Retinal Blood Flow in Normal and Diabetic Dogs

Kent W. Small, Einar Stefánsson, and Diane L. Hatchell

We have found retinal blood flow to be decreased in diabetic dogs 5 months after the onset of diabetes, which is long before they can be expected to develop morphological changes of diabetic retinopathy. Retinal blood flow was determined using radionuclide labelled microspheres. In eight alloxan diabetic dogs without retinopathy, the retinal blood flow was 0.53 ± 0.08 (mean ± SE) ml/min/gm dry tissue weight. This compares with 0.91 ± 0.17 (mean ± SE) ml/min/gm dry tissue weight in seven normal dogs. The decreased blood flow in diabetic retinas is statistically significant ($P = 0.05$). Blood glucose levels did not significantly affect retinal blood flow. This data suggest that changes in retinal blood flow and oxidative metabolism may precede the morphological signs of diabetic retinopathy. Invest Ophthalmol Vis Sci 28:672–675, 1987

While many years frequently elapse before morphological signs of diabetic retinopathy become apparent, it is likely that these are preceded by metabolic or physiological changes in the retina. The discovery and characterization of these changes are important in the search for the etiology of diabetic retinopathy.

Illing and Gray[1] have reported that retinal oxygen consumption is lower in diabetic rabbits than in normal rabbits. Decreased retinal oxygen consumption should lead to a decrease in retinal blood flow via autoregulation in diabetic animals without retinopathy. Recent studies[2,3] suggest that retinal blood flow is elevated in background diabetic retinopathy, and normal, or perhaps even lower than normal, in diabetics without retinopathy. Other researchers demonstrated a correlation between retinal blood flow and the stages of diabetic retinopathy.[4–6] However, it is not clear whether the retinal blood flow changes precede the retinopathy, or if there is a causal relationship.

This study measures retinal blood flow in dogs with short term diabetes to find whether blood flow changes precede the retinopathy. The effect of blood glucose levels on retinal blood flow in normal and diabetic dogs is also studied.

From the Department of Ophthalmology, Duke University Medical Center and the Research Service, Veterans Administration Medical Center, Durham, North Carolina.

Presented in part at the Annual Meeting of the Association for Research in Vision and Ophthalmology in Sarasota, Florida, May 9, 1985.

Supported by Veterans Administration Medical Research Funds; a Core Grant for Vision Research P30 (EYO5722) from the National Eye Institute, NIH, Bethesda, Maryland; by a Recognition Award from the Alcon Research Institute (Dr. Hatchell); and a generous gift from Dr. and Mrs. Harris Vernick.

Submitted for publication: December 4, 1985.

Reprint requests: Dr. Kent Small, Duke University Eye Center, Box 3802, Durham, NC 27710.

Materials and Methods

Ten mongrel dogs weighing 12–15 kg were given 50 mg/kg alloxan intravenously to induce chronic insulin-dependent diabetes mellitus. Blood glucose levels were determined by venipuncture every other morning in all ten dogs using an Ames (Downer's Grove, IL) glucometer. One dog did not develop insulin-dependent diabetes and was not used in the study. NPH insulin was given subcutaneously, a mean of 9 ± 2.3 (mean ± 1 SE) units, every morning as required to prevent development of diabetic ketoacidosis. The mean morning blood glucose level was 317 ± 117 (mean ± 1 SE) mg/dl. The dogs remained insulin-dependent for a mean of 5.3 ± 0.2 (mean ± 1 SE) months at which time they underwent blood flow studies. One diabetic dog had a blood glucose of 900 at the time of the study. The data from this dog is not included. These eight diabetic dogs were otherwise treated in an identical fashion to the eight control nondiabetic dogs weighing 13–15 kg. One of the control dogs developed technical difficulties during the study, which were reflected in the data from this dog. This data was not included in the analysis. All animals were treated in accordance with the ARVO Resolution on the use of animals in Research. During the course of the study some of the dogs were found to have heartworms.

The dogs were anesthetized with 20 mg/kg intravenous pentobarbital and 2 mg/kg succinylcholine chloride, intubated and ventilated with a Bennett (Santa Monica, CA) MA-1 respirator. A femoral artery catheter was placed for continuous monitoring of systemic blood pressure and arterial blood sampling. Arterial blood oxygen tension (PaO_2) was maintained between 95–110 mm Hg with the dog breathing air with 21–30% O_2. A limb lead electrocardiogram was continuously monitored.

A median sternotomy was performed, and a pericardial cradle was created. A purse string suture was placed in the left atrial appendage. A silastic catheter was inserted into the left atrium and used for injecting radionuclide-labelled microspheres. The femoral artery catheter was connected to a calibrated Harvard pump (Harvard Instruments Inc., Ayer, MA), which acted as a surrogate organ, collecting blood at a constant rate of 14 ml/min. As 6–12 million microspheres (Nuclear Products Division, 3-M Company, St. Paul, MN) were injected into the left atrium, blood collection from the Harvard pump was started, and continued for 2 min to collect blood with the radioactive microspheres. Four different radionuclide-labelled (^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb) microspheres were used.

Prior to injection of a radionuclide-labelled microsphere, several parameters were recorded and/or corrected to normal levels. Heart rate was monitored and blood pressure was recorded as mm Hg. A blood gas meter (pH/Blood Gas Analyzer 713, Instrumentation Laboratory Inc., Wilmington, MA) was used to record and correct arterial blood pH to 7.35–7.45, $PaCO_2$ to 33–43 mm Hg, and PaO_2 to 95–100 mm Hg. A Nova 6 Electrolyte Analyzer (Nova Biomedical, Newton, MA) was used to record and/or correct serum sodium to 137–145 mM, potassium to 3.5–4.5 mM, and calcium to 0.9–1.1 mM. Arterial blood was also collected for glucose determination by a Beckman Glucose Analyzer (Fullerton, CA).

Serum glucose was altered by injecting 50% dextrose or regular insulin intravenously. Once all the previously mentioned parameters were again stabilized, another 6–12 million microspheres were injected into the left atrium. This procedure was repeated for two to four different glucose levels in each dog using differently-labelled microspheres each time. The dog was then killed with intravenous barbituate injection, the eyes enucleated, and the retinal tissues dissected and placed in counting vials. These tissues along with the reference blood collected by the surrogate organ were placed in a gamma counter (Packard Auto-gamma Scintillation Counter, Packard Instrument Co., Downer's Grove, IL). Using a PDP 11/23 Digital (Digital Equipment Co., Maynard, MA) computer, the raw radioactive counts were converted into blood flow in units of ml/min/g of tissue dry weight. The retinal samples were dried for 24–48 hr at 55° C and the dry weight measured.

Analysis of Data

In order to determine whether changing blood glucose affected retinal blood flow in diabetic and normal dogs, a hybrid repeated measures two-factor analysis of variance-regression model was used. The design was unbalanced, and sequential sums of squares were used in all tests.

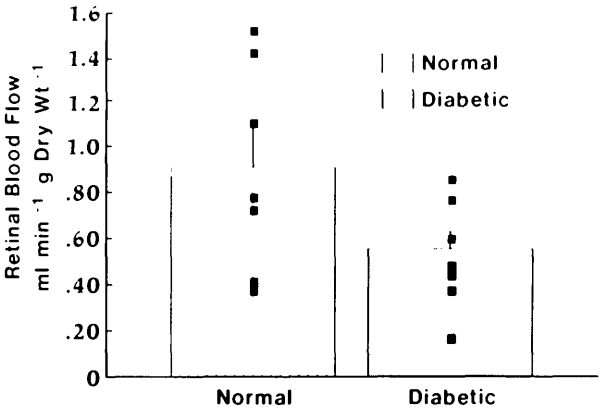

Fig. 1. Retinal blood flow (ml/min/gm dry weight) in normal and diabetic dogs without retinopathy (standard error bar on each column), and average blood flow for individual dogs shown as points on the bar graph.

Because no statistically significant effect of changing blood glucose levels was found, the average retinal blood flow for each dog was determined by averaging the three to four measurements of both retinas in each dog. The retinal blood flow of the diabetic dogs was then compared with the flow of normal dogs. Analysis was performed using the two-tailed student's t-test.

Results

The mean retinal blood flow in the diabetic dogs was 0.53 ± 0.08 (mean ± 1 SE) ml/min/gm of dry tissue weight. The mean retinal blood flow for the normal dogs was 0.91 ± 0.17 (mean ± 1 SE) ml/min/gm of dry tissue weight (Fig. 1). The diabetic retinal blood flow was significantly less than the normal (student's t-test $P = 0.05$).

Changes in blood glucose level had no statistically significant effect on retinal blood flow in the diabetic or the normal dogs.

Discussion

These results demonstrate that retinal blood flow in diabetic dogs without retinopathy is significantly below normal. Yoshida et al[2] found a similar trend in retinal blood flow in human subjects. They found retinal blood flow to be 9.8 arbitrary units in normal patients, decreasing to 8.0 units in diabetics without retinopathy, and then rising significantly to 11–13 units in diabetics with background retinopathy. However, Grundwald et al[7] did not find this same change in retinal blood flow in early diabetic retinopathy. Recently, Feke et al,[3] using the bidirectional laser Doppler technique, demonstrated a significantly-reduced retinal blood flow in diabetic patients with minimal or no retinopathy.

A likely explanation for the decreased retinal blood flow in diabetes without retinopathy is decreased ox-

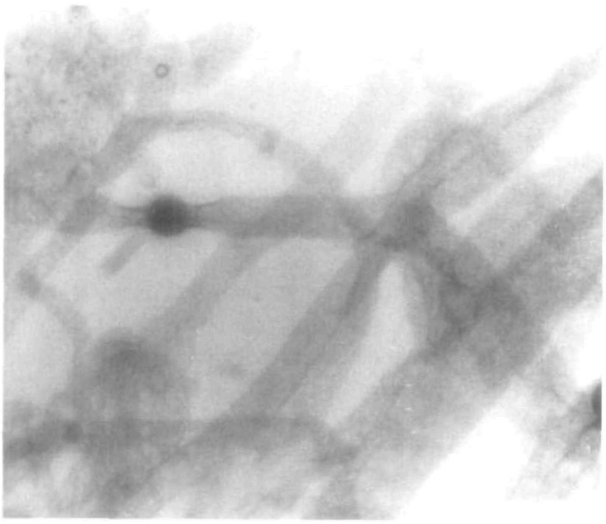

Fig. 2. Flat mount of a retina showing a 10 μm sphere trapped in retinal blood vessels (×125).

ygen consumption. This was originally shown by Illing et al,[1] and recently confirmed in normal and diabetic rabbits in our laboratory.[8] Decreased oxygen consumption would raise the retinal oxygen tension and trigger autoregulatory vasoconstriction in the retina.

Retinal blood flow did not change significantly as a result of changes in blood glucose concentration. This contradicts an earlier report of the effect of hyperglycemia on the retinal circulation in cats.[9] In that report, three groups of cats were treated with intravenous infusion of saline, mannitol, or glucose. In mannitol and saline groups, the baseline retinal blood flow was 13.8 ± 4.9 μl/min (mean ± SE) and 14.0 ± 0.9 μl/min (mean ± SE) respectively. The glucose group had a baseline blood flow of 9.34 ± 0.5 μl/min, which increased to 12 ± 1 μl/min after glucose infusion. Thus, while the retinal blood flow increased after glucose infusion, it remained less than the baseline flow in the other two groups of cats.

We did not see a relationship between blood glucose levels and retinal blood flow in normal or diabetic dogs. However, the variability inherent in the microsphere technique for small tissues impairs its ability to detect small differences in blood flow. Also, measurements were made about 20 min after glucose infusion, and would not show transient changes that may take place immediately following glucose injection.[10]

Ten micrometer microspheres are well-suited for retinal blood flow measurements. The retinal capillaries are smaller than the spheres, and should trap all of the microspheres. Flat mounts of the retina with microspheres showed that most of the microspheres were trapped in precapillary vessels, and 70% of the microspheres were trapped alone in each vessel (Fig. 2). Alm et al[11] found the 9 and 15 μm spheres to be the appropriate size for retinal measurements. Table 1 compares the data from our study with previous reports. Our retinal blood flow values for normal dogs are similar to earlier work in dogs[12] and cats.[13]

There are several requirements for the successful use of microspheres as discussed by Heymann et al.[14] When applying this technique to the measurement of retinal blood flow, special considerations are necessary. Because the retina is a relatively small piece of tissue, the quantity of blood flow to the retina, and the number of microspheres impacting in the retina, will be small. To counter this, a large number of microspheres must be injected. Assuming a Poisson distribution of multiple measurements under identical situations, 384 microspheres in a tissue sample would have a distribution variability within 10% of the mean distribution at the 95% confidence level.[15] Because the glucose level did not effect the blood flow, the multiple blood flow determinations for an individual dog may be averaged together. This value is then equivalent to injecting approximately 18 million microspheres, and having over 400 microspheres in each retina. This easily meets the distribution variability requirements as determined by Buckbreg et al.[15]

The main advantage of the nuclide-labelled microsphere technique is that it gives absolute measurements of retinal blood flow in units of ml/min/gm of tissue. Many of the other methods measure blood flow through a segment of one retinal vessel, and therefore give relative values.[2-7] The major disadvantage of the microsphere technique is that it can only be performed in an experimental animal, and that the animal has to be euthanized.

We used radioactive microspheres to demonstrate a decrease in retinal blood flow in a pre-retinopathic diabetic animal model. We postulate that the decrease may be due to an autoregulatory mechanism secondary to low oxygen consumption of the diabetic retina.

Table 1. Comparison with other reports

Animal	*Reference*	*ml/min/gm dry weight (Mean ± 1 SD)*
		Retina
Dog	This study*	0.91 ± 0.17
Diabetic Dog	This study*	0.53 ± 0.08
Dog	13	0.60 ± 0.35
Cat	14	0.95 ± 0.15

* The dry weight is calculated 0.2 of the wet weight.

Key words: retinal blood flow, diabetes mellitus, radioactive microspheres, alloxan hydrochloride, blood glucose

Acknowledgment

Our appreciation to Dr. Marian Morris for the gift of the dogs.

References

1. Illing EKB and Gray CH: Retinal metabolism in diabetes: The metabolism of retinae of normal and alloxan-diabetic rabbits. J Endocrinol 7:242, 1951.
2. Yoshida A, Feke GT, Morales-Stopello J, Collas GD, Goger DG, and McMeel JW: Retinal blood flow alterations during progression of diabetic retinopathy. Arch Ophthalmol 101:225, 1983.
3. Feke GT, Tagawa H, Buzney SM, Deupree DM, Verstraaeten TC, Coger DG, Weiter JJ, McMell JW, and Gabbay KH: Retinal blood flow alterations in early diabetic retinopathy. ARVO Abstracts. Invest Ophthalmol Vis Sci 27(Suppl):145, 1986.
4. Kohner EM: Dynamic changes in the microcirculation of diabetes as related to diabetic microangiopathy. Acta Med Scan (Suppl) 578:41, 1975.
5. Cunha-Vaz JG, Fonesca JR, de Abreu JRF, and Lima JJP: Studies on retinal blood flow. II. Diabetic retinopathy. Arch Ophthalmol 96:809, 1978.
6. Blair NP, Feke GT, Morales-Stoppello J, Riva CE, Goger DG, Collas G, and McMell JW: Prolongation of the retinal mean circulation time in diabetes. Arch Ophthalmol 100:764, 1982.
7. Grundwald JE, Riva CE, Brucker AJ, Sinclair SH, and Petrig BL: Altered retinal vascular response to 100% oxygen breathing in diabetes mellitus. Ophthalmology 91:1447, 1984.
8. Sutherland FS, Hatchell DL, Stefánsson E, Reiser HJ, and Hatchell MC: In vitro oxygenation of normal and diabetic rabbit retina. ARVO Abstracts. Invest Ophthalmol Vis Sci 27(Suppl): 309, 1986.
9. Atherton A, Hill DW, Keen H, Young S, and Edwards EJ: The effect of acute hyperglycemia on the retinal circulation of the normal cat. Diabetologia 18:233, 1980.
10. Read JS, Ernest JT, Goldstick TK, Raichand M, and Petersen AC: Hyperglycemia and the retinal circulation in man. Invest Ophthalmol Vis Sci 19:168, 1980.
11. Alm A, Tornquist P, and Stjernschantz J: Radioactively labelled microspheres in regional ocular blood flow determinations. Bibl Anat 16:24, 1976.
12. Keough EM, Wilcox LM, Connolly RJ, and Hotte CE: The effect of complete tenotomy on blood flow to the anterior segment of the canine eye. Invest Ophthalmol Vis Sci 19:1355, 1980.
13. Alm A and Bill A: The oxygen supply to the retina. II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. Acta Physiol Scan 84:306, 1972.
14. Heymann MA, Payne DO, Hoffman JIE, and Rudolph AM: Blood flow measurements with radionuclide labelled particles. Prog Cardiovas Diseases 20:55, 1977.
15. Buckberg GC, Luck JC, and Payne BD: Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 31:598, 1971.