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The effect of normobaric hyperoxia in a radiation retinopathy case study

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**THE EFFECT OF NORMOBARIC HYPEROXIA IN A RADIATION
RETINOPATHY CASE STUDY**

by

ELISE PHAM

B.A., University of California, Berkeley, 2014

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requirements for the degree of
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Approved by

First Reader

Aaron W. Young, Ph. D.
Assistant Professor of Physiology and Biophysics

Second Reader

Jorge G. Arroyo, M.D., M.P.H.
Associate Professor of Ophthalmology
Harvard Medical School

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ABSTRACT

Background: Radiation retinopathy is a progressive ocular disease that is a common complication following exposure to ionizing radiation. Despite current treatments, some patients continue to lose vision due to retinal ischemia and retinal edema, necessitating further investigation into therapeutical alternatives. Pilot studies of supplemental oxygen and hyperoxia therapy have recently demonstrated improved outcomes in vision and reduction of symptoms in ocular disorders involving diseases of the retinal vasculature such as diabetic retinopathy and retinal vein occlusion. Hyperbaric oxygen therapy (HBOT) has been reported to improve complications associated with radiation retinopathy, but HBOT poses challenges regarding affordability and availability of hyperbaric chambers. Normobaric hyperoxia (NBH) therapy presents a more cost-effective and accessible alternative that has been successfully shown to ameliorate edema and improve visual outcomes in retinopathies with similar clinical presentations as radiation retinopathy and thus may be an effective treatment for complications due to radiation retinopathy.

Objective: In this case study, we investigated whether administration of NBH could reduce retinal edema and improve visual acuity in a patient with retinal edema and vision loss associated with radiation retinopathy.

Methods: Three patients with radiation retinopathy were initially screened for NBH treatment. Of those, one patient proceeded with treatment and completed two 3-hour NBH sessions and one 3-hour normobaric normoxia (NBN) session which served as a control. Before and after each session, best corrected distance visual acuity (VA) was assessed using Early Treatment Diabetic Retinopathy Study testing, and retinal thickness was imaged using optical coherence tomography. Retinal thickness measurements were taken at five points along the area of edema and average differences and percent changes in thickness were calculated. A student's one-tailed t-test was used to analyze statistical significance.

Results: Retinal thickness in the area of edema was reduced by an average of $27 \pm 7.14 \mu\text{m}$ with an average percent change in thickness of $4.35 \pm 1.01\%$, and VA improved from $20/63^{-2}$ (LogMAR=0.54) to $20/63^{+1}$ (0.48) after the first NBH trial. After the second NBH trial, retinal thickness in the area of edema was reduced by an average of $20.2 \pm 2.59 \mu\text{m}$ with an average percent change in thickness of $4.42 \pm 0.58\%$, and VA was improved from $20/50$ (0.4) to $20/32^{-1}$ (0.22). Following the NBN control session, retinal thickness was reduced by an average of $5.8 \pm 0.84 \mu\text{m}$ and had a percent change of $1.71 \pm 0.23\%$ with relatively little change in VA. Retinal thickness was found to be significantly reduced following each NBH trial when compared to NBN measurements ($p = 0.001$ and 0.0002).

Conclusion: In a patient with radiation retinopathy, NBH treatment demonstrated improved outcomes in VA and reduction of retinal edema. Addition of NBH therapy to existing disease management plans may expand the treatment repertoire for patients suffering from radiation retinopathy and lend further support for the use of NBH therapy as a treatment option for additional ophthalmic disorders.

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LIST OF ABBREVIATIONS

BIDMC	Beth Israel Deaconess Medical Center
BM	Bruch's membrane
CO ₂	Carbon dioxide
CPT	Center point thickness
CRAO	Central retinal artery occlusion
ETDRS.....	Early Treatment Diabetic Retinopathy Study
FiO ₂	Fraction of inspired oxygen
HBOT.....	Hyperbaric oxygen therapy
ILM	Internal limiting membrane
NBH	Normobaric hyperoxia
NBN	Normobaric normoxia
OCT.....	Optical coherence tomography
VA.....	Visual Acuity
VEGF	Vascular endothelial growth factor

INTRODUCTION

Visual impairments and ocular disorders affect over one billion individuals worldwide and are a major cause of global productivity loss (GBD 2019 Blindness and Vision Impairment Collaborators, & Vision Loss Expert Group of the Global Burden of Disease Study, 2021). According to a 2019 report by the World Health Organization, the economic burden incurred from blindness and vision loss impacting the ability to work exceeds \$200 billion (World Health Organization, 2019).

In the United States, reduced visual ability consistently ranks among the top ten disabilities reported by adults over the age of eighteen, and the prevalence of ocular disease is expected to more than double from 2005 to 2050 as certain epidemiological risk factors such as obesity continue to increase among the aging populace (Saaddine et al., 2008). Decreased visual ability can directly impinge upon many aspects of daily living, such as maintaining or seeking employment and navigating a daily routine. Reduction in vision has been shown to be correlated with increased risk of developing other comorbidities, including but not limited to depression, diabetes, stroke risk, and cognitive decline (Rein et al., 2006).

Given the wide-ranging impacts that maintaining visual health has, addressing and treating visual disorders can not only improve individual quality of life but also benefit societal functionality (Saaddine et al., 2003). Several common causes of ocular disorders and diseases, such as errors in refraction, diabetic retinopathy, and glaucoma, are largely treatable and in some cases preventable (Congdon et al., 2004). Although other, less

common diseases and disorders of the eye such as radiation retinopathy impact a smaller fraction of the general populace, the complications stemming from such ophthalmies can result in visual impairment and loss with equal or greater severity than their more common counterparts (World Health Organization, 2019). Research efforts must still be devoted to characterizing, understanding, and improving or developing novel therapies for less common ophthalmies in order to ensure a broad and wide-reaching response to reduce the prevalence of worldwide vision loss.

Anatomy and Physiology of the Human Eye

The eyes are a pair of spherical sensory organs located in the orbital cavities of the skull and situated bilaterally on the front of the face. In conjunction with parts of the central nervous system, they comprise the human visual system and confer the ability to receive and detect light and visual stimuli (Riordan-Eva, 2017). Development of the eye begins at a very early stage of fetal development, around three weeks post-fertilization when neurulation has occurred, and the neural folds are closing. Optic grooves are formed on either side of the neural folds in the cephalic region of the embryo. Cells of these grooves continue to proliferate, differentiate, and develop into the optic cup before ultimately assuming the general structure that will later become the adult eye. After birth, the eye continues to grow and mature until about seven to eight years of age (Tamm et al., 2012).

The overall eyeball is referred to as the globe and measures approximately twenty-four millimeters in diameter in an average adult. Covering the anterior surface of the globe is the conjunctiva, which is a thin and clear mucous membrane that serves to protect and

lubricate the eye to prevent irritation (Figure 1). Reduction of friction between the globe and the surrounding eyelid is an essential function of the conjunctiva. Encapsulating much of the outermost layer of the eye is a dense, fibrous structure called the sclera, which is white in appearance (Figure 1). The sclera is predominately composed of collagen and, to a lesser extent, elastic tissue (Riordan-Eva, 2017).

Posterior to the conjunctiva at the most anterior surface of the eye in descending order are the cornea, aqueous humor, iris, lens, and ciliary body (Figure 1). These structures outline the anterior chamber of the eye, which is the space extending from the cornea to the iris and filled by the aqueous humor. The cornea is a transparent tissue composed of five cellular layers that play a major role in the eye's focusing power. Remaining optical power is contributed by the lens, a biconvex and translucent capsule which is anchored to the ciliary body. Aqueous humor is produced by the ciliary body in the posterior chamber of the eye located between the iris and the lens which then flows outward to the anterior chamber. Ciliary muscles can adjust the shape of the lens to vary focusing power, while the iris acts as an aperture to control the amount of light entering the eye (Riordan-Eva, 2017).

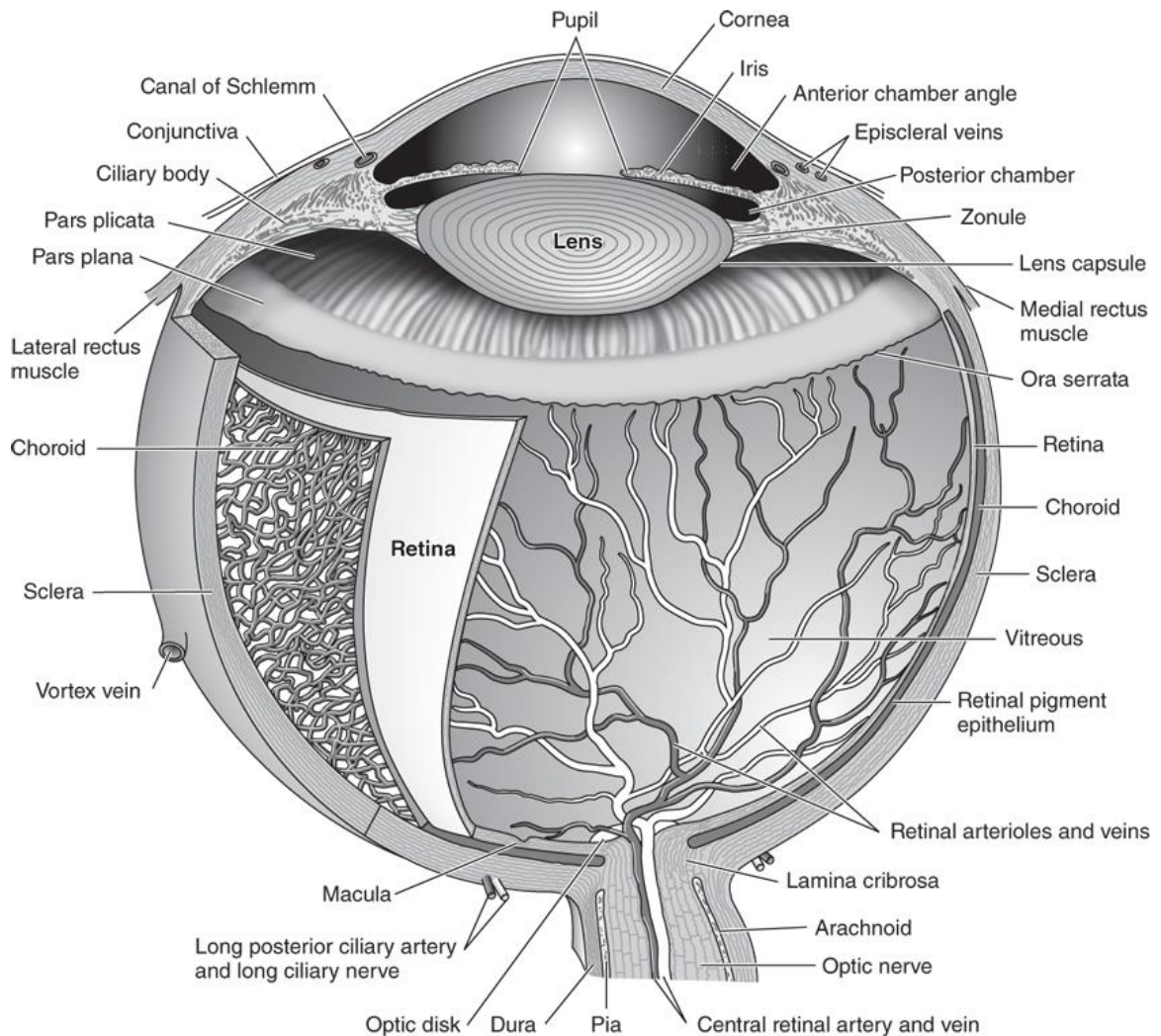


Figure 1. Internal structures of the human eye.

An anatomical diagram depicting a cross-sectional view detailing the internal tissues and structures found in an adult human eye. The sclera, choroid, and retina are illustrated as peeled-away layers to emphasize the extent of fundus coverage by these structures. Reprinted from *Vaughan & Asbury's General Ophthalmology, 19e*, by P. Riordan-Eva, 2017, McGraw-Hill Education. Copyright 2018 by McGraw-Hill Education, Inc.

Internal to the anterior and posterior chambers are the vitreous humor, retina, and the choroid. The vitreous humor is a hydrated, gelatinous, and clear substance constituting the majority of the volume of the globe. Lining the inner, posterior surface of the eye, also

referred to as the fundus, are the choroid, retina, and retinal pigment epithelium. These structures extend from the optic nerve disc to the ciliary body. Clarity of the cornea, aqueous humor, lens, and vitreous humor is essential to allow the transmittance of light through the eye to the retina, where the detection of light and activation of visual sensory receptors occurs (Riordan-Eva, 2017).

The Retina

The retina is a complex collection of neurons and associated cells arranged in multiple, distinct layers (Figure 2). Beginning from the most interior aspect and ending at the innermost side of Bruch's membrane (BM) in the choroid, ten layers have been defined by current literature: the internal limiting membrane (ILM), the nerve fiber layer, the ganglion cell layer, the inner plexiform layer, the inner nuclear layer, the outer plexiform layer, the outer nuclear layer, the external limiting membrane, the photoreceptor layer, and the retinal pigment epithelium (Gupta, 2015). The order, structure, and composition of the retinal layers is crucial to ensure light traveling through the eye is received and converted into an electrical signal that is relayed to the optic nerve at the optic disc and then to the brain for further processing (Masland, 2012).

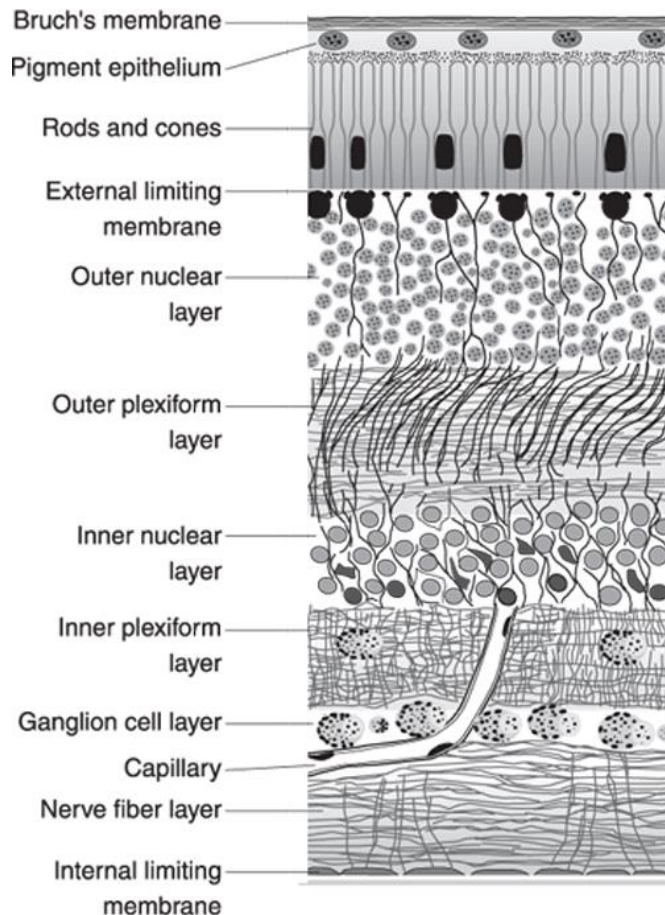


Figure 2. Layers of the retina.

A schematic view of the microscopic structure comprising the retina. The ten, distinct cellular layers are visible, beginning at the innermost aspect with the internal limiting membrane and ending at the pigment epithelium at the outermost aspect. Bruch's membrane is part of the choroid and demarcates the basement membrane of the retina. A capillary is depicted to illustrate blood flow supplied from the central retinal artery to the inner retina. Re-printed from *Vaughan & Asbury's General Ophthalmology, 19e*, by P. Riordan-Eva, 2017, Mc-Graw-Hill Education. Copyright 2018 by McGraw-Hill Education, Inc.

At the center of the retina are the macula and the fovea, which are responsible for conferring sharp, central, and highly detailed color vision. The fovea contains only cone photoreceptors, making it the thinnest part of the retina. As a result, the fovea is visible

upon ophthalmoscopic examination and scans as a small depression in the retina. Along the outer aspect of the retina is the retinal pigment epithelium, which is securely adhered to the sensory portion of the retina at the ciliary body and the optic disc. Throughout the rest of the retina, while the sensory retina and the retinal pigment epithelium are in close proximity with one another, the layers may become separated and lead to the creation of a subretinal space often seen in many retinal pathologies or retinopathies (Riordan-Eva, 2017).

Because the retina is responsible for sensation of light and visual stimuli, aberrations in the structure of the retina such as thickness changes can lead to direct and immediate consequences in vision. Disruption of the retinal structure can deprive cells of the various layers from properly receiving signals and nutrients, which may then lead to stress or death of crucial photoreceptors. Since the retina lacks the ability to regenerate further receptors and neurons, the repercussions of an abnormal or impaired retina may often lead to irreversible blindness if left untreated (Vetter and Hitchcock, 2017).

Vasculature and Nutrient Supply

Many of the tissues comprising the eye are avascular and derive nutrients and oxygen via diffusion through nearby vascular beds, aqueous or vitreous humors, or directly from the atmosphere itself as in the case of the cornea. An episcleral vascular bed overlays the scleral stroma and supplies oxygen and nutrients to the surrounding tissue. More internal structures of the eye rely on passive diffusion through the humors or nearby tissues

from the vascular or uveal structures, which are comprised of the iris, ciliary body, and choroid (Riordan-Eva, 2017).

In spite of the lack of extensive vasculature through the entirety of the retina, retinal cells and photoreceptors rank among the top oxygen metabolizing tissues in the body. The demand for oxygen is fulfilled in part by choroidal capillaries, termed the choriocapillaris, lying along Bruch's membrane. This arrangement is sufficient to deliver required metabolites to the outer third of the retina beginning from the retinal pigment epithelium and reaching up to the outer plexiform layer via the action of diffusion. The remaining inner two-thirds of the retina, except for the fovea, are fed via capillaries branching off the central retinal artery. Nutrients and oxygen diffuse from vascularized areas to remaining avascular areas such as the fovea (Schmidt et al., 2003).

Inner retinal blood vessels lack fenestrations, creating a barrier to ensure more control over fluid movement between the vasculature and the retina since the consequences of abnormal fluid movement and separation of retinal layers could be catastrophic to visual sensory ability, especially if the macula and fovea are involved. At the outer aspect, the choriocapillaris do not share the same wall endothelial architecture as the retinal capillaries and exhibit fenestrations (Nakao et al., 2018).

Radiation Retinopathy

Radiation retinopathy is a progressive ocular complication of the retina with mild to severe consequences and typically develops following retinal exposure to ionizing radiation used for the treatment of malignancies in or around the head (Archer, 1993). The

disease was first observed and described in 1933 by Dr. Hyla Bristow Stallard after noting changes in the retina following radiotherapy for ocular tumors, a novel treatment for eye-related malignancies at the time (Stallard, 1933). Symptoms and clinical presentation of radiation retinopathy are not immediate. Disease onset typically begins around eighteen months post radiation exposure, but the appearance of symptoms may begin appearing as early as only a few months or as late as several years later (Yu and Scheffler, 2020).

Very few studies investigating the overall prevalence of radiation retinopathy in the general populace have been conducted, and incidence of radiation retinopathy remains an active area of research. Current estimates have placed incidence of developing radiation retinopathy following ionizing radiation treatment for ocular malignancies at 42-85% of patients within five years of exposure. Out of 1,300 patients who had undergone plaque radiotherapy at a single care center in Philadelphia, Pennsylvania, about 42% of patients were found to have developed radiation retinopathy within five years of treatment (Gündüz et al., 1999). At the same care center, a 2010 study estimated that about 7% of patients developed proliferative radiation retinopathy, a more severe form, within ten years of completing treatment (Bianciotto et al., 2010). In 2016, another study conducted on patients at a separate care center in Berlin, Germany found that up to 85% of patients treated with proton beam irradiation for ocular malignancies had developed radiation retinopathy within five years (Seibel et al., 2016).

Pathophysiology

Pathophysiological manifestations of radiation retinopathy primarily involve disruption or developmental irregularities of the ocular vasculature leading to effects upon the retina (Archer, 1993). Ionizing radiation can cause chromosomal malformations and the formation of free, oxygen-carrying radicals through excitation of water molecules ubiquitous in the cellular and extracellular compartments. The extreme reactivity of these free radicals damages the molecular stability of many biomolecules, eliciting inflammatory changes (Citrin and Mitchell, 2017).

Endothelial cells lining the blood vessels of the eye are most susceptible to damage from disease progression and are forced to undergo apoptosis in far greater proportion to the pericytes, the supportive mural cells encompassing the capillaries, because endothelial cells are directly exposed to oxygenated blood, and thus free radicals to a larger degree than pericytes (Archer et al., 1991). One differentiating feature between radiation retinopathy and diabetic retinopathy, which bear very similar to identical clinical presentations, is that endothelial cells are often spared from damage while pericytes are disproportionately affected in diabetic retinopathy. This selective sparing is in contrast to the disease progression of radiation retinopathy. Ultimately, however, many of the clinical outcomes and physical symptoms are shared between the two diseases (Maguire and Schachat, 2006).

Microvascular changes associated with radiation retinopathy are similar to those found in diabetic retinopathy, such as angiogenesis, neovascularization, microaneurysms, and enlargement of the foveal avascular zone and capillary non-perfusion. The latter two

processes result in inner retinal hypoxia, macular edema, and subsequent cell death as the retinal structure is disrupted (Archer et al., 1991). Radiation damage causing endothelial cell death prompts surrounding, unaffected endothelial cells to respond and proliferate in order to restore lost segments of the retinal vasculature. Often, the compensatory efforts are insufficient, and the new, resulting vessels lack adequate structure. Additionally, retinal hypoxia induces production of vascular endothelial growth factor (VEGF) to further stimulate production of new blood vessels, which further exacerbates disease symptoms (Pe'er et al., 1995).

Proper filtration and reabsorption of fluid through the capillary beds is impaired due to the inadequate mural architecture of the new vessels, leading to increased accumulation of extracellular fluid and materials in the interstitial space between layers of the retina. As the sensory retina and the retinal pigment epithelium are not strongly attached to one another besides at the optic disc and the border along the ciliary body, fluid accumulation manifests as retinal or subretinal edema separating the neuronal retinal layers. Platelet aggregation and the clotting cascade are also prompted to activate in response to the leaky blood vessels, which in turn can cause blockages and ischemia to occur in the retina (Spielberg et al., 2013).

Obstructions to proper blood flow deprives photoreceptors and other neuronal cells from receiving survival factors and metabolites, predominately oxygen, necessary for their continued survival. The combination of leaky and obstructed vessels leads to reduced perfusion and a hypoxic environment which can have devastating outcomes on vision if prolonged. Since the ability of the retina to detect and react to light stimuli is directly tied

to the thickness and the structural integrity of each neuronal and cellular layer being in close proximity with one another, changes that cause deviations in retinal thickness and separation of the layers are often reported as distorted, blurred, or loss of vision depending on the location of and the extent to which the retina is impaired. If action is not taken to preserve or restore retinal integrity, permanent blindness can result from untreated radiation retinopathy (Spielberg et al., 2013).

Diagnosis

Prior history of radiation exposure, particularly for head and neck malignancies, is often the strongest predictor of eventual development of radiation retinopathy or radiation papillopathy. The most common symptoms of radiation retinopathy include retinal microaneurysms, hemorrhaging, telangiectases, hard exudates, retinal edema, and cotton-wool spots. Other symptoms may also include neovascularization, atrophy of the retina or optic nerve head, or occlusions of the vasculature leading to ischemia (Yanoff and Duker, 2018).

Disease onset and clinical presentation of radiation retinopathy often manifest similarly to presentations of diabetic retinopathy and begin with changes in the retinal vasculature. Radiation retinopathy, like diabetic retinopathy, can be classified as nonproliferative or proliferative depending on whether neovascularization is present. If large areas of the retina are not adequately perfused, the likelihood of new and aberrant angiogenesis and development of proliferative radiation retinopathy increases. Proliferative radiation retinopathy can be identified by the presence of vitreous

hemorrhaging, neovascularization of the iris and other ocular structures, and glaucoma (Yanoff and Duker, 2018).

Many of these symptoms are apparent upon ophthalmic examination, but further diagnostic studies can be ordered to study the extent and elucidate the degree of pathological changes in greater, microscopic detail. Optical coherence tomography (OCT), OCT angiography, and fluorescein angiography are often employed to image the fundus, retina, and vasculature of the fundus. OCT is a common diagnostic technique that can be used to not only record current measurements of retinal thickness but also changes in retinal thickness during repeat follow-up evaluations, which can be instrumental to monitor disease changes, progression, and responses to treatment over time (Gabriele et al., 2011; Figure 3). Spectral domain OCT allows visualization of the layers of the retinal, and subretinal fluid can be detected with high resolution (Kiernan et al., 2011; Figures 4-5).

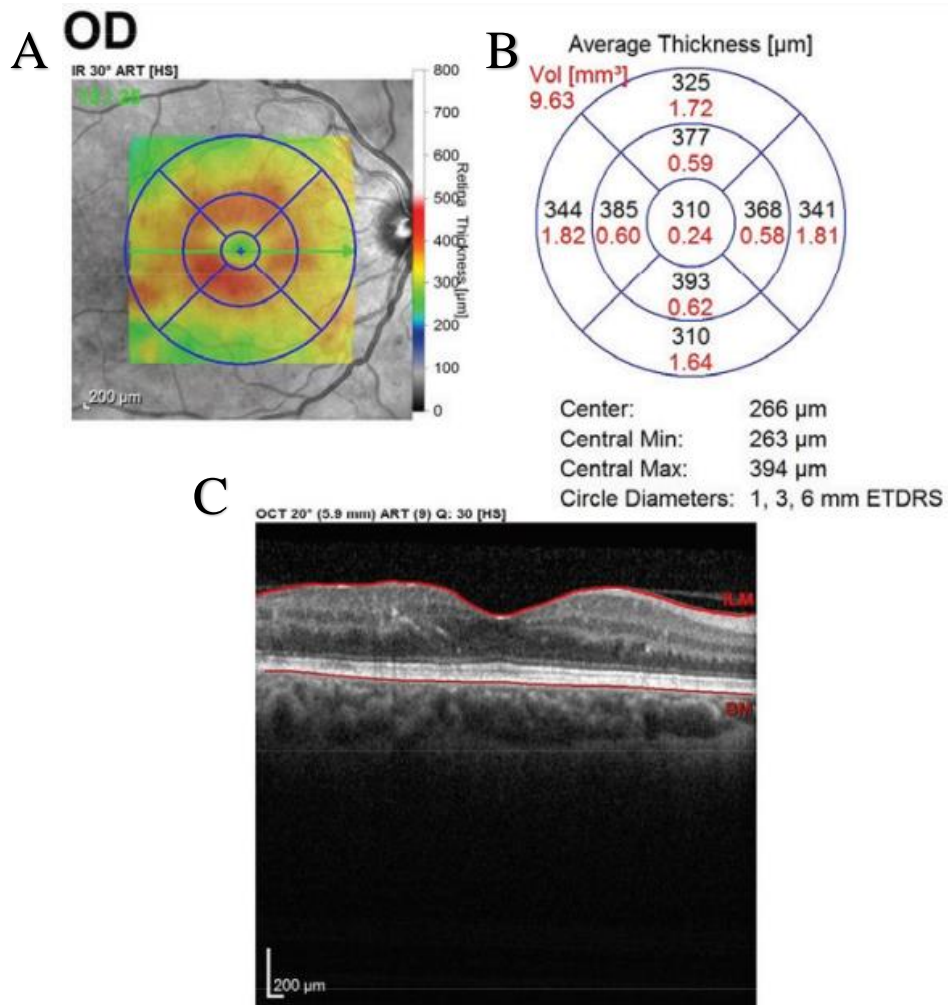


Figure 3. An OCT scan and thickness map of the right eye.

A sample printout of an OCT retinal thickness map using Heidelberg Spectralis instrumentation and software. The right eye shown here exhibits mild thickening of the macula due to complications from diabetes mellitus. (A) A heat map overlaying an image of the fundus indicates relative retinal thickness at the region encompassed within the diameter of the central point thickness circle. (B) Values of average thickness are automatically calculated and displayed in quadrants of the circles along with reference values of the center point thickness, the central minimum, central maximum, and diameter of the circles. (C) A scan through the retina visualizing the various layers and elucidating the structure of the retina. The ILM and BM are outlined in red and demarcate the boundaries of the retina. Re-printed from S.D. Nicoară, 2018.

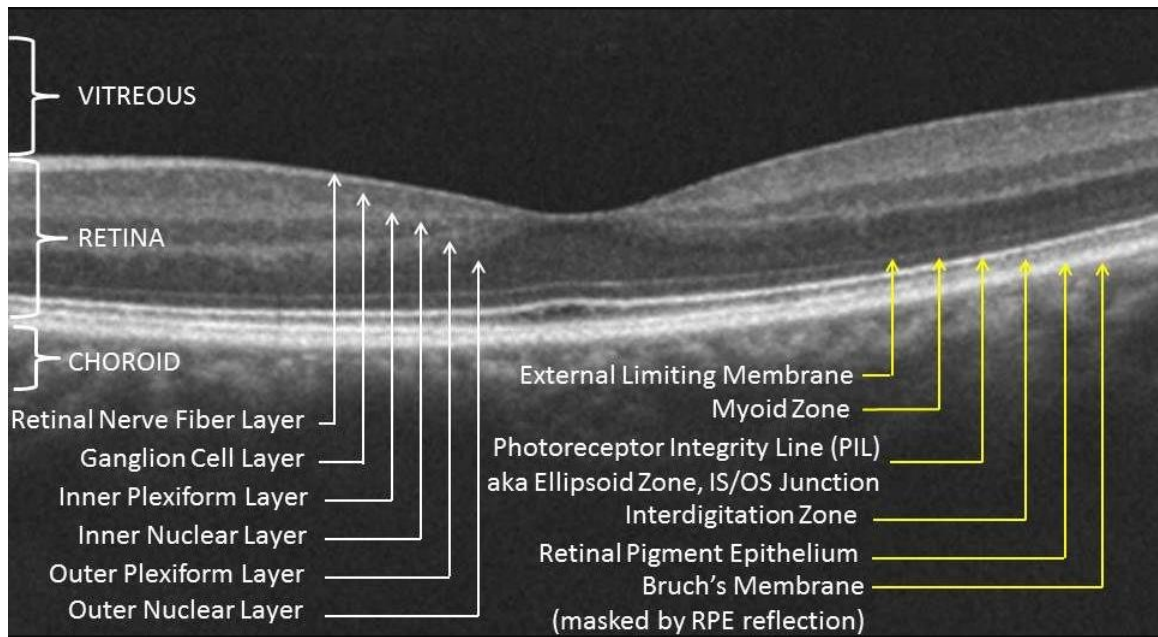


Figure 4. OCT of the retinal layers.

A labelled section of an optical coherence tomography scan with arrows indicating the various layers of the retina along with associated structures. The fovea is visible as an indentation present at the area with the most diminished total retinal thickness but greatest outer nuclear layer thickness. Re-printed from E. Biffi, New England College of Optometry, 2019.

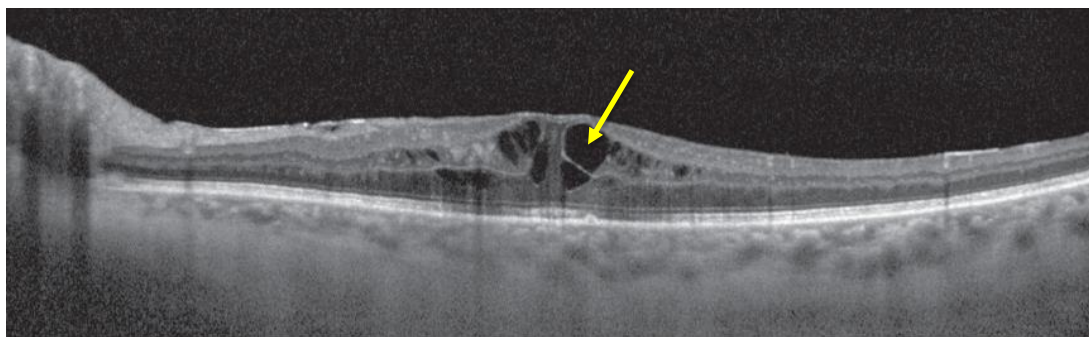


Figure 5. OCT scan of macular edema seen in a retina with radiation retinopathy.

An OCT scan through the retina at the level of the fovea with evidence of macular edema. A yellow arrow points to a space indicating presence of fluid between layers of the retina. Several similar spaces can be observed adjacent to the indicated site. Adapted from Gündüz et al., 2019.

Because clinical presentations of radiation retinopathy are very similar and nearly identical to diabetic retinopathy, care must be taken to obtain a complete medical history in order to differentiate between the two diagnoses. Although history of retinal exposure to ionizing radiation is a strong predictor for the development of radiation retinopathy in many cases, it does not absolutely ensure that disease progression will occur. Complete medical history including preexisting comorbidities must be taken into account before other differential diagnoses can first be ruled out. Other similar retinopathies and ophthalmopathies that share some clinical presentation with radiation retinopathy are branch and central retinal vein occlusions, retinal telangiectasia, hypertensive retinopathy, Coats' disease, and human immunodeficiency virus retinopathy (Kon Graversen, 2016).

Management

Currently, no standard treatments for radiation retinopathy have been established by the United States Food and Drug Administration. Because of the oftentimes nearly identical clinical presentations of radiation retinopathy and diabetic retinopathy, many therapeutics and techniques used to treat diabetic retinopathy are frequently employed to manage complications of radiation retinopathy with varying degrees of success (Yu and Scheffler, 2020). Treatment options range from laser photocoagulation therapy to pharmacological agents and surgical techniques (Reichstein, 2015).

Conventional laser photocoagulation techniques involve use of high-powered pulses of light targeted onto affected areas of the retina. High-powered lasers cause a photothermal reaction causing conversion of light energy into heat to vaporize and

coagulate retinal tissue, which is believed to limit the spread of macular edema and downregulate factors that may promote neovascularization (Kozak and Luttrull, 2015). Laser therapy has been employed to treat radiation retinopathies that present with either or both retinal and macular edema, though the greatest benefit regarding the use of laser therapy seems to be as a preventative measure rather than a reliable solution for long-term resolution of symptoms (Yu and Scheffler, 2020).

An interventional case series by Finger and Kurli found that symptoms of radiation retinopathy had resolved in 64.4% of patients treated with laser photocoagulation. In the same study, sixteen patients had been given prophylactic laser treatment following irradiation for choroidal melanomas and before development of clinically evident symptoms of radiation retinopathy. Out of the sixteen patients, only three exhibited continued disease progression, though it is not known whether all sixteen patients would have definitively developed radiation retinopathy without prophylactic laser treatment (Finger and Kurli, 2005). A prospective chart review of nineteen patients described regression of macular edema in five patients within six months following focal laser therapy, and eight patients demonstrated greater than one Snellen line improvement in visual acuity. However, upon follow-up evaluation two years later, visual acuity had diminished to levels not significantly different from patients who had not undergone laser therapy (Hykin et al., 1998).

Anti-VEGF agents have been widely studied as pharmacological options to treat a variety of ophthalmopathies and have shown effectiveness in managing retinal edema and vasculopathy. Patients who have prior history of uveal melanomas often carry higher

concentrations of VEGF-A and are likely to respond to anti-VEGF agents (Missotten et al., 2006). A 2016 study of 120 patients observed that 80% had stabilized or improved visual acuity following treatment using anti-VEGF therapy. Projections of retaining stable visual acuity five and eight years later were 69% and 38%, respectively. In addition to reduction or regression of either or both macular and retinal edema, cotton-wool spots were also resolved following anti-VEGF therapy (Finger et al., 2016). Common anti-VEGF agents include bevacizumab, aflibercept, and ranibizumab. Triamcinolone acetonide, a corticosteroid, does not target VEGF but has been used alone or in conjunction with anti-VEGF agents and has demonstrated reduced macular edema (Shah et al., 2013).

Surgery is not employed as often as other techniques, but it may be considered in order to alleviate either or both vitreous hemorrhaging and retinal detachment observed in some serious cases of radiation retinopathy. Pars plana vitrectomy is traditionally recommended when other options such as intravitreal injections or laser therapy are not indicated to be effective enough to manage disease complications. Surgical options are not without significant risk, however, and patients may be more susceptible to developing additional complications such as increased risk of infection, retinal tears or further detachments, macular holes, or macular puckers (Yu and Scheffler, 2020).

A 2015 study observed that eight out of sixteen cases that had undergone vitrectomy to treat vitreous hemorrhaging and retinal detachment associated with radiation retinopathy reported improvement in vision following surgery. Nine cases exhibited anatomic resolution of symptoms (Chia et al., 2015). Surgical intervention has also been investigated as a prophylactic measure to abate potential side effects and reduce risk of retinopathy from

radiation scatter during treatment for uveal melanomas. Out of twenty patients who underwent pars plana vitrectomy with silicone injection simultaneously with treatment via iodine-125 plaque brachytherapy, roughly two-thirds of cases reported “good” visual outcomes following combined surgery and brachytherapy. In contrast, about a quarter of control patients who did not undergo vitrectomy and completed brachytherapy only reported “good” visual outcomes (McCannel et al., 2016). Vitrectomy has been found to increase the oxygen levels in the vitreous after surgery (Stefánsson, 2001).

Hyperoxia Therapy

Hyperoxia therapy refers to the administration of supplemental oxygen in concentrations higher than normally breathed values (Mach et al., 2011). Exposure to an excessive amount of oxygen can result in increased risk of oxidative damage and oxygen toxicity; however, in cases of hypoxia and hypoxemia, hyperoxia therapy has become a useful treatment to increase the amount of dissolved oxygen in the blood and restore oxygenation to affected cells and tissues (Casillas et al., 2019).

Hyperoxia therapy can be divided into hyperbaric oxygen therapy (HBOT) and normobaric hyperoxia (NBH). HBOT is the administration of supplemental oxygen at both elevated concentrations and pressures compared to normal environmental and physiological conditions. NBH, in contrast, involves supplemental oxygen at higher than normal concentrations but at one atmosphere of total pressure (Bennett et al., 2015).

Fraction of Inspired Oxygen

Fraction of inspired oxygen (FiO₂) refers to an estimate of the proportion of inhaled oxygen within the inspired gaseous mixture. Breathing normal, atmospheric air results in a FiO₂ of 21%, which is reflective of the amount of oxygen in atmospheric air regardless of altitude. Values of FiO₂ bear consequence on the partial pressure of oxygen available in the alveoli (P_{AO2}) and ultimately the systemic arterial circulation (P_{aO2}). These values are quantifiable through the alveolar gas equation: $P_{AO2} = (P_{ATM} - P_{H2O} \times FiO_2) - (P_{aCO2} / R_q)$. Assuming no pathological barriers to diffusion exist in the lung, increasing FiO₂ thus increases P_{aO2} and the amount of free molecular oxygen dissolved in the blood. A greater amount of dissolved oxygen available in the circulatory system has clinical significance as a potential solution to restore oxygenation for patients who suffer from partial or systemic hypoxia (Fuentes and Chowdhury, 2021).

Use in Animal Models of the Retina

Several studies of hyperoxia in animal models of normal and diseased retinas have presented evidence for the potential therapeutic benefit of hyperoxia for treating ocular disorders. In 1989, Linsenmeier and Yancey first characterized the interaction between retinal metabolism and increased oxygenation by measuring the distribution and consumption of oxygen in cat retinas during conditions of hyperoxia. During hyperoxia, the majority of the oxygen tension in the retina was found to be contributed by oxygen diffusing from the choroid instead of the central retinal artery (Linsenmeier and Yancey, 1989). A later study by Wang and Linsenmeier also showed that hyperoxia administration

was able to improve photoreceptor survival rates in detached cat retinas and likely conferred a protective effect against further damage to the remaining photoreceptors (Wang and Linsenmeier, 2007).

A 1989 study by Pournaras et al. supported Linsenmeier and Yancey's 1989 findings by demonstrating that rates of oxygen diffusion across pig retinas improved following administration of hyperoxia. Miniature pigs with normal and ischemic retinas were anesthetized and exposed to conditions of hyperoxia while partial pressure of oxygen in the retinal tissue was measured using microelectrodes. When compared to control values obtained during conditions of normoxia, the authors found that hyperoxia provided sufficient oxygenation to allow the choroid to supply oxygen through the entire thickness of the ischemic retina (Pournaras et al., 1989). In a similar model conducted on a primate model of retinal ischemia, Pournaras et al. also determined that hyperoxia lowered the expression of VEGF while restoring oxygenation to the retina (Pournaras et al., 1997).

The combinatorial effect of both normobaric hyperoxia and VEGF inhibitors on mouse models of oxygen-induced retinal ischemia was investigated in a 2011 experiment by Zhang et al. Hyperoxia treatment was shown to ameliorate vascular repair and facilitate inhibition of neovascularization during the pre-proliferative phase of ischemic retinopathy, providing further evidence to support the use of hyperoxia for the treatment of ophthalmopathies (Zhang et al., 2011).

Clinical Use of Hyperoxia for Retinopathies

Many retinopathies involve a disruption in the ability to properly oxygenate the retina due to thickening, separation of retinal layers, or vascular disruption causing edema and detachment. Often these complications create a hypoxic environment, but increased oxygen availability in the choroid and, to a lesser extent, the retinal artery during hyperoxia treatment can deliver enough available oxygen to replenish most of the metabolic demands of the retina (Murphy-Lavoie et al., 2012).

Diabetic Retinopathy

Several works describing the beneficial effects of hyperoxia on diabetic retinopathy have been published. The role of hyperoxia to treat symptoms of diabetic retinopathy was first described in 1996 study of twelve patients suffering from ocular complications due to diabetes. Participants were given 100% O₂ at normal atmospheric pressure for fifteen minutes, increasing end tidal partial pressure of oxygen up to 700 mmHg. Following hyperoxia administration, contrast sensitivity was found to have significantly improved in the hyperoxia cohort when compared to controls (Harris et al., 1996). Five patients who received 36% FiO₂ (4 L/min O₂) at normal atmospheric pressure continuously over the course of three months as part of a pilot study exhibited reduced thickness at the fovea by about 43.5% while excessive macular volume was reduced by 54% (Nguyen et al., 2004). HBOT also ameliorated breakdown of the blood-retinal barrier in diabetic retinopathy (Chang et al., 2006). A 2012 study on the acute effects of hyperoxia on diabetic macular

edema in eleven patients showed a 1.2% reduction in the volume of the macula two hours following treatment (Vinten et al., 2012).

Retinal Artery and Vein Occlusions

Cases of central retinal artery occlusion (CRAO) and retinal vein occlusion have also shown improvements in symptoms following hyperoxia treatment. Administration of supplemental hyperoxia within twenty-four hours of first reported loss of vision is a well-established treatment to facilitate restoration of vision and prevent permanent damage due to hypoxia-induced cell death of the retina in patients with central retinal artery occlusions (Olson, 2016). NBH therapy results in treatment effectiveness similar to HBOT in many cases, although more severe cases of CRAO are noted to respond better to hyperbaric treatments (Murphy-Lavoie et al., 2012). In a recent study by Arroyo et al., participants diagnosed with macular edema from complications due to retinal vein occlusion were given 40% FiO₂ (5 L/min O₂) NBH for three hours. Retinal thickness measurements were collected before and after supplemental oxygen administration and compared. Mean central macular thickness was decreased by 4.64% and maximum macular thickness decreased by an average of 7.10% (Arroyo et al., 2021).

Radiation Retinopathy

Two case studies have been published investigating the use of hyperbaric oxygen therapy (HBOT) for the treatment of radiation retinopathy in human patients. A 2007 article by Gall et al. described a 63-year-old woman who had been diagnosed with severe

radiation retinopathy in the left eye four years after treatment of a choroidal melanoma with ruthenium-106 brachytherapy. Evidence of hard exudates on the retina surrounding the optic nerve were discovered upon examination of the fundus. Visual field testing showed that she could only perceive visual stimuli in the upper and lower margins of her field of view. The patient had a history of adverse reactions to steroid medication and was recommended to trial HBOT as an alternative treatment option. Twenty sessions of two-hour 100% O₂ at a pressure of two atmospheres were performed. Two months post HBOT, follow-up examination revealed complete resolution of hard exudates upon the retina and visual field testing showed that a significant portion of the lower half of her field of vision had been restored (Gall et al., 2007).

In a 2010 case report by Haji and Frenkel, a 62-year-old man presented with radiation-induced macular edema in his left eye. Over the course of one month, the patient completed eighteen HBOT sessions at 100% O₂ at a pressure of two atmospheres. Following HBOT, visual acuity measurements showed marked improvement from the 20/100 level to 20/50. Macular thickness was stabilized, indicating that retinal edema had not worsened, and fluorescein angiography showed some evidence of improved perfusion of the macula. As a caveat, the investigators noted that the improvement in vision and symptoms could have been attributed to the effects of an intravitreal triamcinolone injection administered prior to beginning HBOT. Alternatively, improvement in vision and radiation retinopathy symptoms could also have been due to a combinatorial effect of HBOT complimenting the actions of intravitreal triamcinolone (Haji and Frenkel, 2010).

To date, no studies investigating the effects of normobaric hyperoxia on radiation retinopathy have been reported. Since similar retinopathies have seen improvement in symptoms following NBH therapy when compared to HBOT studies, it can be hypothesized that NBH treatment will elicit beneficial responses in cases of radiation retinopathy.

SPECIFIC AIMS

Given the previous success of hyperoxia treatment for retinopathies involving retinal vascular disorders, we hypothesized that retinal edema associated with radiation retinopathy will also benefit from normobaric hyperoxia treatment. Our study sought to determine whether low dose normobaric hyperoxia therapy reduced retinal edema and improved visual acuity in a case study of a patient with radiation retinopathy. We hope that our findings will further expand current knowledge regarding the use of normobaric hyperoxia in the clinical ophthalmologic setting as a potential disease management option for radiation retinopathy and lend support for the use of hyperoxia as a novel treatment for less common ocular disorders involving retinal vascular disease.

METHODS

Study Recruitment and Design

Three patients were initially screened for eligibility and invited to participate in our study of short-term NBH treatment. Inclusion criteria consisted of several parameters: patients must have received confirmed diagnoses of radiation retinopathy at the Retina Service at Beth Israel Deaconess Medical Center (BIDMC), presented with evidence of vision loss and retinal or macular edema, and had symptoms that did not resolve or respond well to previous intravitreal injections or laser therapy. Patients who had been diagnosed with chronic obstructive pulmonary disorder, diabetes with ocular complications, and any other disease involving the retinal vasculature were excluded from consideration in order to reduce potential confounding variables. This study was approved by the BIDMC Institutional Review Board. Following an explanation of the study's investigative purpose and possible side effects and consequences, the patients signed consent forms prior to beginning NBH trials (Appendix, Figure A).

Of the three participants initially recruited to the study, one participant was able to maintain follow-up appointments and complete all NBH and NBN trials. Two three-hour 40% FiO₂ NBH trials were conducted five months apart. Before each trial, an ophthalmic examination was performed. VA measurements and OCT scans were taken before and after each trial (Appendix, Figure B). In between trials, the patient returned to BIDMC for additional routine ophthalmic evaluations to monitor for any significant or adverse changes during the course of the study. To determine whether the observed changes in visual acuity, contrast sensitivity, and retinal thickness following each session were not due to random

chance or a separate confounding factor, one three-hour 20% FiO₂ normobaric normoxia (NBN) session was performed one month after the second NBH session and served as the study's control (Appendix, Figure C).

Ophthalmologic Examination

At the beginning of each examination, the participant's chief complaints and all associated symptoms were recorded. Past medical history, including current medications and family history, were confirmed. Best-corrected visual acuity and intraocular pressures were measured for both eyes.

Topical anesthetic eye drops were administered to both eyes prior to intraocular pressure measurements in order to prevent discomfort from the handheld tonometer. Sympathomimetic eye drops (2.5% phenylephrine) and anticholinergic eye drops (0.5% tropicamide) were then applied to both eyes to enable rapid and wide dilation.

Basic slit lamp biomicroscopy and a binocular indirect ophthalmoscope were used to conduct the examination, which was performed by the same attending ophthalmologist at each appointment. A complete assessment of the fundus was completed and any changes or significant findings from the last visit were recorded.

Normobaric Oxygen Administration

An EverFlo portable oxygen concentrator manufactured by Philips Respironics was used to deliver normobaric hyperoxia through a Hudson facemask fitted over the participant's nose and mouth. For each NBH trial, a constant stream of 40% FiO₂ (5 L/min

O₂) at normal, atmospheric pressure was administered to the participant over the course of three hours. For the NBN control session, a sham portable oxygen concentrator was used to deliver room air at normal, atmospheric pressure through the same Hudson facemask that had been used for the NBH trials.

The participant was awake and seated for the duration of each session in a temperature-controlled, clinical examination room illuminated by ambient lighting. A researcher and attending physician were present to monitor the participant for signs of adverse reactions or distress during the duration of the supplemental oxygen administration.

Visual Acuity

A back-illuminated Early Treatment Diabetic Retinopathy Study (ETDRS) chart composed of rows of successively smaller letters was used to assess best-corrected distance visual acuity at twenty feet. Each eye was tested separately before topical anesthetic or dilating eye drop application. During NBH and NBN sessions, visual acuity was measured immediately prior to and following supplemental oxygen administration. The same ETDRS chart and clinical examination room was used for each repeat follow-up appointment and supplemental oxygen session. Visual acuity measurements were scored as fractions with superscripts denoting how many additional or fewer letters the participant was able to identify in the next or prior lines, respectively. Fractional values were then converted to LogMAR values for quantitative analysis.

Optical Coherence Tomography

A single operator conducted all OCT scans on the participant before and after each NBH or NBN session. A Heidelberg Spectralis instrument manufactured by Heidelberg Engineering (Heidelberg, Germany) was used to perform OCT scans. Images were taken in follow-up mode to ensure fidelity of measurements along the same area of maximal edema. The operator ensured that the participant's head and pupils were aligned correctly with the instrument and that the fundus was optimally focused and illuminated before proceeding with each scan.

Scans were centered on the fovea and included the optic nerve head, encompassing both macular and peripapillary (i.e. surrounding the optic nerve) edema. Horizontal, vertical, and volume scans through the retina were performed. To obtain measurements of center point thickness (CPT) along the site of maximal edema, a circle of six millimeters in diameter was manually moved to five points chosen around the optic disc. Calculations of volume, thickness, and retinal parameters were performed automatically by the Heidelberg Spectralis software, and data was exported for further analysis.

Statistical Analysis

CPT values were collected from OCT measurements at five points centered along the area of maximal retinal edema before and after each NBH and NBN session. The CPT values from each session were averaged to give a mean retinal thickness with standard deviations before and after each trial of supplemental oxygen. Mean retinal thickness following NBH was subtracted from mean retinal thickness prior to NBH administration

to give a mean difference in retinal thickness. Percent differences and standard deviation in retinal thickness following each supplemental oxygen study were also calculated using the same CPT values that were used to determine mean retinal thickness. Standard deviations were also calculated for each percent difference.

Mean differences and percent differences in retinal thickness for each NBH measurement were compared against NBN measurements from the same participant along the same points of maximal edema, with the measurements from the NBN study serving as a control. A student's one-tailed t-test was used to analyze statistical significance and determine whether any significant reduction in mean retinal thickness existed. Significance was defined as p-values below a threshold of $\alpha=0.05$.

RESULTS

Case History and Presentation

A 32-year-old, Caucasian male was referred to the Retina Service at BIDMC in 2001 by his optometrist following a routine eye exam for myopic astigmatism after the discovery of a choroidal nevus in the left eye. Prior medical history included asthma, high cholesterol, generalized anxiety disorder, and previous back surgery, but no other known ocular conditions aside from myopic astigmatism were reported. The patient had never been diagnosed with diabetes or hypertension. His father had previously been diagnosed with prostate cancer while his sister had been diagnosed with breast cancer. Regarding prior family history of eye diseases and disorders, he reported that his maternal grandmother had suffered from age-related macular degeneration, while his mother and father both had a history of cataracts. When a history of present illness was conducted, the patient denied the presence of flashes, floaters, or ocular pain. He denied knowledge of any allergies and reported no current medications.

Upon ophthalmic examination, the lesion was found to be flat and measured three millimeters by six millimeters. Best-corrected visual acuity was found to be 20/20 in both eyes following Snellen chart testing. Intraocular pressures were normal. The growth was indicated to be benign but with the potential to grow into a melanoma. His right eye was found to be unremarkable for retinal changes. Routine follow-ups were recommended every six months to monitor for any significant changes.

Approximately fourteen years later (2015), the patient returned to the Retina Service at BIDMC with complaints of distorted vision in his left eye. His retinal exam revealed that his choroidal lesion had grown laterally in size as well as elevation. Evidence of new subretinal fluid suggested recent growth, and the patient was diagnosed with uveal melanoma in the left eye. The patient was referred to Massachusetts Eye and Ear for proton beam irradiation to treat the malignancy, which was performed one month later. Intravitreal injections of bevacizumab (1.25 mg/0.5 mL) in the left eye every two months were also prescribed to prevent radiation retinopathy.

Despite anti-VEGF therapy, the patient was diagnosed with radiation retinopathy four months following irradiation (2016). Intravitreal bevacizumab injections were continued every two months but visual acuity in the left eye continued to steadily worsen to the 20/60⁻² level. Approximately two years after the radiation retinopathy diagnosis (2018), OCT scans revealed evidence of new retinal edema located superiorly around the optic disc. Prominent peripapillary fluid was observed while some mild subretinal fluid was found in the foveal area. Inferonasal capillary dropout was also noted. Intravitreal triamcinolone acetonide (2 mg) was recommended and intravitreal bevacizumab was discontinued. At a follow-up appointment three months later, OCT imaging revealed increased intraretinal fluid and the patient was prescribed 2 mg intravitreal aflibercept (Eylea) for the left eye every two months. He was then referred back to the Retina Service at BIDMC for continued care.

At the time of presentation, his medications included 10 mg fluoxetine, 10 mg montelukast, 10 mg omeprazole, and 10 mg simvastatin. He was status post ten intravitreal

bevacizumab injections, one intravitreal triamcinolone acetonide injection, and two intravitreal aflibercept injections (Figure 6). The patient denied any new flashes or floaters and reported no ocular pain. Intraocular pressures were normal. OCT imaging revealed evidence of severe peripapillary edema in the left eye (Figure 7). His best-corrected visual acuity was 20/20 in the right eye and 20/80⁺² in the left eye.

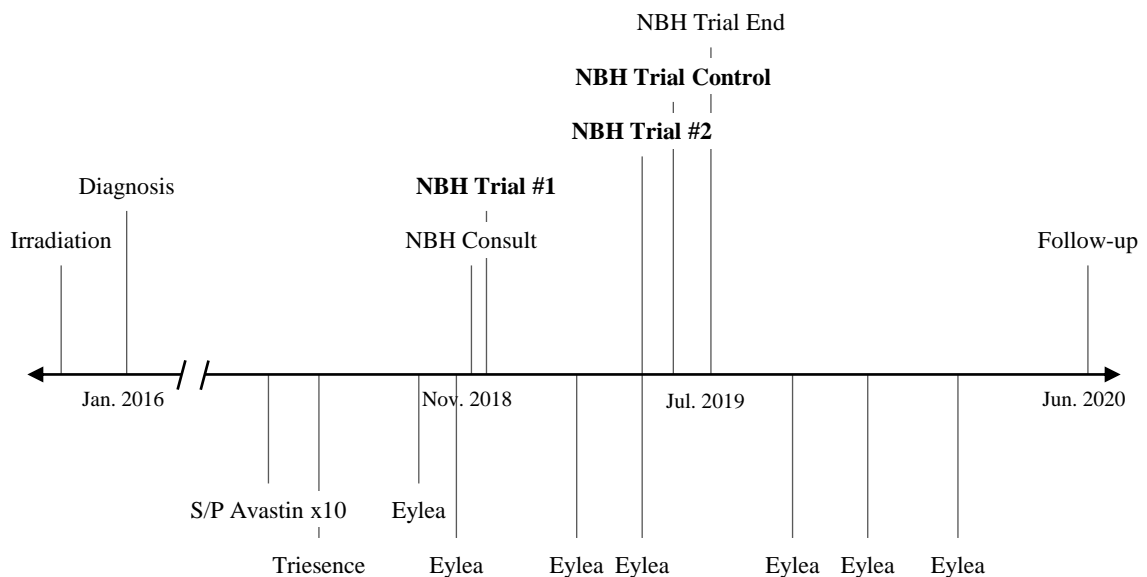


Figure 6. Timeline of case presentation.

A timeline delineating the course of the patient’s case history from time of irradiation and radiation retinopathy diagnosis to final follow-up appointment. Notable clinical events are indicated by lines above the horizontal axis while timing of intravitreal injections are represented lines below the horizontal axis, both of which have been spaced at intervals relative to their time of occurrence. The NBH trials and control session are indicated in bold.

Normobaric Hyperoxia and Normoxia Trials

Retinal thickness along the area of edema was found to be reduced by an average of $27 \pm 7.14 \mu\text{m}$ after the first NBH trial with a mean percent reduction in retinal thickness of $4.35 \pm 1.01\%$ (Table 1, Figures 8-9). Best-corrected VA was improved from $20/63^{-2}$ (LogMAR=0.54) prior to supplemental oxygen administration to the $20/63^{+1}$ (0.48) level directly after the first NBH trial (Table 2, Figure 10).

After the second NBH trial, average retinal thickness along the area of edema was reduced by an average of $20.2 \pm 2.59 \mu\text{m}$ with a mean percent reduction in retinal thickness of $4.42 \pm 0.58\%$ (Table 1, Figures 8-9). Best-corrected VA was improved from $20/50$ (0.4) prior to the session to $20/32^{-1}$ (0.22) immediately after (Table 2, Figure 10).

Following the NBN control session, average retinal thickness was observed to have decreased by $5.8 \pm 0.84 \mu\text{m}$ following the session, and the mean percent reduction in retinal thickness was found to be $1.71 \pm 0.23\%$ (Table 1, Figures 8-9). Best-corrected VA remained relatively the same at $20/40^{-1}$ (0.32) prior to beginning the NBN trial and $20/40$ (0.3) directly following conclusion of the NBN session (Table 2, Figure 10).

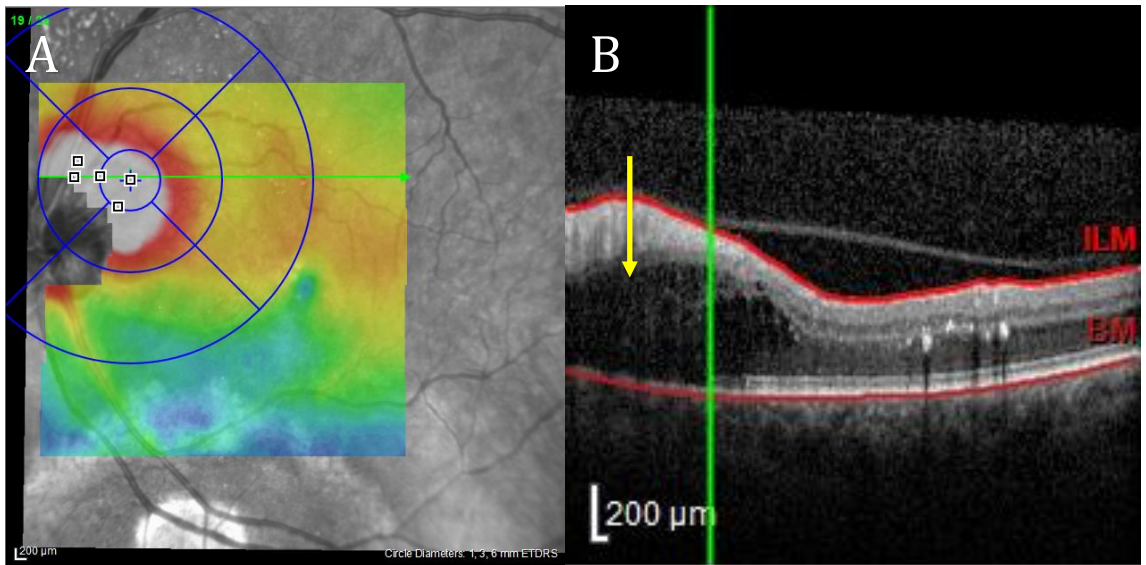


Figure 7. OCT scan of the fundus and retina.

(A) Optical coherence tomography thickness map image of the fundus of the left eye. Each square represents the location of center point thickness measurements that were taken along the area of maximal edema, outlined by the region in red. (B) A vertical scan through the retina at the site of maximal edema demonstrating accumulation of fluid between retinal layers. ILM and BM denote the boundaries of the retina while the green vertical line represents the axis of the scan. A yellow arrow points to evidence of edema.

Table 1. Measurements of retinal thickness before and after supplemental oxygen.

A summary of the mean retinal thickness measured by OCT scans in micrometers before and after each NBH or NBN (control) session, the average difference in retinal thickness, and the percent reduction in retinal thickness following administration of oxygen therapy.

	NBH 1		NBH 2		Control	
	Before	After	Before	After	Before	After
Mean (μm)	617.8	590.8	457.4	437.2	338.4	332.6
Difference (μm)		27 \pm 7.14		20.2 \pm 2.59		5.8 \pm 0.84
% Change		4.35 \pm 1.01		4.42 \pm 0.58		1.71 \pm 0.23

Table 2. Best-corrected visual acuity before and after supplemental oxygen.

Measurements of ETDRS visual acuity and their corresponding LogMAR values taken before and after each NBH and NBN (control) session. A lower LogMAR value corresponds to higher visual acuity.

	Before		After	
	ETDRS	LogMAR	ETDRS	LogMAR
NBH 1	20/63 ⁻²	0.54	20/63 ⁺¹	0.48
NBH 2	20/50	0.4	20/32 ⁻¹	0.22
Control	20/40 ⁻¹	0.32	20/40	0.3

Retinal thickness was found to be significantly reduced following each NBH trial when compared to NBN measurements ($p=0.001$ and 0.0002 , Figure 7). The patient showed a significant percent decrease in retinal thickness along the area of edema after hyperoxia treatment (Figure 8) while VA was seen to have changed from the 20/80 level before the trials to 20/40 in the left eye after the trials (Table 2, Figure 10).

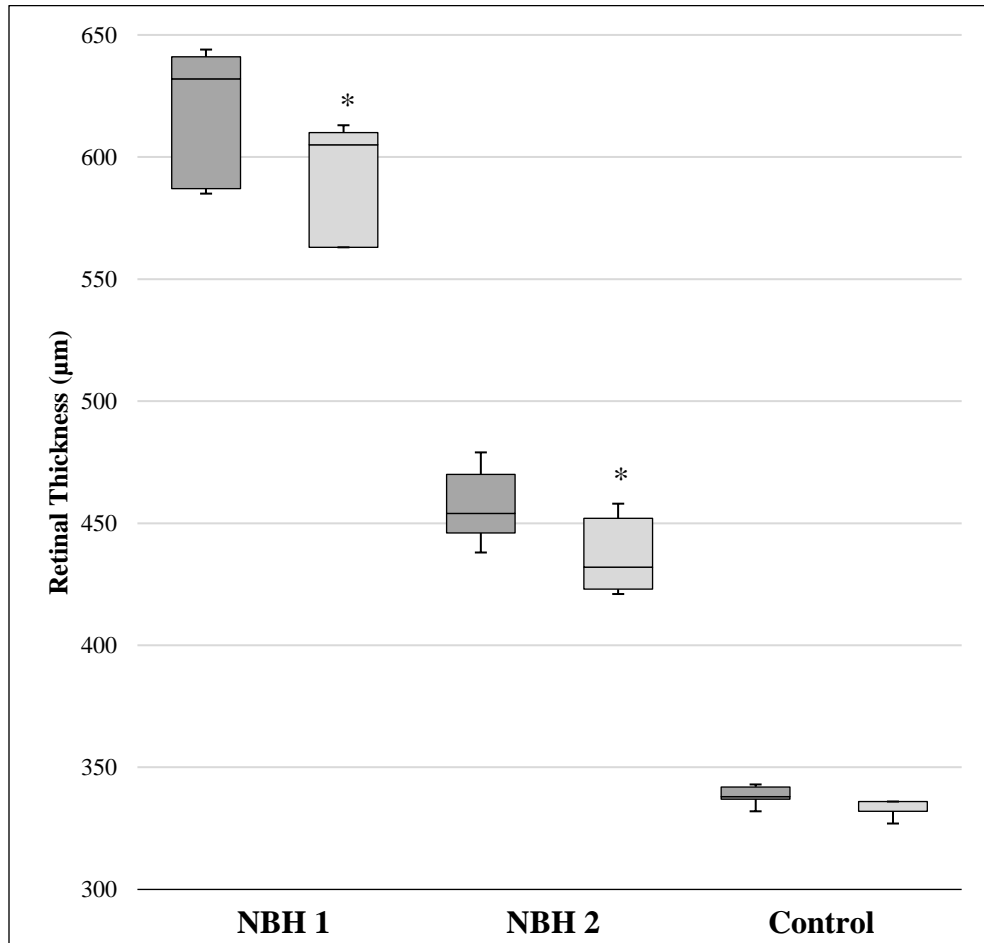


Figure 8. Retinal thickness measurements before and after supplemental oxygen. Darker colored box and whisker plots represent retinal thickness measurements taken before administration of NBH or NBN. Lighter colored box and whisker plots represent retinal thickness measurements after each session. Asterisks denote statistically significant reduction when compared to control values.

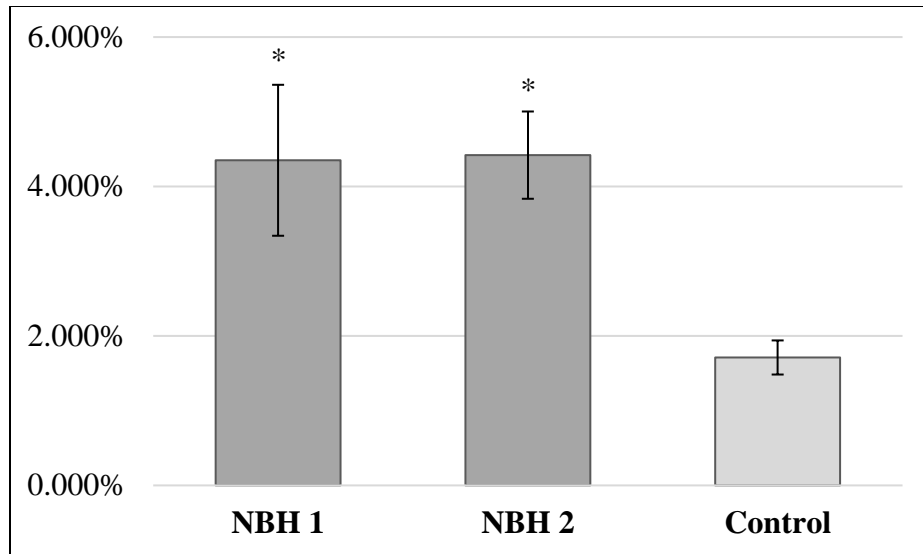


Figure 9. Percent reduction in retinal thickness following supplemental oxygen.

Average percent reduction in retinal thickness following administration of NBH or NBN (control). Standard deviation is represented by error bars. Asterisks denote a statistically significant reduction in retinal thickness when compared to the control.

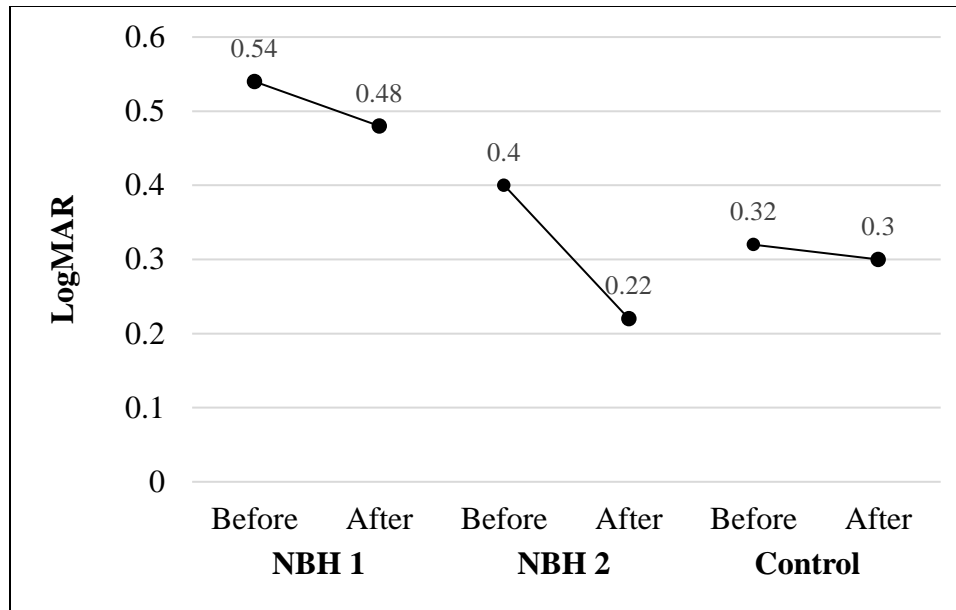


Figure 10. Visual acuity measurements before and after supplemental oxygen.

ETDRS measurements were converted to LogMAR values for quantitative analysis. Values are given above each point. Lower LogMAR values correspond to greater visual acuity.

The patient tolerated hyperoxia treatment well with no signs of adverse reactions or events. No instances of incomplete treatment or deviations from protocol were recorded.

Outcomes Post-Trials

One month after completing the NBH study, the patient returned to the Retina Service at BIDMC for a follow-up visit. No significant changes in VA or retinal edema were noted from the last visit. Best-corrected VA in the left eye was 20/40⁺². OCT scans indicated no changes in retinal edema, which was no longer recurring. No further treatments and continued observation were recommended, and the patient was referred back to Massachusetts Eye and Ear for routine care.

The patient completed three more intravitreal aflibercept injections, spaced two months apart, in the left eye. At a follow-up appointment one year after the conclusion of the low dose NBH study (2020), the patient's retinal edema was shown to have fully regressed, and best-corrected visual acuity was stabilized at the 20/40⁻² level in the left eye and 20/20 in the right eye.

DISCUSSION

Retinal thickness was found to be significantly reduced and visual acuity improved following administration of three hours of 40% FiO₂ NBH. This is the first report of low-dose NBH therapy contributing to a rapid and significant reduction in retinal edema and an improvement in VA when compared to sham treatment in a patient with radiation retinopathy.

Our findings support and parallel the results of other NBH studies conducted in patients with diabetic macular edema and retinal vein occlusion that also found rapid, significant reduction in macular edema and immediate improvement in vision (Nguyen et al., 2004; Arroyo et al., 2021). Given that the pathophysiology of all three of these diseases results in inner retinal ischemia, our findings regarding the use of supplemental NBH to treat an oculopathy involving disorder of the retinal vasculature are not unexpected.

In our patient, maximal retinal edema was located around the optic disc with some minimal edema involving the fovea. The macula and the fovea are known to constitute the majority of central vision, while the periphery of the retina contributes in varying degrees to visual perception (Riordan-Eva, 2017). Many ocular disorders and retinopathies that cause the greatest impairment of vision often involve the fovea and the macula instead of the optic disc. If the area of maximal retinal edema had involved the macula and fovea, we might have expected a greater and more consistent improvement in visual acuity following treatment with supplemental oxygen. Though our patient demonstrated improved visual acuity following NBH treatment, further repeat trials with larger groups of participants are

needed in order to calculate whether any visual acuity changes associated with NBH represent statistically significant improvements.

Furthermore, while oxygen delivery at higher than atmospheric pressures in hyperbaric chambers has been shown to improve vision in patients with radiation retinopathy, hyperbaric oxygen treatment is associated with significantly greater cost, less availability, and greater risk of systemic complications. High operating costs and resources required to outfit or maintain hyperbaric chambers may incur financial burden to a care facility not already equipped with one (Lipsky and Berendt, 2010; Oguz and Sobaci, 2008). In contrast, NBH treatments present a more affordable, accessible, and safer alternative to HBOT (Singhal et al., 2005). Portable oxygen concentrators can be more easily obtained by clinics and hospitals and prescribed for home use, negating the need for patients to return to care facilities for continued treatment.

The long-term, systemic effects of NBH for use in the treatment of ophthalmic disorders has not yet been well characterized. HBOT has been implicated as a cause for myopia and cataract formation, though whether NBH may elicit the same risks in patients is yet to be studied (Palmquist et al., 1984). Experimentally, exposure to normobaric hyperoxia for four hours a day over the course of one week was shown to increase incidence of nuclear cataract formation in rabbit lenses (Wang et al., 2009). It is unclear whether these effects may be replicated in vivo in human eyes.

While NBH is a common therapy employed to manage hypoxemic and hypoxic conditions, increased exposure to greater than normal FiO_2 over an extended period of time in an individual not suffering from oxygen deficiency may lead to toxicity. Exposure to

higher than atmospheric levels of oxygen have been shown to increase production of reactive oxygen species in the body, which potentially could lead to inflammatory changes and oxidative damage to tissues (Mach et al., 2011). Our study employed low dose NBH at a flow rate recommended to minimize risk of developing complications due to hyperoxia (Mach et al., 2011). Further long-term follow-up studies, however, are needed to fully characterize potential risks and benefits of extended NBH treatment.

To understand the efficacy of NBH and its applicability to wider patient populations suffering from radiation retinopathy, repeat studies with larger cohorts of patients should be performed. In addition, future studies may vary the duration of NBH treatment to determine whether longer periods of hyperoxia may exhibit more marked effects or confer additional benefit to patients. Alternatively, the duration of NBH treatment could be reduced to determine whether shorter periods of supplemental hyperoxia administration may be sufficient to elicit similar reductions in edema and improvements in visual acuity.

Two normobaric hyperoxia trials were performed in our study, and it is unknown how the patient and his symptoms would have responded to further sessions. Repeat studies should test whether further sessions of NBH would continue to correspond to reduction of edema and improvement of visual acuity or if there exists an upper limit to the efficacy of NBH in treating ocular vasculopathies. It is worth noting that even after the NBN control session, some reduction in retinal edema was seen. This reduction may have been due to increased rebreathing of CO₂ from the Hudson facemask, which would have led to slightly elevated blood CO₂ levels (Campkin et al., 1993). In response to elevated partial pressures of CO₂, central chemoreceptors and homeostatic mechanisms will act immediately to

increase cerebral blood flow in order to transport and eliminate excess CO₂ (Battisti-Charbonney et al., 2011; Jensen et al., 1991). Increased blood flow to edematous regions has been shown to be associated with reduced interstitial fluid accumulation by increasing venous return in those areas (Burgess et al., 2019; Hettrick, 2009). Greater blood flow to the head region will ultimately also increase flow to the ocular vasculature, which may have contributed to the reduction in edema observed in the NBN trial.

During the course of this study, the patient had begun intravitreal aflibercept treatment two months prior to the study and had received two additional injections between NBH and NBN sessions. Though the patient's retinal edema did not respond to earlier intravitreal triamcinolone acetonide and intravitreal bevacizumab treatments, it is not unlikely that the decrease in mean retinal thickness at the site of maximal edema between the time of the first and second NBH studies and the NBN study was due to continued aflibercept treatment. We cannot conclude whether NBH administration helped contribute to or compliment the effects of aflibercept treatment leading to eventual resolution of retinal edema and stabilization of visual acuity following completion of the study.

Further studies regarding the potential interactions between NBH treatment and current treatments, such as laser therapy and intravitreal injections, should be performed to determine the efficacy of utilizing NBH alone, whether and how hyperoxia may affect the actions of common therapeutic techniques and medications used to treat radiation retinopathy, and the degree of effectiveness in combining these treatment options. NBH therapy also presents a non-invasive alternative to intravitreal injections and may be prescribed for use at home. If proven sufficient, this option would then reduce burden on

patients to return for repeat laser procedures or injections, which often necessitate monthly or weekly appointments over the course of several months (Sivaprasad and Oyetunde, 2016).

Our findings suggest that the role of NBH in ophthalmic care warrants continued investigation. There is not yet enough evidence to indicate that use of NBH may be superior to or adequately replace current practices for treating radiation retinopathy, but the results of our study suggest that NBH may be a beneficial addition to existing treatment repertoire to manage complications due to radiation retinopathy.

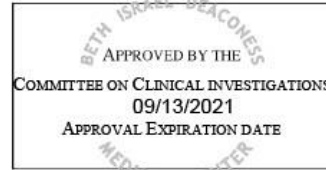
CONCLUSION

In a patient with radiation retinopathy, short-term 40% FiO₂ NBH treatment resulted in improved visual acuity and significant reduction of retinal edema when compared to sham treatment. NBH therapy is a simple, safe, and ubiquitous treatment that may expand therapeutic options for patients suffering from radiation retinopathy. For these reasons, we believe the role of NBH in ophthalmic care warrants continued investigation.

APPENDIX



****For CCI Use ONLY****
**Approved by the Beth Israel Deaconess Medical Center
Committee on Clinical Investigations:**
Consent Approval Date: 9/14/2020
Protocol Number: 2018P000495



INFORMED CONSENT FORM TO TAKE PART IN A RESEARCH STUDY

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR: Jorge Arroyo, MD, MPH
PROTOCOL NUMBER: 2018P000495

INTRODUCTION:

- This is a research study;
- Your participation is voluntary;
- A research study includes only people who choose to take part;
- You may or may not benefit from participating in the study. However, your participation may help others in the future as a result of knowledge gained from the research;
- You may leave the study at any time;
- If you choose not to take part, or if you leave the study, your decision will in no way harm your relationship with any member of the research team or any other individuals at Beth Israel Deaconess Medical Center.

Please read this consent form carefully and ask the investigators or study staff to explain any words or information that you do not clearly understand. Once you read this consent form and understand what your participation in this study will involve, you will be asked to sign this form if you wish to take part. You will be given a signed copy of the form to keep for your records.

DISCLOSURE OF SPECIAL INTERESTS OF BIDMC AND INVESTIGATORS

This study is being conducted by Dr. Jorge Arroyo. There is no funding or monetary support for this study. Neither BIDMC nor Dr. Jorge Arroyo has any additional interests in this research project.

WHOM TO CONTACT IF YOU HAVE QUESTIONS OR PROBLEMS

If you have any questions, concerns or complaints about this research or experience any problems, you should contact Dr. Jorge Arroyo at [617] 667-3391.

PURPOSE

The main purpose of this study is to test the impact and safety of oxygen therapy for your condition of retinal ischemia, which includes any medical problem in which the primary concern is a lack of oxygen to your retina. In addition, we hope to learn what kind of side effects, if any, will be caused by using

Figure A – Part 1. Page 1 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
PROTOCOL #: <u>2018P000495</u>

 APPROVED BY THE COMMITTEE ON CLINICAL INVESTIGATIONS 09/13/2021 APPROVAL EXPIRATION DATE
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oxygen therapy for this purpose, and the severity of any side effects that may occur. This means that you may experience the side effects listed in the risks section of this consent form. As it stands, the current standard of treatment, anti-VEGF drugs, are meant to treat the symptoms of your condition rather than the cause (lack of oxygen). After promising animal models and clinical experience, we are conducting this study with the intent to get information that will help us plan a larger definitive study in the future.

Participants in this study will breathe in a higher concentration of oxygen overnight while they sleep, using a machine called an oxygen concentrator equipped with a face mask. The treatment involved in this study, supplemental oxygen delivered through an oxygen machine to treat retinal ischemia, is investigational. This means that the study therapy is still being tested in research studies and is not approved by the Food and Drug Administration [FDA] for the way that it is being used in this study. Supplemental oxygen therapy has been approved by the FDA for use in many other diseases or conditions, but we do not yet know if it is useful or safe as a treatment for retinal ischemia.

STUDY PARTICIPANTS

You have been asked to be in the study because you have been diagnosed with a condition involving retinal ischemia. Approximately 100 people will take part in this study at Beth Israel Deaconess Medical Center.

DESCRIPTION OF STUDY DETAILS

If you agree to be in this study, you will be asked to read and sign this consent form. After you sign the consent form, the following things will happen:

1. **Screening Procedures:** Screening procedures are tests and procedures that will be done to determine if you are eligible to take part in the research study. For this research study, the screening procedures include: An eye exam – including vision testing and pressure measurements, non-invasive imaging and assessment of eligibility by Dr. Arroyo. Screening for enrollment will occur within 28 days prior to Day 1 of oxygen treatment.
2. **Research Procedures:** If you qualify to take part in this research study, you will undergo these research procedures: You will be given a prescription for a portable oxygen concentrator and face mask, which will be used at night to deliver oxygen at a rate of 5 liters per minute for 6 months. Research staff will go over how to use your oxygen concentrator upon your consent to participate in this research study and will be available by phone if any assistance is required.

You will rent the oxygen concentrator for the duration of the study and will be responsible for covering the costs for the rental (approximately \$75 / month for a total of \$450). If you plan on traveling, please inform us before you leave. It is acceptable to miss some days of

Figure A – Part 2. Page 2 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
PROTOCOL #: <u>2018P000495</u>

 APPROVED BY THE COMMITTEE ON CLINICAL INVESTIGATIONS 09/13/2021 APPROVAL EXPIRATION DATE
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oxygen therapy but please keep track of your use and inform us of any gaps in treatment when you see us next. The more you use the oxygen, the more likely it is to work. Please try to use it as much as you can.

3. Monitoring/Follow-Up Procedures. Procedures performed to evaluate the effectiveness and safety of the treatment are called "monitoring" or "follow-up" procedures. Other than your use of the oxygen concentrator, you will receive no change in care. For this research study, the monitoring/follow-up procedures include follow-up visits at 1, 3, 6, and 12 months. At each follow-up, you will undergo dilated eye examination with vision testing and intraocular pressure (IOP) measurement. Retinal anatomy will be evaluated by SD-OCT imaging/angiography and fundus photography. These visits, and the tests done during these visits, are done in all patients with retinal ischemia, whether or not you are part of this study. They are part of your usual standard medical care.

We may also need to access your medical records to get an accurate sense of your history with us. Accessed data may include: age, eye diagnoses and medications, other related medical conditions, number and frequency of previous eye visits and/or procedures including surgeries and injections. These records are routinely accessed for your standard clinical care, but may be used so that we can accurately judge the impact of the Supplemental Oxygen Therapy.

RISKS AND DISCOMFORTS

As a result of your participation in this study, you are at risk for side effects listed in this section. You should discuss these with the investigator and with your regular doctor if you choose.

More Common: Dry nose and mouth, irritation around mask site

Less Common: Chest tightness, loss of sleep and psychological stress due to sleeping with a mask overnight

Rare: Chest pain, damage to airways, and CO₂ retention resulting in breathing issues

LOSS OF CONFIDENTIALITY

There is the potential for loss of confidentiality by participating in this study. Every effort will be made to protect the confidentiality of your identifiable information. However, if your participation becomes known, it could create a problem or hardship for you depending upon the type of information disclosed.

CONFIDENTIALITY

Information learned from your participation in this study and from your medical record may be reviewed and photocopied by the Food and Drug Administration (FDA) and/or other federal and

Figure A – Part 3. Page 3 of the study consent form.

SUBJECT'S NAME:
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PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
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state regulatory agencies, and by accreditation agencies, the Committee on Clinical Investigations, the Human Subjects Protection Office and others involved in research administration of the Beth Israel Deaconess Medical Center with protection of confidentiality so far as permitted by applicable law. Information resulting from this study and from your medical record may be used for research purposes and may be published; however, you will not be identified by name in such publications.

POSSIBLE BENEFITS

It is not possible to predict whether you will benefit directly from participation in this study. However, your participation may help others in the future as a result of knowledge gained from the research.

OTHER AVAILABLE OPTIONS

Taking part in this study is voluntary. Instead of being in this study, you have the following options:

It is important to note that it is possible to get an oxygen concentrator even if you do not take part in the study. Supplemental oxygen therapy delivered via oxygen concentrator has not been approved by the FDA for treatment of your condition, however, many doctors in the community commonly prescribe the drug to treat lung conditions such as emphysema or Chronic Obstructive Pulmonary Disease (COPD). Please be aware that not all doctors may agree to prescribe this therapy for you, and that not all health insurance companies will pay for the therapy when it is prescribed for retinal disorders.

It is also important to know that your refusal to take part in this study will not affect your care in any way. You can always opt to continue with only anti-VEGF therapy or whatever treatment course is suitable for your condition.

This research study is not meant to diagnose or treat medical problems. Participation in this research study does not take the place of routine physical examinations or visits to your regular doctor.

We recommend that you discuss these and other options with the investigator and your regular doctor so that you can make a well-informed decision about participating in this study.

IF YOU DECIDE NOT TO TAKE PART IN THE STUDY

Participation in this study is voluntary. You have the right to decide not to take part in this study. If you choose to participate, you have the right to leave the study at any time. Your decision to not participate will not result in any penalties or loss of benefits to you. The investigators will tell you about new information that may affect your willingness to stay in this study.

If you decide not to participate in the study or decide to leave the study early, your decision will not affect your relationship with the research team or any other individual at Beth Israel Deaconess Medical Center.

Figure A – Part 4. Page 4 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
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INVESTIGATORS RIGHT TO STOP THE STUDY

The investigators have the right to end your participation in this study if they determine that you no longer qualify to take part, or if it would be dangerous for you to continue, or if you do not follow study procedures as directed by the investigators. Beth Israel Deaconess Medical Center or the funding source may stop the study at any time.

COSTS AND/OR PAYMENTS TO YOU

COSTS COVERED BY STUDY

You will be responsible for the costs of the oxygen used in this research study (\$75 a month, \$450 total). You and your insurance company will also be charged for other tests, procedures or medications of this study that are considered standard treatment for your medical condition, including the study visits and testing at 1, 3, 6 and 12 months.

CO-PAYMENT/DEDUCTIBLE STATEMENT

You will be responsible for any co-payments or deductibles that are standard for your insurance coverage.

PAYMENTS TO YOU:

You will not receive any payments for participating in this study.

COST OF RESEARCH RELATED INJURY:

If you are injured as a direct result of your participation in this study you should contact the Investigator at the number provided under the section "Whom to Call if You Have Questions" in this form. You will be offered the necessary care to treat your injury. You or your insurance company will be billed for medical care and/or hospitalization related to this injury. You will be responsible for all co-payments and deductibles required under your insurance. BIDMC will consider reimbursement of injury related expenses not covered by your insurance on a case-by-case basis. At this time there is no plan to reimburse you for items such as lost wages or lost time from work. By signing this consent form you have not given up any legal rights.

AUTHORIZATION FOR USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION

As part of this study, we will be collecting, using and sharing with others information about you. Please review this section carefully as it contains information about the federal privacy rules and the use and disclosure of your information.

PROTECTED HEALTH INFORMATION [PHI]

By signing this informed consent document, you are allowing the investigators and other authorized personnel to use [internally at BIDMC] and disclose [to people and organizations outside the BIDMC

Figure A – Part 5. Page 5 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
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workforce identified in this consent] health information about you. This may include information about you that already exists (for example: your medical records and other sources of health information, demographic information, the results of any laboratory tests, and mental health records if applicable) as well as any new information generated as part of this study. This is your Protected Health Information.

PEOPLE/GROUPS AT BIDMC WHO WILL SHARE AND USE YOUR PROTECTED HEALTH INFORMATION

Your Protected Health Information may be shared with and used by investigators working on this study, including the supporting research team (such as research assistants and coordinators, statisticians, data managers, laboratory personnel, pharmacy personnel, and administrative assistants), and may also be shared and used by other health care providers at BIDMC who have treated you in the past and have information relevant to the research, or who provide services to you in connection with the research. Your Protected Health Information may also be shared with the members and staff of the Committee on Clinical Investigations of Beth Israel Deaconess Medical Center, which is responsible for reviewing studies for the protection of the research subjects.

PEOPLE/GROUPS OUTSIDE OF BIDMC WITH WHOM YOUR PROTECTED HEALTH INFORMATION WILL BE SHARED

We will take care to maintain confidentiality and privacy about you and your Protected Health Information. We may share your Protected Health Information with the following groups so that they may carry out their duties related to this study:

- The other hospitals and medical centers taking part in this study and research collaborators at those institutions
- Any external health care providers who provide services to you in connection with this research
- Laboratories not affiliated with BIDMC that are involved in conducting tests related to the research
- Statisticians and other data monitors not affiliated with BIDMC
- The members and staff of any other IRBs (beyond the BIDMC Committee on Clinical Investigations) that oversee the research
- Centralized data collectors
- Your health insurance company
- The Food and Drug Administration [FDA], the Department of Health and Human Services [DHHS], the National Institute of Health [NIH], the Office for Human Research Protections [OHRP], and other federal and state agencies that may have jurisdiction over the research
- Hospital and Clinical Research Accrediting Agencies

Those who receive your Protected Health Information during the course of the research may not be required by the federal privacy regulations to protect it, and they may make further disclosures to others and use your information without being subject to penalties under those laws.

Figure A – Part 6. Page 6 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
PROTOCOL #: <u>2018P000495</u>



WHY WE ARE USING AND SHARING YOUR PROTECTED HEALTH INFORMATION

The main reason for using and sharing your Protected Health Information is to conduct and oversee the research as described in this Informed Consent Document. There are many other reasons beyond the research for which BIDMC may use or disclose your Protected Health Information. Not all of these reasons require your express written authorization. For example, we will use and share your Protected Health Information to ensure that the research meets legal, institutional and accreditation requirements and to conduct public health activities. The various ways in which BIDMC may use and disclose your protected health information without your authorization are explained in a document called the Notice of Privacy Practices. If you have not received a copy of BIDMC's Notice of Privacy Practices, please ask us for one and review it before signing this form. In addition to signing this document, you may also be asked to sign a BIDMC General Agreement form acknowledging that you have received the BIDMC Notice of Privacy Practices.

NO EXPIRATION DATE – RIGHT TO WITHDRAW AUTHORIZATION

Your authorization for the use and disclosure of your Protected Health Information in this Study shall never expire. However, you may withdraw your authorization for the use and disclosure of your Protected Health Information at any time provided you notify the Principal Investigator in writing. If you would like to take back your authorization so that your Protected Health Information can no longer be used in this study, please send a letter notifying the Principal Investigator of your withdrawal of your authorization to Dr. Jorge Arroyo at 330 Brookline Ave. CC-5, Boston, MA 02215. Please be aware that the investigators in this study will not be required to destroy or retrieve any of your Protected Health Information that has already been used or disclosed before the Principal Investigator receives your letter, and they are permitted to continue to use and disclose your previously collected information as necessary to complete the research.

REFUSAL TO SIGN

Your clinical treatment may not be conditioned upon whether you sign the Authorization for Research. However, if you choose not to sign this informed consent document and authorization for the use and disclosure of your Protected Health Information, you will not be allowed to take part in the research study.

RIGHT TO ACCESS AND COPY YOUR PHI

If you wish to review or copy your Protected Health Information as it is made part of your medical record, you may do so after the completion or termination of the study by sending a letter to the Principal Investigator requesting a copy of your Protected Health Information. You may not be allowed to inspect or copy your Protected Health Information until this study is completed or terminated.

ADDITIONAL CONTACT FOR QUESTIONS OR CONCERNS

Figure A – Part 7. Page 7 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
PROTOCOL #: <u>2018P000495</u>

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You may contact the Human Subjects Protection Office at [617] 975-8500 in the event that you would like to obtain information or to offer input about the research study. This office is independent of the investigator or investigator's research staff and can also assist with questions relating to your rights as a participant in research, which may include questions, concerns or complaints about your participation in the study.

Figure A – Part 8. Page 8 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
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THE FOLLOWING PARAGRAPHS CONTAIN SOME STANDARD INFORMATION WHICH GENERALLY APPLIES TO INDIVIDUALS PARTICIPATING IN A RESEARCH STUDY.

CONSENT FORM FOR CLINICAL RESEARCH

I have read the previous page[s] of the consent form and the investigator has explained the details of the study. I understand that I am free to ask additional questions.

If I wish additional information regarding this research and my rights as a research subject, or if I believe I have been harmed by this study, I may contact the Human Subjects Protection Office (HSPO).

I am aware that this is a research project and that unforeseen side effects may occur.

I understand that the Beth Israel Deaconess Medical Center has no formal program for compensating patients for medical injuries arising from this research. Medical treatment will be provided for injuries at the usual charge to me or to my insurer unless payment is otherwise provided for in this consent form.

I understand that participation in this study is voluntary and I may refuse to participate or may discontinue participation at any time without penalty, loss of benefits, or prejudice to the quality of care which I will receive.

I acknowledge that no guarantees have been made to me regarding the results of the treatment involved in this study, and I consent to participate in the study and have been given a copy of this form.

Signature of Subject or Legally Authorized Representative (Parent if the subject is a minor)

Date

Relationship of Legally Authorized Representative to Subject

The subject has been given the opportunity to read this consent form and to ask questions before signing, and has been given a copy.

SIGNATURE OF INVESTIGATOR/Co-Investigator DATE

PRINT INVESTIGATOR'S/Co-Investigator's NAME

A signing co-investigator must be listed on the study's approved Research Staffing Form at the time of consent.

Figure A – Part 9. Page 9 of the study consent form.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: Supplemental Oxygen Therapy for Retinal Ischemia
PRINCIPAL INVESTIGATOR'S NAME: JORGE ARROYO, MD, MPH
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THE FOLLOWING SECTIONS ARE NOT NEEDED FOR ALL STUDIES AND SHOULD BE UTILIZED AS INDICATED:

If the subject is able to speak and understand English but is not able to read or write

<p>I was present during the entire oral presentation of the informed consent and witnessed the subject's agreement to participate in the study.</p> <p>Signature of Witness: _____</p> <p>Printed Name of Witness: _____</p> <p>Date: _____</p>

If the subject is able to understand English but is not physically able to read or write or see

<p>I was present during the entire oral presentation of the informed consent and witnessed the subject's agreement to participate in the study.</p> <p>Signature of Witness: _____</p> <p>Printed Name of Witness: _____</p> <p>Date: _____</p>

If the subject is not English speaking and signed the translated Short Form in lieu of the English consent document.

<p>As someone who understands both English and the language spoken by the subject, I interpreted, in the subject's language, the researcher's presentation of the English consent form. The subject was given the opportunity to ask questions.</p> <p>Signature of Interpreter: _____</p> <p>Printed name of Interpreter: _____</p> <p>Date: _____</p>

Figure A (Parts 1 – 10). Study consent form.

A copy of the consent form that was provided to each potential participant explaining the purpose, terms, and possible consequences of the study.

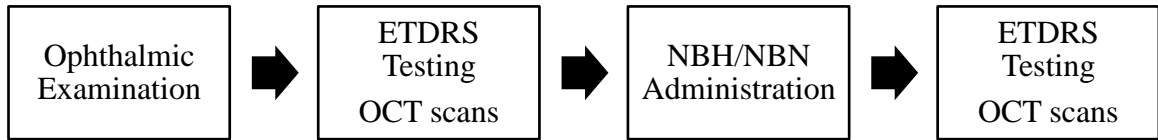


Figure B. Order of events during each trial.

A flowchart representation of the processes conducted during each NBH and NBN trial. An ophthalmic examination was performed first by the same attending physician, then VA measurements and OCT scans were taken. NBH or NBN was then administered for three hours, and VA measurements and OCT scans were taken again immediately following conclusion of NBH or NBN administration.

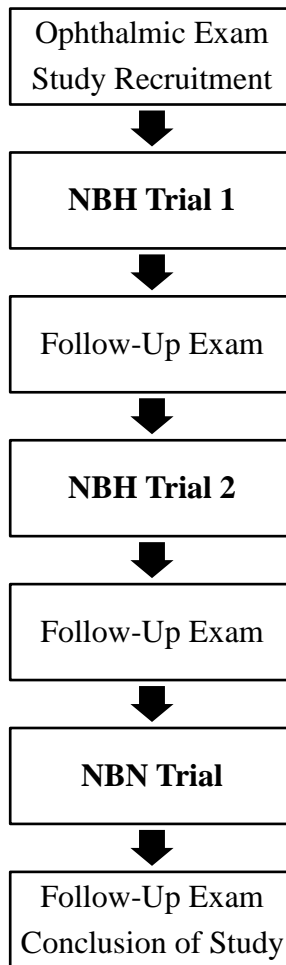


Figure C. Order of appointments and trials during the course of the study.

A flowchart depicting the order of appointments and trials conducted during the study. Bolded items indicate an NBH or NBN trial, and non-bolded items indicate a routine, follow-up ophthalmic examination between trials to monitor for any adverse or significant changes.

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VITA

