

Measuring the Effect of Pars Plana Vitrectomy on Vitreous Oxygenation Using Magnetic Resonance Imaging

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PURPOSE. To study the effect of pars plana vitrectomy (PPV) on vitreous oxygenation (pO_2) using magnetic resonance imaging (MRI).

METHODS. Patients due to undergo PPV for either macular hole or epiretinal membrane were recruited. MRI scanning was performed 1 week before and at least 3 months after PPV. MRI T_1 mapping was performed using an inversion recovery-true fast imaging with steady-state precession (TrueFISP) sequence at several inversion times, from a single slice positioned through the center of both eyes in the axial oblique plane. Additional phantom data were measured in porcine vitreous, to define the relationships between T_1 relaxation times and balanced salt solution (BSS), to simulate human vitreous and aqueous, respectively, for a suitable pO_2 range (5–70 mm Hg). Pre-PPV pO_2 was also measured intraoperatively using a polarographic oxygen probe.

RESULTS. Eleven participants (age range 59–84) were recruited; two declined the post-PPV scan. Corrected T_1 times indicated that the mean (\pm SD) pO_2 increased significantly following PPV, from 13.2 ± 5.8 to 34.5 ± 8.0 mm Hg ($P < 0.001$). In the nonsurgical (control) eye, pO_2 did not change significantly from the first to second MRI scan (13.7 ± 7.8 vs. 16.3 ± 8.7 mm Hg, $P = 0.239$). Mean pO_2 measured intraoperatively was 7.2 ± 0.6 mm Hg ($n = 10$).

CONCLUSIONS. These results confirm that vitrectomy substantially increases vitreous pO_2 . MRI is a noninvasive technique that can be used to study vitreous oxygenation in both vitrectomized and nonvitrectomized eyes. (*Invest Ophthalmol Vis Sci*. 2013;54:2028–2034) DOI:10.1167/iops.12-11258

The role of the vitreous in the pathogenesis of conditions such as epiretinal membrane and macular hole has been established for several decades,¹ however, it is only in more recent years that evidence has emerged showing that the vitreous can influence other eye conditions, especially those characterized by a degree of retinal ischemia. Pars plana vitrectomy (PPV), and to a lesser degree posterior vitreous detachment (PVD), have been reported to be associated with improved visual and anatomical outcome in a number of

conditions, such as diabetic macular edema (DME),² exudative age-related macular degeneration (wet AMD),^{3–8} central retinal vein occlusion (CRVO),^{9–11} and branch retinal vein occlusion (BRVO).^{12–14}

A number of hypotheses have been proposed to explain why PPV, or PVD, may have a beneficial effect in these conditions, including reduction of vitreomacular traction (VMT), increased diffusion of intravitreal molecules such as pro-inflammatory cytokines and VEGF, and increased vitreous oxygenation.^{15–20} PPV, performed in patients with diabetic retinopathy, can increase iris neovascularization²¹ and conversely decrease retinal neovascularization,²² leading to the hypothesis that intraocular oxygen distribution is altered following surgery. Stefánsson and co-workers found that combined vitrectomy and lensectomy in cat eyes significantly reduced the partial pressure of oxygen (pO_2) in the anterior chamber,^{23,24} and, furthermore, with an induced BRVO, the preretinal pO_2 was higher in eyes that had undergone vitrectomy compared with those that had not.²⁵ Considering all the available evidence it seems plausible that by removing the vitreous, intravitreal pO_2 increases.

Until recently, the measurement of intravitreal pO_2 in humans has been limited to the use of an intraocular probe at the time of surgery. Maeda and Tano measured pO_2 at various locations in the vitreous cavity of vitrectomized and non-vitrectomized diabetic patients, and concluded that following PPV, pO_2 levels equalize throughout the vitreous cavity.²⁶ Hølekamp et al. measured vitreous pO_2 at the time of PPV in diabetic and nondiabetic patients and reported nondiabetic patients had a statistically significant higher oxygen tension in the mid vitreous cavity and adjacent to the lens.²⁷ Similarly, Williamson et al. investigated vitreous pO_2 levels in patients with CRVO, finding them significantly lower than controls. Both groups also reported immediate “post-PPV” pO_2 measurements at the conclusion of surgery,^{28,29} which have to be interpreted with caution as the measurements take place in an eye that has just been infused with fluid exposed to atmospheric conditions, where oxygen tension is much higher.

Owing to the invasive nature of current measurement techniques, measuring the vitreous pO_2 of a patient who had previously undergone a vitrectomy could only be done at the time of a secondary intraocular intervention. In the large series of patients that had intraocular pO_2 measured by Hølekamp et al., 8 out of the 69 eyes had had one or more previous vitrectomies, with a mean time between first and last vitrectomy of 10 months (range 3–20 months).²⁸ They reported a significantly higher mid vitreous pO_2 in the eyes that had undergone previous surgery, compared with a group of eyes that were chosen as controls (13.3 ± 0.8 vs. 8.4 ± 0.7 mm Hg, $P < 0.003$). However, there have been no longitudinal studies on the same group of patients, investigating changes in vitreous pO_2 following PPV over an extended period of time, and not just before and immediately after surgery.

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Retinal oximetry can be used to noninvasively measure retinal vessel oxygen saturation.³⁰ It has successively shown oxygen saturation changes in the retinal vessels of a number of diseases characterized by ischemia,^{31–33} however, it has not been used in the context of PPV. Furthermore, it is not known to what degree vitreous pO₂ is affected by retinal vessel oxygenation. It would seem from the evidence stated above that vitreous pO₂ is reduced in the presence of retinal ischemia in retinal vein occlusion and diabetic retinopathy, although the exact details of the relationship between the two is not known.

The accurate measurement of magnetic resonance imaging (MRI) T₁ relaxation times enables pO₂ calculation, as these times decrease with increasing levels of paramagnetic O₂.^{34–37} Berkowitz et al. provided proof of principle that MRI could provide a noninvasive, indirect measurement of pO₂ in the eye.³⁵ The technique is able to rapidly capture images, and provide precise measurement of T₁, without the image artifacts that would usually result from ocular movements and the blink reflex.³⁸ In pure water at magnetic field strength of 1.5 tesla (T) and 37°C, T₁ is 4.74 seconds, and it decreases by 47 ms, a reduction of 1.14%, for an increase in pO₂ of 10 mm Hg.³⁷ The current accuracy of this technique enables measurement of vitreous pO₂ to within 8 mm Hg.³⁸ The spatial resolution is dependent on image reconstruction that is determined by the scanner during the acquisition stage, and is currently at 0.9 mm².

T₁ relaxation times are influenced by the presence of paramagnetic constituents in the tissue being scanned. Following PPV, the vitreous gel is replaced by aqueous fluid produced by the ciliary body. The T₁ time in vitreous gel, for a given pO₂, is different to the T₁ time from aqueous. In order to calculate pO₂ changes following PPV using MRI it is important to determine the magnitude of this effect. We measured T₁ times at various oxygen levels in porcine vitreous and balanced salt solution (BSS) to allow calibration of the pre- and post-PPV pO₂ measurements.

We hypothesized that PPV increases vitreous pO₂, which is suggested by the current available literature. We aimed to use the described MRI technique to noninvasively measure the vitreous pO₂ before and after PPV.

METHODS

Ethical approval for the study was obtained from both the Brighton West Research Ethics Committee and the Brighton and Sussex Medical School Research Governance and Ethics Committee. The study was conducted in accordance to the tenets of the Declaration of Helsinki.

Patients, aged 18 years and over, who were due to undergo a vitrectomy for either epiretinal membrane (ERM) removal or macular hole (MH) were recruited from a vitreoretinal unit in a United Kingdom teaching hospital after informed consent was obtained.

Exclusion criteria were significant cardiovascular or respiratory disease, diabetes mellitus, or anemia.

Magnetic Resonance Imaging Scanning Protocol

MRI was performed on all patients 1 week before PPV. Imaging was performed on a Siemens Avanto 1.5T MRI scanner (Siemens, Erlangen, Germany). The scanning protocol used an inversion recovery (IR) true fast imaging with steady-state precession (TrueFISP) imaging sequence that was repeated using 17 inversion times (TI) in the range 0.7 to 30 seconds. The order of the inversion times was initially randomized to mitigate potential drift effects in the scanner. This same order was then used for all scans, to enable all participants to experience identical scanning conditions. A single slice was positioned through the center of both eyes in the axial oblique plane. Other TrueFISP parameters were: repetition time = (20 + TI) seconds, echo time = 1.52 ms, flip

angle = 80°, matrix = 256 × 256, and voxel dimensions = 0.9 × 0.9 × 4 mm³. The total T₁ measurement time was approximately 15 minutes. Participant eye fixation was only required for the duration of k-space acquisition for each of the 17 TIs, which is approximately 1 second each time. For this short period, the participant was verbally instructed to keep their eyes open and fixate on a target attached to the scanner room wall (a small black circle on a white piece of paper), which was visible via a mirror attached to the head coil. In between the k-space acquisition times, participants were allowed to move their eyes and blink.

At least 3 months following PPV, participants underwent a second MRI scan, using the same scanning protocol.

MRI data analysis was performed under masked conditions, with the images having been randomized before being presented to a single MRI physicist (ND). Vitreous cavity T₁ relaxation times were calculated from a predetermined central vitreous cavity region of interest (RoI) using anatomical landmarks, from both the vitrectomized (experimental) and nonvitrectomized (control) eye for both MRI scans. Figure 1 is a representative TrueFISP image used for anatomical visualization. The Figure includes the hexagonal RoI from where T₁ is calculated. The overall mid vitreous T₁ was an average of the pixel strength within the RoI. T₁ mapping involved a pixel-by-pixel, three-parameter fit of the signal intensity S (at each TI) to the following equation that is used in magnetic resonance physics to calculate relaxation time³⁸:

$$S(TI) = A + B e^{-TI/T_1} \quad (1)$$

A and B are parameters that account for inversion pulse flip angle, equilibrium signal intensity, and repetition time. Since flip angle is included, the technique is resilient to B₁ errors. Figure 2 is a representative T₁ map of both eyes obtained using this technique. Absolute pO₂ was determined using the phantom data obtained as part of this study, which quantifies the dependence of oxygenation on T₁ in both vitreous and aqueous as detailed below.

Comparing the Relationship of pO₂ and T₁ in Vitreous and Aqueous

Porcine vitreous and BSS were selected to emulate human vitreous and aqueous, respectively. Porcine vitreous has been shown to be the most appropriate animal model because of its greater homology to human vitreous compared with other species.³⁹ BSS was chosen as an appropriate model for human aqueous, in the context of MRI, as both fluids contain a number of the same constituents at relatively similar concentrations, and are therefore likely to possess comparable paramagnetic profiles. Elements such as sodium (BSS 155.7 mmol versus aqueous 162.9 mmol), potassium (10.1 mmol versus 2.2–3.9 mmol), calcium (3.3 mmol versus 1.8 mmol), magnesium (1.5 mmol

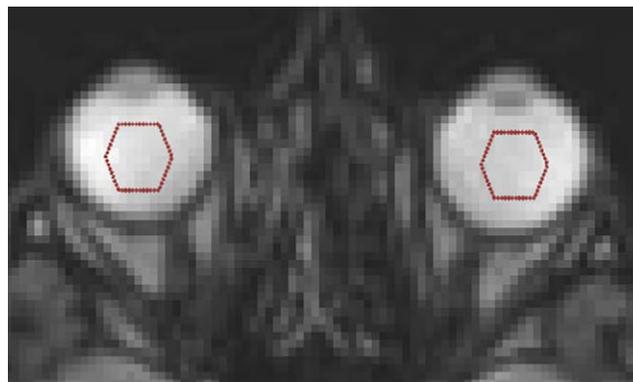


FIGURE 1. An MRI TrueFISP image allowing anatomical visualization from a single slice through the center of both eyes in an axial oblique plane. The mid vitreous cavity dotted hexagons are the regions of interest from where T₁ times were measured.

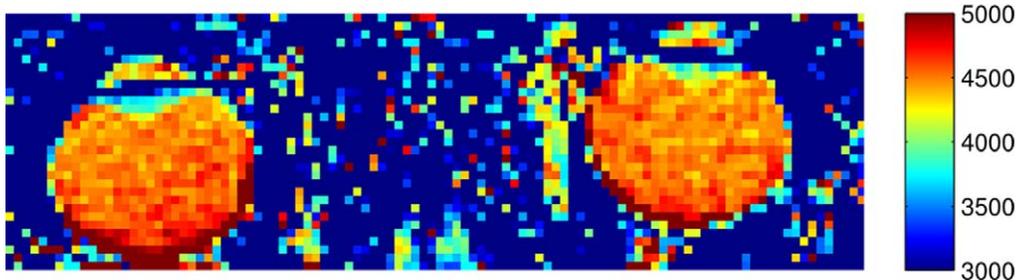


FIGURE 2. An MRI T_1 map of both eyes.

versus 1.1 mmol), and chloride (128.9 mmol versus 131.6 mmol) exist in similar concentrations.⁴⁰ Aqueous contains small amounts of bicarbonate, phosphate, lactate, glucose, and ascorbate, which BSS does not contain. Conversely, there are higher concentrations of citrate and acetate in BSS compared with aqueous. These differences are unlikely to change T_1 times significantly. Both fluids have a similar osmolality (BSS 298 mM versus aqueous 304 mM) and pH (7.6 vs. 7.38).⁴⁰

The test objects (phantoms) were glass vials (50 mL volume), containing either the porcine vitreous or BSS. The contents of each vial were deoxygenated by gentle bubbling with 100% nitrogen gas, during which time the oxygenation of the porcine vitreous or BSS was monitored. Minimum oxygen levels obtained using this technique for both porcine vitreous (~5 mm Hg) and BSS (~2.5 mm Hg) were reached by 2 minutes, after which time no further reduction in oxygenation could be achieved. The vials were then immediately placed inside a covered polystyrene water bath. The water bath temperature was monitored using a thermometer, and hot water was added to the bath when necessary to maintain a temperature of 30°C and above. The phantoms were left in place for at least 30 minutes to allow the temperature of their contents to equilibrate with the water bath. A flexible polarographic electrode oxygen probe (the same model used for oxygen measurement during PPV), connected to a Licox CMP monitor (Integra Neurosciences, Andover, UK), was used to measure the pO_2 of the vitreous or BSS. To restrict the ingress of air, the probe was passed through a fine gauge needle that was inserted through the rubber top of each vial. The pO_2 was recorded following a 5 minute period of equilibration, along with the water bath temperature, and the probe and needle were then immediately withdrawn to reseal the vial. All the vials underwent MRI, using the same scanning protocol used on the patients. The pO_2 and water bath temperature were remeasured following the scan to identify any changes that may have occurred during the period of T_1 measurement. T_1 times were adjusted to a standard temperature of 35°C, to simulate vitreous cavity temperature,^{41,42} by using a linear fitting of $1/T_1$ against temperature data by Hindman et al., who investigated in detail the relationship of T_1 relaxation times to a wide range of temperatures.⁴³ The pO_2 of each vial was increased between successive T_1 measurements by removing the lid for approximately 1 second, to allow a small amount of air to enter the vial. In this way, T_1 was measured in vitreous and BSS with pO_2 ranging from 5 mm Hg to 70 mm Hg.

Pars Plana Vitrectomy and Intraoperative pO_2 Measurement

All patients were operated on by a single vitreoretinal surgeon (EH) in a standardized way. All participants underwent the operation by local anesthetic. All patients had 99% to 100% oxygen saturation measured by pulse oximetry during surgery. Three sclerotomies were prepared, and an infusion line was inserted but not switched on. A flexible polarographic electrode oxygen probe (Integra Neurosciences) was inserted into the vitreous cavity and connected to a Licox CMP monitor. The probe was held in place for 5 minutes to allow the

measurements to equilibrate, and the mid cavity pO_2 was recorded. All patients then underwent a standard three port 20 gauge PPV, with management of their ERM or MH.

Statistical Analysis

Data are shown as mean \pm SD. To compare the data obtained between the first and second MRI scans in both the experimental and control eyes, dependent (paired) Student's *t*-tests were performed. Independent (unpaired) Student's *t*-tests were used to compare data between the experimental and control eyes. *P* less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS v20 (IBM, Armonk, New York).

RESULTS

Calibration of Vitreous and Aqueous T_1 Relaxation Times

The measurement of pO_2 , using the polarographic oxygen probe, before and after T_1 measurement, showed pO_2 remained almost constant, altering by a maximum of 1%, which equates to less than a 1 mm Hg change throughout the measured pO_2 range. During scanning, the water bath temperature reduced by approximately 2°C, and the midpoint temperature was used for further calculations. Recent work performed by our team has shown that the plot of relaxation rate R_1 ($R_1 = 1/T_1$) against measured pO_2 is linear.⁴⁴ The porcine vitreous and BSS relaxation rates were plotted against measured pO_2 (Fig. 3). The data may be expressed by the equation

$$R_1 = A + B \cdot pO_2 \quad (2)$$

where $A = 0.218 \text{ s}^{-1}$, $B = 3.47 \times 10^{-4} \text{ s}^{-1} \text{ mm Hg}^{-1}$ for porcine vitreous, and $A = 0.210 \text{ s}^{-1}$, $B = 3.64 \times 10^{-4} \text{ s}^{-1} \text{ mm Hg}^{-1}$ for BSS. Figure 3 shows that for a given R_1 , vitreous pO_2 is lower compared to BSS pO_2 .

MRI Vitreous pO_2 Measurement

Pre-PPV MRI was performed on 11 patients (7 female, 4 male). Two (1 female, 1 male) patients declined the post-PPV scan because of illness. The postoperative MRI was performed at a median of 103 days following PPV (mean 104.8 ± 10.8 ; range 91–124). Patient ages ranged from 59 to 84 years, with a median of 73 years.

Undistorted T_1 maps of the eyes were obtained with estimated Bland-Altman⁴⁵ within-subject variability of T_1 less than 1% (equivalent to $pO_2 \sim 8 \text{ mm Hg}$), and between subject variability of T_1 less than 1.5% (equivalent to $pO_2 \sim 12 \text{ mm Hg}$). The preoperative MRI scans revealed no difference in pO_2 between the experimental and control eyes (13.2 ± 5.8 vs. $13.7 \pm 7.8 \text{ mm Hg}$, $P = 0.872$). Following PPV, there was a

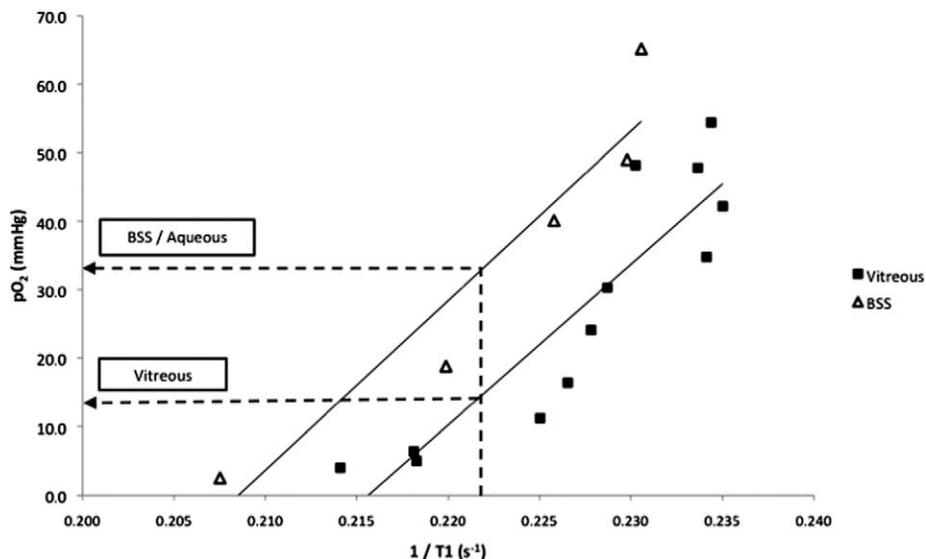


FIGURE 3. The relationship of rate of relaxation ($1/T_1$) at different pO_2 levels in both porcine vitreous and BSS, with temperature corrected to 35°C. The trendline equations were used to calibrate the pre- and post-PPV scans. For a given pO_2 , vitreous has a higher T_1 relaxation rate compared with BSS/aqueous.

significant increase in mean pO_2 from 13.2 ± 5.8 to 34.5 ± 8.0 mm Hg ($P < 0.001$) in the experimental eyes. In the control eyes, there was no statistical difference in mean pO_2 between the first and second MRI scans (13.7 ± 7.8 vs. 16.3 ± 8.7 mm Hg, $P = 0.239$). The Table and Figure 4 summarize these findings. Further analysis of the control eye of each patient, revealed a mean difference between the first and second scans of 7.7 ± 4.9 mm Hg.

Pars Plana Vitrectomy pO₂ Measurement

No patients experienced complications as a result of PPV. Intra-operative oxygen measurements with the probe were available in 10 patients. pO_2 values ranged from 6.5 to 8.1 mm Hg with a mean of 7.2 ± 0.6 (median 7.1) mm Hg.

DISCUSSION

The data obtained from this study suggest that vitrectomy increases vitreous oxygenation, a finding that is consistent with previous, invasive animal and human studies.²³⁻²⁹ We provide the first longitudinal data in the same subjects suggesting that this is not a temporary effect, and that oxygen levels in the vitreous remain raised at least 3 months following PPV.

An increase in vitreous oxygenation is one possible mechanism for the improved clinical outcome reported following PPV in diseases associated with retinal ischemia, such as diabetic retinopathy,² neovascular AMD,³⁻⁸ CRVO,⁹⁻¹¹ and BRVO.¹²⁻¹⁴ There is evidence that the retina receives a

small amount of oxygen directly from the vitreous cavity.^{46,47} This supply is unlikely to be of significance in eyes with an intact retinal blood supply, however, in ischemic retinal or macular diseases, it is possible that the oxygen present in the vitreous cavity becomes more valuable. The vitreous gel itself may also consume a small amount of oxygen,⁴⁸ and with its removal, increase the oxygen available to the retina.

Diffusion characteristics in the vitreous cavity alter substantially after PPV.^{15,20} The vitreous gel is approximately 300 to 2000 times more viscous than aqueous, and following PPV there is a similar increase in the diffusion coefficients of intravitreal molecules, including O_2 .^{15,49} In a nonvitrectomized and nonischemic eye, a pO_2 gradient exists, with lower levels adjacent to the lens, compared with the retina.⁵⁰ The increased diffusion of O_2 following PPV reduces this gradient, possibly facilitating oxygen flow from less ischemic parts of the retina to areas where it is needed the most. Higher levels of oxygen are measurable at the lens following PPV, providing a possible mechanism for increased promotion of cataract formation.^{28,48,50}

The MRI scanning protocol that we have used is suitable for measuring relative changes in pO_2 within the magnitudes of change expected following PPV. Any absolute pO_2 values derived from this technique have to be interpreted with caution because of current maximum accuracy of T_1 acquisition (~ 40 ms, or 5 mm Hg). Prior work undertaken by our team on healthy volunteers showed this technique could measure vitreous pO_2 to within approximately 8 mm Hg.³⁸ In this study, we analyzed the control eye of each patient in both

TABLE. Vitreous Oxygenation Data

	Intraoperative pO ₂ mm Hg, n = 10	Pre-PPV MRI		Post-PPV MRI		
		T ₁ ms, n = 11	pO ₂ mm Hg, n = 11	T ₁ ms, n = 9	pO ₂ mm Hg, n = 9	ΔpO_2 mm Hg, n = 9
Experimental (PPV) eye	7.2 ± 0.6	4497.7 ± 40.7	13.2 ± 5.8	4495.4 ± 58.5	34.5 ± 8.0	+21.3
Control eye	n/a	4494.5 ± 54.1	13.7 ± 7.8	4476.1 ± 60.3	16.3 ± 8.7	+2.6

Data were measured intra-operatively with an oxygen probe, and with MRI 1 week before, and 3 months after vitrectomy, correcting for vitreous to aqueous shift. All values are mean \pm SD. Δ , change.

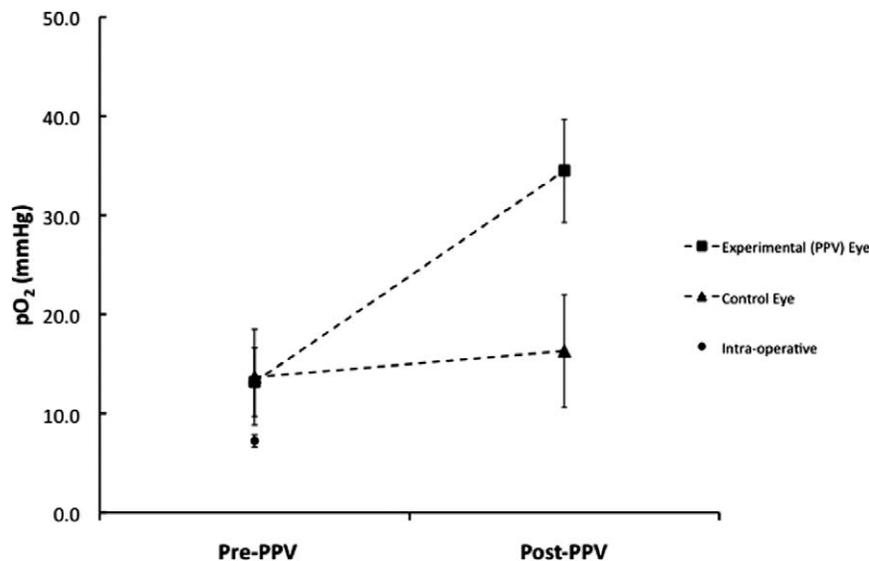


FIGURE 4. The effect of pars plana vitrectomy on vitreous oxygenation. Values are mean and error bars are SD.

the first and second scans, and found a mean difference of 7.7 mm Hg, which closely compares to our previous work.

A recent study by Muir et al. used a similar MRI scanning technique to investigate vitreous pO₂ in distilled water, ex vivo animal vitreous, and three healthy volunteers.⁵¹ They measured R₁ (1/T₁) in distilled water at various oxygen levels, and in the vitreous of two intact globes (one baboon and one rabbit), at three different temperatures, 34°C, 37°C, and 40°C. They reported comparable R₁ intercepts (i.e., representing fully deoxygenated states) to our study, for both water (we used BSS), 0.205 s⁻¹ vs. 0.210 s⁻¹, and vitreous, 0.209 s⁻¹ vs. 0.218 s⁻¹. These small differences in the reported R₁ intercepts between the two studies, which are moderately significant when converted to pO₂, are likely to be due to two main factors: temperature and to a far lesser extent, MR field strength (3 T versus 1.5 T). The R₁ intercepts from Muir et al. were for a temperature of 37°C, compared with our temperature of 35°C. T₁ measurements are highly sensitive to temperature change, and it is for this reason that the absolute pO₂ values are more variable, and presumably less accurate, than direct intraocular measurement. For example, a change of 1°C alters the T₁ time by approximately 100 ms, which equates to a pO₂ of 13 mm Hg in BSS. Previous studies have shown that the in vivo temperature of the vitreous cavity may range from 33.9°C to 36.6°C, with a likely temperature gradient existing.^{41,42} For this reason, the T₁ relaxation times measured from the phantom porcine vitreous and BSS were temperature corrected to 35°C to simulate, as accurately as possible, the temperature in the mid vitreous cavity. Similarly, when calibrating the T₁ times for either vitreous or aqueous, the assumption was made that the patients' mid vitreous cavity temperature at the time of their scans was 35°C. We are confident that the temperature used for the calculations was close to the actual temperature because we found only a mean difference of 6.0 mm Hg between the pO₂ readings of the pre-PPV MRI and the pre-PPV intraocular probe (which at these levels is only minimally influenced by temperature). Given that, using MRI, each 1°C change in temperature equals approximately 13 mm Hg, the 6.0 mm Hg difference found would mean that the vitreous temperature was within 0.5°C of 35°C.

We adjusted our T₁ times to a standard temperature of 35°C using data from Hindman et al.⁴³ A fundamental characteristic of MR physics is that magnetic field strength affects the

absolute T₁ value.⁵² The relationship between temperature, field strength, and T₁ is complex. However, Hindman et al. conducted their experiments at 1.4 T (60 MHz), which is similar to the 1.5 T magnet (64 MHz) used in our study, meaning their measurements are particularly relevant to our work. In addition, the temperature correction that we performed was over a relatively small range (5°C), further mitigating any potential errors arising due to the small difference in field strength of Hindman's magnet and our own.

The final factor that reduces the accuracy of this technique is the difference in the T₁ time in vitreous and aqueous for a given pO₂. We attempted to correct for this by performing the calibration work on porcine vitreous and BSS as described, to simulate human vitreous and aqueous, respectively.

The maximum accuracy that T₁ can be measured, in addition to the temperature dependence of this technique, introduces a certain amount of error into the absolute pO₂ measurements. Although these errors are reflected in the difference in SDs between the MRI-derived vitreous pO₂ and the intraoperative vitreous pO₂, the mean MRI value obtained is comparable to several other studies.^{50,53} The combined pre-PPV mean pO₂, from both the experimental and control eyes, was 13.4 ± 6.7 mm Hg, which is higher, although not markedly, than the measurements taken in the patients at the time of surgery, 7.2 ± 0.6 mm Hg. One of the first studies that measured vitreous pO₂ with a polarographic electrode oxygen probe, reported a mid vitreous pO₂ of 15.9 ± 2.8 mm Hg.⁵³ Shui et al. measured oxygenation at various points in the vitreous cavity of rabbits and reported a similar mid cavity pO₂ of 13 ± 2 mm Hg.⁵⁰ Park et al. recently published a study comparing probe designs for determining intraocular oxygen distribution both in vivo and in vitro, and raised issues relating to the accuracy of polarographic electrodes, as used in our study and several mentioned beforehand, to measure pO₂ in small areas such as the vitreous cavity.⁵⁴ However, our intraoperative pO₂ measurements were highly consistent, and closely matched other human data in which the potentially more accurate optical fluorescence fiber optic oxygen probes were used. For example, Holekamp et al. found a mean mid vitreous pO₂ of 7.1 ± 0.5 mm Hg in 69 eyes²⁸, and 8.5 ± 0.6 mm Hg in a further 29 eyes.²⁷ In addition, we have performed preliminary work comparing the pO₂ readings from polarographic and optical fluorescence fiber optic probes in BSS, and found no difference (Dowell NG, unpublished data, 2011). The

previously described study by Muir et al. reported a mid vitreous pO₂ of 18.3 mm Hg, which is comparable to our result (13.4 ± 6.7 mm Hg).⁵¹ Apart from the variability inherent to the technique's limitations, the reasons for the small difference between the results are likely to be the same that accounted for the differences in both studies phantom experiments, namely temperature and magnetic field strength. The fact that our result is closer to our own intraocular measurements, and to the large number of eyes measured by Holekamp et al.,^{27,28} would suggest that the mid vitreous temperature is lower than Muir's assumed temperature of 37°C temperature assumption used by Muir et al.⁵¹ However, interestingly Muir et al. investigated T₁ changes with eyes open and closed (in our study the participants had their eyes open) and found a difference that suggested vitreous temperature increased a small amount with closed eyes. Although it is not explicitly stated in their article, the 18.3 mm Hg mid vitreous pO₂ reported could be for closed eyes, or an average of open and closed, thereby, increasing the temperature closer to their assumed value, which could explain why the difference between pO₂ in both our studies was only 4.9 mm Hg, far less than would be expected given the 2°C difference in our temperature assumptions.

Due to spatial resolution limitations, this technique is currently only able to measure the average pO₂ within the central vitreous cavity. We took a standardized central vitreous region and averaged the pixel-by-pixel T₁ signal from within this region. It is hoped that future improvements in the technique will allow pO₂ mapping from different areas of the vitreous, such as the preretinal and retrolenticular regions.

Although the absolute values derived using this MRI technique are not as accurate as intraocular measurements, it possesses several advantages. It is noninvasive and, therefore, essentially risk free. In addition, it is free from surgical artifact that may influence vitreous pO₂, such as infusion fluid oxygenation. It allows the investigation of longitudinal changes in vitreous pO₂, not only following PPV, but also in a number of retinal conditions that affect vitreous oxygenation.

In conclusion, this study provides further evidence that vitrectomy increases vitreous pO₂. This information can be used to help develop different treatment strategies for patients with retinal ischemia. This MRI technique can be used in future studies, to further elucidate vitreous oxygenation changes following vitrectomy, and also in ischemic retinal diseases, in a longitudinal and safe manner.

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