cataracts develop much earlier in vitro than in vivo, and study time is considerably shorter.\textsuperscript{10,16,21,22} The purpose of the study reported here was to determine responses of canine and feline lenses to incubation in a medium with a high glucose concentration.

Materials and Methods

Lens incubation—We used paired lenses from 25 dogs and 17 cats euthanatized at the Department of Small Animals, University of Zürich, for reasons other than eye diseases. Selection criteria were blood glucose concentrations within reference ranges (dogs, 4.4\textendash}5.9 mmol/L; cats, 4.0\textendash}9.0 mmol/L) and ophthalmoscopically normal eyes. Mean ± SD ages of the dogs and cats were 5.39 ± 4.78 and 8.16 ± 6.14 years, respectively. Breed distribution was mixed-breed dog (n = 9), Beagle (6), German Shepherd Dog (3), Golden Retriever (2), Miniature Poodle (1), Bernese Mountain Dog (1), Jack Russell Terrier (1), Airedale Terrier (1), Dachshund (1), Domestic Shorthair (1), and Persian cat (3). Globes were euthanatized within 3 hours after euthanasia and sectioned transversely behind the equator. Lenses were carefully dissected free and rinsed with balanced salt solution.\textsuperscript{4} Thereafter, lenses were weighed and individually preincubated for 24 hours in 6-well tissue culture plates with 10 mL of control medium in each well. As outlined by Korte,\textsuperscript{9} 1 L of stock medium was composed of 9.8 g of M199, 6.0 g of HEPES-buffer, 0.1 g of L-glutamine, 2.2 g of sodium hydrogen carbonate, 12 mL of penicillin-streptomycin-amphotericin B solution, 60 mL of lamb serum, and dH2O added to final volume. Glucose concentration in stock solution was ≤ 6 mmol/L, and the pH was 7.4. High-glucose medium (30 mmol/L) was produced by supplementing the stock solution with 24 mmol of D(–)fructose/L. Control medium (6 mmol/L) was produced by supplementing the stock solution with 24 mmol of (–)fructose/L to obtain the same toxicity of 300 ± 2 mmol in both media. Fructose was used as an osmolyte in control medium, because it is not a substrate of aldose reductase, and it has no adverse effect on incubated lenses.\textsuperscript{10,17,20} All lenses were checked for capsular lesions or preexisting fiber alterations by use of a dark-field microscope immediately after dissection and 24 hours after incubation in control medium. Lenses selected for this study had neither damaged capsules nor extensive fiber alterations. Lenses of older animals (>4 years) with isolated fiber lesions were included, because these minor alterations were seen in 39 of 44 lenses of older animals, either on the first day or within the first 2 to 5 days of incubation. After the first 24-hour evaluation, paired lenses were incubated for another 13 days in a water-jacketed incubator (37°C; 5% CO\textsubscript{2} humidified atmosphere); in each pair, 1 lens was incubated in control medium and the other lens in high-glucose medium. Lenses were fixed in 4% buffered formaldehyde for 24 to 48 hours, embedded in paraffin, sectioned, and stained with H&E for light microscopic examinations.

Statistical analyses—Lenses were distributed in 2 age groups: group 1 contained lenses of animals ≤ 4 years of age, and group 2 contained lenses of animals > 4 years of age. This age distribution was chosen on the basis of morphologic lens changes in experimental medium. Aldose reductase activity and glucose concentration in control and experimental lenses within each species were compared between the 2 age groups by use of a paired 2-tailed Student t test. Aldose reductase activity and glucose concentration within each age group were compared between species for control and experimental lenses by use of an unpaired 2-tailed Student t test. Aldose reductase activity and glucose concentration within each species were compared between control and experimental lenses by use of a paired 2-tailed Student t test (control and experimental lenses were paired lenses from the same animal). Comparison of bw was also made between control and experimental lenses within each species by use of a paired Student t test. Values of P ≤ 0.001 were considered significant.

Results

Morphologic changes in incubated lenses—After the 14-day incubation period, mean ± SD bw of experimental lenses (canine lenses, 703.91 ± 114.92 mg; feline lenses, 1017.46 ± 131.78 mg), but this difference was not significant (P = 0.2). Many lenses of older animals (> 4 years) had isolated, swollen subcapsular fibers either before incubation (15/44) or within the first 2 to 5 days of incubation (12/44); during the incubation period, this finding was similar in degree between control and experimental lenses. Because of their localization (posterior subcapsular, centrally located, slowly extending to the periphery) and appearance (swollen fibers radially oriented), these lesions were easily distinguishable from glucose-induced vacuole formation.

Glucose concentrations—Glucose concentrations in lens homogenates were determined with an enzymatic method that used hexokinase.\textsuperscript{1} In this reaction, glucose is phosphorylated by hexokinase to glucose-6-phosphate, which is oxidated by glucose-6-phosphatase dehydrogenase, thereby generating NADH. The amount of NADH is proportional to the glucose concentration and is estimated photometrically.\textsuperscript{11}

Histologic examinations—Histologic examinations were performed on 2 pairs of canine control and experimental lenses and 2 pairs of feline control and experimental lenses. Lenses were fixed in 4% buffered formaldehyde for 24 to 48 hours, embedded in paraffin, sectioned, and stained with H&E for light microscopic examinations.

Statistical analyses—Lenses were distributed in 2 age groups: group 1 contained lenses of animals ≤ 4 years of age, and group 2 contained lenses of animals > 4 years of age. This age distribution was chosen on the basis of morphologic lens changes in experimental medium. Aldose reductase activity and glucose concentration in control and experimental lenses within each species were compared between the 2 age groups by use of a paired 2-tailed Student t test. Aldose reductase activity and glucose concentration within each age group were compared between species for control and experimental lenses by use of an unpaired 2-tailed Student t test. Aldose reductase activity and glucose concentration within each species were compared between control and experimental lenses by use of a paired 2-tailed Student t test (control and experimental lenses were paired lenses from the same animal). Comparison of bw was also made between control and experimental lenses within each species by use of a paired Student t test. Values of P ≤ 0.001 were considered significant.
Canine and feline experimental lenses developed glucose-related opacities, whereas control lenses did not (Fig 1). Glucose-induced alterations differed between cats and dogs regarding localization and extent and varied with age in feline lenses.

**Canine lenses**—Twenty-two of 25 lenses incubated in high-glucose medium developed large vacuoles (compared with those in feline lenses) arranged in chains in the equatorial region (Fig 2–4). Onset of vacuole formation was observed at 3 to 11 days (mean, 7 days). Histologically, 2 canine experimental lenses of dogs that were 1.3 and 3 years old had equatorial vacuoles, lens fiber swelling, local distinct eosinophilic globules, and vacuole formation in the lens epithelium mainly restricted to the equatorial region. Lesions were not detected in the 2 control lenses.

**Feline lenses**—The 17 lenses incubated in high-glucose medium had age-dependent alterations. All 5 lenses from young cats (0.16 to 3.2 years old) developed marked lens fiber swelling with prominent suture lines, extensive opacities consisting of tiny vacuoles, and distinct eosinophilic globules distributed in multiple layers and located mainly in the peripheral posterior cortex (Fig 3–7). In contrast, 4 lenses from middle-aged cats (4.5 to 8 years old) remained clear except for isolated punctate opacities at 1 or 2 ends of the suture lines, which regressed during the 14-day incubation period in the 8-year-old lens. None of the 8 lenses of old cats (10 to 18 years old) had any glucose-induced opacities at any time. Of 9 feline experimental lenses that developed opacities, 8 did so after 1 to 4 days (mean, 2 days) and only 1 after 8 days. The 2 feline experimental lenses examined histologically had fiber

**Figure 1**—Photomicrograph of a section of lens from a healthy 1-year-old cat. H&E stain; bar = 50 μm.

**Figure 2**—Stereoscopic photograph of a lens from a 1.3-year-old dog after lens incubation for 14 days in a high-glucose medium. Notice vacuoles in chain formation at the equatorial region. Unstained; bar = 2 mm.

**Figure 3**—Dark-field photomicrograph of a portion of the same lens as in Figure 2. Notice vacuoles in chain formation at the equatorial region. Unstained; bar = 163 μm.

**Figure 4**—Photomicrograph of a portion of the same lens as in Figure 2. Notice vacuoles in the equatorial region and in epithelial cells, as well as swollen lens fibers. H&E stain; bar = 50 μm.

**Figure 5**—Stereoscopic photograph of a lens from a 1-year-old cat after lens incubation for 14 days in a high-glucose medium. Notice extensive opacities consisting of tiny vacuoles in the posterior cortex, swollen lens fibers, and a marked suture line. Unstained; bar = 2 mm.
swelling and vacuole formation in the posterior cortex that was much more extensive in the lens of the 2-month-old cat, compared with the lens of the 1-year-old cat. In the 2-month-old cat, lens fiber swelling and fiber rupture were also seen in the anterior cortex. The epithelial cells contained vacuoles and had minor focal hyperplasia. However, in the 1-year-old cat, lens glucose-induced alterations were mainly restricted to the posterior cortex, and vacuole formation in epithelial cells was restricted to the equatorial region. Histologic examination of the 2 control lenses did not reveal any alterations, except for minor focal epithelial hyperplasia in the 2-month-old lens, similar to the 2-month-old experimental lens.

Aldose reductase activity—Measurement of aldose reductase activity revealed significantly (P ≤ 0.001) greater overall activity (experimental and control lens values combined) in lenses of young (≤ 4 years) dogs (27.27 ± 2.66 U/100 mg lww) and cats (33.82 ± 8.13 U/100 mg lww) than in lenses of older (> 4 years) dogs (21.74 ± 3.50 U/100 mg lww) and cats (14.41 ± 1.50 U/100 mg lww).

Comparing older (> 4 years) lenses (experimental and controls combined), canine lenses had significantly (P ≤ 0.001) greater aldose reductase activity (21.74 ± 3.50 U/100 mg lww) than feline lenses (14.41 ± 1.50 U/100 mg lww) did. There was no significant difference in aldose reductase activity between lenses incubated in control medium and their corresponding lenses incubated in high-glucose medium, for either canine or feline lenses. Activity of aldose reductase was similar in freshly frozen lenses and control lenses.

Glucose concentration—Mean glucose concentrations in canine (n = 23) and feline (15) experimental lenses (6.26 ± 0.45 and 6.45 ± 1.68 mmol/L) were significantly (P ≤ 0.001) greater than mean glucose concentrations in their matching canine and feline control lenses (2.44 ± 0.35 and 1.71 ± 0.46 mmol/L). A significant difference in glucose concentration was not detected between the 2 age groups or between canine and feline experimental lenses. However, in the control group, feline lenses had significantly (P ≤ 0.001) lower glucose concentrations, compared with canine lenses.

Discussion

In this study, canine and feline lenses developed opacities when exposed to a high glucose concentration. In canine lenses, this phenomenon did not appear to be age dependent, whereas development of lenticular opacities in cats was restricted to lenses of young cats. Intralenticular glucose concentration was significantly increased in a similar manner in experimental lenses of dogs and cats and young and old animals, indicating an overall similar glucose uptake in lenses incubated in experimental medium. This process depends on a carrier transport stereospecific for D-glucose, because free glucose cannot pass through lipid membranes.14 Once inside the lens, glucose is primarily phosphorylated to glucose-6-phosphate by hexokinase to enter the glycolytic pathway and the pentose phosphate pathway. However, high glucose-6-phosphate concentrations inhibit hexokinase activity in order to prevent excess production of lactate.15,16,35 Therefore, increased lenticular concentrations of glucose increase aldose reductase activity, which causes glucose to become metabolized via the sorbitol pathway.17,18

In the present study, aldose reductase activity was similar in paired experimental and control lenses, although glucose concentrations were significantly higher in overall experimental lenses, compared with control lenses. In lenses of rats, the activity of aldose reductase increases with the onset of diabetes.17,36,37 However, in human lenses, aldose reductase activity is significantly lower in diabetics than in nondiabetics. It is suggested that a direct effect of the glycemic status on the lens enzyme is masked by a loss of enzyme secondary to the development of cataract.39 Although aldose reductase activity has been reported to be increased in some tissues of diabetic rats and humans, the effects of various concentrations of glucose on the enzyme activity in vivo are unknown.39,40 Studies14-15 on erythrocytes of diabetic humans reveal no correlation between fasting plasma glucose concentrations and aldose reductase activity, and acute increase of blood glucose concentration does not affect enzyme activity, although aldose reductase activity is significantly
increased in erythrocytes of diabetic patients, compared with nondiabetic subjects. In rats fed galactose or lenses incubated in glucose, the concentration of aldose reductase mRNA appears to increase 2-fold.44 However, this increase is, in some part, due to increased cell proliferation of the epithelial cell layer of the lens.49 Concurrently, aldose reductase mRNA concentrations decreased to low concentrations in the underlying fiber cells, consistent with maturation of cataracts, presumably because of cell disintegration resulting from the galactose insult.44 In contrast to epithelial cells in cultured rat lenses, dog lens epithelial cells exposed to high glucose concentration had no substantial increase in aldose reductase mRNA.49 However, high glucose concentration in vivo may induce aldose reductase expression (translational or post-translational regulation) without increased aldose reductase mRNA.49 An important question concerns the extent to which alterations in aldose reductase mRNA concentrations cause alterations in tissue aldose reductase activities. One study that examined the effect of galactosemia in various tissues revealed that changes in aldose reductase mRNA concentrations in response to galactose feeding vary between tissues and are not accompanied by parallel changes in tissue activities of aldose reductase.49 Factors other than aldose reductase mRNA concentrations, notably aldose reductase turnover and enzyme activation, may determine tissue enzyme activities.50,51 Another important fact is that lenses may have loss of glutathione (GSH) during culturing.49 Glutathione is present in large amounts in normal lenses and plays a major role in antioxidant defenses. The protective action of GSH is linked to its oxidation to glutathione disulfide (GSSG). Recovery of GSH occurs through the NADPH-dependent enzyme glutathione reductase, which reduces GSSG back to GSH. In virtually all types of cataracts, GSH decreases significantly in the lens, usually prior to opacification.52 Bovine lens aldose reductase incubated in the presence of GSSG has progressive loss of enzyme activity. The extent of aldose reductase oxidation is dependent on the GSH-to-GSSG ratio only and is not influenced by the absolute concentration of GSH.52 We conclude that there are many factors influencing aldose reductase activity. Therefore, a decisive explanation for our observation of similar aldose reductase activity in paired experimental and control lenses, despite increased glucose concentrations in experimental lenses, cannot be given.

In healthy lenses, only 5% of glucose is metabolized via the sorbitol pathway, whereas in lenses from diabetic animals, 10 to 33% of glucose enters this pathway.53,54 Measurement of aldose reductase in our study revealed that its activity was significantly higher in lens of younger animals than in lenses of older animals. Decreased aldose reductase activity with age has been described in dogs, humans, and rats.9,11,30,49 However, the significantly lower aldose reductase activity in lenses of older cats, compared with lenses of dogs and young cats, may explain why lenses of older cats remained free of opacities when exposed to high glucose concentration in vitro. Furthermore, age-dependent reactions of feline lenses to high glucose concentration in vitro seem to reflect the situation in diabetic cats in vivo. Diabetes mellitus in young cats is uncommon, but was recently reported to induce diabetic cataracts.13 Aldose reductase activity is high in the rat, rabbit, guinea pig, octagon degu, and aldose reductase-transgenic mice, in which sugar cataracts develop rapidly. However, aldose reductase activity is low in mice and may explain why diabetic mice do not develop cataracts.9,11,55 A similar mechanism could prevent cataract formation in old cats. Furthermore, the difference in localization of vacuole formation detected between canine and feline lenses in our study could be attributable to different compartmentalization of the enzyme aldose reductase. Immunohistochemical studies on lenses of diabetic rats and humans reveal a distinct increase in staining for aldose reductase, compared with healthy lenses, which is associated with glucose-derived vacuoles and water clefts. In lenses of diabetic rats, vacuoles are concentrated in the subepithelial and deep cortical equatorial region, whereas in lenses of diabetic humans, vacuoles develop in the superficial anterior and posterior cortex.56 The pathogenesis of diabetic cataracts appears to be complex, and the physiologic role of the sorbitol pathway is not yet fully understood. In diabetes, fluctuations in aqueous humor glucose concentrations are potentially damaging to the lens, because a double osmotic shock is involved at each change.9 The first is a hyperosmotic shock (hyperglycemia induces hyperosmolarity of aqueous humor) that leads to an initial decrease in lens hydration and, thus, in lens volume. The second osmotic effect is attributable to the ensuing net movement of glucose into the lens, accompanied by water influx and increased membrane permeability.57 The lens tries to compensate for altered membrane permeability by increasing Na/K-ATPase activity to preserve Na and K concentrations.14,52 The normal lens usually maintains the same volume despite changes in the osmolarity of the surrounding medium. The initial rapid phase of volume regulation is achieved by a change in membrane permeability and increased cation pump activity. This process is accompanied by a change in content of intracellular osmolytes, which requires hours or days.58,59 Because the lens also has to maintain an osmotic equilibrium with its environment, osmotic stress (from high glucose concentrations in the aqueous humor) induces increased aldose reductase activity, leading to sorbitol accumulation.6,22,28,31 With increasing sorbitol concentrations, the lens must deplete other osmolytes, such as taurin, myoinositol, amino acids, and glutathione, for osmotic compensation.10,11 With an abrupt decrease of glucose concentration and osmolarity (following each insulin administration), the lens may remain hypertonic because accumulated sorbitol is not able to pass cell membranes. With marked and frequent osmotic changes, the lens is likely to loose osmolytes and therefore to become unable to adapt. Furthermore, reduction of myoinositol leads to an altered phosphoinositoid turnover (which is important for membrane integrity) and thus to a loss in Na/K-ATPase activity.24 When the cation pump is not able to compensate further, an increase in electrolyte develops, leading to water influx, fiber swelling, and
rupture.\textsuperscript{27} During hyperglycemia, complex metabolic shifts develop, which are indicated by suppression of the glycolytic pathway and increased activity of the pentose phosphate and sorbitol pathways, thus leading to altered concentrations in NAD\textsuperscript{+}NADH, NADP\textsuperscript{-}NADPH, ATP, metabolic intermediates, and antioxidants.\textsuperscript{23,25,26,46} Moreover, crystalline lens proteins become modified because of glycation, fragmentation, aggregation, and increased proteolysis, and membrane lipids become peroxidized because of increased reactive oxygen species.\textsuperscript{29,61-63}

In our study, canine and feline lenses incubated in high-glucose medium for 14 days had equal uptake of glucose, independent of age. Because glucose stress (glucose uptake) was the same in all experimental lenses at any age, we conclude that the difference in extent of morphologic lesions that developed in response to high glucose concentrations between canine and feline lenses was at least partly attributable to different aldose reductase activity and an unequal decrease in aldose reductase activity with age. This lens incubation model appears to be well suited to explore the pathogenesis of diabetic cataracts and the different reaction patterns to glucose stress in different species.

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\textsuperscript{BSS ADATO, Bausch \& Lomb Surgical GmbH, Heidelberg, Germany.}

\textsuperscript{Medium 199, Category No. M3769 powder, Sigma, Buchs, Switzerland.}

\textsuperscript{Olympus Tokyo CK, Volketswil, Switzerland.}

\textsuperscript{Polymermatta GmbH, Luzern, Switzerland.}

\textsuperscript{Dibasic sodium phosphate, Fluka, Buchs, Switzerland.}

\textsuperscript{COBAS MIRA automated photometer, Hoffman-LaRoche, Basel, Switzerland.}

\textsuperscript{COBAS INTEGRA Glucose HK Liquid, Roche, Basel, Switzerland.}


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