Microvascular Retinopathy in the Zucker Diabetic Fatty Rat

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Purpose. To determine if quantifiable morphometric signs of retinopathy occur in the Zucker diabetic fatty rat (ZDF/Gmi^fa, formerly designated ZDF/Drt), a partially inbred strain in which the genetic propensity for diabetes is only expressed in obese males.

Methods. Retired diabetic (ZDF/Gmi^fa) and control lean Zucker (fa/+ ) breeder rats were examined for quantifiable evidence of microvascular changes of the retinal capillaries by gross examination, trypsin digestion of retinal vessels, and transmission electron microscopy.

Results. Gross examination of retinas and trypsin digestion of capillaries revealed no differences. Quantitative assessment of capillary cell nuclear density showed that diabetic retinas were hypercellular compared to lean rats (3.888 ± 0.041 versus 3.304 ± 0.046 nuclei per 100 μm (mean ± SE), P = 0.0042). Transmission electron microscopic analysis of retinal capillary basement membrane thickness demonstrated thicker measurements in diabetic animals (mean thickness 21% greater in diabetic rats, P = 0.0307).

Conclusions. This model may be useful for pharmacologic intervention studies because it is naturally and severely non-insulin-dependent diabetic, there are quantifiable retinal vascular changes, and same-sex litter mates can be used as controls. Invest Ophthalmol Vis Sci 1993;34:2367-2371.

The obese Zucker rat (fa/fa) genotype displays glucose intolerance and hyperinsulinemia without gross hyperglycemia. Recently, ocular findings in the obese Zucker rat have been described that include focal nodules of the basement membranes of retinal capillaries (without general thickening), pericyte loss, and increased endothelial intercellular junctions.1 The Zucker diabetic fatty rat (ZDF/Gmi^fa, initially designated ZDF/Drt) is a partially inbred strain derived from the fa/fa line, which is born normoglycemic and normoinsulinemic but becomes frankly hyperglycemic at 6–7 wk of age.2,3 Neuropathy and nephropathy eventually develop4,5 and death usually occurs by 1 yr of age. The animals can be maintained without treatment on a standard rat chow diet with average blood glucose values of more than 500 mg/dl throughout their lives. The ZDF/Gmi^fa rat has been proposed as a model of type 2 diabetes mellitus.6 The retinas of retired breeder ZDF/Gmi^fa rats as well as lean litter mates used as controls were analyzed to investigate the occurrence of retinopathic changes.

METHODS

The eyes of four pairs of litter mates were analyzed. Four male retired breeder ZDF/Gmi^fa rats and four male lean retired breeder Zucker (fa/+ ) rats, each a litter mate of one of the ZDF/Gmi rats, were obtained from the colony maintained at Indiana University School of Medicine. Treatment of animals adhered to the ARVO resolution on the Use of Animals in Research. Each pair of litter mates was between 6 and 7 mo of age. Rats were fed a diet of Purina 5008 rat chow (Ralston Purina, St. Louis, MO) ad libitum.
average blood glucose value was more than 500 mg/dl for diabetic rats and 120 mg/dl for lean rats by glucose oxidase assay at the time of death. The animals were killed by injection with intracardiac potassium; after enucleation one globe from each rat was randomly selected for fixation in 4% glutaraldehyde and the other globe was placed in 10% buffered formalin.

Glutaraldehyde-fixed retinas were dissected free of sclera and choroid, then trimmed and postfixed in 1% osmium tetroxide. After dehydration and embedding in epoxy resin, thin sections were stained with uranyl acetate and lead citrate. Cross-sectional portions of capillaries in the inner nuclear layer of the retina were examined. Vessels were photographed if they were of capillary caliber and their outer diameter had a maximum:minimum ratio of less than 1.5 to approximate a perpendicular cross sectioning. Photographs were obtained at 8000X and printed on 8 × 10" paper for a final magnification of 21,600X. Each photograph was assigned a coded number and 25 capillary cross sections from each animal were presented in random order to a masked observer. The observer rejected some photographs for technical inadequacy, presence of noncapillary elements or for nonperpendicular cross sectioning. The minimum two-point measurement technique was applied to estimate basement membrane thickness using a micrometer rule.

Formalin-fixed retinas were hemisectioned sagitally and processed for trypsin digestion according to the technique of Kuwabara and Cogan. After air drying, specimens were stained with hematoxylin and periodic acid-Schiff reagent and coverslipped. The hemiretinal specimens were photographed under low-power light microscopy. From the low-power photograph, six regions were chosen in each retina by a random selection method employing an acetate overlay with randomly distributed dots. Regions within 1 mm of the optic nerve head or the far vascular periphery were excluded. These areas were then photographed at 100X magnification. Noncapillary vessels were excluded and marked out of the photograph. A count of nuclei in retinal capillaries was performed by direct counts from the trypsin digest preparation and documented on the photograph. After initial attempts to resolve endothelial cells from pericytes in the trypsin digested specimens under high-power light microscopy, an unacceptably high number of nuclei was found to be of indeterminate identity; this agrees with data from other studies showing the unreliability of this measurement in mouse retinas. Therefore, the total number of both pericyte and endothelial cell nuclei were counted in each region, with an average of 1400 nuclei total per retina. The summed length of all capillaries in each photograph was measured using a computer mouse with a digitizing board and planimeter software (Easydij 5.0, Geocomp Inc., Boulder, CO). The number of nuclei per unit length of capillary was calculated and this figure was averaged from each of the six analyzed regions for each rat.

Each retinal specimen was examined completely and extensively under medium-power light microscopy (400X magnification) for evidence of microaneurysms and acellular capillaries. A search was made for pericyte ghosts using oil immersion (1000X magnification) and at least 30 such high power fields in each specimen were examined.

Data were analyzed using a random effect mixed analysis of variance model. This analysis corrected for differences in numbers of observations from each retina.

RESULTS

Diabetic animals had mean retinal capillary basement membrane thickness 21% greater than the lean litter mate rats (mean diabetic thickness 113.4 nm versus 89.0 nm for lean, P = 0.0067) (Figure 1). The degree of significance was not changed appreciably by excluding several outlier very thick measurements from a diabetic rat. No differences between groups were noted in regard to basement membrane nodularity (Figure 2). Pericyte degeneration was not noted in any cross section specimen.

Trypsin-digested retinal capillaries demonstrated hypercellularity in diabetic rats compared to lean rats (3.888 ± 0.041 per 100 μm (mean ± SE) for diabetic rats versus 3.304 ± 0.046 per 100 μm for lean, P = 0.0042) (Figure 3). This hypercellularity was not
DISCUSSION

We have described quantifiable retinal vascular changes consisting of retinal capillary basement membrane thickening and hypercellularity of retinal capillaries in obese ZDF/Gmi<fa/-fa retired male breeder rats compared to their lean (fa/+) litter mates. These microvascular changes are also among those seen in diabetic retinopathy in humans and in other diabetic rat models.\textsuperscript{10,11} Although the precise significance of these changes is unclear regarding the overall pathogenesis of diabetic retinopathy, it is likely that these changes are important because they reflect alterations in the most fundamental structural elements of the capillaries.

We did not encounter other lesions more typical of diabetic retinopathy in humans, such as pericyte degeneration, microaneurysms, and acellular capillaries. This may be attributable to species differences, because microaneurysms have only rarely been described in the rat retina. It may be that we have evaluated a very early stage of retinopathy, and such changes might occur if the animals survived long enough. In addition, it may be that the retinopathy is multifactorial in cause and that obesity, hyperinsulinenia, and other unknown traits associated with the Zucker diabetic fatty phenotype play a role in the production of the described changes.

The hypercellularity of retinal capillaries noted in the trypsin-digested specimens of the ZDF/Gmi rat is most likely attributable to endothelial cell proliferation, as described in the hyperinsulinemic fa/fa rat\textsuperscript{1} and in streptozotocin and BB diabetic rats.\textsuperscript{10,11} We found light microscopy to be unreliable in differentiating retinal capillary endothelial cells from pericytes in rats, as have others in the mouse,\textsuperscript{9} and we therefore...
did not determine the relative contribution to the increased density from the two cell types. Pericyte degeneration was not noted either in trypsin digestion preparations or in electron microscopic cross sections. Less than one cell nucleus is encountered on the average in cross sections of rat capillaries. A very large number of electron microscopic cross sections would have to be examined from each retina to resolve a difference in endothelial cell to pericyte ratio or to obtain accurate information about pericyte density. Such a study was not undertaken in these animals. We therefore cannot state whether pericyte loss is a feature of the retinopathy in the ZDF/Gmi animals, or what relationship exists between pericyte populations and the hypercellularity noted on trypsin digestion.

Thickening of the retinal capillary basement membrane was noted in the ZDF/Gmi rats, which is consistent with other animal models of diabetes as well as in galactosemic animals. Although the basement membranes were not thickened in a study of retinal vessels of the Zucker fa/fa rat, thickening has been noted in the skeletal muscle capillaries of the same strain. In contrast to the study of Dosso fa/fa rats, we did not find a greater prevalence of nodularity of the basement membranes in the ZDF/Gmi rats. The ability to easily detect significant microvascular changes in a relatively small number of animals indicates the potential utility of this strain for studies of microangiopathy related to diabetes. The availability of same-sex litter mate controls in naturally-occurring diabetic rats suggests that this may be a useful model for the testing of therapeutic modalities.

**Key Words**
diabetic retinopathy, animal models, inbred strains, trypsin digestion, capillary basement membrane

**Acknowledgments**
The authors thank Richard G. Peterson, PhD, Department of Anatomy, Indiana University School of Medicine for making available the ZDF/Gmi rats for this project. Statistical consultation was provided by Amita Manatunga, PhD, Department of Medicine, Indiana University School of Medicine and the Regenstrief Health Institute, Indianapolis, Indiana.

**References**
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