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Research Report

Optically Improved Mitochondrial Function Redeems Aged Human Visual Decline

Harpreet Shinhmar, MSc,[1] Manjot Grewal, BSc,[1] Sobha Sivaprasad, MBBS, PhD,[1] Chris Hogg,[1] Victor Chong, MBBS, PhD,[2] Magella Neveu, PhD,[1] and Glen Jeffery, DPhil[1,*]

[1]Institute of Ophthalmology, University College London, UK. [2]Boehringer Ingelheim, Germany.

*Address correspondence to: Glen Jeffery, DPhil, Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V 9EL, UK. E-mail: g.jeffery@ucl.ac.uk

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Abstract

The age spectrum of human populations is shifting toward the older with larger proportions suffering physical decline. Mitochondria influence the pace of aging as the energy they provide for cellular function in the form of adenosine triphosphate (ATP) declines with age. Mitochondrial density is greatest in photoreceptors, particularly cones that have high energy demands and mediate color vision. Hence, the retina ages faster than other organs, with a 70% ATP reduction over life and a significant decline in photoreceptor function. Mitochondria have specific light absorbance characteristics influencing their performance. Longer wavelengths spanning 650–>1,000 nm improve mitochondrial complex activity, membrane potential, and ATP production. Here, we use 670-nm light to improve photoreceptor performance and measure this psychophysically in those aged 28–72 years. Rod and cone performance declined significantly after approximately 40 years of age. 670-nm light had no impact in younger individuals, but in those around 40 years and older, significant improvements were obtained in color contrast sensitivity for the blue visual axis (tritan) known to display mitochondrial vulnerability. The red visual axis (protan) improved but not significantly. Rod thresholds also improved significantly in those >40 years. Using specific wavelengths to enhance mitochondrial performance will be significant in moderating the aging process in this metabolically demanding tissue.

Keywords: Aging, Color vision, Photobiomodulation

Human aging is a major societal problem and the retina ages faster than other organs, partly due to its high metabolic rate (1,2). Here, 30% of central rods die and cones have reduced function by 70 years of age (3–5). The pace of aging is partly controlled by the cells metabolism regulated by its mitochondria that produce adenosine triphosphate (ATP) to fuel cell function. When mitochondria decline, they have reduced membrane potential and ATP synthesis. When this occurs, mitochondria can increase production of reactive oxygen species that increases systemic inflammation and can signal cell death (6).

Mitochondrial density is greatest in photoreceptors and their decline can be linked to reductions in retinal function and the onset of age-related disease (7). However, aged mitochondrial performance can be improved optically because mitochondria absorb longer wavelengths, including those beyond the limits of human vision and this is often termed photobiomodulation. Light-induced improvements in mitochondrial function are associated with an increase in mitochondrial membrane potential and ATP production (8,9). Further, long-wavelength light can improve retinal and general central nervous system function that has declined due to age or mitochondrial insult (10–12). It has also been shown that photobiomodulation can improve aged murine retinal function (13). However, the mouse retina lacks a peak in cone density centrally and ages very differently from primates (14). Further, rodents commonly avoid light and do not use vision as their primary sensory modality.

Here, we use longer wavelengths to determine if this treatment can improve aged human retinal function. Specifically, we test the hypothesis that relatively brief daily exposures to 670 nm for 2 weeks can significantly improve retinal function in those over approximately 40 years of age, particularly in the cones mediating the tritan visual axis, which we see as blue.

Method

Twenty-four healthy participants of both sexes were used with University College London ethical approval. They ranged from 28 to 72 years. The cutoff point between younger and older groups was >38 years, with age as the only significant variable. Different participants were used to measure rod (scotopic) thresholds and color contrast sensitivity (CCS), which were undertaken at different times. There were 12 individuals in each group. In the CCS group there were six younger (five female and one male) and six older (four female and two male) participants, and in the scotopic threshold group there were six younger (four females and two males) and six older (four female and two males) participants. 670-nm light devices were based on simple commercial DC torches with ten 670-nm LEDs mounted behind a light diffuser embedded in a tube that was 4 cm in diameter. Energies at the cornea were approximately 40 mW/cm^2 which often resulted in a mild green after image for approximately 5–10 seconds. Participants were asked to use the light to illuminate their dominant eye every morning for 3 minutes and to repeat this daily for 2 weeks. These metrics were selected because they fell within the range used in a large number of animal experiments. 670-nm illumination was largely confined to the central retina comprising the peaks in rod and cone density.

Cone Function

Color contrast sensitivity was assessed by measuring color contrast thresholds across the protan (red visual axis) and tritan (blue visual axis) axes using a computer graphics system, "Chromatest," on 12 healthy participants of progressive ages with no known ocular or systemic diseases (mean age, 43.1 years; range, 28–68 years; standard deviation = 13.7). For CCS measurements, participants were seated at a fixed distance from the stimulus monitor such that the optotype letters subtended a 1.3-degree angle on the retina. The letters were displayed on a randomized noise background to ensure stimulus equiluminance. They appeared either in red or blue to test protan and tritan axes, respectively. The software utilizes binary search algorithm to determine thresholds. Three baseline recordings were taken prior to onset of 670-nm exposure and after. Final CCS recordings were taken on the last day of treatment. Results were analyzed from initial baseline recordings and final recordings taken after 670-nm exposure. Test–retest variability was 0.5% for protan thresholds and 1.4% for tritan thresholds.

Rod Function

Rod thresholds were also determined before and after 670-nm exposure in 12 healthy participants that were different from the CCS group. 670-nm application times were as above (mean age, 47.8 years; range, 29–72 years; standard deviation = 16.5). Participants had their pupils dilated to maximize photon capture and were dark-adapted for 40 minutes. Retinal sensitivities were measured with the Medmont dark-adapted chromatic perimeter (Medmont, Australia) with a stimulus size of 1.73° (Goldmann size V) using 3 decibel (dB) steps (6-3 staircase threshold strategy). Light stimuli (505 nm presented for 200 ms) were presented at 17 test points distributed within the central 24° of the retina at 4°, 6°, 8°, and 12° eccentricity to the fovea. Appropriate lens correction was used for a viewing distance of 30 cm. Participants were instructed to fixate on a central target and were monitored during testing using an in-built infrared camera. Because measurements were in dB, higher values represent improved detection. Test–retest variability was within 1.5 dB.

Statistical Analysis

Data were graphed and analyzed using GraphPad Prism 6 (GraphPad, San Diego, CA) with a Wilcoxon matched-pairs signed rank test for significance. Measurements comparing younger and older groups were analyzed with a Mann–Whitney *U* test for significance.

Results

In each of the three visual functions examined at baseline there were signs of decline from approximately 40 years of age. However, this needs qualification as data presented are not displayed with age as a linear variable. In the tritan axis, baseline CCS increased significantly over about 40 years compared to younger participants by maximum of 47% and an average of around 20% (Figure 1A, $p \leq .0001$).

Over the total group spanning 28–68 years of age there was a significant improvement in tritan thresholds after exposure to 670 nm by 14% ($p \leq .01$) represented by lower values. However, when the total group was divided into younger and older, the improved thresholds were clearly the result of positive shifts in those over the age of 38 where there was a 22% improvement ($p \leq .001$), while younger individuals showed no change (Figure 1B, $p > .05$). Hence, 670-nm treatment in older participants improved tritan thresholds bringing them toward levels found in younger individuals. However, significant differences remained between the younger baseline and older treated ($p \leq .01$). Protan thresholds showed no change after 670-nm exposure in the younger group (Figure 1C, $p > .05$), but it did improve by approximately 10% in the older participants, although this did not reach statistical significance (Figure 1D, $p > .05$).

Scotopic thresholds were measured in decibels (dB) where improved sensitivity results in increased numerical values. Improved sensitivity was found in 8 of the 12 participants, 3 of which were under the age of 40 (Figure 1E). But there were no statistically significant changes in the younger group ($p > .05$). However, in line with the group tested for tritan thresholds, older individuals showed significant improvement (Figure 1F, $p \leq .05$). While significant improvements in photoreceptors function were found for both rods and cones, participants did not report any subjective changes in their vision.

Discussion

We show improved aged human photoreceptor function following relatively brief exposure to 670-nm light that modulates mitochondria, but there were differences between protan and tritan thresholds reflecting different features specific to the two cone color systems. The tritan system improved by approximately 22% in older participants. The tritan axis is partly based on short-wavelength-sensitive cone function. These cells are relatively frail and suffer disproportionality in age and disease including diabetes (15). Further, we have recently shown in old world primates that S-cones contain fewer mitochondria than other cone classes and are more reliant on glycolysis for ATP production of ATP. This may increase their pace of aging (16). In explaining the relative increased magnitude of improvement in tritan function over protan, it is possible that there is a smaller margin of error in S-cone mitochondrial function due to their reduced number. Hence, 670 nm may have

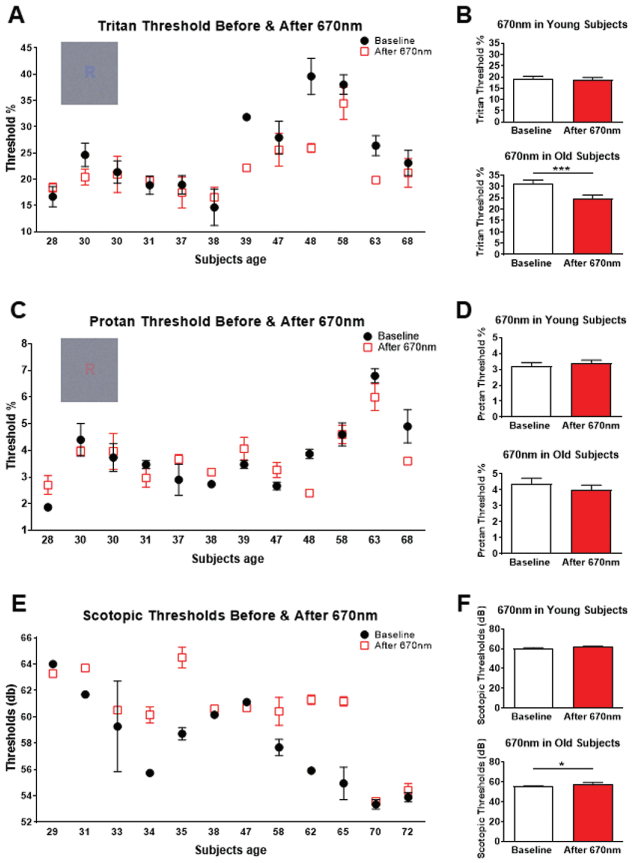

Figure 1. Color contrast sensitivities and scotopic thresholds. Black circles represent baseline measurements and red boxes are those in the same individuals measured after 2 weeks 670-nm exposure. **(A)** Thresholds for tritan function in individuals 28–68 years. Baseline measurements increase over approximately 40 years of age, consistent with reduced function. Overall changes in the group are significant after treatment as lower values represent improved function. **(B)** However, when the group is divided into younger and older, only changes in the older group are significant. **(C)** Thresholds for protan function in individuals 28–68 years. 670-nm exposure showed no effect to protan function. The blue R in (A) and the red R in (C) are examples of the target to be identified as it appeared on the screen. **(D)** Measurements in protan function only produce reduced thresholds in the older group but this was not significant. **(E)** Scotopic thresholds for individuals 28–72 years, thresholds are measured in decibels (dB) where improvements result in greater numerical values. **(F)** 670-nm had no impact on scotopic thresholds in younger individuals, but there was a significant improvement in older participants. Wilcoxon test for across all age groups, Mann–Whitney *U* for histograms. Statistical significance abbreviations: $*p \leq .05$, $***p \leq .001$. Error bars are *SEM*.

greater impact on them. However, the protan system also improved by around 10% in older participants even though this was not statistically significant.

A potential factor influencing tritan sensitivity is the progressive yellowing of the human lens with age, which will absorb shorter wavelengths and theoretically has the potential to affect our measurements (17). However, it is now known that there is compensation for these changes with mechanisms that stabilize color appearance independent of changes in the lens (18). Although, there is no evidence that there are significant changes in lens opacity between 35 and 40 years that could explain the differences in baseline between our younger and older groups. Also, changes in lens opacity would not undermine the value of the results obtained following 670-nm exposure over baseline.

The ability to improve cone function is of importance because these cells, unlike rods, do not die with age in primates including humans (3). This includes blue cones that share molecular markers with rods and have known vulnerabilities (15,16,19,20). Cones appear to survive, although with reduced function (5,21). That they survive even though there is a significant decline in retinal ATP represents a challenge in our concepts of aging. Because if they can be refueled by improving mitochondrial function, as implied by our findings, it may affect positively on aged vision. The importance of this is re-enforced by our increased reliance on artificial lighting resulting in the retina rarely being fully dark-adapted. Hence, rods remain commonly saturated. In this environment human vision becomes cone-dependent, independent of time of day.

Improved photoreceptor function was not confined to cones, as aged rod function also showed a positive outcome. Similar improved rod function was found in mice after 670-nm exposure. The mouse retina is more rod-dominated than the human (22) and here 670-nm exposure significantly improves the magnitude of the aged electroretinogram, which is the physiological response of the retina recorded by electrodes on the ocular surface (13). However, there are many qualifications here that do not undermine the data in mouse or human but make parallels between them difficult. An additional factor that limits the impact of 670 nm on rods is that in human they are lost with age by approximately 30% by 70 years of age (3). Hence, improvements in the older group occur even though the photoreceptor population examined is declining.

In spite of the differences between photoreceptor classes there is clear evidence for improved aged visual function using long-wavelength light that is known to affect positively on mitochondria (8). While much of the data are on models of induced pathology, including that of the visual system (10) there is a growing literature on its influence in aging. The rationale for its use here has a degree of clarity based upon the mitochondrial theory of aging. This argues that reactive oxygen species production, which is often in reciprocal relationship with ATP, drives aging (6). However, this is not without qualification (23). While the outer retina has the greatest metabolic rate in the body, the central nervous system consumes large quantities of ATP in the maintenance of its ionic membrane pumps, and this declines significantly with age (1). Hence, the aged retina and brain move toward an ATP deficit that undermines function.

While there is general acceptance that longer wavelengths improve aged or damaged central nervous system function (10), its route of action remains partly obscured. We do not know exactly what mitochondrial elements absorb these wavelengths, their dynamics or downstream interactions. Further, it is now clear that they have a temporal domain because long wavelengths have different impacts at different times of the day. These temporal differences relate to the changing state of mitochondria over 24 hours where their complex activity and ATP production vary markedly. Within this context, it has been argued that long-wavelength light absorbance is only effective when mitochondria are not working at maximum output, but have spare capacity in their complexes and ATP production. At times of the day when mitochondrial complex activity peaks, longer wavelengths appear to be ineffective (24). However, our understanding of the temporal variations in the efficacy of long-wavelength light usage is limited and needs considerable exploration before its use can be applied to maximal effect.

This pilot study has limitations due to its sample size, but the results reveal significant improvement in both rod and cone function in an aged cohort but not in younger individuals. This difference is presumably because age-related mitochondrial decline has not yet affected the younger individuals. Widespread positive results using long-wavelength light in aging and disease in animals have provided an impetus for their clinical application in full-scale clinical trials for diabetic retinopathy (NCT03866473) and age related macular degeneration (NCT02725762, 03878420). However, a recently published study on AMD patients has failed to show any improvement in retinal function in this disease (25). Consequently, there is much that we still need to understand regarding the advantages and limits of this therapeutic route.

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Conflict of Interest

None declared.

References

1. Wong-Riley MT. Energy metabolism of the visual system. *Eye Brain*. 2010;2:99–116. doi:10.2147/EB.S9078
2. Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Prog Retin Eye Res*. 2001;20(2):175–208. doi:10.1016/s1350-9462(00)00027-6
3. Curcio CA, Millican CL, Allen KA, Kalina RE. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. *Invest Ophthalmol Vis Sci*. 1993;34(12):3278–3296.
4. Owsley C. Aging and vision. *Vision Res*. 2011;51(13):1610–1622. doi:10.1016/j.visres.2010.10.020
5. Neveu MM, Dangour A, Allen E, et al. Electroretinogram measures in a septuagenarian population. *Doc Ophthalmol*. 2011;123(2):75–81. doi:10.1007/s10633-011-9282-1
6. Harman D. About "Origin and evolution of the free radical theory of aging: a brief personal history, 1954-2009". *Biogerontology*. 2009;10(6):783. doi:10.1007/s10522-009-9253-z
7. Eells JT. Mitochondrial dysfunction in the aging retina. *Biology (Basel)*. 2019;8(2):1–10. doi:10.3390/biology8020031
8. Gkotsi D, Begum R, Salt T, et al. Recharging mitochondrial batteries in old eyes. Near infra-red increases ATP. *Exp Eye Res*. 2014;122:50–53. doi:10.1016/j.exer.2014.02.023
9. Kokkinopoulos I, Colman A, Hogg C, Heckenlively J, Jeffery G. Age-related retinal inflammation is reduced by 670 nm light via increased mitochondrial membrane potential. *Neurobiol Aging*. 2013;34(2):602–609. doi:10.1016/j.neurobiolaging.2012.04.014
10. Fitzgerald M, Hodgetts S, Van Den Heuvel C, et al. Red/near-infrared irradiation therapy for treatment of central nervous system injuries and disorders. *Rev Neurosci*. 2013;24(2):205–226. doi:10.1515/revneuro-2012-0086
11. Powner MB, Salt TE, Hogg C, Jeffery G. Improving mitochondrial function protects bumblebees from neonicotinoid pesticides. *PLoS One*. 2016;11(11):e0166531. doi:10.1371/journal.pone.0166531
12. Begum R, Calaza K, Kam JH, Salt TE, Hogg C, Jeffery G. Near-infrared light increases ATP, extends lifespan and improves mobility in aged *Drosophila melanogaster*. *Biol Lett*. 2015;11(3):20150073. doi:10.1098/rsbl.2015.0073
13. Sivapathasuntharam C, Sivaprasad S, Hogg C, Jeffery G. Aging retinal function is improved by near infrared light (670 nm) that is associated with corrected mitochondrial decline. *Neurobiol Aging*. 2017;52:66–70. doi:10.1016/j.neurobiolaging.2017.01.001
14. Volland S, Esteve-Rudd J, Hoo J, Yee C, Williams DS. A comparison of some organizational characteristics of the mouse central retina and the human macula. *PLoS One*. 2015;10(4):e0125631. doi:10.1371/journal.pone.0125631
15. Greenstein VC, Hood DC, Ritch R, Steinberger D, Carr RE. S (blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. *Invest Ophthalmol Vis Sci*. 1989;30(8):1732–1737.
16. Kam JH, Weinrich TW, Sangha H, Powner MB, Fosbury R, Jeffery G. Mitochondrial absorption of short wavelength light drives primate blue retinal cones into glycolysis which may increase their pace of aging. *Vis Neurosci*. 2019;36:E007. doi:10.1017/S0952523819000063
17. Pokorny J, Smith VC, Lutze M. Aging of the human lens. *Appl Opt*. 1987;26(8):1437–1440. doi:10.1364/AO.26.001437
18. Tregillus KE, Werner JS, Webster MA. Adjusting to a sudden "aging" of the lens. *J Opt Soc Am A Opt Image Sci Vis*. 2016;33(3):A129–A136. doi:10.1364/JOSAA.33.00A129
19. Craft CM, Huang J, Possin DE, Hendrickson A. Primate short-wavelength cones share molecular markers with rods. *Adv Exp Med Biol*. 2014;801:49–56. doi:10.1007/978-1-4614-3209-8_7
20. Weinrich TW, Powner MB, Lynch A, Jonnal RS, Werner JS, Jeffery G. No evidence for loss of short-wavelength sensitive cone photoreceptors in normal ageing of the primate retina. *Sci Rep*. 2017;7(1):46346. doi:10.1038/srep46346
21. Silvestre D, Arleo A, Allard R. Healthy aging impairs photon absorption efficiency of cones. *Invest Ophthalmol Vis Sci*. 2019;60(2):544–551. doi:10.1167/iovs.18-25598
22. Carter-Dawson LD, Lavail MM. Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. *J Comp Neurol*. 1979;188(2):245–62. doi:10.1002/cne.901880204
23. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153(6):1194–1217. doi:10.1016/j.cell.2013.05.039
24. Weinrich TW, Kam JH, Ferrara BT, Thompson EP, Mitrofanis J, Jeffery G. A day in the life of mitochondria reveals shifting workloads. *Sci Rep*. 2019;9(1):13898. doi:10.1038/s41598-019-48383-y
25. Grewal MK, Sivapathasuntharam C, Chandra S, et al. A pilot study evaluating the effects of 670 nm photobiomodulation in healthy ageing and age-related macular degeneration. *J Clin Med*. 2020;9(4):1001. doi:10.3390/jcm9041001