EFFECTS OF LUTEIN AND DOCOSAHEXAENOIC ACID ENRICHED EGG CONSUMPTION ON VISUAL FUNCTION IN OLDER ADULTS: IMPLICATIONS FOR AGE-RELATED MACULAR DEGENERATION

By

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Abstract

Despite ample research has shown the ability of lutein, zeaxanthin and omega-3 docosahexaenoic acid (DHA) to benefit retina function, research utilizing whole foods as a vehicle for these nutrients is lacking. As age-related macular degeneration (AMD) is common among Caucasian older adults, this study investigated whether DHA enriched egg consumption can improve electrophysiological retina function in this population. A total of thirty (male: n=11, female: n=19) healthy Caucasian older adults (64.0 ± 3.4 years) consumed two lutein and DHA enriched eggs (0.87 mg lutein/day, 220 mg DHA/day) daily for six weeks, while avoiding supplements and foods high in eye related nutrients. Rod and cone cell function was assessed by full field electroretinogram. Plasma and red blood cell (RBC) DHA, plasma lutein, lipid profiles, and lipoprotein subfractions were assessed at day 0 (PRE) and 6 wks (POST). Following 6 weeks of egg treatment, the maximum amplitude (log cds/m^2) of scotopic a-wave (PRE: 234.6 ± 9.4, POST: 270.4 ± 8.7) and photopic b-wave (PRE: 72.0 ± 3.3, POST: 101.5 ± 5.8) significantly ($p<0.001$) increased. During this period, plasma DHA levels significantly increased by 35.4% ($p < 0.001$). No significant alterations in plasma Chol (mmol/L), HDL-Chol, LDL-Chol, triacylglycerides (TAG, mmol/L), plasma lutein and RBC DHA were present. A significant increase in large HDL particles (PRE: 30.9% (±1.3); POST: 34.1% (±1.5)) and a decrease in intermediate sized particles (PRE: 54.4% (±1.2); POST 51.5% (±1.0)) occurred, while no changes were identified in LDL sub-fractions. This study suggests that consuming two lutein and DHA enriched eggs daily may improve electrophysiological retina function without adversely altering plasma Chol and TAG in Caucasian older adults. Overall, the consumption of eggs containing lutein and DHA may be a simple nutritional strategy for preventing age related vision deterioration including AMD.

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Abbreviations

AI	Adequate intake
ALA	α-Linolenic acid (C18:3n-3)
AMD	Age-related macular degeneration
AREDS	Age Related Eye Disease Study
CoA	Coenzyme A
CRP	C-reactive protein
DHA	Docosahexaenoic acid (C22:6n-3)
ELOVL4	Elongation of very long chain fatty acids 4
EPA	Eicosapentaenoic acid (C20:5n-3)
ERG	Electroretinogram
HPLC	High performance liquid chromatography
HDL-C	High-density lipoprotein cholesterol
LA	Linoleic acid (C18:2n-6)
LCPUFA	Long chain polyunsaturated fatty acid
LDL-C	Low-density lipoprotein cholesterol
MPOD	Macular pigment optical density
OCT	Optical coherence tomography
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
RDA	Recommended dietary allowance
RPE	Retinal pigment epithelium
TAG	Triacylglycerol
TC	Total cholesterol
UL	Upper limit
VEGF	Vascular endothelial growth factor
VLCPUFA	Very long chain polyunsaturated fatty acids (C>24)
VLDL	Very low density lipoproteins

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Chapter 1: Literature Review

Introduction

It is estimated that 285 million individuals are visually impaired worldwide and 82% of individuals living with blindness are over the age of 50 (WHO, 2014). Visual impairment is associated with an overall reduction in quality of life due to reduced functional ability, increased risk of falls and an increase in symptoms of depression. The main vision related diseases associated with aging include glaucoma, cataracts, diabetic retinopathy and age-related macular degeneration (AMD). AMD is a degenerative condition affecting the macular region of the retina, and is the leading cause of severe vision loss among older adults in developed countries. AMD is characterized by the death and dysfunction of photoreceptors in the retina, including rods and blue-light sensitive cones. The retina and retinal pigment epithelium (RPE) are associated with the greatest loss of photoreceptors during the aging process, making these particular regions highly vulnerable to degeneration. As the function of the RPE continues to decline the flow of nutrients and waste products becomes obstructed, leading to the production of basal laminar deposits known as drusen. As the size and quantity of drusen increases, further deterioration leads to the eventual loss of central vision. Currently, ocular laser surgery has been accepted as the most successful treatment option for individuals suffering from AMD but is invasive and is non-curative. Considering the limited treatment options for AMD, preventative measures such as nutritional therapeutic strategies to prevent and/or treat AMD may provide individuals with a simple, non-invasive and cost-effective strategy to treat and prevent visual impairment with little to no risk involved.

Eggs are a well-known source of vital nutrients including lecithin (choline), unsaturated fatty acids, essential amino acids, folate, carotenoids, and a number of other vitamins and minerals. Additionally through the alteration of chicken feed, eggs can be

enriched to provide greater concentrations of nutrients such as docosahexaenoic acid (DHA, C22:6n-3), lutein and zeaxanthin. Both DHA, lutein and zeaxanthin have been extensively studied regarding their effects on retina health and for the prevention of diseases such as AMD. Many of these studies have concluded that these particular nutrients are highly important for maintaining the health of the retina. While the effects of these individual nutrients have been studied mainly in their supplemental forms, the synergistic effect of these nutrients within the yolk of the egg has not yet been tested in relation to retina function. Considering the egg is a rich source of eye related nutrients, it is of interest to determine whether the consumption of lutein and DHA enriched eggs can significantly improve visual function among the aging population, who are at risk of developing eye related diseases such as AMD.

The present review will focus on the retina, AMD and how specific nutrients including lutein, zeaxanthin, and DHA may have the potential to be consumed synergistically in the form of a chicken egg to improve retina health and prevent the formation of AMD.

Introduction to the Retina

The retina is a constituent of the central nervous system and is the highly light sensitive and innermost layer of the eye. Although the average human eye is about 22 mm in diameter, the total area of the retina is approximately 1,100 mm^2 (Kolb, 2007). Despite its modest size, the retina is essential for the processing of visual information. When light enters the eye through the cornea, the retina is responsible for conversion of light to electrical potentials. These electrical potentials travel through the retinal layers and enter the visual cortex for processing via the optic nerve. The retina is comprised of nine layers differing in cellular distribution and function (Kolb, 2003). These retinal layers and their cellular components are displayed in Table 1-1.

Table 1-1. Layers of the retina and their cellular components.

Retinal Layer	Cellular Components
Outermost Surface of the Retina	
Retinal Pigment Epithelium	
Photoreceptor Layer	Outer and inner segments of rods and cones
Outer Limiting Membrane	
Outer Nuclear Layer	Cell bodies of rods and cones
Outer Plexiform Layer	Axons of rods and cones, dendrites of horizontal and bipolar cells
Inner Nuclear Layer	Nuclei of horizontal, bipolar and amacrine cells
Inner Plexiform Layer	Axons of bipolar and amacrine cells, dendrites of ganglion cells
Layer of Ganglion Cells	Nuclei of ganglion cells
Layer of Optic Nerve Fibers	Fibers of ganglion cells
Innermost Surface of the Retina	

The RPE is the outermost layer of the retina located between the outer segments of photoreceptor cells and the choroid. Separating the RPE from the choriocapillaris is Bruch's Membrane, a thin layer of tissue and source of oxygen for the RPE (Boulton and Dayhaw-Barker, 2001). The highly pigmented cells of the RPE perform numerous functions that are crucial for maintaining the health and normal functioning of photoreceptor cells and for the prevention of retinal disease and degeneration (Boulton and Dayhaw-Barker, 2001). The RPE is an environment highly saturated in oxygen, as it is continuously exposed to photo-oxidative energy and a continuous flow of oxygen via the choroidal blood vessels (Strauss, 2005). This level of oxygen consumption within the retina paired with high concentrations of lipids such as polyunsaturated fatty acid, DHA, leads to an environment highly susceptible to oxidative damage (Boulton and Dayhaw-Barker, 2001; Jeffrey et al., 2001). In response to the levels of oxidative stress present within the retina, many functional roles of the RPE are responsible for mitigating this stress and maintaining the overall health of this sensitive environment. The various

functions of the RPE include absorption of light energy and protection against photo-oxidation; transportation of ions, metabolites and fluid at the blood-retinal barrier; delivery of nutrients such as glucose, retinol and fatty acids to the photoreceptor cells; the exchange of retinal between the RPE and the photoreceptors; and the phagocytosis of debris formed from the constant regeneration of photoreceptor outer segments (Boulton and Dayhaw-Barker, 2001; Strauss, 2005).

The photoreceptor cells are responsible for the capture of light energy and the process of phototransduction (Jeffrey et al., 2001). Photoreceptor cells can be classified as either rod or cone cells, both of which consist of an inner and outer segment (Kolb, 2012). The inner segments of photoreceptor cells are essential for cellular metabolism, while the outer segments are responsible for the absorption of light energy in the form of photon particles (Kolb, 2012). The occupancy of rods and cones is unevenly distributed, as the human retina contains roughly 90 million rods and 4 to 5 million cones (Ryan et al., 2013). Rods are located within the peripheral region of the retina and are responsible for scotopic (dark) vision. Cones, mainly situated in the center of the retina, are responsible for photopic (light) vision and colour perception, as these cells are sensitive to light of various wavelengths. There are three forms of visual pigments among cone receptors including red (long wavelengths), green (medium wavelengths) and blue (short wavelengths) (Kolb, 2012). The red, green and blue cone receptors peak at the wavelengths of 564 nm, 533 nm and 437 nm, respectively (Kolb, 2012). In response to light entering through the corneal surface, the hyperpolarization of rods and cones creates an electrical potential that travels through the cellular components of the retina. From the photoreceptors, signals are transmitted through the inner plexiform layer including the horizontal, bipolar, and amacrine cells (Kolb, 2003). Lastly, the electrical synapses continue through the ganglion cells to the optic nerve to be processed by the visual cortex (Kolb, 2003). To allow for interaction between cells via dendritic synapses, the outer

plexiform layer separates the outer nuclear layer and the inner nuclear layer, while the inner plexiform layer separates the inner nuclear layer and the ganglion cell layer (Ryan, 2013).

At the center of the retina is the macula lutea (macula), a highly pigmented yellow spot containing the fovea centralis (fovea). The fovea is heavily concentrated with cones and contains nearly zero rod cells. The fovea is recognized as the area of highest visual acuity, providing overall sharpness to vision (Kolb, 2012). As the macula lutea and the central fovea are highly significant to the provision of clear central vision, any degeneration of this area can have serious consequences and lead to loss of central vision or total blindness. AMD is a primary example of a retinal disease affecting the macula, leading to the gradual loss of vision over time or the sudden loss of vision in months to weeks in advanced forms of the disease.

Measuring Retina Function with Full Field Electroretinography

The full field eletroretinogram (ERG) is a highly sensitive electrophysiological test of retina function. The test administers flashes of light to assess the efficiency of photoreceptors and inner retinal cells to obtain an objective measurement. By providing a stimulus in the form of light, photoreceptors and inner retinal cells are stimulated to obtain an electrical response. This response travels through the retinal layers to the surface of the cornea where it is captured by an electrode located directly on the surface of the eye.

The test begins with the administration of a 1% tropicamide for pupil dilation to allow for the attainment of accurate test results. Following 30 minutes of dark adaptation, a wire electrode is placed on the surface of the cornea under the bottom eyelid to measure the electrical response produced by the cells of the retina. A reference electrode is placed on the forehead and a ground electrode on the ear lobe. All three electrodes are then

connected to an amplifier and computer. At this time, the participant is placed in a comfortable position and the chin is rested on the Ganzfield dome. Once the participants face is comfortably within the Ganzfield dome, the ERG will begin producing flashes of light and the scotopic, (dark-adapted data) is collected. Once completed, the test is repeated without dark adaptation and the photopic (light adapted) data is obtained.

The result of the test is presented as a biphasic waveform that represents the responses produced by the cells of the retina. Data is analyzed by the dissection of the waveform into three main constituents including the a-wave, b-wave and oscillatory potentials (OP) (Creel, 2015). Additionally, each constituent is measured by amplitude and response time referred to as 'implicit time' in ERG terminology. The a-wave represents the overall health of the photoreceptor cells and is measured by the amplitude and implicit time from baseline to the negative-most trough of the waveform (Creel, 2015). As previously mentioned, the ERG test is performed in a scotopic and photopic state. As the scotopic test is completed post dark adaptation, this particular a-wave is representative of rod cell function. The a-wave obtained during the photopic test is indicative of cone cell function as the participant is exposed to 3.0 $cd \cdot sec \cdot m^{-2}$ background white light throughout the duration of the test (Creel, 2015). The b-wave exemplifies the health of the inner retinal cells including the Müller and bipolar cells and is measured by the amplitude and implicit time of the negative-most trough to the positive-most peak of the waveform (Creel, 2015). Lastly, the OP represents the health of the inner retinal amacrine cells and is a measure of the amplitude and implicit time of the ascending limb of the b-wave (Creel, 2015).

Due to the ability of the ERG test to differentiate between groups of retinal cells, the test allows for specificity in determining potential areas of concern within the retina. Furthermore, the sensitivity and objectivity of this test can allow researchers and clinicians to monitor minute changes within the retinal cells over time. Considering these

advantages, the full field ERG is an exemplary test of retina function, beneficial for determining the retinal changes associated with interventional research.

Changes in Visual Function Associated with Aging

As with many areas of the human body, there are numerous changes in visual function associated with aging, leading to an increased risk of visual impairment in later stages of life (Carter, 1994). According to the World Health Organization (WHO, 2014), about 65% of individuals over the age of 50 have some form of visual impairment. These age-related changes include but are not limited to; presbyopia, a reduction in the ability to focus on close objects; decreased contrast sensitivity; decreased dark to light adaptation; and slowed glare recovery (Carter, 1994). Along with these age related vision changes, the risk of numerous vision related diseases and conditions increases with age including glaucoma, cataracts, diabetic retinopathy and AMD. All of these conditions are associated with a gradual or sudden loss of vision, permanently reducing overall quality of life. Other health related complications pertaining to vision loss include loss of balance, increased incidence of falls, exercise reduction, frailty, depression, loss of independence, reduced mobility and social isolation (CNIB, 2015; Legood et al., 2002).

Eye Diseases Related to Aging

Glaucoma.

According to the Glaucoma Research Foundation (2015), glaucoma is the second leading cause of blindness globally with over 60 million cases of glaucoma worldwide. Individuals of African descent are six to eight times more likely to develop glaucoma when compared to Caucasian individuals (Glaucoma Research Foundation, 2015). Other risk factors include individuals over 60 years of age, previous eye related injury and individuals with a genetic predisposition to the disease (Glaucoma Research Foundation,

2015). There are numerous forms of glaucoma such as angle-closure glaucoma, normal-tension glaucoma and the most common form of the disease, open-angle glaucoma (Glaucoma Research Foundation, 2011). At least 90% of cases of glaucoma are of the open-angle variety. Open-angle glaucoma is associated with an increase in intraocular pressure that forms due to the reduction or inhibition of proper fluid drainage out of the eye (Glaucoma Research Foundation, 2011). A prolonged increase in intraocular pressure damages the retinal nerve fibers leading to a gradual loss of vision (Carter, 1994). Open-angle glaucoma is often asymptomatic in early stages of the disease. Symptoms of advanced glaucoma include reduced functional ability in dim light, contrast sensitivity, dark adaptation, central acuity and finally eventual blindness if untreated (Glaucoma Research Foundation, 2011; Carter, 1994).

Cataract.

Cataract is an eye disease associated with the opacification of the lens often related to aging. Cataract is responsible for 51% of blindness worldwide (WHO, 2016). The opaque cloudiness of cataract occurs due to a number of age-related changes or degradation to the eye over time including; metabolic changes; prolonged exposure to UV light; substance abuse such as alcohol and cigarette smoke; nutritional deficiencies; and deterioration secondary to disease such as diabetes mellitus (Carter, 1994; WHO, 2016). Cataract is associated with a wide range of symptoms including reduced contrast sensitivity and color perception, sensitivity to glare and loss of vision (Carter, 1994). To prevent the formation of cataract, there are numerous lifestyle modifications an individual can undergo to reduce their risk. These modifications include maintaining overall eye health such as reducing UV light exposure and avoiding alcohol, smoking and narcotic abuse. In advanced stages of the disease, treatment may include replacement of the

crystalline lens with an artificial intraocular lens through surgical extraction (Carter, 1994; WHO, 2016).

Diabetic Retinopathy.

Diabetic retinopathy is another age-related disease occurring in one third of individuals diagnosed with type 1 and 2 diabetes mellitus (WHO, 2016). In 2002, diabetic retinopathy was responsible for 5% of total blindness globally (WHO, 2016). Currently, an average 347 million individuals worldwide have diabetes, a number that is increasing at epidemic proportions (WHO, 2016). Diabetic retinopathy damages the delicate vasculature of the retina due to prolonged hyperglycemia. Other risk factors of diabetic retinopathy include pregnancy, hypertension, hyperlipidemia, and nephropathy (Moreno et al., 2013). In early stages of diabetic retinopathy, symptoms include vascular blockages and deterioration of retinal veins, intraretinal hemorrhage, the formation of hard exudates and macular edema (Carter, 1994). These symptoms can progress to proliferative retinopathy with neovascularization, vascular fibrosis and of course blindness (Carter, 1994; WHO, 2016).

Age-Related Macular Degeneration.

Prevalence.

AMD is the leading cause of severe vision loss in developed countries, debilitating roughly 1 million Canadians and 2 million Americans (American Academy of Ophthalmology, 2011; CNIB, 2015; National Eye Institute, 2010). The prevalence and severity of AMD increases with age and it is estimated from 2010 to 2050, US prevalence rates will more than double from 2.1 to 4.4 million (National Eye Institute, 2010). Evidently, this estimation is directly correlated to the world's aging population as the World Health Organization (WHO, 2016) estimates that the prevalence of individuals

over 60 will likely increase from 12% to 22% by the year 2050. This relationship between AMD and aging was exhibited by the Beaver Dam Eye Study (Klein et al., 2002), which monitored the prevalence of visual impairment among Wisconsin residents including the presence of AMD over a 20-year period. In participants under the age of 55, virtually no AMD was present. On the contrary, the prevalence of AMD increased to 4.1% in individuals over 75 with a total of 12.1% suffering from early AMD and 2.1% with late stage AMD (Klein et al., 2002). Additionally, of the 190 participants diagnosed with AMD prior to the trial, 12.6% of these individuals advanced to late stage AMD, 8.4% advanced to exudative macular degeneration, and 4.2% developed pure geographic atrophy. In comparison, the Rotterdam Eye Study (Vingerling et al., 1995) performed in the Netherlands, observed the prevalence of AMD among individuals 55 and older. Comparable to the findings of the Beaver Dam Eye Study (Klein et al., 2002), only 0.1% of individuals 55 to 64 years of age had some form of AMD, which increased to 3.7% of individuals over the age of 75. Among participants with AMD, 1.7% suffered from advanced forms of the disease including atrophic or neovascular AMD (Vingerling et al., 1995). These findings provide substantiated evidence that the prevalence and severity of AMD is directly proportional to aging. Considering the progressive global inflation of individuals over the age of 60, AMD has the potential to intensify as a public health concern of which preventative strategies are of the utmost importance.

Risk factors.

Other than of course aging, there are an abundance of risk factors related to the development and progression of AMD. These risk factors include gender, race, genetic, environmental and lifestyle components. Although AMD occurs in both gender groups, 65% of cases occur among females. Additionally, nearly 90% of all cases of AMD occur among the Caucasian population when compared to all other racial and ethnic groups

(Coleman et al., 2008; National Eye Institute, 2010). Although not definitive, researchers have proposed a correlation between numerous genetic aberrations and the development of AMD, potentially contributing to 70% of cases (Holz et al., 2012). Potential AMD related genes include complement factor H on chromosome 1q31 and ARMS2/HTRA1 (Holz et al., 2012). Environmental risk factors include exposure to ultraviolet blue light, oxidative stress and numerous behavioural and lifestyle factors such as smoking can double ones risk of developing AMD (Seddon et al., 2006). Among 4000 European men and women over the age of 65, Chakravarthy et al. (2007) determined that current smokers were 5 times more likely to develop geographic atrophy and 2.5 times more likely to develop neovascular AMD. Lastly, a nutrient deficient diet low in vitamins A, C, E, zinc, lutein, zeaxanthin and omega-3 fatty acids such as DHA can lead to the development of AMD later in life.

Symptoms.

While the majority of degeneration related to AMD occurs in the macula lutea, the deterioration can also affect surrounding tissues including the RPE, Bruch's membrane and the choriocapillaris (Coleman et al., 2008). According to the Age Related Eye Disease Study (AREDS) group (2013, 2001), AMD can be classified into 4 categories. These categories include no AMD, early, intermediate and late AMD. The characteristics and symptoms of each category are summarized in Table 1-2.

Table 1-2. Age Related Eye Disease Study (AREDS) Classification System of AMD

Stage and Classification	Characteristics
No AMD (AREDS Category 1)	None or a few small drusen (<63 μm in diameter)
Early AMD (AREDS Category 2)	Any or all of the following: Multiple small drusen (63-124 μm in diameter), or RPE abnormalities
Intermediate AMD (AREDS Category 3)	Any or all of the following: extensive intermediate drusen, and at least one large drusen (≥125 μm in diameter), or geographic atrophy not involving the center of the fovea
Late AMD (AREDS Category 4)	Characterized by one or more of the following in one eye (in the absence of other causes): - Geographic atrophy of the RPE and choriocapillaris involving the center of the fovea - Neovascular maculopathy such as; - Choroidal neovascularization - Serous and/or hemorrhagic detachment of the sensory retina or RPE; - Retinal hard exudates (a secondary phenomenon resulting from chronic leakage from any source); - Sub-retinal and sub-RPE fibrovascular proliferation; - Disciform scar

(Age-Related Eye Disease Study Research Group, 2001)

Early and intermediate AMD.

Early stages of AMD are characterized by the presence of drusen, yellow or white deposits that accumulate between the RPE and Bruch's membrane. Drusen is formed due to the inability of the RPE to dispose of waste products including the frequently regenerated outer segments of photoreceptor cells (Weikel et al., 2012; Cheung and Eaton, 2013). Drusen may be present in various sizes, textures and density. The presence of drusen that is large, soft and closely packed together is characteristic of advanced

forms of the AMD (Weikel et al., 2012). Drusen that is small in size, ranging in 63 to 124 μm in diameter is distinctive of early AMD (Age-Related Eye Disease Study Research Group, 2001). The presence of drusen may be without symptoms or cause visual impairment. However, an increase in the number and size of drusen leads to geographic atrophy and/or neovascular maculopathy, both of which have the ability to advance early AMD to late forms of AMD in a very minimal period of time (Age-Related Eye Disease Study Research Group, 2001; Binns, 2011). Mild symptoms occur in early AMD including mild blurred vision and impaired dark adaptation or patients may be completely asymptomatic (Binns, 2011; Cheung and Eaton, 2013).

Late AMD: Geographic atrophy and neovascularization.

Late AMD can be categorized as either non-exudative dry AMD (geographic atrophy), or the exudative wet form of AMD (neovascular AMD). Dry AMD or geographic atrophy is the hypo or hyperpigmentation of the RPE involving the center of the fovea, due to the presence of large drusen (≥125 μm in diameter) (Age-Related Eye Disease Study Research Group, 2001). These pigmentary abnormalities lead to eventual RPE cell death, atrophy and an increase in the visibility of choroidal blood vessels (Weikel et al., 2012). The escalation of cell death and degeneration of the RPE and photoreceptor cells increases waste product production, increasing the production of drusen and further advancing the disease (Weikel et al., 2012). Geographic atrophy is also associated with the loss of central vision, a debilitating symptom that may progress significantly within months to years (Binns, 2011).

Neovascular maculopathy is prevalent among 10% of individuals with AMD, and is responsible for up to 90% of severe vision loss in AMD patients (Mogk, 2013). Neovascular AMD is characterized by the uncontrolled growth of abnormal blood vessels that begin in the choroid, progress into Bruch's membrane and begin to develop under the

RPE (Weikel et al., 2012; Binns, 2011). These aberrant blood vessels are exceedingly fragile and highly at risk for leakage and subretinal hemorrhage, leading to serious complications including fluid exudation, lipid deposition, serous or hemorrhagic detachment of the retina or RPE and disciform scars (Cheung and Eaton, 2013; American Academy of Opthamology, 2011). Neovascular AMD can alter vision by making straight lines appear curved or initiate the sudden loss of central vision occurring over the duration of just days or weeks (Cheung and Eaton, 2013).

Current treatment options for AMD.

Currently there is no treatment to regress the retinal damage associated with AMD however, there are a number of options that may slow the progression or reduce symptoms of the disease. Unfortunately, all treatments options are only available to slow the progression of wet or neovascular AMD, not dry AMD. These options include photocoagulation therapy, photodynamic therapy and anti-VEGF (vascular endothelial growth factor) therapy.

Photocoagulation therapy.

Photocoagulation therapy is a treatment option for patients with neovascular AMD that seals aberrant blood vessels to prevent further leakage with the use of an argon green or krypton laser. This treatment option reduces long-term severe vision loss and improves vision by up to 21% (Macular Photocoagulation Study Group, 1986). Although photocoagulation therapy has been effective in reducing vision loss in some patients with wet AMD, there are severe risks involved including vision loss from laser-induced retinal damage. Furthermore, this particular therapy has a low rate of vision gain and recurrence rates of up to 50% (Lim et al., 2012). Due to these high risk complications, photodynamic

therapy has replaced photocoagulation therapy as the standard treatment for neovascular AMD (Stone, 2007; Coleman et al., 2008).

Photodynamic therapy.

Photodynamic therapy is initiated with an intravenous injection of verteporfin, a photosensitizing dye that is administered into the blood stream over a time period of ten minutes (Coleman et al., 2008). Following the injection, irradiation with a low energy laser light is performed, inducing the production of reactive oxygen species that damages the endothelial tissue and temporarily seals the abnormal blood vessels (Coleman et al., 2008;). Photodynamic therapy may reduce the risk of vision loss by up to 20% while decreasing the risk of severe side effects (Wormald et al., 2007). Risks involved in photodynamic therapy include photosensitivity, and acute severe visual loss in 3-4% of patients (Coleman et al., 2008).

Anti-vascular endothelial growth factor therapy.

The final and most commonly utilized treatment option for AMD includes anti-VEGF therapy. VEGF is responsible for signaling angiogenesis of choroidal blood vessels (Stone, 2007). This particular therapy consists of intravitreal injections of VEGF inhibitors such as pegatanib, bevacizumab, or ranibizumab and is the current standard of care for patients with neovascular AMD (Coleman et al., 2008). The provision of VEGF inhibitors intravitreally reduces the growth of aberrant blood vessels in the retina and reduces the progression of neovascular AMD with limited risk of serious side effects (Stone, 2007, Rosenfeld et al., (2006)). The MARINA study implemented by Rosenfeld et al. (2006) assessed the effects of monthly intravitreal injections of ranibizumab for 24 months in individuals with neovascular AMD. Results of this study determined that ranibizumab at 0.3 to 0.5 mg significantly improved visual acuity. Additionally, among

477 participants receiving wither 0.3 or 0.5 mg injections monthly, only 2.3% demonstrated adverse ocular effects. Anti-VEGF therapy can be performed in combination with photodynamic therapy and may be beneficial to further enhance the improvement and maintenance of visual acuity (Yoshida et al., 2013).

The treatment options available for AMD are limited and only available for individuals with the wet form of the disease. Currently, anti-VEGF therapy is the current standard of care and demonstrates beneficial improvements in visual acuity for patients suffering from neovascular AMD (Rosenfeld et al., 2006). However, as only 10% of individuals with AMD suffer from the wet form of the disease, is of at utmost importance that researchers develop a greater understanding of the etiology of AMD and to discover preventative methods to reduce the prevalence of this incapacitating disease (Mogk, 2013).

Visual Function and Quality of Life

There are a number of life changes that occur in conjunction with vision loss that have the ability to reduce overall quality of life for individuals suffering from degenerative eye diseases such as AMD. Vision loss leads to daily restrictions and reduced functional ability, increased risk of falls and an increase in the prevalence of depression (Alma et al., 2011). In a study performed by Alma et al. (2011) on visually impaired individuals over 55 years of age, 94% of individuals experienced restrictions to daily tasks and 16% of individuals reduced their visits to public places. These restrictions are often due to fear of injury (Alma et al., 2011). Falls among older adults can lead to extreme consequences and are the leading cause of death due to injury (Lamoureux et al., 2011). The visually impaired are twice as likely to fall, have recurrent falls and experience fractures when compared to individuals with no visual restrictions (Lamoureux et al., 2011). Furthermore, visually impaired individuals may be at an

increased risk of depression due to reduced functional ability and the inability to perform everyday tasks and leisurely activities (Lamoureux et al., 2011). For example, Rovner and Casten (2008) demonstrated that in individuals suffering from AMD, a correlation existed between the severity of vision loss and a reduction in daily valued activities such as reading, driving and crafts. This inability to perform leisurely activities was directly related to an increase in depression among participants.

Nutrients Involved in Retina Function

As with all areas of the human body, good nutrition is essential for optimal health and disease prevention, and the retina is no exception. Considering the limited treatment options for individuals with AMD and the risks related to these treatments, prevention strategies such as nutritional modifications are essential to reduce the prevalence of age-related eye disease and vision deterioration. In the past decade, a wealth of researchers have dedicated their time to understanding the role of nutrition in the functioning and overall health of the retina and visual system. Some of the nutrients in question include; the antioxidants vitamin C and vitamin E; zinc; n-3 long chain polyunsaturated fatty acids (LCPUFA) such as DHA; and the carotenoids β-carotene, lutein and zeaxanthin. A primary example of research performed in this area includes the AREDS 1 and 2 (2013, 2001). The objective of this research was to examine the effect of nutrient supplementation on the prevention and progression of AMD including vitamin C, vitamin E, beta-carotene and zinc in AREDS and with the addition of lutein and zeaxanthin, DHA and eicosapentaenoic acid (EPA) in AREDS 2. (Age-Related Eye Disease Study Research Group, 2001; 2013). Along with the addition of lutein, zeaxanthin , DHA and EPA, beta-carotene was removed from the original AREDS formulation due to the relationship of beta-carotene and an increase in the incidence of lung cancer in smoking individuals (Age-Related Eye Disease Study Research Group, 2013). Results of the

AREDS 1 study indicated that the supplementation of antioxidant nutrients including vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg) zinc oxide (80 mg) and cupric oxide (2 mg) could significantly decelerate the progression of AMD to advanced stages over a duration of 5 years. These benefits were only demonstrated in individuals at a high risk of developing advanced forms of AMD, along with individuals with intermediate or advanced AMD in one eye. In this group, overall risk of progression to advanced forms of AMD was reduced by about 25%. Moreover, risk of vision loss related to advanced AMD was reduced by about 19%. Unfortunately, no benefits were elicited in individuals with early stages of the disease or without AMD altogether. Additionally, AREDS 2 added 10 mg of lutein, 2 mg of zeaxanthin, 350 mg of DHA and 650 mg of EPA to the original AREDS formulation to determine if any further risk reduction was achievable. Unfortunately, no further significant benefit was demonstrated regarding the addition of these supplements to the original AREDS formula. Additionally, the lack of beta-carotene in the AREDS formulation demonstrated no effects on AMD risk or progression, however a greater incidence of lung cancer in smokers was demonstrated with beta-carotene supplementation. Beta-carotene has since been removed from the commercially available AREDS2 formula.

Considering the potential for nutritional modifications to benefit the health of the retina and ward off disease, it is of interest to determine if the inclusion of supplemental or dietary sources of eye related nutrients are of any benefit to the health of the retina with aging and for the prevention of disease. The following is a review of specific eye related nutrients of interest including lutein, zeaxanthin and DHA.

Xanthophylls: Lutein and zeaxanthin.

Lutein and zeaxanthin are xanthophylls, a family of oxygenated carotenoids that can only be obtained through dietary consumption. Lutein ($C_{40}H_{52}O_2$) and zeaxanthin

($C_{40}H_{56}O_2$) are chemical isomers consisting of 11 conjugated double bonds, an ionone ring and a hydroxylated cyclic structure located at both ends of a 40 carbon skeleton (Carpentier et al., 2009). Structurally, lutein and zeaxanthin differ only in the positioning of one double bond present on the ionone ring of each molecule. Lutein consists of a β-ionone ring and a ε-ionone ring, whereas zeaxanthin consists of two β-ionone rings (Abdel-Aal et al., 2013). The structure of lutein and zeaxanthin are displayed in Figure 1-1.

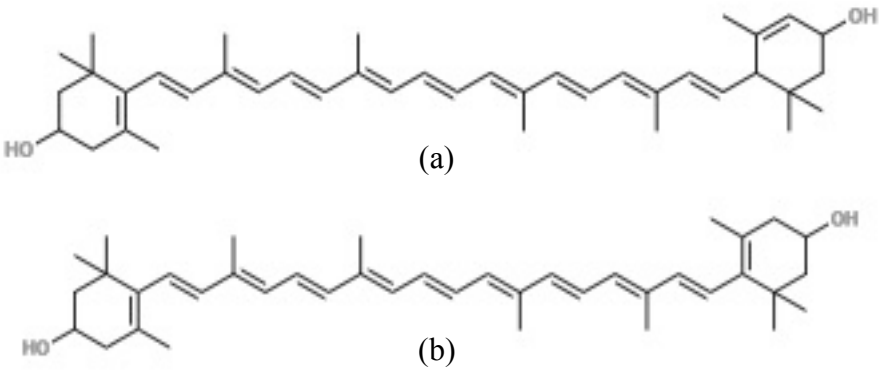

Figure 1-1. Structure of lutein (a) and zeaxanthin (b)

Dietary sources of lutein and zeaxanthin.

Although there is currently no Recommended Dietary Allowance (RDA) for lutein and zeaxanthin, the average daily consumption of the carotenoids in North America is an average of 1 to 2 mg per day (Dawczynski et al., 2013). Dietary sources of lutein and zeaxanthin consist of chicken egg yolk and mainly leafy green vegetables including kale, lettuce and spinach. The leafy green vegetables that contain the greatest quantity of lutein and zeaxanthin include kale (48.0-114.7 μg/g), parsley (64-106.5 μg/g), spinach (59.3-79.0 μg/g), lettuce (10.0-47.8 μg/g) and broccoli (7.1-33.0 μg/g) (Abdel-Aal et al., 2013). Trace amounts of lutein and zeaxanthin may also be obtained through the consumption of

wheat and grain products such as corn, einkorn wheat, and durum wheat.

Absorption and metabolism of lutein and zeaxanthin.

Following the ingestion of lutein and zeaxanthin, the carotenoids are absorbed via enterocytes of the small intestine. Together with other lipid soluble nutrients, lutein and zeaxanthin are packaged into chylomicrons and transported to liver hepatocytes via the lymphatic system (Kijlstra, Tian, Kelly, and Berendschot, 2012). As lutein and zeaxanthin are lipophilic nutrients, the absorption and bioavailability of xanthophylls is largely dependent on the quantity of lipids ingested at the time of consumption, and the matrices of food consumed. From the liver, lutein and zeaxanthin are incorporated into lipoproteins and are released into the blood stream for transport to extrahepatic tissues including the retina (Kijlstra et al., 2012). High-density lipoprotein cholesterol (HDL-C) is considered to be the primary transport lipoprotein responsible for delivering lutein and zeaxanthin to the eye for uptake into the retina (Kijlstra et al., 2012). The mechanistic action for the retinal uptake of lutein and zeaxanthin is unclear at this time.

Lutein and zeaxanthin in the retina.

Unlike α-carotene, β-carotene and β-cryptoxanthin, lutein and zeaxanthin are not provitamin A carotenoids however, they are essential for optimal functioning of the retina. Lutein, zeaxanthin and meso-zeaxanthin, a metabolite of lutein, are exceedingly concentrated in the highly pigmented macula, located in the center of the retina. Due to their highly concentrated presence within the macula, the three xanthophylls are also termed macular pigment. A photomicrograph image of this highly pigmented yellow region can be viewed in Figure 1-2. Macular pigment performs a variety of functions in the protection and maintenance of the retina and in the prevention of retina related disease including AMD. Macular pigment is responsible for two primary protective

mechanisms within the retina including its powerful antioxidant abilities and by reducing the penetration of harmful blue light into the retinal tissues. Visible wavelengths within the range of 400 to 500 nm induce photo-oxidative damage to the retina including the photoreceptor cells (Carpentier et al., 2009). Macular pigment is responsible for the absorption of blue light between the wavelengths of 445 to 472 nm, protecting the retina from lipid peroxidation, scavenging free radicals and quenching reactive oxygen species (Dawczynski et al., 2013; Abdel-Aal et al., 2013; García-Layana et al., 2013). Due to the high concentrations of PUFA and their abundance of conjugated double bonds within the retinal tissues, the retina is exceedingly susceptible to oxidative damage (Kijlstra et al., 2012; Carpentier et al., 2009).

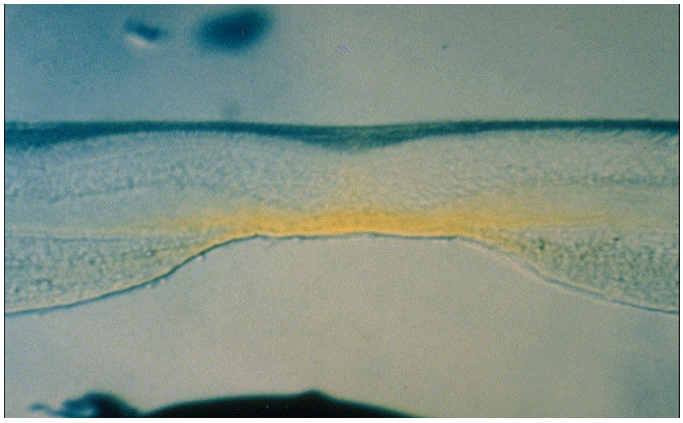

Figure 1-2. Photomicrograph image of macular pigment (Snodderly et al., 1984)

Human trials.

A wide variety of research has focused on the effects of lutein and zeaxanthin consumption on serum concentrations, visual performance measures and macular pigment optical density (MPOD). Many of these studies have demonstrated a positive association between the consumption of lutein and zeaxanthin and their potential benefit to retinal health and for the prevention of AMD. These studies include the use of

supplemental forms of lutein and zeaxanthin often in conjunction with other supplements such as DHA and antioxidant vitamins (vitamins C and E), and the use of dietary sources including eggs and leafy green vegetables.

Supplemental lutein and zeaxanthin.

A number of recent studies have investigated the effects of supplemental lutein and zeaxanthin on serum concentrations and MPOD in individuals with early to late AMD. In individuals diagnosed with dry AMD, Dawczynski et al. (2013) analyzed serum concentrations of lutein and zeaxanthin and MPOD in 172 participants from 60 to 80 years of age. Participants of this study were randomly assigned to one of three treatment groups for a total of 12 months. Group one consumed 10 mg of lutein with 1 mg of zeaxanthin in combination with 255 mg of fish oils (100 mg DHA and 30 mg EPA) and antioxidants (vitamins C, E, zinc and copper). Group 2 consumed 20 mg of lutein with 2 mg of zeaxanthin and 500 mg of fish oils (200 mg DHA and 60 mg EPA) and group 3 consumed a placebo. Both treatment groups were successful in significantly increasing MPOD parameters. However, the higher dosage did not increase MPOD more effectively then the lower dosage, suggesting the potential for a plateau effect as lutein and zeaxanthin may become saturated within the retinal tissues. These findings were replicated by Arnold et al. (2013). This particular study supplemented 145 older adults with dry AMD to determine the effects of lutein and zeaxanthin on plasma fatty acids, antioxidant capacity and MPOD. Participants were randomly assigned to one of three groups; the placebo group; group 1 who consumed 10 mg of lutein, 1 mg of zeaxanthin, 100 mg of DHA and 30 mg of EPA; and group 2 who consumed 20 mg of lutein, 2 mg of zeaxanthin, 200 mg of DHA and 60 mg of EPA. Following one month of supplementation, plasma concentrations of lutein and zeaxanthin significantly increased and stabilized for the remaining 11 months. MPOD displayed a significant increase in

both treatment groups, however no differences were displayed in dose variations. Results of these studies suggest that improvements in MPOD may be obtained with a once daily dosage of lutein and zeaxanthin at 10 mg and 1 mg, respectfully. Unfortunately, no measure of retina function was administered in either trial.

García-Layana et al., (2013) compared MPOD, best-corrected visual acuity, contrast sensitivity and macular thickness of 44 individuals with early stage AMD. Participants were randomly assigned to a placebo group or an intervention group who consumed 12 mg of lutein, 0.6 mg of zeaxanthin and 280 mg of DHA per day. After 1 year of supplementation, participants in the intervention group displayed a significant increase in MPOD when compared to the placebo group. However, visual acuity, contrast sensitivity and macular thickness did not improve. In comparison, Akuffo et al. (2015) demonstrated similar results in individuals with AMD. No beneficial effects were present upon measuring best-corrected visual acuity and contrast sensitivity with various degrees of supplementary lutein, zeaxanthin and meso-zeaxanthin for three years. As to be expected, MPOD improved in all intervention groups however, no benefits to disease progression were evident.

Further analysis of supplementary lutein and zeaxanthin on MPOD was carried out by Huang et al. (2008), who examined lutein and zeaxanthin supplementation in 40 participants from the ages of 64 to 86 with and without various degrees of AMD. All 40 participants consumed a supplement containing 10 mg of lutein and 2 mg of zeaxanthin daily for 6 months. Twenty of the participants consumed an additional 350 mg DHA and 650 mg of EPA. Consistent with the findings of Arnold et al. (2013) and Dawczynski et al. (2013), serum lutein and zeaxanthin concentrations increased significantly throughout the first month of the trial and stabilized for the remaining 6 months. Following a 3-month cessation period initiating after the 6 month trial, serum concentrations of lutein and zeaxanthin returned to baseline, indicating a constant dietary supply of the

xanthophylls is required to maintain heightened levels of serum concentrations. Interestingly, in a comparison of individuals with and without AMD, the increase in serum lutein and zeaxanthin concentrations were lower among individuals with AMD. However, these findings were not replicated by Meagher et al. (2013) in a comparison of serum response to supplementation in individuals with and without early AMD. Over 8 weeks of supplementation of various doses of xanthophyll carotenoids, serum xanthophyll levels were comparable between both groups.

Recent research has now included the supplementation of meso-zeaxanthin, along with lutein and zeaxanthin to improve MPOD and other visual function parameters. As previously mentioned, meso-zeaxanthin is non-dietary derivative of lutein and it is suggested that adequate levels of meso-zeaxanthin must be present within the retina for macular pigment to adequately uphold its antioxidant and light filtration properties. Two recent studies analyzed three supplementation patterns with various combinations of the 3 carotenoids on individuals with early AMD (Sabour-Pickett et al., 2014; Akuffo et al., 2015). Both supplements containing either lutein and zeaxanthin or lutein, zeaxanthin and meso-zeaxanthin were beneficial in improving MPOD and contrast senstivity, however the greatest improvements were demonstrated upon the inclusion of meso-zeaxanthin (Sabour-Pickett et al., 2014; Akuffo et al., 2015).

Based on results from a wide variety of research, the protective capabilities of macular pigment for retinal health seems to be highly beneficial for the prevention of retinal degeneration. However based on these findings, the ability of macular pigment to successfully digress the symptoms of retinal degeneration is unclear. According to Huang et al. (2015), this lack of knowledge regarding the improvement of MPOD may be due to insensitive measures of retina and visual function such as visual acuity and contrast sensitivity. Based on this hypothesis, Huang et al., (2015) performed a study in 112 Chinese individuals with early AMD over the age of 50. Participants were randomly

assigned to one of three groups and received either 10 mg of lutein, 10 mg of lutein and 10 mg of zeaxanthin or placebo daily over the span of 2 years. To determine the effects of supplementation on macular pigment and retinal sensitivities, MPOD, multifocal ERG and microperimeter-determined mean retinal sensitivity were measured. As to be expected, MPOD significantly improved in both treatment groups. Furthermore, multifocal ERG peak to trough amplitude significantly improved in measures of the central retina (ring 1 and 2). Lastly, a significant increase in microperimeter-determined mean retinal sensitivity was demonstrated in both treatment groups. These findings suggest that lutein and zeaxanthin potentially improves retina sensitivity in patients with early AMD, along with the protective effects demonstrated in a wide variety of studies as mentioned above.

Dietary lutein and zeaxanthin.

As previously mentioned, lutein and zeaxanthin are present in a number of natural food sources such as egg yolk and leafy green vegetables including kale, spinach, lettuce and parsley. In the past decade, a number of studies have focused on the beneficial effects of food sources of lutein and zeaxanthin on retina function and for the prevention of AMD.

Delcourt et al. (2006) performed a prospective study on 640 individuals over the age of 60 with various forms of AMD to determine if a correlation exists between dietary lutein and zeaxanthin consumption and the presence of the disease. Visual functioning of participants was measured by ophthalmic examinations and photographic grading was utilized to assess the association of serum lutein and zeaxanthin concentrations with the presence of AMD. Plasma lutein and zeaxanthin concentrations displayed a strong inverse relationship with the presence of AMD. These findings are consistent with the previously mentioned research performed by Huang et al. (2008). Subjects with high

levels of serum lutein and zeaxanthin displayed a 79% reduced risk of developing AMD compared to individuals with low serum concentrations. Lastly, further support of these findings was demonstrated in The Blue Mountains Eye Study (Tan et al., 2008), a population based cohort examining vision and vision related disease in 3654 individuals over the age of 49. Individuals consuming the most dietary lutein and zeaxanthin were at a significantly lower risk of developing drusen or AMD.

More specifically, a variety of research regarding eggs as a source of lutein and zeaxanthin has emerged in relation to retina function and MPOD. Vishwanathan et al. (2009), investigated the effects of egg consumption on MPOD in 56 subjects who were over the age of 60 and consistently taking cholesterol lowering medications. Following a one-month baseline period, participants were instructed to consume 2 eggs a day for 5 weeks. After a second wash out period of 4 weeks, participants consumed 4 eggs a day for 5 weeks. Serum lutein and zeaxanthin concentrations substantially increased by 16% and 36% following the consumption of 2 eggs and 24% and 82% following the consumption of 4 eggs a day for 5 weeks, respectively. No significant alterations in MPOD were observed however, when subjects were categorized based on baseline MPOD levels, researchers identified that low baseline MPOD was associated with a 6% increase in MPOD following the 2 egg phase and a 48% increase in MPOD after the 4 egg phase. In comparison, Kelly et al. (2014) divided 100 healthy participants into 5 groups who received either one normal egg, a lutein enriched egg-yolk based beverage, a lutein enriched egg, a zeaxanthin enriched egg or placebo daily for 90 days. Serum lutein concentrations significantly increased in individuals consuming the lutein enriched egg-yolk based beverage and the lutein enriched egg, and serum zeaxanthin significantly increased in participants consuming the zeaxanthin enriched egg. No alterations in MPOD levels occurred in any groups. These findings suggest that eggs are capable of increasing serum xanthophylls much more efficiently then supplemental lutein and

zeaxanthin as an enriched egg contains roughly 0.1 mg of lutein and is increasing serum concentrations at a rate that is comparable to 5-10 mg of supplemental lutein.

In further support regarding the enhanced bioavailability of lutein and zeaxanthin when consumed in the form of an egg, Chung et al. (2004) investigated the absorptive capacity of lutein and zeaxanthin from eggs compared to plant and supplemental sources. In ten healthy male subjects from 26 to 75 years of age, 6 mg of lutein was consumed daily in the form of a frittata. The supplement group consumed a plain egg white frittata with a lutein or lutein ester supplement; the spinach treatment group consumed an egg white frittata made with frozen spinach; and the egg group consumed an egg frittata made with high lutein eggs. All treatment meals were consumed once daily for the duration of 2 weeks. On day 10, a statistically significant increase in serum lutein concentrations was indicated in all three treatment groups. Participants consuming lutein enriched eggs demonstrated the greatest increase in serum lutein concentrations (67.3 ± 8.2 nmol) when compared to spinach (31.7 ± 4.6 nmol) and supplemental lutein (21.7 ± 3.5 nmol).

As mentioned previously, researchers believe there is a relationship between MPOD and HDL-C, as lutein and zeaxanthin likely travel from hepatic tissues to the retina as a component of these lipoproteins. This hypothesis is supported by the research of Wenzel et al. (2006) in an analysis of the effect of egg consumption on MPOD in 24 healthy females from 24 to 59 years of age. Participants were randomly assigned to one of three groups for the duration of the 12-week study who consumed either 6 treatment eggs per week containing 331 μg of lutein and zeaxanthin per egg yolk; 6 treatment eggs a week containing 964 μg of lutein and zeaxanthin per egg yolk; or a placebo. Serum lutein concentrations significantly increased in group 1 and serum zeaxanthin concentrations and MPOD significantly improved in group 1 and 2. Additionally, a positive correlation between HDL-C levels, serum lutein and zeaxanthin concentrations and MPOD was indicated. Furthermore, Loane et al. (2010) replicated these findings as

an analysis of cholesterol and serum lutein concentrations in 302 participants found a significant association between serum HDL-C and serum lutein concentrations. No relationship between serum low-density lipoprotein cholesterol (LDL-C) and serum lutein concentrations was indicated. Considering the wide array of research demonstrating the ability of egg consumption to increase serum HDL-C concentration, eggs may be an exceptional source of lutein and zeaxanthin to benefit protection and overall health of the retina function (Pearce et al., 2011; Farrel, 1998; Andersen et al., 2013; Blesso et al., 2013; Green et al., 2006).

Animal trials.

There is limited research performed on animal models regarding the effect of lutein and zeaxanthin on retina function however, the evidence of the following studies supports the protective function of macular pigment as a blue light filter and potent antioxidant. Kowluru et al. (2008) monitored the protective abilities of zeaxanthin against retinal oxidative damage in diabetic rats. Rats were fed diets with 0.02% or 0.01% zeaxanthin for a total of 2 months. Zeaxanthin supplementation displayed a significant protective effect on the inhibition of diabetes induced retinal oxidative damage however, it is unknown whether retina function improved as no test was administered to analyze this parameter.

In xanthophyll deficient rhesus monkeys, Barker et al. (2011) evaluated the effect of lutein and zeaxanthin supplementation for a total of 22 to 28 weeks. Rhesus monkeys were fed 2.2 mg of lutein and zeaxanthin per kg of body weight per day or a standard control diet. The animals who consumed lutein and zeaxanthin were less sensitive to blue light induced retinal damage when compared to the control group. The lutein and zeaxanthin deficient animals displayed minimal levels of macular pigment compared to the intervention group. Similarly, another study performed on 18 xanthophyll deficient

rhesus monkeys assigned one of two diets including a stock diet and a diet supplemented with 2.2 mg of lutein and zeaxanthin per kg of bodyweight (Neuringer et al. 2004). Following diet consumption for a total of 24 to 56 weeks, serum lutein and zeaxanthin increased substantially in the first 4 weeks. From weeks 24 to 32, a continuous increase in MPOD was apparent, however MPOD levels plateaued for the remaining 32 to 56 weeks. These studies performed in xanthophyll deficient rhesus monkeys demonstrated that dietary lutein and zeaxanthin had a substantial effect on increasing MPOD levels, suggesting that diet interventions in even the most severely deficient cases may still allow for improvement in retina function and potentially for the prevention of retinal disease.

A number of studies have utilized the avian species to analyze the accumulation and uptake of carotenoids into the retina with the supplementation of lutein and zeaxanthin. This research is relatable to humans as birds such as quail, have comparable retina concentrations of lutein and zeaxanthin to the human retina. In the house finch, Toomey and McGraw (2010) provided one of three diets including a low (0.078 ± 0.031 μg/g), a medium (10 μg/g) and a high carotenoid diet (30 μg/g), to determine if lutein and zeaxanthin consumption effects retinal carotenoid accumulation. The birds that were fed a medium and high carotenoid diet displayed significantly higher retinal carotenoids by week 4 including galloxanthin, an avian retinal carotenoid found only in the eye. In leghorn chicks, Wang et al. (2007) compared the effect of a diet that included 5.2 and 1.7 mg of lutein and zeaxanthin per kg of body weight per day with a xanthophyll deficient diet. Plasma and tissue concentrations were measured at day 1, day 14 and 28. Interestingly, while plasma and tissue concentrations of lutein and zeaxanthin had depleted by up to 90%, lutein and zeaxanthin in the retina was minimally reduced from 4.4 to 4.0 μg/g and 5.6 to 5.7 μg/g, respectively. The ability of the chick to maintain such levels suggests the potential essentiality of the xanthophylls to the health of retina in this animal model.

In quail, consumption of a carotenoid deficient diet was compared to the consumption of a standard turkey diet by Toyoda et al. (2002). Serum concentrations of lutein and zeaxanthin in quail fed the standard turkey diet were more than double the serum concentrations in carotenoid deficient quail. It was also determined that the dominant retinal xanthophylls were lutein and zeaxanthin and zeaxanthin was absorbed to a much higher extent when compared to lutein levels. In comparison, a similar study administered by Thomson et al. (2002) determined that quail fed zeaxanthin had three to five times more retinal zeaxanthin than birds fed a carotenoid deficient diet. Additionally, mean lutein and zeaxanthin concentrations were higher in retinas of females than in males suggesting sex related differences in absorption and utilization. Results of these studies suggest that zeaxanthin may be highly important in relation to retina function of avian animal models due to their ability to maintain levels of zeaxanthin in the retina after a period of xanthophyll deprivation.

Evidently, it is clear to see that dietary lutein and zeaxanthin display promising effects on the maintenance of retina health and for the prevention of retinal disease in the human and animal model. As displayed by the ability of lutein and zeaxanthin to increase MPOD, these xanthophylls exhibit a protective role against AMD by reducing the absorption of harmful blue light and reducing oxidative stress. However, the ability of lutein and zeaxanthin to reduce symptoms and regress the development of AMD is questionable and yet to be elucidated. Furthermore, both supplemental lutein and zeaxanthin and food sources of the carotenoids have exhibited beneficial effects on MPOD. Specifically, lutein enriched eggs have proven to be an excellent source of lutein and zeaxanthin. Findings of the research previously mentioned propose that the lipid matrix of the egg yolk and the ability of eggs to increase HDL-C levels may provide an optimal environment for the absorption and utilization of lutein and zeaxanthin, especially for use in the retina (Abdel-Aal et al., 2013).

Long chain polyunsaturated fatty acids - docosahexaenoic acid.

LCPUFAs consist of 18 to 20 carbons and greater than 2 double bonds. LCPUFAs are commonly categorized based on the location of the first double bond from the methyl (omega) end of the molecule (Sangiovanni et al., 2009). Omega-3 LCPUFAs such as α-linolenic acid (ALA, C18:3n-3) have a double bond present on the third carbon from the methyl end of the acyl chain. An omega-3 LCPUFA of great interest among lipid researchers is DHA (Δ4,7,10,13,16,19-DHA; $C_{22}H_{32}O_2$). A wide array of research has linked DHA to numerous essential bodily processes including cardiovascular health, brain development, cancer prevention and inflammation reduction (Siriwardhana et al., 2012). DHA is present within all tissues but is highly concentrated in the brain, testes and retina as phospholipids, mainly as a component of phosphatidylethanolamine (PE) and phosphatidylserine (PS) (Sangiovanni et al., 2009). In comparison to other DHA containing tissues, the outer segments of photoreceptor cells are exceptionally concentrated with DHA.

Sources of docosahexaenoic acid.

There are two mechanisms by which the human body can obtain DHA. First, DHA can be synthesized in vivo by the enzymatic elongation and desaturation of the essential fatty acid ALA (18:3n-3) (Abedi and Sahari, 2014). The majority of the conversion of ALA to DHA occurs in the liver hepatocyte. In the endoplasmic reticulum, ALA is converted to tetracosahexaenoic acid (24:6 n-3) and then transported to the peroxisome to undergo β-oxidation to DHA (Sprecher et al., 2009; Domenichiello et al., 2015). A visual representation of this pathway is provided in Figure 1-3. Currently, there is no RDA for DHA due to its ability to be synthesized from ALA. However, adequate intake (AI) values have been established for ALA, which are provided in Table 1-3. As

humans tend to have a limited capacity for the conversion of ALA to DHA, obtaining adequate DHA through ALA conversion is often insufficient (Abedi and Sahari, 2014). Dietary sources of ALA include vegetable oils, nuts, seeds and soy products. Examples of these foods include walnuts (2.30 g/60 mL), tofu (2.02 g/150 g), and flaxseed (2.50 g/15 mL).

Additionally, DHA can also be obtained through the consumption of seafood and fatty fish. Dietary sources that contain high levels of DHA include anchovies (1.54 g/75 g), caviar (4.90 g/75 g), mackerel (0.90-1.49 g/75 g), mussels (0.59 g/75 g), salmon (1.40-1.61 g/75 g), and sardines (0.74-1.05 g/75 g). As fish may be lacking in the diets of many individuals, supplemental fish oils are another option that contain high concentrations of DHA including herring oil (0.48g/5 ml), salmon oil (1.43g/5 ml) and sardine oil (0.95/5 ml). Both DHA and ALA can also be obtained through the consumption of eggs at 0.03 to 0.12 g of ALA and 0.04 g of DHA per 100 g (about 2 large eggs) or in DHA enriched eggs at 0.26 g of DHA and 0.54 g of ALA per 100 g.

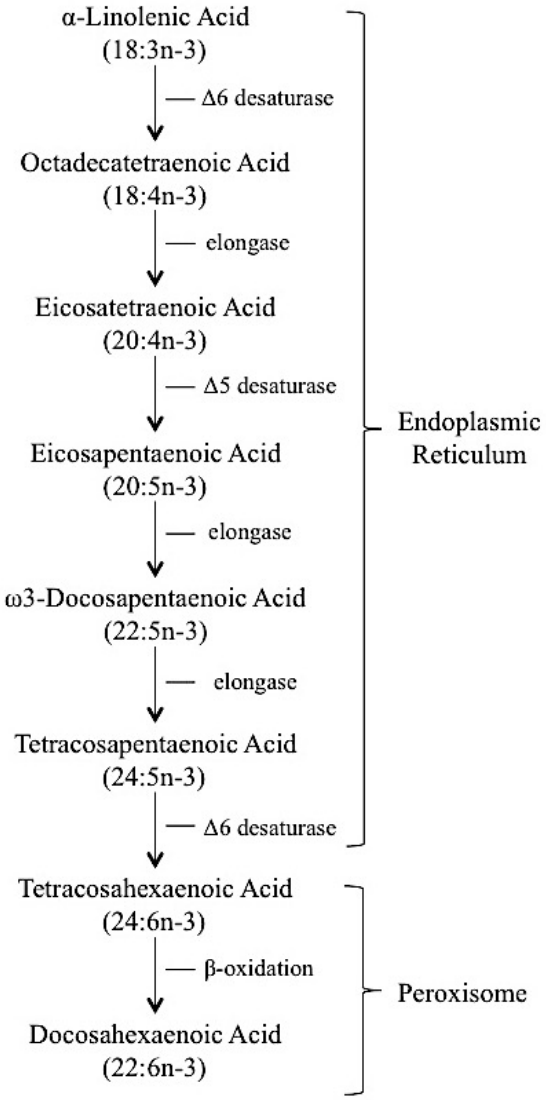

Figure 1-3. Conversion of α-linolenic acid to docosahexaenoic acid.

Table 1-3. Adequate Intake Values for Linoleic Acid and α-Linolenic Acid

Age	Linoleic Acid (n-6) g/day		α-Linolenic Acid (n-3)g/day	
	AI	UL	AI	UL
Infants				
0-6 mo	4.4	ND	0.5	ND
7-12 mo	4.6	ND	0.5	ND
Children				
1-3 y	7	ND	0.7	ND
4-8 y	10	ND	0.9	ND
Males				
9-13 y	12	ND	1.2	ND
14-18 y	16	ND	1.6	ND
19-30 y	17	ND	1.6	ND
31-50 y	17	ND	1.6	ND
51-70 y	14	ND	1.6	ND
>70 y	14	ND	1.6	ND
Females				
9-13 y	10	ND	1.0	ND
14-18 y	11	ND	1.1	ND
19-30 y	12	ND	1.1	ND
31-50 y	12	ND	1.1	ND
51-70 y	11	ND	1.1	ND
>70 y	11	ND	1.1	ND
Pregnancy				
<18 y	13	ND	1.4	ND
19-30 y	13	ND	1.4	ND
31-50 y	13	ND	1.4	ND
Lactation				
<18 y	13	ND	1.3	ND
19-30 y	13	ND	1.3	ND
31-50 y	13	ND	1.3	ND

AI: Adequate Intake, UL: Upper Limit, ND: No Data (Institute of Medicine, 2010)

Absorption and metabolism of docosahexaenoic acid.

DHA obtained through dietary sources is mainly present in foods esterified as triacylglycerols (TAGs) (Sangiovanni and Chew, 2005). Upon entering the small intestine, TAGs are hydrolyzed at the *sn*-1 and *sn*-3 positions by pancreatic lipases to form 2 free fatty acids and a monoacylglycerol. Via membrane translocation, the free

fatty acids and monoacylglycerols enter the enterocyte and are then re-esterified into TAGs and phospholipids. The TAGs and phospholipids are combined with apoproteins to be assembled into chylomicrons and very low-density lipoproteins (VLDL) to prepare for transport via the lymphatic system to the liver (Mahan et al., 2012).

As previously mentioned, DHA can also be synthesized, mainly in the liver, from ALA obtained through the diet. Once ALA enters the hepatocyte it is activated by coenzyme A (CoA) and progresses through enzymatic elongation and saturation reactions to form DHA-CoA. DHA-CoA is further esterified to phospholipids and packaged with lipoproteins to be transported with dietary DHA by chylomicrons and VLDLs. Once DHA is present within the inner segments of photoreceptor cells of the retina, DHA is esterified to phosphatidic acid through the activation of CoA to be utilized by the outer segments of photoreceptor cells (Sangiovanni and Chew, 2005).

Docosahexaenoic acid and long chain polyunsaturated fatty acids in the retina.

In comparison to other tissues within the human body, the retina contains the greatest concentration of LCPUFAs. The five major fatty acids in the human retina consist of DHA, arachidonic acid (20:4n-6), stearic acid (18:0), oleic acid (18:1), and palmitic acid (16:0) (Futterman and Andrews, 1964). The majority of these fatty acids are present in the retina as phospholipids including PE, phosphatidcylcholine (PC), PS and phosphatidylinositol (PI) (Gulcan et al., 1993). Gulcan et al. (1993) reported that the human macula consisted of 38.6% PE, 38.5% PC, 13.5% PS and 10.3% PI. Among one of the most abundant LCPUFAs in the retina is DHA (Sangiovanni and Chew, 2005). DHA constitutes anywhere from 8-20% of total retinal fatty acids and up to 30% in the outer segments of photoreceptor cells (Jeffrey et al., 2001). Considering DHA constitutes only 1-5% of total fatty acids within the human body, the exceptionally high

concentrations within the retina suggests that DHA is potentially of great importance to retina function and development (Querques et al., 2011; Liu, Chang, Lin et al., 2010). Unfortunately, the functional role of DHA and other LCPUFAs in the retina is not well understood although various theories exist. It has been hypothesized that DHA may partake in numerous roles within the retina including in the development of the visual system; in the prevention of cell apoptosis and oxidative damage; in the development and maintenance of photoreceptor membranes and neurotransmitters; in rhodopsin activation; and in rod and cone development (Liu et al., 2010). DHA is a major structural component of membranes within the retina (SanGiovanni, 2005; Querques et al., 2011; Suh et al., 2009). The numerous double bonds in the structure of DHA allows for membrane fluidity by enhancing the area between adjacent lipids, creating an optimal environment that is beneficial for intracellular communication, maintaining the renewal of photoreceptor outer segments and producing a favorable environment for photo-transduction (SanGiovanni, 2005; Querques et al., 2011).

Although varying DHA concentrations within the retina has been linked to numerous retinal diseases including AMD, the precise role of omega-3 fatty acids in the prevention of AMD is yet to be elucidated (Gerstenblith et al., 2013). In 2010, Liu et al. (2010) analyzed fluctuations in the concentrations of LCPUFAs and very long chain polyunsaturated fatty acid (VLCPUFAs) that occurred in human donor eyes with aging. DHA levels in individuals with AMD were significantly lower than individuals with no AMD, suggesting that DHA plays an integral role in the normal functioning of the retina. The following studies provide further evidence that relate DHA to AMD and overall retina health in human and animal models including the consumption of DHA in dietary and supplemental forms.

Human trials.

Supplemental forms of docosahexaenoic acid.

The ability of DHA consumption to prevent retinal degeneration in humans is a topic not well understood. However, a variety of research has analyzed the effects of supplemental DHA in individuals with various forms of AMD. Unfortunately, results have demonstrated DHA to be of little benefit in improving visual function in these individuals. For example, a study performed by Gerstenblith et al. (2013), analyzed the effect of supplemental DHA on electrophysiological retina function in individuals over the age of 50 with early to intermediate dry AMD. Participants of this study consumed 4 g of omega-3 fatty acids including 840 mg of EPA and 2520 mg of DHA for a duration of 6 months. No significant improvement in electrophysiological retina function occurred, nor were there any effect on visual acuity and overall retina function. Additionally as previously discussed regarding the results of AREDS II, there was no benefit to the addition of 10 mg of lutein, 2 mg of zeaxanthin, 350 mg of DHA and 650 mg of EPA to the original AREDS formula of 500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene, 80 mg of zinc oxide and 2 mg of cupric oxide as reported by the Age Related Eye Disease Study Group (2013).

Souied et al. (2013) investigated the ability of DHA supplementation to prevent or delay the progression of exudative AMD. Patients included were over the age of 55 with exudative AMD in one eye and symptoms of early AMD in the other. Participants were randomly assigned to receive a placebo or consume 840 mg of DHA and 270 mg of EPA daily for a total of three years. The ability of DHA to reduce ones risk of developing exudative AMD was measured by time to occurrence of choroidal neovascularization, the incidence of neovascular AMD, changes in visual acuity, and the development and progression of drusen. No significant differences were demonstrated between the DHA supplemented group and the placebo group in time to occurrence and incidence of

neovascular AMD. Moreover, no significant effects on visual acuity, progression and development of drusen or the presence of geographic atrophy occurred. However, high concentrations of red blood cell membrane DHA and EPA was directly correlated with a reduced risk of the development of neovascular AMD.

Over the span of four years, Hoffman et al. (2014) monitored the effects of DHA supplementation on individuals diagnosed with X-linked retinitis pigmentosa. A total of 60 individuals received a daily multivitamin and 33 were randomly assigned to consume 30 mg of DHA per kg body weight per day. To assess whether DHA reduced the progression of the disease in these subjects, participants underwent a full-field ERG at baseline and annually for 4 years. Results demonstrated no significant difference between groups in functional loss of cone cells, rod function, maximum ERG amplitude or cone implicit time. Additionally, further detailed analysis carried out on the same subjects by Hoffman et al. (2015) included a measure of visual acuity, shape discrimination, dark-adapted thresholds, fundus imaging, visual field sensitivity, and optical coherence tomography (OCT). Following DHA supplementation, no significant group differences were elicited in visual acuity, shape discrimination, fundus imaging, and OCT. However, a statistically significant improvement in dark-adapted thresholds and visual field sensitivity occurred over the 4-year intervention. As DHA present within erythrocytes significantly increased from baseline, Hoffman et al. (2015) concluded a direct relationship between erythrocyte DHA concentrations and the improvement in dark-adapted thresholds and visual field sensitivity. As X-linked retinitis pigmentosa is a rare genetic disease often diagnosed in childhood or adolescence, it is unclear as to whether the results of this study are applicable to age related eye disease, or the retina health of the general population. On the contrary, research has demonstrated lower than average plasma and erythrocyte DHA concentrations among individuals with X-linked retinitis pigmentosa and a reduction in retinal DHA among individuals with AMD (Liu et al.,

2010). Although unclear, DHA's role in the retina is likely essential for optimal retina health.

Dietary docosahexaenoic acid.

In a cross-sectional population based study performed by Augood et al. (2008) of individuals over the age of 65, participants completed a food frequency questionnaire and underwent fundus photography to determine if fatty fish consumption was related to a reduced risk of AMD. In comparison to individuals consuming fatty fish less than once per week, individuals consuming fatty fish once per week were 50% less likely to develop neovascular AMD. This risk was further reduced in subjects consuming fatty fish more than once per week. However, there was no association between consuming non-oily fish such as white fish and a reduction in the risk of developing AMD, suggesting that the DHA present in fatty fish may be preventative against retinal degeneration. In comparison, the Blue Mountains Eye Study included 3654 Australian participants and demonstrated a similar relationship between dietary fatty acids and the 10-year incidence of AMD (Tan et al., 2009). One serving of fatty fish per week compared to less than one serving per week displayed a significant reduction in the presence of early AMD. Another important finding of this study determined that one or two servings of nuts per week, compared to less than one serving, was associated with a reduced risk of AMD and the presence of drusen. These findings are interesting as nuts such as walnuts are a rich source of ALA, which can be converted to DHA within the body. Moreover, results of the Women's Health Study (Christen et al., 2011) further supported these findings. Individuals consuming one or more serving of fatty fish per week, compared to less than one serving per month, reduced their risk of AMD by 42% (Christen et al., 2011). Furthermore, participants with the highest intake of DHA were at a 38% lower risk of developing AMD as compared to individuals with the lowest intake.

Animal trials.

Although research is limited, researchers have studied the protective ability of DHA for the prevention of vision related diseases including AMD in various animal models. In comparison to the clinical trials to date, the research performed on animal models continues to support the evidence that DHA contributes to the protection of the retina from retinal degeneration. However, research in animal models substantiates the limited benefit of DHA supplementation to improve symptoms in the previously damaged or disease-ridden retina. Chucair et al. (2007) investigated the combined effects of DHA, lutein, zeaxanthin and beta-carotene on retina function in rats. To measure the effects of these nutrients, the retinal neurons of rats were analyzed to determine any variations in cellular apoptosis, preservation of mitochondrial membrane potential, cytochrome *c* translocation and opsin expression. DHA, lutein and zeaxanthin did not demonstrate a beneficial effect in preventing oxidative damage and apoptosis of photoreceptor cells. Although, these nutrients did aid in the preservation of mitochondrial membrane potential, prevented cytochrome *c* release from mitochondria and enhanced photoreceptor cell differentiation.

Research executed by Dornstauder et al. (2012) monitored the effects of DHA supplementation in transgenic mutant human elongation of very long chain fatty acids 4 (ELOVL4): E4 mice. This unique breed of mouse is unable to convert LCPUFAs to VLCPUFAs in the retina, due to the absence of the ELOVL4 enzyme. In humans, the absence of this enzyme leads to numerous detrimental health effects including juvenile visual deterioration. In this study, the mice were fed a diet consisting of either 0% DHA or 1% DHA manipulated at 1 to 3, 3 to 6, 6 to 12 and 12 to 18 months. To evaluate retina health, electrophysiological retina function was assessed by ERG analysis and A2E accumulation, a phototoxic metabolite of lipofuscin that is present in the RPE in aging individuals with AMD. Although electrophysiological retina function did not improve, no

further degradation occurred following DHA supplementation. Additionally, at 18 months, DHA was also associated with reduced concentrations of accumulated A2E. Considering ELOVL4 transgenic and wildtype mice naturally experience a decline in retina function with age, these findings imply that DHA may potentially prevent further degeneration in retina function in this animal model. Comparatively in the $Ccl2^{-/-}|Cx3cr1^{-/-}$ mouse model, Tuo et al. (2009) evaluated the effects of DHA on retina function. This particular breed of mouse is of interest, as it tends to develop retinal diseases similar to AMD in humans. Mice were assigned to consume either a high omega-3 fatty acid diet or a low omega-3 fatty acid diet. Fundus photography was utilized to assess any lesions that developed over the span of the trial. Results of this study demonstrated a reduction in the formation of lesions, and a greater improvement of existing lesions over time among animals consuming a diet rich in omega-3 fatty acids. Additionally, omega-3 fatty acids were also inversely correlated with the accumulation of lipofuscin in RPE cells and A2E accumulation.

Upon reviewing the evidence concerning DHA consumption and its benefit to retina health among animal, clinical and population based cohort studies, findings suggest that adequate DHA consumption may be required to reduce ones risk of developing retinal degeneration. In individuals who have already experienced retinal degeneration, DHA has demonstrated limited benefit in reducing the damage associated with the disease however, results are inconclusive regarding DHA's ability to slow the progression and worsening of the previously damaged or diseased retina. Although further research is required to make this assumption, it is of interest as to whether DHA metabolism is altered in individuals with AMD, resulting in a deficiency and a reduction in the protective capacity of DHA in photoreceptor cell and RPE maintenance. This potential for DHA deficiency and mechanistic dysfunction could lead to further damage in the retina and loss of vision. Based on the potential protective nature of DHA, future

research should focus on determining AI levels of DHA for retina health in healthy individuals to reduce one's risk of developing retinal degeneration and improve overall eye health.

Enriched Eggs as a Source of Eye Related Nutrients

Eggs are an affordable and nutrient dense source of protein and are often recommended as part of a healthy, balanced diet. Among a number of other vital nutrients including vitamin A, vitamin D, riboflavin, folate, vitamin B12, choline, zinc and iron, one large 50 g egg consists of 72 Kcal and 6.28 g of protein (United States Department of Agriculture. N.D). Eggs provide all essential amino acids required to promote proper muscle growth and development in children and adolescents, and prevent muscle wasting and frailty among older adults (Ruxton et al., 2010).

Over the past 50 years, there has been a paradigm shift regarding how much dietary cholesterol is safe for consumption in relation to cardiovascular health. In the 1960's the American Heart Association recommended that individuals consume no more than 3 eggs per week and less than 300 mg of cholesterol daily. However to date, a variety of research has demonstrated that egg consumption does not adversely affect blood lipid parameters. In a review of epidemiological research and controlled prospective studies concerning the association with egg consumption and the risk of cardiovascular disease, results concluded that egg and dietary cholesterol consumption by the general population is not adversely associated with blood cholesterol (Fuller et al., 2015). Additionally, a meta-analysis completed by Rong et al. (2013) analyzed literature regarding the relationship between egg consumption and coronary heart disease and stroke. The review included 8 studies with 263,938 participants for risk of coronary heart disease and 210,404 participants for risk of stroke. Results suggested no association between egg consumption and coronary heart disease or stroke in the general population,

and that consumption of one egg per day does not increase one's risk for cardiovascular disease. Based on findings such as these, the recent American Heart Association (2015) and the Canadian Heart and Stroke Foundation (2015) dietary guidelines contain no restriction on dietary cholesterol and state that one egg per day is not associated with cardiovascular disease.

Although the cholesterol content of eggs in often viewed in a negative manner, the lipid distribution of the egg yolk provides a highly unique lipid matrix optimal for the absorption and utilization of lipid soluble nutrients. A study by Chung et al. (2004) compared four different sources of lutein, providing 6 mg of lutein per day. These lutein sources included lutein and lutein ester supplements, spinach and lutein enriched eggs. On day two of the trial, all serum lutein concentrations increased significantly from day one. However, serum lutein concentrations in individuals consuming eggs demonstrated the greatest improvement when compared to the other dietary interventions. Despite the fact that eggs contain a considerably lower quantity of lutein and zeaxanthin compared to plant sources such as kale and spinach, the lipid matrix of the egg yolk increases the absorptive power of lutein and zeaxanthin. Additionally as previously discussed, HDL-C is the main transport lipoprotein for lutein and zeaxanthin (Loane et al., 2010). A variety of research has demonstrated that egg consumption increases HDL-C levels, potentially further improving the transport of lutein and zeaxanthin to various bodily tissues such as the retina (Anderson et al., 2013; Blesso et al., 2013; Greene et al., 2006; Schnohr et al., 1994; Vishwanathan et al., 2009; Pearse et al., 2010; Farrel, 1998).

Although there is limited to no research linking egg consumption to retina health, the results obtained by Viswanathan et al. (2009) and Wenzel et al. (2006) demonstrate promising outcomes and raise further questions. As previously mentioned, both studies demonstrated a positive correlation between egg consumption and MPOD. As MPOD is associated with a reduction in oxidative damage to the cellular components within the

retina, it may be that enriched egg consumption could potentially be of benefit to maintaining the health of the retina.

On average, 100 g of egg (about 2 large eggs) provides 503 µg of lutein and zeaxanthin and 58 mg of DHA however, variations exist dependent on the nutrient composition of the chicken feed provided and various environmental variables (American Egg Board, 2013). Eggs can be enriched by supplementing laying hens with fish oils, alfalfa and corn to obtain greater concentrations of DHA and lutein providing 235.8 mg and 0.94 mg per 100g serving, respectively (United States Department of Agriculture. N.D.; American Egg Board, 2013; Burnbrae Farms, N.D.). Based on the potential of the eye related nutrients at question to benefit retina health and the unique lipid matrix of the egg to act as a vehicle for these lipid soluble nutrients, there is a need for research analyzing the synergistic effects of these nutrients within the egg to benefit the health of the retina and reduce ones risk of developing age-related eye disease.

Chapter 2: Research Plan

Rationale

It is estimated that 65% of visually impaired individuals are over the age of 50 (World Health Organization, 2016). Visual impairment is associated with an overall reduction in quality of life due to reduced functional ability, increased risk of falls and an increase in symptoms of depression (CNIB, 2015; Legood et al., 2002). The main vision related diseases associated with aging include glaucoma, cataracts, diabetic retinopathy and age-related macular degeneration (AMD) (National Eye Institute, 2016). According to the World Health Organization (2015), the population of individuals over the age of 60 is likely to double from 12 to 22% from 2015 to 2050. With an influx of older adults, comes an increase in age related eye disease such as AMD. Due to this increase in older adults, it is imperative to find preventative measures such as nutritional therapeutic strategies to prevent and/or treat age related vision deterioration.

The consumption of specific nutrients has shown to be of potential benefit for the health of the retina, such as the antioxidant vitamins and minerals, n-3 long chain polyunsaturated fatty acids (LCPUFA) such as DHA and the carotenoids, β-carotene, lutein and zeaxanthin. Based on the findings of the Age-Related Eye Disease Study (Age-Related Eye Disease Study Research Group, 2001), antioxidant nutrient supplementation including vitamin C (500 mg), vitamin E (400 IU), β-carotene (15 mg) zinc oxide (80 mg) and cupric oxide (2 mg) is currently recommended for individuals with AMD to digress the progression of the disease. In participants at high risk of developing advanced forms of AMD, risk of advancement of the disease and risk of vision loss was decreased by 25% and 19% respectively, over 5 years of supplementation.

Regarding lutein and zeaxanthin consumption, several studies have demonstrated improvements in MPOD, enhancing the ability of the retina to protect itself against harmful blue light penetration and oxidative damage (Dawczynski et al., 2013; Abdel-Aal

et al., 2013; García-Layana et al., 2013). Moreover, cohort studies focusing on DHA in various population groups have found a significant positive correlation between the consumption of oily fish and a reduction in the incidence of AMD (Christen et al., 2011; Tan et al. 2009; Augood et al., 2008). A substantial concentration of DHA is uniquely found within the retina tissues, specifically in outer segments of photoreceptor cells. Despite the fact that the physiological function of DHA within the retina is not fully understood, researchers suggest that DHA may have protective abilities to reduce retina damage with age (Gerstenblith et al., 2013). Unfortunately, limited findings propose a benefit of lutein, zeaxanthin and DHA to improve retina function in individuals previously diagnosed with AMD (Gerstenblith et al., 2013; Age Related Eye Disease Study Group, 2013; Souied et al., 2013).

Although considerable research has focused on the beneficial properties of supplemental nutrients for retina function, research regarding the use of whole foods to act as a vehicle for these nutrients is currently lacking. Eggs are an affordable, well-known source of vital nutrients including a variety of lipid soluble vitamins, minerals and essential amino acids. Regular chicken eggs contain minimal amounts of lutein, zeaxanthin and DHA, however eggs can be enriched to contain greater concentrations of these nutrients through the alteration of the feed of laying hens. As reported by Burnbrae Farms (2016), enriched eggs contain roughly double the amount of lutein and zeaxanthin and quadruple the amount of DHA as compared to normal eggs. Moreover, the unique lipid matrix and cholesterol content of the egg yolk provides a highly bioavailable source of lipid soluble nutrients to enhance the absorption and utilization of lutein, zeaxanthin and DHA. Considering the unique nutrient composition of the lutein and DHA enriched egg, it is of interest to determine whether the consumption of lutein and DHA enriched eggs can improve retina function among Caucasian older adults to enhance retina function and reduce the risk of developing retinal disease such as AMD.

Research Objective

The overall objective of this research is to investigate the effect of lutein and DHA enriched egg consumption by Caucasian older adults on electrophysiological retina function and blood lipid parameters.

The specific research objectives include:

1. To determine if lutein and DHA enriched egg consumption improves self-assessed health and vision status
2. To examine if lutein and DHA enriched egg consumption improves electrophysiological retina function
3. To measure the effects of lutein and DHA enriched egg consumption on lipid profiles including:
    a. Plasma total cholesterol (TC), LDL-C, HDL-C and TAGs
    b. LDL-C and HDL-C particle size
    c. Plasma and erythrocyte fatty acids
4. To determine whether lutein and DHA enriched egg consumption improves plasma lutein concentrations

Research Hypothesis

The working hypothesis of this research is that lutein and DHA enriched egg consumption will improve retina function in Caucasian older adults by increasing egg-nutrient associated blood profiles while not adversely affecting plasma lipid profiles. More specifically, in Caucasian older adults, lutein and DHA enriched egg consumption will:

1. Improve self-assessed health and vision status
2. Improve electrophysiological retina function

3. Not influence TC and TAG concentrations
4. Not influence HDL-C/LDL-C particle size
5. Increase DHA in plasma and erythrocytes
6. Increase lutein in plasma

Chapter 3. Experimental Design and Methods

Experimental Design

Study design.

All study protocols were approved by the University of Manitoba Office of Research Ethics and Compliance Joint-Faculty Research Ethics Board. Recruitment was initiated through the distribution of recruitment fliers on the University of Manitoba campus and surrounding areas in clinics, apartment blocks and fitness facilities. Advertisements were posted on numerous occasions in the Winnipeg Metro, and in the University of Manitoba Centre on Aging weekly newsletter. This intervention trial commenced with a four-week pre-washout period where participants were instructed to avoid the consumption of eggs along with supplements and foods high in vitamin A, beta-carotene, zinc, DHA/fish oil, and carotenoids (lutein and zeaxanthin). A list of foods to be avoided was provided to participants including eggs; oysters; liver (chicken, beef, veal, etc.); sweet potato; pumpkin; carrots; kale; fish and caviar; spinach; supplements that contain zinc, choline, vitamin A, lutein, zeaxanthin and DHA**.** Participants were also asked to limit their intake of foods moderately high in eye related nutrients including pork ham, bacon, beef, lamb, butternut squash, collard greens, turnip greens, baked beans and green peas. Following this four-week period, commencement of a repeated measure design using PRE (week 0)-DURING (week 3)-POST (week 6) assessment was implemented to observe the effects of enriched egg consumption on electrophysiological retina function and blood parameters in Caucasian older adults. The 6-week intervention was chosen based on preexisting literature regarding egg consumption and previous research performed in our lab demonstrating minor changes in retina function in only 4 weeks of dietary intervention (Vishwanathan et al., 2009; Chung et al., Murray, 2014). Preexisting literature indicated that 6 weeks was ample time to allow for a dietary

intervention to alter blood and retina parameters and allow for the safe consumption of 2 eggs per day in relation to adverse changes in lipid profiles (ie. Plasma TC, TAGs etc.) (Andersen et al., 2013; Blesso, et al., 2013; Katz et al., 2005; Mayurasakorn et al., 2008). To analyze the effects of enriched egg consumption on visual function and blood parameters, fasting blood and urine samples were collected at PRE, DURING and POST assessment. Additionally, a questionnaire was completed at the same time points that included a demographic section and a variation of the validated National Eye Institute's (NEI) Visual Functioning Questionnaire (Mangione et al., 2001) to assess any changes in self-reported health and vision status. A 3-day food record was collected at POST assessment to gain an understanding of the overall diet and the average intake of vision related nutrients among older adults. Lastly, a full-field ERG measurement was performed at PRE and POST assessment to objectively measure alterations in retina function following the 6-week dietary intervention.

To ensure all potential participants were deemed appropriate for this study, a screening tool was administered to ensure potential participants fit strict inclusion and exclusion criteria. Inclusion criteria included; i) individuals between 50-80 years of age; ii) of Caucasian ethnicity; iii) with good English written and oral communication skills. Exclusion criteria included; i) individuals with an egg allergy; ii) individuals diagnosed with a chronic disease (ie. diabetes, cardiovascular disease etc.); iii) individuals diagnosed with mental cognitive disorders; iv) individuals diagnosed as hypercholesterolemic; v) individuals taking statins and other lipid lowering medications; vi) individuals diagnosed with eye related disease (ie. glaucoma, AMD, retinitis pigmentosa, etc.).

Participants.

A total of 150 individuals demonstrated interest in the study. Seventy four

potential participants completed the screening questionnaire and 49 were deemed eligible to take part in this study. Reasons for ineligibility included; not within target age range (2.9%); not of Caucasian ethnicity (2.9%); current smoker (5.9%); history of cardiovascular disease (8.8%); type 1 or 2 diabetes mellitus (2.9%); history of vision related disease (20.6%); elevated blood cholesterol levels (23.5%); and individuals currently taking statin or lipid lowering medications (26.5%). Of the eligible 49, a total of 19 participants decided to not partake for a variety of personal reasons. Finally, a total of 30 individuals were deemed eligible and willing to commit to completing the 6-week trial.

All 30 individuals completed the trial, however one individual missed their appointment for post assessment blood and urine collection due to travel and 2 participants did not complete their 3-day food records due to unknown reasons. As one participant began taking lipid-lowering medications during the trial, this data was excluded from the final analysis.

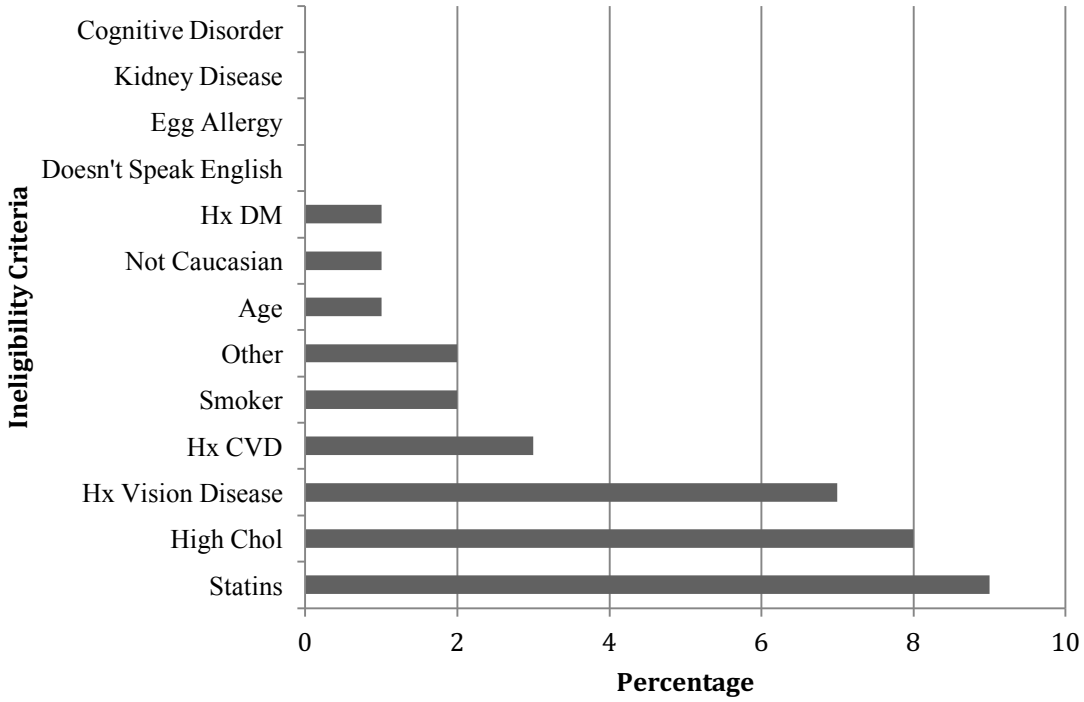

Figure 3-1: Ineligibility criteria of screened individuals. Figure expressed as a percentage of n=25 ineligible individuals.

Lutein and docosahexaenoic acid enriched eggs.

Medium lutein and DHA enriched eggs were generously donated by Burnbrae Farms (Winnipeg, Manitoba, CA). Eggs were collected from Burnbrae Farms Supply Center (500 Pandora Avenue West, Winnipeg Manitoba, Canada, R2C 1N1) 3 days prior to distribution to participants at PRE and DURING assessment dates. Fresh eggs were provided to participants every 3 weeks and stored at 4ºC to maintain nutrient composition and prevent spoilage.

To enhance the concentrations of lutein and DHA within the yolk of the egg, the feed is altered to supply greater concentrations of these nutrients to laying hens. To increase the concentration of omega-3 fatty acids in the egg, flaxseed is incorporated into the feed along with fish oils to improve DHA content (Burnbrae Farms, 2016). Alfalfa and corn are introduced to enhance levels of lutein and zeaxanthin (Burnbrae Farms, 2016). The nutrient composition in a 92 g serving of lutein and DHA enriched eggs (2

medium eggs) according to Burnbrae Farms (2016) is provided in Table 3-1 and 3-2.

To assess the fatty acid distribution and lutein concentration of the treatment eggs, a total of 8 lutein and DHA enriched eggs and 4 regular eggs were randomly selected during the 6-week trial and stored at -80ºC until further analysis. The eggs underwent lipid and carotenoid extraction followed by gas chromatography and high-performance liquid chromatography (HPLC). The results of the fatty acid and lutein analysis is displayed in Table 3-3 and Figure 3-2, respectively. For further information regarding these analyses, please see the Experimental Methods section for details.

Table 3-1: Macronutrient composition of 92g of lutein and DHA enriched eggs

Nutrient	**Content**
Kilocalories	121.51 Kcal
Fat	8.68 g
Saturated Fat	2.60 g
Trans Fat	0 g
Polyunsaturated Fat	1.74 g
n-6	1.39 g
n-3	0.69 g
DHA	217.0 mg
Monounsaturates	3.47 g
Cholesterol	338.49 mg
Sodium	112.83 mg
Carbohydrates	1.74 g
Fibre	0 g
Sugars	0 g
Protein	10.42 g

Burnbrae Farms (2016)

Table 3-2: Micronutrient composition of 92g of lutein and DHA enriched egg

Nutrient	Nutrient Content	RDA (Persons 2 years of age or older)	Percent RDA
Lutein	0.87 mg	NA	NA
Vitamin A	173.58 RE	1000 RE	17.4 %
Vitamin C	0 mg	60 mg	0.0 %
Calcium	38.19 mg	1100 mg	3.5 %
Iron	1.46 mg	14 mg	10.4 %
Vitamin D	2.17 µg	5 µg	43.4 %
Vitamin E	8.68 mg	10 mg	86.8 %
Thiamine	0.05 mg	1.3 mg	3.5 %
Riboflavin	0.42 mg	1.6 mg	26.0 %
Niacin	3.19 NE	23 NE	13.9 %
Vitamin B6	0.06 mg	1.8 mg	3.5 %
Folate	114.57 µg	220 µg	52.1 %
Vitamin B12	2.78 µg	2 µg	138.9 %
Pantothenate	2.43 mg	7 mg	34.7 %
Phosphorus	114.57 mg	1100 mg	10.4 %
Magnesium	8.68 mg	250 mg	3.5 %
Zinc	1.25 mg	9 mg	13.9 %
Iodide	41.66 µg	160 µg	26.0 %
Selenium	30.38 µg	50 µg	60.8 %

RDA: Recommended Dietary Allowance (Burnbrae Farms, 2016; National Institute of Health, 2010)

Table 3-3. Fatty acid percent distribution of lutein and DHA enriched eggs versus regular eggs

Fatty Acid (% w/w)	Lutein and DHA Eggs (n=8)	Regular eggs (n=4)
SFA	37.05 (±0.3)	37.34 (±0.2)
MUFA	41.94 (±0.3)	44.83 (±0.5)
PUFA	21.01 (±0.4)	17.84 (±0.4)
n-3	7.54 (±0.2)	1.21 (±0.1)
ALA	5.27 (±0.1)	0.45 (±0.0)
DHA	1.96 (±0.1)	0.67 (±0.1)
n-6	0.23 (±0.0)	0.61 (±0.0)
LA	0.09 (±0.0)	0.10 (±0.0)

Mean (±SEM)

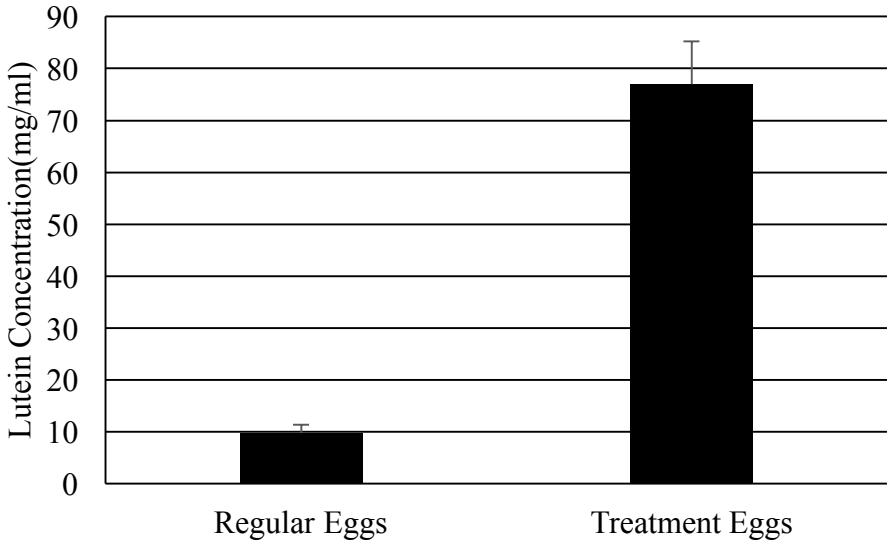

Figure 3-2: Comparison of the lutein concentration of regular eggs versus enriched eggs. Data expressed as mean ± SEM (n=3 regular eggs, n=7 treatment eggs).

Experimental Methods

Questionnaire.

A questionnaire was administered to assess variations in self-perceived health and vision status at PRE, DURING and POST assessment. The questionnaire included two sections including one related to demographics and another regarding self-perceived visual functioning.

Demographics.

The demographics portion of the questionnaire included basic questions regarding gender, age and self- assessed health and vision status. Self- reported height and weight were used to calculate BMI, which was categorized as obese (≥30.0), overweight (25.0-29.9), normal weight (18.5-24.9), and underweight (<18.5). Participants were asked to rate their overall health and eyesight as either excellent, very good, good, fair or poor. Pain and discomfort of the eyes was categorized as none, mild, moderate, severe or very severe and participants were asked to list any symptoms related to this irritation (ie. burning, itching, aching). Lastly, a question regarding time spent worrying about one's

eyesight was included and reported as none of time, a little of the time, some of the time, most of the time and all of the time.

National Eye Institute's visual functioning questionnaire.

A modified version of the National Eye Institute Visual Functioning Questionnaire was utilized to measure participants' self- assessed perception of their eye related symptoms (Mangione et al., 2001). This questionnaire was completed at PRE, DURING and POST assessment to determine if variations in self perceived visual functioning occurred throughout the span of the 6-week trial and enriched egg intervention.

Three-day food record.

To obtain information regarding the average dietary intake of Caucasian older adults, participants were asked to complete a three-day food record between PRE and POST assessment for 3 consecutive days including 2 days during the week (Monday to Friday), and one day on the weekend (Saturday or Sunday). Verbal and written instructions were provided at DURING assessment regarding serving sizes and level of specificity required for the proper completion of the record. Records were collected at POST assessment and analyzed using Dietitians of Canada's eaTracker.ca (Dietitians of Canada, 2016), a nutrient database utilizing data from the Canadian Nutrient File (Health Canada, 2012).

Blood parameters.

Peripheral fasted blood samples were collected via venipuncture of the antecubital fossa from fasting participants at PRE, DURING, and POST assessment in K2EDTA. Blood samples were centrifuged at 3200 RPM for 10 minutes (Sorvall Legend RT,

Thermo Scientific, Waltham, MA) at 4ºC and plasma, leukocytes and erythrocytes were distributed in 1.5 and 2.0 ml Eppendorf tubes. A total of 10 ml of urine was collected in a 15 ml centrifuge tube. Both the urine and blood samples were stored at -80ºC until further analyses.

Plasma lipid profiles and high sensitivity c-reactive protein.

Plasma lipid profiles including TC, HDL-C, LDL-C, TAGs and high sensitivity CRP Samples were analyzed using a Cobas C 111 Analyzer (Roche Diagnostics, Risch-Rotkreuz, CH) at the Canadian Centre for Agri-Food Research in Health and Medicine at St. Boniface Hospital in Winnipeg, Manitoba.

Full-field electroretinogram.

The full field electroretinogram (ERG) UTAS-4000 data system (LKC Technologies, Inc., Gaithersburg, MD) was used to compare any variations in retina function from PRE (baseline) to POST assessment (following 6 weeks of intervention). Prior to the test, the participant was placed in a comfortable sitting position and standard dilating drops (1% Tropicamide) were administered to both eyes to ensure maximum retina sensitivity. For the next twenty minutes, the participant and technician sat in a dark room to allow for dark adaptation prior to commencement of the test. Following dark adaptation, the technician used dim red light to prepare participants for administration of the ERG. To ensure a clean surface to prevent movement of electrodes and maximum conductance, a 70% isopropyl alcohol swab was used to clean the forehead, right earlobe, and the inner and outer regions of the eye. A DTL plus electrode was placed gently on the surface of the cornea under the bottom of both eyelids, and held in place by adhesive pads on the inner and outer regions of the eye. An electrocardiograph (ECG) electrode was placed on the center of the forehead and a ground electrode on the right earlobe. The

participant then underwent three bilateral ERG tests to measure inner and outer retina function including mixed scotopic response, photopic response and photopic flicker response, all of which were previously established by our laboratory and discussed in Suh et al. (2009) and Murray et al. (2014).

Dark and light adapted electroretinogram.

The first test administered is the mixed scotopic response to obtain dark-adapted, rod driven responses. A series of flash stimuli was elicited by a Ganzfield Dome, which gave off a white flash (6500 K, xenon bulb) 10 μsec in duration. This flash occurred three times consecutively to obtain an average response and ensure accurate and consistent results. This series of flash stimuli repeated itself for a total of 14 steps with a slight increase in intensity occurring at each step. Intensity of illumination ranged from -3.7 to 1.89 log sc cd-sec/m^2 (logarithm of scotopic candela sec/meter square). As the intensity of the illumination stimulus gradually increases, the amount of time between stimuli increases ranging from 10 seconds to 2 minutes at its longest point. These inter-stimulus intervals allow for maximal rod recovery prior to each stimulus to obtain accurate readings of rod responses at each step.

Immediately following the dark adapted ERG, an exposure to a background light of 30 sc cd-sec/m^2 was applied to prepare for the administration of the photopic response test, measuring the cone-driven intensity responses. Similar to the mixed scotopic response test, the Ganzfield dome elicits a white flash (6500 K, xenon bulb) 10 μsec in duration to obtain an average of five responses. This series of flash stimuli repeated itself for a total of 9 steps, with a slight increase in intensity occurring at each step ranging from -1.22 to 2.39 log sc cd-sec/m^2 (logarithm of scotopic candela sec/meter square).

The result of the rod and cone driven intensity responses obtained at each step is a biphasic waveform that can be dissected into three main constituents including the a-

wave, b-wave and oscillatory potential (OP). The constituents of the waveform are analyzed by two principal measurements including amplitude and implicit time. The a-wave is a representation of the physiological health of the photoreceptor cells and is a measure of the amplitude and the time interval from the onset of the stimulus at baseline (0 sec) to the lowest point of the negative trough of the waveform. The b-wave represents the health of the inner retinal cells such as the müller and ON bipolar cells. B-wave is a measure of the amplitude and the time interval from onset of the stimulus from the lowest point of the negative trough of the a-wave to the most positive point of the waveform. Lastly, the OP is representative of amacrine cell function of the inner retina, measured on the ascending limb of the b-wave. The summed amplitude of OPs are obtained through calculations completed by commercial software (EMWin from LKC Technologies Inc., Gaithersburg, MD).

Analysis of plasma, erythrocyte and enriched egg fatty acids.

Sample preparation for plasma and erythrocyte fatty acid analysis.

Analysis of plasma fatty acids and erythrocytes was performed in a manner similar to that of the simplified method described by Kang and Wang (2005). A total of 50 µl of plasma and roughly 25 mg of erythrocytes were distributed into 8 ml glass methylation tubes along with 5 µl of an internal standard of C21:0. To cleave fatty acids present within the plasma, saponification was carried out by adding 0.5 ml of a 0.5 M aqueous solution of methanolic potassium hydroxide. The saponified sample then underwent a methylation procedure. At this time, 1 ml of distilled hexane and 1 ml of 14% boron trifluoride in methanol was added and the samples were heated in a sandbath for one hour at 110°C. Following a cooling period of 10 minutes, 1 ml of double distilled water was added and the samples were vortexed prior to 5 minutes of centrifugation at 3200 RPM (Sorvall Legend RT, Thermo Scientific, Waltham, MA). The upper hexane

layer containing fatty acid methyl esters was removed, dried down under a nitrogen gas stream, and then reconstituted with 30 µl and 50 µl of distilled hexane for erythrocyte and plasma samples, respectively. Samples were stored at -80°C prior to further analyses by gas chromatography.

Sample preparation of enriched egg fatty acid analysis.

Whole eggs were frozen at -80°C and freeze-dried (Virtis 25XL Freezemobile, SP Scientific, Gardiner NY, US) prior to extraction. A total of 0.5 g of freeze dried egg yolk was extracted in duplicate using 20 ml of chloroform:methanol (2:1, vol/vol). Following a 30-second vortex and a 20-minute sonication (Brandon 3510, Danbury, CT), 5 ml of sample was removed for fatty acid analysis. Saponification was carried out through the addition of 1.5 ml of a 0.5 M aqueous solution of methanolic potassium hydroxide. The remaining methylation procedure was identical to the method carried out for plasma and erythrocyte fatty acid analysis with a final volume of 1.5 ml of distilled hexane.

Gas chromatography run conditions.

Separation of plasma and erythrocyte fatty acids was carried out on a SGE BPX-70 column (10 m X 0.10 mm diameter and 0.2 m film thickness), using a Bruker 450 gas chromatography instrument coupled to a flame ionization detector (Bruker, Corporation, CompassXport 3.0, Massachusetts, US). The temperature program was 120°C for 0 min, then was raised to 180°C at 10°C/min, held for 0.2 min, raised to 250°C at 15°C/min, held for 0 min, and finally raised to 270°C at 6°C/min and held for 2 min. Total run time was 16.2 min, and samples were run for 0.3 min with a 1:1 split ratio, then 100:1 split ratio for the remaining run time with a 0.4 ml/min column flow. Hydrogen was used as the carrier gas for the method. The injector/detector temperatures were 280°C and 290°C, respectively and hydrogen was used as the carrier gas. Lastly, the injector/detector

temperatures were 280°C and 290°C, respectively.

Separation of enriched egg fatty acids was carried out on an Agilent DB-225MS column (30 m X 0.25 mm diameter and 0.25 mm film thickness) using a Bruker 450 gas chromatography instrument (Bruker, Corporation, Compass Xport 3.0, Massachusetts, US) coupled to a flame ionization detector. The initial temperature was 110°C for 1 min, then was raised to 205°C at 30°C/min, held for 1 min, raised to 220°C at 10°C/min, held for 10 min, finally was raised to 240°C at 20°C/min and held for 25 min. Total run time was 42.67 min, and samples were run at a 40:1 split ratio, column flow of 0.3 ml/min, and 1 ul of sample was injected. Hydrogen was used as the carrier gas for the method. The injector/detector temperatures were 270°C and 290°C, respectively.

Analysis of lipoprotein subfraction distribution.

The analysis of plasma HDL and LDL subfraction distribution was carried out by polyacrylamide gel electrophoresis using the Lipoprint Lipoprotein Subfractions Testing System (Quantimetrix, California, US). All protocols were followed as provided by the system instructions and duplication of samples was implemented.

Plasma and enriched egg carotenoid composition measurement.

Chemicals, materials and preparation of standard stock solutions.

All solutions utilized in this experiment were ≥95.0% HPLC grade and were purchased from Sigma-Aldrich (Oakville, Ontario, CA) including LC-MC grade water, methanol, and methyl-t-butyl ether. Additionally, all standard stock solutions required for analysis of carotenoids in plasma and egg samples were purchased from Sigma-Aldrich (Oakville, Ontario, CA) including lutein, retinyl acetate and trans-β-Apo-8'-carotenal. To ensure limited degradation of standard stock solutions, all solutions were prepared with HPLC grade acetone on the same day of extraction and stored at -80°C.

Sample preparation.

Egg samples.

To ensure limited degradation of carotenoids, all sample preparations occurred under dim red light. To determine the lutein content of enriched and non-enriched egg samples, the extraction method was similar to that of the method previously developed in our laboratory to determine the carotenoid content of carrot powder (McClinton, 2012; Murray, 2014). A total of 0.5 g of lyophilized egg yolk was extracted in duplicate using 20 ml of chloroform:methanol (2:1, vol/vol). Following a 30 sec vortex and a 20 min sonication (Brandon 3510, Danbury, CT), 12 ml of hexane, 1 ml of double distilled water and an internal standard of 0.5 µg trans-β-Apo-8'-carotenal were added. The internal standard was included to assess carotenoid recovery and any losses that may have occurred throughout the extraction process. Samples were then vortexed for 30 sec and centrifuged at 1200 x g for 15 min (Sorvall Legend RT, Thermo Scientific, Waltham, MA). The upper phase was then collected and the extraction procedure was repeated again once more. Once the upper phase of the second extraction was collected, the pooled organic phases were evaporated under a nitrogen gas stream. Once completely dry, the samples were saponified using 2 ml of a 10% aqueous solution of potassium hydroxide in ethyl alcohol (w/v) and heated at 60°C for 60 min. Following heating, the samples were placed in an ice bath and 2 ml of double distilled water and 5 ml of hexane were added. The upper phase was collected and the process was repeated two more times. The upper organic phases were dried down under a nitrogen gas stream. Lastly, the residue was redissolved in 1.5 ml of acetone. A total of 30 µl was injected into the C30 Carotenoid (3µm; 4.6 cm x 250 mm, Waters Ltd, Lachine, QC) reverse-phase column.

Plasma samples.

The extraction of lutein from plasma was based on the method previously described by McClinton (2012) and Murray (2014). In short, 500 µl of plasma was extracted in 2 ml of chloroform:methanol (2:1, vol/vol), vortexed for 30 sec and sonicated for 20 min (Brandon 3510, Danbury, CT). An additional 3 ml of hexane, 0.01% butylated hydroxytoulene (BHT) in ethanol and an internal standard of 0.5 µg of retinyl acetate were added to each sample. Following a 30 second vortex and 15 minutes of centrifugation at 2400 x g (Sorvall Legend RT, Thermo Scientific, Waltham, MA), the upper phase was collected and the extraction was repeated again once more. The pooled upper phases were dried under a stream of nitrogen gas and the residue was re-dissolved in 100 µl of acetone.

Chromatographic conditions.

The chromatographic run conditions were based on methods previously completed in our lab by Murray (2014) and McClinton (2012). The chromatography was performed on a Agilent Technologies 1100 Series system equipped with Agilent Technologies ChemStation software (Santa Clara, CA). The column oven and autosampler temperatures were maintained at 25.5°C and 8°C, respectively. The autosampler door was covered to minimize light exposure. Carotenoids were separated using a C30 Carotenoid (3µm; 4.6 cm x 250 mm, Waters Ltd, Lachine, QC) reverse-phase column, with a gradient mobile phase at 0.75 mL/min flow rate consisting of methanol:methyl t-butyl ether:HPLC grade water (81:15:4, by vol, solvent A) and methanol:methyl t-butyl ether:HPLC grade water (7:90:3, by vol, solvent B).

Carotenoid run conditions.

Run conditions for carotenoids were as follows: Initial, 100% A, held until 35

min; gradient to 39% A, 61% B from 35 min to 40 min; re-equilibration to initial composition of 100% A from 40 min, with a hold at 100% A for 5 min. The total run time was 45 min. Detection wavelength was set at 326-460 nm for all standards and carotenoid compounds. Identification of carotenoids was determined by comparing retention times and visible spectra with corresponding standards. To quantify lutein, a calibration curve with known amounts of the standards was used. The reference peak area was used to plot the quantity of each standard compound. Using the linear regression equation established from the calibration curve, lutein concentrations were established in egg and plasma samples. The linear regression value (R^2) for lutein was >0.99. The calibration curve for lutein is displayed in Figure A-1. To ensure intra- and inter-day reproducibility, duplicates of samples were run at various time points throughout the day via HPLC.

Statistical Analysis

The effect of enriched egg consumption on visual function and blood parameters was analyzed by one-way analysis of variance (ANOVA) with repeated measures and Duncan's Multiple Range Test and by paired t-test. To assess the relationship between plasma lipids and lutein with ERG results a Pearson correlation coefficient was determined and classified as moderate (0.40-0.59), strong (0.60-0.79) or very strong (0.80-1.0). All statistical analysis was carried out using SAS 9.2 (SAS Institute Inc., Toronto, ON) and a p-value of ≤ 0.05 was deemed statistically significant.

Chapter 4. Results

Basic Demographic Data

Demographic and self- perceived health and vision status.

The average age of participants included in this study was 64.0 (±0.72) and ranged from 53 to 80 years of age. Of 30 participants, 36.7% (n=11) were male and 63.3% were female (n=19). Based on self-reported height and weight, no significant changes in BMI occurred from PRE to POST assessment (PRE, 28.1(±0.94), POST 28.0(±0.82)), implying the dietary intervention had no effect on body weight. Based on BMI (based on self-reported weight and height) at PRE assessment, 0.0% of individuals were underweight, 30.0% were normal weight, 30.0% were overweight and 40.0% were obese. At POST assessment, 0.0% were underweight, 26.7% were normal weight, 40.0% were overweight and 33.3% were obese. When participants were asked to rate their overall health status an improvement occurred from PRE to POST assessment, however this did not reach statistical significance. At PRE assessment 30.0% rated their overall health as excellent, 40.0% very good, 23.3% good, 6.7% fair, and 0% poor. At POST assessment 26.7% answered excellent, 63.3% very good, 3.33% good, 6.67% fair, and 0% poor.

No statically significant alterations occurred in self-perceived health and vision status from PRE to POST assessment. At PRE and POST assessment, 13.3% of participants rated their overall vision status as excellent, 56.7% as very good, 30.0% as good and 0.00% as fair and poor. Further questioning regarding eye related pain and discomfort determined that no pain and discomfort was experienced by 70% of individuals, mild discomfort in 16.7%, moderate in 6.67%, severe in 1% and very severe in 0% of participants at PRE assessment. This distribution changed slightly over the duration of the 6-week trial and is displayed in Table 4-1. Lastly, the time participants

spent worrying about their eyesight was categorized as none of the time, a little of the time, some of the time, most of the time and all of the time. Interestingly, this distribution altered slightly in a comparison of PRE and POST assessment with 40.0%, 43.3%, 10.0%, 3.33% and 3.33% stating none, a little, some, most, and all of the time at PRE assessment and 53.3%, 30.0%, 16.7%, 0% and 0% at POST assessment, respectively.

Table 4-1: Participant demographics and self-perceived health and vision status

Characteristic	Participants (n=30)			Significant Effects ($p<$)
Age (yrs)	64.0(±3.4)			
Gender (%)				
• Male	11 (36.7)			
• Female	19 (63.3)			
Height (m)	1.66(±0.1)			
	Pre	During	Post	
Weight (kg)	78.6(±3.4)	76.3(±2.5)	77.7(±2.9)	NS
BMI (kg/m^2)	28.1(±0.9)	27.8(±0.8)	28.0(±0.8)	NS
• Underweight (<18.5)	0.0	0.0	0.0	
• Normal (18.5-24.9)	30.0	31.0	26.7	
• Overweight (24.9-29.9)	30.0	31.0	40.0	
• Obese (>30)	40.0	36.7	33.3	
Health Status (%)				NS
• Excellent	30.0	26.7	26.7	
• Very Good	40.0	43.3	63.3	
• Good	23.3	26.7	3.33	
• Fair	6.67	3.33	6.67	
• Poor	0.0	0.0	0.0	
Vision Status (%)				NS
• Excellent	13.3	20.0	13.3	
• Very Good	56.7	46.7	56.7	
• Good	30.0	30.0	30.0	
• Fair	0.0	3.3	0.0	
• Poor	0.0	0.0	0.0	
Eye Related Pain and Discomfort (%)				NS
• None	70.0	66.7	63.3	
• Mild	16.7	23.3	20.0	
• Moderate	6.67	3.3	13.3	
• Severe	3.3	0.0	3.3	
• Very Severe	0.0	0.0	0.0	
Time Worrying about Eyesight (%)				NS
• None of the Time	40.0	43.3	53.3	
• A Little of the Time	43.3	33.3	30.0	
• Some of the Time	10.0	16.7	16.7	
• Most of the Time	3.33	6.7	0.0	
• All of the Time	3.33	0.0	0.0	

Age, height, weight and BMI expressed as mean ± SEM. Gender, BMI classes, health status, vision status, eye related pain, and time worrying expressed as percent of participants (%). NS, not significant.

The Effects of Enriched Egg Consumption on Self-Perceived Visual Function

Self-perceived visual function was assessed utilizing questions from the National Eye Institute's Visual Functioning Questionnaire (Mangione et al., 2001) to determine if enriched egg consumption improved self-perceived visual function.

Self-perceived visual function and daily activities.

When participants were asked how difficult performing daily activities were due to their vision at PRE, DURING and POST assessment, enriched egg consumption had no significant effect on the level of difficulty completing everyday tasks such as reading ordinary print in newspapers (Table 4-2).

Table 4-2. Self-perceived visual function during daily activities at PRE, DURING and POST assessment

Daily Activity	Assessment (%)			Significant Effects ($p<$)
	Pre	During	Post	
1. Reading ordinary print in newspapers				NS
• N/A	0.0	6.7	0.0.	
• No Difficulty	83.3	63.3	73.3	
• A Little difficulty	10.0	26.7	20.0	
• Moderate difficulty	6.7	3.3	6.7	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
2. Reading small print (ie. Medicine bottle, telephone book)				NS
• N/A	0.0	6.7	0.0	
• No Difficulty	50.0	3.3	36.7	
• A Little difficulty	26.7	40.0	36.7	
• Moderate difficulty	10.0	33.3	16.7	
• Very difficult	13.3	13.3	10.0	
• Unable to do	0.0	3.3	0.0	
3. Reading large print (ie. Numbers on a telephone)				NS
• N/A	0.0	3.3	0.0	

• No Difficulty	100.0	96.7	100.0	
• A Little difficulty	0.0	0.0	0.0	
• Moderate difficulty	0.0	0.0	0.0	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
4. Doing work or hobbies that require you to see up close (ie. Sewing, using tools)				NS
• N/A	0.0	3.3	0.0	
• No Difficulty	63.3	63.3	56.7	
• A Little difficulty	23.3	16.7	33.3	
• Moderate difficulty	13.3	13.3	6.7	
• Very difficult	0.0	3.3	0.0	
• Unable to do	0.0	0.0	0.0	
5. Finding something on a crowded shelf				NS
• N/A	0.0	6.7	0.0	
• No Difficulty	83.3	86.7	90.0	
• A Little difficulty	13.3	6.7	6.7	
• Moderate difficulty	3.3	0.0	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
6. Cooking				NS
• N/A	0.0	3.3	0.0	
• No Difficulty	93.3	96.7	86.7	
• A Little difficulty	3.3	0.0	6.7	
• Moderate difficulty	0.0	0.0	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
7. Watching television				NS
• N/A	3.3	10.0	3.3	
• No Difficulty	80.0	80.0	83.3	
• A Little difficulty	10.0	6.7	10.0	
• Moderate difficulty	3.3	0.0	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	3.3	3.3	3.3	
8. Reading street signs or names of stores				NS
• N/A	0.0	3.3	0.0	
• No Difficulty	70.0	80.0	76.7	
• A Little difficulty	26.7	10.0	20	
• Moderate difficulty	3.3	6.7	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
9. Going down stairs or curbs in dim light				NS
• N/A	0.0	6.7	0.0	
• No Difficulty	73.3	66.7	66.7	

• A Little difficulty	20.0	23.3	26.7	
• Moderate difficulty	6.7	3.3	6.7	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
10. Noticing objects off the side while walking along				NS
• N/A	0.0	6.7	0.0	
• No Difficulty	86.7	76.7	90.0	
• A Little difficulty	10.0	16.7	6.7	
• Moderate difficulty	3.3	0.0	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
11. Seeing how people react to things you say				NS
• N/A	0.0	10.0	0.0	
• No Difficulty	93.3	86.7	96.7	
• A Little difficulty	6.7	0.0	0.0	
• Moderate difficulty	0.0	3.3	3.3	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
12. Picking out and matching your own clothes				NS
• N/A	0.0	10.0	0.0	
• No Difficulty	96.7	86.7	93.3	
• A Little difficulty	3.3	0.0	3.3	
• Moderate difficulty	0.0	0.0	0.0	
• Very difficult	0.0	0.0	0.0	
• Unable to do	0.0	0.0	0.0	
13. Visiting with people in their homes at parties or in restaurants				NS
• N/A	0.0	6.7	0.0	
• No Difficulty	93.3	90.0	90.0	
• A Little difficulty	6.7	0.0	3.3	
• Moderate difficulty	0.0	0.0	3.3	
• Very difficult	0.0	3.3	0.0	
• Unable to do	0.0	0.0	0.0	
14. Going out to see movies, plays or sports events				NS
• N/A	3.3	10.0	3.3	
• No Difficulty	86.7	83.3	90.0	
• A Little difficulty	3.3	3.3	0.0	
• Moderate difficulty	3.3	3.3	3.3	
• Very difficult	3.3	0.0	3.3	
• Unable to do	0.0	0.0	0.0	

15. Driving during the daytime				NS
• N/A	20.0	20.0	20.0	
• No Difficulty	76.7	73.3	73.3	
• A Little difficulty	3.3	0.0	3.3	
• Moderate difficulty	0.0	3.3	3.3	
• Very difficult	0.0	3.3	0.0	
• Unable to do	0.0	0.0	0.0	
16. Driving at night				NS
• N/A	23.3	20.0	20.0	
• No Difficulty	40.0	43.3	40.0	
• A Little difficulty	26.7	23.3	33.3	
• Moderate difficulty	6.7	6.7	3.3	
• Very difficult	3.3	3.3	3.3	
• Unable to do	0.0	3.3	0.0	
17. Driving in difficult conditions (ie. Bad weather, rush hour, on the highway)				NS
• N/A	23.3	20.0	20.0	
• No Difficulty	60.0	50.0	53.3	
• A Little difficulty	10.0	13.3	20.0	
• Moderate difficulty	6.7	13.3	3.3	
• Very difficult	0.0	0.0	3.3	
• Unable to do	0.0	3.3	0.0	

Data expressed as percent of participants (%). NS, not significant.

Self-perceived visual function and driving ability.

Results of the questionnaire demonstrated that 80% of participants operated a vehicle on a daily basis. Additionally, 10.0% of participants gave up driving although; this was not related to eyesight alone. No significant variations were present regarding driving ability related to vision from PRE to POST assessment (Table 4-3).

Table 4-3. Self-perceived visual function and driving ability at PRE, DURING and POST assessment.

Driving ability	Assessment (%)			Significant Effects ($p<$)
	Pre	During	Post	
18. Are you currently driving, at least once in a while?				NS
• Yes	80.0	80.0	80.0	
• No	20.0	20.0	20.0	
19. Have you never driven a car or have you given up driving?				NS
• Never Drove	6.7	6.7	6.7	
• Gave up	10.0	13.3	10.0	
20. If you GAVE UP driving: What was the main reason?				NS
• Mainly eyesight	0.0	0.0	0.0	
• Mainly other reasons	6.7	13.3	10.0	
• Both eyesight and other reasons	3.3	0.0	0.0	

Data expressed as percent of participants (%). NS, not significant.

Self-perceived visual function and barriers to daily living.

Following 6 weeks of enriched egg consumption, no significant alterations occurred regarding vision related barriers to daily living. The majority of participants (80-90%) demonstrated very little concern regarding these issues. When asked "Do you accomplish less than you would like to because of your vision?" 80.0% and 86.7% answered none of the time at PRE and POST assessment, respectively. These results are displayed in Table 4-4.

Table 4-4. Self-perceived visual function and barriers to daily living at PRE, DURING and POST assessment.

Response to vision problems	Assessment (%)			Significant Effects ($p<$)
	Pre	During	Post	
1. Do you accomplish less than you would like to because of your vision?				NS
• All of the time	0.0	0.0	0.0.	
• Most of the time	0.0	0.0	3.3	
• Some of the time	13.3	6.7	3.3	
• A little of the time	6.7	6.7	6.7	
• None of the time	80.0	86.7	86.7	
2. Are you limited in how long you can work or do other activities because of your vision?				NS
• All of the time	0.0	0.0	0.0	
• Most of the time	3.3	3.3	3.3	
• Some of the time	10.0	10.0	3.3	
• A little of the time	3.3	6.7	10.0	
• None of the time	83.3	80.0	83.3	
3. How much does pain or discomfort in or around your eyes (burning, itching, aching) keep you from doing what you would like to be doing?				NS
• All of the time	0.0	0.0	0.0	
• Most of the time	6.7	0.0	0.0	
• Some of the time	0.0	3.3	3.3	
• A little of the time	6.7	13.3	13.3	
• None of the time	86.7	83.3	83.3	

Because of my eyesight...	Assessment (%)			Significant Effects ($p<$)
	Pre	During	Post	
4. I stay home most of the time				NS
• Definitely true	0.0	0.0	0.0	
• Mostly true	0.0	3.3	0.0	
• Not sure	0.0	0.0	3.3	
• Mostly false	10.0	3.3	6.7	
• Definitely false	90.0	86.7	90.0	
5. I feel frustrated a lot of the time				NS
• Definitely true	0.0	3.3	0.0	
• Mostly true	6.7	0.0	0.0	
• Not sure	0.0	3.3	3.3	
• Mostly false	6.7	6.7	6.7	
• Definitely false	86.7	83.3	90.0	

6. I have much less control over what I do				NS
• Definitely true	0.0	0.0	0.0	
• Mostly true	3.3	0.0	3.3	
• Not sure	3.3	6.7	3.3	
• Mostly false	6.7	10.0	6.7	
• Definitely false	83.3	80.0	86.7	
7. I have to rely too much on what other people tell me				NS
• Definitely true	0.0	0.0	0.0	
• Mostly true	3.3	0.0	3.3	
• Not sure	0.0	3.3	0.0	
• Mostly false	3.3	6.7	3.3	
• Definitely false	93.3	86.7	93.3	
8. I need a lot of help from others				NS
• Definitely true	3.3	0.0	0.0	
• Mostly true	0.0	0.0	3.3	
• Not sure	3.3	3.3	0.0	
• Mostly false	0.0	6.7	0.0	
• Definitely false	93.3	86.7	96.7	
9. I worry about doing things that will embarrass myself or others				NS
• Definitely true	0.0	0.0	0.0	
• Mostly true	0.0	3.3	0.0	
• Not sure	0.0	0.0	0.0	
• Mostly false	6.7	6.7	6.7	
• Definitely false	93.3	86.7	93.3	

Data expressed as percent of participants (%). NS, not significant.

Three-Day Food Record

Participants were asked to complete a three-day food record within the three weeks following DURING assessment and prior to POST assessment, to monitor compliance to dietary restrictions and determine average intake patterns among this highly specific population group.

Macronutrient consumption.

A total of 28 three-day food records were collected at POST assessment. The average caloric consumption in males was 2425 (±98) Kcal and in females was 1774

(±59) Kcal. Both males and females fell within the Acceptable Macronutrient Distribution Ranges (AMDR) for protein (10-35% of total energy consumption), carbohydrates (45-65% of total energy consumption) and fat (20-35% of total energy consumption) as recommended by the Institute of Medicine (2010). Although both men and women consumed within the recommended range for total fat consumption, both genders were among the high range at 35.0% for women and 34.6% for men. Additionally, the American Heart Association (2016) recommends that less than 7% of total energy derive from saturated fat, yet both males and females exceeded this recommendation consuming 10.7% and 11.8% of calories from saturated fat, respectively. Lastly, it is recommended that less than 1% of total energy consumption derive from trans fats. Both male and female participants remained within this recommendation.

As fat consumption was among the high range of the AMDR, carbohydrate consumption was among the low range for both males and females at 47.5% and 46.8%. Both males and females did not consume their recommended fibre intake, which is 30 g for males and 21 g for females daily for individuals over 51 years of age (Institute of Medicine, 2010). Lastly, the World Health Organization (2015) recommends that less than 10% of total energy consumption be from added sugars and consuming <5% is associated with even greater health benefits. Both males and females exceeded this recommendation as 16.8% and 19.5% of total energy consumption came from added sugar. The macronutrient distribution of participants is displayed in Table 4-5**.**

Table 4-5: Macronutrient consumption of participants

Nutrients	Participants	
	Males (n=11)	**Females (n=19)**
Calories (kcal)	2424.8 (±98.0)	1773.2 (±58.6)
Fat (g)	93.3 (±4.7)	68.9 (±2.9)
Sat Fat (g)	28.8 (±1.7)	23.2 (±1.4)
Trans Fat (g)	1.1 (±0.2)	1.2 (±0.2)
Chol (mg)	533.9 (±19.8)	495.3 (±12.8)
Carbohydrate (g)	287.9 (±16.5)	207.6 (±9.0)
Fibre (g)	25.9 (±1.8)	18.0 (±0.9)
Sugars (g)	102.0 (±8.8)	86.3 (±4.7)
Protein (g)	96.3 (±4.1)	79.8 (±2.9)

Mean (±SEM)

Micronutrient consumption.

Micronutrient consumption patterns among participants were not extraordinary. However, both males and females exceeded the Recommended Dietary Allowance (RDA) of sodium, which is 1300 mg for males and females from 51-70 years of age and 1200 mg for individuals over 70 (Institute of Medicine, 2010). Although females managed to stay below the Tolerable Upper Intake Level (UL) of sodium (2300 mg/day) at 2146.7 mg (±100.4), males exceeded this recommendation at 3026.7 mg (±254.4) (Institute of Medicine, 2010). In both males and females, the RDA or Adequate Intake Level (AI) was not reached for calcium, vitamin D and E. Additionally, females did not reach the RDA/AI for potassium and vitamin A. Results of the micronutrient consumption patterns of participants are displayed in Table 4-6**.**

Table 4-6. Micronutrient consumption of participants

Micronutrients	Participants			
	Males (n=11)	**RDA/AI**	**Females (n=19)**	**RDA/AI**
Sodium (mg)	3026.7 (±254.4)	1200-1300	2146.7 (±100.4)	1200-1300
Potassium (mg)	4045.1 (±187.6)	4700	3091.2(±111.7)	4700
Vit A (RAE)	775.1 (±41.0)	900	787.3(±42.3)	700
Vit C (mg)	143.4 (±15.8)	90	117.7(±9.2)	75
Calcium (mg)	978.5 (±80.8)	1000-1200	970.8(±54.7)	1200
Iron (mg)	17.3 (±0.7)	8	12.8(±0.6)	8
Vit D (mcg)	6.0 (±0.7)	15-20	13.1(±2.1)	15-20
Vit E (mg)	8.6 (±0.43)	15	7.7(±0.3)	15
Thiamin (mg)	2.0 (±0.1)	1.2	1.5(±0.1)	1.1
Riboflavin (mg)	2.9 (±0.2)	1.3	2.6(±0.1)	1.1
Niacin (NE)	45.1 (±2.1)	16	36.5(±1.7)	14
Folate (DFE)	580.6 (±30.3)	400	448.3(±20.6)	400
Vit B6 (mg)	2.6 (±0.4)	1.7	1.6(±0.1)	1.5
Vit B12 (mcg)	5.1 (±0.4)	2.4	4.6(±0.2)	2.4

Mean (±SEM). RDA, recommended dietary allowance; AI, adequate intake. (Institute of Medicine, 2010

Effects of Enriched Egg Consumption on Retina Function

The full field ERG was used to measure variations in electrophysiological retina function from PRE to POST assessment following 6 weeks of enriched egg consumption by Caucasian older adults.

Effects of enriched egg consumption on scotopic (dark-adapted) responses.

Enriched egg consumption significantly ($p<0.001$) increased scotopic a-wave maximum amplitude (rod photoreceptor cell function) from PRE to POST assessment (PRE: 234.58 μV (±9.4), POST: 270.39 μV (±8.7)). However, no significant improvement occurred in rod photoreceptor driven inner retinal cell function including scotopic b-wave maximum amplitude or oscillatory potentials from PRE to POST assessment. Additionally, no significant improvements in scotopic a- and b-wave implicit time occurred following the 6-week dietary intervention. These results are displayed in

Figure 4-1.

To assess the relationship between scotopic responses and blood parameters, Pearson correlation coefficients were calculated (Figure 4-3). Following enriched egg consumption for 6 weeks, a moderate negative correlation existed between scotopic a-wave maximum amplitude (rod photoreceptor response) and HDL cholesterol (r=-0.45), compared to no correlation between these parameters at PRE assessment (r=0.01). Additionally, a moderate positive correlation between rod-driven oscillatory potential maximum amplitude and total cholesterol (r=0.46) was present however again; no correlation was demonstrated at PRE assessment (r=0.01).

Upon assessing the relationship between scotopic responses and plasma lutein concentrations, only weak positive correlations between scotopic a-wave maximum amplitude (rod photoreceptor response) (PRE: r=0.03, POST: r=0.38), b-wave maximum amplitude (PRE: r=0.26, POST: r=0.03) and rod-driven oscillatory potential maximum amplitude (PRE: r=0.08, POST: r=0.32) with plasma lutein concentrations were determined at both PRE and POST assessment (Figure 4-4).

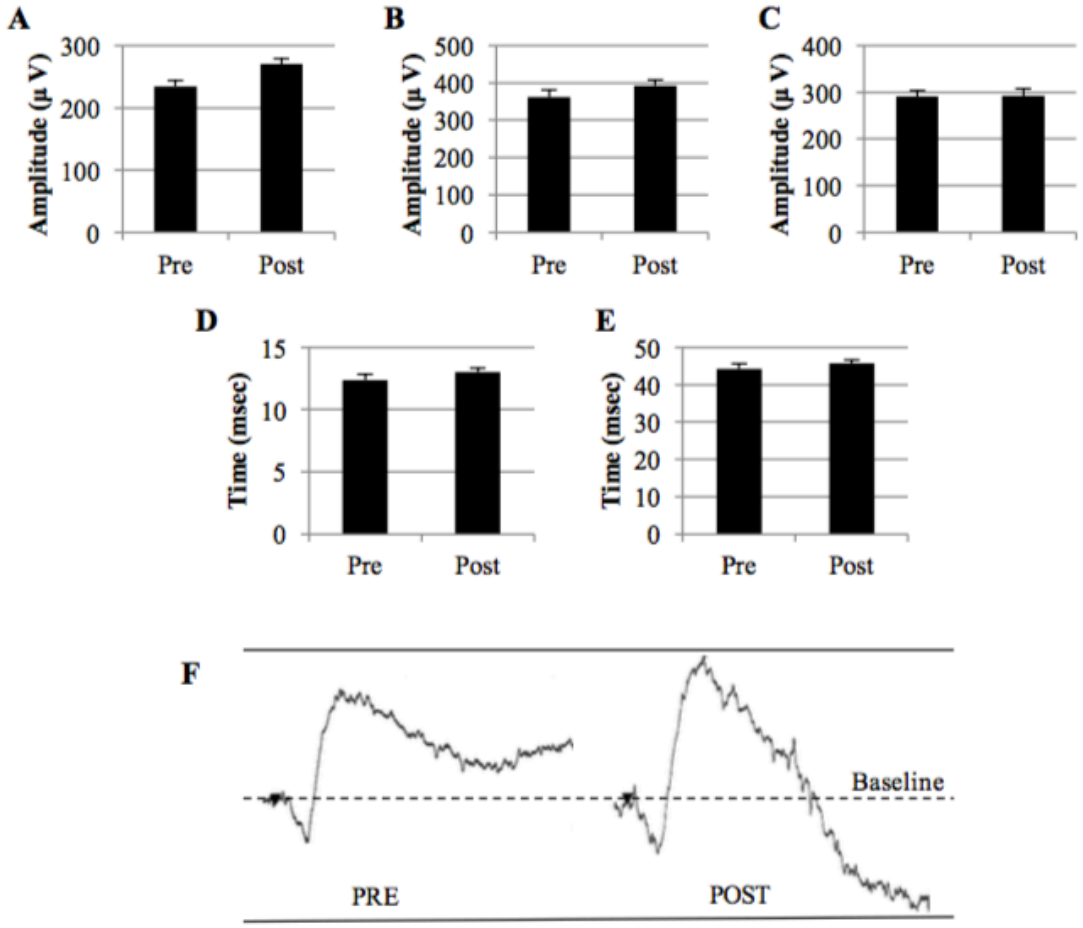

Figure 4-1: Effects of enriched egg consumption on scotopic (dark-adapted) responses. Data expressed as mean ± SEM (n=28 participants). Paired t-test demonstrated a statistically significant increase in (A) a-wave maximum amplitude from PRE to POST assessment. No significant changes occurred in (B) b-wave maximum amplitude; (C) oscillatory potential maximum amplitude; (D) a-wave implicit time at intensity 1.4 log sc cd•sec/m^2; (E) b-wave implicit time at intensity 1.4 log sc cd•sec/m^2; (F) Representative scotopic ERG measurement at -0.02 log cd.sec/m^2.

Effects of enriched egg consumption on photopic (light-adapted) responses.

Enriched egg consumption significantly (p<0.001) improved photopic b-wave maximum amplitude (cone-driven bipolar cell function) from PRE to POST assessment (PRE: 71.97 μV (±3.3), POST: 101.47 μV (±5.8)). No significant alterations occurred in cone- driven oscillatory potentials or b-wave implicit time (Figure 4-2).

To assess the relationship between scotopic responses and blood parameters,

Pearson correlation coefficients were calculated (Figure 4-3). At POST assessment, cone-driven oscillatory potential maximum amplitude was positively correlated with both TC (r=0.55) and LDL-C (r=0.57) however, only a weak relationship existed with TC (r=0.21) and LDL-C (r=0.12) at PRE assessment. Furthermore, at PRE assessment a strong positive correlation was present with plasma lutein concentrations and b-wave maximum amplitude (cone-driven bipolar cell function) (r=0.73) and a moderate positive correlation with cone-driven oscillatory potential maximum amplitude (r=0.52). However, only a weak relationship existed at POST assessment between plasma lutein concentrations and b-wave maximum amplitude (cone-driven bipolar cell function) (r=0.32) and cone-driven oscillatory potential maximum amplitude (r=0.30) (Figure 4-4).

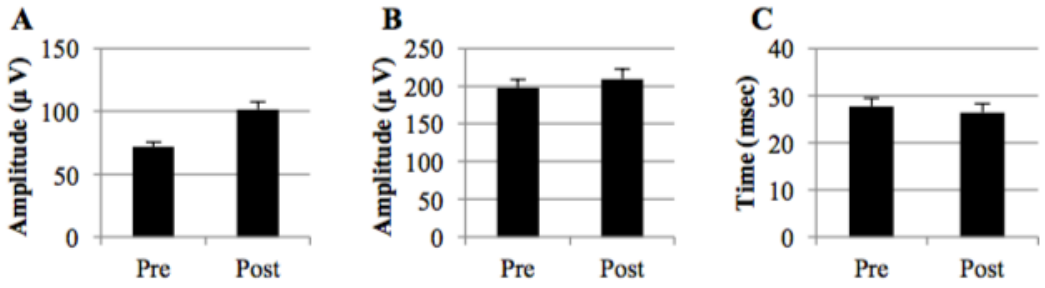

Figure 4-2: Effects of enriched egg consumption on photopic (light-adapted) responses. Data expressed as mean ± SEM (n=28 participants). Paired t-test demonstrated a statistically significant increase in (A) b-wave maximum amplitude from PRE to POST assessment. No significant changes occurred in (B) oscillatory potential maximum amplitude; (C) b-wave implicit time at intensity 1.4 log sc•cdsec/m^2.

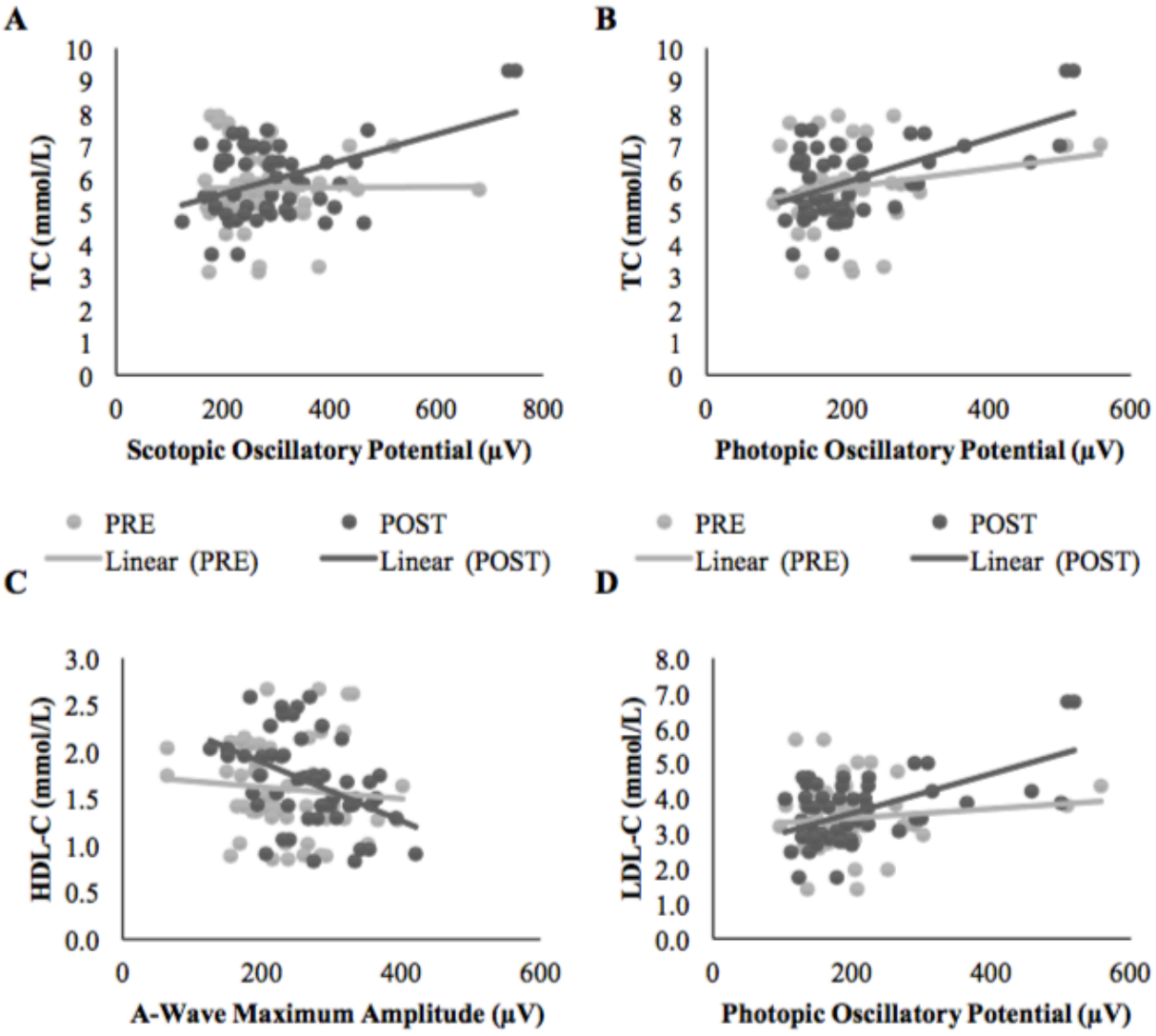

Figure 4-3: Correlation between electrophysiological retina function and plasma lipid profiles. A Pearson correlation coefficient was calculated for PRE and POST assessment. (A) Total cholesterol (TC) versus scotopic oscillatory potential (PRE: $y = 0.00009x + 5.7092$, $r^2 = 0.000061$, $r = 0.008$; POST: $y = 0.0045x + 4.6412$, $r^2 = 0.209$, $r = 0.457$); (B) Total cholesterol (TC) versus photopic oscillatory potential (PRE: $y = 0.0029x + 5.168$, $r^2 = 0.043$, $r = 0.207$; POST: $y = 0.0066x + 4.5915$, $r^2 = 0.307$, $r = 0.554$); (C) High density lipoprotein cholesterol (HDL-C) versus a-wave maximum amplitude (PRE: $y = -0.0006x + 1.7505$, $r^2 = 0.008$, $r = -0.090$; POST: $y = -0.0032x + 2.5209$, $r^2 = 0.199$, $r = -0.446$); (D) Low density lipoprotein cholesterol (LDL-C) versus photopic oscillatory potential (PRE: $y = 0.0013x + 3.1885$, $r^2 = 0.015$, $r = 0.121$; POST: $y = 0.0056x + 2.4545$, $r^2 = 0.327$, $r = 0.572$).

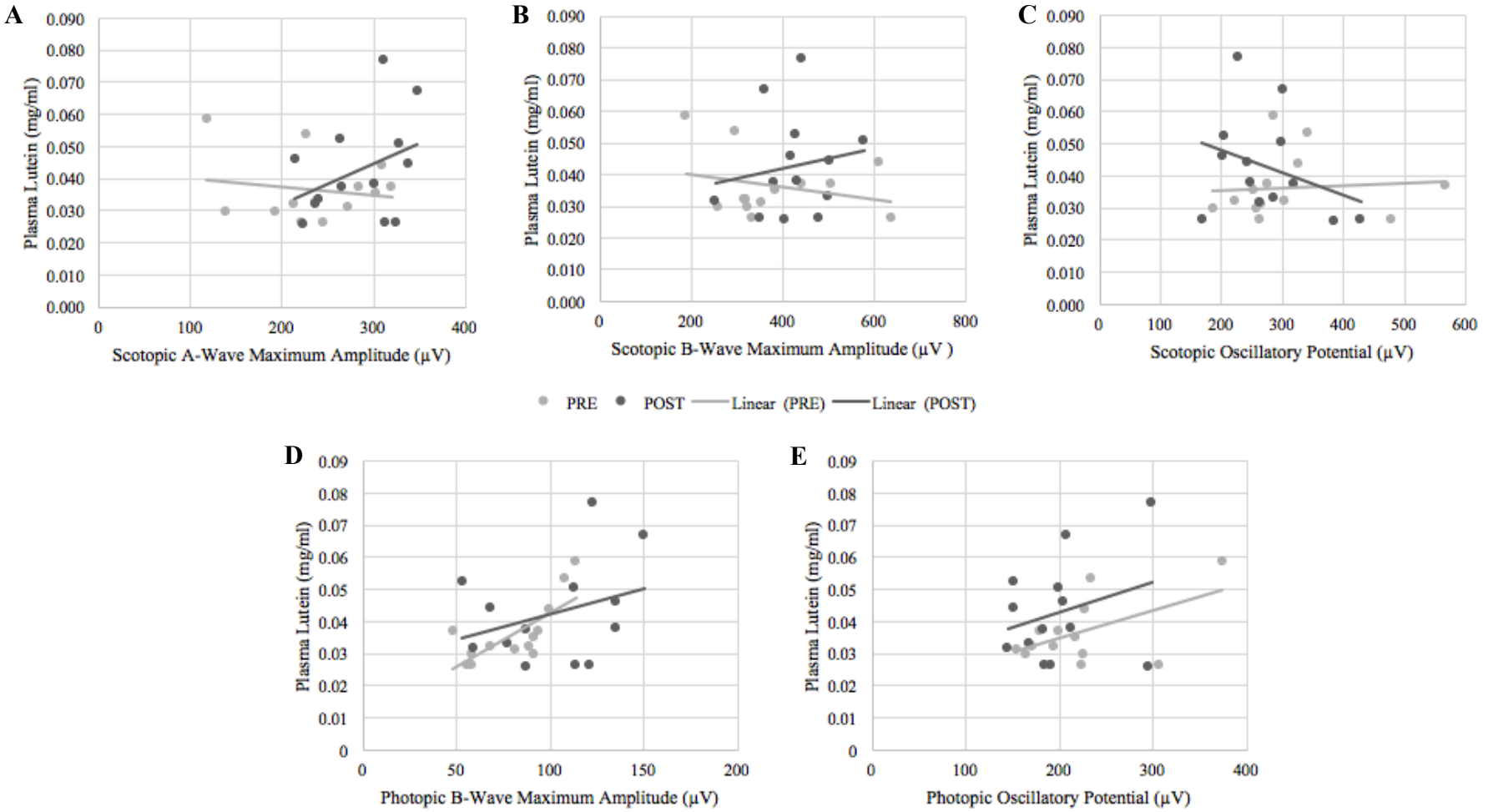

Figure 4-4: Correlation between electrophysiological retina function and plasma lutein concentrations. A Pearson correlation coefficient was calculated for PRE and POST assessment. (A) scotopic a-wave maximum amplitude (PRE: $y = - 0.00005x + 0.043$, $r^2 = 0.270$, $r = -0.172$; POST: $y = 0.0001x + 0.0065$, $r^2 = 0.142$, $r = 0.377$); (B) scotopic b-wave maximum amplitude (PRE: $y = - 0.0005x + 0.044$, $r^2=0.068$, $r= -0.260$; POST: $y=0.00005x + 0.0298$, $r^2=0.025$, $r= 0.159$); (C) scotopic oscillatory potential (PRE: $y = 0.000000006x + 0.0342$, $r^2=0.006$, $r= 0.078$; POST: $y = -0.000000005x+ 0.062$, $r^2=0.105$, $r= -0.324$); (D) photopic b-wave maximum amplitude (PRE: $y = 0.0003x + 0.088$, $r^2=0.535$, $r= 0.732$; POST: $y=0.0002x + 0.0267$, $r^2=0.101$, $r= 0.317$); photopic oscillatory potential (PRE: $y = 0.00000000005x + 0.0175$, $r^2=0.270$, $r= 0.519$; POST: $y=0.004x + 0.0238$, $r^2=0.087$, $r= 0.295$).

Effects of Enriched Egg Consumption on Plasma Lutein Concentrations

Lutein concentrations within plasma was analyzed to assess whether enriched egg consumption could increase lutein concentrations in plasma to subsequently enhance uptake into tissues including the retina. Due to unforeseen complications with the HPLC equipment, an n of 14 was used for the plasma lutein analysis.

Although not statistically significant, an increase of 19.4% in plasma lutein concentrations occurred from PRE (0.036 mg/ml (±0.003)) to POST (0.043 mg/ml (±0.004)) assessment (Figure 4-5).

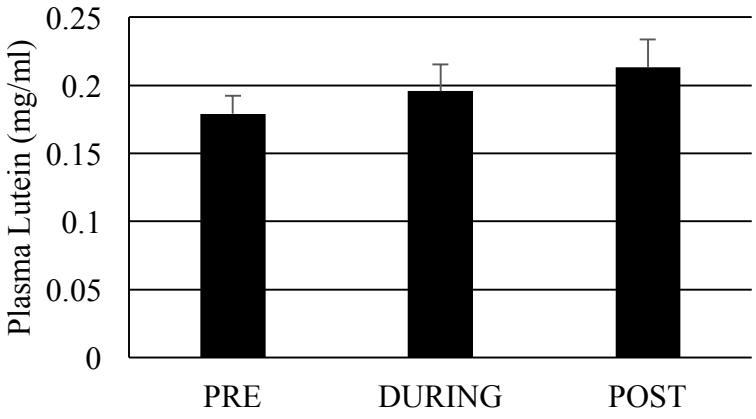

Figure 4-5. Effects of enriched egg consumption on plasma lutein concentrations. Data expressed as mean (±SEM) (n=14 participants). One-way ANOVA with repeated measurement identified no statistically significant effects of enriched egg consumption on plasma lutein concentrations.

Effects of Enriched Egg Consumption on Plasma Lipid Profiles and C-Reactive Protein

Plasma lipid profiles were analyzed to determine the effects of enriched egg consumption on plasma TAGs, cholesterol, c-reactive protein (CRP) and LDL/HDL particle size.

Plasma cholesterol, triacylglycerols and c-reactive protein.

Following 6 weeks of enriched egg consumption, no statistically significant alterations in total-C, HDL-C, LDL-C, CRP and plasma TAGs were present (Fig. 4-4; Fig. 4-5; Table 4-7). However, a minute increase in total-C (Pre: 5.72 mmol/L (±0.2); (Post: 6.01 mmol/L (±0.2)), HDL-C (Pre: 1.60 mmol/L (±0.1); (Post: 1.66 mmol/L (±0.1)), LDL-C (Pre: 3.44 mmol/L (±0.2); Post: 3.69 mmol/L (±0.2)) and CRP (Pre: 1.88 mg/L (±0.3); Post: 1.78 mg/L (±0.4)) occurred from PRE to POST assessment, with a minimal reduction in plasma TAGs (Pre: 1.56 mmol/L (±0.1); Post: 1.50 mmol/L (±0.1)).

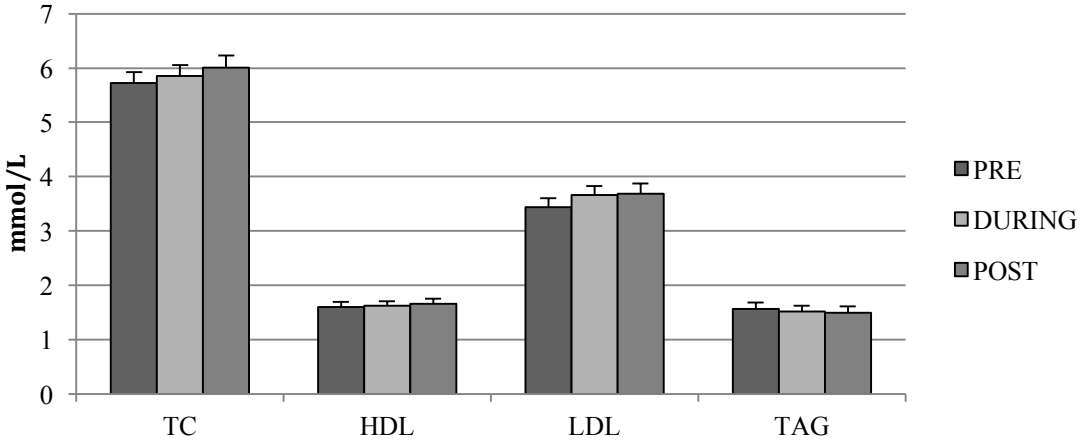

Figure 4-6. Effects of enriched egg consumption on plasma lipid profiles. Data expressed as mean (±SEM) (n=28 participants). One-way ANOVA with repeated measurement identified no statistically significant effects of enriched egg consumption on plasma total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TAG).

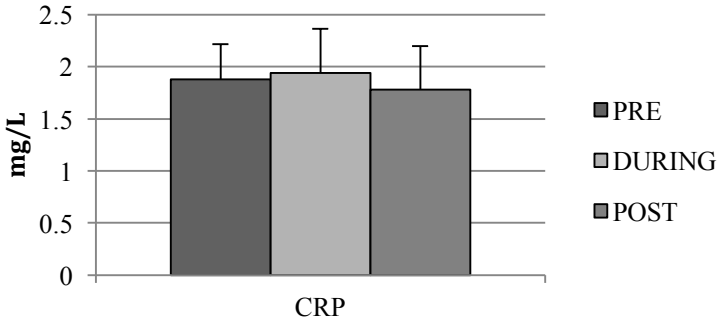

Figure 4-7. Effects of enriched egg consumption on plasma C-reactive protein (CRP). Data expressed as mean (±SEM) (n=28 participants). One-way ANOVA with repeated measurement demonstrated no statistically significant effects of enriched egg consumption on plasma c-reactive protein (CRP).

Table 4-7. Plasma blood profiles at PRE, DURING and POST assessment.

	PRE	DURING	POST	Significant Effects ($p<$)
TC (mmol/L)	5.72(±0.2)	5.85(±0.2)	6.01(±0.2)	NS
HDL (mmol/L)	1.60(±0.1)	1.62(±0.1)	1.66(±0.1)	NS
LDL (mmol/L)	3.44(±0.2)	3.66(±0.2)	3.69(±0.2)	NS
TAGs (mmol/L)	1.56(±0.1)	1.52(±0.1)	1.50(±0.1)	NS
CRP (mg/L)	1.88(±0.3)	1.94(±0.4)	2.17(±0.4)	NS

Data expressed as mean (±SEM). No statistically significant effects on blood lipid profiles from enriched egg consumption were identified by one-way ANOVA with repeated measurement. TC, total cholesterol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TAGs, triacylglycerols; CRP, high sensitivity c-reactive protein.

Effects of enriched egg consumption on lipoprotein particle size.

To further assess the effects of enriched egg consumption on plasma cholesterol in Caucasian older adults, LDL-C and HDL-C particle size was analyzed among participants who experienced greater than or equal to a 10% decrease or increase in plasma total-C levels. A total of 11 participants experienced an increase, and 6 participants experienced a decrease greater than or equal to 10% following the 6-week intervention. A total of 17 participants were included in the lipoprotein subfraction analysis.

Low-density lipoprotein particle size distribution.

The consumption of enriched eggs for 6 weeks had no effect on LDL-C particle size. Moreover, the distribution among participants included greater concentrations of the LDL-1 large particle, and the smallest concentration of LDL-7 small particle. The distribution demonstrated among participants follows a healthy normal distribution (Type A) as recommended in the literature (Austin et al., 1994). The distribution of LDL-C particle size is displayed in Figure 4-8**.**

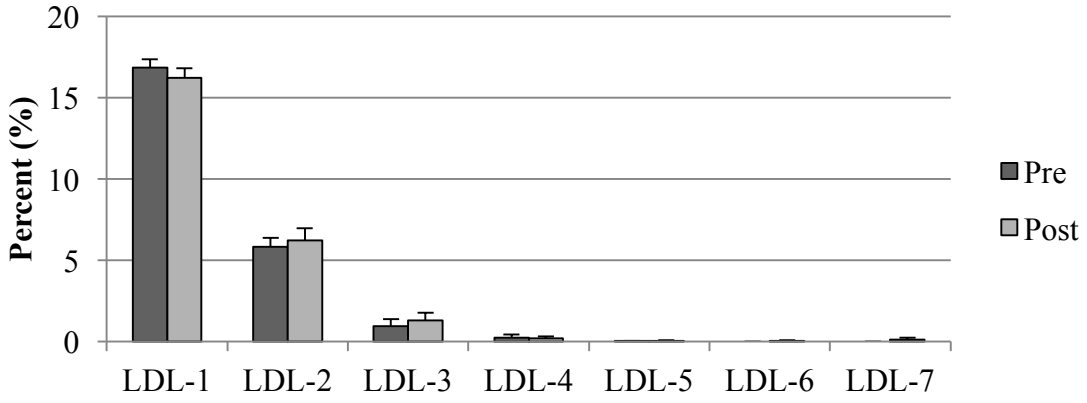

Figure 4-8. Effects of enriched egg consumption on low-density lipoprotein (LDL) particle size. Data expressed as mean (±SEM) (n=17 participants). The paired-t test model demonstrated no significant changes from PRE to POST assessment.

High-density lipoprotein particle size distribution.

Following the 6-week intervention, a statistically significant increase in the concentration of the large HDL-2 particles occurred from PRE (8.49 mg/dl (±0.8)) to POST (10.09 mg/dl (±0.0.8)) assessment. No other significant changes were present among any other HDL-particles when analyzed individually. Upon grouping particles in large (HDL-1, HDL-2, HDL-3), intermediate (HDL-4, HDL-5, HDL-6, HDL-7) and small (HDL-8, HDL-9, HDL-10) particle classes, a significant increase in the distribution of large HDL-particles (PRE: 30.94% (±1.3); POST: 34.10% (±1.5)) and a significant

decrease in the distribution of intermediate HDL-particles (PRE: 54.43% (±1.2); POST 51.51% (±1.0)) occurred (Figure 4-9).

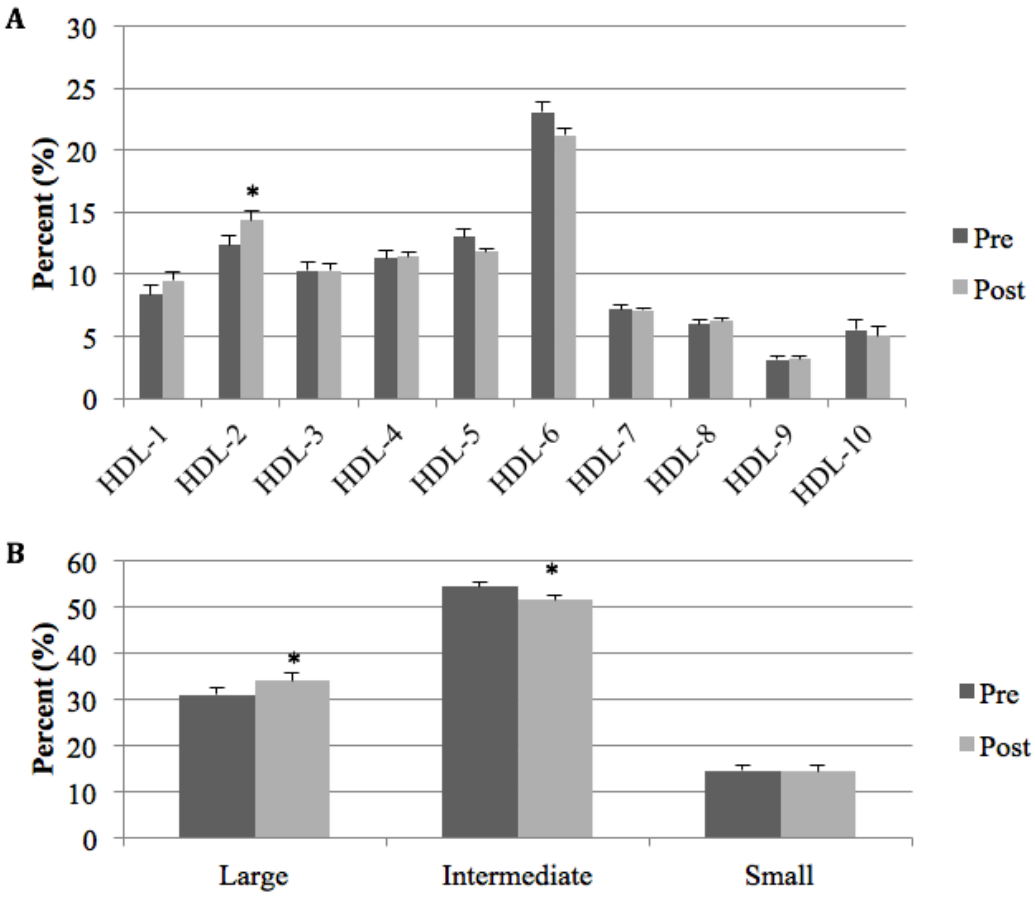

Figure 4-9. Effects of enriched egg consumption on high-density lipoprotein (HDL) particle size. Data expressed as mean (±SEM) (n=17 participants). The paired-t test model determined a significant ($p<0.05$) increase in the distribution of HDL-2 particles (Large: HDL-1, HDL-2, HDL-3; Intermediate: HDL-4, HDL-5, HDL-6, HDL-7; Small: HDL-8, HDL-9, HDL-10) (A) and large HDL particles (B) and a significant ($p<0.05$) reduction in intermediate HDL particles (B).

Effects of Enriched Egg Consumption on Plasma and Erythrocyte Fatty Acids

To determine the absorption and bioavailability of DHA present within the yolk of the enriched egg following 6-weeks of daily consumption, both plasma and erythrocyte fatty acids were assessed.

Plasma fatty acids.

Enriched egg consumption for 6 weeks had no significant effect on saturated, monounsaturated (MUFA), polyunsaturated (PUFA), n-3, n-6, n-7 and n-9 fatty acids. Additionally, no significant alterations in the essential fatty acids α-linolenic acid and linoleic acid were present. As expected, DHA concentrations significantly increased from PRE (1.86% (±0.1)) to DURING (2.57% (±0.1)) assessment and this increase was maintained into POST (2.52% (±0.1)) assessment. Results of the plasma fatty acid analysis are displayed in Table 4-8.

To assess the relationship between electrophysiological retina function and plasma DHA concentrations, Pearson correlation coefficients were calculated (Figure 4-10). At POST assessment, a weak negative correlation was present among scotopic a-wave maximum amplitude (rod photoreceptor function) and plasma DHA concentrations (r=0.23) however; this correlation was not present at PRE assessment (r=0.07). Additionally, a weak negative correlation existed among photopic b-wave maximum amplitude (cone-driven bipolar cell function) and plasma DHA concentrations at both PRE (r=-0.26) and POST (r=-0.25) assessment. Lastly, between cone-driven oscillatory potential maximum amplitude and plasma DHA, a weak positive correlation existed at both PRE (r=0.21) and POST (r=0.29) assessment.

Table 4-8. Effects of enriched egg consumption on plasma fatty acids.

Fatty Acid (% w/w)	PRE	DURING	POST	Significant Effects ($p<$)
SFA	34.35 (±0.3)	34.10 (±0.5)	34.44 (±0.3)	NS
MUFA	26.70 (±0.5)	24.86 (±1.0)	25.82 (±0.5)	NS
PUFA	38.88 (±0.5)	40.96 (±0.8)	39.65 (±0.6)	NS
n-3	4.30 (±0.3)	5.06 (±0.3)	4.66 (±0.1)	NS
ALA	0.86 (±0.1)	0.92 (±0.1)	0.89 (±0.0)	NS
DHA	1.86 (±0.1)[b]	2.57 (±0.1)[a]	2.52 (±0.1)[a]	0.001
n-6	28.15 (±0.5)	29.01 (±0.7)	28.24 (±0.5)	NS
LA	25.95 (±0.5)	26.86 (±0.7)	26.25 (±0.5)	NS
n-7	3.62 (±0.3)	2.96 (±0.2)	3.93 (±0.7)	NS
n-9	21.00 (±0.5)	19.55 (±1.0)	19.73 (±0.9)	NS

Data expressed as mean (±SEM) (n=30 participants). One way ANOVA with repeated measurement and Duncan's multiple range test demonstrated a significant increase in saturated fatty acids from PRE to DURING assessment, and a significant reduction from DURING to POST assessment. Plasma DHA significantly increase from PRE to DURING assessment. No significant changes in MUFA, PUFA, n-3, n-6, n-7, n-9 fatty acids, ALA and LA occurred.

Erythrocyte fatty acids.

Following the 6-week intervention, no significant modifications to the fatty acid distribution of erythrocytes occurred. A statistically significant increase in MUFAs occurred from PRE to DURING assessment however, these values returned to baseline from DURING to POST assessment. No significant alterations occurred in SFA, PUFA, n-3, n-6 fatty acids or LA, ALA, EPA and DHA concentrations, however a small increase of 8.94% occurred in erythrocyte DHA concentrations from PRE (3.02% ± 0.5) to POST (3.29% ± 0.5) assessment. Results of the erythrocyte fatty acid analysis are summarized in Table 4-9.

To assess the relationship between electrophysiological retina function and erythrocyte DHA concentrations, Pearson correlation coefficients were calculated (Figure 4-8). Interestingly, a weak negative correlation occurred between scotopic a-wave maximum amplitude (rod photoreceptor function) and erythrocyte DHA concentrations at POST assessment (r=-0.34). This relationship was not present at PRE assessment (r=-0.08).

Table 4-9. Effects of enriched egg consumption on erythrocyte fatty acids.

Fatty Acid (% w/w)	PRE	DURING	POST	Significant Effects ($p<$)
SFA	46.52(±2.7)	44.86 (±2.4)	45.75 (±2.3)	NS
MUFA	12.63^{b} (±1.0)	9.44^{a} (±0.7)	13.10^{b} (±0.9)	0.05
PUFA	40.84 (±2.6)	45.69 (±2.2)	41.16 (±2.2)	NS
n-3	17.57 (±2.6)	24.35 (±2.7)	17.22 (±2.3)	NS
ALA	0.30 (±0.1)	0.29 (±0.1)	0.34 (±0.1)	NS
EPA	14.16 (±2.7)	20.71(±2.8)	13.47 (±2.7)	NS
DHA	3.02 (±0.5)	3.25 (±0.5)	3.29 (±0.5)	NS
n-6	13.87 (±0.9)	12.22 (±0.8)	14.35 (±0.8)	NS
LA	6.36 (±0.4)	5.32 (±0.4)	7.05 (±0.7)	NS

Data expressed as mean (±SEM) (n=30 participants). One-way ANOVA and Duncan's multiple range test demonstrated a significant increase in MUFA concentrations from PRE to DURING assessment, and values normalized from DURING to POST assessment. No significant changes in SFA, PUFA, n-3, n-6 fatty acids, ALA, LA, EPA and DHA occurred.

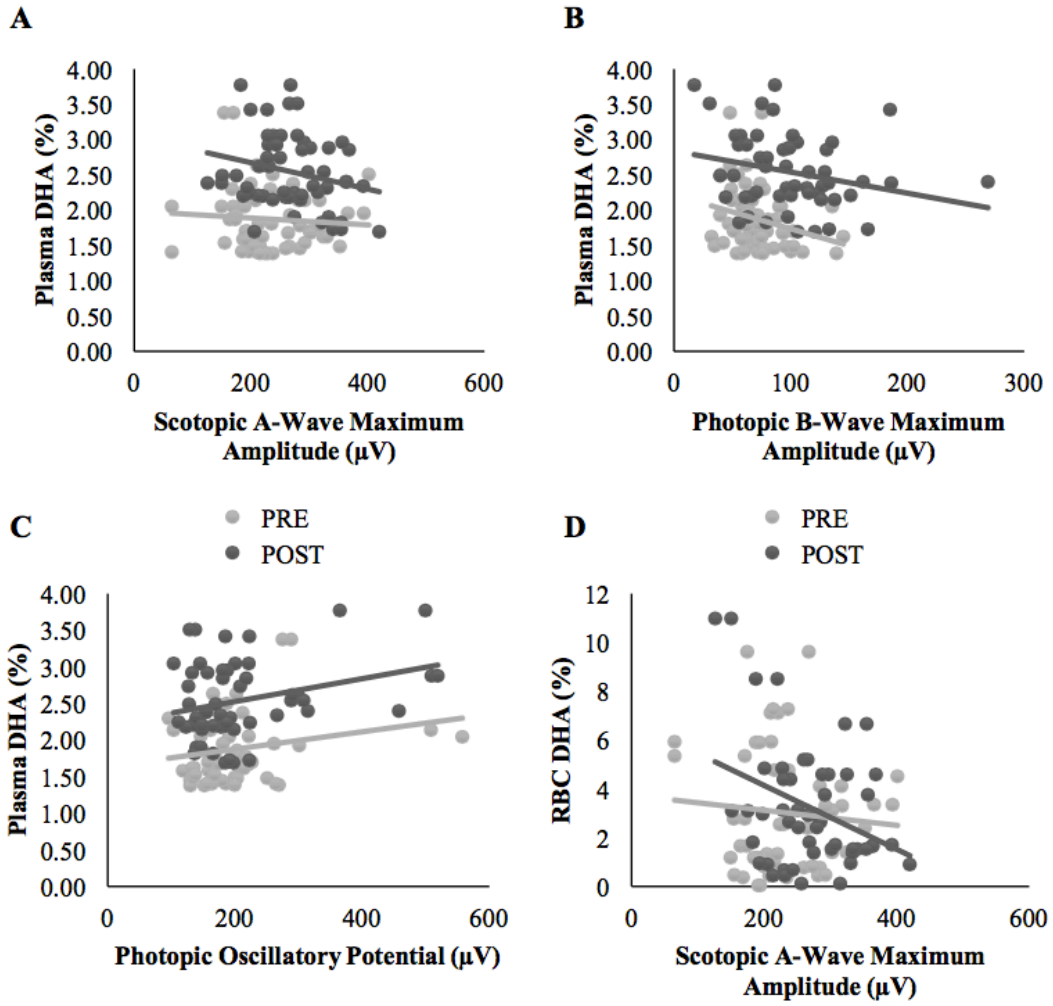

Figure 4-10: Correlation between electrophysiological retina function and plasma and erythrocyte docosahexaenoic acid. A Pearson correlation coefficient was calculated for PRE and POST assessment. (A) Plasma docosahexaenoic acid (DHA) versus scotopic a-wave maximum amplitude (PRE y = -0.0005x + 1.9812, r^2 = 0.005, r = -0.073; POST y = -0.0019x + 3.0503, r^2 = 0.005, r = -0.235); (B) Plasma DHA versus photopic b-wave maximum amplitude (PRE y = -0.0049x + 2.2196, r^2 = 0.00.068`, r = -0.260; POST y = -0.003x + 2.8406, r^2 = 0.062, r = -0.248); (C) Plasma DHA versus photopic oscillatory potential (PRE y = 0.0012x + 1.6348, r^2 = 0.0.043, r = 0.208; POST y = 0.0016x + 2.2151, r^2 = 0.085, r = 0.292); (D) Erythrocyte DHA versus scotopic a-wave maximum amplitude (PRE y = -0.003x + 3.7303, r^2 = 0.008, r = -0.087; POST y = -0.0131x + 6.7451, r^2 = 0.113, r = -0.344).

Chapter 5. Discussion

This is the first study to examine the effects of daily lutein and DHA enriched egg consumption on self-perceived health and vision status, electrophysiological retina function and related blood parameters in older adults. It was hypothesized that in Caucasian older adults, lutein and DHA enriched egg consumption would improve electrophysiological retina function; not adversely influence plasma lipid profiles and HDL/LDL particle distribution; increase DHA concentrations in plasma and erythrocytes; and increase lutein and zeaxanthin concentrations in plasma. Results of the full-field electroretinography indicated a significant ($p<0.001$) improvement in rod-photoreceptor cell function (scotopic a-wave maximum amplitude) and cone-driven inner retinal cell function (photopic b-wave maximum amplitude) of 15.3% and 41.0%, respectively. On the contrary, enriched egg consumption demonstrated no significant effects on rod-driven inner retinal cell function; or oscillatory potentials and implicit time in both dark and light adapted measures. While enriched egg consumption improved retina function, no adverse alterations to plasma lipids were indicated including plasma TC, HDL-C, LDL-C and TG. Moreover in participants whose TC increased or decreased by 10%, enriched egg consumption improved the distribution of HDL particles as a 10.2% increase in large particles and a 5.4% decrease in intermediate sized particles occurred from PRE to POST assessment. No alterations in LDL particle distribution was elicited. Lastly, plasma concentrations of DHA significantly increased by 35.4% from PRE to POST assessment however, erythrocyte DHA concentrations remained unchanged.

Effects of Enriched Egg Consumption on Retina Function

The consumption of two lutein and DHA enriched eggs daily for 6-weeks was beneficial in improving rod-photoreceptor cell function (scotopic a-wave maximum

amplitude) and cone-driven inner retinal cell function (photopic b-wave maximum amplitude). Although research utilizing the full-field ERG to analyze the effects of whole foods on retina function is a topic unstudied, the effects of lutein and zeaxanthin supplementation on MPOD and retina function measured by multifocal ERG has been previously studied (Berrow et al., 2012). In recently published research by Berrow et al. (2016), the effect of nutrient supplementation on multifocal electroretinographic retina function was assessed. A total of 52 participants were randomly assigned to receive either a placebo or a supplement containing 12 mg of lutein, 0.6 mg of zeaxanthin, 150 mg of vitamin C, 15 mg of vitamin E, 400 μg of copper, 20 mg of zinc, and 1080 mg of n-3 fatty acids for 40 weeks. As expected, MPOD significantly increased among treated participants. Additionally, a significant reduction in inner retinal P1 implicit time occurred following supplementation. No alterations were elicited in amplitude or measures of peripheral retina function. Moreover, in studies utilizing only supplemental lutein and zeaxanthin (Huang et al 2015; Ma et al. 2012), multifocal ERG results demonstrated that N1P1 response densities of the central retina improved with limited alterations detected in the peripheral retinal regions. As the N1P1 signal obtained from the multifocal ERG is largely dependent on the functioning of bipolar cells, and the inner retina is highly concentrated with cone photoreceptor cells, it is reasonable to assume that lutein and zeaxanthin consumption improved cone-driven bipolar cell function in these participants (Hood et al. 2003; Huang et al 2015; Ma et al. 2012). Although MPOD was not assessed in the present study, results from both Huang et al. (2015) and Ma et al. (2012) attributed the improvement in multifocal ERG results of the central retina to the increase in MPOD. Both lutein and zeaxanthin are the main constituents of macular pigment. The greatest concentrations of lutein and zeaxanthin are present within the outer and inner plexiform layers of the fovea (as cited in Ma et al., 2012). Based on previous

research, it can be assumed that the improvement in cone-driven bipolar cell function may be attributed to the lutein and zeaxanthin provided in the form of enriched eggs.

It is commonly noted that there are two mechanisms by which lutein and zeaxanthin protect the cells of the retina including; by acting as a highly efficient antioxidant to reduce oxidative damage; and reducing the influx of harmful blue light into retinal tissues. The powerful ability of macular pigment to act as an antioxidant is highly efficient in reducing oxidative damage by scavenging reactive oxygen species and reducing lipid peroxidation in a region highly concentrated in PUFAs. Additionally, macular pigment has been linked to an improvement in photostress recovery. Photostress recovery can be defined as an assessment of visual function measuring the time required for photopigment to regenerate following bleaching, due to bright light exposure (Scripsema et al., 2015). It is suggested that the light absorptive capacity of macular pigment reduces the exposure of photoreceptor cells to short wavelength, high-energy light, allowing for a reduction in photostress recovery time and improving visual function (Scripsema et al., 2015). Hammond et al. (2014) analyzed the effects of lutein and zeaxanthin supplementation on MPOD and photostress recovery by measuring time required to recover vision following 30 seconds of intense xenon white light exposure. Following 1 year of supplementation with 10 mg of lutein and 2 mg of zeaxanthin daily in 115 healthy subjects, MPOD and photostress recovery significantly improved among treated individuals. Although implicit time was not altered in the current study, an increase in macular pigment concentrations following egg consumption may have improved photostress recovery, thereby increasing the amplitude by which the rod photoreceptor, and cone-driven inner retinal cells could respond.

Although many studies have attributed improvements in visual function following nutrient supplementation to lutein and zeaxanthin, DHA has consistently demonstrated its essentiality for maintaining optimal retina function. In rod photoreceptor cells, DHA

consist of 50-60% of total fatty acids (Querques et al., 2011). As rod outer segments undergo continuous oxidative damage and membrane renewal, a continuous supply of DHA is required to allow for the regeneration of membranous discs and maintain membrane fluidity (Querques et al., 2011). Currently, research regarding DHA consumption and electrophysiological retina function is highly lacking in human. However in a mouse model, a diet containing 0% or 1% DHA was analyzed for its effects on electrophysiological retina function and A2E accumulation. DHA consumption was attributed to a reduction in age-related functional losses and A2E accumulation in both wild type and E4 transgenic STGD3 mice. This transgenic mouse model is lacking the ELOVL4 gene required for the elongation of very long chain fatty acids. Nevertheless, it is important to note that ERG results demonstrated that DHA preserved visual function with age but did not improve visual functioning. This inability of DHA to improve the functioning of the previously damaged retina has been demonstrated in humans with various forms of AMD. Gerstenblith et al. (2013) evaluated the effects of daily EPA and DHA supplementation for 6 months in individuals over 50 years of age with early to intermediate dry AMD. Results of this study were inconsistent with the findings of the current study as Gerstenblith et al. (2013) found no benefit of n-3 supplementation on best-corrected visual acuity and multi-focal electroretinographic testing. Furthermore, the addition of 350 mg of DHA and 650 mg of EPA to the original AREDS supplement formulation of vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg) zinc oxide (80 mg) and cupric oxide (2 mg) demonstrated no added benefit to individuals with various forms of AMD (Age-Related Eye Disease Study Research Group, 2001; 2013).

As there is no research regarding the effects of DHA consumption on retina function in healthy subjects, it is exceedingly difficult to compare the findings of the current study to research including individuals with various forms of retinal degeneration. Furthermore, it is difficult to compare a supplement containing only EPA and DHA or

lutein and zeaxanthin with a whole food such as an egg, which contains lutein, zeaxanthin and DHA among many other nutrients. Nevertheless, the consumption of lutein and DHA enriched eggs for 6 weeks appears to improve retina function in Caucasian older adults. As DHA is required for the constant maintenance of photoreceptor cells, and macular pigment is essential to protect the retina from harmful blue light and oxidative damage, it is most likely both of these nutrients played a synergistic role in the outcome of the current study.

Effects of Enriched Egg Consumption on Plasma Lutein Concentration

Following enriched egg consumption for 6 weeks, plasma lutein (n=14) was not significantly altered from PRE to POST assessment. However, a trend towards improvement was demonstrated as a 19.4% (p=0.096) increase was present upon comparing PRE and POST plasma lutein concentrations. Results of the current study are comparable to findings of related literature regarding egg consumption and plasma lutein concentrations. Vishwanathan et al., (2009) monitored plasma lutein following 5 weeks of the consumption of either 2 or 4 non-enriched egg yolks per day. Egg yolk consumption led to a significant 16% and 24% (p<0.05) increase in plasma lutein with 2 and 4 egg yolks daily. Additionally, Wenzel et al. (2006) compared enriched egg with non-enriched egg consumption and a placebo to determine any variations in plasma lutein concentrations. Participants consumed 6 treatment eggs per week or placebo for the duration of the 12-week trial period. Similar to findings of the current study, a trend towards increasing plasma lutein concentrations was present with a non-significant 23% and 26% increase with non-enriched and enriched egg consumption. Lastly, a recent 14 week cross-over intervention study assessed the consumption of 0 to 3 non-enriched eggs daily for 4 weeks (DiMarco et al., 2017). One egg per day demonstrated no significant effects on plasma lutein concentrations. In contrast, 2 to 3 eggs daily significantly

increased plasma lutein concentrations at 4 weeks with a 20% and 31% increase, respectively.

As previously mentioned, lutein is present among various plant based sources including kale and spinach in much greater concentrations as compared to egg yolk. Considering the lipid-soluble nature of lutein, a variety of research has demonstrated an enhanced bioavailability of the carotenoid when consumed in the lipid rich yolk of the chicken egg to promote nutrient absorption and utilization (Chung et al., 2004). As hypothesized, it was expected that plasma lutein concentrations would significantly increase following enriched egg consumption. Albeit the results of the current study did not align with this expectation, considering the trend towards improvement and results of similar studies, it is likely that egg consumption for a longer duration or analysis with a larger sample size would lead to a significant increase.

Effects of Enriched Egg Consumption on Plasma Lipid Profiles

Plasma cholesterol and triacylglycerols.

The consumption of two lutein and DHA enriched eggs per day for 6-weeks did not adversely alter plasma TC, HDL-C, LDL-C, TAG or CRP in Caucasian older adults. The effects of egg consumption on plasma lipid profiles is a topic commonly studied and results of the current study expand on existing knowledge. Although eggs were once viewed as potentially dangerous for individuals at risk of cardiovascular disease, the American Heart Association (2015) and Canadian Heart and Stroke Foundation's (2015) dietary guidelines include no restriction on dietary cholesterol and state that the consumption of one egg per day is not associated with the risk of cardiovascular disease. Although there is a wide variety of conflicting evidence of the effects of egg consumption on cardiovascular risk factors, studies have found no correlation between the consumption of 2-3 eggs daily with cardiovascular risk factors in healthy adults (Andersen et al., 2013; Blesso et al., 2013; Katz et al., 2005; Njike et al., 2010). Results

of the current study can be compared to results obtained from Njike et al. (2010). Participants were randomly assigned to consume either two medium hardboiled eggs per day or an egg substitute. Following six weeks of dietary intervention, no adverse alterations in TC, HDL-C, LDL-C and TAG were present. Additionally, Katz et al., (2005) analyzed alterations in LDL-C and TC following the consumption of two eggs per day. The age of participants ranged from 36 to 73 with an average of 55.7 years. Following six weeks of egg consumption, no alterations were present regarding TC and LDL-C among treated individuals.

Data from the Third National Health and Nutrition Examination Survey (NHANES III) included 6833 healthy males and 8113 healthy females over the age of 17, to assess the relationship between egg consumption and the risk of cardiovascular disease (Scrafford et al., 2011). After adjusting for health, lifestyle and dietary factors, the consumption of ≥7 eggs per week was not positively correlated with mortality due to coronary heart disease (Scrafford et al., 2011). On the contrary, research suggesting a positive relationship between egg consumption and cardiovascular disease does exist. For example, a meta-analysis including 14 studies involving 320,778 individuals found a positive relationship between egg consumption and the development of cardiovascular disease and diabetes (Li et al., 2013). Results of this meta-analysis concluded that for every additional 4 eggs per week consumed, ones risk of cardiovascular disease increased by 6% (Li et al., 2013). However, as cardiovascular disease is highly multifactorial, the 14 studies included in this meta-analysis corrected for various confounding factors but these included factors differed among independent studies. Therefore, it is exceedingly difficult to fully conclude that egg consumption alone can increase ones risk of cardiovascular disease without adjusting for all related risk factors.

Although the evidence is highly conflicting regarding the safety of egg consumption, the diet and lifestyle chosen among individuals consuming eggs is of the

utmost importance. As previously mentioned, there are numerous other dietary factors that can lead to adverse alterations in blood lipids including the over consumption of trans and saturated fat; high-energy diets; and the inadequate consumption of fibre, fruit, vegetables and whole grains. Additionally, other lifestyle behaviours such as a sedentary lifestyle, smoking, and excess weight gain are greatly related to ones risk of developing cardiovascular disease. When egg consumption is paired with these unfavorable behaviours, it is most likely one will see adverse changes to blood lipid parameters. Based on the findings of the current study and various other studies regarding the effects of egg consumption of blood lipid parameters, egg consumption is safe among healthy individuals making healthy lifestyle choices and is therefore a safe nutrition intervention to improve the retina health of Caucasian older adults.

Lipoprotein particle size distribution.

Both LDL and HDL particle size distribution was analyzed in individuals who experienced greater than or equal to a 10% decrease or increase in plasma total-C levels (n=17). Enriched egg consumption had no effect on LDL-particle size distribution. On the contrary, enriched egg consumption favorably altered the distribution of HDL particles. A significant increase in the large HDL particles with a significant decrease among intermediate HDL particles occurred following the enriched egg intervention. Results of the current study align with the results obtained by Mutungi et al. (2010). In 21 male subjects from 40 to 70 years of age consuming 3 eggs per day, large HDL particles significantly increased by 51.6% along with a significant decrease in intermediate sized HDL particles. Similar to the current study, no changes occurred among small HDL particles. Results by Mutungi et al. (2010) also exhibited a significant 42.0% increase in large LDL particles with egg consumption. Although a normal LDL particle distribution was seen among the majority of participants in the current study, no modifications

occurred among large LDL particles. Findings by Greene et al. (2006) further support the ability of eggs to increase the number of large HDL particles. A total of 29 women and 13 men from 50-80 years of age consumed 3 eggs per day for 30 days. In comparison to the current study, no alteration to LDL particle distribution was elicited, however a significant increase in large HDL particles occurred. Upon grouping participants as either hyper-responders (response to dietary cholesterol > 2.2 mg/dL for each additional 100 mg of dietary cholesterol) or hypo-responders (response to dietary cholesterol < 2.2 mg/dL for each additional 100 mg of dietary cholesterol), hyper-responders demonstrated a significant increase in large LDL and HDL particles following egg consumption.

Both LDL and HDL particle distributions have been described as either pattern A or B distributions. Pattern A distributions consist of mainly large, buoyant lipoprotein particles including either LDL or HDL-1 and -2 particles, with minute concentrations of small dense particles such as LDL or HDL-3 to -7 (Superko, 2009). Individuals with pattern B distributions of LDL cholesterol are three times more likely to develop congestive heart disease when compared to pattern A individuals (Superko, 2009). This is due to the fact that small LDL particles are much more susceptible to oxidative damage, increasing ones risk for atherosclerotic plaque production. Additionally, pattern B distributions of HDL cholesterol have also been correlated with an increase in ones risk of developing cardiovascular disease (Superko, 2009). As HDL-C is responsible for transferring circulating cholesterol to the liver for excretion, small dense HDL particles are often less efficient in removing cholesterol from the blood stream, increasing the risk of atherosclerotic plaque development (Greene et al., 2006). Considering enriched egg consumption improved the distribution of HDL cholesterol among participants by increasing large and decreasing intermediate sized particles, enriched egg consumption may be beneficial in improving retina health while promoting a healthy HDL particle distribution.

Effects of Enriched Egg Consumption on Plasma and Erythrocyte Fatty Acids

Plasma fatty acids.

Following only 3 weeks of enriched egg consumption, plasma DHA concentrations significantly increased by 38.2% from PRE to DURING assessment. Following week 3 of enriched egg consumption a plateau effect occurred, however the 38.2% increase in plasma DHA was maintained into POST assessment. Existing literature regarding the effects of DHA enriched egg consumption on plasma fatty acids have elicited similar results. Gillingham et al. (2005) monitored the alterations in blood lipid parameters following 21 days of DHA enriched egg consumption. In nearly half the duration of the present study, results demonstrated a 22% increase in serum phospholipid DHA concentrations. In comparison, research measuring the effects of supplemental DHA on plasma lipid concentrations has exhibited similar findings. Vandal et al. (2008) recruited 10 older adults with an average age of 74 to consume 680 mg of DHA and 320 mg of EPA daily in the form of a fish oil supplement. Plasma fatty acids were analyzed following the 21-day supplementation period. At day 21, plasma DHA concentrations increased by 46%, while all other omega-3 fatty acid concentrations remained unchanged. Interestingly, the dose of DHA administered in the current study was only 31.9% of that provided in the study executed by Vandal et al. (2008). However compared to DURING assessment in the present study, DHA concentrations were only 7.8% lower than the concentrations published by Vandal et al. (2008). Additionally, both studies included older adults with highly comparable baseline plasma DHA concentrations at 1.86 ± 0.1 and 1.60 ± 0.6 in the present and Vandal et al. (2008) study, respectively. These findings suggest that the unique lipid matrix of the egg provides a highly bioavailable environment for the absorption of DHA. As a PUFA, DHA is highly prone to peroxidation and over supplementation may lead to detrimental health effects.

Enriched egg consumption provides only one third of the DHA present in supplements, yet eggs have demonstrated exceptional absorptive capacity and have the potential for numerous health benefits including the improvement of retina function. Therefore, eggs may be a much safer alternative to supplemental DHA, more specifically for the health of the retina.

Erythrocyte fatty acids.

The consumption of lutein and DHA enriched eggs did not significantly alter erythrocyte fatty acids statistically, suggesting DHA supplementation did not reach adequate levels for a sufficient duration to increase erythrocyte DHA concentrations. However, a minor increase of 8.94% was present in erythrocyte DHA concentrations from PRE to POST assessment. Pre-existing literature related to this topic has found conflicting results. In comparison, Payet et al. (2004) provided 150 mg of DHA daily in the form of an egg product, and erythrocyte fatty acids were measured every 3 months for a total of 9 months. Participants in this study were older adults from 63 to 92 years of age. Following 3 months of dietary intervention, erythrocyte membrane DHA concentrations significantly increased by 57.2%. In the present study, membrane lipids were not analyzed independently, however the significant increase demonstrated in erythrocyte DHA concentrations by Payet et al. (2004) does not align with the current findings. Considering a daily intake of only 150 mg of DHA could increase erythrocyte DHA concentrations in 3 months of supplementation, it is unclear as to why no significant alterations occurred in the present study. As the fatty acid composition of erythrocyte reflects long-term intake, it is possible that the 4-week washout phase prior to the dietary intervention affected the erythrocyte uptake of DHA.

To determine whether a dose response exists for DHA supplementation, 26 participants were recruited and randomly assigned to receive either a daily dose of 0, 2, 4

or 6 g of fish oil providing 0, 0.52, 1.04 and 1.56 g of DHA per day, respectively (Milte et al., 2008). In all three-treatment groups, erythrocyte DHA substantially increased. Additionally, a dose response was determined as erythrocyte DHA concentrations increased proportionately with each larger dose by up to 78% in the group consuming 6g per day (Milte et al., 2008). Considering the substantial increase in plasma DHA concentrations with only 217 mg of DHA per day, consumption of lutein and DHA enriched eggs for a longer duration of time would most likely lead to greater uptake of DHA into erythrocytes.

Lastly, although erythrocyte DHA concentrations did not significantly increase, beneficial effects to retina function were still present. As erythrocyte lipid concentrations reflect long-term dietary intake, it can be assumed that erythrocyte DHA concentrations would be more representative of membrane lipid concentrations including the retina in comparison to plasma concentrations. However in the present study, it is possible that only minute increases in retinal DHA concentrations lead to significant effects on the retina, or plasma DHA concentrations are also representative of DHA concentrations within the retina.

Conclusions

The consumption of 2 commercially available lutein and DHA enriched eggs per day for 6 weeks improved rod photoreceptor and cone-driven inner retinal cell function in healthy Caucasian older adults. In doing so, egg consumption did not undesirably alter blood lipid parameters. Moreover, HDL-C particle distribution was favourably altered, increasing large HDL particles, which are more efficient in transferring circulating cholesterol to the liver for excretion and tissues such as the retina. The outer segments of rod photoreceptor cells contain vast amounts of DHA, required for the constant regeneration of membranous discs. Additionally, lutein or macular pigment is

exceedingly concentrated in the cone dominated central region of the retina. Macular pigment reduces light induced oxidative damage and has been linked to improving photostress recovery. Considering both DHA and lutein are required for optimal maintenance and functioning of the retina, it is likely that both nutrients are responsible for the improvements exhibited in this study. Furthermore, the unique lipid matrix of the egg allows for an increase in the absorptive capacity of lipid soluble nutrients including lutein and DHA. Therefore, although eggs contain minimal amounts of lutein and DHA compared to nutritional supplements, enriched eggs provide a nutritious and bioavailable source of these nutrients along with protein, energy and various other vitamins and minerals. In conclusion, enriched eggs are a safe and affordable nutrition strategy to improve the health of the retina in Caucasian older adults. Additionally, the incorporation of lutein and DHA enriched eggs into ones diet may prevent degeneration of the retina associated with ageing, sequentially reducing the risk of age related eye disease such as AMD.

Chapter 6. Overall Summary

The aim of this intervention trial with a repeated measure design was to assess whether DHA and lutein enriched egg consumption could improve electrophysiological retina function in health Caucasian older adults. Moreover, as eggs contain substantial concentrations of cholesterol, it was of interested to determine whether the consumption of 2 eggs daily for 6 weeks would lead to any adverse alterations in plasma lipids in this highly specific population. At POST assessment following 6 weeks of dietary treatment, enriched eggs positively altered retina function among participants. Scotopic a-wave maximum amplitude significantly increased from PRE to POST assessment, representing an improvement of the functional ability of the rod photoreceptor cells. Furthermore, a significant alteration in photopic b-wave maximum amplitude was present at POST assessment, depicting an improvement in the functioning of cone driven inner retinal cells including bipolar cell function. Previous literature has demonstrated the ability of lutein and zeaxanthin consumption to enhance cone driven bipolar cell function and potentially increase the amplitude by which these cells response by improving photostress recovery. Furthermore, as DHA is exceedingly concentrated in the outer segments of rod photoreceptor cells, and macular pigment is most concentrated among the inner retinal cells of the macula, it is most likely both of these nutrients provided in the form of an enriched egg were responsible for the improvements in retina function. However, further research regarding the effects of these nutrients on electrophysiological retina function in healthy subjects is required to further assess these outcomes and to determine the precise roles of these individual nutrients in the healthy aging retina.

Not only did enriched egg consumption improve retina function, it did so without eliciting negative effects to related blood lipid parameters as TC, HDL-C, LDL-C, TAG and CRP remained unchanged. Furthermore, although the distribution of LDL particles

was not altered following the dietary intervention, the majority of participants followed a healthy pattern A distribution. Correspondingly, the most common distribution of HDL particles among participants was also pattern A, which was further improved following enriched egg consumption. Enriched egg consumption led to a significant increase in large HDL particles, with a significant decrease in intermediate HDL particles. Both of these alterations are favourable in reducing ones risk of developing cardiovascular disease. Lastly, considering the improvements to retina function demonstrated in the current study and the limited increases in erythrocyte DHA concentrations, plasma DHA concentrations may be representative of DHA concentrations within the retina.

Overall based on the current findings, enriched eggs may be a beneficial nutrition strategy to improve retina function while potentially improving blood lipid profiles among healthy older adults. As this is the first study of its kind, further research should be conducted to confirm these findings.

Strengths and Limitations

This study has a number of strengths and limitations that must be addressed. As the majority of research focuses on the functional ability of supplemental nutrients, this study aimed to do so with a nutritious whole food easily incorporated into the diet of the average individual. Nutrient supplements are costly and often provide a less bioavailable source of very specific nutrients. Eggs are an affordable and nutritious source of complete protein providing all essential amino acids and a wide variety of vitamins and minerals. This unique lipid matrix also creates an eminently bioavailable environment for the absorption and utilization of lipid soluble nutrients. By utilizing commercially available lutein and DHA enriched eggs in this study, the corresponding results are conveniently applicable to the dietary choices of individuals looking for a simple nutrition strategy to enhance retina function. Additionally, to mimic the normal daily consumption patterns of

the general population, participants were allowed to consume the treatment eggs in whichever form they prefer (ie. boiled, fried, scrambled). However although this practice is positive in assessing the effects of enriched egg consumption in general daily living practices, various forms of egg preparation may be viewed as a limitation due to the alterations or loss of nutrients that may occur during cooking.

Several limitations are present among the design of this study. First off, this study was not randomized and a control group was not incorporated to evaluate if the consumption of enriched eggs was responsible for any alterations in retina function and blood parameters. Due to the extensive exclusion criteria implemented to recruit healthy older adults to partake in this study, recruitment of an adequate number of participants to ensure robust data with both a treatment and control group was not possible due to time constraints. To assess the effects of enriched egg consumption, a repeated measure design was implemented to allow for participants to act as their own control. Secondly, although the goal of this research was to recruit healthy non-hyperlipidemic participants, the average TC among participants at PRE assessment was 5.72 mmol/L. According to the Medical Council of Canada (2013) normal TC is less than 5.2 mmol/L, demonstrating that the majority of participants included in this study were hyperlipidemic at baseline. To minimize this limitation in the future, screened participants should undergo blood work to assess eligibility based on exclusion and inclusion criteria. Unfortunately, this was not a viable option in the present study and exclusion criterion was based on self-reported health status.

An additional limitation to this study was the free-living study design. Although participants were asked to strictly follow the dietary intervention in question, it is impossible to ensure full compliance. This may be greatly impactful on the results of this study if participants were consuming other foods or supplements high in retina related nutrients, or avoiding the consumption of treatment eggs. To reduce the impact of this

limitation, a three-day food record was collected to assess the dietary patterns of participants. Additionally, participants were provided with strict instructions regarding which foods and supplements to avoid and egg preparation methods were recorded and submitted at both DURING and POST assessment. To mitigate the effects of this limitation, further research utilizing a full-feeding approach would be highly beneficial to ensure enriched eggs are responsible for the positive study outcomes related to retina function. Lastly, eggs naturally contain a wide variety of nutrients. Although eggs were enriched with lutein and DHA, it is impossible to ensure any alterations to retina function are due to these particular nutrients and not a variety of multiple compounds working in a synergistic fashion. Among many other vitamins and minerals eggs are rich in zinc and choline, both of which are related to optimal functioning of the retina.

Recommendations for Future Research

As this study is the first of its kind, further research regarding the effects of lutein and DHA enriched egg consumption on retina function and related blood parameters is essential to further understanding this relationship. Currently, there is a lack of knowledge regarding normative data for the full-field ERG in this particular population. Future research should focus on obtaining data regarding normal healthy ERG values in healthy older adults to create normative standards for future research. These standards could be utilized to compare full-field ERG values of other population subgroups and further understand the effects of nutrient consumption on retina function.

Secondly, the majority of research utilizing the ERG to measure retina function is done so in participants with varying degrees of retinal disease including AMD. Nutrients including lutein and DHA are often studied for their ability to reduce symptoms related to AMD. Although lutein has demonstrated some benefit in slowing the progression of AMD, DHA supplementation has shown to be of limited benefit. It is recommended that

future research focus on the preventative effects of these nutrients to reduce ones risk of developing retinal disease. By administering these nutrients to healthy individuals, researchers can further understand the preventative mechanisms of each specific nutrient and how they relate to improving retina function. Furthermore, this study included only Caucasian individuals as roughly 90% of cases of age-related macular degeneration occur among these individuals (National Eye Institute, 2010). Future research could include participants of various ethnicities to determine how retina function in these individuals is affected by enriched egg consumption and how this compares to the retina function of Caucasians.

The full-field ERG provides an objective measurement of the functioning of major cellular components within the retina. To assess these individual groups of cells, the waveform elicited following a stimulus in the form of bright light can be dissected into different regions, each representing the functioning of a specific cellular group. However, the full-field ERG cannot decipher which region of the retina these cellular responses originate from and how they differ from one another. For example, if cells of the peripheral retina are responding in a healthy manner, the functioning of cells in the central or foveal region may be deficient. To assess the retina function in various regions of the retina, a multi-focal ERG is required. Future research regarding how the health of the retina responds to enriched egg consumption and how this relates to the prevention of AMD, should utilize the multi-focal ERG. As AMD mainly affects the macula, a multi-focal ERG would analyze any variations of central retina functioning following enriched egg consumption.

AMD is a disease highly related to oxidative stress within the sensitive cells of the retina due to the exceptionally high concentrations of PUFAs such as DHA in retinal tissues. As the enriched eggs included in this study provide both lutein, a potent antioxidant, and DHA, an omega-3 LCPUFA associated with anti-inflammatory

properties, it is of interest to assess the effects of enriched egg consumption on oxidative stress. Future research should consider analyzing markers of oxidative stress such as malondialdehyde and 8-iso-Prostaglandin F2alpha in plasma. Assessment of these biomarkers may determine any antioxidant capabilities of the consumption of lutein and DHA enriched eggs in Caucasian older adults.

Lastly as previously mentioned, a randomized controlled trial with a crossover design would be optimal in further evaluating the results obtained in this study. As described in previous research, participants in cholesterol related studies are often grouped as either hyper- or hypo-responders (Clark et al., 2006). These individuals may respond to a dietary intervention including eggs differently, as varying degrees of absorption dietary cholesterol and alterations in circulating plasma lipids have been reported (Clark et al., 2006). By administering a study with a crossover design participants act as their own control, controlling for any genetic variants that may alter the results of the study. Moreover, a full-feeding trial to directly monitor the diet and compliance of participants would be the gold standard to understand the relationship between enriched egg consumption, retina function and related blood parameters.

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Appendix A – High-Performance Liquid Chromatography of Lutein

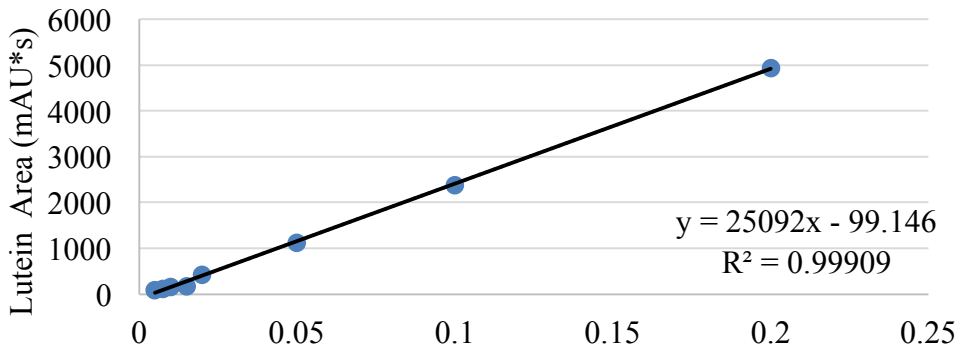

Figure A-1: Calibration curve for lutein used for plasma and egg carotenoid determination.