

# A Model for Graded Retinal Ischemia in Rats

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**Purpose:** Retinal ischemic injury depends on grade and duration of an ischemic insult. We developed a method to induce ischemic injury in rats permitting: (1) Variable grades of retinal blood flow (F) reduction, (2) controllable duration of F reduction, (3) injury without collateral neural damage, and (4) optical measurements of F and O<sub>2</sub>-related factors: O<sub>2</sub> delivery (DO<sub>2</sub>), O<sub>2</sub> extraction fraction (OEF), and metabolic rate of O<sub>2</sub> (MO<sub>2</sub>).

**Methods:** In five anesthetized rats the left common carotid artery (CA) was ligated and the right CA was exposed. A variable clamp having a backstop and a rod mounted on a micromanipulator straddled the right CA. Advancing the rod with the micromanipulator produced graded compressions of the CA. F and O<sub>2</sub>-related factors were measured with established optical techniques.

**Results:** Four to seven grades of F for at least 10 minutes were achieved per rat. F decreased only with compressions of over 60%. DO<sub>2</sub> changed in proportion to F, particularly at low F. As F decreased, OEF initially changed little, but then rose steeply to its maximum of 1 when F was approximately 4 μL/min. MO<sub>2</sub> was stable with reduced F until OEF maximized, after which it decreased progressively.

**Conclusions:** This model in rats permits acute, graded inner retinal ischemia that is reversible after prescribed durations, does not otherwise injure the eye and allows optical measurement of important physiologic factors during ischemia.

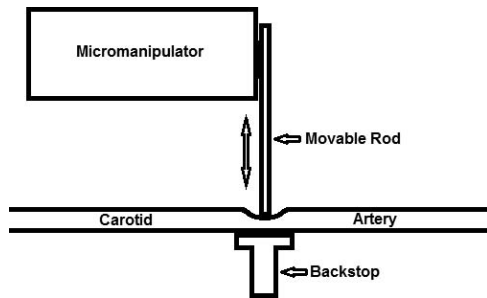
**Translational Relevance:** This model will allow improved understanding of retinal ischemic injury and enable better management of this common, sight-threatening affliction.

## Introduction

Retinal ischemia is a major cause of visual loss in several common eye diseases, including diabetic retinopathy and retinal vessel occlusions. Much information about retinal ischemic injury has been derived from animal models. Two main methods have been used to induce retinal ischemia in animals. First, ischemia can be induced by compressing the wall of a vessel. Ligation has been used to compress and close the common carotid artery (CA),<sup>1</sup> pterygopalatine artery,<sup>2</sup> ophthalmic vessels,<sup>3</sup> and central retinal artery.<sup>4</sup> Additionally, retinal vessel walls can be compressed by an intraocular rod.<sup>5</sup> Second, the vessel lumen can be obstructed by emboli,<sup>6</sup> thrombosis,<sup>7</sup> an intraluminal suture,<sup>8</sup> diathermy,<sup>9</sup> or photocoagulation.<sup>10</sup> Elevation of the intraocular pressure (IOP)

also has been used in many studies to interrupt the ocular blood flow,<sup>11</sup> but there now is good evidence that other factors, including mechanical effects, contribute substantially to the resulting injury; thus, limiting its use to characterize the effects of ischemia.<sup>12–14</sup>

Despite the multiplicity of methods, two major knowledge gaps remain. The first gap in knowledge is that the ischemic retinal insult depends on the level of reduced blood flow and the duration of that reduction. Nevertheless, only IOP elevation, with the limitations noted above,<sup>13,14</sup> has been used to induce levels of reduced inner retinal blood flow (F). In fact, most of the aforementioned models induce total cessation of F, often permanently. When attempts have been made to vary the ischemic insult, the approach usually has been to vary the duration of



**Figure 1.** Schematic diagram of apparatus for graded compression of the CA in rats. The micromanipulator allows for precise positioning of the movable rod in the directions indicated by the *double-headed arrow*. This permits controllable compression of the CA between the movable rod and backstop.

total F cessation. The assumption underlying these attempts has been that the ischemic insults induced by these durations would be equivalent to the insults induced by varying the grade of F reduction. Nonetheless, this assumption has not been verified, and the relative ischemic effects of these two approaches may well differ. For example, abrupt, total ischemia may preclude certain metabolic responses to ischemia that may have time to develop during partial (graded) ischemia. In any case, reduction as opposed to cessation of F appears to be more similar to the clinical situation, since some retinal blood flow tends to be retained in patients with vascular occlusions.

The second gap in knowledge is that the roles of several key factors in the pathophysiology of ischemic retinal injury have not been determined given the lack of models for graded retinal ischemia in which they could be measured. These factors include F, blood  $O_2$  concentration,  $O_2$  delivery ( $DO_2$ , the product of F and arterial  $O_2$  concentration,  $O_{2A}$ ), the inner retinal  $O_2$  extraction fraction (OEF), and the inner retinal rate of  $O_2$  metabolism ( $MO_2$ ). These  $O_2$ -related factors (i.e.,  $DO_2$ , OEF, and  $MO_2$ ) are particularly crucial because  $O_2$  is the substance with the largest net flux between the blood and retinal cells.<sup>15</sup> Accordingly, the first deficiency to occur during an ischemic insult is inadequate  $DO_2$ . Clearly, the impact of combinations of levels and durations of F and  $O_2$ -related factors on retinal ischemic injury cannot be determined in models in which there is total cessation of F.

We addressed these two gaps in knowledge by devising a method for graded retinal ischemia in rats. This model is based on previous work revealing that ligation of one CA led to minimal retinal changes, whereas ligation of both CAs caused more severe

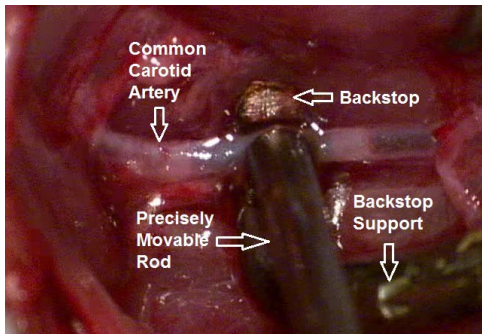
retinal changes.<sup>1,16</sup> We tested the hypothesis that by ligating one CA and variably compressing the other CA we could induce controlled grades of acute retinal ischemia and measure changes in the  $O_2$ -related factors  $DO_2$ , OEF, and  $MO_2$ .

## Methods

### Animals

The study was performed on five male Long Evans rats, which were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the Animal Care Committee at the University of Illinois at Chicago. Mean age  $\pm$  standard deviation (SD) was  $78 \pm 5$  days (range, 71–84). The rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg) with additional injections given to maintain anesthesia as necessary. The right pupil was dilated with 2.5% phenylephrine and 1% tropicamide. The femoral artery was cannulated and a catheter was attached. An incision then was made parallel to and to the left of the midline in the neck and, using blunt dissection, the salivary glands, lymph nodes, and ventral neck muscles were retracted to expose the left CA. The branches of the sympathetic nerve close to the CA were carefully dissected from the artery so that the CA was ligated without involving the nerve. A similar incision was made to the right of the midline, and the right CA was isolated.

The rats were placed on an animal holder that had copper tubing perfused with heated water to maintain body temperature. A custom-built clamp, consisting of a rod mounted on a micromanipulator and a backstop (Fig. 1), was positioned to straddle the right CA (Fig. 2). The positions of the rod and backstop were adjusted so that both were in contact with the CA without compressing it. The backstop was fixed in location using a flexible holder that could be locked accurately in position (Flexbar Machine Corporation, Islandia, NY). Graded compressions of the CA were achieved by rotating the knob of the micromanipulator to advance the rod towards the backstop with micrometer resolution. A glass cover slip with 1% hydroxypropyl methylcellulose was applied to the right cornea to minimize its refractive power and prevent dehydration. Retinal vascular  $O_2$  tension ( $PO_2$ ) and blood flow imaging were performed in the right eye of each rat, starting 10 minutes after CA compressions that varied from 0% (full flow) to 100%



**Figure 2.** Backstop and precisely movable rod on the exposed CA in a rat.

(minimal/no flow). The time needed for making measurements was approximately 10 minutes. For  $PO_2$  imaging, an oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT), was administered through the femoral arterial catheter (20 mg/kg). For retinal blood flow imaging, 2- $\mu$ m polystyrene fluorescent microspheres (Invitrogen, Grand Island, NY) were injected through the catheter.

## Imaging

The methods to measure  $F$  and  $O_2$ -related factors were similar to those reported previously.<sup>17,18</sup> Retinal vascular  $PO_2$  was measured using our optical section phosphorescence lifetime imaging system.<sup>17,19</sup> Briefly, a vertical laser line (532 nm) was projected onto the retina, and phosphorescence lifetimes in the retinal vessels were determined by imaging analysis using a frequency-domain approach<sup>19,20</sup> and converted to  $PO_2$  measurements using the Stern-Volmer equation. Mean arterial ( $PO_{2A}$ ) and venous ( $PO_{2V}$ ) values were calculated from the individual artery and vein measurements in each animal, respectively.

Our previously described blood flow imaging system<sup>17</sup> was used to measure retinal venous blood velocity and retinal arterial and venous vessel diameters. Briefly, a diode laser beam (488 nm) was projected on the retina after intravenous injection of fluorescent microspheres. Image sequences of the intravascular motion of the microspheres were acquired, and blood velocity in all individual veins ( $V$ ) was measured by manually tracking displacements of the microspheres over time.<sup>17</sup> Correspondingly, diameters of all individual major retinal veins ( $D_V$ ) were measured using dedicated software applied to red-free retinal images.<sup>17</sup> Blood flow ( $F$ ) in each vein was calculated according to  $F = (V \cdot \pi \cdot D_V^2) / 4$

and summed among all veins to determine total venous blood flow.

## Global Inner Retinal Oxygen Delivery, Extraction Fraction, and Metabolism

The  $O_2$  of blood in the retinal arteries ( $O_{2A}$ ) and veins ( $O_{2V}$ ) was calculated as the sum of oxygen bound to hemoglobin and dissolved in blood:  $O_2 = SO_2 \cdot C \cdot HGB + PO_2 \cdot k$ , where  $SO_2$  is the oxygen saturation (%),  $C$  is the oxygen-carrying capacity of hemoglobin (1.39 mL  $O_2$ /g),<sup>21</sup>  $HGB$  is the hemoglobin concentration (13.8 g/dL), and  $k$  is the oxygen solubility in blood (0.003 mL  $O_2$ /dL·mm Hg).<sup>22</sup>  $SO_2$  was calculated using the measured  $PO_2$  and the hemoglobin oxygen dissociation curve in rats.<sup>18,23,24</sup> The arteriovenous oxygen content difference was calculated as:  $O_{2A-V} = O_{2A} - O_{2V}$ .

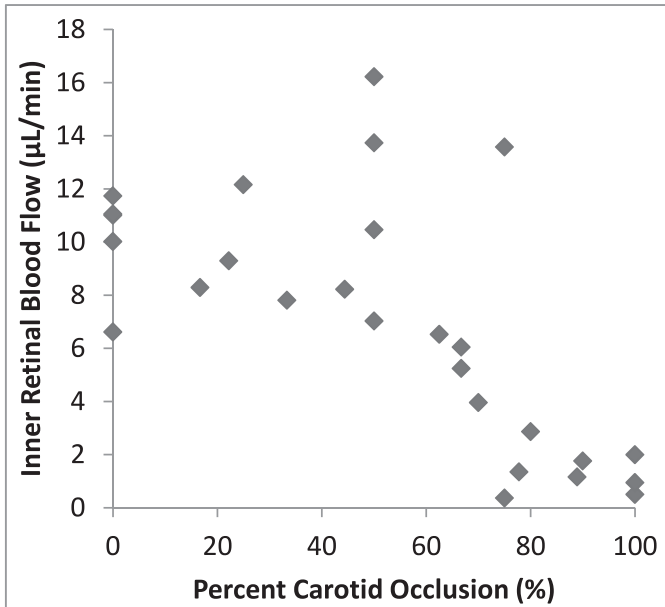
$DO_2$  was defined as the rate that oxygen enters the retinal circulation, and it was calculated as the product of  $F$  and  $O_{2A}$ .  $MO_2$  was defined as the rate that oxygen is metabolized by the inner retinal tissue, and under steady state it equals the rate  $O_2$  is extracted from the retinal circulation. Thus,  $MO_2$  was calculated as the product of  $F$  and  $O_{2A-V}$ . In the steady state, OEF equals the ratio of  $MO_2$  to  $DO_2$  and was calculated as  $O_{2A-V} / O_{2A}$ .<sup>25</sup> By definition, OEF varies between 0 and 1.

The primary outcome of applying these methods was the induction of varying grades of retinal ischemia. The secondary outcome was measurement of the  $O_2$ -related factors  $DO_2$ , OEF, and  $MO_2$  at the induced grades of ischemia.

## Results

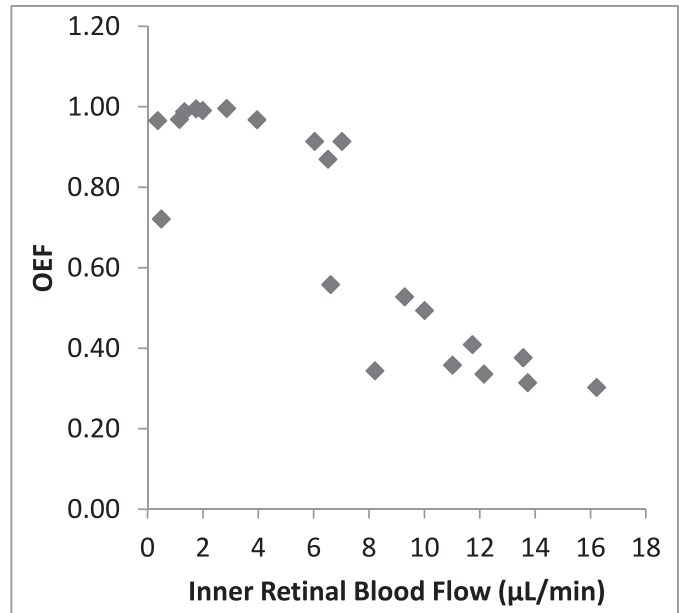
Using the rod attached to the micromanipulator, we were able to make precise compressions of the CA. Between 4 and 7 grades of compression per rat were induced for a total of 27 compressions. Progressive compression was related to progressive reduction of  $F$  as displayed in Figure 3. However, the relation was nonlinear in that there were minimal or no changes in  $F$  at CA compressions less than approximately 60%, whereas  $F$  tended to decrease progressively as the CA compression increased above 60%.

Since the animal model allowed measurement of  $F$  and  $PO_2$  during the periods of graded ischemia, we were able to measure the  $O_2$ -related factors over a range of  $F$  values.  $DO_2$  declined progressively with reduction of  $F$  (Fig. 4). A linear relationship was expected since the variation of  $O_{2A}$  was small and  $DO_2$



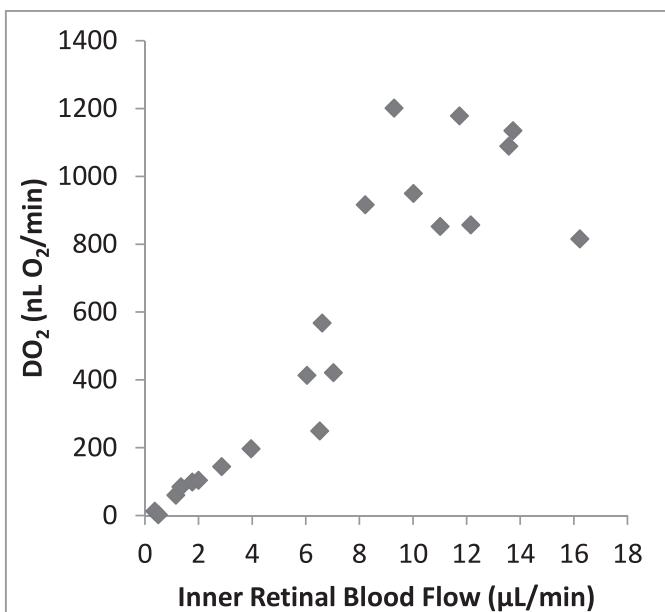
**Figure 3.** F by percent CA occlusion. Note that no clear changes in F are apparent at occlusions of less than approximately 60%, whereas F tended to decrease progressively as the occlusion increased above 60%.

is the product of F and  $O_{2A}$ . With reductions of F, OEF initially remained relatively constant and then increased progressively to a maximum approximating 1.0 when F was approximately 4  $\mu\text{L}/\text{min}$  (Fig. 5). Since all  $O_2$  in the blood is removed when OEF equals 1.0, it

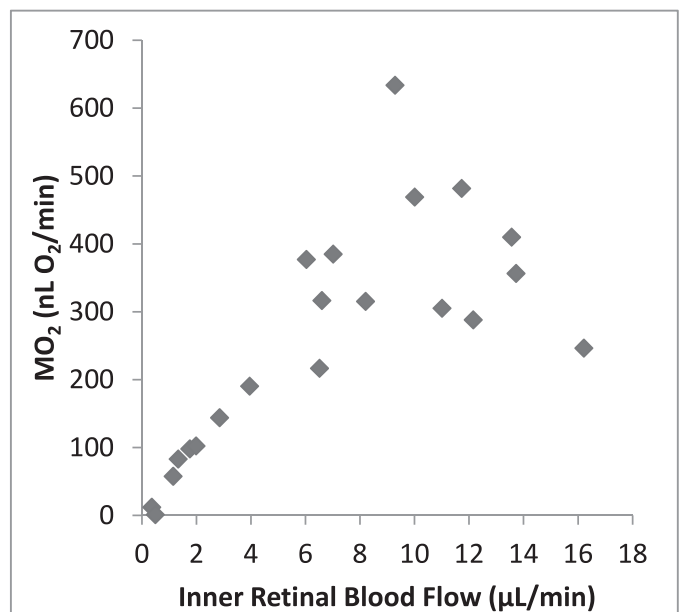


**Figure 5.** Retinal OEF by F. With reductions of F, OEF initially changed little and then increased progressively to a maximum approximating 1.0 when F was approximately 4  $\mu\text{L}/\text{min}$ . OEF remained essentially constant with further reduction of F.

did not change with further reduction of F. Figure 6 indicates that  $MO_2$  declined minimally as F decreased to approximately 5  $\mu\text{L}/\text{min}$ , but it declined steeply as the reduction of F progressed. We noted that  $MO_2$  began to decline as OEF approached the maximum.



**Figure 4.** Retinal  $DO_2$  by F.  $DO_2$  declined progressively with reduction of F in a way that was consistent with a linear relationship.



**Figure 6.** Retinal  $MO_2$  by IF.  $MO_2$  declined minimally as F decreased to approximately 5  $\mu\text{L}/\text{min}$ , but it declined steeply as the reduction of F progressed.

## Discussion

We presented a rat model that provides graded, temporally controllable acute, retinal ischemic insults. This could allow correlation of these two key prognostic factors, namely grade and duration, with the resulting ischemic retinal injury. While the many models of retinal ischemia<sup>1–11</sup> have various advantages and have permitted discovery of many features of retinal ischemic injury, only elevated IOP can produce graded ischemic insults. He et al.<sup>13,14</sup> produced multiple grades of retinal ischemia by progressively increasing IOP in rats. They calculated OEF (called OER) from ocular laser Doppler flow and preretinal PO<sub>2</sub> measurements taken between major retinal vessels. However, their F, PO<sub>2</sub>, and OEF measurements included contributions from the choroidal circulation, whereas the values in our study were derived only from the retinal circulation. Furthermore, they showed that a substantial component of the injurious effects of elevated IOP was not related to ischemia. In contrast, our method does not add injurious effects to those of ischemia, manipulate or distort the eye, or cause corneal clouding, which precludes clear imaging of the ocular posterior segment and measurement of O<sub>2</sub>-related factors.

Our model permitted preliminary noninvasive measurements of F, DO<sub>2</sub>, OEF, and MO<sub>2</sub> during graded ischemia. To our knowledge, there have been no previous reports in which a comparable combination of these inner retinal O<sub>2</sub>-related factors were measured in experimental graded ischemia. Of note, we showed for the first time to our knowledge in retinal ischemia that OEF reaches 1 (indicating that all vascular oxygen is being extracted), even while there still is substantial F. The relationship of F to the percent CA occlusion appeared to be nonlinear in which changes in F occurred mainly with compressions greater than 60%. This was the result of retinal vascular compensatory vasodilation, which eventually became maximized, and, probably, a nonlinear relationship between CA compression and the resulting cross-sectional area of the vessel. We interpreted the findings of DO<sub>2</sub>, OEF, and MO<sub>2</sub> in the following manner. At mildly reduced levels of F and DO<sub>2</sub>, MO<sub>2</sub> is maintained by extracting a higher percentage of the O<sub>2</sub> in the blood, that is, by increasing OEF. With further reductions in the F and DO<sub>2</sub> levels, OEF eventually reaches its maximum value of 1 when all O<sub>2</sub> in the blood is extracted. At more severely reduced levels of F and DO<sub>2</sub>, MO<sub>2</sub> decreases proportionately

and, when maintained for long enough durations, energy failure and the complex metabolic sequences of ischemic retinal injury must supervene, which terminate in cell death and loss of visual function.<sup>26</sup>

At 100% occlusion of the CA there may have been a small amount of F, at least in some rats. This is consistent with findings of other studies with long-term total bilateral CA occlusion that showed various electroretinographic, biochemical, and histologic changes.<sup>27–32</sup> In fact, with total CA occlusion, the circle of Willis may provide some blood flow to the ophthalmic artery either directly into the orbit<sup>33</sup> or by way of retrograde flow through the internal carotid artery and then antegrade flow into the pterygopalatine artery (this artery is the normal blood supply to the eye in rats). Nonetheless, in the present short-term study, MO<sub>2</sub> was essentially reduced to zero with maximal compression of one CA and ligation of the other CA.

Our study had limitations. First, this method reduced flow in the retinal and choroidal circulations, which differs from clinical retinal vascular occlusions, which do not involve the choroid. Thus, findings here of DO<sub>2</sub>, OEF, and MO<sub>2</sub>, with F may differ from those that occur during retinal vascular occlusions only. However, it does mimic carotid occlusive disease and ophthalmic artery occlusion. Second, we made measurements during progressive grades of occlusion. Accordingly, some effects of the previous levels of occlusion probably were continuing at each grade of compression, as depicted in the presented data variability. However, we were particularly interested in the general pattern of how DO<sub>2</sub>, OEF, and MO<sub>2</sub> related to F over a wide range. Future studies designed to collect data in groups of animals at each F reduction level will introduce less variability and better delineation of the relationships. Third, the CA blood flow was not measured. Thus, there may have been some variability among animals in the actual amount of compression. This also would contribute to measurement variability. Fourth, we did not measure the arterial pH, which can be reduced in ischemia. Lowering the pH shifts the oxygen hemoglobin dissociation curve to the right and causes SO<sub>2</sub> to become lower for a given PO<sub>2</sub> value. While our SO<sub>2</sub> and O<sub>2</sub> content values may be somewhat high, it is unlikely that this would have a major impact on the overall relationships among the O<sub>2</sub> biomarkers. Fifth, the sample size was small. Nonetheless, it was adequate to demonstrate the feasibility of generating the model and obtaining data.

In conclusion, we described a new model for acute

retinal ischemia in rats that can be delivered with controlled grade and duration without otherwise injuring the eye. We also showed that the methods we described previously to measure O<sub>2</sub>-related factors can be applied, and we presented preliminary combined measurements in this model of retinal ischemia.

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