Early Supernormal Retinal Oxygenation Response in Patients with Diabetes

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PURPOSE. To determine whether the human retinal oxygenation response (ΔPO2) to a hyperoxic provocation is abnormal in patients with type I diabetes.

METHODS. Magnetic resonance imaging (MRI) was used to measure ΔPO2 during 100% oxygen breathing in patients with type I diabetes who had either no clinically detectable retinopathy (n = 5) or mild to moderate background diabetic retinopathy (BDR; n = 5) and in age-matched healthy control subjects (n = 7).

RESULTS. Both the patients with diabetes and the control subjects exhibited a significant (P < 0.05) increase in the preretinal vitreous signal intensity on changing from room air breathing to oxygen inhalation (i.e., 5 minutes). However, only diabetic patients demonstrated significant (P < 0.05) increases in ΔPO2 between measurements made at 5 minutes of oxygen inhalation and measurements at longer durations of hyperoxia (15, 25, and 35 minutes). Furthermore, ΔPO2 was significantly (P < 0.05) greater in patients with diabetes than in control subjects, but there was no significant difference in ΔPO2 (P > 0.05) between patients with diabetes, with or without retinopathy. Age and ΔPO2 correlated significantly (P < 0.05) in control subjects but not in patients with diabetes. In control subjects, ΔPO2 was relatively uniform panretinally, whereas in the diabetic group, changes in oxygenation response were spatially inhomogeneous.

CONCLUSIONS. These results demonstrate, for the first time, that MRI ΔPO2 detects a significant supernormal retinal oxygenation response in patients with type I diabetes, even before the appearance of retinopathy. This study raises the possibility of an abnormal vasoregulatory response in patients with type I diabetes, even before the development of clinically evident retinopathy. Whether the MRI retinal oximetry technique could be optimized to provide an accurate, sensitive, quantitative, early, and robust physiological biomarker of diabetic retinovascular complications.

D iabetes is an insidious disease in which irreparable structural organ damage often occurs before symptoms of the disease are recognized.1 In the United States alone, approximately 18 million people, or approximately 6.3% of the population, have diabetes.2 The most common complication associated with diabetes is diabetic retinopathy, which is the leading cause of legal blindness among working-age adults in the United States.3 After 15 years of diabetes, almost all patients with type I diabetes and more than 60% of patients with type II diabetes have retinopathy.2–4 Unfortunately, in many cases, retinopathy goes undetected and untreated until visual acuity is affected, by which time irreversible retinal damage often has occurred.5

Currently, there are no widely accepted, noninvasive surrogate markers that either reliably detect early retinal vascular dysfunction, accurately predict retinopathy progression, or measure therapeutic effectiveness in patients with diabetes.5 Instead, the detection and diagnosis of diabetic retinovascular disease generally is based on visual inspection of the retina (examination and/or contact lens biomicroscopy) with confirmation by nonquantitative and subjective methods, such as angiography and/or fundus photography. These techniques are more useful for detecting established damage than for predicting the potential for the development or progression of damage. Therefore, it is critical to develop a biomarker that can be used to detect the abnormal vascular-function–associated diabetic retinopathy at earlier stages. The availability of this type of biomarker would facilitate earlier intervention and enhance drug treatment monitoring, thereby increasing the potential for preventing visual loss.

Recently, it has become possible to assess the retinal oxygenation response to a hyperoxic challenge directly and noninvasively with magnetic resonance imaging (MRI). In this technique, pioneered by Berkowitz (see Ref. 6 for a review) and referred to here as MRI retinal oximetry, the retinal oxygenation response is measured as a difference in signal intensity between T1-weighted MRI images obtained during room air breathing and during either an oxygen or a carbogen inhalation challenge. Berkowitz et al.7–9 and Zhang et al.10,11 have confirmed in animal models that changes in preretinal vitreous PO2 during hyperoxia are an accurate measure of retinal oxygenation response. In experimental studies of both retinopathy of prematurity7–11 and diabetic retinopathy12–15 these investigators demonstrated that retinal oxygenation can be reliably measured using MRI. There is a strong association of early abnormalities in retinal oxygenation with the development of histopathology associated with preproliferative diabetic retinopathy and drug treatment responses.12–19 This finding has led to the hypothesis that retinal disease is associated with an inability of the retinovascular system to regulate oxygen supply and demand adequately.

Taken together with evidence that patients with diabetes exhibit an impairment in vascular reactivity (autoregulation),20 these results suggest strongly that MRI retinal oximetry could provide a sensitive surrogate maker of retinal vascular dysfunction in human diabetes and a quantitative method to monitor drug treatment efficacy in patients with diabetes. Preliminary data demonstrate that MRI can be used to measure the retinal oxygenation response in healthy human volunteers; however, the effect in that proof-of-concept experiment was small.21 Therefore, the present study was designed to determine whether the MRI retinal oximetry technique could be optimized to provide an accurate, sensitive, quantitative, early, and robust physiological biomarker of diabetic retinovascular complications.
METHODS AND MATERIALS

Three groups of participants were recruited from the Retina Service of the Department of Eye Care Services at Henry Ford Hospital: diabetic individuals who exhibited either no detectable retinopathy, those who had or mild to moderate background diabetic retinopathy (BDR), and healthy individuals of similar age (control subjects). All the diabetic individuals were insulin-dependent (type I). Staging of diabetic retinopathy was based on fundus photography (seven-field stereo photos) and fluorescein angiography. Fundus photographs were taken and classified using the Early Treatment Diabetic Retinopathy Study extension of the Modified Airlie House procedure.\textsuperscript{24} Definition of the stages of diabetic retinopathy was based on the CDS guide for Primary Care Practitioners\textsuperscript{25} and the American Academy of Ophthalmology Preferred Practice Pattern.\textsuperscript{26} Patients with diabetes who exhibited retinal microaneurysms, occasional blot hemorrhages, hard exudates, or one or two soft exudates in the absence of any evidence of more advanced retinopathy were classified as having BDR. Patients with diabetes with none of these changes were included in the no-retinopathy group. Patients with diabetes who exhibited new vessels on the disc, new vessels elsewhere on the retina, or preretal or vitreous hemorrhage or fibrous tissue proliferation were classified as having proliferative diabetic retinopathy and excluded from the study. Fundus photographs and angiograms were graded in a masked fashion by two retina specialists. In cases in which there were disagreements, a third retinal specialist served as an adjudicator. Patients with diabetic cataract were not excluded.

All volunteers received a complete ophthalmic examination directed by a fellowship-trained retinal specialist. The examination included a medical history, best correct visual acuity, ocular alignment corrected by a fellowship-trained retinal specialist. The examination in- cluded a visual acuity test, slit lamp biomicroscopy, keratometry, fundus and optic disc examination, tonometry, and stereo photography of the optic disc. All participants had visual acuity of 20/50 or better, IOP between 11 and 19 mm Hg, and no history of either ocular disease or any systemic disease with known ocular complications other than diabetes.

Nine visually normal individuals and 13 patients with diabetes originally volunteered to participate in this study. Two of the volunteers (one control and one patient with diabetes) were unwilling to complete the entire test series because of discomfort in the magnet, whereas three other volunteers (one control and two patients with diabetes) completed the test series, but their data were unusable because of excessive eye or head movement. Consequently, seven of the visually normal volunteers (mean age, 42 ± 10 years) and 10 of the patients with diabetes (mean age, 37 ± 12 years; mean duration of diabetes, 22 ± 13 years) completed testing and provided usable data. Five of the patients with diabetes had no detectable retinopathy (mean age, 34 ± 13 years; mean duration of diabetes, 21 ± 16 years), whereas the other five patients had mild to moderate BDR (mean age, 40 ± 10 years, mean duration of diabetes, 18 ± 12 years).

All aspects of the study complied with the tenets of the Declaration of Helsinki and were approved by the Institutional Review Boards of both Henry Ford Hospital and Wayne State University. The MRI retinal oximetry studies were performed with a 1.5-T system (Sonata; Siemens, Iselin, NJ). Before the MRI examination, all participants had consented and reviewed a metal-safety screening form with a nurse or technologist, to assure the participants’ safety. The technologist then positioned the patient on the magnet, to assure the subject’s comfort. The technologist then positioned the subject’s eye to be tested. The contralateral eye was patched. Eye movements were minimized during the scan by instructing the subject to fixate on a dim, continuously lit, fiber-optic source placed in the center of the surface coil. Adjustments were made so that the subject’s eye was centered in the surface coil, and the fiber optic fixation point was comfortably visualized. During the functional MRI testing, all subjects initially breathed room air and then were switched to pure oxygen. Breathing was regulated with a two-way nonrebreathing face mask (Hans Rudolf, Inc., Kansas City, MO) placed over the subject’s nose and mouth. This mask allowed the subjects to talk and breathe freely while insuring appropriate gas inhalation. In preliminary studies, we confirmed, using a transcutaneous probe that provides a continuous readout of arterial blood gas changes, that this mask setup reliably increased baseline PaO2 by a factor of at least 4 without substantial alterations in PaCO2, as long as the mask fit snugly to the face so that there were no leaks. This allowed us to achieve a PaO2 >350 mm Hg and PaCO2 between 35 and 45 mm Hg during 100% oxygen breathing (Trick GL, Berkowitz BA, 2003). Head movements were minimized by comfortably securing the subject’s head to the imaging table in an individually fitted head mold (KGF Enterprises, Inc., Chesterfield, MO) that was fabricated before the scan. Once the subject was inside the magnet, all lights except the fixation light were turned off, to aid the subject in fixating the fiber-optic source. Subjects were then instructed to refrain from blinking during a 15-second, single-slice, spoiled-gradient, recalled-echo sequence (TR 75 ms, TE 2.79 ms, flip angle 22°, in-plane resolution 0.39 × 0.39 mm, slice thickness 5 mm, with the slice positioned to center on the optic nerve and center of the lens) and to blink only during the interleaved 5-second rest period. This no-blink/blink cycle was repeated sequentially 40 times for each image set. The image acquisition parameters were somewhat different from those in previous studies\textsuperscript{11,12} and were chosen based on the work of Paul Tofts and Mark Haacke (personal communication, 2003) to optimize the contrast-to-noise ratio based on computer simulations (data not shown).

A sequential series of six image sets (10 minutes’ acquisition per data set) were collected. Two image sets were collected during room air breathing, followed by four image sets collected during the 100% oxygen inhalation challenge. For each subject, image sets were sequentially collected during 10, 20, 30, and 40 minutes of the hyperoxic challenge. Taking the center of these acquisition times, we designate the time points as 5, 15, 25, and 35 minutes, respectively. Between these image sets, localizer images were acquired to ensure that the eye had not moved out of the slice. If it had, the slice was repositioned.

Data Analysis

All images were analyzed by the same procedure. First, to correct for any movement within the slice plane, without distorting the image, a segmented-image coregistration was performed on a computer with software written in-house. After coregistration, the MRI data were transferred to a computer (Power Mac G4; Apple Computer, Cupertino, CA) and analyzed (NIH IMAGE; a freeware program developed by Wayne Rasband, National Institutes of Health, Bethesda, MD, available at http://rsb.info.nih.gov/ihimage/). In each data set, images free of motion artifacts were averaged, to improve the signal-to-noise ratio. ΔPO2 was determined on a pixel-by-pixel basis as follows. For each pixel, the fractional signal enhancement E is calculated as $E = (S(t) - S_0)/S_0$, where $S(t)$ is the pixel signal intensity at time (t) after starting the gas inhalation and $S_0$ is the signal intensity at the same pixel spatial location during the baseline room air breathing condition. As a function of ΔPO2, modeled with an oxygen relaxivity of $2 \times 10^{-4}$ s$^{-1}$/mm Hg O$_2$ measured at 4.7 T.\textsuperscript{13} This relaxivity had been measured in a salmine phantom, which is a reasonable model of vitreous (98% water).\textsuperscript{15} The relationship between relaxivity and field is nonlinear (e.g., $2.5 \times 10^{-4}$ s$^{-1}$/mm Hg O$_2$ at 0.16 T and $3.5 \times 10^{-4}$ s$^{-1}$/mm Hg O$_2$ at 0.56 T), and we estimate that the relaxivity at 4.7 T is probably not more than approximately 30% lower than the value at 1.5 T. Thus, the value at 4.7 T seems reasonable for the purposes of the present work. E is linear up to at least 200 mm Hg, where it has the value 12.4%. With these constants, ΔPO2 can be obtained from E using the simplified equation $\Delta$PO$_2 = 16 \cdot E$, derived by fitting to a linear model.\textsuperscript{21} The ΔPO2 parameter image was analyzed as follows: The pixels along a 1-pixel-thick line at the boundary of the retina-choroid and vitreous are set to black. The values in another 1-pixel-thick line drawn
in the preretinal vitreous immediately anterior to the black pixels are then extracted to create a spatially calibrated ΔPO₂ band for each subject. This procedure minimizes the potential that retina–choroid pixel values will contaminate those used in the final analysis (‘pixel bleed’) and insures that similar regions in the preretinal vitreous space are sampled for each image. From these pixel values, either signal intensity changes or ΔPO₂ bands were extracted for statistical analysis. Data were initially summarized by computing panretinal changes (i.e., including both the temporal and the nasal regions). Comparison of retinal ΔPO₂ between control and experimental groups at the 5-, 15-, 25-, and 35-minute time points was performed using a generalized estimating equation (GEE) approach. This method performs a general linear regression analysis with the mean temporal and nasal values for each subject and accounts for temporal within-subject correlation. For purposes of local analysis, however, data were average in clusters of three sequential pixels, with each cluster representing a 0.39 × 1.17-mm retinal sector. In the local analysis, changes in signal intensity between respective pixel clusters in the images obtained at baseline (i.e., during room air breathing) and the images obtained during the hyperoxic inhalation challenge were compared with t-tests. Because interpretation of the raw data can be confounded by unequal variance at different spatial loci, this statistical analysis was used to take into account both the magnitude and the variance of the difference at each position. Consequently, the resultant spatial maps provide a strictly descriptive analysis of the changes in signal intensity associated with each duration of the hyperoxic challenge based on statistical significance (analogous to using z-scores).

**RESULTS**

**Clinical Characteristics**

The clinical characteristics of the participants are summarized in Table 1. The control group included 5 women and 2 men, whereas 7 of the 10 patients in the diabetic group were men. However, the gender difference was not significantly significant (two-tailed Fisher exact test; \( P = 0.153 \)). The mean age of the patients with diabetes (37 ± 12 years) was slightly younger than the control group (43 ± 10 years), but this difference was not statistically significant (\( P = 0.258 \)). There was no significant difference (\( P > 0.05 \)) in either age or duration of diabetes between the patients without retinopathy and the patients with mild to moderate diabetic retinopathy. All the participants in the control group had normal visual fields, whereas 7 of the 10 patients with diabetes exhibited significant visual field defects (Table 2).

**MRI Results**

**Before Hyperoxia.** At baseline, no significant difference in signal intensity was found between the control subjects (ΔPO₂ ± 2 arbitrary units [AU], mean ± SEM) and the diabetic subjects (ΔPO₂ ± 3 AU; \( P = 0.39 \)) or the diabetic patients with no retinopathy (ΔPO₂ ± 5 AU) and the diabetic patients with BDR (ΔPO₂ ± 4 AU; \( P = 0.89 \)). In other words, without the hyperoxic provocation, MRI of the vitreous produced consistent data across groups that were insensitive to the presence of diabetes.

**During Hyperoxia.** During the initial phase of the hyperoxic challenge, signal intensity increased rapidly in both the control subjects and the patients with diabetes. Consequently, at the 5-minute time point ΔPO₂ (i.e., the change in PO₂ during the hyperoxic challenge) averaged 105 ± 23 mm Hg in the control group and 114 ± 30 mm Hg in the patients with diabetes, which in both cases represented significant elevations in signal intensity from baseline (\( P = 0.0006 \) and \( P = 0.0012 \), respectively). The difference between the two groups was not statistically significant (\( P = 0.75 \)).

As the duration of hyperoxia increased, signal intensity remained elevated in both groups (Figs. 1, 2, 3). Visual inspection of the enhancement images for the different groups revealed distinct patterns at the 35-minute time point (Fig. 1). These differences were then qualitatively compared. In the control group (Fig. 2) ΔPO₂ increased slightly during the hyperoxic challenge, reaching 144 ± 29 mm Hg by the 25-minute time point, but then leveled off so that the increase approached, but did not attain, statistical significance (ANOVA, \( P > 0.05 \)). Furthermore, in the control group there was no statistically significant difference between ΔPO₂ at 5 minutes or at any other duration (Fig. 2). In contrast, in the diabetic patients, ΔPO₂ increased continuously throughout the duration of the hyperoxic challenge (Fig. 3), and this trend was statistically significant (ANOVA, \( F = 3.08, P < 0.05 \)).

**TABLE 1.** Summary of the Characteristics of the Control and Patient Groups

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**TABLE 2.** Summary of the Visual Field Findings in the Control Subjects and the Patients with Diabetes

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<th>GHT</th>
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MD, mean deviation; PSD, pattern standard deviation; GHT, glaucoma hemifield test; WNL, within normal limits.

* \( P < 5% \).
† \( P < 2% \).
cally significant differences between ΔPO₂ at 5 minutes and at 15, 25, and 35 minutes were noted in the diabetic patients (Fig. 3). The elevation in ΔPO₂ during the hyperoxic challenge was comparable for both groups of patients with diabetes, and no significant differences were evident (Fig. 4).

To evaluate local changes in ΔPO₂, data were averaged in 3-pixel clusters extending 20 mm temporal and nasal from the center of the optic disc, with each cluster representing the average ΔPO₂ in a 0.39 × 1.17-mm retinal sector. After 5 minutes of hyperoxic inhalation challenge, the control group exhibited significant elevations in signal intensity across the entire image, with no systematic variations in the significance level of the changes in signal intensity (Fig. 5, left). After 35 minutes of hyperoxic inhalation challenge the significance of the signal-intensity changes in the control group increased, but in an essentially uniform pattern (Fig. 5, right). In the diabetic patients, after 5 minutes of hyperoxic inhalation challenge significant elevations in signal intensity also were evident across the entire image; however, the most significant increases in signal intensity were grouped in a region that extended from the macula to 10 mm temporally (Fig. 5, left). By 35 minutes of hyperoxic inhalation challenge the region of most highly significant increases in signal intensity in diabetic patients had expanded and extended from 10 mm nasal to 17 mm temporal to the macula (Fig. 5, right).

**Effect of Age**

In the control group, the ΔPO₂ determined from the 35-minute time point and from the time-averaged data across all four time points was found to correlate significantly with age ($r^2 = 0.86$, $P < 0.005$, and $r^2 = 0.592$, $P < 0.05$). Unlike in the control group, no significant correlations between ΔPO₂ and patient age were observed in the diabetic patients ($P > 0.05$ in all cases).

**FIGURE 1.** Enhancement images (35-minute time point) of representative subjects (two control subjects and two patients with diabetes). Black line in the posterior segment shows the border of the vitreous and retina and another (bottom right of images) shows the center of the optic nerve (for reference). Note the increased signal in the diabetic patient with no detectable retinopathy (NR) as well as in the diabetic patient with BDR, compared with age-matched control subjects. Note also the considerable mixing of the oxygen signal throughout the vitreous, although this effect was minimal in the younger control subjects.

**FIGURE 2.** The increase in ΔPO₂ as a function of the duration of the hyperoxic challenge is plotted for the control group. In response to the hyperoxic challenge, visually normal subjects initially exhibited a ΔPO₂ of 104.7 mm Hg (5 minutes) and this value increased to approximately 143.9 mm Hg during the hyperoxic challenge. Error bars, SEM.

**FIGURE 3.** The increase in ΔPO₂ as a function of the duration of the hyperoxic challenge is plotted for the patients with diabetes. In response to the hyperoxic challenge, the diabetic subjects initially exhibited a ΔPO₂ of approximately 112 mm Hg, which was comparable to the initial ΔPO₂ of the control group. However, in the patients with diabetes, this value elevated more rapidly during the hyperoxic challenge, eventually reaching more than 210 mm Hg. Error bars, SEM.
Automated perimetry was conducted on all participants. None of the healthy control subjects exhibited visual field defects; however, visual field abnormalities were detected in 7 of the 10 patients with diabetes. The visual field defects most often were superior or inferior arcuate scotomas, and similar defects were seen in the diabetic patients with no retinopathy and the diabetic patients with BDR. The association between elevated ΔPO$_2$ and visual field dysfunction was evaluated by determining the correlation between ΔPO$_2$ averaged over the duration of the hyperoxic challenge and two global indices of visual field sensitivity, mean deviation, and pattern standard deviation. No significant correlation was found between ΔPO$_2$ and either mean deviation ($r = 0.09$, $P > 0.35$) or pattern standard deviation ($r = 0.29$, $P > 0.10$).

**DISCUSSION**

The results in this study demonstrate, for the first time, that an MRI retinal oximetry technique similar to that validated in preclinical studies by Berkowitz$^6$ is practical for use clinically as an early physiological biomarker of diabetic retinopathy. Functional MRI testing was successfully completed on 7 (78%) of the 9 control subjects and 10 (77%) of the 13 patients with diabetes who volunteered to participate. The primary reason participants failed to generate useful data was suboptimal eye or head stabilization, which generated excessive motion artifacts; however, some participants withdrew before completing the MRI study because of discomfort in the magnet. The 78% success rate is similar to what we reported earlier in a dynamic contrast-enhanced MRI of diabetic macular edema$^{25}$ that involved a less complicated setup with no facemask. Because of the long imaging times involved with both the dynamic contrast-enhanced MRI protocol and the retinal oximetry procedure, the two approaches have not been conducted together. We realize that the 22% attrition rate appears high for a clinical test, but it must be recognized that this was the initial application of MRI testing to a group of patients. Therefore, it is difficult to compare the attrition rate in this study with what might be obtained with more routine clinical procedures (e.g., visual field testing, electroretinography) that have been standardized over decades. In general, approximately 1% to 10% of patients are intolerant to MRI procedures because of anxiety or claustrophobic responses. However, in our procedure the patients also had a magnetic coil over one eye, the contralateral...
eye patched, and the head secured in a custom head mold with a face mask placed over the nose and mouth. These factors also contributed to the discomfort and anxiety as well as possible motion artifacts. Methods to reduce anxiety and discomfort (e.g., hypnosis, teaching the use of coping strategies, sedation) can be tailored to the needs of the patient but were not used in this study. The use of these methods, together with the use of a lighter disposable facemask to reduce pressure on the face and a modification of the timing of the image series (e.g., collecting only three images during hyperoxia with 5-minute rest periods between each imaging sets) can be expected to increase our success rate. Nevertheless, our results suggest that a level of 70% to 80% success can be reasonably expected for functional MRI studies of retinovascular physiology. We speculate that the demonstrated ability to collect high-resolution and blink-artifact-free human data will motivate functional MRI retinal oximetry studies in other retinopathies and ocular disorders, including glaucoma, ARMD, ocular oncology, and sickle cell retinopathy.

The human retinovascular system undergoes a range of changes during the normal aging process, and there is substantial evidence that anatomic and physiologic changes associated with advancing age could upset the balance between retinal oxygen supply and demand.20–34 The precise influence of normal aging on retinal oxygenation is poorly understood. In this study there was a 27-year age range in the control subjects and a 30-year age range in the patients with diabetes. The oldest participant was 54 years of age. Consequently, no elderly persons were tested. However, despite this truncated age range, which could obscure an association, a correlation between \( \Delta P_{O_2} \) and age was detected in the control group with the retinal oxygenation response increasing approximately linearly as a function of age (0.26 mm Hg per year). It is possible that a more subtle association might be found in the patients with diabetes if a larger age range were studied. Nevertheless, this finding suggests that, during the hyperoxic challenge, there was an imbalance between \( O_2 \) supply and demand, perhaps resulting in a greater oxygen surplus, in the older, nondiabetic individuals. The mechanism underlying this age-related imbalance is not known. A range of structural changes, including a reduction in arteriolar and venular diameters along with the development of anomalous vessel branching geometry, occur in the human retinovascular system during the normal aging process.34,35 Age-related reductions in blood velocity,26,28 blood volume,26 and blood flow,26,27 as well as increases in vascular resistance,36 have all been reported to occur in the elderly. Based on these patterns of retinovascular changes, it might be expected that \( \Delta P_{O_2} \) would decrease with age. Clearly, this was not the case in the present study. Alternatively, retinovascular reactivity (autoregulation) declines during normal aging,37,38 and this decline would be expected to result in an increase in \( \Delta P_{O_2} \) as a function of age. Therefore, the present results appear most consistent with an age-dependent loss of retinovascular reactivity. In this case, there would be relatively less vasoconstriction in older subjects than in younger patients during the hyperoxic challenge, thereby resulting in a greater accumulation of \( O_2 \) in the preretinal vitreous.

In this study, we found that \( \Delta P_{O_2} \) was significantly super-normal in patients with diabetes and that this was true in patients with no clinically detectable retinopathy as well as in patients with BDR. These results are similar to previous reports of an abnormal retinal oxygenation response in experimental diabetes in rodent models of diabetic retinopathy well before the appearance of retinal histopathology.16,18,19 In these rodent models, drug treatments that corrected the early subnormal retinal \( \Delta P_{O_2} \) were found also to minimize the late-stage development of retinal histopathology. In addition, therapies that did not correct early abnormal response also were ineffective in halting the development of subsequent histopathology. These data raise the possibility that using functional MRI to measure the increase in \( \Delta P_{O_2} \) associated with a hyperoxic inhalation challenge could provide a useful technique for the early evaluation of drug treatment efficacy in diabetes. The small sample size in this study makes it difficult to interpret the absence of a significant difference between the no DR and BDR groups. However, we believe that the supernormal retinal oxygenation response observed in patients with diabetes is likely to represent an abnormality in retinovascular reactivity that occurs in association with the development of diabetes and, therefore, presages the development of retinopathy.

It should be noted that two of the patients had had diabetes for more than 30 years without development of retinopathy (slow progression), whereas one of the patients had it develop relatively quickly (2.5 years, rapid progression). This raises the question of whether patients with either slower or more rapid progression to retinopathy exhibit an atypical \( \Delta P_{O_2} \). Within the context of the present study, this question is difficult to answer fully because of the relatively small sample. However, an examination of the \( \Delta P_{O_2} \) values for each of these patients revealed that at no time point did their results vary significantly (i.e., defined as more than 2 SD from the mean of their respective subgroup). Additional studies are needed to investigate this question more fully.

The mechanism mediating the supernormal \( \Delta P_{O_2} \) in patients with diabetes remains unclear. Given the well-documented retinal neurodegeneration-associated diabetes, one possible explanation is that there is a decreased demand for \( O_2 \) in the diabetic retina due to a loss of neural elements. In this case, more choroidal oxygen than normal may transverse the retina, leading to a supernormal preretal venous response. This possibility cannot be completely ruled out at present. However, we note that the failure to find a difference between the patients with diabetes with and those without retinopathy together with the lack of a correlation between the increase in \( \Delta P_{O_2} \) and the extent of the visual field defect in the patients with diabetes argues against this alternative because the degree of cell loss should be associated with both the degree of retinopathy and the extent of the visual dysfunction. These considerations suggest that the supernormal oxygenation response is due to impaired vascular reactivity.

Several studies have shown that hyperoxia elicits significant (~50%) constriction in retinal arteries and veins during a hyperoxic challenge in healthy subjects, but there is disagreement in these studies concerning the spatial uniformity of the vasoconstriction. Some reports suggest that vasoconstriction in the vessels in the temporal retina exceeds the vasoconstriction in the nasal retina,26,50 but other reports describe a relatively uniform vasoconstriction.40 In this study, we found that, in healthy subjects, \( \Delta P_{O_2} \) was essentially equivalent in the temporal and nasal regions of the image. Therefore, if the elevation in \( \Delta P_{O_2} \) observed in this study is a function of vascular reactivity, then our local measurements suggest that, in healthy control subjects, the vasoconstriction associated with a hyperoxic challenge would be comparable in vessels in the temporal and nasal retina. One alternative possibility is that the mixing of oxygen in the vitreous (Fig. 1) that occurs due to eye movements might equalize temporal and nasal differences. However, based on the same assumption, our local measurements also suggest that, in diabetics, vasoconstriction would be greater in vessels in the nasal than in temporal retina. More work is needed to resolve this issue.

There is an extensive body of literature describing the visual dysfunction associated with the development and progression of diabetic retinopathy. Patients with diabetes often exhibit dyschromatopsia,41–45 contrast sensitivity deficits,45,44 electro-
retinographic abnormalities \(^{45-47}\) and visual field loss \(^{45,48-50}\). All these deficits may occur before the development of detectable retinopathy. The relationship between these functional deficits and diabetes-induced abnormalities in retinal oxygenation remains unclear, although at least one study has described a partial reversal of diabetic dyschromatopsia during hyperoxia. Further research into the link between these deficits and retinal oxygen is clearly warranted.

In conclusion, this study supports the use of MRI as a routine measure of retinal oxygenation response to a hyperoxic inhalation challenge in patients with diabetes as well as in healthy humans. The retinal oxygenation response to a hyperoxic provocation appears elevated in older relative to younger healthy individuals and becomes supernormal before and during the appearance of retinopathy in type 1 diabetes. We speculate that both of these effects are consistent with impaired autoregulation. Functional MRI of the human retinal oxygenation response appears to be a powerful method that provides new and relevant information concerning diabetic retinopathy and possibly other retinal diseases.

References


