Published in final edited form as:

Exp Eye Res. 2016 February ; 143: 141–147. doi:10.1016/j.exer.2015.09.018.

The Effect of Intravitreal Vascular Endothelial Growth Factor on Inner Retinal Oxygen Delivery and Metabolism in Rats

Norman P. Blair^a, Justin Wanek^a, Pang-yu Teng^{a,1}, and Mahnaz Shahidi^a

Norman P. Blair: npblair@uic.edu; Justin Wanek: wanek_justin@yahoo.com; Pang-yu Teng: pangyuteng@gmail.com; Mahnaz Shahidi: mahnshah@uic.edu

^aDepartment of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 West Taylor Street, Chicago IL 60612, United States of America

1.0 Introduction

Vascular endothelial growth factor (VEGF) is a signal protein that has potent mitogenic and survival activity for endothelial cells and powerful vascular permeability-enhancing properties. (Ferrara, 2004; Nishijima et al., 2007) Thus, it plays an important role in embryonic retinal vascular development, pathologic retinal vascular leakage, and neovascularization of the choroid, optic nerve, retina, and iris. (Ferrara, 2004) Accordingly, inhibitors of VEGF are now the treatments of choice for many cases of macular edema, (Brown et al., 2011; Campochiaro et al., 2011; Diabetic Retinopathy Clinical Research et al., 2010; Nguyen et al., 2010) choroidal neovascularization (Gragoudas et al., 2004; Rosenfeld et al., 2006) and neovascularization of the retina, optic nerve and iris. (Ernst et al., 2012; Grisanti et al., 2006; Martinez-Zapata et al., 2014)

Retinal hypoxia/ischemia, via hypoxia inducible factor (HIF), (Ozaki et al., 1999) is a major stimulator of VEGF. (Aiello et al., 1995; Miller et al., 1994; Pe'er et al., 1995) This suggests that VEGF may play a role in counteracting hypoxia and the injurious effects that supervene if the hypoxia is severe enough. There are several ways by which VEGF could mitigate hypoxic effects. First, by stimulating endothelial proliferation and vessel growth, VEGF could lead to better perfusion and alleviate retinal hypoxia. However, neovascularization often fails to vascularize ischemic tissue and instead can lead to deleterious effects such as hemorrhage and fibrosis. Furthermore, elevated VEGF over time can stimulate excessive endothelial proliferation that can occlude capillaries. (Hofman et al., 2001; Tolentino et al., 2002) Second, VEGF has been shown to have a survival role on retinal neural cells. (Saint-Geniez et al., 2008) Third, VEGF could increase retinal blood flow and oxygen delivery to the inner retina (DO_2). VEGF has been shown to cause retinal vasodilation, vascular tortuosity and increased blood flow in animals. (Ali Rahman et al., 2011; Ameri et al., 2007; Arana et al., 2012; Clermont et al., 1997; Ozaki et al., 1997) Several studies are available on

Correspondence to: Norman P. Blair, npblair@uic.edu.

¹UCLA Radiological Sciences, Suite 650, 924 Westwood Boulevard, Los Angeles, CA 90024

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

retinal vascular changes after treatment of patients with VEGF inhibitors. A reduction in vessel diameters (Fontaine et al., 2011; Papadopoulou et al., 2009) and reductions in retrobulbar flow velocities have been observed. (Bonnin et al., 2010; Hosseini et al., 2012; Mete et al., 2010; Toklu et al., 2011) Nitta and coworkers found that the retinal blood flow measured by laser speckle flowgraphy decreased in patients treated for diabetic macular edema with an anti-VEGF agent. (Nitta et al., 2014) These observations imply that prior to treatment VEGF had increased retinal blood flow and DO_2 . On the other hand, Fontaine and coworkers found no change in retinal blood flow by laser Doppler flowmetry after anti-VEGF therapy for age-related macular degeneration even though the retinal arterioles became narrower. (Fontaine et al., 2011) Barak and coworkers found increased blood velocity with the retinal function imager after anti-VEGF treatment, and they attributed this to suppression of vasodilation. (Barak et al., 2012) We have not found in the literature any measurements of DO_2 after the introduction of VEGF into the vitreous. Fourth, VEGF could down-regulate inner retinal oxygen metabolism (MO_2), as HIF does, (Kim et al., 2006) so that the tissue can adapt to mild reductions in oxygen availability. Reduced MO_2 might be expected to contribute to ischemic preconditioning and the suppression of cell death pathways, processes in which VEGF is thought to play a role. (Evans et al., 2008; Jin et al., 2001; Parcellier et al., 2003; Wick et al., 2002) However, we have not found any measurements in the literature on the effects of VEGF on inner retinal MO_2 .

We hypothesized that VEGF increases DO_2 , which could be advantageous in conditions characterized by hypoxia, but that it does not alter MO_2 . We tested these hypotheses using the methods we have developed to measure DO_2 and MO_2 in rats. (Wanek et al., 2011; Wanek et al., 2013, 2014)

2.0 Methods

2.1 Animals

Ten Long Evans pigmented rats (weight: 483 ± 127 g, mean $\pm$ SD) were used in the study. The rats were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg). Following the method of Miyamoto and co-workers, the right eye of each rat received an intravitreal injection of VEGF A (R & D Systems, Minneapolis, MN) while the fellow eye received vehicle (BSS) alone. (Miyamoto et al., 2000) The dose was increased from the concentration employed by Miyamoto et al (50 ng in 5 μ l) to 250 ng in 5 μ l of BSS because of the extremely rapid clearance of VEGF from the vitreous as recently reported. (Lee et al., 2010) Data acquisition was performed 24 hours after VEGF injection. The rats were reanesthetized with intraperitoneal injections of ketamine and xylazine. Additional injections of ketamine (20 mg/kg) and xylazine (1 mg/kg) were given to maintain anesthesia as required. Rats were ventilated mechanically with room air with the use of an endotracheal tube connected to a small animal ventilator (Harvard Apparatus, Inc., South Natick, MA). To monitor the animal's physiological condition, the femoral artery was cannulated and a catheter was attached to draw blood and connect a pressure transducer. Systemic arterial oxygen tension (P_aO_2), carbon dioxide tension (P_aCO_2), and pH were measured with a blood gas analyzer (Radiometer, Westlake, OH) 5 to

10 minutes after initiation of ventilation. Ventilation parameters, including the respiratory rate and minute volume, were adjusted until the $P_a\text{CO}_2$ was within the normocapnic range. (West, 2007) Hemoglobin concentration (Hgb) was also measured with a hematology system (Siemens, Tarrytown, NY) from arterial blood. Blood pressure (BP) and heart rate (HR) were monitored continuously with a data acquisition system (Biopac Systems, Goleta, CA) linked to the pressure transducer.

Before imaging, the rat was placed in an animal holder with a copper tubing water heater, which maintained the body temperature at 37°C. The pupils were dilated with 2.5% phenylephrine and 1% tropicamide. A glass cover slip with 1% hydroxypropyl methylcellulose was applied to the cornea to eliminate its refractive power and prevent dehydration. For retinal vascular PO_2 imaging, an oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT), was dissolved (12 mg/mL) in bovine serum albumin solution (60 mg/mL) and administered through the femoral arterial catheter (20 mg/kg). For retinal blood velocity imaging, 2- μm polystyrene fluorescent microspheres (Invitrogen, Grand Island, NY) were injected through the catheter. Typically, two to three injections of the microspheres were given, and each injection was approximately 0.4 mL (10^5 microspheres/mL).

2.2 Retinal Hemodynamics Imaging

Our previously described prototype blood flow imaging system (Wanek et al., 2011) was used for red-free and fluorescent microsphere imaging to assess venous blood vessel diameter and velocity, respectively. A slit lamp biomicroscope with standard light illumination (Carl Zeiss, Oberkochen, Germany) was equipped with a green filter (540 ± 5 nm; Edmund Optics, Barrington, NJ) for red-free retinal imaging, and a 488-nm diode laser (Melles Griot, Carlsbad, CA), coupled with an emission filter (560 ± 60 nm; Spectrotech, Inc., Saugus, MA) for fluorescent microsphere imaging. Images were captured with a high-speed electron multiplier charge coupled device camera (QImaging, Surrey, Canada). Red-free retinal images were obtained using the full resolution of the camera (1002×1004 pixels). For fluorescent images, the camera sensor was binned to maximize the frame rate to 108 Hz, allowing the motion of the microspheres to be resolved in time, but with lower spatial resolution (248×250 pixels). Multiple image sequences, each 5 seconds in duration, were recorded over several minutes.

Diameters of all individual retinal arteries (D_{Aind}) and veins (D_{Vind}) were measured from red-free images over a fixed vessel length (200 μm) that extended between approximately 300 and 500 μm from the center of the optic disk. D_{Aind} and D_{Vind} were obtained based on the average full width at half maximum of 12 intensity profiles perpendicular to the blood vessel axis. In control eyes 5.2 ± 1.0 arteries and 5.2 ± 1.1 veins were measured, whereas 5.5 ± 1.1 arteries and 5.4 ± 1.0 veins were included from VEGF-injected eyes. For each rat, a mean arterial (D_{A}) and venous diameter (D_{V}) was calculated from all D_{Aind} and all D_{Vind} values, respectively. Blood velocity in all individual veins (V_{ind}) was measured by manually tracking displacements of the microspheres over time, following our previously reported methodology. (Wanek et al., 2011) Typically, three to five image sequences were analyzed to derive V_{ind} , which was determined by averaging 20 – 30 microsphere velocity

measurements in each vein. The number of measurements used to obtain each V_{ind} was contingent on the number of microspheres that could be visualized in the image sequences. In each rat, a mean velocity (V) was derived from all V_{ind} measurements. V was measured in veins because they are less affected by pulsation and have larger diameters as compared with arteries. Blood flow in each major vein was calculated from $D_{V_{ind}}$ and V_{ind} measurements ($V_{ind} * \pi * D_{V_{ind}}^2 / 4$) and summed over all veins to provide a measure of the total venous blood flow in the retinal circulation (F) in that animal. Since the retinal circulation is considered to be an end-artery system, (Levin and Adler, 2011) the venous blood flow was taken to be equal to the total arterial or total retinal blood flow. Measurements of F were obtained approximately 15 minutes following PO_2 imaging, while the physiological state of the animals was relatively stable, as indicated by the continuous monitoring of BP, HR, and the use of constant ventilation parameters.

2.3 Retinal Vascular PO_2 Imaging

Retinal vascular PO_2 was measured using our established optical section phosphorescence lifetime imaging system. (Shahidi et al., 2006; Shahidi et al., 2009) A laser line was projected on the retina after intravenous injection of the Pd-porphine probe and an optical section phosphorescence image was acquired with an intensified charge-coupled device camera. Due to the angle between the excitation laser beam and imaging path, phosphorescence emissions from the retinal vessels were depth-resolved from the underlying choroid. Phosphorescence lifetimes in the retinal vessels were determined using a frequency-domain approach and converted to PO_2 measurements using the Stern-Volmer equation. (Lakowicz et al., 1992; Shonat and Kight, 2003) PO_2 was measured in all major retinal arteries ($PO_{2A_{ind}}$) and retinal veins ($PO_{2V_{ind}}$) at locations within three optic disc diameters ($\sim 600 \mu m$) from the edge of the optic nerve head. Three repeated $PO_{2A_{ind}}$ and $PO_{2V_{ind}}$ measurements were averaged per blood vessel. The mean and SD of the $PO_{2A_{ind}}$ values for arteries (PO_{2A}) and $PO_{2V_{ind}}$ values for veins (PO_{2V}) were calculated for each animal.

The oxygen content of blood (O_{2ind}) was calculated for each individual major retinal artery and vein as the sum of oxygen bound to hemoglobin and dissolved in blood: (Pittman, 2011) $O_{2ind} = SO_2 * C * HgB + PO_2 * k$, where SO_2 is the oxygen saturation (%), C is the oxygen-carrying capacity of hemoglobin (1.39 mL O_2 /g), (Nathan and Singer, 1999) and k is the oxygen solubility in blood (0.003 mL O_2 /dL-mm Hg. (Pittman, 2011) SO_2 was calculated from the hemoglobin dissociation curve in rat (Cartheuser, 1993) by using the measured blood PO_2 and pH values. Last, in each animal mean arterial (O_{2A}) and venous (O_{2V}) oxygen contents were determined from O_{2ind} measurements. The arteriovenous oxygen content difference (O_{2A-V}) was calculated as: $O_{2A-V} = O_{2A} - O_{2V}$.

2.4 Oxygen Delivery and Oxygen Metabolism

DO_2 was determined from the F and O_{2A} measurements according to the following equation: (Pittman, 2011) $DO_2 = F * O_{2A}$. MO_2 was calculated from measurements of F and O_{2A-V} , using Fick's principle: (Pittman, 2011) $MO_2 = F * O_{2A-V}$.

2.5 Statistical Analysis

The data were analyzed with the paired Student's t test. Statistical significance was accepted at P less than 0.05.

3.0 Results

3.1 Systemic Physiologic Factors

BP and HR were 105 ± 17 mm Hg and 235 ± 44 beats/min, respectively. The systemic P_aO_2 , P_aCO_2 and pH were 86 ± 7 mm Hg, 40 ± 9 mm Hg and 7.42 ± 0.06 , respectively. These indicate that the rats had a normal systemic physiologic condition during the experiments.

3.2 Retinal Hemodynamic Factors

The results of the retinal hemodynamic factors are presented in Table 1. D_A in the control and VEGF-injected eyes were 42 ± 6 and 44 ± 5 μ m, respectively ($P=0.03$), an increase of 4%. A greater effect was observed in D_V . The values in the control and eyes receiving VEGF were 50 ± 6 and 61 ± 12 μ m, respectively ($P=0.008$), an increase of 22%. Figure 1 depicts red-free fundus images of normal and VEGF-injected eyes. Vasodilation is visible in the VEGF-injected eyes, especially in the veins. No significant difference was found in V , which were 11.7 ± 2.4 and 10.9 ± 2.6 mm/s in the control and VEGF-injected eyes, respectively ($P=0.21$). The largest effect of VEGF was seen in F . These were 7.3 ± 1.6 and 10.6 ± 3.9 μ L/min without and with VEGF injection, respectively, ($P=0.007$), an increase of 45%.

3.3 Retinal Vascular Oxygenation Factors

The results of the vascular oxygen factors are displayed in Table 2. A statistically significant difference was found between control and VEGF-injected eyes only in O_{2A-V} . PO_{2A} were 46 ± 12 and 45 ± 9 mm Hg in the control and VEGF-injected eyes, respectively ($P=0.41$). PO_{2V} were 28 ± 6 in the control eyes and 31 ± 6 mm Hg in the eyes receiving VEGF ($P=0.11$). In the control and VEGF-injected eyes O_{2A} were 12.9 ± 2.8 and 12.8 ± 2.4 mL O_2 /dl, respectively ($P=0.92$). O_{2V} were 6.9 ± 2.5 and 8.1 ± 2.3 mL O_2 /dl in the control and eyes administered VEGF, respectively ($P=0.12$). O_{2A-V} were 6.0 ± 1.7 and 4.8 ± 1.1 mL O_2 /dl in the control and VEGF-injected eyes, respectively ($P=0.05$).

3.4 Oxygen Delivery and Oxygen Metabolism

The results of oxygen delivery and oxygen metabolism are presented in Table 3 and depicted graphically in Figure 2. This figure shows that in 9 of 10 rats DO_2 was greater in the VEGF-injected eye than in the control eye. DO_2 were 950 ± 340 and 1380 ± 650 nL O_2 /min in the control and eyes receiving VEGF, respectively ($P=0.005$), an increase of 45%. In contrast, no difference was observed in MO_2 between the two groups. MO_2 were 440 ± 150 and 490 ± 190 nL O_2 /min in the control and VEGF-injected eyes, respectively ($P=0.31$).

4.0 Discussion

In the present study VEGF induced an increase in DO_2 while MO_2 remained unchanged. The increase in DO_2 was mainly caused by an increase in F , since we found no change in O_{2A} . The increase in F , in turn, was caused by an increase in D_V , since no change in V was observed. Because VEGF stimulates nitric oxide, (Fukumura et al., 2001) it is not surprising that both the arteries and veins were dilated, as many have found in animals injected with VEGF intravitreally. As expected O_{2A} was unchanged by VEGF because it is dominated by systemic phenomena acting before blood enters the retina, whereas O_{2V} is dominated by the extraction of oxygen during the transit of the blood from artery to vein, namely MO_2 . When DO_2 increases while MO_2 remains unchanged, the amount of oxygen left in the veins to exit the eye must increase, accounting for the smaller O_{2A-V} we observed in the eyes injected with VEGF.

No measurements of DO_2 in the retina have been reported until recently because methods have not been available to measure both F and O_{2A} in the same experiment. However, significant information on DO_2 can be inferred from F measurements because O_{2A} tends to be constant in the absence of systemic changes, such as anemia or low FiO_2 . Clermont and coworkers reported increases in retinal blood flow in rats after intravitreal administration of VEGF. (Clermont et al., 1997) They measured the vessel diameters and the mean circulation time of injected fluorescein to obtain their results in units of pixel^2 per time. Inner retinal blood flow equals the volume of the vascular tree divided by the mean circulation time. (Eberli et al., 1979) Thus, this method gives results that are related to actual blood flow only to the extent that the square of the vessel diameters represents the vascular volume. Several studies have looked at blood flow in patients who had been treated with inhibitors of VEGF. However, the level of VEGF at baseline and the completeness of inhibition at the time of measurement were unknown, so only semi-quantitative conclusions can be drawn about the effect of VEGF on retinal blood flow. None have combined blood flow estimates with oxygen measurements.

Studies on the metabolic effects of VEGF have not suggested a major influence on energy generation. (Evans et al., 2008; Jin et al., 2001; Parcellier et al., 2003; Wick et al., 2002) As we hypothesized, we found no alterations in inner retinal MO_2 , even though DO_2 increased. No previous measurements are available on the effect of VEGF on inner retinal MO_2 . Again, this is related to limitations in methods to acquire this information.

Much important information has been obtained on oxygen in the retina with previous methods. Oxygen consumption can be measured in the outer retina with oxygen sensitive microelectrodes and slit-beam phosphorescent imaging in animals assuming one dimensional oxygen diffusion from the choroid. (Linsenmeier, 1986; Linsenmeier and Braun, 1992; Pournaras, 1995; Wanek et al., 2012; Yu and Cringle, 2001) However, the validity of this assumption is questionable in the inner retina except under certain experimental conditions. (Alder et al., 1990; Braun et al., 1995) Methods to assess retinal oxygenation including MRI and oximetry have yielded great contributions to our understanding of retinal oxygenation, (Choudhary et al., 2013; Hammer et al., 2009; Hardarson, 2013; Kashani et al., 2014; Klefter et al., 2014; Man et al., 2013; Shahidi et al.,

2013; Trick and Berkowitz, 2005; Yi et al., 2013; Zhang et al., 2011) but these do not quantify MO_2 . Recently, Palkovits and coworkers have published results obtained by the combined use of retinal vascular oximetry, vessel caliber analysis and blood velocity measurements using laser Doppler velocimetry in healthy subjects. (Palkovits et al., 2014a; Palkovits et al., 2014b) We anticipate that much useful information will be obtained with these methods in the near future.

Our results on the effects of VEGF on DO_2 and MO_2 may be relevant to sight threatening diabetic retinopathy in which the presence of nonperfusion, hypoxia and elevated VEGF is indisputable. Their significance in the early stages of human diabetic retinopathy and retinopathy in rats with diabetes is less clear. VEGF levels have been found to be elevated in diabetic rats within three months of diabetes. (Gong et al., 2013; Kusari et al., 2010) However, we have reported no changes in DO_2 or MO_2 in rats after 6 weeks of diabetes and no intraretinal hypoxia was found in diabetic rats, at least up to 12 weeks of diabetes. (Lau and Linsenmeier, 2014; Wright et al., 2011). Presumably, in this case VEGF is stimulated by factors other than hypoxia and at levels low enough not to enhance DO_2 .

One limitation of the current study was lack of knowledge about the amount of VEGF in the retina 24 hours after intravitreal injection. This time interval allowed diffusion of VEGF from the vitreous into the retina. The amount of VEGF in the retina depends on the initial vitreal concentration, half-life, and time. The amount of VEGF we injected was 250 ng, which is within the range of the amounts (2–10,000 ng) employed by other investigators. (Ameri et al., 2007; Arana et al., 2012; Clermont et al., 1997; Hofman et al., 2001; Lee et al., 2010; Miyamoto et al., 2000; Tolentino et al., 1996) Loss of VEGF from the vitreous is extremely rapid. The half-life of VEGF in the rabbit vitreous has been reported to be 2.46 hours. (Lee et al., 2010) If VEGF in rats has the same half-life, approximately nine half-lives would have occurred by the time of our measurements at 24 hours after injection. In this case, the VEGF concentration would be reduced to about 1/500 of the initial value, which would be about 10 ng/ml assuming a rat vitreous volume of 50 μ l. This is well within the range of maximal concentrations reported in diseased human eyes (3 – 5000 ng/ml). (Adamis et al., 1994; Aiello et al., 1994; Brooks et al., 2004; Funatsu et al., 2002; Malecaze et al., 1994; Malik et al., 2005; Ogata et al., 2002; Simo et al., 2002; Watanabe et al., 2005) While the half-life of intravitreal VEGF in rats may not be identical to that in rabbits, there still would have been a dramatic reduction of the initial intravitreal concentration of VEGF by the time of our measurements. Further studies are needed to determine the optimal dose and time interval for evaluating VEGF-induced changes in retinal oxygen delivery and oxygen metabolism.

We have formed hypotheses based on the notion that VEGF has effects that counter those of hypoxia. This seems reasonable since VEGF is stimulated by HIF and it induces nitric oxide. Nonetheless, intravitreal administration of VEGF causes a retinopathy resembling ischemic retinopathies characterized by vascular tortuosity, edema, hemorrhages, microaneurysms, capillary occlusion and neovascularization. (Ali Rahman et al., 2011; Ameri et al., 2007; Arana et al., 2012; Hofman et al., 2001; Ozaki et al., 1997; Tolentino et al., 2002; Tolentino et al., 1996) Neovascularization and edema are not surprising based on the known mitogenic and permeability-enhancing actions of VEGF. It appears that with high

levels and time VEGF may actually exacerbate retinal hypoxia, at least locally, by inducing excessive endothelial proliferation that can occlude capillaries and by inducing retinal edema that will lengthen the diffusion distance between the capillaries and cells. Thus, there is complexity to the actions of VEGF in that it can both counteract and exacerbate retinal hypoxia.

Acknowledgements

This study was supported by the National Eye Institute, Bethesda, MD, EY017918 (MS) and EY001792 (UIC), Research to Prevent Blindness, New York, NY, senior scientific investigator award (MS) and an unrestricted departmental award. We acknowledge the technical support of Marek Mori and Tara Nguyen.

References

- Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, Yeo KT. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *American journal of ophthalmology*. 1994; 118:445–450. [PubMed: 7943121]
- Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *The New England journal of medicine*. 1994; 331:1480–1487. [PubMed: 7526212]
- Aiello LP, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Archives of ophthalmology*. 1995; 113:1538–1544. [PubMed: 7487623]
- Alder VA, Ben-Nun J, Cringle SJ. PO₂ profiles and oxygen consumption in cat retina with an occluded retinal circulation. *Investigative ophthalmology & visual science*. 1990; 31:1029–1034. [PubMed: 2354908]
- Ali Rahman IS, Li CR, Lai CM, Rakoczy EP. In vivo monitoring of VEGF-induced retinal damage in the Kimba mouse model of retinal neovascularization. *Current eye research*. 2011; 36:654–662. [PubMed: 21657827]
- Ameri H, Chader GJ, Kim JG, Sadda SR, Rao NA, Humayun MS. The effects of intravitreal bevacizumab on retinal neovascular membrane and normal capillaries in rabbits. *Investigative ophthalmology & visual science*. 2007; 48:5708–5715. [PubMed: 18055823]
- Arana LA, Pinto AT, Chader GJ, Barbosa JD, Morales S, Moreira AT, Maia M, Humayun MS. Fluorescein angiography, optical coherence tomography, and histopathologic findings in a VEGF(165) animal model of retinal angiogenesis. *Graefes's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2012; 250:1421–1428.
- Barak A, Burgansky-Eliash Z, Barash H, Nelson DA, Grinvald A, Loewenstein A. The effect of intravitreal bevacizumab (Avastin) injection on retinal blood flow velocity in patients with choroidal neovascularization. *European journal of ophthalmology*. 2012; 22:423–430. [PubMed: 22081670]
- Bonnin P, Pournaras JA, Lazrak Z, Cohen SY, Legargasson JF, Gaudric A, Levy BI, Massin P. Ultrasound assessment of short-term ocular vascular effects of intravitreal injection of bevacizumab (Avastin((R))) in neovascular age-related macular degeneration. *Acta ophthalmologica*. 2010; 88:641–645. [PubMed: 19563370]
- Braun RD, Linsenmeier RA, Goldstick TK. Oxygen consumption in the inner and outer retina of the cat. *Investigative ophthalmology & visual science*. 1995; 36:542–554. [PubMed: 7890485]
- Brooks HL Jr, Caballero S Jr, Newell CK, Steinmetz RL, Watson D, Segal MS, Harrison JK, Scott EW, Grant MB. Vitreous levels of vascular endothelial growth factor and stromal-derived factor 1 in patients with diabetic retinopathy and cystoid macular edema before and after intraocular injection of triamcinolone. *Archives of ophthalmology*. 2004; 122:1801–1807. [PubMed: 15596583]

- Brown DM, Campochiaro PA, Bhisitkul RB, Ho AC, Gray S, Saroj N, Adamis AP, Rubio RG, Murahashi WY. Sustained benefits from ranibizumab for macular edema following branch retinal vein occlusion: 12-month outcomes of a phase III study. *Ophthalmology*. 2011; 118:1594–1602. [PubMed: 21684606]
- Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, Murahashi WY, Rubio RG. Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. *Ophthalmology*. 2011; 118:2041–2049. [PubMed: 21715011]
- Cartheuser CF. Standard and pH-affected hemoglobin-O₂ binding curves of Sprague-Dawley rats under normal and shifted P50 conditions. *Comparative biochemistry and physiology. Comparative physiology*. 1993; 106:775–782. [PubMed: 7906635]
- Choudhary TR, Ball D, Fernandez Ramos J, McNaught AI, Harvey AR. Assessment of acute mild hypoxia on retinal oxygen saturation using snapshot retinal oximetry. *Investigative ophthalmology & visual science*. 2013; 54:7538–7543. [PubMed: 24159087]
- Clermont AC, Aiello LP, Mori F, Aiello LM, Bursell SE. Vascular endothelial growth factor and severity of nonproliferative diabetic retinopathy mediate retinal hemodynamics in vivo: a potential role for vascular endothelial growth factor in the progression of nonproliferative diabetic retinopathy. *American journal of ophthalmology*. 1997; 124:433–446. [PubMed: 9323935]
- Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, Ferris FL 3rd, Friedman SM, Glassman AR, Miller KM, Scott IU, Stockdale CR, Sun JK. Diabetic Retinopathy Clinical Research, N. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2010; 117:1064–1077. e1035. [PubMed: 20427088]
- Eberli B, Riva CE, Feke GT. Mean circulation time of fluorescein in retinal vascular segments. *Archives of ophthalmology*. 1979; 97:145–148. [PubMed: 103518]
- Ernst BJ, Garcia-Aguirre G, Oliver SC, Olson JL, Mandava N, Quiroz-Mercado H. Intravitreal bevacizumab versus panretinal photocoagulation for treatment-naïve proliferative and severe nonproliferative diabetic retinopathy. *Acta ophthalmologica*. 2012; 90:e573–e574. [PubMed: 22405048]
- Evans IM, Britton G, Zachary IC. Vascular endothelial growth factor induces heat shock protein (HSP) 27 serine 82 phosphorylation and endothelial tubulogenesis via protein kinase D and independent of p38 kinase. *Cellular signalling*. 2008; 20:1375–1384. [PubMed: 18440775]
- Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocrine reviews*. 2004; 25:581–611. [PubMed: 15294883]
- Fontaine O, Olivier S, Descovich D, Cordahi G, Vaucher E, Lesk MR. The effect of intravitreal injection of bevacizumab on retinal circulation in patients with neovascular macular degeneration. *Investigative ophthalmology & visual science*. 2011; 52:7400–7405. [PubMed: 21693608]
- Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, Buerk DG, Huang PL, Jain RK. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98:2604–2609. [PubMed: 11226286]
- Funatsu H, Yamashita H, Ikeda T, Nakanishi Y, Kitano S, Hori S. Angiotensin II and vascular endothelial growth factor in the vitreous fluid of patients with diabetic macular edema and other retinal disorders. *American journal of ophthalmology*. 2002; 133:537–543. [PubMed: 11931788]
- Gong CY, Lu B, Hu QW, Ji LL. Streptozotocin induced diabetic retinopathy in rat and the expression of vascular endothelial growth factor and its receptor. *International journal of ophthalmology*. 2013; 6:573–577. [PubMed: 24195027]
- Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR. Group, V.I.S.i.O.N.C.T. Pegaptanib for neovascular age-related macular degeneration. *The New England journal of medicine*. 2004; 351:2805–2816. [PubMed: 15625332]
- Grisanti S, Biester S, Peters S, Tatar O, Ziemssen F, Bartz-Schmidt KU. Tuebingen Bevacizumab Study, G. Intracameral bevacizumab for iris rubeosis. *American journal of ophthalmology*. 2006; 142:158–160. [PubMed: 16815268]

- Hammer M, Vilser W, Riemer T, Mandecka A, Schweitzer D, Kuhn U, Dawczynski J, Liemt F, Strobel J. Diabetic patients with retinopathy show increased retinal venous oxygen saturation. *Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2009; 247:1025–1030.
- Hardarson SH. Retinal oximetry. *Acta ophthalmologica* 91 Thesis. 2013; 2:1–47.
- Hofman P, van Blijswijk BC, Gaillard PJ, Vrensen GF, Schlingemann RO. Endothelial cell hypertrophy induced by vascular endothelial growth factor in the retina: new insights into the pathogenesis of capillary nonperfusion. *Archives of ophthalmology*. 2001; 119:861–866. [PubMed: 11405837]
- Hosseini H, Lotfi M, Esfahani MH, Nassiri N, Khalili MR, Razeghinejad MR, Nouri-Mahdavi K. Effect of intravitreal bevacizumab on retrobulbar blood flow in injected and uninjected fellow eyes of patients with neovascular age-related macular degeneration. *Retina*. 2012; 32:967–971. [PubMed: 22146127]
- Jin K, Mao XO, Batteur SP, McEachron E, Leahy A, Greenberg DA. Caspase-3 and the regulation of hypoxic neuronal death by vascular endothelial growth factor. *Neuroscience*. 2001; 108:351–358. [PubMed: 11734367]
- Kashani AH, Lopez Jaime GR, Saati S, Martin G, Varma R, Humayun MS. Noninvasive assessment of retinal vascular oxygen content among normal and diabetic human subjects: a study using hyperspectral computed tomographic imaging spectroscopy. *Retina*. 2014; 34:1854–1860. [PubMed: 24732694]
- Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell metabolism*. 2006; 3:177–185. [PubMed: 16517405]
- Klefter ON, Lauritsen AO, Larsen M. Retinal hemodynamic oxygen reactivity assessed by perfusion velocity, blood oximetry and vessel diameter measurements. *Acta ophthalmologica*. 2014
- Kusari J, Zhou SX, Padillo E, Clarke KG, Gil DW. Inhibition of vitreoretinal VEGF elevation and blood-retinal barrier breakdown in streptozotocin-induced diabetic rats by brimonidine. *Investigative ophthalmology & visual science*. 2010; 51:1044–1051. [PubMed: 19710406]
- Lakowicz JR, Szmacinski H, Nowaczyk K, Berndt KW, Johnson M. Fluorescence lifetime imaging. *Analytical biochemistry*. 1992; 202:316–330. [PubMed: 1519759]
- Lau JC, Linsenmeier RA. Increased intraretinal PO₂ in short-term diabetic rats. *Diabetes*. 2014; 63:4338–4342. [PubMed: 25028524]
- Lee SS, Ghosn C, Yu Z, Zacharias LC, Kao H, Lanni C, Abdelfattah N, Kuppermann B, Csaky KG, D'Argenio DZ, Burke JA, Hughes PM, Robinson MR. Vitreous VEGF clearance is increased after vitrectomy. *Investigative ophthalmology & visual science*. 2010; 51:2135–2138. [PubMed: 20019364]
- Levin, LA.; Adler, FH. *Adler's physiology of the eye*. 11th ed.. Edingburg: Saunders/Elsevier; 2011.
- Linsenmeier RA. Effects of light and darkness on oxygen distribution and consumption in the cat retina. *The Journal of general physiology*. 1986; 88:521–542. [PubMed: 3783124]
- Linsenmeier RA, Braun RD. Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *The Journal of general physiology*. 1992; 99:177–197. [PubMed: 1613482]
- Malecaze F, Clamens S, Simorre-Pinatel V, Mathis A, Chollet P, Favard C, Bayard F, Plouet J. Detection of vascular endothelial growth factor messenger RNA and vascular endothelial growth factor-like activity in proliferative diabetic retinopathy. *Archives of ophthalmology*. 1994; 112:1476–1482. [PubMed: 7980139]
- Malik RA, Li C, Aziz W, Olson JA, Vohra A, McHardy KC, Forrester JV, Boulton AJ, Wilson PB, Liu D, McLeod D, Kumar S. Elevated plasma CD105 and vitreous VEGF levels in diabetic retinopathy. *Journal of cellular and molecular medicine*. 2005; 9:692–697. [PubMed: 16202216]
- Man RE, Lamoureux EL, Taouk Y, Xie J, Sasongko MB, Best WJ, Noonan JE, Kawasaki R, Wang JJ, Luu CD. Axial length, retinal function, and oxygen consumption: a potential mechanism for a lower risk of diabetic retinopathy in longer eyes. *Investigative ophthalmology & visual science*. 2013; 54:7691–7698. [PubMed: 24168995]

- Martinez-Zapata MJ, Marti-Carvajal AJ, Sola I, Pijoan JI, Buil-Calvo JA, Cordero JA, Evans JR. Anti-vascular endothelial growth factor for proliferative diabetic retinopathy. The Cochrane database of systematic reviews. 2014; 11:CD008721. [PubMed: 25418485]
- Mete A, Saygili O, Mete A, Bayram M, Bekir N. Effects of intravitreal bevacizumab (Avastin) therapy on retrobulbar blood flow parameters in patients with neovascular age-related macular degeneration. *Journal of clinical ultrasound: JCU*. 2010; 38:66–70. [PubMed: 19953626]
- Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B, et al. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. *The American journal of pathology*. 1994; 145:574–584. [PubMed: 7521577]
- Miyamoto K, Khosrof S, Bursell SE, Moromizato Y, Aiello LP, Ogura Y, Adamis AP. Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). *The American journal of pathology*. 2000; 156:1733–1739. [PubMed: 10793084]
- Nathan AT, Singer M. The oxygen trail: tissue oxygenation. *British medical bulletin*. 1999; 55:96–108. [PubMed: 10695081]
- Nguyen QD, Shah SM, Khwaja AA, Channa R, Hatef E, Do DV, Boyer D, Heier JS, Abraham P, Thach AB, Lit ES, Foster BS, Kruger E, Dugel P, Chang T, Das A, Ciulla TA, Pollack JS, Lim JJ, Elliott D, Campochiaro PA, Group R-S. Two-year outcomes of the ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology*. 2010; 117:2146–2151. [PubMed: 20855114]
- Nishijima K, Ng YS, Zhong L, Bradley J, Schubert W, Jo N, Akita J, Samuelsson SJ, Robinson GS, Adamis AP, Shima DT. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. *The American journal of pathology*. 2007; 171:53–67. [PubMed: 17591953]
- Nitta F, Kunikata H, Aizawa N, Omodaka K, Shiga Y, Yasuda M, Nakazawa T. The effect of intravitreal bevacizumab on ocular blood flow in diabetic retinopathy and branch retinal vein occlusion as measured by laser speckle flowgraphy. *Clinical ophthalmology*. 2014; 8:1119–1127. [PubMed: 24959068]
- Ogata N, Nishikawa M, Nishimura T, Mitsuma Y, Matsumura M. Unbalanced vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor in diabetic retinopathy. *American journal of ophthalmology*. 2002; 134:348–353. [PubMed: 12208245]
- Ozaki H, Hayashi H, Viores SA, Moromizato Y, Campochiaro PA, Oshima K. Intravitreal sustained release of VEGF causes retinal neovascularization in rabbits and breakdown of the blood-retinal barrier in rabbits and primates. *Experimental eye research*. 1997; 64:505–517. [PubMed: 9227268]
- Ozaki H, Yu AY, Della N, Ozaki K, Luna JD, Yamada H, Hackett SF, Okamoto N, Zack DJ, Semenza GL, Campochiaro PA. Hypoxia inducible factor-1 α is increased in ischemic retina: temporal and spatial correlation with VEGF expression. *Investigative ophthalmology & visual science*. 1999; 40:182–189. [PubMed: 9888442]
- Palkovits S, Lasta M, Told R, Schmidl D, Boltz A, Napora KJ, Werkmeister RM, Popa-Cherecheanu A, Garhofer G, Schmetterer L. Retinal oxygen metabolism during normoxia and hyperoxia in healthy subjects. *Investigative ophthalmology & visual science*. 2014a; 55:4707–4713. [PubMed: 25015353]
- Palkovits S, Told R, Schmidl D, Boltz A, Napora KJ, Lasta M, Kaya S, Werkmeister RM, Popa-Cherecheanu A, Garhofer G, Schmetterer L. Regulation of retinal oxygen metabolism in humans during graded hypoxia. *American journal of physiology. Heart and circulatory physiology*. 2014b; 307:H1412–H1418. [PubMed: 25217648]
- Papadopoulou DN, Mendrinou E, Mangioris G, Donati G, Pournaras CJ. Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. *Ophthalmology*. 2009; 116:1755–1761. [PubMed: 19560206]
- Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochemical and biophysical research communications*. 2003; 304:505–512. [PubMed: 12729585]

- Pe'er J, Shweiki D, Itin A, Hemo I, Gnessin H, Keshet E. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. Laboratory investigation; a journal of technical methods and pathology. 1995; 72:638–645.
- Pittman, RN. Regulation of Tissue Oxygenation. San Rafael (CA): 2011.
- Pournaras CJ. Retinal oxygen distribution. Its role in the physiopathology of vasoproliferative microangiopathies. Retina. 1995; 15:332–347. [PubMed: 8545580]
- Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY, Group MS. Ranibizumab for neovascular age-related macular degeneration. The New England journal of medicine. 2006; 355:1419–1431. [PubMed: 17021318]
- Saint-Geniez M, Maharaj AS, Walshe TE, Tucker BA, Sekiyama E, Kurihara T, Darland DC, Young MJ, D'Amore PA. Endogenous VEGF is required for visual function: evidence for a survival role on muller cells and photoreceptors. PloS one. 2008; 3:e3554. [PubMed: 18978936]
- Shahidi AM, Patel SR, Flanagan JG, Hudson C. Regional variation in human retinal vessel oxygen saturation. Experimental eye research. 2013; 113:143–147. [PubMed: 23791637]
- Shahidi M, Shakoor A, Blair NP, Mori M, Shonat RD. A method for chorioretinal oxygen tension measurement. Current eye research. 2006; 31:357–366. [PubMed: 16603469]
- Shahidi M, Wanek J, Blair NP, Mori M. Three-dimensional mapping of chorioretinal vascular oxygen tension in the rat. Investigative ophthalmology & visual science. 2009; 50:820–825. [PubMed: 18824736]
- Shonat RD, Kight AC. Oxygen tension imaging in the mouse retina. Annals of biomedical engineering. 2003; 31:1084–1096. [PubMed: 14582611]
- Simo R, Lecube A, Segura RM, Garcia Arumi J, Hernandez C. Free insulin growth factor-I and vascular endothelial growth factor in the vitreous fluid of patients with proliferative diabetic retinopathy. American journal of ophthalmology. 2002; 134:376–382. [PubMed: 12208249]
- Toklu Y, Cakmak HB, Raza S, Anayol A, Asik E, Simsek S. Short-term effects of intravitreal bevacizumab (Avastin((R))) on retrobulbar hemodynamics in patients with neovascular age-related macular degeneration. Acta ophthalmologica. 2011; 89:e41–e45. [PubMed: 21232081]
- Tolentino MJ, McLeod DS, Taomoto M, Otsuji T, Adamis AP, Luty GA. Pathologic features of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. American journal of ophthalmology. 2002; 133:373–385. [PubMed: 11860975]
- Tolentino MJ, Miller JW, Gragoudas ES, Jakobiec FA, Flynn E, Chatzistefanou K, Ferrara N, Adamis AP. Intravitreal injections of vascular endothelial growth factor produce retinal ischemia and microangiopathy in an adult primate. Ophthalmology. 1996; 103:1820–1828. [PubMed: 8942877]
- Trick GL, Berkowitz BA. Retinal oxygenation response and retinopathy. Progress in retinal and eye research. 2005; 24:259–274. [PubMed: 15610976]
- Wanek J, Blair NP, Shahidi M. Outer retinal oxygen consumption of rat by phosphorescence lifetime imaging. Current eye research. 2012; 37:132–137. [PubMed: 22070458]
- Wanek J, Teng PY, Albers J, Blair NP, Shahidi M. Inner retinal metabolic rate of oxygen by oxygen tension and blood flow imaging in rat. Biomedical optics express. 2011; 2:2562–2568. [PubMed: 21991548]
- Wanek J, Teng PY, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism under normoxia and hypoxia in rat. Investigative ophthalmology & visual science. 2013; 54:5012–5019. [PubMed: 23821203]
- Wanek J, Teng PY, Blair NP, Shahidi M. Inner retinal oxygen delivery and metabolism in streptozotocin diabetic rats. Investigative ophthalmology & visual science. 2014; 55:1588–1593. [PubMed: 24550355]
- Watanabe D, Suzuma K, Suzuma I, Ohashi H, Ojima T, Kurimoto M, Murakami T, Kimura T, Takagi H. Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. American journal of ophthalmology. 2005; 139:476–481. [PubMed: 15767056]
- West, JB. Pulmonary physiology and pathophysiology: an integrated, case-based approach. 2 ed.. Philadelphia: Lippincott Williams & Wilkins; 2007.
- Wick A, Wick W, Waltenberger J, Weller M, Dichgans J, Schulz JB. Neuroprotection by hypoxic preconditioning requires sequential activation of vascular endothelial growth factor receptor and

- Akt. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2002; 22:6401–6407. [PubMed: 12151519]
- Wright WS, McElhatten RM, Harris NR. Increase in retinal hypoxia-inducible factor-2alpha, but not hypoxia, early in the progression of diabetes in the rat. Experimental eye research. 2011; 93:437–441. [PubMed: 21689648]
- Yi J, Wei Q, Liu W, Backman V, Zhang HF. Visible-light optical coherence tomography for retinal oximetry. Optics letters. 2013; 38:1796–1798. [PubMed: 23722747]
- Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. Progress in retinal and eye research. 2001; 20:175–208. [PubMed: 11173251]
- Zhang HF, Puliafito CA, Jiao S. Photoacoustic ophthalmoscopy for in vivo retinal imaging: current status and prospects. Ophthalmic surgery, lasers & imaging: the official journal of the International Society for Imaging in the Eye. 2011; 42(Suppl):S106–S115.

Highlights

- Vascular endothelial growth factor (VEGF) was injected intravitreally in rats.
- VEGF induced vasodilation in retinal arteries and veins.
- Blood velocity and vascular oxygen content were not altered by VEGF.
- VEGF increased retinal blood flow and oxygen delivery.
- Retinal oxygen metabolism was not altered by VEGF.

Figure 1. Red-free images of the two peripapillary areas of a rat, one without (left) and one with (right) intravitreally injected vascular endothelial growth factor (VEGF). Note the increase in vessel diameters, especially in the veins, induced by VEGF.

Figure 2. Inner Retinal Oxygen Delivery (DO₂) and Oxygen Metabolism (MO₂) in Rat Eyes with Intravitreally Injected Vascular Endothelial Growth Factor (VEGF) and in their Control Fellow Eyes. Lines join values from the two eyes of each animal.

Table 1

Retinal Vascular Factors in Rat Eyes with Intravitreally Injected Vascular Endothelial Growth Factor and in their Control Fellow Eyes

	Control	VEGF	P Value
D _A (μm)	42 ± 6	44 ± 5	0.03
D _V (μm)	50 ± 6	61 ± 12	0.008
V (mm/s)	11.7 ± 2.4	10.9 ± 2.6	0.21
F (μl/min)	7.3 ± 1.6	10.6 ± 3.9	0.007

VEGF = Vascular Endothelial Growth Factor, D_A = Retinal Arterial Diameter, D_V = Retinal Venous Diameter, V = Venous Blood Velocity, F = Total Inner Retinal Blood Flow

Table 2

Retinal Vascular Oxygen Factors in Rat Eyes with Intravitreally Injected Vascular Endothelial Growth Factor and in their Control Fellow Eyes

	Control	VEGF	P Value
PO _{2A} (mm Hg)	46 ± 12	45 ± 9	0.41
PO _{2V} (mm Hg)	28 ± 6	31 ± 6	0.11
O _{2A} (mL O ₂ /dl)	12.9 ± 2.8	12.8 ± 2.4	0.92
O _{2V} (mL O ₂ /dl)	6.9 ± 2.5	8.1 ± 2.3	0.12
O _{2A-V} (mL O ₂ /dl)	6.0 ± 1.7	4.8 ± 1.1	0.05

VEGF = Vascular Endothelial Growth Factor, PO_{2A} = Retinal Arterial PO₂, PO_{2V} = Retinal Venous PO₂, O_{2A} = Retinal Arterial Oxygen Content, O_{2V} = Retinal Venous Oxygen Content, O_{2A-V} = Retinal Arteriovenous Content Difference

Table 3

Inner Retinal Oxygen Delivery and Metabolism in Rat Eyes with Intravitreally Injected Vascular Endothelial Growth Factor and in their Control Fellow Eyes

	Control	VEGF	P Value
DO ₂ (nL O ₂ /min)	950 ± 340	1380 ± 650	0.005
MO ₂ (nL O ₂ /min)	440 ± 150	490 ± 190	0.31

VEGF = Vascular Endothelial Growth Factor, DO₂ = inner retinal oxygen delivery, MO₂ = inner retinal oxygen metabolism