THE INFLUENCE OF AGE AND LUTEIN STATUS ON VESTIBULAR AND

AUDITORY FUNCTION

by

JENNIFER CAROLINE WONG

(Under the Direction of Billy R. Hammond, Jr.)

ABSTRACT

Increased oxidative stress may promote increases in neural noise. One immediate defense mechanism for increased oxidative stress is to accumulate exogenous antioxidants, such as the dietary carotenoids. Lutein (L) and zeaxanthin (Z) are two carotenoids found in the macular pigment (MP) and outer layers of the lens. Retinal levels of LZ are positively correlated with levels of LZ in the auditory cortex and cerebellum, regions important for auditory and vestibular function, respectively. The purpose of these studies was to examine (using both longitudinal and cross-sectional designs) the relationship between age, indices of oxidative exposure (MP density, lens density), and sensory function (vestibular and auditory). MP and lens density were assessed psychophysically with a non-invasive method. Vestibular function was assessed with the One Foot Balance test. Auditory function was assessed with a Grason-Stadler Instruments 61 two channel instrument (GSI 61) using the Modified Hughson-Westlake method. Two elderly groups participated in the longitudinal arms of this study. MP density was available for one group that was tested 11 years earlier ($N = 12$, $M = 74.5 \pm 9.1$ years). MP density was significantly ($p < .001$) higher at the second time point.

Vestibular function was also assessed longitudinally. These measures, along with MP density, were available across an eight year time period ($N = 7$, 70.3 ± 4.4 years). Vestibular function was significantly ($p < .05$) higher when assessed at the second time point. MP density was not associated with individual baseline or follow-up vestibular assessments. Fifty non-smokers and 17 smokers, across ages, participated in the cross-sectional study. Age was significantly related to all variables: vestibular and auditory function and MP and lens density. When stratified by age and smoking status, MP density and lens density were significantly related to auditory function only in young healthy individuals.

INDEX WORDS: age, lutein, zeaxanthin, macular pigment, vestibular function, auditory function

THE INFLUENCE OF AGE AND LUTEIN STATUS ON VESTIBULAR AND

AUDITORY FUNCTION

by

JENNIFER CAROLINE WONG

B.S., The University of Georgia, 2005

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2013

THE INFLUENCE OF AGE AND LUTEIN STATUS ON VESTIBULAR AND

AUDITORY FUNCTION

by

JENNIFER CAROLINE WONG

Major Professor: Billy R. Hammond, Jr.

Committee: Philip V. Holmes
                   Gaylen L. Edwards

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

DEDICATION

To my parents who support and believe in me unconditionally.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

One of the more difficult aspects of aging is loss in neural efficiency. At a minimum,

sensory systems must retain the ability to discriminate a signal from noise generated either

externally or internally. Signal detection theory, a technique for measuring thresholds in sensory

research, is largely based on separating signals from noise. The ability to make this

discrimination decreases with age and sensory or neural disease. Despite these accepted axioms,

most clinical assessments in common use (e.g., perimetric evaluation of visual thresholds,

clinical audiometric assessments) are based on measuring absolute thresholds without

considering any other degradation of the signal. This is problematic since absolute thresholds

likely do not adequately reflect underlying loss. For example, humans lose approximately 30

million rod photoreceptors between 20 and 60 years of age, but scotopic sensitivity barely

declines (as noted by Hammond et al., 1998).

In this series of studies, we investigated the relationship between sensory noise, age, and

factors that are thought to influence how well one ages. With respect to the latter, oxidative

stress has consistently been identified as a stressor that accelerates the aging process. The

nervous system uses an abundance of oxygen, and is therefore susceptible to damage by reactive

oxygen species. This is one reason why the nervous system possesses such a high density of

endogenous antioxidants (like superoxide dismutase) and accumulates such large quantities of

exogenous antioxidants (like the tocopherols or carotenoids). With respect to the latter, the

technology exists to measure the concentration of a collection of carotenoids (lutein (L),

zeaxanthin (Z), and *meso*-zeaxanthin) in the macula. Collectively, these pigments are referred to as the macular pigment (MP). LZ are hypothesized to improve visibility by filtering short-wave light (e.g., Wooten & Hammond, 2002; Hammond et al., 2012), act as an antioxidant (e.g., Sujak et al., 1999; Stahl & Sies, 2002), and enhance gap junction communication (e.g., Stahl & Sies, 2001). Retinal levels of LZ reflect levels of these carotenoids throughout the monkey brain (Vishwanathan et al., 2013). Retinal and serum levels of LZ are also significantly related to cognitive function (e.g., Johnson et al., 2008). It therefore follows that neural efficiency may be influenced by changes in LZ concentration. This study was designed to test this hypothesis.

Another question is how all of these variables are related to age itself. Past studies have yielded inconsistent results on the relationship between age and MP density. This inconsistency may be due, in part, to methodological issues (e.g., see Hammond et al., 2005). It may also, however, be due to the fact that mostly cross-sectional data were collected. In the present study, we addressed the methodological confounds by using a validated non-invasive psychophysical method (the "gold standard" in this area of research). To address the possibility of confounding due to a cross-sectional design, we employed a longitudinal design.

To assess long-term oxidative exposure, we measured lens optical density. The crystalline lens in the anterior chamber of the eye is the only tissue in the body that does not undergo biological renewal (the cells are the same throughout life and not replaced via mitosis) but must stay transparent. Oxygen can modify the crystalline proteins within the lens (the major etiology of age-related cataracts) leading to a loss of transparency with time. We quantified the loss non-invasively by measuring lens optical density. Taken together, MP density and lens density should reflect protection from oxidative exposure and long-term oxidative exposure, respectively.

We also measured smoking status, a known oxidative stressor. Smokers have significantly lower MP density compared to non-smokers, even when they have higher levels of carotenoid intake (e.g., Hammond et al., 1996). Smokers also have higher lens density compared to non-smokers (Hammond et al., 1999). Carotenoid levels are lower in the blood circulation of smokers (e.g., Handelman et al., 1996) and cigarette smoke can damage blood vessels (e.g., Boms et al., 2010). Cigarettes also contain pro-oxidants (e.g., Pryor & Stone, 1993; Church & Pryor, 1985) that are damaging to lipid membranes (e.g., Niki et al., 1993) as a result of an increase in the presence of oxygen radicals.

We predicted that, when controlled for age, this collection of disparate factors would relate to endogenous levels of sensory noise. We quantified sensory noise using an auditory paradigm. LZ are found in the auditory cortex and levels there correlate highly with levels in the retina (when measured in monkeys; Vishwanathan, et al., 2013). Past studies (e.g., Mayer, 1876; Carhart & Jerger, 1959) have also measured auditory noise and, hence, we used an auditory paradigm for our primary dependent variable. The specific aims of this project may be summarized as follows:

I. To assess longitudinal changes in MP density.

II. To determine the relationship between longitudinal changes in MP density and vestibular function.

III. To determine differences in sensory noise between young and elderly samples.

IV. To compare sensory noise in smokers and non-smokers.

V. To determine the relationship between sensory noise and our oxidation biomarkers (lens and macular pigment optical density, smoking status).

CHAPTER 2

NEURAL NOISE

The "Neurological Noise hypothesis" states that the ability to detect a signal depends on the signal-to-noise ratio (e.g., Gregory, 1957). With increased age, there is a decrease in the signal-to noise ratio. The decline in the ability to discriminate a signal from noise may be a result of an increase in cell loss, higher levels of intrinsic noise, a decreased ability to compensate for increases in noise (e.g. Gutherie et al., 2006), and many other factors. Gregory (1957) proposed two methods to investigate the influence of age on neural noise. First, aging can be examined by artificially increasing noise in young participants, which makes these young participants "functionally old." Second, noise levels can be examined cross-sectionally. Salthouse and Lichty (1985) found that it took relatively small increases in background noise to lead to significantly longer reaction times and greater thresholds in elderly subjects. This finding was consistent with the hypothesis that older individuals lose the ability to tolerate higher levels of extrinsic noise (e.g., increased background noise).

Noise within the Visual System

Many factors (e.g., light scatter, lens density) can create noise within the visual system, which may disrupt visual function. Gregory (1957) examined noise in the aging visual system by measuring differential intensity thresholds against changes in background intensity. Noise was defined as the slope of the best-fit line (in a basic psychometric function). Gregory (1957) found an age-related increase in the slope, which suggested that older individuals had higher

levels of neural noise. The same paradigm has been used to examine noise in the rod pathways (e.g., Gutherie et al., 2006).

Noise within the Auditory System

Noise within the auditory system has been studied more extensively compared to the visual system. Air- and bone-conduction testing are typical clinical methods used to assess pure-tone thresholds. An audiogram displays a person's thresholds, type of hearing loss (conductive, sensorineural, mixed), degree of loss, and configuration or shape of loss in relation to the speech spectrum. Carhart and Jerger (1959) originally suggested the modified Hughson-Westlake technique, a psychophysical method combining ascending and descending methods for obtaining pure-tone thresholds, as the method for clinical audiometric use because it is simple and is less subject to auditory adaptation (stimulates the on-effect, the firing of auditory neurons) (e.g., Hallpike & Hood, 1951). This technique for obtaining hearing thresholds remains the standard for clinical use today.

In addition to mechanical forces, susceptibility to auditory dysfunction is influenced by genetics (as reviewed by Davis et al., 2003), age-related physiological changes (e.g., Gates & Cooper, 1991), health status (e.g., Gates et al., 1993; Maggi et al., 1998), and environmental factors (e.g., Rosen, 1966; Wallhagen et al., 1997) such as noise exposure (e.g., Patuzzi & Rajan, 1992) and smoking (e.g., Starck et al., 1999).

One way to predict noise-induced hearing loss (NIHL) susceptibility is by assessing one's noise-induced temporary threshold shift (TTS), greater TTS may increase the risk for NIHL (e.g., Humes, 1980). One way to measure TTS is with a threshold of masking (TOM) test, which overloads the auditory system with a masker while the participant tries to detect a pure-tone.

Olsen and Berry (1979) showed that the degree of auditory dysfunction, as defined by hearing loss at the masker frequency, is inversely related to TOM scores. The TOM test can measure sensitivity to cochlear function (e.g., Humes, 1980) and may be a better predictor of TTS recovery rather than pure-tone thresholds (Chermak et al., 1984). Temporary threshold shifts can also serve as an index of noise (e.g., Jerger & Carhart, 1956) and NIHL (e.g., Humes, 1980) susceptibility.

Noise-induced hearing threshold shifts (NITS) are characterized by a noise notch, threshold loss at 3, 4, or 6kHz (e.g., CDC, 1986; Brookhouser et al., 1992; Katz, 1994). Acute (e.g., explosion) or chronic noise exposure can result in NITS. NITS can also reflect changes in oxidative stress. Loud or chronic noise creates oxidative stress in the cochlea (as reviewed in Table 1 of Fechter, 2005), which can increase noise within the auditory system. Antioxidants may prevent or slow the progression of oxidative stress-induced (e.g., aging, noise exposure) hearing loss. Niskar and others (2001) found 597 children met the NITS criteria, and 14.6% of the sample displayed a noise-notch in both ears, suggesting that children are exposed to damaging levels of noise, and the prevalence of NITS increases with age, as expected due to more noise exposure.

CHAPTER 3

OXIDATIVE STRESS

Increased oxidative stress can lead to higher levels of noise, making it difficult to separate a signal from noise. For example, oxidative stress is a common cause of rod death. Remaining rods, in order to occupy the physically space, change their structural (e.g., hypertrophy) and physiological characteristics. The combined result is a cell that is less functionally distinct with age. Oxidative stress, an imbalance of antioxidants and oxidative species, can result from exposure to oxidative radical-producing situations (e.g., light exposure, smoking) or when the body does not produce or accumulate enough antioxidants to combat reactive oxygen species, both of which result in tissue damage (e.g., Sies, 1985; Sies, 1997). Antioxidants are compounds that can delay or inhibit the oxidation of another substrate (e.g., Halliwell & Gutteridge, 1989). Because neural tissue is so metabolically active, it is particularly susceptible to damage via singlet oxygen and/or oxygen radicals. This is one reason why most sensory receptive tissues, like the retina and basilar membrane, are so replete with exogenous antioxidants and antioxidant enzymes.

Empirical evidence, for instance, has shown that oxidative stress can impair auditory function (as noted by Henderson et al., 2006) by inducing glutamate ototoxicity (e.g., Puel et al., 1998), reducing cochlear blood flow (e.g., Hultcrantz, 1979), disrupting ion balance (e.g., Spicer & Schulte, 1996), and loss of cells. Administration of antioxidants reduces ototoxicity (e.g., Wu et al., 2002; Fetoni et al., 2004), by preventing or slowing the progression of oxidative stress-induced auditory dysfunction (e.g., Sergi et al., 2004). Dietary antioxidant deficiencies (e.g.,

vitamin B_{12} deficiencies) can lead to auditory dysfunction and neuronal death (e.g., Wilson et al., 1991) whereas supplementation may prevent or slow the progression of auditory dysfunction (e.g., Quaranta et al., 2004). Lutein and zeaxanthin are xanthophylls that have antioxidative properties (e.g., Sujak et al., 1999; Stahl & Sies, 2002). LZ can be found in the retina, and retinal levels of LZ positively correlate with levels of LZ in the auditory cortex (studied in monkey brains, Vishwanathan et al., 2013). Based on this relation, it is reasonable to hypothesize that retinal levels of LZ (as a proxy for the amount of LZ in, for instance, auditory cortex) would correlate with auditory function. This study is the first to assess that possible link.

CHAPTER 4

MACULAR PIGMENT

Wald (1945) was the first to identify xanthophylls in the human retina, which were later

identified, specifically, as lutein and zeaxanthin in the macula. Collectively, with *meso-*

zeaxanthin, these pigments are referred to as the macular pigment (MP) (e.g., Snodderly et al.,

1984). Lutein and zeaxanthin are hypothesized to improve a variety of visual functions such as

increasing visual range through purely optical mechanisms (e.g., Wooten & Hammond, 2002;

Hammond et al., 2012). They are known also to have physiological effects such as enhancing

gap junction communication by embedding into the phospholipid membrane (e.g., Stahl & Sies,

2001) and quenching free oxidative radicals (e.g., Sujak et al., 1999; Stahl & Sties, 2002).

Retinal LZ is positively correlated with LZ throughout the brain (as studied in monkey brains,

Vishwanathan et al., 2013) and can influence cognitive function (e.g., Johnson et al., 2008; as

reviewed by Johnson, 2012).

Macular Pigment and Aging

MP density varies significantly across individuals (e.g., Pease et al., 1987; Hammond et

al., 1997b), which may be, in large part, a result of modifiable lifestyle factors (e.g., diet,

smoking status). Past studies, primarily cross-sectional, remain inconsistent on the relationship

between age and MP density. Some studies show an inverse relationship between age and MP

density (e.g., Gellerman et al., 2002; Nolan et al., 2010), whereas others show no relationship

(e.g., Bone et al., 1988; Hammond et al., 1997b). This can be misleading as the disparity across

studies is, in large part, methodological. The Raman method, for example, has at least one major confound (lens density) that increases with age (e.g., Gellerman et al., 2002). Furthermore, the magnitude of the decline is important. For example, some studies (e.g., Nolan et al., 2010) have shown statistically significant declines, but the magnitude is small. Taken together, it leaves the question unanswered: does MP density decline with age? This is important since, as noted, higher MP density is related to better visual outcomes and lower disease risk. If the elderly, who are already at higher disease risk, have lower MP density, that risk could potentially be ameliorated by supplementing with LZ.

Methods for Quantifying Macular Pigment Density

There are several methods, both *ex* and *in vivo*, for quantifying MP in terms of optical density (MPOD). *Ex vivo* methods include high performance liquid chromatography (e.g., Bone et al., 1985) and microdensitometry (e.g., Snodderly et al., 1991). *In vivo* techniques can be categorized into physical and psychophysical methods. Physical *in vivo* techniques include fundus reflectometry (e.g., Kilbride et al., 1989; Berendschot et al., 2000), autofluorescence (e.g., Robson et al., 2003; Trieschmann et al., 2006), and Raman spectroscopy (e.g., Bernstein et al., 1998; Bernstein et al., 2004; Neelam et al., 2005). Psychophysical *in vivo* techniques include color matching, motion photometry, detection threshold, and heterochromatic flicker photometry (HFP). HFP is the most common technique used to quantify MP because it is simple to conduct (e.g., it does not require bleaching or pupil dilation like many of the physical methods), valid, and reliable (Hammond et al., 2005).

CHAPTER 5

LENS

The crystalline lens primarily absorbs short-wave light and is transparent to long-wave light. Short-wave light contains more energy and is more damaging compared to long-wave light. Since the lens does not undergo biological renewal, any changes (i.e., higher levels of short-wave light exposure) to the lens are permanent. Therefore, the lens may reflect long-term exposure to oxidative events (e.g., light exposure, smoking).

Lens and Aging

Many biological changes occur within the aging lens (as seen in Figure 3 of Taylor, 1993), which contribute to a denser and more opaque lens. Age-related changes in the concentration of the crystalline proteins (increases in α crystallin and decreases in β and γ crystallins) result in a denser and less transparent lens. A variation in CHIP28, a water protein found more commonly in older lenses, increases the vulnerability of the lens fibers to breakdown in the presence of stress or injury, resulting in opacities (e.g., Gooden et al., 1985). Increases in lens density can contribute to changes in visual function, including decreases in light transmission and increases in light scatter (e.g., Mellerio, 1971).

Opacities can also develop from an increase in oxidation, specifically a site-specific, metal-catalyzed oxidation mechanism (Garland, 1990). A metal ligand (copper or iron) binds to a specific binding site (i.e., protein). The metal ligand loses an oxygen molecule by a reductant (i.e., superoxide radical) to form a reduced metal, which then interacts with hydrogen peroxide to

produce free oxygen radicals. Free oxygen radicals can interact with surrounding protein and alter the composition of the amino acids. The process of metal reduction also generates hydrogen peroxide at the specific binding site, increasing the production of free oxygen radicals. Hence, antioxidants may act as an immediate defense system for protecting the crystalline proteins in the lens by quenching free oxidative radicals. Fiber segregation may be a secondary lens defense mechanism (as reviewed by Taylor, 1993). As fibers become damaged, they separate from the undamaged fibers. This separation defense mechanism fails with age, and damaged fibers no longer separate from the undamaged ones, contributing to the development of opacities.

Actual lens density may deviate from an estimated lens age as a result of exposure to oxidative events. Xu and others (1997) derived lens age by fitting data to past models of lens density, including, a linear model (Savage et al., 1993), an exponential model (Weale, 1988), and a two-factor model (Pokorny et al., 1987. The linear model overestimated lens density in the older sample while it underestimated lens density in the young. The exponential model overestimated lens density in the young and underestimated lens density in the old. The two-factor model fit the data the best, and took into account a linear increase in lens density (0.12 units/decade) prior to age 60 and an exponential increase in lens density (0.40 units/decade) after 60 (Pokorny et al., 1987). Sample and others (1988) found that lens density increased as a function of age ($p < .025$) but showed a stronger significant relationship in individuals between the ages of 60-70 years ($p < .01$), confirming the two-factor model.

Methods for Quantifying Lens Density

There are several methods for quantifying lens optical density (OD), both *in vitro* (e.g., direct light transmission through excised lenses) and *in vivo* (e.g., optical and retinal reflectance). Other *in vivo* techniques include non-invasive psychophysical methods of assessing lens OD (e.g., scotopic sensitivity and color matching). Table 5.1 provides an overview of methods used to assess and quantify lens density in the human lens.

Scotopic sensitivity is one well-studied method to quantify lens OD. Wald (1945) examined scotopic sensitivity in phakics (individuals with lenses) and aphakics (individuals without lenses) to estimate light absorption by the macular pigment and lens. Wald essentially found that, in the absence of a lens, the shape of the scotopic sensitivity spectrum was indistinguishable from the action spectrum of rhodopsin. This provided a non-invasive method of measuring the lens but also showed that some complex visual functions could be explained by relatively low-level physiological processes (in this case, the isomerization of photopigment). Tan (1971), confirming Wald's early observations, found that aphakics have higher scotopic sensitivity compared to phakics and higher absorption of UV light.

One drawback for using scotopic sensitivity to measure lens density is that the method requires a long period of dark adaptation (between 30-40 minutes). One method of decreasing the time required for dark adaptation is to increase the stimulus area (e.g., Hecht et al., 1935; Arden & Weale, 1954; Werner, 1982) and at an even faster rate if the pre-experimental lighting was at lower illumination. Scotopic sensitivity is a well-validated method for deriving lens OD; however, the duration of the experiment can be lengthy because of the dark adaptation period. Wooten and others (2007) suggested a photopic sensitivity method for measuring lens density. Both absolute scotopic sensitivity and relative photopic sensitivity methods yielded valid and

comparable lens density measures ($r = .80$, $p < .001$). Using the photopic sensitivity method is faster since dark adaptation is not required, and this method provides comparable lens density measures as quantified by the scotopic sensitivity method. In the present study, we used the expedited method (as described by Wooten et al., 2007) for our lens density assessments.

Table 5.1. Various methods used to assess lens density.

Study	Study Design	Objective	Method of Measuring Lens Density	Sample Size (*N*)	Results
Wald, 1945	Cross-sectional	To examine scotopic sensitivity in aphakics and phakics	Scotopic sensitivity	24 phakic, 6 aphakic	Aphakics' scotopic sensitivity spectrum was indistinguishable from the action spectrum of rhodopsin
Mellerio, 1971	Ex vivo, human lenses	To examine photometric density per unit of pathlength ex vivo human lenses	Ex vivo, human lenses; Wooster Scanning Microdensitometer used to assess photometric density	20	Young lenses (less than 32 years) were less dense and more transparent compared to old lenses (greater than 46 years).
Tan, 1971	Cross-sectional	To investigate scotopic sensitivity using psychophysical methods in aphakics; To better understand the absorption of UV light in aphakics	Scotopic sensitivity	10 aphakic	Aphakics have higher scotopic sensitivity compared to a normal observer, and also higher absorption of UV light, thus scotopic sensitivity can be used to derive lens OD
Werner, 1982	Cross-sectional	To examine age-related changes in scotopic sensitivity and lens density	Scotopic sensitivity	9 (4.5 months – 66 years), 42 (1 month – 70 years)	Age-related increase in lens optical density
Pokorny, et al., 1987	Re-analysis of Stiles and Burch (1958) color matching data	To determine if age-related changes in lens density are linear or a scalar multiple of a young eye	Re-analysis of Stiles and Burch (1958) color matching data	unknown	The re-analysis of Stiles and Burch (1958) color matching data indicate that lens OD is not a scalar multiple of a young eye. Pokorny and others (1987) developed a two-factor model, suggesting that lens OD increases by 0.12 log units/decade before the age of 60 and 3-fold after the age of 60 (about 0.4 log units/decade).
Sample et al., 1988	Cross-sectional	To examine the effect of aging on the lens	Free view, absolute scotopic thresholds obtained for both eyes	54 (18-78 years), tested both eyes when available (total 84 eyes)	Lens OD increased as a function of age ($p < .025$), and there was a greater increase in lens OD between the ages of 60-70 years ($p < .01$). There were no significant sex differences in lens OD.
Weale, 1988	Ex vivo, human lenses	To examine transmission of the human lens in the UV spectrum and age-related variations in transmission	Perkin-Elmer spectrophotometer	24 excised lenses (0-85 years)	Exponential model of lens absorption- with increased age, the lens becomes denser with age due to the increased light absorption
Leske et al., 1991	Case-Control	To evaluate the risk for the development of lens opacities and the influence of various risk factors (i.e., nutrition, medical history, personal characteristics, and environmental factors)	Slit lamp and lens photography	1428 (40-79 years)	Inverse relationship between opacities and vitamin A, vitamin C, vitamin E, riboflavin, niacin, thiamine, multivitamins, and iron; Positive association between age, non-white race, low education, non-professional occupation, diabetes, use of oral steroids, current smoker and risk for developing lens opacities. Interestingly, there was a decreased risk for developing lens opacities with increased sunlight exposure. Total caloric intake did not influence risk, however, individual nutrient intake did.
Savage et al., 1993	Cross-sectional	To evaluate age-related changes in lens optical density	Scotopic sensitivity	82 (20-79)	Linear model for age-related changes in lens optical density
Hammond et al., 1997c	Cross-sectional	To examine the relationship between	Absolute scotopic thresholds using	51 (7 YF M =30 SD = 3.6 years, 5	There was no relationship between MP and lens OD for the younger group, however, there was a significant relationship between MP and lens OD for

		macular carotenoids and lens optical density to determine "lens health"	Maxwellian view optical system	YM M = 29 SD = 3.7, 23 OF M = 68 SD = 6.3, 16 OM M = 66 SD = 10.6 years)	older group (r = -.42, p < .001). This suggests that MP and lens OD relationship develops with increasing age. Also, there was a slightly stronger relationship between MP and lens OD in females (r = -.34, p < .1) compared to males (r = -.55, p < .003).
Xu et al., 1997	Cross-sectional	To determine lens optical density using retinal of the optic disk reflectance and heterochromatic flicker photometry	Retinal reflectance of the optic disk and heterochromatic flicker photometry in a 2-channel optical system (lights of wavelength 430 and 495nm)	27 phakic (22-74 years); 2 pseudophakic (1F- 69 years, 1M- 70 years)	The linear model overestimated lens density in the older sample while underestimated lens density in the young. The exponential model was the opposite of the linear model, overestimating the lens density in the young and underestimating lens density in the old. The two-factor model fit the reflectance data the best. The HFP estimated lens age showed a positive relationship with chronological age (r = .92). The retinal reflectance estimated lens age also showed a positive relationship with chronological age (r = .90).
Duindam et al., 1998	Ex vivo, donated lenses	To examine the biochemical composition of focal opacities	Confocal Raman microspectroscopy (CRM) to examine the biochemical composition of focal opacities	2 donor lenses (42 and 52 years)	Protein content was constant within and outside of the opacities. Filipin signals, a measure of cholesterol, were more intense at the edges of the opacities. This higher flipin signal suggests that opaque lenses have higher cholesterol levels compared to surrounding tissue. Past studies have shown that cholesterol is synthesized within the lens, and with increasing age, cholesterol synthesis decreases, leading to an increase in opacities. Duindam and others (1998) found that lens opacities showed higher cholesterol levels, contrary to the idea that cholesterol synthesis maintains lens transparency. Lens opacities also displayed higher concentrations of disulfide bonds and tryptophan. All of these biochemical composition changes contribute to the reordering of the lens and the development of focal opacities.
Wooten et al., 2007	Experimental	To examine differences between an absolute scotopic sensitivity method and relative photopic sensitivity method	Absolute scotopic sensitivity using Maxwellian view optical system, relative photopic sensitivity using MP densitometer	Experiment 1 (30, M = 24, SD = 7 years), Experiment 2 (16, M = 30, SD = 14 years)	This study found valid and comparable lens OD measures with both absolute scotopic thresholds and relative photopic thresholds, suggesting that the photopic method can be used to determine lens OD without the use of a long dark adaptation period.

CHAPTER 6

SMOKING STATUS: ANCILLARY MEASURE OF OXIDATIVE STATUS

Macular pigment density and lens density reflect protection from oxidative events and long-term oxidative exposure, respectively. Smoking is one known modifiable lifestyle factor that can increase systemic oxidative stress, and has been shown to influence both MP density and lens density. Smokers have significantly lower MP compared to non-smokers, even when they have higher levels of carotenoid intake (e.g., Hammond et al., 1996). Carotenoid levels are lower in the blood circulation of smokers (e.g., Handelman et al., 1996) and cigarette smoke can damage blood vessels (e.g., Boms et al., 2010). Cigarettes also contain cadmium, which is found in higher concentration in cataractous lenses (e.g., Harding, 1995). Furthermore, cigarettes contain pro-oxidants (e.g., Church & Pryor, 1985; Pryor & Stone, 1993) that are damaging to lipid membranes (e.g., Niki et al., 1993) and may increase the presence of free oxidative radicals and oxidative damage, which can increase the development of opacities (e.g., Flaye et al., 1989). Hammond and others (1999) found a dose-response relationship between smoking and lens density. This dose-response relationship was also present in past smokers, suggesting that smoking cessation did not alleviate the lasting effects of smoking on the lens.

CHAPTER 7

THE LONGITUDINAL INVESTIGATION OF MACULAR PIGMENT DENSITY AND

VESTIBULAR FUNCTION[1]

---

[1] Wong, J.C. and B.R. Hammond. To be submitted.

Abstract

Lutein (L), zeaxanthin (Z), and the stereoisomer *meso*-zeaxanthin comprise the macular pigment (MP). Retinal LZ are positively related to LZ in the cerebellum, a region of the brain important for vestibular function. In this study, we assessed longitudinal changes in MP density and vestibular function in 19 older adults. MP density was assessed non-invasively with heterochromatic flicker photometry. Vestibular function was assessed with the One Foot Balance test. The primary result was that MP density, on average, increased over the time periods studied. Vestibular function, as expected, decreased. MP and vestibular function were not related (likely due to low statistical power).

Introduction

Wald (1945) was the first to identify xanthophylls in the human macula. These xanthophylls were later identified as lutein (L) and zeaxanthin (Z). Collectively with *meso*-zeaxanthin, these pigments are referred to as the macular pigment (MP) (e.g., Snodderly et al., 1984). LZ are hypothesized to improve visibility by filtering short-wave light (e.g., Wooten & Hammond, 2002; Hammond et al., 2012), enhance gap junction communication (e.g., Stahl & Sies, 2001) and act as an antioxidant (e.g., Sujak et al., 1999; Stahl & Sties, 2002). Past studies remain inconsistent on the relationship between age and MP, in part, due to methodological issues. For example, the Raman method is confounded by lens density, which increases with age (e.g., Gellerman et al., 2002). Furthermore, some studies (e.g., Nolan et al., 2010) have shown statistically significant declines, but the magnitude is small.

Retinal LZ is positively correlated with levels of LZ in the cerebellum (as studied in the non-human primate brain, Vishwanathan et al., 2013), a region of the brain important for

vestibular function. Past studies have shown an age-related decline in vestibular function (e.g.,

Bergström, 1973). Increases in oxidative stress in the inner ear can also lead to vestibular

dysfunction; however, antioxidants have been shown to preserve vestibular function (e.g., Sergi

et al., 2004). Renzi and others (2013) also found a positive relationship between MP density and

vestibular function. The present study investigated longitudinal changes in MP density and

vestibular function.

Method

Densitometer

An LED-based Newtonian-view densitometer was used to measure MP density (for a full

description, see Wooten et al., 1999). A 460nm LED (maximally absorbed by the MP) and

550nm wavelength LED (not absorbed by the MP) comprised the one-degree diameter test

stimulus. The LEDs were presented 180 degrees out of phase, which gave the perception of a

flickering stimulus. The stimulus was presented in the fovea and peripheral retina.

Participants

Two groups were recruited from a convenience sample. Participants ($N = 12$, $M = 74.5 \pm$

9.1 years) were recruited from a database of individuals with MP density assessments from 11

years ago. Participants ($N = 7$, 70.3 ± 4.4 years) were recruited from a database of individuals

with MP density and vestibular function assessments from 8 years ago. All participants were in

good general and ocular health. Informed consent was obtained for all participants and the

Tenets of the Declaration of Helsinki were followed.

Procedure

Participants completed general health questionnaires. The retinal distribution of MP density was measured with customized heterochromatic flicker photometry (see Stringham et al., 2008 for a full description) in the right eye only. An initial critical flicker frequency (CFF) value was obtained and used to calculate the initial lowest flicker frequency (LFF) for each stimulus presentation. The participant maintained a central fixation while the experimenter adjusted the blue radiance using an ascending and descending method until the participant perceived the stimulus as stable (not flickering). Each stimulus was presented for five trials. If the blue radiance values differed by more than a range of 100 (on the photometer readout) between each trial, more trials were conducted. Macular pigment optical density (MPOD) was derived from the log difference between the central and peripheral retinal sensitivity. Vestibular function was measured with the One-Foot Balance technique (for a full description, see Ayres, 1980). Vestibular function was defined as the longest duration a person could balance over three trials. Analyses were conducted with SPSS, version 20.

Results

In the 11-year longitudinal study, average baseline MP density ($M = 0.21 \pm 0.17$ OD) was significantly lower ($p < .001$) than follow-up MP density ($M = 0.38 \pm 0.23$ OD) (Figure 7.1). Individual MP density was significantly higher ($p < .001$) at follow-up compared to baseline assessments except for two individuals who displayed decreases in MPOD (Figure 7.2).

Table 7.1 lists the descriptive data for all variables (age, MP density, vestibular function) in the 8-year longitudinal study. MP density was not significantly different between baseline (M

$= 0.40 \pm 0.26$OD) and follow-up ($M = 0.42 \pm 0.20$ OD) assessments in the 8-year study ($t = -.239$, $p = .41$). Baseline vestibular function ($M = 15.2 \pm 16.9$ seconds) was trending higher ($t = -1.808$, $p = .06$) than follow-up vestibular function ($M = 6.2 \pm 4.7$ seconds). Furthermore, there was no association between MP density and vestibular function at either baseline ($r = .62$, $p = .07$) or follow-up ($r = .50$, $p = .13$).

Discussion

Over 83% of the sample increased in MP density over the 11-year period. Furthermore, over 41% of the sample increased their daily fruit and vegetable intake. The increase in MP density (Figure 7.2) may have been a result of the participants' initial exposure to the benefits of LZ. The individuals with the greatest increase in MP density also reported increases in dietary fruit and vegetable intake. Past studies have shown that dietary increases in LZ (which are found in higher concentrations in dark, leafy green vegetables, yellow pigmented fruits and vegetables, and egg yolks) can increase MP density (e.g., Hammond et al., 1997a). This result is significant. It has been argued that MP density changes over time based on physiological changes to the retina itself. For example, although there is significant rod loss, there is very little change in oxygen saturation of the retina over time. Hence, with lower consumption, oxidative stress to the retina becomes higher in the older person. This, combined with a higher concentration of photosensitizers (e.g., A2E) creates a much higher oxidative environment within the elderly retina. Our finding that MP does not decline suggests that improved dietary intake can offset the greater vulnerability of the retina with age.

In the 8-year longitudinal study, MP density was not significantly different between baseline and follow-up assessments. Dietary fruit and vegetable intake were not available for the

baseline assessment in the 8-year longitudinal study; therefore, we cannot conclude if there were any dietary changes. Furthermore, vestibular function was significantly lower at the follow-up assessment. Exclusion of the two youngest individuals from the study (57 and 59 years) eliminates the relationship between age and vestibular function. This is likely due to the limited sample. The small sample size may also explain the absence of a relationship between MP density and vestibular function.
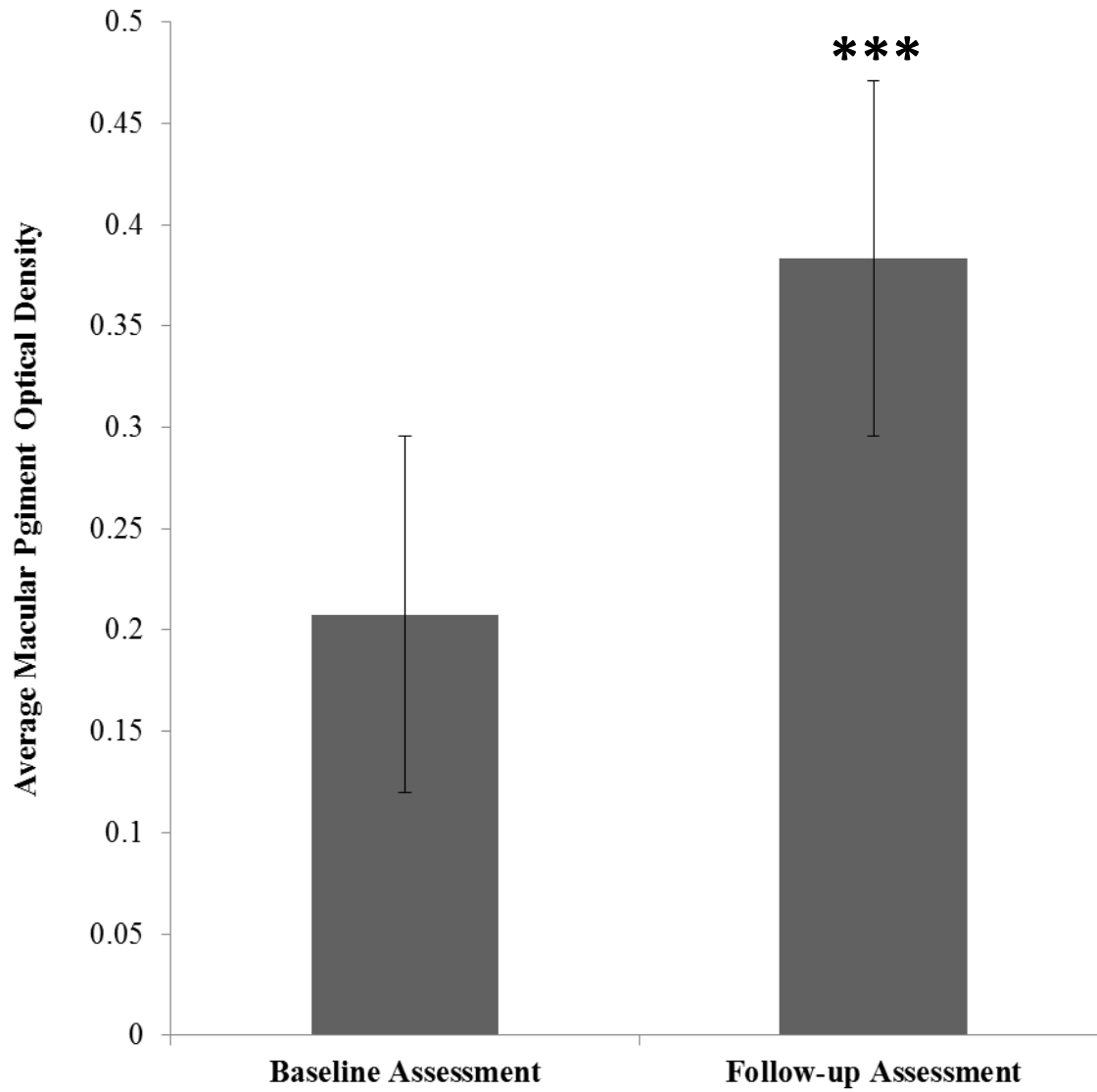
Figure 7.1. Average MP density (mean ± standard deviation) across all participants at baseline and follow-up 11 years later. (***$p < .001$).
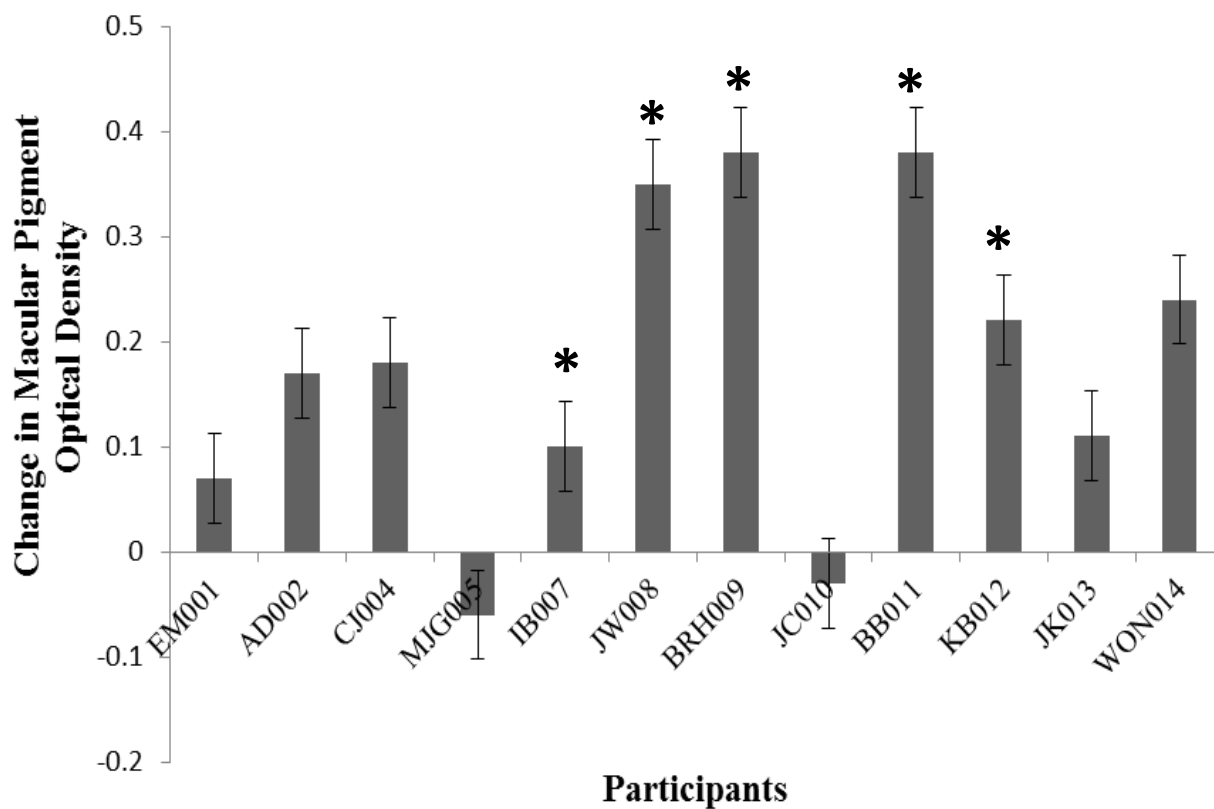
Figure 7.2. Change in individual MP density (mean ± standard deviation) over the 11-year period. (*individuals with increased dietary fruit and vegetable intake over the 11-year period).

Table 7.1. Descriptive data for MP density and vestibular function at baseline and follow-up 8 years later.

Participant	Baseline Age (years)	Baseline MPOD	8-Year Follow-up MPOD	Baseline Vestibular Function (seconds)	8-Year Follow-up Vestibular Function (seconds)	Change in Vestibular Function (seconds)
WON014	69	.37	.25	5.90	5.40	-0.50
JM017	63	.26	.32	4.20	4.50	0.30
EH019	57	.96	.75	34.00	6.29	-27.71
JE021	59	.40	.62	45.00	16.12	-28.88
EM001	63	.35	.33	8.00	4.28	-3.72
KB012	67	.20	.40	4.80	1.30	-3.50
KM018	62	.27	.24	4.80	5.40	-0.60

CHAPTER 8

THE INFLUENCE OF AGE AND LUTEIN STATUS ON VESTIBULAR AND AUDITORY

FUNCTION (CROSS-SECTIONAL STUDIES)[2]

---

[2] Wong, J.C., Kaplan, H.S., and B.R. Hammond. To be submitted.

Abstract

Oxidative stress increases with age. One form of protection from increased oxidative stress is the accumulation of antioxidants (e.g., carotenoids) in stressed tissues. Lutein (L) and zeaxanthin (Z) are two dietary carotenoids found in the retina and lens. Retinal LZ positively correlates with LZ levels in the auditory cortex and cerebellum, regions of the brain important for auditory and vestibular function, respectively. The purpose of this study was to investigate the influence of age and oxidative biomarkers (MP density and lens density) on sensory function (auditory and vestibular) across the lifespan and in smokers and non-smokers. MP density and lens density were assessed with a non-invasive psychophysical technique. Vestibular function was assessed with a balance test. Auditory function was assessed with the Modified Hughson-Westlake method. In the present study, age was significantly related to all variables. Both lens and MP density were related to auditory function (and auditory function in noise) but only for the young non-smokers. All of the other relations were non-significant.

Introduction

Susceptibility to neurodegenerative disease increases with age. One characteristic of neurodegenerative disease is a decrease in neural efficiency. One way to characterize neural efficiency is to assess the ability to discriminate a signal from noise. Higher levels of noise can decrease the ability to separate a signal from noise. Increased oxidative stress can result in tissue damage, which can increase neural noise. One hypothesis advanced by Renzi and Hammond (2010) for increasing neural efficiency is to increase intake of the exogenous antioxidants lutein and zeaxanthin. These pigments are found throughout the nervous system and have been shown to have a variety of direct influences on cellular function such as increasing signaling, enhancing

gap junction communication, structural stability of microtubules, etc. (for a review see Hammond, 2012). Because retinal levels of LZ are positively related to levels of LZ in the auditory cortex and cerebellum (as studied in monkey brains, Vishwanathan et al., 2013), regions of the brain important for auditory and vestibular function, respectively, measures of MP can be used as a proxy for brain concentrations.

Oxygen can also modify the crystalline proteins within the lens, resulting in a denser and less transparent lens. Changes to the crystalline lens are permanent since the lens does not undergo biological renewal. Taken together, MP density and lens density should provide measures of protection from oxidative exposure and long-term exposure to oxidative events, respectively.

Ancillary variables (e.g., smoking status) have been shown to influence both MP density and lens density. Smoking may interfere with the deposition of carotenoids in the retina. Smokers have significantly lower MP density compared to non-smokers, even when they have higher levels of carotenoid intake (e.g., Hammond et al., 1996). Cigarettes also contain pro-oxidants (Pryor & Stone, 1993; Church & Pryor, 1985), which may increase systemic oxidative stress. Cigarettes also contain cadmium, which is found in higher concentration in cataractous lenses (e.g., Harding, 1995). Hammond and others (1999) found that smokers had higher lens density compared to non-smokers. This relationship was also found in past smokers, which suggests that smoking cessation does not alleviate any lasting effects that smoking may have on the lens.

Increased oxidative stress may also contribute to vestibular and auditory dysfunction. Past studies have shown that antioxidants may preserve both auditory and vestibular function (e.g., Sergi et al., 2004). Renzi and others (2013) found a positive relationship between MP

density and vestibular function. However, the relationship between LZ and auditory function has not been previously examined. The purpose of this study was to investigate the relationship between age, oxidative biomarkers (MP density and lens density), and sensory function (vestibular and auditory).

Method

Densitometer

An LED-based Newtonian-view densitometer was used to measure MP density (for a full description, see Wooten et al., 1999). The test stimulus (one-degree diameter), contained a wavelength that was maximally absorbed by the MP (460nm) and a reference wavelength that was not absorbed by the MP (550nm). These wavelengths were presented 180 degrees out of phase, which gave the perception of a flickering stimulus. The stimulus was presented in the fovea and peripheral retina. To measure lens density, the test stimulus consisted of a two-degree circular field comprised of two LEDS (406nm (maximally absorbed by the lens) and 550nm (not absorbed by the lens)). The test stimulus was presented 180 degrees out of phase in the peripheral retina.

Auditory Function

Auditory function was assessed with air-conduction threshold testing. All testing was completed with a clinical audiometer, a Grason-Stadler Instruments 61 two channel instrument (GSI 61) using EAR 3A insert earphones. The audiometric test booth was double-walled and constructed for psychoacoustic testing. The ambient noise level in the room was 31dB, as measured by a private company, Med-Acoustics, Inc. All equipment was calibrated prior to,

during, and at the end of the study (See Appendix A for calibration records for the GSI 61 and clinical room).

Participants

Participants were recruited from an undergraduate psychology research pool and the surrounding community. Participants were separated into three groups: young non-smokers (YNS) ($N = 29$, $M = 21.38 \pm 3.31$ years), young smokers (YS) ($N = 17$, $M = 19.65 \pm 2.29$ years), and older non-smokers (ONS) ($N = 21$, $M = 70.10 \pm 5.57$ years). Smokers were defined as individuals who smoked at least 10 cigarettes/week for a year, equivalent to 26 packs/year. Average smoking pack years was 778.13 ± 1326.27 and the average years smoked was 3.47 ± 3.19 years. Participants with intraocular lenses ($N = 7$) and missing assessments ($N = 5$) were excluded from analyses. All participants were in good general, ocular, and aural health. Informed consent was obtained for all participants, and the Tenets of the Declaration of Helsinki were followed.

Procedure

Participants completed general health questionnaires. The retinal distribution of MP density was measured with customized heterochromatic flicker photometry (see Stringham et al., 2008 for a full description) in the right eye only. An initial critical flicker frequency (CFF) value was obtained and used to calculate the initial lowest flicker frequency (LFF) for each stimulus presentation. The participant maintained a central fixation while the experimenter adjusted the blue radiance using an ascending and descending method until the participant perceived the stimulus as stable (not flickering). Each stimulus was presented for five trials. If the

photometric values differed by more than 100 (for the 460nm LED) between each trial, more

trials were conducted. Macular pigment optical density (MPOD) was derived from the log

difference between the central and peripheral retina sensitivity.

Lens density was quantified in the right eye using photopic flicker photometry (see

Wooten et al., 2007 for a complete description). Two LEDs, 406nm (maximally absorbed by the

lens) and 550nm (not absorbed by the lens,) were presented 180 degrees out of phase at a

frequency between 1.5-3.0Hz. The participant maintained a peripheral fixation while the

experimenter adjusted the violet radiance using an ascending and descending method until the

participant perceived the stimulus as stable (not flickering). The stimulus was presented ten

times. If the photometric values differed by more than 200 (for the 406nm LED) between each

trial, more trials were conducted. Lens optical density was derived by taking the log energy

difference between the 406nm and 550nm wavelengths. An industrial fiber optics photometer

was used to measure energy values of the 406nm LED. The photometer head was placed

directly in front of the LED. Energy values were recorded for arbitrary positions on the

participant knob (See Appendix B for the log energy values of the 406nm LED with respect to

the participant knob position).

Vestibular function was measured with the One-Foot Balance Technique (see Ayres,

1980 for a full description). Vestibular function was defined as the duration a participant could

balance with eyes closed, averaged across three trials. Participants also completed a standard air-

conduction hearing test and otoscopic check conducted by a licensed audiologist. All testing was

conducted in a calibrated audiometric suite. All participants passed the otoscopy screening as

both tympanic membranes were visible. For the standard hearing test, participants' pure-tone

thresholds were assessed using the Modified Hughson-Westlake procedure (See Carhart &

Jerger, 1959 for a full description) at the following frequencies (Hz): 250, 500, 1000, 2000,

3000, 4000, 6000, and 8000. Following the standard hearing test, participants completed the

noise paradigm. The noise paradigm introduced a broadband white noise at 30dB, 50dB, and

60dB simultaneously with a 1000Hz pure-tone (frequency least susceptible to age-related and

noise-induced hearing loss). For each level of white noise, the pure-tone was first presented at

10dB above the level of the white noise, and then the Modified Hughson-Westlake method was

used to obtain threshold. Pure-tone and auditory thresholds in noise were obtained in the right,

left, and both ears. Auditory stimuli presentation were counterbalanced in order to eliminate

order effects. Binaural assessments were used for analyses since right and left ear thresholds

were not statistically different. Threshold shift was calculated by subtracting the pure-tone

threshold from the auditory threshold obtained in the presence of white noise. The white noise

paradigm was tested for reliability in a sample of young, healthy, non-smokers ($N = 9$, $M = 19.6$

± 1.2 years), see Appendix C. Analyses were conducted with SPSS, version 20.

Results

In the present study, age was significantly related to all variables (Appendix D) except

for auditory thresholds obtained in the presence of white noise (Appendix E). Since age was a

covariant, all subsequent analyses were controlled for age. Table 8.1 lists the descriptive data for

all of the variables (MP density, lens density, vestibular function, and auditory function). Table

8.2 lists the Pearson product moment correlations and significance values for all of the variables

in a sample of non-smokers ($N = 50$, 18-81 years). No associations were found between the

variables when statistically controlling for age (suggesting the possibility that there could be a

fundamentally different relation in young subjects versus old), therefore, we also examined

relations between the variables when stratified by age and smoking status (Table 8.3). MP density and lens density were not associated with vestibular function when stratified by age and smoking status. However, MP density and lens density were significantly related to auditory thresholds in young non-smokers. In young non-smokers, MP density was inversely related to pure-tone thresholds ($r = -.35$, $p < .05$). MP density was also inversely related to auditory thresholds obtained in the presence of 50dB of white noise ($r = -.36$, $p < .05$). Furthermore, lens density was inversely related to pure-tone thresholds ($r = -.36$, $p < .05$) in young non-smokers. Lens density was also inversely related to auditory thresholds obtained in the presence of 30dB ($r = -.36$, $p < .05$) and 50dB ($r = -.47$, $p < .01$) of white noise.

Table 8.4 provides the descriptive data for all of the variables when control-matched for age and MP density in a sample of young smokers and non-smokers. Vestibular function and lens density were not significantly different between smokers and non-smokers. However, pure-tone thresholds were significantly higher in smokers ($p < .05$) compared to non-smokers. MP density was inversely related ($r = -.48$, $p < .05$) to pure-tone thresholds in non-smokers (Figure 8.1). Furthermore, MP density was inversely related to auditory thresholds obtained in 50dB of white noise ($r = -.50$, $p < .05$). Auditory thresholds in noise were not significantly different between non-smokers and smokers. Lens density was inversely related to pure-tone thresholds and auditory thresholds obtained in noise (Table 8.5). Lens density was inversely related to pure-tone thresholds in smokers ($r = -.65$, $p < .01$). Lens density was also positively associated with threshold shifts at each level of white noise in smokers ($p < .05$).

Discussion

When statistically controlling for age, no associations were found between the oxidative biomarkers (MP density) and sensory function (vestibular and auditory). Therefore, we stratified the data by age and smoking status. When this analysis was conducted, we found that the oxidative biomarkers were significantly related to auditory function in young healthy individuals. Lens density reflects long-term oxidative exposure, and with increased oxidative events, there should be increased levels of intrinsic noise, resulting in higher auditory thresholds. However, our finding refutes this hypothesis. Therefore, we separated the young non-smokers into tertiles based on lens density. We found that the individuals with the highest lens density also had higher levels of MP density (Table 8.6). Therefore, LZ in the retina as a proxy of LZ in the auditory cortex may be contributing to the lower auditory thresholds. Unlike past studies (e.g., Renzi et al., 2013), we did not find a relation between MP density and balance ability. The sample size and assessment was similar so it is unclear why this result failed to replicate.

Smoking, a known oxidative stressor, may also confound the relations between the oxidative biomarkers and sensory function. Therefore, we examined the associations between the oxidative biomarkers and sensory function between smokers and nonsmokers when control-matched for age and MP density. When control-matched for age and MP density, vestibular function was not significantly different between young smokers and non-smokers. Lens density was also not significantly different between the two groups, which is inconsistent with past studies (e.g., Hammond et al., 1999). One explanation is that the smokers in this study may not been exposed to high levels of oxidative stress. The smokers in this study were not heavy smokers, nor had they smoked for a long duration.

When control-matched for age and MP density, pure-tone thresholds were significantly lower in non-smokers compared to smokers ($t = -2.062$, $p < .05$). Our data is consistent with past studies that have found a positive association between cigarette smoking and hearing loss (e.g., Siegelaub et al., 1974; Rosenhall et al., 1993; Cruickshanks et al., 1998; Itoh et al., 2001). Of note, MP density was related to pure-tone thresholds ($r = -.51$, $p < .05$) in non-smokers. Antioxidants have been shown to preserve auditory function (e.g., Sergi et al., 2004). However, antioxidants may serve some other function in smokers.

This study provided cross-sectional data on the relationships between the oxidative biomarkers (MP density and lens density) and sensory function (auditory and vestibular) when controlled for age. The oxidative biomarkers were related to auditory thresholds only in young healthy individuals. The absence of relations in older individuals and young smokers may explained by the presence of other age-related factors or smoking-induced oxidative stress, respectively.

Table 8.1. Descriptive data (mean ± standard deviation) for age, MP density, lens density, vestibular function, and auditory function as stratified by age and smoking status.

	Young Non-Smokers (*N* = 29)	Young Smokers (*N* = 17)	Older Non-Smokers (*N* = 21)
Age (years)	21.38 ± 3.31	19.65 ± 2.29	70.10 ± 5.57
MP Density	0.51 ± 0.23	0.46 ± 0.20	0.70 ± 0.32
Lens Density	1.17 ± 0.09	1.16 ± 0.07	1.37 ± 0.23
Vestibular Function (seconds)	18.44 ± 20.91	18.62 ± 26.77	3.68 ± 2.48
Auditory Function			
Pure-tone Thresholds (dB)	7.41 ± 5.80	8.53 ± 5.66	16.31 ± 7.27
Auditory Threshold in 30dB of White Noise (dB)	31.38 ± 3.51	31.47 ± 3.86	32.62 ± 2.56
Auditory Threshold in 50dB of White Noise (dB)	50.00 ± 7.32	50.59 ± 4.96	52.14 ± 2.99
Auditory Threshold in 60dB of White Noise (dB)	61.55 ± 3.80	61.76 ± 4.98	62.62 ± 3.01
Threshold Shift in 30dB of White Noise (dB)	23.97 ± 6.77	22.94 ± 5.74	16.31 ± 7.44
Threshold Shift in 50dB of White Noise (dB)	42.59 ± 8.11	42.06 ± 6.01	35.83 ± 7.60
Threshold Shift in 60dB of White Noise (dB)	54.14 ± 5.95	53.24 ± 6.95	46.31 ± 7.69

Table 8.2. Pearson product moment correlations ($r \mid p$) between all variables when statistically controlling for age in a sample of non-smokers.

	Auditory Function							
	Vestibular Function (seconds)	**Pure-tone Threshold (dB)**	**Threshold Shift in 30dB of White Noise (dB)**	**Threshold Shift in 50dB of White Noise (dB)**	**Threshold Shift in 60dB of White Noise (dB)**	**Auditory Threshold in 30dB of White Noise (dB)**	**Auditory Threshold in 50dB of White Noise (dB)**	**Auditory Threshold in 60dB of White Noise (dB)**
MP Density	.05 | .36	-.17 | .12	.21 | .08	.01 | .48	.12 | .21	.10 | .24	-.18 | .11	-.09 | .27
Lens Density	-.03 | .43	.08 | .30	-.12 | .21	-.19 | .10	-.19 | .10	-.11 | .23	-.17 | .13	-.22 | .06

*$p < .05$, **$p < .01$, ***$p < .001$

Table 8.3. Pearson product moment correlations ($r \mid p$) between each variable when stratified by age and smoking status.

Young Non-Smokers ($N = 27$)

		Auditory Function						
	Vestibular Function (seconds)	Pure-tone Thresholds (dB)	Threshold Shift in 30dB of White Noise (dB)	Threshold Shift in 50dB of White Noise (dB)	Threshold Shift in 60dB of White Noise (dB)	Auditory Thresholds in 30dB of White Noise (dB)	Auditory Thresholds in 50dB of White Noise (dB)	Auditory Thresholds in 60dB of White Noise (dB)
MP Density	.10 | .31	-.35 | .03*	.34 | .04*	-.09 | .33	.16 | .21	.07 | .37	-.36 | .03*	-.29 | .06
Lens Density	.05 | .41	-.36 | .04*	.12 | .27	-.17 | .19	.20 | .16	-.37 | .03*	-.13 | .26	-.24 | .12

Young Smokers ($N = 17$)

		Auditory Function						
	Vestibular Function (seconds)	Pure-tone Thresholds (dB)	Threshold Shift in 30dB of White Noise (dB)	Threshold Shift in 50dB of White Noise (dB)	Threshold Shift in 60dB of White Noise (dB)	Auditory Thresholds in 30dB of White Noise (dB)	Auditory Thresholds in 50dB of White Noise (dB)	Auditory Thresholds in 60dB of White Noise (dB)
MP Density	-.28 | .15	-.08 | .39	-.17 | .27	.14 | .30	.08 | .39	-.38 | .08	.08 | .38	.02 | .48
Lens Density	.39 | .07	.25 | .17	-.19 | .24	-.54 | .02*	-.33 | .11	.10 | .36	-.37 | .08	-.17 | .26

Older Non-Smokers ($N = 21$)

		Auditory Function						
	Vestibular Function (seconds)	Pure-tone Thresholds (dB)	Threshold Shift in 30dB of White Noise (dB)	Threshold Shift in 50dB of White Noise (dB)	Threshold Shift in 60dB of White Noise (dB)	Auditory Thresholds in 30dB of White Noise (dB)	Auditory Thresholds in 50dB of White Noise (dB)	Auditory Thresholds in 60dB of White Noise (dB)
MP Density	.06 | .40	.01 | .49	.02 | .46	.06 | .40	.02 | .47	.09 | .36	.18 | .23	.06 | .40
Lens Density	-.32 | .08	.23 | .17	-.26 | .13	-.18 | .22	-.34 | .07	-.12 | .31	.09 | .35	-.33 | .08

*$p < .05$, **$p < .01$, ***$p < .001$

Table 8.4. Descriptive data (mean ± standard deviation) for age, MP density, lens density, vestibular function, and auditory function when control-matched for age and MP density.

	Young Non-Smokers (*N* = 17)	Young Smokers (*N* = 17)
Age (years)	19.53 ± 1.59	19.65 ± 2.29
MP Density	0.47 ± 0.19	0.46 ± 0.20
Lens Density	1.12 ± 0.10	1.16 ± 0.07
Vestibular Function (seconds)	14.74 ± 11.20	18.62 ± 26.77
Auditory Function		
Pure-tone Thresholds (dB)	6.03 ± 4.76	8.47 ± 5.50
Auditory Threshold in 30dB of White Noise (dB)	32.50 ± 2.58	31.47 ± 3.86
Auditory Threshold in 50dB of White Noise (dB)	48.76 ± 9.22	50.59 ± 4.96
Auditory Threshold in 60dB of White Noise (dB)	61.86 ± 4.03	61.76 ± 4.98
Threshold Shift in 30dB of White Noise (dB)	26.32 ± 5.46	22.94 ± 5.74
Threshold Shift in 50dB of White Noise (dB)	42.79 ± 8.92	42.06 ± 6.01
Threshold Shift in 60dB of White Noise (dB)	55.74 ± 4.82	53.24 ± 6.95

Table 8.5. Pearson product moment correlations ($r \mid p$) between each variable when control-matched for age and MP density.

Non-Smokers ($N = 17$)

| | Vestibular Function (seconds) | Auditory Function | | | | | | |
		Pure-tone Thresholds (dB)	Threshold Shift in 30dB of White Noise (dB)	Threshold Shift in 50dB of White Noise (dB)	Threshold Shift in 60dB of White Noise (dB)	Auditory Thresholds in 30dB of White Noise (dB)	Auditory Thresholds in 50dB of White Noise (dB)	Auditory Thresholds in 60dB of White Noise (dB)
MP Density	-.21 | .21	-.48 | .03*	.55 | .01**	-.25 | .17	.42 | .05*	.27 | .15	-.50 | .02*	-.07 | .40
Lens Density	-.17 | .27	-.40 | .06	.11 | .35	-.41 | .06	.23 | .20	-.50 | .02*	-.60 | .01**	-.21 | .22

Smokers ($N = 17$)

| | Vestibular Function (seconds) | Auditory Function | | | | | | |
		Pure-tone Thresholds (dB)	Threshold Shift in 30dB of White Noise (dB)	Threshold Shift in 50dB of White Noise (dB)	Threshold Shift in 60dB of White Noise (dB)	Auditory Thresholds in 30dB of White Noise (dB)	Auditory Thresholds in 50dB of White Noise (dB)	Auditory Thresholds in 60dB of White Noise (dB)
MP Density	-.29 | .13	-.07 | .39	-.07 | .40	.07 | .39	-.03 | .45	-.21 | .21	.00 | .49	-.13 | .32
Lens Density	.37 | .07	.25 | .17	-.17 | .26	-.54 | .01**	-.34 | .09	.12 | .33	-.37 | .07	-.19 | .24

*$p < .05$, **$p < .01$, ***$p < .001$
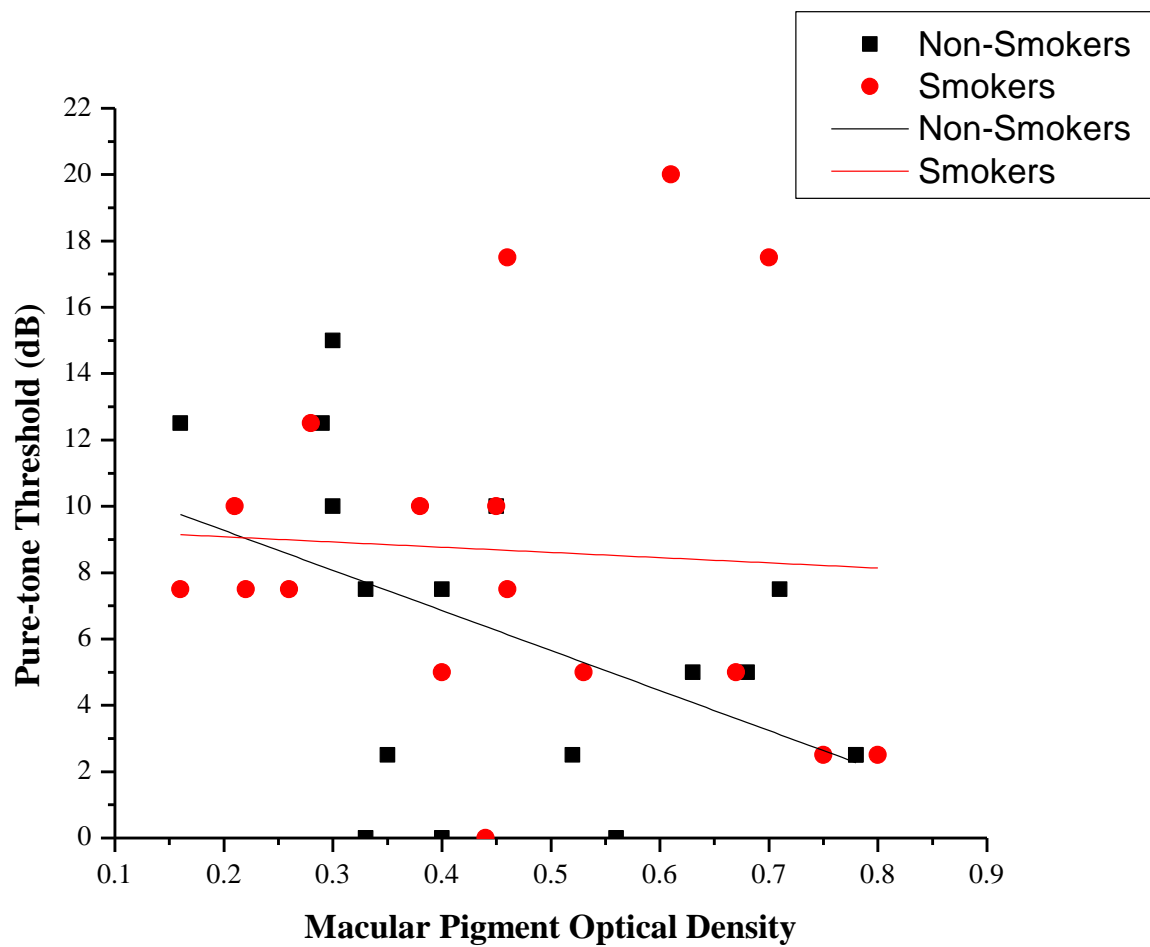
Figure 8.1. Association between MP density and pure-tone thresholds between smokers and non-smokers when control-matched for age and MP density.

Table 8.6. Young non-smokers separated into tertiles based on lens density.

	Mean ± Standard Deviation
Low Lens Optical Density	**1.07 ± 0.03**
Macular pigment optical density	0.44 ± 0.27
Pure-tone Threshold (dB)	9.17 ± 5.00
Auditory Thresholds in 30dB of Noise (dB)	32.78 ± 2.64
Auditory Thresholds in 50dB of Noise (dB)	53.33 ± 2.50
Auditory Thresholds in 60dB of Noise (dB)	63.33 ± 2.50
Mid Lens Optical Density	**1.15 ± 0.36**
Macular pigment optical density	0.56 ± 0.19
Pure-tone Threshold (dB)	6.11 ± 3.33
Auditory Thresholds in 30dB of Noise (dB)	31.11 ± 3.33
Auditory Thresholds in 50dB of Noise (dB)	48.33 ± 5.59
Auditory Thresholds in 60dB of Noise (dB)	60.00 ± 5.00
High Lens Optical Density	**1.26 ± 0.03**
Macular pigment optical density	0.52 ± 0.20
Pure-tone Threshold (dB)	5.28 ± 4.23
Auditory Thresholds in 30dB of Noise (dB)	30.56 ± 4.64
Auditory Thresholds in 50dB of Noise (dB)	51.67 ± 4.33
Auditory Thresholds in 60dB of Noise (dB)	61.67 ± 3.54

CHAPTER 9

CONCLUSION

This collection of studies had a few major outcomes. The first was the finding that MP density tends to either not change with age or increase when assessed using a longitudinal design. That is important since it argues against the interpretation that facets of aging must cause declines in pigment density (e.g., as argued in many papers by Gellerman and Bernstein). The second major finding was that MP and lens density (our variables for assessing antioxidant and oxidant exposure) was related to noise but only for younger non-smokers; both smoking and increasing age seem sufficient to swamp any effects of the dietary carotenoids on our assessment of neural efficiency. This is not surprising since age and smoking are probably the biggest contributors to neural loss (and hence, likely swamp most variables). The fact, however, that we do find an effect when controlling for smoking and age (via stratification analysis) is consistent with the idea that they do improve the fidelity of neural function.

How might they do this? Craft and others (2004) originally found that L and Z are the dominant carotenoids within the brain comprising some 66-77% of the total carotenoids in the brain and concentrating in white matter tracts. Within the neuron itself, L and Z are known to associate with the microtubules of axons. Microtubules are cylindrical protein lattices that form the cytoskeleton of neurons. The walls of microtubules are formed by subunit proteins (i.e., tubulin). Whereas traditionally microtubules have been regarded as purely structural in nature, more recent evidence (e.g., Glanz, 1997; Maniotis, 1997a,b) has shown that they may serve mechanical signaling and communicative functions (e.g., by influencing second-messenger

systems). Indeed, the amount of information processing inside neurons is a burgeoning topic in Neuroscience and has even been used as a postulated basis for consciousness. Bernstein and others (1997) originally identified tubulin as a possible binding protein for lutein and zeaxanthin. Crabtree and others (2001) confirmed tubulin as the most likely binding protein for retinal L and Z (using stoichiometry) and suggested that a major function of the macular carotenoids, previously unconsidered, is to modulate the dynamic instability of microtubules.

The idea that the MP carotenoids might aide neural metabolism has also been postulated. Dartnall and Thomson (1949) originally suggested that L and Z facilitate retinal oxygen respiration. The xanthophylls do often serve this function in anoxic situations in other animals and plants (e.g, Karnaukhov, 1990). Dwyer et al. (2001) originally suggested that L could influence blood flow (most probably including retinal blood flow) by preventing atherosclerotic changes through inhibition of LDL-induced migration of plaque-forming monocytes to artery walls.

Another possibly relevant feature of carotenoids is their ability to enhance gap junctional communication (e.g., Stahl & Sies, 2001). Gap junctions increase intracellular communication between glial and neuronal cells. Acting as portals, they influence propagation of action potentials, second-messenger systems and the movement of metabolites and electrolytes. Within the retina, gap junctions are crucial to light processing via lateral connections (e.g., Cook et al, 1995; Vaney, et al., 1998).

Evidence from such diverse sources suggests that, indeed, L and Z could influence the actual activity of neurons. Since these are major food components within the brain, further study of their effects on the brain are warranted.

REFERENCES

Arden, G.B., & Weale, R.A. (1954). Nervous mechanisms and dark adaptation. *J. Physiol., 125*:
417-426.

Ayres, A.J. (1980). Southern California sensory integration tests: manual (rev.) Los Angeles:
Western Psychological Services.

Berendschot, T.T.J.M., Goldbohm, R.A., Klöpping, W.A.A., van de Kraats, J., van Norel, J., &
van Norren, D. (2000). Influence of lutein supplementation on macular pigment, assessed with
two objective techniques. *Investigative Ophthalmology & Visual Science, 41*(11): 3322-3326.

Bergström, B. (1973). Morphology of the vestibular nerve: II. The number of myelinated
vestibular nerve fibers in man at various ages. *Acta Oto-laryngologica, 76*: 173-179.

Bernstein, P.S., Balashov, N.A., Tsong, E.D., & Rando, R.R. (1997). Retinal tubulin binds
macular carotenoids. *Investigative Ophthalmology & Visual Science, 38*(1): 167-175.

Bernstein, P.S., Yoshida, M.D., Katz, N.B., McClane, R.W., & Gellerman, W. (1998). Raman
detection of macular carotenoid pigments in intact human retina. *Investigative Ophthalmology &
Visual Science, 39*(11): 2003-2011.

Bernstein, P.S., Zhao, D., Sharifzadeh, M., Ermakov, I.V., & Gellerman, W. (2004). Resonance raman measurement of macular carotenoids in the living human eye. *Biochemistry and Biophysics, 430*: 163-169.

Boms, N., Yonai, Y., Molnar, S., Rosengarten, B., Bornstein, N.M., Csiba, L., & Olah, L. (2010). Effect of smoking cessation on visually evoked cerebral blood flow response in healthy volunteers. *Journal of Vascular Research, 47*: 214-220.

Bone, R.A., Landrum, J.T., Fernandez, L., & Tarsis, S.L. (1988). Analysis of the macular pigment by HPLC: human retinal distribution and age study. *Investigative Ophthalmology & Visual Science, 29*(6): 843-849.

Bone, R.A., Landrum, J.T., & Tarsis, S.L. (1985). Preliminary identification of the human macular pigment. *Vision Res, 25*(11): 1531-1535.

Brookhouser, P.E., Worthington, D.W., & Kelly, W.J. (1992). Noise-induced hearing loss in children. Laryngoscope, 102: 645-655.

Carhart, R., & Jerger, J.F. (1959). Preferred method for clinical determination of pure-tone thresholds. *Journal of Speech and Hearing Disorders, 24*(4): 330-345.

CDC. (1986). Leading work-related diseases and injuries—United States. MMWR Morb Mortal Wkly Rep, 35: 185-188.

Chermak, G.D., Dengerink, J.E., & Dengerink, H.A. (1984). Threshold of octave masking as a predictor of temporary threshold shift following repeated noise exposure. Journal of Speech and Hearing Disorders, 49: 303-308.

Cook, J.E., & Becker, D.L. (1995). Gap junctions in the vertebrate retina. *Microscopy Research and Technique, 31*(5): 408-419.

Crabtree, D.V., Ojima, I., Geng, X., & Adler, A.J. (2001). Tubulins in the primate retina: evidence that xanthophylls may be endogenous ligands for the paclitaxel-binding site. *Bioorganic & Medicinal Chemistry, 9*: 1967-1976.

Craft, N.E., Haitema, T.B., Garnett, K.M., Fitch, K.A., & Dorey, C.K. (2004). Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *Journal of Nutrition, Health & Aging, 8*(3): 156-162.

Church, D.F., & Pryor, W.A. (1985). Free-radical chemistry of cigarette smoke and its toxicological implications. *Environmental Health Perspectives, 64*: 111-126.

Cruickshanks, K.J., Wiley, T.L., Tweed, T.S., Klein, B.E., Klein, R., et al. (1998). Prevalence of hearing loss in older adults in Beaver Dam, Wisconsin. The epidemiology of hearing loss study. Am J Epidemiol, 148: 879-886.

Dartnall, H.J.A., & Thomson, L.C. (1949). Retinal oxygen supply and macular pigmentation. *Nature, 164*(41777): 876.

Davis, R.R., Kozel, P., & Erway, L.C. (2003). Genetic influences individual susceptibility to noise: a review. *Noise and Health, 5*(20): 19-28.

Dwyer, J.H., Navab, M., Dwyer, K.M., Hassan, K., Sun, P., Shircore, A., Hama-Levy, S.,…Fogelman, A.M. (2001). Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation, 103*: 2922-2927.

Duindam, J.J., Vrensen, G.F.J.M., Otto, C., & Greve, J. (1998). Cholesterol, phospholipid, and protein changes in focal opacities in the human eye lens. *Invest Ophthalmol Vis Sci, 39*: 94-103.

Fechter, L.D. (2005). Oxidative stress: a potential basis for potentiation of noise-induced hearing loss. Environmental Toxicology and Pharmacology, 19: 543-546.

Fetoni, A.R., Sergi, B., Ferraresi, A., Paludetti, G., & Troiani, D. (2004). α-Tocopherol protective effects on gentamicin ototoxicity: an experimental study. International Journal of Audiology, 43: 166-171.

Flaye, D.E., Sullivan, K.N., Cullinan, T.R., Silver, J.H., & Whitelocke, R.A.F. (1989). Cataracts and cigarette smoking: The city eye study. *Eye, 3*: 379-384.

Garland, D. (1990). Role of site-specific, metal-catalyzed oxidation in lens aging and cataract: a hypothesis. *Exp Eye Res., 50*: 677-682.

Gates, G.A., Cobb, J.L., D'Agostino, R.B., & Wolf, P.A. (1993). The relation of hearing in the elderly to the presence of cardiovascular disease and cardiovascular risk factors. Arch Otolaryngol Head Neck Surg, 119: 156-161.

Gates, G.A., & Cooper, J.C. (1991). Incidence of hearing decline in the elderly. Acta Otolaryngol, 111: 240-248.

Gellerman, W., Ermakov, I.V., Ermakova, M.R., & McClane, R.W. (2002). In vivo resonant Raman measurement of macular carotenoid pigments in the young and the aging human retina. *J Opt Soc Am A, 19*(6): 1172-1186.

Glanz, J. (1997). Force-carrying web pervades living cell. *Science, 276*(5): 678-679.

Gooden, M., Rintoul, D., Takehana, M., & Takemoto, L. (1985). Major intrinsic polypeptide (MIP26K) from lens membrane: reconstitution into vesicles and inhibition of channel forming activity by peptide antiserum. *Biochemical and Biophysical Research Communications, 128*(2): 993-999.

Gregory, R.L. (1957). Increase in "neurological noise" as a factor in ageing. *Proceedings from the 4th Congress International Association of Gerontology,* 314-324.

Gutherie, A.H., McDowell, J.E., & Hammond, B.R. (2006). Scotopic sensitivity in schizophrenia. *Schizophrenia Research, 84,* 378-385.

Halliwell, B. & Gutteridge, J.M.C. (1989). *Free Radicals in Biology and Medicine,* 2nd ed. Clarendon Press, Oxford, UK.

Hammond, B.R. (2012). The dietary carotenoids in lutein and zeaxanthin in pre- and postnatal development. *Functional Food Reviews, 4*(3): 130-137.

Hammond, Jr., B.R., Johnson, E.J., Russell, R., Krinsky, N.I., Yeum, K., Edwards, R.B., & Snodderly, D.M. (1997a). Dietary modification of human macular pigment density. *Investigative Ophthalmology & Visual Science, 68*(9): 1795-1801.

Hammond, B.R., Wooten, B.R., Engles, M., & Wong, J.C. (2012). The influence of filtering by the macular carotenoids on contrast sensitivity measured under simulated blue haze conditions. *Vision Research, 63*: 58-62.

Hammond, B.R., Wooten, B.R., Nanez, J.E., & Wenzel, A.J. (1999). Smoking and lens optical density. *Ophthal. Physiol. Opt., 19*(4): 300-305.

Hammond, Jr., B.R., Wooten, B.R., & Smollon, B. (2005). Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optometry & Vision Science, 82*(5): 387-404.

Hammond, Jr., B.R., Wooten, B.R., & Snodderly, D.M. (1996). Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. Vision Res, 36(18): 3003-3009.

Hammond, Jr., B.R., Wooten, B.R., & Snodderly, D.M. (1997b). Individual variations in the spatial profile of human macular pigment. *JOSA A, 14*(6): 1187-1196.

Hammond, Jr., B.R., Wooten, B.R., & Snodderly, D.M. (1997c). Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. *Optometry and Vision Science, 74*(7): 499-504.

Hammond, Jr., B.R., Wooten, B.R., & Snodderly, D.M. (1998). Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest Ophthalmol Vis Sci, 39*(2): 397-406.

Handelman, G.J., Packer, L., & Cross, C.E. (1996). Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J Clin Nutr, 63*: 559-565.

Harding, J.J. (1995). Cigarettes and cataract: cadmium or a lack of vitamin C? *British Journal of Ophthalmology, 79*: 199-201.

Hecht, S., Haig, C., & Wald, G. (1935). The dark adaptation of retinal fields of different size and location. *J. Gen. Physiol., 19*: 321-337.

Henderson, D., Bielfeld, E.C., Harris, K.C., & Hu, B.H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear and Hearing, 27*(1): 1-19.

Hultcrantz, E. (1979). The effect of noise on cochlear blood flow in the conscious rabbit. *Acta Physiologica Scandinavica, 106*(1): 29-37.

Humes, L.E. (1980). Temporary threshold shift for masked pure tones. *Audiology, 19*: 335-345.

Itoh, A., Nakashima, T., Arao, H., Wakai, K., Tamakoshi, A., et al. (2001). Smoking and drinking habits as risk factors for hearing loss in the elderly: epidemiological study of subjects undergoing routine health checks in Aichi, Japan. Public Health, 115: 192-196.

Jerger, J.F., & Carhart, R. (1956). Temporary threshold shift as an index of noise susceptibility. *Journal of the Acoustical Society of America, 28*(4): 611-613.

Johnson, E.J. (2012). A possible role for lutein and zeaxanthin in cognitive function in the elderly. *Am J Clin Nutr, 96*(suppl): 1161S-1165S.

Johnson, E.J., McDonald, K., Caldarella, S.M., Chung, H., Troen, A.M., & Snodderly, D.M. (2008). Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutritional Neuroscience, 11*(2): 75-83.

Karnaukhov, V.N. (1990). Carotenoids: recent progress, problems and prospects. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 95*(1): 1-20.

Katz, J. (Ed.). (1994). Handbook of Clinical Audiology. Baltimore, MD: Williams & Wilkins.

Kilbride, P.E., Alexander, K.R., Fishman, M., & Fishman, G.A. (1989). Human macular pigment assessed by imaging fundus reflectometry. *Vis Res, 29*: 663-674.

Leske, M.C., Chylack, L.T., & Wu, S. (1991). The lens opacities case-control study. *Arch Ophthalmol, 109*: 244-251.

Maggi, S., Minicuci, N., Martini, A., Langlois, J., Sivicro, P. et al., (1998). Prevalence rates of hearing impairment and comorbid conditions in older people: the Veneto Study. J Am Geriatr Soc, 46: 1069-1074.

Maniotis, A.J., Bojanowski, K., & Ingber, D.E. (1997). Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. *Journal of Cellular Biochemistry, 65*: 114-130.

Maniotis, A.J., Chen, C.S., & Ingber, D.E. (1997b). Demonstration of mechanical connections between integrins cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci., 94*: 849-854.

Mayer, A.M. (1876). Researches in acoustics. Phil, Mag. *2*: 500-507.

Mellerio, J. (1971). Light absorption and scatter in the human lens. *Vision Research, 11*: 129-141.

Neelam, K., O'Gorman, N., Nolan, J., O'Donovan, O., Wong, H.B., Eong, K.G.A., & Beatty, S. (2005). Measurement of macular pigment: raman spectroscopy versus heterochromatic flicker photometry. *Investigatvie Ophthalmology & Visual Science, 46*(5): 1023-1032.

Niki, E., Minamisawa, S., Oikawa, M., Komuru, E. (1993). Membrane damage from lipid oxidation induced by free radicals and cigarette smoke. Annals of the New York Academy of Science, 686: 29-37.

Niskar, A.S., Kieszak, S.M., Holmes, A.E., Esteban, E., Rubin, C., & Brody, D.J. (2001). Estimated prevalence of noise-induced hearing threshold shifts in children 6 to 19 years of age: The third national health and nutrition examination survey, 1988-1994, United States. *Pediatrics, 108*(1): 40-43.

Nolan, J.M., Kenny, R., O'Regan, C., Cronin, H., Loughman, J., Connolly, E.E., Kearney, P., Loane, E., & Beatty, S. (2010). Macular pigment optical density in an ageing irish population: the irish longitudinal study on ageing. *Ophthalmic Research, 44*: 131-139.

Olsen, C.C., & Berry, G.A. (1979). Further interpretation of the threshold of masking (TOM) test. *Scandinavian Audiology, 8*(4): 217-223.

Patuzzi, R., & Rajan, R. (1992). Additivity of threshold evaluations produced by disruption of outer hair cell function. *Hearing Research, 60*(2): 165-177.

Pease, P.L., Adamas, A.J., & Nuccio, E. (1987). Optical density of human macular pigment. *Vision Res., 27*(5): 705-710.

Pokorny, J., Smith, V.C., & Lutze, M. (1987). Aging of the human lens. *Applied Optics, 26*(8): 1437-1440.

Pryor, W.A., & Stone, K. (1993). Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. Annals of the New York Academy of Science, 686: 12-27.

Puel, J.L., Ruel, J., d'Aldin, C.G., & Pujol, R. (1998). Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. *Neuroreport, 9*(9): 2109-2114.

Quaranta, A., Scaringi, A., Bartoli, R., Margarito, M.A., & Quaranta, N. (2004). The effects of 'supra-physiological' vitamin B12 administration on temporary threshold shift. International Journal of Audiology, 43: 162-165.

Renzi, L.M., Bovier, E.R., & Hammond, B.R., (available online March 4, 2013). A role for the macular carotenoids in visual motor response. *Nutritional Neuroscience.* Retrieved from http://dx.doi.org/10.1179/1476830513Y.0000000054.

Renzi, L.M., & Hammond, B.R. (2010). The relation between the macular carotenoids, lutein and zeaxanthin, and temporal vision. *Ophthal. Physiol. Opt., 30*: 351-357.

Robson, A.G., Moreland, J.D., Pauleikhoff, D., Morrissey, T., Holder, G.E., Fitzke, F.W., Bird, A.C., & van Kuijk, F.J.G.M. (2003). Macular pigment density and distribution: comparison of fundus autofluorescence with minimum motion photometry. *Vision Research, 43*: 1765-1775.

Rosen, S. (1966). Hearing studies in selected urban and rural populations. Trans NY Acad Sci, 29: 9-21.

Rosenhall, U., Sixt, E., Sundh, V., & Svanborg, A. (1993). Correlations between presbyacusis and extrinsic noxious factors. Audiology, 32: 234-243.

Salthouse, T.A., & Lichty, W. (1985). Tests of the neural noise hypothesis of age-related cognitive change. *Journal of Gerontology, 40*(4): 443-450.

Sample, P.A., Esterson, F.D., Weinreb, R.N., & Boynton, R.M. (1988). The aging lens: in vivo assessment of light absorption in 84 human eyes. *Investigative Ophthalmology & Visual Science, 29*(8): 1306-1311.

Savage, G.L., Haegerstrom-Portnoy, G., Adams, A.J., & Hewlett, S.E. (1993). Age changes in the optical density of human ocular media. *Clin. Vision Sci., 8*: 97-108.

Sergi, B., Fetoni, A.R., Ferraresi, A., Troiani, D., Azzena, G.B., Paludetti, G., & Maurizi, M. (2004). The role of antioxidants in protection from ototoxic drugs. *Acta Oto-laryngologica, 124*(S552): 42-45.

Siegelaub, A.B., Friedman, G.D., Adour, K., & Seltzer, C.C. (1974). Hearing loss in adults: relation to age, sex, exposure to loud noise, and cigarette smoking. Arch Environ Health, 29: 107-109.

Sies, H. (1985). Oxidative stress: introductory remarks. In *Oxidative Stress*, ed. Sies, H. pp. 1-8. Academic Press, London.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology, 82*: 291-295.

Snodderly, D.M., Auran, J.D., & Delori, F.C. (1984). The macular pigment. II. Spatial distribution in primate retinas. *Investigative Ophthalmology Visual Science, 25*(6): 674-685.

Snodderly, D.M., Handelman, G.J., Adler, A.J. (1991). Distribution of individual macular pigment carotenoids in central retina of macaque and squirrel monkeys. *Invest Ophthalmol Vis Sci, 32*: 268-279.

Spicer, S.S., & Schulte, B.A. (1996). The fine structure of spiral ligament cells relates to ion return to the stria and varies with place frequency. *Hearing Research, 100*: 80-100.

Stahl, W., & Sies, H. (2001). Effects of carotenoids and retinoids on gap junctional communication. *Biofactors, 15*: 95-98.

Stahl, W., & Sies, H. (2002). Antioxidant effects of carotenoids: implication in photoprotection in humans. In: cadenzas E, Packer L, eds. Handbook of Antioxidants, 2nd ed. New York: Marcel Dekker; 223-233.

Starck, J., Toppila, E., & Pyykkö, I. (1999). Smoking as a risk factor in sensory neural hearing loss among workers exposed to occupational noise. *Acta Oto-laryngologica, 119*(3): 302-305.

Stringham, J.M., Hammond, B.R., Nolan, J.M., Wooten, B.R., Mammen, A., Smollon, W., & Snodderly, D.M. (2008). The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Experimental Eye Research, 87*: 445-453.

Sujak A., Gabrielska, J., Grudzinski, W., Borc, R., Mazurek, P., & Gruszecki, WI. (1999). Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. Arch Biochem Biophys, 371: 301-307.

Tan, K.E.W.P. (1971). *Vision in the ultraviolet* (Doctoral dissertation). Retrieved from http://dds.crl.edu/CRLdelivery.asp?tid=13464

Taylor, A. (1993). Cataract: Relationships between nutrition and oxidation. *Journal of the American College of Nutrition, 12*(2): 138-146.

Trieschmann, M., Heimes, B., Hense, H.W., & Pauleikhoff, D. (2006). Macular pigment optical density measurement in autofluorescence imaging: comparison of one- and two-wavelength methods. *Graefe's Arch Clin Exp Ophthalmol, 244*: 1565-1574.

Vaney, D.I., Nelson, J.C., & Pow, D.V. (1998). Neurotransmitter coupling through gap junctions in the retina. *Journal of Neuroscience, 18*(24): 10594-10602.

Vishwanathan, R., Neuringer, M., Snodderly, D.M., Schalch, W., & Johnson, E. (2013). Macular lutein and zeaxanthin are realted to brain lutein and zeaxanthin in primates. *Nutritional Neuroscience, 16*(1): 21-29.

Wald, G. (1945). Human vision and the spectrum. *Science, 101*: 653-658.

Wallhagen, M.I., Strawbridge, W.J., Cohen, R.D., & Kaplan, G.A. (1997). An increasing prevalence of hearing impairment and associated risk factors over three decades of the Alameda County Study. Am J Public Health, 87: 440-442.

Weale, R.A. (1988). Age and the transmittance of the human crystalline lens. *J. Physiol., 395*: 577-587.

Werner, J.S. (1982). Development of scotopic sensitivity and the absorption spectrum of the human ocular media. *J. Opt. Soc. Am., 72*(2): 247-258.

Wilson, J., Braunwald, E., Isselbacher, K., Petersdorf, R., Martin, J.B., Fauci, A.S., & Root, R.K. (1991). Harrison's Principles of Internal Medicine, Vol. 2, 12th edn, New York, NY: McGraw Hill, pp. 1523-1529.

Wooten, B.R., & Hammond, B.R. (2002). Macular pigment: influences on visual acuity and visibility. *Progress in Retinal and Eye Research, 21*: 225-240.

Wooten, B.R., Hammond, B.R., Jr., Land, R.I., & Snodderly, D.M. (1999). A practical method for measuring macular pigment optical density. *Investigative Ophthalmology Visual Science, 40*(11): 2481-2489.

Wooten, B.R., Hammond, B.R., & Renzi, L.M. (2007). Using scotopic and photopic flicker to measure lens optical density. *Ophthal. Physiol. Opt., 27*: 321-328.

Wu, W.J., Sha, S.H., & Schacht, J. (2002). Recent advances in understanding aminoglycoside ototoxicity and its prevention. *Audiology and Neurotology, 7*(3): 171-174.

Xu, J., Pokorny, J., & Smith, V.C. (1997). Optical density of the human lens. *J. Opt. Soc. Am. A., 14*(5): 953-960.

Appendix A. Calibration of the audiometer and clinical chamber.


MED-ACOUSTICS, INC.
(A Trilogy Audiometrics Company)
1685 East Park Place Blvd.
Stone Mountain, GA 30087
(770) 498-8075 / (800) 749-8075
FAX (770) 498-9246

NOISE ANALYSIS
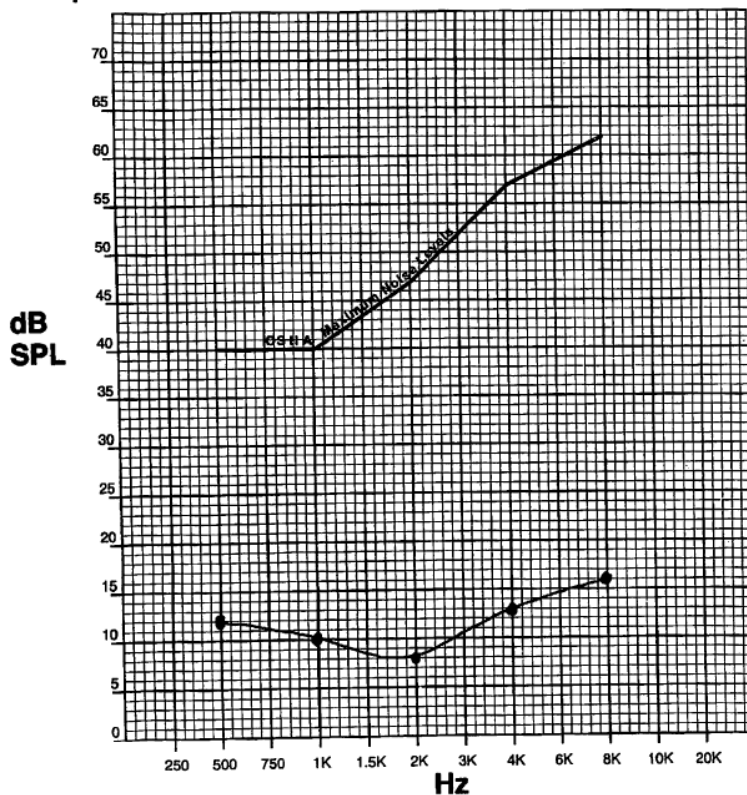
CLIENT _UGA_

DATE _12-10-2012_

LOCATION _Booth - Right - Big_

TYPE _✓_ AMBIENT ____ CLICKS ____ OTHER ____

TECHNICIAN _FRANK Scott_

S.L.M. TYPE _LD 824_

SN _3988_

MIC TYPE _LD 2575_

SN _1900_

TN # _106362_

NOTES ____ DbA= _31db_

| Hz	MAXIMUM ALLOWABLE NOISE LEVELS	MEASURED NOISE LEVELS
250		
500	40 db	12db
750		
1K	40 db	10db
1.5K		
2K	47 db	8db
3K		
4K	57 db	13db
6K		
8K	62 db	16db

Appendix B. Log energy values of the 406nm LED at arbitrary participant knob position.
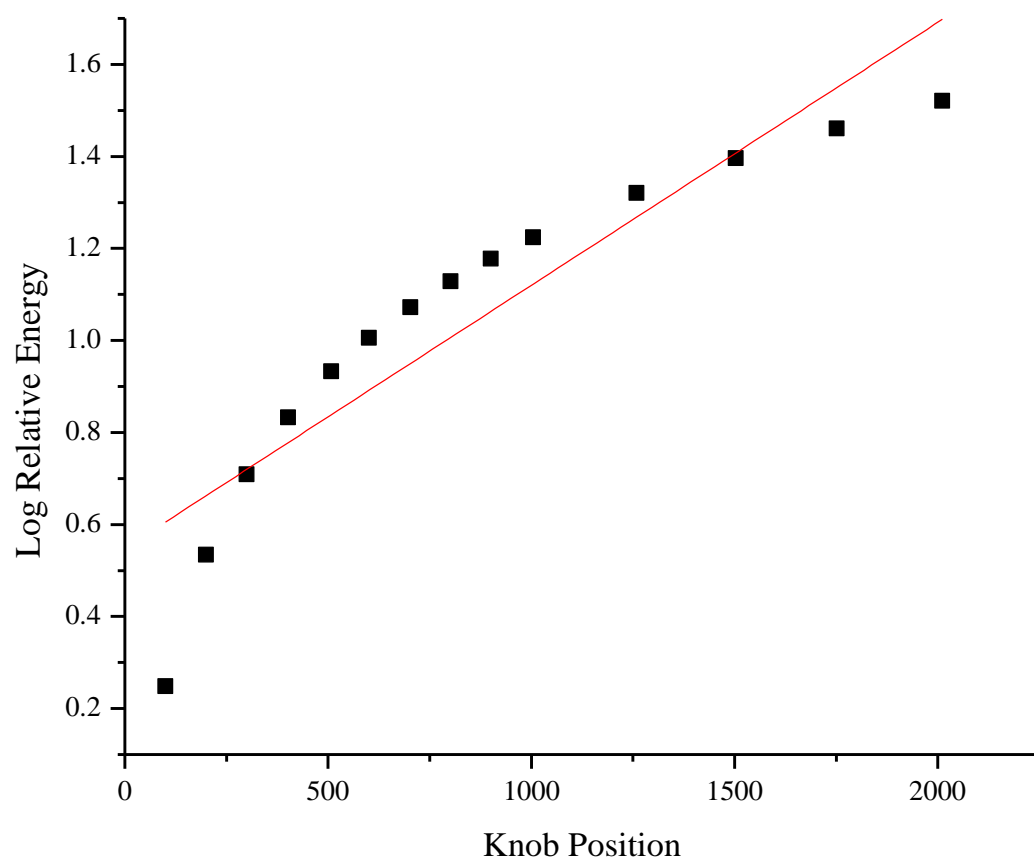
Appendix C. Binaural pure-tone thresholds and threshold shifts in white noise were not statistically different across two trials in a sample of young non-smokers. ($N = 9$, $M = 19.6 \pm 1.2$ years).
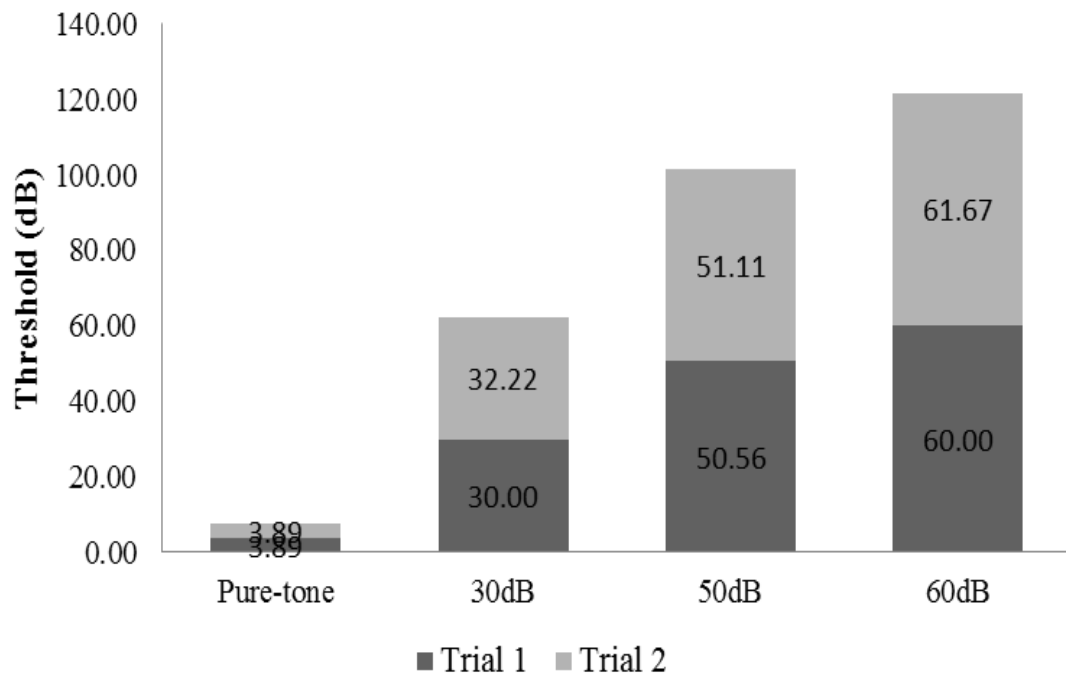
Appendix D. Age was a covariant for all variables. A.) MP density was trending higher in older individuals ($t = -1.629$, $p = .06$) B.) Lens density was significantly higher in older individuals ($t = 2.770$, $p < .01$). C.) Vestibular function was significantly lower in older individuals ($t = -2.927$, $p < .01$). D.) Pure-tone thresholds were significantly higher in older individuals ($t = -4.944$, $p < .01$).

A.)

C.)

B.)

D.)
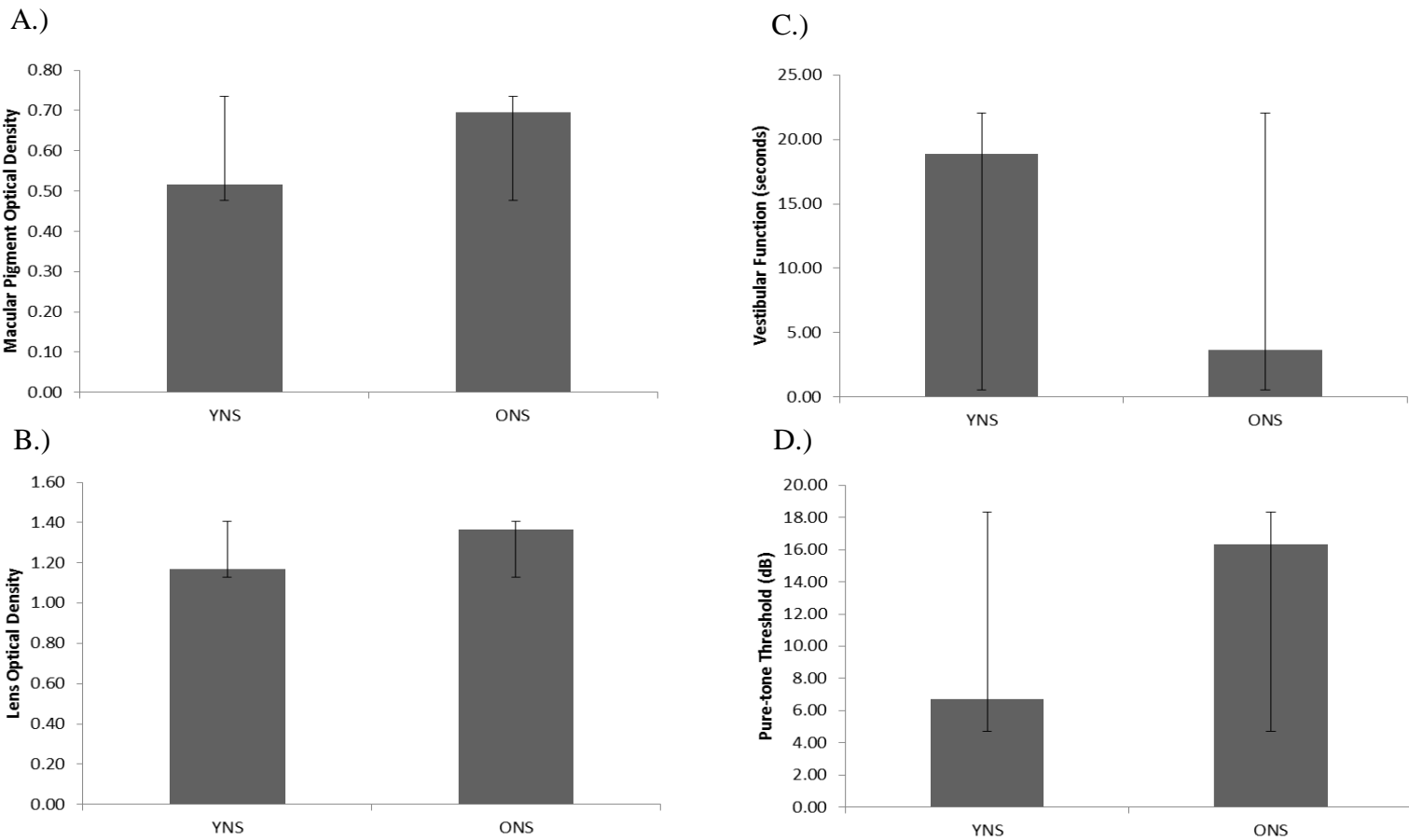
Appendix E. In a sample of non-smokers, age was not related to auditory thresholds obtained in the presence of noise.