The effect of systemic hyperoxia and hypoxia on scotopic thresholds in people with early and intermediate age-related macular degeneration.

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Abstract

Purpose

Morphological retinal changes combined with functional evidence implicate hypoxia in the pathogenesis of age-related macular degeneration (AMD). However, the role of hypoxia in the scotopic threshold deficit reported in AMD has not been investigated. This study compared scotopic thresholds in participants with early and intermediate AMD recorded under conditions of systemic hypoxia, hyperoxia and normoxia.

Materials and Methods

Over two sessions scotopic thresholds were measured with participants breathing 21% and 60% oxygen (n= 12 early AMD, n= 11 age-similar controls) or 21% and 14% oxygen (n=16 early AMD, n=20 age-similar controls). Thresholds were measured using a 'white', annular 12 degrees stimulus, using a QUEST procedure.

Results

There was no statistically significant change in scotopic thresholds within the AMD or control group when breathing the hyperoxic gas mixture (60% oxygen) or the hypoxic gas mixture (14% oxygen) when compared to the normoxic condition (21% oxygen). There was also no statistically significant difference in scotopic thresholds between groups under the hyperoxic or hypoxic gas conditions. The difference between groups under the normoxic condition was not statistically significant for the hyperoxia study (p= 0.70), but did reach significance in the hypoxia study (p=0.05).

Conclusion

This study provided no evidence that breathing that breathing 14% or 60% oxygen altered scotopic thresholds in those with early AMD when compared to controls. However, the lack of elevated scotopic thresholds in the AMD group of the hyperoxia study is of note, as it is unlikely that hyperoxia would reduce thresholds which were not significantly raised at baseline, regardless of whether hypoxia was a factor in the disease pathogenesis. The findings of this study do not rule out a role for hypoxia in early AMD, but this needs to be assessed in future experiments using measures that differ significantly between people with AMD and controls.

Key words: Age-related Macular Degeneration; hyperoxia; hypoxia; scotopic thresholds; visual function

Introduction

Age-related macular degeneration (AMD) is a disease characterised by progressive central vision loss due to photoreceptor degeneration at the macula. It is the leading cause of certified visual loss in the UK [1] and is predicted to affect 196 million people globally by 2020 [2].

The pathogenesis of AMD remains elusive but oxidative stress [3,4], inflammation [5], and hypoxia [6,7] have all been implicated. Morphological changes to the retina and associated structures implicate hypoxia in the disease process. For example, changes to the choroid seen in AMD include a decreased choroidal blood flow [8], choriocapillaris drop out [9], and changes in choroidal vascularity index [10]. A reduced choroidal blood supply might be associated with a reduced oxygen supply to the outer retina, especially in view of animal studies suggesting that the oxygen supply is only just sufficient to meet the needs of the photoreceptors under dark adapted conditions [11]. In addition, age related thickening of Bruch's membrane [12] and the formation of basal linear deposits associated with AMD [13,14], increase the distance over which oxygen must diffuse to reach the retina from the choroidal circulation. According to Fick's law, this results in a reduction in the diffusion flux [7], which may also impact on oxygen availability at the outer retina. Immunohistochemical studies have found increased levels of VEGF in early AMD [15], lending support to the notion that the hypoxia is a feature of early AMD. However, it should be noted that a range of other factors may also lead to elevated levels of VEGF, including inflammation, acidosis, and other growth factors, such as TGF-α,TGF-β, insulin-like growth factor-1, FGF and platelet-

derived growth factor [16,17]. VEGF up-regulation alone is not, therefore, a strong indicator of the presence of hypoxia.

There is also circumstantial evidence to suggest that hypoxia may affect visual function in AMD. Visual deficits have been reported early in the disease process with colour vision [18,19], contrast sensitivity [20], dark adaptation [21], and scotopic thresholds [22] all altered in early AMD. These same functions are also reduced in a comparable way in normal participants experiencing a hypoxic episode [23-27], suggesting that hypoxia could contribute to the dysfunction seen in AMD. There is evidence from prior studies that systemic hyperoxia may overcome deficits in retinal function in diabetic retinopathy [28-29], but this has not been investigated in AMD.

Despite the anatomical and functional evidence implicating hypoxia in the pathogenesis of AMD, the relationship between abnormal visual function and hypoxia in AMD has not yet been investigated. This study aimed to address this by comparing scotopic thresholds in participants with early AMD recorded under conditions of systemic hyperoxia, hypoxia and normoxia. The hypotheses were as follows

- If hypoxia were responsible for the elevated scotopic thresholds associated with early AMD, breathing oxygen enriched air would result in a selective reduction in scotopic thresholds in people with AMD.
- If hypoxia were responsible for the elevated scotopic thresholds associated with early AMD, breathing oxygen diminished air would result in a selective rise in scotopic thresholds in people with AMD, which would be greater than that observed in healthy controls.

Materials and Methods

Participants

All participants were recruited from patients attending the University Hospital Wales Ophthalmology outpatients' clinic and Cardiff University Eye Clinic, or directly from local optometrists in the South Wales area. All participants were aged 55 years or older with a corrected visual acuity of 6/12 or better, a refractive error of < 6.00 DS in the most powerful meridian, and clear ocular media in the test eye (less than grade 2 on the LOCSIII grading scale [30] for all criteria). Exclusion criteria included: smoking, lung conditions, (such as emphysema), systemic health conditions or medication known to affect vision, or the presence of ocular disease other than AMD. Participants with a fundus appearance graded as early to intermediate AMD in one or both eyes (who had medium to large drusen with or without focal pigmentary changes) [31] were assigned to the AMD group. This group also contained patients who were being treated for neovascular AMD (nAMD) in the non-test eye who had no, or only early AMD in the test eye. The control group comprised age matched participants with no sign of AMD in both eyes [31]. Written consent was obtained before participation and all procedures adhered to the tenets of the Declaration of Helsinki. The study was approved by South East Wales Research Ethics Committee (reference number 12/WA/0266).

Apparatus and psychophysical methods

All stimuli for the psychophysical studies were presented on a gamma corrected high-resolution monitor (LS902UT, Liyama, Hoofddorp, Netherlands) driven by an 8-bit (Geforce 9, nVIDIA, Santa Clara, USA) graphics board under the control of MATLAB (Mathworks Inc, Natick, MA, USA). Scotopic thresholds were measured in response to

a ‘white’, annular stimulus which had a radius of 12 degrees and a width of 0.5 degrees. Stimulus threshold was determined using the QUEST Bayesian adaptive procedure [32] written with Psychophysics Toolbox extensions [33]. The outputs of the program were scotopic threshold and its standard deviation. A pulse oximeter (Crucial Medical Systems CMS 50E) was used to monitor peripheral blood oxygen saturation (SpO_2) in all experiments. Air was respired by the patient through a 60% ventimask (Intersurgical, Wokingham, UK) which mixes the contents of a gas cylinder in a 1:1 ratio with ambient air. Hence, a cylinder containing 100% oxygen delivered 60% oxygen, medical air provided 21% oxygen and a canister containing 8% oxygen provided 14% oxygen for inspiration.

Experimental design

Baseline examinations including patient history, distance monocular logMAR visual acuity (Early Treatment Diabetic Retinopathy Study), visual field screening (SITA fast 24-2, Humphrey Field Analyzer) and colour vision testing (desaturated D15) were completed at the start of the visit. All procedures involving bright lights being presented to the eyes (binocular indirect fundus examination, fundus photography, slit lamp examination and OCT) were carried out at the end of each visit, to avoid excessive photopigment bleaching before the measurement of dark-adapted retinal function. The fundus appearance of all participants was classified by Optometrist observer TC according to the Beckman Initiative grading scale (Ferris et al., 2013), and also assigned a risk factor for progression to advanced AMD according to the AREDS simplified scale [34], whereby individuals are assigned a risk point (up to a maximum of

4) for factors including the presence of large drusen in one or both eyes, the presence of pigmentary changes, and the presence of nAMD in the fellow eye.

At the first visit, the effect of hyperoxia was assessed to address the first hypothesis. The timeline of the experiment is shown in Figure 1. After a one-hour period of dark adaption, each participant took part in two trial QUEST runs, each consisting of 20 presentations. Participants proceeded to the main study only if the thresholds of both practice runs were within 1 standard deviation of one another. If this was not the case then a third practice run was undertaken, if this threshold was not within 1 standard deviation of the first two practice runs then the study was stopped at this point.

The first of two randomly assigned gases, either medical air (control) or the experimental (hyperoxic) gas was then turned on. The participant was masked to which gas was being inhaled and the randomisation of the gas order was balanced so that the same number of individuals received each gas type first. The first gas was inhaled for 10 minutes and, within the last 5 minutes, scotopic threshold was determined. There then followed a 5-minute wash out period where no gas was given. The second of the two gases was then turned on for 10 minutes with the scotopic threshold recorded again within the final 5 minutes. The participant's SpO_2 was monitored throughout the duration of the experiment, and recorded at one-minute intervals with the experiment terminated if SpO_2 dropped below 85%.

[Location of Figure 1]

Participants returned on a different day to take part in the evaluation of the effect of hypoxia. The timeline of the study was identical on the second visit, with the only

variation being the oxygen content of the experimental gas condition. All baseline tests were repeated to ensure correct classification.

Statistical analysis

There were no previous studies, to our knowledge, available to inform power calculations hence this was an exploratory randomized controlled trial. Distributional assumptions were tested using the Shapiro-Wilkes test. Paired t-tests (Wilcoxon signed rank test for non-normally distributed data) were carried out to determine whether SpO_2 and scotopic thresholds were significantly different between gas conditions within each group. Independent samples t-tests (or Mann-Whitney U test as a non-parametric alternative) were carried out to determine whether SpO_2 and scotopic thresholds differed significantly between controls and people with AMD under each gas condition, and also to compare change in threshold between gas conditions in people with and without AMD. In order to test for order effects, a paired t-test was used to compare scotopic thresholds between the gas presented first and second.

Results

All participants taking part in the first study, evaluating the effect of hyperoxia on scotopic thresholds were invited to take part in the second study to assess the effect of hypoxia, but the final cohort differed between studies. In the hyperoxic study there were 11 control participants (mean age 69 years ±6SD, 36% female), and 12 individuals with AMD (mean age 74 years±6SD, 50% female). In the hypoxia study there were 20 control participants (mean age 68 years ±5SD, 40% female), and 16 with AMD (mean age 73 years ±5SD, 38% female). There was no significant

difference in age between the AMD group and the control group for either study ($p>0.05$). The participant characteristics of the people with early and intermediate AMD are shown in Table 1. It is notable that 18 of the 22 participants had high risk AMD features in the fellow eye. One participant was excluded from the hypoxia study due to their SpO_2 dropping below 85%. One participant was excluded from the hypoxia study due to their SpO_2 dropping below 85%. Hence, data were collected from 35 participants in total for this study.

[Location of Table 1]

Hyperoxia and Scotopic thresholds

SpO_2 results

Figure 2 (Panel A) shows the group mean SpO_2 levels for the AMD and control participants, with confidence intervals across ten minutes. Statistical analysis for the last 5 minutes of the data confirmed that the difference in mean SpO_2 readings between breathing 60% and 21% oxygen was significant in people with early AMD (Wilcoxon signed rank $p < 0.01$) and in the control group (Wilcoxon signed rank $p < 0.01$). The median SpO_2 (averaged over the last 5 minutes of breathing each gas type) under normoxic conditions was 98% (96.5% to 98%) in the participants with AMD, and 97% (97% to 98%) in the control group. This difference, whilst small, was statistically significant (Mann-Whitney $p<0.01$). The median SpO_2 under hyperoxic conditions was 99% (99% to 99%) in the participants with AMD, and 99% (99% to 99%) in the control group. This did not differ significantly between the two groups (Mann-Whitney $p = 0.94$).

[Location of Figure 2]

Scotopic threshold results

Scotopic threshold data were obtained for all participants. As an example, the output of the QUEST procedure is shown in Figure 3 panels a and c for a control participant alongside the SpO_2 readings per minute over the 10 minutes of gas inhalation (Figure 3 panels b and d). The gas order for this participant was 21% oxygen (Figure 3 top panels) followed by 60% oxygen (Figure 3 bottom panels). The QUEST output shows the convergence of the procedure towards a final threshold which is the mode of the final probability density function.

[Location of Figure 3]

The scatter plot in Figure 4, panel A, shows the difference in scotopic threshold between hyperoxic and normoxic gas conditions for each individual in the AMD group (a) and control group (b). It can be seen that the proportion of individuals showing a reduced threshold when breathing 60% oxygen was equivalent between groups. Approximately half of each group showed an increase in threshold, and half showed a decrease under the hyperoxic gas condition.

[Location of Figure 4.]

Figure 5, panel A, shows the group mean scotopic thresholds for controls and people with AMD under hyperoxic and normoxic conditions. There was no statistically significant difference between the scotopic thresholds when breathing 21% and 60% oxygen for the AMD group (-3.89 log cd/m^2 ±0.31SD vs. -3.89 log cd/m^2 ±0.30SD; paired t-test, P= 0.976), or the control group (-3.95±0.38 SD log cd/m^2 vs. -4.01±0.27SD log cd/m^2; paired t test, P=0.249). There was no statistically significant difference in scotopic thresholds between the AMD and control groups when breathing 60%

oxygen (independent samples t- test; *P*=0.31), or 21% oxygen (independent samples t- test; *P*=0.702). There was no statistically significant difference in the change in scotopic thresholds from the 21% to the 60% oxygen condition between the two participant groups (0.002 log cd/m^2 ±0.17SD vs. 0.06 log cd/m^2±0.17SD; Mann-Whitney; *P*=0.413). There was also no statistically significant difference in the scotopic thresholds between the first and second gases across the whole dataset (independent t test; *P*= 0.61).

[Location of Figure 5]

Hypoxia and scotopic thresholds

SpO2 results

Figure 2 (panel B) shows the mean SpO_2 levels per minute for the AMD and control participants, with confidence intervals. There was a statistically significant decrease in SpO_2 in the final 5 minutes when breathing the hypoxic 14% oxygen gas compared to the normoxic 21% oxygen gas in people with early AMD (Wilcoxon signed rank *P*=<0.0001) and in the control group (Wilcoxon signed rank p<0.0001). The median (interquartile range) SpO_2 under normoxic conditions was 97% (95%-99%) in the participants with AMD, and 98% (97%-99%) in the control group (averaged over the last 5 minutes of breathing each gas type). This was statistically significant (Mann-Whitney *P*=<0.005). The median SpO_2 under hypoxic conditions was 93% (90%-96%) in the participants with AMD, and 94% (90%-98%) in the control group. This was also statistically significant (Mann-Whitney; p<0.0001)

Hypoxia and scotopic threshold results

Scotopic threshold data were analysed for 33 out of 35 participants, as one participant (015) did not proceed to the main trial after failing the requirement for the scotopic threshold from each of the practice trials to be within one standard deviation of each other. The data for one control participant (022) had to be removed from analysis due an abnormal OCT indicating a pre-existing ocular condition. Figure 4, panel B shows a scatter plot of change in scotopic thresholds for all individuals within the control and AMD groups. Again, there was a similar proportion of individuals showing an increase in threshold when breathing 14% oxygen, as there was showing a decrease in threshold compared to medical air.

Figure 5 panel B shows the group mean thresholds for each participant group, under each gas condition, with 95% confidence intervals. There was no significant difference in mean scotopic thresholds when breathing 14% oxygen compared to when breathing 21% oxygen within the AMD group (-4.02 log cd/m^2 ± 0.11 vs -4.00 log cd/m^2 ± 0.07; paired t-test, *P*=0.781) or the control group (-4.14 log cd/m^2±0.09SD vs. -4.14 log cd/m^2 ±0.10; paired t-test, *P*= 0.950). There was also no significant difference in the change in thresholds under different gas conditions between AMD and control groups (0.03 log cd/m^2 IQR 0.13 vs. -0.02 log cd/m^2 IQR 0.12; Mann-Whitney, *P*= 0.509). ~~Between groups, there was no statistically significant difference in mean scotopic threshold between the two groups when breathing 14% oxygen (independent samples t test, *P*= 0.130).~~ There was, however, a statistically significant difference in mean scotopic threshold between the two groups when breathing 21% oxygen (independent samples t-test, p=0.049) but not when breathing 14% oxygen (independent samples t test, p= 0.130), with the AMD group showing a significantly higher threshold than the control group under

normoxic conditions. Finally, there was no effect of the order of presented gas (paired samples t-test, *P*=0.196).

Discussion

This is the first study to investigate the effect of variable oxygen levels on scotopic thresholds in people with AMD. Contrary to the initial hypotheses, there was no evidence that breathing 14% or 60% oxygen significantly altered scotopic thresholds in those with early to intermediate AMD or in controls, therefore the scotopic thresholds of participants with AMD were not affected differently to control participants by breathing different oxygen concentrations.

One possible conclusion of this study is that hypoxia is not responsible for the functional deficit seen in early AMD. However, there are other experimental factors which may have contributed to this finding. One explanation for the lack of effect of breathing 60% oxygen on scotopic thresholds in participants with early AMD is that, due to the eligibility criteria for the study which excluded individuals with existing lung conditions, no participants in the study were systemically hypoxic i.e. both groups showed a mean SpO_2 in excess of 97% when breathing 21% oxygen. Therefore, mean SpO_2 was close to saturation in both groups under the medical air condition, and any increase in mean SpO_2 attributable to breathing 60% oxygen was limited by the ceiling effect to only 1-2%. As the accuracy of the pulse oximeter, according to the manufacturer's guidelines, is +/- 2% of the reading given (Contec, *CMS-50E User manual*), the statistically significant change in oxygen saturation induced by the hyperoxic gas condition was within the measurement error of the machine. However, SpO_2 is an indirect measure of the proportion of haemoglobin bound to oxygen. The

oxygen tension in the choroid and blood plasma was not measured directly in this study. This is relevant as retinal oxygen tension has been shown to be markedly elevated in systemic hyperoxia even in conditions of near blood oxygen saturation [36]. Tissue oxygen saturation in the outer retina is particularly sensitive to changes in inspired oxygen levels as the choroidal circulation does not autoregulate and there is no change in choroidal resistance in response to the metabolic demands of the retina, or as a consequence of hypoxia or hyperoxia [11,36].

It is also possible that, despite the near 100% SpO_2 under normal breathing conditions, structural changes to the subretinal structures cause a localized reduction in oxygen supply to the photoreceptors which is not reversible by the inhalation of supplementary oxygen in an individual who is not systemically hypoxic. For example, according to Fick's law [7], age-related thickening of Bruch's membrane [12] as well as the formation of basal linear deposits associated with AMD [13,14], will lead to reduced oxygen flux to the outer retina as a result of the increased diffusion path. The decreased choroidal blood flow [8], choriocapillaris drop out [9], and changes in choroidal vascularity index [10] associated with AMD may also exacerbate localized hypoxia of the outer retina.

Another important factor to consider when interpreting these results is that there was no statistically significant difference in scotopic thresholds between the early AMD and control groups when breathing 21% oxygen in the hyperoxia study. This is in contrast to previous work which has found evidence that scotopic thresholds are higher in participants with AMD when compared to age-matched controls [21,22,37-40]. There are several possible explanations for this discrepancy. Firstly, our group of

participants with early AMD included individuals at an earlier stage of the disease than those included in previous studies. Notably, participants with late stage AMD were included by Brown et al. [40] and Owsley et al.[22,37] which may have resulted in increased scotopic thresholds in their cohort. Further analysis of the data of Owsley et al. [37] comparing only the participant group with early AMD to the control group (by calculating the 95% confidence interval), demonstrated no significant difference in scotopic thresholds between the two groups. It is also possible that the lack of a significant elevation in threshold in the current study was attributable to the large annular stimulus used. Owsley et al. [21,22, 37] sampled the retina at a similar distance from the fovea (10-12° in the inferior visual field) but used a spot stimulus (1.7° diameter). The larger area covered by the annular stimulus will have resulted in a greater level of spatial summation of rod responses. This has the benefit of reducing variability in thresholds where the disease being investigated is characterized by localised abnormalities (i.e. it is heterogeneous), however, it does mean that the deficit measured could be reduced by allowing healthier regions of the retina to compensate for localised areas of abnormality. Finally, it is worth noting that the mean threshold of the AMD group *was* marginally higher than that of the control group under normoxic conditions (see Figure 5), but that this difference failed to reach statistical significance. In the second part of the study, evaluating the effect of systemic hypoxia on thresholds, a small but significant difference was seen between AMD and control groups under the 21% oxygen condition. As this second study had a larger sample size, it is possible that the hyperoxia study was insufficiently powered to detect the small deficit in threshold present in the AMD group. The relevance of the lack of elevated scotopic thresholds in the AMD group of the hyperoxia study is

that it is unlikely that hyperoxia will be found to reduce thresholds which are not significantly elevated in the first place, regardless of whether hypoxia is a factor in the disease pathogenesis.

Another explanation for the lack of effect of either gas condition on thresholds could be that the 10 minute periods of hyperoxia and hypoxia were insufficient to induce sufficient localized retinal changes in oxygenation to affect retinal function. This would also explain the finding that hyperoxia and hypoxia did not alter scotopic thresholds in older participants with healthy retinas. Whilst this is in agreement with literature on the effect of systemic hyperoxia on scotopic thresholds [26], it is in contrast to other previous hypoxia studies which have suggested that hypoxia raises thresholds in normal participants [25,41-45]. However, in earlier studies the oxygen concentration supplied to create a hypoxic episode was lower than that used in this study [25,42,44,45], whilst in other studies which used a similar level of oxygen [27,35], the duration of breathing the air was longer. Preliminary studies by our lab found that SpO_2 stabilised after 8-11mins of breathing air with an altered oxygen concentration. This is illustrated by the data in figure 2, in which a plateau in SpO_2 is demonstrated within the 10 minute timeframe of the experiment. The levels of SpO_2 achieved were also consistent with those reported by previous studies using a 14% oxygen test gas with a longer period of pre-adaptation (15 minutes), suggesting that the test duration was sufficient to reach an equilibrium of SpO_2 [27,35]. However, given the widespread use of longer adaptation times in other studies investigating the effect of systemic periods of hyperoxia and hypoxia [25,27,35,42,44,45], future work in this area may benefit from allowing a prolonged period of adaptation (in excess of 15 minutes) to the altered oxygen conditions to ensure that respiratory equilibrium has been

achieved. Whilst a greater level of hypoxia could also have been induced by breathing air with a lower oxygen concentration, the 14% oxygen used to induce hypoxia was in line with other studies (e.g. Connolly et al. [25, 40]), and ethical considerations prevented us from taking this approach.

In this study, only one aspect of visual function was assessed, scotopic thresholds. It is possible that other aspects of visual function may have shown a greater effect of changing systemic oxygenation. For example, rates of dark adaptation [25,26], colour vision [24] and contrast sensitivity [35] have been shown to be affected in healthy controls when breathing air with a reduced oxygen concentration. In this study, scotopic function was chosen for evaluation as hypoxia is hypothesized to be at its greatest in the outer retina under scotopic conditions, when the rod current is active [11]. Hence, it is unlikely that a greater effect would have been observed under photopic or mesopic conditions. However, it may be of particular interest in future work to evaluate the effect of systemic hypoxia on rates of dark adaptation. The kinetics of dark adaptation have been shown to be affected differentially to scotopic thresholds by early AMD, and are likely to reflect a different aspect of the disease mechanism [21,37]. It is possible that elevated scotopic thresholds reflect the loss of rod photoreceptors reported to occur in early AMD [46], and so may not be reversible by short term manipulation of blood oxygenation. Delays in dark adaptation associated with AMD have previously been shown to be mitigated by vitamin A supplementation [47], suggesting that they may be more amenable to reversal by manipulation of physiological conditions.

It might be argued that this study was limited by the exclusion of individuals with advanced nAMD in the test eye. The evidence of molecular markers of hypoxia such as VEGF and hypoxia inducible factor is much stronger in these individuals than in those with early/intermediate AMD [15,48-50]. Thus, it may follow that the likelihood of finding a significant effect in this study would be increased by the inclusion of participants with nAMD. One reason for focusing on people with early and intermediate AMD was that understanding the mechanisms which drive the early stages of AMD is likely to have more meaningful treatment implications. Mitigation of the factors causing early AMD to advance may bring about delayed progression to late stage disease. Once choroidal neovascularisation has occurred, substantial and irreversible structural changes to the macula have already taken place. Another rationale is that the changes in visual function which have occurred in people with advanced AMD are less likely to be reversible by changes in systemic oxygenation than the milder functional losses associated with early stage disease. In this study, the majority of eyes with early or intermediate AMD (18/22) had a status in the fellow eye which put them at a relatively high risk of progression to a more advanced disease state in the test eye [34] (see Table 1). In all cases, this was due to neovascular changes in the fellow eye. Other studies have tested the non-neovascular eye of participants with unilateral nAMD as a means of determining the effects of a more severe subtype of early/intermediate AMD on visual function [51-55]. The lack of evidence of hypoxia in this cohort is, perhaps, of more clinical significance than a similar finding in individuals with bilateral early changes would be as it suggests that even in a more severe subtype of early AMD there is no clear evidence of systemic hypoxia. However, it remains the case that evaluation of the effects of hyperoxia and

hypoxia on individuals with nAMD may produce a different outcome and would be of interest in future work.

A possible limitation of this study was the use of a fixed termination after 40 presentations rather than a dynamic termination in the QUEST procedure, used due time constraints for safety. Studies comparing dynamic and fixed stopping criterion in Bayesian procedures concluded that there was little evidence to recommend the use of the dynamic over a fixed criterion [56]. An additional limitation was that the effects of breathing reduced levels of oxygen were measured by changes in SpO_2, which is a systemic measure. It may be that it takes longer to create localised retinal hypoxia than it does to induce systemic changes. A further limitation of this study was the relatively small sample size which introduces the possibility of a type II error in the negative findings of this study.

In conclusion, this study did not provide evidence to the support the hypothesis that hypoxia is responsible for functional deficits in early AMD, but did not exclude the possibility that localised retinal hypoxia may be a contributory factor. The understanding of the aetiology of AMD is key in the development of new treatments. If hypoxia plays a part in the pathogenesis of early AMD then treatments aimed at reducing hypoxia could have therapeutic value.

Disclosure Statement

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References

1. Bunce C, Zekite A, Walton S, Rees A, Patel PJ. Certifications for sight impairment due to age related macular degeneration in England. Pub Health. 2015;129(2):138–142.

2. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2(2):e106-16.

3. Jarrett SG, Boulton ME. Consequences of oxidative stress in age-related macular degeneration. Mol Aspects Med. 2012;33(4):399–417.

4. Blasiak J, Petrovski G, Vereb Z, Facskó A, Kaarniranta K. Oxidative Stress, Hypoxia, and Autophagy in the Neovascular Processes of Age-Related Macular Degeneration. Biomed Res Int. 2014: Article number 768026.

5. Ambati J, Atkinson JP, Gelfand BD. Immunology of age-related macular degeneration. Nat Rev Immunol. 2013;13(6), pp.438–51.

6. Feigl B. Age-related maculopathy - linking aetiology and pathophysiological changes to the ischaemia hypothesis. Prog Retin Eye Res. 2009; 28(1):63–86.

7. Stefánsson E, Geirsdóttir A, Sigurdsson H. Metabolic physiology in age related

macular degeneration. Prog Retin Eye Res. 2011;30(1):72–80.

8. Grunwald JE, Metelitsina TI, DuPont JC, Ying G-S, Maguire MG. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. Invest Ophthalmol Vis Sci. 2005; 46(3): 1033–8.

9. Seddon JM, McLeod DS, Bhutto IA, Villalonga MB, Silver RE, Wenick AS, Edwards MM & Lutty GA. Histopathological Insights Into Choroidal Vascular Loss in Clinically Documented Cases of Age-Related Macular Degeneration. JAMA Ophthalmol. 2016;134(11):1272.

10. Wei X, Ting DS, Ng WY, Khandelwal N, Agrawal R, Cheung CMG. Choroidal Vascularity Index: A Novel Optical Coherence Tomography Based Parameter in Patients With Exudative Age-Related Macular Degeneration. Retina. 2017;37(6):1120–1125.

11. Wangsa-Wirawan ND, Linsenmeier RA. Retinal Oxygen. JAMA Ophthalmol. 2003;121(4):547–557.

12. Pauleikhoff D, Harper A, Marshall J, Bird AC. Aging changes in Bruch's membrane. A histochemical and morphologic study. Ophthalmology. 1990;97:171–8.

13. Curcio CA, Johnson M, Rudolf M, Huang J-D. The oil spill in ageing Bruch membrane. Br J Ophthalmol. 2011;95:1638-1645.

14. Curcio CA. Imaging Maculopathy in post mortem eyes. Vis Res. 2005; 45:3496–3503

15. Kliffen M, Sharma HS, Mooy CM, Kerkvliet S, de Jong PT. Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol.

1997;81(2):154–62.

16. Ferrara, N., Gerber, H. & LeCouter, J. The biology of VEGF and its receptors. Nat Med. 2003;9:669–676.

17. Fukumura D, Xu L, Chen Y, Gohongi T, ZSeed B, Jain RK. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res. 2001;61(16):6020-6024.

18. Arden GB, Wolf JE. Colour vision testing as an aid to diagnosis and management of age related maculopathy. Br J Ophthalmol. 2004;88(9):1180–5.

19. O'Neill-Biba M, Sivaprasad S, Rodrguez-Carmona M, Wolf JE, Barbur JL. Loss of chromatic sensitivity in AMD and diabetes: a comparative study. Ophthal Physiol Opt. 2010;30(5):705–16.

20. Midena E, Degli Angeli C, Blarzino MC, Valenti M & Segato T. Macular function impairment in eyes with early age-related macular degeneration. Invest Ophthalmol Vis Sci. 1997;38(2):469–77.

21. Owsley C, Jackson GR, White M, Feist R, Edwards D. Delays in rod-mediated dark adaptation in early age-related maculopathy. Ophthalmology. 2001;108(7):1196–202.

22. Owsley C, Jackson GR, Cideciyan AV, Huang Y, Fine SL, Ho AC, Maguire MG,Lolley V, Jacobson SG. Psychophysical evidence for rod vulnerability in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2000;41(1):267–73.

23. Connolly DM. Spatial Contrast Sensitivity at Twilight: Luminance, Monocularity,

and Oxygenation. Aviat Space Environ Med. 2010;81(5):475–483.

24. Willmann G, Ivanov IV, Fischer MD, Lahiri S, Pokharel RK, Werner A, Khurana TS. Effects on colour discrimination during long term exposure to high altitudes on Mt Everest. Br J Ophthalmol. 2010;94(10):1393–7.

25. McFarland RA, Forbes WH. The Effects of Variations in the Concentration of Oxygen and of Glucose on Dark Adaptation. J Gen Physiol. 1940; 24(1):69–98.

26. Connolly DM, Hosking SL. Aviation-related respiratory gas disturbances affect dark adaptation: a reappraisal. Vision Res. 2006;46(11):1784–93.

27. Connolly DM, Barbur JL, Hosking SL, Moorhead IR. Mild hypoxia impairs chromatic sensitivity in the mesopic range. Invest Ophthalmol Vis Sci. 2008;49(2):820–7.

28. Harris A, Arend O, Danis RP, Evans D, Wolf S, Martin BJ. Hyperoxia improves contrast sensitivity in early diabetic retinopathy.Br J Ophthalmol. 1996 Mar; 80(3):209–213

29. Drasdo N, Chiti Z, Owens DR, North RV. Effect of darkness on inner retinal hypoxia in diabetes. Lancet. 2002;359(9325);2251-2253.

30. Chylack LT Jr(1), Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, Friend J, McCarthy D, Wu SY. The lens opacities classification system III. the Longitudinal study of cataract study group. Arch Ophthalmol. 1993;111(6):831-6.

31. Ferris FL 3rd1, Wilkinson CP, Bird A, Chakravarthy U, Chew E, Csaky K, Sadda SR. Beckman Initiative for Macular Research Classification Committee. Clinical

classification of age-related macular degeneration. Ophthalmology. 2013;120(4):844-51

32. Watson AB, Pelli DG. QUEST: a Bayesian adaptive psychometric method. Percept Psychophys. 1983;33(2):113–20.

33. Brainard DH. The Psychophysics Toolbox. Spatial vision. 1997:10(4):433–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9176952

34. Ferris FL, Davis MD, Clemons TE, Lee L-Y, Chew EY, Lindblad AS, Milton RC, Bressler SB, Klein R. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. Arch Ophthalmol. 2005;123(11):1570–1574.

35. Connolly DM, Hosking SL. Oxygenation and gender effects on photopic frequency-doubled contrast sensitivity. Vision Res. 2008;48(2): 281–8.

36. Linsenmeier RA, Zhang HF. Retinal oxygen: from animals to humans. Prog Retin Eye Res. 2017;58:115-151

37. Owsley C, McGwin Jr G, Jackson GR, Kallies K, Clark M. Cone- and rod-mediated dark adaptation impairment in age-related maculopathy. Ophthalmol. 2007;114(9):1728–35.

38. Chen C, Wu L, Wu D, Huang S, Wen F, Luo G, Long S. The local cone and rod system function in early age-related macular degeneration. Doc Ophthalmol. 2004;109(1):1–8.

39. Steinmetz RL, Haimovici R, Jubb C, Fitzke FW, Bird AC. Symptomatic

abnormalities of dark adaptation in patients with age-related Bruch's membrane change. Br J Ophthalmol. 1993;77(9):549–54

40. Brown B, Adams AJ, Coletta NJ, Haegerstrom-Portnoy G. Dark adaptation in age related maculopathy. Ophthal Physiol Opt. 1986;6(1):81–4.

41. McDonald R, Adler F. Effect of anoxemia on the dark adaptation of the normal and of the vitamin A-deficient subject. Arch Ophthalmol. 1939;37:368–378.

42. Wald G, Harper P. Respiratory effects upon the visual threshold. J Gen Physiol. 1942;25(6):891–903.

43. Kobrick LJ, Zwick H, Devine JA. Effects of extended hypoxia on night vision. Aviat Space Environ Med. 1984;55(3):191–5.

44. Ernest JT, Krill AE. The effect of hypoxia on visual function. Psychophysical studies. Invest Ophthalmol Vis Sci. 1971;10(5):323–8.

45. Hecht S, Hendley C. Anoxia and brightness discrimination. J Gen Physiol. 1946;29:335–351.

46. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci. 1996;37:1236–1249.

47. Owsley C, McGwin G, Jackson GR, et al. Effect of short-term, high-dose retinol on dark adaptation in aging and early age related maculopathy. Invest Ophthalmol Vis Sci. 2006;47:1310–8.

48. Amin R,Puklin JE,Frank RN.Growth factor localization in choroidal neovascular membranes of age-related macular degeneration. Invest Ophthalmol Vis Sci. 1994;35:3178–88.

49. Reddy MR, Zamora RL, Kaplan HJ. Distribution of growth factors in subfoveal neovascular membranes in age-related macular degeneration and presumed ocular histoplasmosis sysndrome. Am J Ophthalmol. 1995;120:291–301.

50. Sheridan CM, Pate S, Hiscott P, Wong D, Pattwell DM, Kent D. Expression of hypoxia-inducible factor-1alpha and -2alpha in human choroidal neovascular membranes. Graefes Arch Clin Exp Ophthalmol. 2009;247:1361-1367.

51. Eisner A, Fleming SA, Klein ML, Mauldin WM. Sensitivities in older eyes with good acuity: eyes whose fellow eye has exudative AMD. Invest Ophthalmol Vis Sci. 1987;28(11): 1832-1837.

52. Eisner A, Stoumbos VD, Klein ML, Fleming SA. Relations between fundus appearance and function. Eyes whose fellow eye has exudative age-related macular degeneration. Invest Ophthalmol Vis Sci. 1991;32(1): 8-20.

53. Sandberg MA, Weiner A, Miller S, Gaudio AR. High-risk characteristics of fellow eyes of patients with unilateral neovascular age-related macular degeneration. Ophthalmology. 1998;105(3): 441-447.

54. Dimopoulos IS, Tennant M, Johnson A, Fisher S, Freund PR, Sauve Y. Subjects With Unilateral Neovascular AMD Have Bilateral Delays in Rod-Mediated Phototransduction Activation Kinetics and in Dark Adaptation Recovery. Invest Ophthalmol Vis Sci. 2013;54(8): 5186-5195

55. Hogg R, Rufino S, Staurenghi G, Murphy G, Santos AR, Rosina C, Chakravarthy U. Clinical Characteristics of Reticular Pseudodrusen in the Fellow Eye of Patients

with Unilateral Neovascular Age-Related Macular Degeneration. Ophthalmology. 2014;121(9): 1748-1755.

56. Anderson AJ. Utility of a dynamic termination criterion in the ZEST adaptive threshold method. Vision Res. 2003;43(2):165–170.

Table 1 Characteristics of participants with early AMD. AMD status score relates to the AREDS simplified scale score, and indicates the number of risk factors per eye.[34]

Age (years)	Gender	AMD status test eye	AMD status fellow eye	Combined status	VA test eye (LogMAR)	VA fellow eye (LogMAR)	Hypothesis tested (1=hyperoxic gas, 2 = hypoxic gas, 3 = both)
69	F	1	2	3	0.28	0.5	1
71	M	0	2	2	-0.16	0.12	1
67	F	1	2	3	0.12	-0.14	1
86	F	0	2	2	0.18	0.54	1
83	F	1	1	2	-0.2	-0.18	1
78	F	1	2	3	0	-0.1	1
77	M	1	2	3	0.00	0.02	2
78	M	2	2	4	0.2	0.82	3
74	M	1	2	3	0.08	0.52	2
76	M	1	2	3	-0.06	0	3
69	F	1	2	3	0.02	0.48	2
82	M	1	2	3	0.24	0.3	2
69	M	1	2	3	0.02	1.4	3
71	M	0	2	2	0	0.2	3
78	F	1	2	3	0	0	3
72	F	1	1	2	0	0.06	2
67	M	1	2	3	0.02	0	3
69	F	1	2	3	0.2	1	2
74	M	1	0	1	0	-0.08	2
68	F	1	2	3	-0.01	0.1	2
79	F	1	1	2	0.1	0.14	2
64	M	0	2	2	-0.1	0.02	2

Figure legends

Figure 1. Timeline of experimental protocol. This was the same for both the hyperoxia and the hypoxia study. 'Gas 1' and 'gas 2' refer to the two gases used in random order in each visit. One of these was always medical air.

Figure 2. Mean SpO2 results per minute for each group (controls and AMD, with 95% confidence intervals). Results are compared under conditions of 60% oxygen (diamonds) vs. 21% oxygen (squares) (panel A a,b) and 14% oxygen (diamonds) vs. 21% oxygen (squares) (panel B a,b). Both experimental gases has a significant effect on group averaged SpO_2 data, with SpO_2 readings stabilizing after 5 minutes of breathing the gas.

Figure 3. Sample scotopic threshold QUEST output from one individual with standard deviations (a,c), with corresponding SpO2 recordings (b,d) during data collection. Top panels show data when breathing 21% oxygen and bottom panels when breathing 60% oxygen. All data collected from control participant 017.

Figure 4. Scatter plot showing change in scotopic threshold under different gas conditions for all participants. Panel A shows the difference in thresholds when breathing 60% oxygen compared to breathing 21% oxygen. Panel B shows the difference in thresholds when breathing 14% oxygen compared to breathing 21%

oxygen. A negative value indicates a lower threshold for the experimental gas than the medical air.

Figure 5 Group mean scotopic thresholds with 95% confidence intervals for the AMD group (a) and for the control group (b) under 21% and 60% oxygen conditions (Panel A) and 21% and 14% conditions (Panel B, a and b). There were no significant differences in threshold between gas conditions for either group.