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Effect of diabetes and hyaluronidase on the retinal endothelial glycocalyx in mice

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Abstract

We sought to investigate the effects of diabetes and hyaluronidase on the thickness of the endothelial glycocalyx layer in the mouse retina. In our study, the retinal circulation of diabetic Ins2(Akita) mice and their nondiabetic littermates were observed via intravital microscopy. The endothelial glycocalyx thickness was determined from the infusion of two fluorescently labeled plasma markers, one of which was a high molecular weight rhodamine dextran (MW=155,000) excluded from the glycocalyx, and the other a more permeable low molecular weight sodium fluorescein (MW=376). In nondiabetic C57BL/6 mice, the glycocalyx thickness also was evaluated prior to and following infusion of hyaluronidase, an enzyme that can degrade hyaluronic acid on the endothelial surface. A leakage index was used to evaluate the influence of hyaluronidase on the transport of the fluorescent tracers from the plasma into the surrounding tissue, and plasma samples were obtained to measure levels of circulating hyaluronic acid. Both diabetes and hyaluronidase infusion significantly reduced the thickness of the glycocalyx in retinal arterioles (but not in venules), and hyaluronidase increased retinal microvascular leakage of both fluorescent tracers into the surrounding tissue. However, only hyaluronidase infusion (not diabetes) increased circulating plasma levels of hyaluronic acid. In summary, our findings demonstrate that diabetes and hyaluronidase reduce the thickness of the retinal endothelial glycocalyx, in which hyaluronic acid may play a significant role in barrier function.

Keywords

endothelial glycocalyx; retina; diabetes; Ins2(Akita); hyaluronic acid; hyaluronidase

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1. Introduction

Diabetic retinopathy involves microvascular complications, in which retinal microvessels are characterized by microaneurysms, leukocyte-endothelial cell adhesion, hemorrhages, increased permeability, capillary occlusion, and neovascularization. It is possible that an underlying contributor to these microvascular complications is the loss of the glycocalyx (endothelial surface layer). The endothelial glycocalyx is a dense layer of molecules including glycoproteins, proteoglycans, glycosaminoglycans, and associated proteins, and performs several critical functions including docking of cytokines; physically facilitating the interactions between plasma molecules and their endothelial receptors; inhibiting thrombosis; shielding against leukocyte-endothelial cell adhesion; transducing mechanical shear stress into intracellular signaling cascades (e.g., the production of nitric oxide); and acting as a permeability barrier.

To date, almost nothing is known about diabetes-induced changes in the endothelial glycocalyx of the retina, with (to our knowledge) one report using electron microscopy of rat retinal sections (Kumase, Morizane et al. 2010) showing a reduced glycocalyx thickness (by ~50%) at one week following induction of streptozotocin-induced diabetes, and one report from humans with diabetes (Broekhuizen, Lemkes et al. 2010) showing the same percentage reduction in the retinal circulation of the plasma protein exclusion zone that is thought to represent glycocalyx thickness. In the latter study, the decrease in the exclusion zone was partially reversed by a strategy of dietary supplementation of glycosaminoglycans. However, the specific glycocalyx components lost from the diabetic retina, and the mechanisms responsible for the loss have yet to be determined.

Among the glycosaminoglycans in the endothelial glycocalyx are heparan sulfate, hyaluronic acid, and chondroitin sulfate. All three of these glycosaminoglycans have been found to be responsible for substantial exclusion of macromolecules from the glycocalyx volume (Gao and Lipowsky 2010). The wholebody glycocalyx volume in mice has been found to decrease by more than 50% with a single infusion of hyaluronidase (VanTeeffelen, Brands et al. 2013), an enzyme that can be produced by endothelial cells (Chajara, Raoudi et al. 2000, Chowdhury, Hemming et al. 2016), and which degrades hyaluronic acid. Hyaluronidase has been found to enhance the rate of plasma protein leakage across the blood brain barrier (Zhu, Li et al. 2017), but to date, it is unknown whether the same is true for the blood retinal barrier. Both hyaluronic acid and hyaluronidase are found in elevated concentrations in plasma in diabetes (Nieuwdorp, Mooij et al. 2006, Broekhuizen, Lemkes et al. 2010).

In the current study, we tested several hypotheses including that: a diabetesinduced loss of the retinal endothelial glycocalyx will be found in a genetic, Ins2(Akita) mouse model of type 1 diabetes; hyaluronidase infusion also will induce a loss of glycocalyx in mice; hyaluronic acid levels will be elevated in diabetic mouse plasma; and hyaluronidase infusion will alter the endothelial blood retinal barrier.

2. Materials and Methods

2.1. Animal groups

Breeding pairs of male heterozygous Ins2(Akita) mice with female C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Plasma glucose values were obtained via a drop of blood from a tail vein prick using a sterile needle, with an AlphaTrak 2 meter (Pompano Beach, FL). Heterozygous Ins2(Akita) mice (confirmed via genotyping) with plasma glucose levels higher than 300 mg/dl at 3–4 months of age comprised the diabetic group; wild-type littermates with no Ins2 mutation comprised the corresponding nondiabetic controls. A total of 12 diabetic Akita mice were included (median glucose values at 3–4 months of age = 600 mg/dl) along with 11 nondiabetic controls (median glucose = 187 mg/dl; $p < 0.0001$ vs diabetic). Median glucose values are provided since two diabetic Akita mice had values exceeding the meter limit of 750 mg/dl. Twenty-one male C57BL/6 mice (~2–4 months old), obtained from Jackson Laboratories, were used in experiments investigating the effects of hyaluronidase infusion. The experimental protocols were approved by the Institutional Animal Care and Use Committee of LSUHSC-S and performed according to the criteria outlined by the NIH Guide for the Care and Use of Laboratory Animals, and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Ins2(Akita) genotyping

Akita mouse genotyping was performed using polymerase chain reaction (PCR) followed by a restriction enzyme digest protocol based on a protocol from Jackson Laboratories. Briefly, DNA was extracted from tail snips using REDExtract-N-Amp Tissue PCR Kit (Sigma, MO) followed by PCR to amplify the DNA using the primers: Fwd: 5' TGCTGATGCCCTGGCCTGCT 3' and Rev: 5' TGGTCCCACATATGCACATG 3'. Amplification was performed by pre-heating at 94°C for 3 min, 12 cycles of 94°C for 20 sec-64°C for 30 sec with a 0.5°C decrease per cycle-72°C for 35 sec, 25 cycles of 94°C for 20 sec-58°C for 30 sec-72°C for 35 sec, and 2 min hold at 72°C and storage at 4°C. Restriction digest as per manufacturer's protocol was carried out with restriction enzyme Fnu4HI (NEB, MA) with 1 hr incubation at 37°C. 2% agarose gel electrophoresis was used to obtain the WT allele (140bp) and mutated allele (280bp), imaged using Chemidoc and Image Lab 4.1 (Biorad, CA).

2.3. Surgery and preparation for intravital microscopy

Mice were anesthetized with a combination of ketamine (50 mg/kg) and pentobarbital (50 mg/kg), with the animals kept warm on a heating pad for the remainder of the experiment. During surgery, the eyes were moistened with phosphate-buffered saline. An incision was made along the shaved lower abdomen, and the femoral vein was cannulated with polyethylene tubing (PE10) filled with heparinized saline (25 U/ml). The pupil of the left eye was dilated with a drop of 1% tropicamide ophthalmic solution followed by a drop of 2.5% hypromellose ophthalmic solution, with the eye then covered with a 5 mm circular glass coverslip.

2.4. Intravital video recording of fluorescent dyes in the retinal circulation

Under low magnification (4x), the retina of the left eye was brought into focus using a Nikon Eclipse E600FN microscope and fluorescein filter, with a video recorded using Elgato Video Capture (Munich, Germany). After switching the microscope to a dual fluorescein/rhodamine filter, solutions of tetramethyl rhodamine isothiocyanate-labeled dextran (155 kD MW; Sigma T1287; 70 mg/kg) and sodium fluorescein (MW 376; Sigma F6377; 1 mg/kg) were infused i.v. approximately 1 minute apart, with the timing of the dye arrival used to differentiate arterioles from the venules (arterioles filling first). Each of the arterioles and venules around the optic disk (typically 4–7 each per retina) were brought into focus and recorded with a 10x objective over the following 5–10 minutes.

2.5. Measure of glycocalyx thickness and vascular leakage

From the intravital video recording focused on each arteriole and venule, at least seven video frames per vessel were analyzed to determine the diameter filled by both sodium fluorescein and 155 kD TRITC-dextran. The video frames were separated by ImageJ (National Institutes of Health) into green and red components (Figure 1A-B), and intensity plot profiles (Figure 1C-D) were created from a line 50 μm wide and 125 μm long, subjected to a 7-point moving average. The diameters were calculated as the distance between the two edges of the vessel, defined as the highest gradients in intensity (7-point moving average) between the vessel and surrounding tissue (Figure 1E-F). The glycocalyx thickness was calculated as the difference (divided by 2) between the two diameters.

In selected experiments, the plasma-to-tissue leakage of the tracers were quantified as a leakage index, calculated as the ratio of the fluorescent intensities in defined regions outside vs inside the vessels. The tissue regions (averaged from both sides of the vessel) were 20 μm from the vessel edges, 5 μm in width and 20 μm in length (the latter parallel to the vessel). The vessel region (also 5 $\times$ 20 μm , longer dimension along the direction of flow) was taken from the vessel midline. In one group of C57BL/6 mice, hyaluronidase (12 mg/kg; Sigma H3884; from bovine testes) was infused i.v. into the femoral vein 30 minutes prior to the infusion of the plasma tracers for the measure of vascular leakage. It should be noted that this hyaluronidase can cleave β -N-acetylhexosamine-[1 $\rightarrow$ 4] glycosidic bonds not only in hyaluronic acid, but also in chondroitin sulfate.

2.6. Plasma hyaluronic acid and hyaluronidase activity

Plasma samples were obtained from each group of mice to assay for hyaluronic acid and hyaluronidase activity. Hyaluronic acid (HA) concentrations in plasma were measured using an ELISA kit (amsbio, Cambridge, MA) according to the manufacturer's instructions. Briefly, wells in the plate were coated with HA, followed by the addition of standards and samples in duplicates containing HA and biotin-HA binding protein (HABP). Horseradish peroxidase (HRP)-avidin was added to the wells and incubated, and HABP was detected by chromophoric substrate. The optical density (OD) was measured with a spectrophotometer at a wavelength of 450 ± 2 nm, and the plasma HA concentrations were interpolated using the standard curve.

From the same mice, plasma samples were analyzed for hyaluronidase activity using a modified zymography protocol (Puissant, Gilis et al. 2014). Laemmli buffer was added to plasma samples in non-denaturing, non-reducing conditions, and incubated at room temperature for 1 hour. The samples were run in a 10% polyacrylamide gel containing 0.17 mg/ml hyaluronic acid (R&D Systems GLR002; Minneapolis, MN). Following electrophoresis, the gels were incubated in 3% Triton X-100 for 2 hours at room temperature to renature the proteins, and then for 22 hours at 37°C in developing buffer on a rotating shaker. After washing with water, the gels were incubated for 2 hours at 37°C in 20 mM Tris/HCl, pH 8.8, containing 0.1 mg/ml of pronase (Sigma-Roche PRON-RO), then washed in water. The gels were stained in 0.5% Alcian blue staining solution in 2% acetic acid solution for 1 hour, then destained in 2% acetic acid solution, changed every hour until bands of hyaluronidase digestion could be observed and gel images collected (ChemiDoc; Biorad).

2.7. Statistics

Statistical comparisons between two groups were performed using Student's t-tests and among multiple groups using ANOVA with Tukey's post-hoc corrections (GraphPad Prism; La Jolla, CA). Parametric tests were performed except in the case of plasma glucose, where values could exceed the limit of the meter. Values are presented as means $\pm$ standard error, with the exception of medians for plasma glucose.

3 Results

3.1. Glycocalyx loss in retinal arterioles of diabetic Ins2(Akita) mice

The retinal endothelial glycocalyx was measured in both arterioles and venules of Ins2(Akita) mice (Figure 2). The average thickness of the arteriolar glycocalyx in diabetic mice ($1.34 \pm 0.12 \mu\text{m}$; $N=7$) was significantly smaller (by 35%; $p<0.01$) than in the nondiabetic controls ($2.06 \pm 0.16 \mu\text{m}$; $N=7$); however, no statistical difference was found between the two groups when comparing the thickness of the venular glycocalyx ($1.44 \pm 0.21 \mu\text{m}$ diabetic; $1.73 \pm 0.11 \mu\text{m}$ nondiabetic). Both males and females were included in the glycocalyx measurements (4 nondiabetic males, 3 nondiabetic females, 3 diabetic males, 4 diabetic females); no statistical differences between sexes were observed.

3.2. Plasma hyaluronic acid and hyaluronidase activity

Plasma levels of hyaluronic acid were significantly higher (by >4 -fold; $p<0.01$; Figure 3A) following an intravenous infusion of 12 mg/kg hyaluronidase into nondiabetic C57BL/6 mice ($N=5$) compared with untreated controls ($N=4$). However, no change was observed in diabetic Ins2(Akita) mice ($N=5$) compared with nondiabetic controls ($N=4$; Figure 3B). The same pattern was found with plasma hyaluronidase activity, with the activity apparent via zymography in mice infused with the enzyme (plasma taken 10 minutes following the infusion), but barely detectable in untreated C57BL/6 mice and in both groups of Ins2(Akita) mice (Figure 4).

3.3. Glycocalyx loss in retinal arterioles of mice infused with hyaluronidase

With hyaluronidase infusion into nondiabetic C57BL/6 mice, the average thickness of the arteriolar glycocalyx ($0.92 \pm 0.12 \mu\text{m}$; $N=6$) was significantly smaller (by 44%; $p<0.01$) than in the untreated controls ($1.64 \pm 0.12 \mu\text{m}$; $N=6$) as shown in Figure 5; however, no statistical difference was found between the two groups when comparing the thickness of the venular glycocalyx ($0.92 \pm 0.20 \mu\text{m}$ hyaluronidase; $1.28 \pm 0.15 \mu\text{m}$ control).

3.4. Increased retinal vascular permeability with hyaluronidase infusion

The rate of vascular leakage of sodium fluorescein from the retinal microvasculature of untreated control C57BL/6 mice is shown in Figure 6A, with a 3.6-fold increase in this rate found with the infusion of hyaluronidase (Figure 6B). The rate of leakage of the larger 155 kD TRITC-albumin was essentially insignificant in untreated controls (Figure 6C), but increased substantially with the infusion of hyaluronidase (Figure 6D).

4. Discussion

Virtually nothing is known concerning the retinal endothelial glycocalyx, in either physiological or pathological situations. To our knowledge, this is the first report of the thickness and partial constituency of the retinal endothelial glycocalyx in mice, and the loss of glycocalyx in a mouse model of type 1 diabetes. Our results indicate that diabetes affects the arteriolar glycocalyx to a greater extent than the venular glycocalyx, and that hyaluronic acid is a normal constituent of the retinal glycocalyx that may contribute to the inner blood retinal barrier.

Increases in plasma hyaluronic acid and hyaluronidase activity have been detected in both human type 1 and type 2 diabetes (Nieuwdorp, Mooij et al. 2006, Broekhuizen, Lemkes et al. 2010), and a cause and effect relationship between these increases can be speculated, since the enzyme can cleave hyaluronic acid from the endothelial surface. However, we did not find an increase in either hyaluronidase activity or hyaluronic acid in the plasma of Ins2(Akita) mice, suggesting that additional studies will be required to confirm the identity of the glycocalyx molecules that are lost in this model. However, a loss of hyaluronic acid from the glycocalyx in the Ins2(Akita) mice cannot be ruled out, as it is possible that endothelial-derived hyaluronidase could gradually, and locally, degrade hyaluronic acid in the glycocalyx without resulting in a high plasma concentration or activity of the enzyme or of hyaluronic acid.

If hyaluronic acid is lost from the diabetic retinal glycocalyx, other enzymes besides hyaluronidase could be considered. Thrombin, cathepsin B, elastase, proteinase 3, and plasmin have all been found to be capable of releasing hyaluronic acid from the glycocalyx, for example, from isolated, perfused guinea pig hearts (Becker, Jacob et al. 2015); although these enzymes were found to be far less potent than hyaluronidase. It also could be considered that reactive oxygen species (ROS) could be at least partially responsible for a diabetes-induced loss of hyaluronic acid from the glycocalyx, given the role of ROS in diabetic retinopathy (Eshaq, Wright et al. 2014) and their specific targeting of hyaluronic acid compared with sulfated glycosaminoglycans (Moseley, Waddington et al. 1997).

With both diabetes and hyaluronidase infusion, we detected a greater change in the endothelial glycocalyx on the arteriolar side of the retinal circulation, with less consistent and extensive changes on the venular side. We are unaware of any other such reports of segment-specific effects of diabetes (or hyaluronidase) on glycocalyx structure. However, it should be noted that a previous study (Yen, Cai et al. 2012) was unable to detect heparan sulfate on arterioles of the rat mesentery or mouse cremaster in a technique involving arteriolar cannulation by micropipettes delivering an immunolabel. In the one published study investigating the effect of diabetes on the retinal glycocalyx in humans (Broekhuizen, Lemkes et al. 2010), retinal vessels with diameters of $90\text{ }\mu\text{m}$ (non-capillaries) were used, but no separation of the measurements into arterioles and venules was mentioned. Additionally, only capillaries were studied in the single study in rats investigating the effect of diabetes on the retinal endothelial glycocalyx (Kumase, Morizane et al. 2010).

If our observation of a greater effect of diabetes and hyaluronidase on the arteriolar versus venular side can be interpreted as a greater concentration of hyaluronic acid on the arteriolar surface, then a relevant issue to assess is that of function. It has been reported (Mochizuki, Vink et al. 2003) that in isolated canine femoral arteries, the level of nitric oxide (NO) production is linearly related to the perfusion rate, with the NO production almost completely eliminated with simultaneous exposure to hyaluronidase. Also, in cultured endothelial cells, flow induces a doubling of hyaluronic acid expression compared to static conditions (Gouverneur, Spaan et al. 2006). Therefore, it is possible that hyaluronic acid can act as a mechanosensor helping regulate arteriolar control of retinal blood flow.

Hyaluronic acid, formed in the cell membrane to a size exceeding 1 million daltons (Lennon and Singleton 2011) and incorporated into the glycocalyx in part by binding membrane CD44, has also been shown to create matrices in the glycocalyx and act as a sieving barrier (Henry and Duling 1999). Using the hamster cremaster microcirculation, a previous study (Henry and Duling 1999) has found that hyaluronidase increased the permeation of 70 and 145 kD dextrans into the glycocalyx, which potentially could allow a faster leakage into the surrounding tissue after traversing the retinal endothelial tight junctions. Similar findings of hyaluronidase-induced increases in glycocalyx permeation were obtained for 70 kD dextran in the rat mesentery (Gao and Lipowsky 2010). Our results from hyaluronidase infusion experiments demonstrate a significantly higher rate of plasma leakage of both low molecular weight sodium fluorescein and 155 kD dextran into the surrounding tissue, consistent with a significant role for hyaluronic acid in blood retinal barrier function.

In summary, we have demonstrated that diabetes and hyaluronidase reduce the thickness of the retinal endothelial glycocalyx, in which hyaluronic acid may play a significant role in barrier function. Future studies are warranted to more fully elucidate the complete structure and function of the retinal glycocalyx, in health and disease states such as diabetic retinopathy.

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References

- Becker BF, Jacob M, Leipert S, Salmon AH and Chappell D (2015). "Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases." *Br J Clin Pharmacol* 80(3): 389–402. [PubMed: 25778676]
- Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, Schlingemann RO, Nieuwdorp M, Stroes ES and Vink H (2010). "Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus." *Diabetologia* 53(12): 2646–2655. [PubMed: 20865240]
- Chajara A, Raoudi M, Delpech B, Leroy M, Basuyau JP and Levesque H (2000). "Increased hyaluronan and hyaluronidase production and hyaluronan degradation in injured aorta of insulin-resistant rats." *Arterioscler Thromb Vasc Biol* 20(6): 1480–1487. [PubMed: 10845861]
- Chowdhury B, Hemming R, Faiyaz S and Triggs-Raine B (2016). "Hyaluronidase 2 (HYAL2) is expressed in endothelial cells, as well as some specialized epithelial cells, and is required for normal hyaluronan catabolism." *Histochem Cell Biol* 145(1): 53–66. [PubMed: 26515055]
- Eshaq RS, Wright WS and Harris NR (2014). "Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy." *Redox Biol* 2: 661–666. [PubMed: 24936440]
- Gao L and Lipowsky HH (2010). "Composition of the endothelial glycocalyx and its relation to its thickness and diffusion of small solutes." *Microvasc Res* 80(3): 394–401. [PubMed: 20600162]
- Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD and Vink H (2006). "Fluid shear stress stimulates incorporation of hyaluronan into endothelial cell glycocalyx." *Am J Physiol Heart Circ Physiol* 290(1): H458–452. [PubMed: 16126814]
- Henry CB and Duling BR (1999). "Permeation of the luminal capillary glycocalyx is determined by hyaluronan." *Am J Physiol* 277(2 Pt 2): H508–514. [PubMed: 10444475]
- Kumase F, Morizane Y, Mohri S, Takasu I, Ohtsuka A and Ohtsuki H (2010). "Glycocalyx degradation in retinal and choroidal capillary endothelium in rats with diabetes and hypertension." *Acta Med Okayama* 64(5): 277–283. [PubMed: 20975760]
- Lennon FE and Singleton PA (2011). "Hyaluronan regulation of vascular integrity." *Am J Cardiovasc Dis* 1(3): 200–213. [PubMed: 22254199]
- Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA and Kajiya F (2003). "Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release." *Am J Physiol Heart Circ Physiol* 285(2): H722–726. [PubMed: 12730059]
- Moseley R, Waddington RJ and Embury G (1997). "Degradation of glycosaminoglycans by reactive oxygen species derived from stimulated polymorphonuclear leukocytes." *Biochim Biophys Acta* 1362(2–3): 221–231. [PubMed: 9540853]
- Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JA, Ince C, Holleman F, Diamant M, Heine RJ, Hoekstra JB, Kastelein JJ, Stroes ES and Vink H (2006). "Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes." *Diabetes* 55(4): 1127–1132. [PubMed: 16567538]
- Puissant E, Gilis F, Dogne S, Flamion B, Jadot M and Boonen M (2014). "Subcellular trafficking and activity of Hyal-1 and its processed forms in murine macrophages." *Traffic* 15(5): 500–515. [PubMed: 24502338]
- VanTeeffelen JW, Brands J, Janssen BJ and Vink H (2013). "Effect of acute hyaluronidase treatment of the glycocalyx on tracer-based whole body vascular volume estimates in mice." *J Appl Physiol* (1985) 114(9): 1132–1140. [PubMed: 23449940]
- Yen WY, Cai B, Zeng M, Tarbell JM and Fu BM (2012). "Quantification of the endothelial surface glycocalyx on rat and mouse blood vessels." *Microvasc Res* 83(3): 337–346. [PubMed: 22349291]
- Zhu J, Li X, Yin J, Hu Y, Gu Y and Pan S (2017). "Glycocalyx degradation leads to blood-brain barrier dysfunction and brain edema after asphyxia cardiac arrest in rats." *J Cereb Blood Flow Metab* 10.1177/0271678X17726062.

- Diabetes and hyaluronidase degrade the retinal endothelial glycocalyx.
- Arteriolar, rather than venular, glycocalyx is more sensitive to the glycocalyx loss.
- The retinal glycocalyx is comprised, in part, by hyaluronic acid.
- Hyaluronidase infusion increases retinal vascular leakage of plasma tracers.

Figure 1.

Method of in vivo glycocalyx measure. Video frames are separated into green (sodium fluorescein, panel A) and red (155 kD TRITC-dextran, panel B) components. Vessel edges and diameters are obtained from the intensity plot profiles (panels C, D) as defined as the sharpest gradients between the plasma intensity and the surrounding background (panels E, F), with the glycocalyx thickness being one-half the difference between the measures of diameter.

Figure 2.

The glycocalyx of retinal arterioles (A) in diabetic Ins2(Akita) mice (N=7) was significantly smaller than in nondiabetic controls (N=7; ** $p<0.01$), but no statistical difference was found between the two groups in the venules (B).

Figure 3. Plasma hyaluronic acid levels were higher in C57BL/6 mice infused with hyaluronidase (A, ** $p < 0.01$) than in controls, but not in diabetic Ins2(Akita) mice (B).

Figure 4. Zymography of plasma hyaluronidase activity in the four experimental groups. Elevated activity was seen only in the mice infused with hyaluronidase. The displayed sections of the gels are from ~50 kD and higher as indicated by the MW markers.

Figure 5.

The glycocalyx of retinal arterioles (A) in C57BL/6 mice infused with hyaluronidase was significantly smaller than in untreated controls (N=6 each; ** $p<0.01$), but no statistical difference was found between the two groups in the venules (B).

Figure 6.

Tissue/vessel leakage index of sodium fluorescein (NaFl) in untreated control C57BL/6 mice (A) and in hyaluronidase-infused C57BL/6 mice (B), and for 155-kD TRITC-dextran in the same two groups, respectively (C, D).