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Modifiable and Non-Modifiable Factors Related to the Macular Pigment Optical Density (MPOD) in a Population of College-Aged Adults

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MODIFIABLE AND NON-MODIFIABLE FACTORS RELATED TO THE MACULAR PIGMENT OPTICAL DENSITY (MPOD) IN A POPULATION OF COLLEGE-AGED ADULTS

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in The School of Nutrition and Food Sciences

by
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B.S., Escuela Agrícola Panamericana, El Zamorano, 2014
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<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic Acid</td>
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<tr>
<td>ALA</td>
<td>Alpha Linolenic Acid</td>
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<td>AMD</td>
<td>Age Related Macular Degeneration</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>AREDS</td>
<td>Age-Related Eye Disease Study</td>
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<td>AREDS 2</td>
<td>Age-Related Eye Disease Study 2</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>DHA</td>
<td>Docosahexaenoic Acid</td>
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<tr>
<td>DHA+EPA</td>
<td>Docosahexaenoic Acid + Eicosapentaenoic Acid</td>
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<td>EPA</td>
<td>Eicosapentaenoic Acid</td>
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<td>FA</td>
<td>Fatty Acids</td>
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<td>HFP</td>
<td>Heterochromatic Flicker Photometry</td>
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<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>LA</td>
<td>Linoleic Acid</td>
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<tr>
<td>LC-PUFAs</td>
<td>Long-chain Polyunsaturated Fatty Acids</td>
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<tr>
<td>LED</td>
<td>Light Emitting Diodes</td>
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<tr>
<td>L+Z</td>
<td>Lutein + Zeaxanthin</td>
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<tr>
<td>MPOD</td>
<td>Macular Pigment Optical Density</td>
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<tr>
<td>NEI</td>
<td>National Eye Institute</td>
</tr>
<tr>
<td>NDSR</td>
<td>Nutrition Data System for Research Program</td>
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<tr>
<td>OFF</td>
<td>Optimal Flicker Frequency</td>
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<td>RPE</td>
<td>Retinal pigment epithelium</td>
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ABSTRACT

Age-related macular degeneration (AMD) is one of the leading causes of blindness among the elderly worldwide. Retinal problems, specifically in the macula, hold potential for development of AMD. Macular pigment optical density (MPOD) gives a measure of the thickness of the macula and therefore health of the macula. Some of the populations at risk for development of AMD include being white, female, and having light eye color. Factors related to the development of the disease have been divided into modifiable and non-modifiable; non-modifiable include a genetic predisposition. Control of modifiable factors, including body mass index (BMI) and diet [dietary intake of lutein+ zeaxanthin (L+Z) and docosahexaenoic acid + eicosapentaenoic acid (DHA, 22:6n3 +EPA, 20:5n3)], have been associated with a reduction in prevalence of the disease. To date, few studies have evaluated MPOD in young adults, and to the best of our knowledge no studies have evaluated the relationship of MPOD to those factors in that population. We posed the question: What factors (diet, BMI, gender, ethnicity, eye color) affect macular health in a young adult population? MPOD was measured for 475 young adults (18-28 years old) using a macularmetrics densitometer. Dietary information was collected using a food frequency questionnaire and a 24-hour dietary recall. BMI for each subject was calculated.

Young females had lower MPOD values than males (0.3295 vs 0.3659). There was no difference with BMI or eye color. There were no differences among normal, overweight and obese BMI groups (p > 0.05). However, combined normal+ overweight subjects vs obese had higher MPOD (p= 0.032). Employing sequential regression, the addition of DHA+EPA and L+Z improved the model beyond that provided by gender and BMI.
Dietary intake of DHA+EPA and L+Z was higher in males than in females. However, in general, the consumption of those nutrients was low in both males and females. In conclusion, female gender and obese BMI seem to be related to MPOD in this young population. Dietary information points to a necessity for early education about eye health, nutrition and body weight for young adults.
1. INTRODUCTION

Age-related macular degeneration (AMD) is a multifactorial disease that may be linked to factors such as body mass index (BMI), gender, ethnicity, eye color and dietary patterns [1]. AMD is one of the main causes of blindness among the elderly and is primarily characterized by damage or degeneration of a vital segment of the inner layers in the retina, specifically the macula [2]. Treatments and recommendations to prevent late stage AMD are limited and most treatments serve to primarily delay the progression of the disease [3]. Age has been considered the most significant common factor related to prevalence of the disease [4], but there are other both modifiable and non-modifiable factors that influence the development and progression of the disease. Based on this evidence, it is proposed that some of those factors could show effects on macular health early in life.

Factors related to AMD that can be changed to delay the progression of the disease are known as modifiable factors. Control of modifiable factors that affect the progression of the disease including sun exposure, smoking, high BMI, and dietary intake of docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), lutein and zeaxanthin [4] are considered the primary focus of prevention. However, the prevalence of AMD also seems to be affected by non-modifiable factors. Evidence points to a genetic predisposition for AMD, and ethnicity, eye color, gender, family history of the disease are also identified as susceptibility biomarkers of AMD [1]. However, to the best of our knowledge, none of these factors have been evaluated as potential risks in a young population. An evaluation of some of these factors was explored in the current study to determine their relationship with macular health, specifically density of the macular pigment.
1.1 Justification

Factors related to macular health early in life have not been evaluated to the best of our knowledge [5]. Non-modifiable factors that point to a genetic predisposition, such as age, ethnicity, and eye color may increase the risk for developing some eye diseases such as AMD [1]. On the other hand, there are also modifiable factors, such as BMI, nutrition, sun exposure, and smoking. These factors could be controlled, and together with creating awareness early in life about macular health and AMD hold the potential for reducing the risk for developing this disease.

Nutrition, specifically DHA, plays an important role as a structural component in the outer segment of the photoreceptor cells [6]. Lipoproteins have a high affinity for DHA and deliver DHA to the eyes and the brain [7]. Also, the xanthophyll carotenoid pigments, which comprise the macular pigment, play an important role as they act as antioxidants and filters in the macula to protect the photoreceptors against damage [8]. Adipose tissue, which has a higher affinity for lipid membranes, tends to compete with the retina for xanthophyll carotenoid pigments [9]. It has been shown that higher BMI is related to lower macular pigment [10]. Previous research has determined that individuals with BMI values in the obese category [11], female gender [10], white skin, and light iris color [12] are related to a higher prevalence and risk for development of AMD.

1.2 Assumptions

It is assumed that participants in the study will give correct information about their health status, smoking habits, and food consumption collected with a 24-hour dietary recall. Also, it is expected that participants will follow the instructions for the eye health screening test, and it is
assumed that the researchers will document the correct information following the procedures of the study.

1.3 Research Hypothesis

- Non-modifiable and modifiable factors related to development of AMD in the elderly will be associated with macular pigment density early in life.

- Study subjects who are obese (BMI ≥ 30) will have lower macular pigment, assessed as macular pigment optical density (MPOD), defined and discussed in the “Literature Review”.

1.4 Objectives

- Evaluate if macular pigment density early in life is related to dietary intake of lutein+zeaxanthin (L+Z) and DHA+ EPA (22:6n-3, 20:5n-3), BMI, gender, ethnicity and iris color.

- Estimate dietary intake of L+Z and DHA+ EPA among participants.

1.5 Limitations

- MPOD measures were obtained once per participant.

- Dietary intake was assessed once using one 24-hour dietary recall.

- Health histories that included demographic and nutrition information were self-reported.

- Previous pregnancy was not included as an exclusion criterion.
2. LITERATURE REVIEW

2.1 The Eye: Anatomy and Physiology

The eye is a notable specialized organ capable of picking up light and converting it to electrical signals that are sent to the brain in a process known as photo transduction. The primary function of the eye is to clarify images in our environment and send those images to the brain through the optic nerve [13]. The eye is composed of three outer coats: the outer fibrous coat, the uvea tract or middle vascular layer, and the neural or inner layer [8, 13]; the neural layer is further subdivided into the anterior and posterior segments [14]. For the current study, the focus was on the neural/inner layer.

The neural layer contains the specialized retinal photoreceptor cells, the rods and cones, which are responsible for differentiation of colors in bright and dim light [13, 14]. The outer and middle layers include the cornea, the sclera, the iris and the choroid structures. These structures play a secondary role in the photo transduction process and are necessary for both focusing and transmitting light to the retina [8, 13]. The eye’s intraocular fluids include aqueous humor, vitreous humor, and blood, all of which play important functional roles [13]. The aqueous humor supplies nutrients and oxygen to the cornea and lens, and it maintains the shape of the internal eyeball [14]. The vitreous humor is responsible for filling the vitreous chamber which is the largest cavity of the eye and covers the space between the lens and the retina [8, 14]. Blood plays a role in maintaining the intraocular pressure and retinal nutrition [13].

2.2 The Retina

The retina, considered the ‘photographic film’ of the eye, is located at the innermost area of the three layers of the eye. Photoreceptors (cones and rods) are the specialized retinal cells responsible for converting information, shown as images, into signals that are sent to the brain
The retina is composed of two main layers: the retinal pigment epithelium (RPE) and the inner neurosensory layer [15]. The RPE is the outer layer of the retina, composed of millions of cells [14]; it is the only layer with a high pigmented color capable of absorbing excess light [15]. The RPE also produces important eye growth factors, protects against oxidative damage, and provides support to photoreceptors; hence it is critical to visual function. Positioned behind the RPE is a semipermeable membrane, Bruch’s membrane. Bruch’s membrane regulates the exchange of nutrients and other essential substances between the choroid and the outer layers of the retina [14, 15]. The choroid, a large network of blood vessels, supplies the retina with nutrients [8, 14, 15] (Figure 1).

![Figure 1. Schematic picture of the human eye showing the location of the RPE, Bruch’s membrane, choroid and the macula. Reprinted and reproduced with permission from John Wiley and Sons [16]](image)

The thickness of Bruch’s membrane tends to change with age, affecting both the permeability and transport of nutrients. As a result of those changes, Bruch’s membrane tends to be impacted by oxidative stress, chronic inflammation [14], and the presence of retinal drusen [8, 15, 17] which are sub-retinal lesions or deposits [14, 17]. Retinal drusen show different
characteristics including a sharply defined shape and a yellowish color [17]. These changes in the eye’s structure and architecture result in an increased risk for development of early stages of AMD that may progress to late stages of the disease [17].

2.3 The Macula and MPOD

The macula is a circular area in the central retina subdivided into two segments, the fovea and the foveola [18]. The macula contains the densest concentration of photoreceptors, and it’s responsible for high resolution vision which permits the conduct of daily activities [19]. The photoreceptors in the macula are exclusively narrow and elongated cones, located in a wide zone, the fovea [8], which is the peak of clearest vision, allowing color perception and contrast sensitivity [8, 14, 15]. The central area of the fovea is known as the foveola [18].

The macula is about 5.5 mm in diameter, and it presents as a yellow color due to the existence of xanthophyll carotenoid pigments, primarily lutein, zeaxanthin, and meso-zeaxanthin [8, 9, 14, 15, 20, 21]. Those carotenoids represent approximately 36, 18 and 18%, respectively, of the total carotenoids in the retina [20] and together are referred to as the macular pigment. The concentration of xanthophyll carotenoid pigments is higher in the retina than in any other tissue, with a concentration of $10^{-3}\text{ M}$ in the fovea [21]. These carotenoid pigments serve as a filter against ultraviolet irradiation damage [8], acting as an antioxidant against reactive oxygen species that are generated as a by-product of metabolism [9, 20]. It has been demonstrated that a denser macular pigment may improve visual function and also reduce risk for age-related eye disease [9].

Lutein and zeaxanthin are considered unusual xanthophyll carotenoid pigments due to the presence of hydroxyl groups at the end of each molecule [9]. These xanthophyll carotenoid pigments come from the diet, having been synthesized by algae, bacteria and plants [20]. Dietary
sources include food from animal origin such as salmon, but the primary sources are in fruits and colored vegetables, especially green leafy varieties [9, 21, 22]. Macular pigment concentrations vary across populations and individuals as a result of differences in both diet and the varying affinity of different tissues to store these pigments [21]. Based on previous research, there are many other variables that can affect the level of macular pigment such as age [5], ethnicity [23, 24], body fat [25-28], gender [29, 30] and probably others [21].

MPOD is a quantitative measure of the attenuation of blue light by the macular pigment and is used clinically to determine macular health based on lutein and zeaxanthin concentrations [9]. Those pigments are concentrated in the macula, and they represent the primary carotenoids that make up the macular pigment [31]. Measures of MPOD are obtained by measuring the central retina (fovea) and the periphery of the macula (parafovea), which result in a measure of thickness of the macular pigment [32, 33]. Measures are shown as density units (du) and vary from 0 to 1 in the human macula [9]. Techniques to assess MPOD in vivo include heterochromatic flicker photometry (HFP) which has been used widely [32] since 1999 [34]. For purposes of this study, measures were obtained using a macular metric densitometer, an instrumentation validated by Wooten et al [34] in 1999, that operates based on the HFP technique. The instrumentation and technique are detailed in the “Materials and Methods” section of Chapter 3.

2.4 Age Related Macular Degeneration

AMD is a degenerative, multifactorial, chronic disease that affects the central retina, and it is one of the leading causes of blindness worldwide [8, 35-38]. Loss of vision is associated with photoreceptor cell degradation in the center of the retina, the macula [2]. Damage in the macula and reduction of macular carotenoid pigments are initial indicators of possible risk for developing AMD [9]. AMD is recognized as the leading cause of blindness in adults over 65
years of age in developing countries [31]. This eye disease can be divided into three stages: early, intermediate, and late stages. The early and intermediate classifications of AMD are based on the size of the drusen in the retinal area. The late stages are characterized by the presence of geographic atrophy or neovascular age-related macular degeneration [36].

The pathogenesis of AMD involves degenerative changes in the outer portion of the retina, RPE, Bruch’s membrane, and the choroid [36]. The factors responsible for these damages are poorly understood, but include metabolic, genetic and environmental mechanisms [8]. These factors have been divided into modifiable, those we can control to prevent the disease, and the non-modifiable which are linked to age and genetics [39]. Based on results from a meta-analysis in 2012, the estimated prevalence of early AMD is 6.8% and late AMD is 1.5% in White populations aged 40 years and older [36]. Consequently, Wong et al [40] estimated, based in a systematic literature review of the all population–based studies of AMD, that 8.7% of the worldwide population has AMD. These authors projected that in 25 years, 288 million people will be affected by AMD [40]. Currently there is no effective cure for AMD; prevention is the best approach to controlling/preventing vision loss as result of AMD.

2.5 Factors Associated with AMD Development

Diverse risk factors are associated either directly or indirectly with the development of AMD. The most significant factors are age and genetic predisposition [4]; age by itself is considered the major risk factor for developing AMD [35, 36, 41]. Age-related changes at the cellular level, along with modifiable factors such as smoking and sunlight exposure, increase the susceptibility for developing AMD [41]. Genetic predisposition is a factor that has been linked with ethnicity [1, 24, 39] and iris color [42], considered non-modifiable factors. Modifiable factors have been studied for the control and prevention of AMD. Those factors include BMI,
smoking, cumulative sunlight exposure, dietary intake, and cardiovascular disease [4]. The following non-modifiable and modifiable factors: iris color, ethnicity, gender, BMI and dietary patterns are considered here.

2.5.1 Iris Color

Iris color is considered a factor related to the risk of developing AMD [1, 42]. Individuals with light-colored irises (blue/gray) have a significantly lower MPOD compare to those with dark irises (brown/black) [42, 43]. Intermediate iris colors (hazel/green) do not present differences in MPOD compared to light-colored and dark irises [42, 43]. A lower MPOD is therefore associated with a higher risk of developing AMD.

A study by Frank et al [42], reported a higher prevalence of AMD in White subjects with light-colored irises, compared to those with dark-colored irises. Consequently, Nicolas et al [12] found that individuals with light-color irises have a strong tendency to AMD progression compared to those with dark-colored irises. Both studies concluded that differentiation between AMD prevalence/progression and iris color may be associated with genetic and environmental differences among ethnic groups [12, 42].

The tendency of light colored eyes for susceptibility to lower MPOD and AMD development has been associated with some factors which include the accumulation of melanin and carotenoids and higher amounts of light passing through light colored eyes. Melanin regulates the light that reaches the retina and it is responsible for iris color. Higher concentrations of melanin reduce the risk for damage to the retina. The dark brown iris contains a higher concentration of melanin compared to the light blue iris which contains less [44].
2.5.2 Ethnicity

Ethnicity has been reported to be an important factor explaining risk for the development of AMD [1]. Some studies have shown a higher prevalence and risk for developing AMD (lower MPOD) in White individuals compared to other ethnic groups including Black and Latinos [10, 23, 24, 42, 45]. A study performed by Wolf-Schnurbsch et al [24] evaluated macular pigment differences between white non-Hispanics and Africans. They demonstrated a significantly lower macular pigment in the white, non-Hispanic individuals compared to the Africans.

A study by Wong et al [40] reported the prevalence of AMD by ethnicity using a population-based study approach. Those investigators showed that of the participants with AMD, 43.5% were of European ancestry, 12.4% were of African ancestry, 33.1% were of Asian ancestry, 9.7% were of Hispanic ancestry, and 1.3% were of other ancestries. Difference in eye disease prevalence, including AMD, among different ethnicities, points to a genetic component [24, 39]. It is further proposed that this differentiation could be linked to exogenous factors that are impacted by the culture such as diet and smoking [24].

2.5.3 Female Gender

Female gender has been associated with increased risk for development AMD in some studies [10, 30]. A study by Hammond et al [30] in a population of 88 non-smoking subjects (19-79 years of age) found a significantly lower macular pigment in females compared to males. The investigators suggested that the reduced macular pigment in females might be associated with a higher risk for AMD. Differentiation between genders may be linked to the longevity of females compared to males, which makes them more likely to develop age-related diseases [29, 46]. Also, life-style related factors such as diet, smoking or sun exposure have been associated with this gender differentiation [29]. It has also been reported that sex-dependent biological
differences could directly affect the pathogenic mechanism of some diseases, including AMD [29]. Shaw [47] found that women with more than one pregnancy were more likely to be diagnosed with AMD. Also, she reported that BMI and parity interact together as a predictor for AMD in women.

Female gender by itself may not be a risk factor for developing the disease, as AMD has been associated with other risk factors such as age and diet, among others [1]. The National Eye Institute (NEI), using data from 2010, reported the prevalence rate of AMD cases by gender. They stated that 65% of AMD cases were in women compared to 35% of cases in men [48]. Also, in a systematic review based on the world population up to 2010, Stevens et al [49] reported a higher prevalence of blindness in women than in men worldwide. They determined that of the 32.4 million blind people globally, 60% were women. This gender differentiation may also be linked to the fact that most of the eye disease studies have been carried out in older populations and, according to Zetterberg [29], predominantly in populations between 65-74 years of age.

2.5.4 Body Mass Index (BMI)

BMI has been utilized as a general indicator of obesity in several research studies related to AMD [25-28, 50]. Significant associations have been found between higher BMI and lower MPOD and higher risk for development of AMD [10, 26, 50]. A study by Seddon et al [26] stated that a higher BMI increases the risk for progression to advanced forms of AMD. They concluded that obesity may be a modifiable factor that can reduce the risk for developing late stages of AMD. Also, Hammond et al [50] showed an inverse relationship between BMI and MPOD in a population of 682 subjects with an average age of 29 years. They reported that individuals usually considered as obese have the lowest MPOD measures.
On the other hand, BMI has been considered an imperfect measure due to the lack of information regarding individuals’ body composition [27]. The relationship between individuals/populations in the BMI obese group and AMD has been inconsistent; some studies have found no associations, while others have found association but in specific population subgroups [4]. This inconsistency may be related to the fact that BMI is not a proxy for % body fat, and it may be further affected by factors such as ethnicity and differences in body build [51].

Body fat may have an impact on carotenoid supply to different tissues [52]. Research by Bovier et al [53] hypothesized that the adipose tissues may compete with the retina for xanthophyll carotenoid pigments as adipose tissue contains the highest concentration of carotenoids in the human body [52]. Therefore, a higher body fat percentage may be related to a lower MP [53] due to less uptake of carotenoids in the retina [50]. It has also been reported that individuals with high BMI’s perform less physical activity than those with lower BMI’s. Therefore, decreased physical activity may be linked to reduced risk for developing eye diseases, including AMD [26].

2.5.5 Dietary Patterns and Nutrition

Dietary patterns are considered a component of lifestyle [54]. It has been suggested by Chiu et al [54] that a diet high in healthy foods could be optimal for reducing AMD risk. Some of the dietary patterns associated with a decreased trend in the prevalence of AMD include the Caribbean, Oriental, Breakfast and Salad diets [54]. These patterns include fish, seafood, fruit, vegetables, and legumes, all of which contain important nutrients previously related with reduced prevalence of AMD [31, 54].

Nutrition has been considered one of the most significant modifiable factors for controlling and preventing the development of AMD [31]. Nutrients can modulate oxidative
damage to the retina caused by daily sunlight exposure [9] and metabolic activity [31]. The retina contains many lipids with double bonds, which makes it more susceptible to damage by reactive oxygen species [31]. On the other hand, evidence points to a potential positive role of long chain omega-3 fatty acids (FA) and macular xanthophyll pigments in the prevention of AMD [31, 55]. Long chain omega-3 fatty acids and fish intake were evaluated together in the Age-Related Eye Disease Study (AREDS) by SanGiovanni et al [56]. Results suggested a beneficial influence of dietary lipids at some stages of AMD. They concluded that dietary ω-3 long-chain polyunsaturated fatty acids may decrease the risk of progression from drusen to late stages of AMD [56]. On the other hand, the AREDS study demonstrated preventive benefits for the progression of AMD by daily oral supplementation with antioxidants, vitamins and zinc. The formulation used in the supplement was composed of vitamin C, Vitamin E (d-alpha-tocopherol), zinc, copper and vitamin A (beta-carotene) [31].

The Age-Related Eye Disease Study 2 (AREDS 2) evaluated L+Z together with antioxidants and vitamins to evaluate their relationship with AMD progression. The results demonstrated no significant relationship with AMD progression, and it was recommended that further exploration for evaluation of L+Z together with antioxidants and vitamins be performed [55]. Years later a study by Wu et al [57] evaluated the intake of lutein and zeaxanthin and other carotenoids with prevalence of AMD. These investigators concluded that the risk to advanced AMD was reduced and recommended an increased dietary consumption of a wide variety of fruits and vegetables [57].
2.6 Lipids and Essential Fatty Acids

Lipids are generally defined as a group of fatty substances important to living organisms [58]. Included in the group are phospholipids, integral components of cell membranes, [59] and triglycerides which include fats and oils. Fat molecules are composed of FA which are monocarbonic acids encountered in food in more than 40 different forms [60]. Fat in the diet is indispensable, because it provides us with essential FA required by the body [61].

Essential FA are those that cannot be synthetized de novo, that’s why they have to be consumed in the diet [62]. Linoleic acid (LA), an omega-6 FA, and the \( \omega \)-linolenic acid (ALA), an omega-3 FA [59, 61-63] (Figure 2) are considered essential. ALA is only found in plants, mainly unicellular algae, which tend to be consumed by different types of fish that provide these important omega-3 FA to the human diet [59]. On the other hand, LA can be found in cereal grains and products from the vegetable oil industry [64]. After a series of elongations and desaturations, ALA and LA are converted into DHA or EPA and arachidonic acid (AA), respectively (Figure 2) [59, 62, 63]. In humans, the rate of conversion of ALA to EPA and DHA is quite slow thus, the primary source of the long-chain polyunsaturated fatty acids (LC-PUFAs) is seafood [65]. The Dietary Guidelines for Americans 2015-2020 recommends for the general population about 8 ounces per week of a variety of seafood [66]. The ratio of EPA to DHA found in fish differs, because there are differences in fish metabolism, diets, water temperatures, and other factors [63].

EPA and DHA are known as omega-3 LC-PUFAs and are essential components of human nutrition [67]. Conversion of omega-3 LC-PUFAs from ALA is in direct competition with the production of AA from LA, because of the use of the same enzyme (\( \Delta6 \) - desaturase) (Figure 2) [63]. Prevalence of LA in the human diet, mainly in the Western industrialized
countries, has increased the ratio of omega-6/omega-3 fatty acids [63, 64]. Consequently, this imbalanced ratio has increased the presence of pro-inflammatory metabolic products from AA, such as prostaglandins, thromboxane, and others [64]. These metabolic products from AA in large quantities promote allergic and inflammatory disorders [64], cardiovascular disease, diabetes, and other afflictions [68].

Figure 2. Biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) using the precursors LA and ALA. (Reproduced from [65])
Increasing the consumption of food high in ALA, or the LCPUFAs (DHA and EPA) is important in the promotion of health [64]. A diet based primarily on dietary sources of ALA could lead to a deficiency of essential fatty acids (Table 1) because of the direct competition for Δ6-desaturase between ALA and LA. Therefore, it is important to consume foods from animal origins and algae that contain the omega-3 LCPUFAs in that form [68] (Table 2).

Table 1. Selected natural sources of ALA [69, 70]

<table>
<thead>
<tr>
<th>Food</th>
<th>ALA g/serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaxseed oil*</td>
<td>7.26</td>
</tr>
<tr>
<td>Chia seeds•</td>
<td>5.06</td>
</tr>
<tr>
<td>Flaxseed, whole*</td>
<td>2.35</td>
</tr>
<tr>
<td>Canola oil*</td>
<td>1.28</td>
</tr>
<tr>
<td>Soybean oil*</td>
<td>0.92</td>
</tr>
<tr>
<td>Black walnuts•</td>
<td>0.76</td>
</tr>
<tr>
<td>Mayonnaise*</td>
<td>0.74</td>
</tr>
<tr>
<td>Edamame, frozen,</td>
<td>0.28</td>
</tr>
<tr>
<td>Refried beans, canned,</td>
<td>0.21</td>
</tr>
<tr>
<td>Tilapia, cooked•</td>
<td>0.04</td>
</tr>
<tr>
<td>Kidney beans, canned</td>
<td>0.10</td>
</tr>
<tr>
<td>Baked beans, canned</td>
<td>0.07</td>
</tr>
<tr>
<td>Ground beef, 85% lean,</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Serving sizes: *1 tbsp., • 1 ounce, • 1/2 cup, • 3 ounces

Table 2. Selected natural sources of EPA and DHA [69, 70]

<table>
<thead>
<tr>
<th>Food</th>
<th>EPA g/serving</th>
<th>DHA g/serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon, Atlantic,</td>
<td>0.59</td>
<td>1.24</td>
</tr>
<tr>
<td>Salmon, Atlantic, wild</td>
<td>0.35</td>
<td>1.22</td>
</tr>
<tr>
<td>Herring, Atlantic</td>
<td>0.77</td>
<td>0.94</td>
</tr>
<tr>
<td>Sardines, canned in</td>
<td>0.45</td>
<td>0.74</td>
</tr>
<tr>
<td>Mackerel, Atlantic</td>
<td>0.43</td>
<td>0.59</td>
</tr>
<tr>
<td>Salmon, pink, canned</td>
<td>0.28</td>
<td>0.63</td>
</tr>
<tr>
<td>Trout, rainbow, wild</td>
<td>0.40</td>
<td>0.44</td>
</tr>
<tr>
<td>Oysters, eastern, wild</td>
<td>0.30</td>
<td>0.23</td>
</tr>
<tr>
<td>Sea bass</td>
<td>0.18</td>
<td>0.47</td>
</tr>
<tr>
<td>Tuna, light, canned in</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Tilapia, cooked</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Cod, Pacific</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Scallops</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Tuna, yellowfin</td>
<td>0.01</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*3 ounces
2.7 DHA in the Retina

DHA is an important nutrient which is highly concentrated in the phospholipids of retinal photoreceptor outer segments [6]. The transport of DHA to the photoreceptors starts with the consumption of DHA (22:6) and ALA (18:3, n-3) from the diet (Figure 3) [6, 7]. Subsequently, ALA is elongated and desaturated in the liver for conversion to DHA. Docosahexaenoic acid is esterified into phospholipids, which are secreted as lipoproteins and delivered to the RPE, passing through the choroid (Figure 3). The details of the mechanism used in this transport are poorly understood [7], but it is notable that phospholipids rich in DHA are taken from the sub-retinal circulation for deposition in the RPE [71]. Therefore, there is a high affinity of lipoproteins with acetylated DHA for delivery of DHA in to RPE and brain compared to other tissues [7].

![Figure 3. Pathway of DHA transport from the diet to the photoreceptor cells. Adapted from [72, 73]](image)

Transport of DHA from the liver to the RPE comes from the long loop (Figure 3) [71]. The first part of transport is accomplished using the inter-photoreceptor matrix to send the DHA to the inner segment of the photoreceptor. Docosahexaenoic acid is then taken from the inner
segment to be used for phospholipid biosynthesis or for other photoreceptor membranes [71].
DHA moves through the connecting cilium to the outer segment of the photoreceptor for
biogenesis of disk membranes [7, 73]. As new disks are synthesized in the base of the outer
membrane, old disks are pushed to the RPE [71].

Docosahexaenoic acid is continually replenished in part by recycling. This process is
carried out by the short loop which transports DHA to the outer segment via the inner segment to
be reused for new disk membranes [71] (Figure 3).
3. MATERIALS AND METHODS

3.1 Subject Recruitment

Young adults, 18-28 years of age, who were students at Louisiana State University (LSU) were recruited to participate in this study. The subjects were excluded from participating if they smoked or had been diagnosed with diabetes or eye diseases, or were underweight (BMI< 18.5). For recruiting purposes, flyers were distributed on the LSU campus. A digital version of the flyer was distributed to professors and they were asked to share the information in classes. It was also published on Facebook among different LSU student groups. Subjects who expressed interest in being a study participant were contacted via e-mail using an email account dedicated to the eye health study. Eligible subjects who agreed to participate were scheduled for a one-time appointment at the Eye Health Study Laboratory, Knapp Hall at LSU. During the visit, subjects completed a non-invasive eye test as well as a questionnaire regarding general health and foods consumed. It is important to mention that Institutional Review Board (IRB) approval was obtained before the start of the study (Appendix A).

A total of 516 participants 18 – 28 years old were recruited between 2012 and 2017 to assess MPOD. Most participants were female, had brown/black eyes, were white, and had a normal BMI [11] (Table 3). Of the 516 subjects who were recruited 31 were excluded from analysis due to apparent technical problems with the macular metrics densitometer. Six participants were also excluded from the analysis because the MPOD values showed a possible misunderstanding of equipment use by the participants. Missing data during analysis led to the exclusion of participants.
Table 3. Characteristic and nutrition (DHA+EPA, L+Z) of the subjects in the eye health study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subjects (n = 475)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.69 ± 0.12</td>
</tr>
<tr>
<td>Gender [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>300 (63)</td>
</tr>
<tr>
<td>Male</td>
<td>175 (37)</td>
</tr>
<tr>
<td>Irides Color [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Brown/Black</td>
<td>247 (52)</td>
</tr>
<tr>
<td>Blue</td>
<td>113 (24)</td>
</tr>
<tr>
<td>Green/Hazel</td>
<td>115 (24)</td>
</tr>
<tr>
<td>Ethnicity [n (%)]</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>307 (65)</td>
</tr>
<tr>
<td>Black</td>
<td>71 (15)</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>46 (10)</td>
</tr>
<tr>
<td>Asian</td>
<td>34 (7)</td>
</tr>
<tr>
<td>Alaska Native</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Biracial</td>
<td>9 (2)</td>
</tr>
<tr>
<td>BMI (kg/m²) [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5 to &lt;25.0)</td>
<td>217 (46)</td>
</tr>
<tr>
<td>Overweight (25.0 to &lt;30)</td>
<td>157 (33)</td>
</tr>
<tr>
<td>Obese (30.0 or higher)</td>
<td>101 (21)</td>
</tr>
<tr>
<td>MPOD (du)</td>
<td>0.343 ± 0.01</td>
</tr>
<tr>
<td>Family Hx of ED [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53 (11)</td>
</tr>
<tr>
<td>No</td>
<td>422 (89)</td>
</tr>
<tr>
<td>Consume F&amp;V high in L+Z [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>98 (21)</td>
</tr>
<tr>
<td>No</td>
<td>377 (79)</td>
</tr>
<tr>
<td>Consume seafood high in DHA+EPA [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>224 (47)</td>
</tr>
<tr>
<td>No</td>
<td>251 (53)</td>
</tr>
<tr>
<td>Total L+Z (dietary + supplement mcg)</td>
<td>1880.09 ± 149.56</td>
</tr>
<tr>
<td>Total DHA+EPA (dietary + supplement mg)</td>
<td>148.69 ± 17.06</td>
</tr>
</tbody>
</table>

Abbreviations: MPOD= macular pigment optical density, BMI= body mass index [11], Hx= history, ED= eye diseases, F&V= fruits and vegetables, DHA+EPA= docosahexaenoic acid and eicosapentanoic acid, L+Z= lutein+ zeaxanthin, du= density units. Mean ± standard error, n (%) percentage based on number of participants analyzed
*Recommendation by Dietary Guidelines for American 2010-2020, 250 mg/day of DHA+EPA [66]
3.2 Study Design

Eligible subjects were scheduled for a one-time (1-1.5 hours) appointment at the Eye Health Study Laboratory, Knapp Hall. A reminder email with the day and time was sent to all participants the day before the appointment. At the appointment, participants read and signed the consent form and completed a dietary survey and a health history form (Appendix B) which documented age, height (measured in the laboratory), weight (measured in the laboratory), ethnicity, eye color, family history of eye disease, diagnosis of eye disease, high cholesterol or blood pressure, and information about other health conditions. The health history form also provided information about nutrient supplementation, location where consented participants routinely ate, the frequency of fruit and vegetable consumption (weekly, monthly or yearly) and those most commonly consumed, frequency of consumption of seafood (weekly, monthly or yearly), kind of seafood consumed, and reasons for not consuming fruits, vegetables and seafood.

Information regarding the consumption of foods high in L+Z and DHA was obtained through the health history form and by using lists of foods (Appendix D) [70]. These lists reported the amount of L+Z in an extended list of foods (mainly vegetables and fruits) and information about the amount of DHA contained in different seafood.

Anthropometric measurements, including height and weight, were obtained using standard techniques. Height was measured using a stadiometer; subjects were instructed to stand without shoes and with heels together. Weight was measured using the TANITA Body Composition Analyzer Model TBF-300A. BMI was calculated using a web page of the National Institutes of Health [74], introducing height and weight of the participant in standard measures.
Brief explanations about eye physiology (macula) and the objectives of the study were provided to the subjects. A maculametrics instructional video was shown to the participants to explain how to use the instrument. For example, a section of the video explained how the subjects must rotate the knob on the instrument and when they needed to stop the rotation to permit recording of the data by the research assistant. Any additional questions about the use of the macular metric instrument were answered before the trial started. Participants completed 10 trials in two different stimuli (2 and 5). Usual dietary intake was recorded using a 24-hour dietary recall the same day of the appointment using the Nutrition Data System for Research program (NDSR) 2016. During the 24-hour dietary recall participants provided information (food and amounts) about their dietary intake in the previous day.

3.3 Assessment of MPOD Using the Macular Metrics Densitometer

MPOD was assessed using the instrument ‘Macular Metrics Densitometer’ which was calibrated daily for each participant to adjust the blue and green light emitting diodes (LEDs) based on the maculametrics instructions. This adjustment allowed the participants to see an even mixture of both LEDs, which is perceived as a stable target [72]. MPOD was measured in one eye based on the preference of the participants; previous research has shown that the amount of MP is the same in both eyes [75]. MPOD was measured with the HFP technique which generates an MP profile using green (550 nm; not absorbed) and blue (460 nm; maximally absorbed) LEDs in an inverse-yoked manner [34]. This means that if one of the lights increases, the other decreases, and vice versa. This manner avoids subject confusion during the test because the luminance of the target stays stable [32, 33].
Each participant customized the optimal flicker frequency (OFF) to minimize variances in the luminance readings. An increment or decrease of 1 Hz was made to the OFF if the subject did not perceive the null flicker area [32, 33]. During the test participants perceived a flickering target produced by the absorption of blue light luminance in the macular pigment. Then, they were instructed to rotate the subjects’ knob to adjust the amount of blue light and find the null flickering area, in which the target is perceived as stable [32, 33].

To measure macular pigment, the densitometer was set at a target location of 0.5 degree (stimuli #2) retinal eccentricity which is a located stimulus with a central fixation spot [32]. This stimulus measures the amount of blue light necessary to achieve null flicker at the fovea [33]. A reference location at 7 degrees (stimuli #5) was used to measure the amount of blue light necessary to achieve null flicker in the parafovea where macular pigment is presumed to be 0 [32, 33]. This stimulus employs an eccentrically located red LED as the fixation point [32]. The measurement value was manually documented by the experimenter and the subject’s knob was readjusted to not perceive the null flickering area. This process was repeated to complete a total of ten trials in each stimulus #2 and #5. Outliers were removed and were defined as values varied for the trial (±400). In those cases, participants were requested to complete additional readings. Subjects were directed to take breaks during the assessment to readjust their vision.

Manual measurements were entered electronically into the computer data base from which the stimuli’s measures were used to calculate the MPOD.

\[
\text{MPOD} = \log_{10}\left(\frac{\text{blue radiance for target 2}}{\text{green radiance for target 2}}\right) - \log_{10}\left(\frac{\text{blue radiance for target 5}}{\text{green radiance for target 5}}\right)
\]

[72]
3.4 Statistical Analyses

Data were analyzed using SPSS IBM Statistics 24. Levene’s test of equality of variances was conducted to assess homogeneity of variances. Together with Levene’s test, an independent-samples t-test was conducted to determine if differences existed between MPOD and the following factors: gender, ethnicity and BMI groups. Differences between MPOD and the following factors: eye colors and combined BMI (normal+ overweight vs obese) was examined using an analysis of variance (ANOVA). The difference between MPOD and eye color was also evaluated using a Welch’s adjusted F ratio because the Ho was not supported. The relationship between MPOD and the diet was examined using a sequential linear regression in which combined BMI and gender were added as predictive factors to the model.

An independent-samples t-test and a Levene’s Test of Equality of Variances were conducted to determine if differences existed between dietary intake of L+Z and DHA+EPA and the following factors: gender and ethnicity. Differences between dietary intake of L+Z and DHA+ EPA and BMI groups was examined using an analysis of variance (ANOVA). The level of significance was set at p≤0.05.
4. RESULTS

MPOD, Modifiable and Non-Modifiable Factors

Results obtained by the comparison of MPOD and the modifiable and non-modifiable factors are follows. Females had significantly lower MPOD (M= 0.3295, SE= 0.01) compared to males (M= 0.3659, SE= 0.01), t (473) = -2.336, p= 0.028. There were no differences in MPOD for white subjects compared to all other ethnicities (p= 0.093). There were also no differences in MPOD for eye color (blue/gray, green/hazel, brown/black) F (2, 472) = 0.398, p > 0.05.

Considering BMIs there were no differences among BMI groups (normal, overweight, obese) for MPOD, F (2, 472) = 2.374, p > 0.05. However, on average, the obese group had lower MPOD (M= 0.3117, SE= 0.02) compared to normal (M= 0.3488, SE= 0.01) and overweight (M= 0.3548, SE= 0.01) or to the combined groups (normal+ overweight) (M= 0.3513, SE= 0.01).

There were differences between the combined groups (normal+ overweight) and obese group t (473) = 2.152, p= 0.032.

Table 4. Multiple regression of MPOD and dietary intake (diet and supplementation) of DHA+EPA and L+Z with categorical predictor factors (gender and BMI).

<table>
<thead>
<tr>
<th>Model 1</th>
<th>B</th>
<th>SE</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.33</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.04</td>
<td>0.02</td>
<td>0.11*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th>B</th>
<th>SE</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.38</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.03</td>
<td>0.02</td>
<td>0.10*</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>-0.04</td>
<td>0.02</td>
<td>-0.10*</td>
</tr>
<tr>
<td>Total Intake of DHA+EPA (mg)</td>
<td>3.26E-05</td>
<td>0.00</td>
<td>0.07</td>
</tr>
<tr>
<td>Total Intake of L+Z (mcg)</td>
<td>-5.37E-07</td>
<td>0.00</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Note: R²= 0.01 for Model 1, ΔR² = 0.02 for Model 2 (p < 0.05). *p < 0.05

DHA= docosahexaenoic acid, EPA= eicosapentaenoic acid, L+Z=lutein+ zeaxanthin
Employing sequential regression, the addition of DHA+EPA and L+Z improved MPOD beyond that provided by differences in gender and BMI. Table 4 displays the regression coefficients (B) and intercept, and the standardized regression coefficients (β) after adding all independent variables to this model. Gender and BMI contributed significantly to the model; the adjusted R² value of 0.019 indicates that approximately 2% of the variability in MPOD values is predicted by the dietary intake of DHA+EPA and L+Z. This model is a significant fit of the data overall (p= 0.012).

Diet

Males reported significantly higher dietary intake of DHA + EPA (mg) (M= 213.47, SE= 35.14) compared to females (M= 110.91, SE= 17.273), t (473) = -2.620, p= 0.009. When normalized for dietary DHA, based on kcal/day, there were no differences for females (M= 45.16, SE= 7.23) compared to males (M= 67.78, SE= 11.22) (p= 0.091). On the other hand, females reported on average a lower consumption (43%) for seafood high in DHA + EPA, such as salmon and tuna, compared to males (54%) (Figure 4). Only 8% of females and 19% of males from the population of 475 subjects consumed the amount of DHA + EPA per day that is recommended by the Dietary Guidelines for Americans 2015-2020 (250 mg/day) (Figure 6). In general, just 12% of the participants were meeting the recommendation of consuming 250mg DHA+EPA/day.

There were also no differences in dietary intake of L+Z (mcg) for females (M= 1661.08, SE= 165.73) compared to males (M= 2255.53, SE= 288.44) (p= 0.075). When dietary intake of L+Z (mcg) was normalized based on kcal/day, there were no differences (p= 0.402) between females and males. Preference for fruit and vegetables rich in L + Z, such as spinach and kale, was slightly higher in females (22%) compared to males (18%) (Table 4) and (Figure 5).
Participants reported consuming 7 portions of fruits and vegetables per week, compared to the recommendation by the Dietary Guidelines for Americans 2015-2020, of $2\frac{1}{2}$ cup of vegetables per day and 2 cups of fruits.

Figure 4. Percentage of participants by gender consuming seafood high in docosahexaenoic acid+ eicosapentaenoic acid (DHA + EPA)

Figure 5. Percentage of participants by gender consuming fruits and vegetables (F&V) high in lutein+ zeaxanthin (L+Z)

Figure 6. Percentage of females vs males meeting docosahexaenoic acid+ eicosapentaenoic acid (DHA+EPA) Dietary Guidelines for Americans 2015-2020 [66]

There were no differences in dietary intake of DHA+ EPA (mg) for white subjects compared to the other ethnicities (p= 0.068). On average, white subjects consumed less DHA+
EPA (M= 123.68, SE= 18.74) compared to the other ethnicities (M= 194.41, SE= 33.76). There were also no differences in dietary intake of L+ Z for white subjects compared to the other ethnicities (0.712). On average white subjects consumed more L+ Z (M= 1920.97, SE= 184.07) than the other ethnicities (M= 1805.38, SE= 256.93).

On the other hand, there were no differences for BMI groups and dietary intake of DHA+EPA [F (2, 472) = 0.038, p > 0.05] and L+ Z [F (2, 472) = 2.488, p > 0.05]. However, on average the normal BMI group reported a higher dietary intake of L+ Z (M= 2242, SE= 253.11) compared to overweight (M= 1599.25, SE= 222.81) and obese (M= 1539.07, SE= 275.23) groups.
5. DISCUSSION

Few studies have evaluated MPOD in young adults to the best of our knowledge [76-78]. The uniqueness of the current study lies in the fact that MPOD was evaluated in a young population and related to known modifiable and non-modifiable factors associated with the development of AMD. Modifiable factors evaluated in the current study included BMI and diet; non-modifiable factors included eye color, ethnicity and gender [1, 4]. The current study showed that MPOD values in this college-aged adult population averaged 0.343 ± 0.01, which is similar to values reported in previous studies in healthy subjects (age range: 18-30 years) using heterochromatic photometry range (0.3 to 0.45) [76, 77].

In the current study, females had lower MPOD values compared to males. This finding supports previous study findings that point to gender differentiation in MPOD, with women at higher risk of developing AMD [30]. Previous work has attributed this difference in risk to various aspects including longevity (age), life-style factors (diet, smoking) [29], and pregnancy [47]. The current study controlled for age and smoking based on exclusion criteria. However, pregnancy (current or previous) was not a recruitment criterion, and this has been a factor previously linked to prevalence and development of AMD [47]. It was suggested by Shaw [47] that more pregnancies may increase the risk to develop AMD on the basis of lower MPOD values. This risk is based on the transfer of DHA from the mother to the fetus, which lowers the supply of DHA for the mother’s tissues [47]. Previous research has also reported a higher risk and prevalence of AMD mainly in white subjects [23, 24]. Our results did not find differences among ethnicities and MPOD.

Differentiation in the development and progression of AMD has been reported for variation in eye color [12, 42]. This differentiation has been linked to the amount of melanin and
carotenoids in the eye [43], with the brown iris containing the highest amount. Melanin reduces damage to the retina by regulating the amount of light passing through the eye [44]. In the current study, our findings do not suggest differentiation in MPOD among participants’ eye colors, which could be linked to the fact that most of the subjects in the study had black/brown eyes (52%) (Table 3); or it could be linked to the young age of the participants.

BMI as a modifiable factor also has been linked to the prevalence and development of AMD in several studies [10, 26, 50]. This assumption is founded on the relationship observed between high BMI values and % body fat. Body fat could directly affect the supply of carotenoids in the retina, since adipose tissue contains the highest concentrations of carotenoids in the human body and this leads to a direct competition with the retina for these carotenoids [52]. Our findings do not suggest a differentiation among BMI groups and MPOD. However, we found differentiation in MPOD between the obese group versus the combined normal weight + overweight group. This differentiation underscores the previous assumption which stated that people with higher BMI could have higher % body fat, which could lead to a higher risk for development of AMD. Based on our data, we proposed that high BMI values (obese category) may affect macular health early in life. This difference is supported by studies by Hammond et al [50] and Zhang et al [79] who found relationships between high BMI values (obese category) and MPOD in the prevalence or incidence of AMD. Additionally, in the current study MPOD varied with dietary intake of DHA+EPA and L+Z included along with gender and BMI as possible predictors of the model. The model was positively affected by BMI and gender and it revealed that just 2% of the variability was related to DHA+EPA and L+Z.

In the current study, young adult females had a lower dietary intake of DHA+EPA compared to males, and just 43% of them routinely consumed seafood high in DHA + EPA. The
recommendation of the Dietary Guidelines for Americans 2015-2020 for a daily average of 250 mg of DHA+EPA [66] was met by only 8% of females and 19% of males (Figure 6), and in general by just 12% of the participants in the study.

Dietary consumption of L+Z by females was low and this may be the result of low consumption of fruits and vegetables rich in those carotenoids (Figure 5). Just 22% of females reported consuming fruits and vegetables high in L+Z. Dietary Guidelines for Americans 2015-2020 recommends the consumption of \(2\frac{1}{2}\) cup of vegetables per day and 2 cups of fruits, based on a diet of 2000 kilocalories. Our findings suggest that on average this young adult population consumed 7 fruit or vegetables per week, which is equivalent to 1 fruit or vegetable per day.

Consumption of dietary DHA+EPA for this young adult population was similar across the ethnicities evaluated. White subjects consumed slightly more L+Z than the other ethnicities, which points to a higher consumption of fruits and vegetables by this population. This finding supports the study by Dubowitz et al [80], who found that white subjects, on average, tend to consume higher amounts of fruits and vegetables compared to other ethnicities.

This exploratory study provided important data that can guide us in future studies of eye health, diet, and BMI in young adults. Further studies can focus on the following: (i) evaluating MPOD and its relationship with % body fat in similar populations; (ii) increasing the number of subjects with light eye color and from other ethnicities; (iii) recording more 24-hours recalls for average dietary intake of nutrients; and (iv) exploring the benefits of increasing DHA+EPA and L+Z as an intervention for MPOD in young adults.
6. CONCLUSION

The purpose of this study was to explore macular health by examining MPOD in a young adult population and MPOD’s relationship to factors known to be associated with developing AMD. This exploratory study points to female gender and high BMI as variables related to MPOD. However, as there were fewer participants in some BMI categories (normal = 217 vs overweight = 157 vs obese = 101), it is important to increase the number of subjects in those BMI groups, to assess if there is a difference.

The Dietary Guidelines for Americans 2015-2020 recommend that adults consume 250 mg DHA+EPA/day because of the benefits for general health. Also, it is recommended to consume $\frac{9}{2}$ cups of vegetables and 2 cups of fruits per day, based on a 2000 kcal/day diet. Consumption of seafood and fruits and vegetables as sources of DHA+EPA and L+Z respectively, will promote benefits in retinal and macular health.

Furthermore, because dietary consumption of DHA+EPA and L+Z was low for both females and males in the current study, it is concluded that early education about protection of the eye, eye health, diet and BMI should be provided to young adults to make them aware of the long-term benefits.
LITERATURE CITED


APPENDIX A: IRB APPROVAL FORM

To: Carol J. Lammi-Keefe, Ph.D., R.D.
    Alma Beth Clark Professor and Head
    Human Nutrition and Food

From: Michael Keenan, Ph.D.
      Chair, LSU AgCenter IRB

Re: Changes in IRB Protocols HE07-2a and H08-1

Date: January 9, 2009

Your amendments to your two protocols are approved. Protocol #HE07-2a: “Evaluation of eye health as it relates to foods usually eaten” with 18-30 year old female subjects only, has been amended to “Evaluation of eye health as it relates to foods usually eaten by college-aged students” and includes both male and female students ages 18-28 years old. Protocol #H08-1: “Fats of breast milk of women with or without gestational diabetes mellitus” has been amended to increase the age range of the female subjects from 18-35 years of age to 18-40 years of age.
Health History Form

Name: _______________________
Code #: ________

Date: ________________________

Age: ________

Weight: ________ lbs. (to be completed by investigator)

Height: ________ inches (to be completed by investigator)

BMI: ________ (to be completed by investigator)

1. Ethnicity (check one):
   - □ American Indian or Alaska Native
   - □ Asian
   - □ Black or African American
   - □ Native Hawaiian or Other Pacific Islander
   - □ White
   - □ Hispanic or Latino
   - □ Others (specify): ______________________

2. Eye color:
   - □ Blue
   - □ Brown
   - □ Green
   - □ Hazel
   - □ Black
3. Do you have a family history of eye disease?  ______Yes ______ No
   If yes, name disease if known ______________________________

4. Have you been diagnosed with eye disease?  ______ Yes ______ No
   If yes, name disease ______________________________

5. Do you have high cholesterol or high blood pressure?  _____ Yes _____ No

6. Do you have any other health conditions not mentioned above? (For example, have you been diagnosed with diabetes, hyper(hypo)thyroidism, heart disease, etc). If yes, please list.
   ______ Yes ______ No
   List:_____________________________________________

7. Do you smoke cigarettes, cigars or pipes?  ______ Yes ______ No
   If yes, how many per day ______ per week ______ per month ______

8. Do you drink alcoholic beverages?  ______ Yes ______ No
   If yes, how many per day ______ per week ______ per month ______

9. Do you use any nutritional supplements?  _____ Yes _____ No
   If yes, which ones? (Include brand name if known)________________________

10. Do you eat most of your meals away from home?  _____ Yes _____ No
    If yes, where do you usually eat? ________________________________
11. How many times do you eat fruits and vegetables?

   _____ per week

   (or) _____ per month

   (or) _____ per year

12. List the fruits and vegetables most commonly eaten

   1. ____________________

   2. ____________________

   3. ____________________

   4. ____________________

13. Below, please circle the fruits and vegetables that you eat:

   Kale
   Turnip Greens
   Collards
   Spinach
   Broccoli
   Corn
   Peas
   Romaine Lettuce (also called cos lettuce)
   Carrots
   Brussels sprouts
   Green Beans
   Tangerines and tangerine juice
   Oranges and orange juice
   Okra
   Celery
   Tomatoes and Tomato juice
   Melons
   Peaches
   Papayas

14. How many times do you eat seafood (fresh, frozen or canned)?

   _____ per week

   (or) _____ per month

   (or) _____ per year
15. List the type of seafood most commonly eaten (if fish, what kind, example: salmon or catfish?)

1. ____________________
2. ____________________
3. ____________________
4. ____________________

16. If you don’t consume fresh fruits and vegetables frequently what are the main reasons?

☐ Too expensive
☐ I don’t know how to prepare them
☐ I don’t not buy my own groceries
☐ I don’t like them
☐ They don’t have long shelf life
☐ Other: ______________________

17. If you don’t consume seafood frequently what are the main reasons?

☐ Too expensive
☐ I don’t know how to prepare them
☐ I don’t not buy my own groceries
☐ I don’t like them
☐ They don’t have long shelf life
☐ Other: ______________________
## APPENDIX C: SELECT FOOD SOURCES HIGH IN DHA AND L+Z

[https://ndb.nal.usda.gov/ndb/search/list](https://ndb.nal.usda.gov/ndb/search/list)

<table>
<thead>
<tr>
<th>Food Description</th>
<th>DHA (mg) per 3 oz. (85 g)</th>
<th>Preparation Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon, Atlantic</td>
<td>1238</td>
<td>[farmed, cooked, dry heat]</td>
</tr>
<tr>
<td>Whitefish</td>
<td>1025</td>
<td>[mixed species, cooked, dry heat]</td>
</tr>
<tr>
<td>Herring, Atlantic</td>
<td>939</td>
<td>[cooked, dry heat]</td>
</tr>
<tr>
<td>Trout</td>
<td>575</td>
<td>[mixed species, cooked, dry heat]</td>
</tr>
<tr>
<td>Bass</td>
<td>473</td>
<td>[mixed species, cooked]</td>
</tr>
<tr>
<td>Squid</td>
<td>323</td>
<td>[mixed species, cooked, fried]</td>
</tr>
<tr>
<td>Drum</td>
<td>313</td>
<td>[freshwater, cooked, dry, heat]</td>
</tr>
<tr>
<td>Snapper</td>
<td>232</td>
<td>[mixed species, cooked, dry heat]</td>
</tr>
<tr>
<td>Tuna</td>
<td>201</td>
<td>[skipjack, fresh, cooked, dry heat]</td>
</tr>
<tr>
<td>Oyster</td>
<td>178</td>
<td>[eastern, wild, cooked, dry heat]</td>
</tr>
<tr>
<td>Shrimp</td>
<td>120</td>
<td>[crustaceans, shrimp, mixed species, cooked, moist heat]</td>
</tr>
<tr>
<td>Catfish</td>
<td>116</td>
<td>[channel, wild, cooked, dry heat]</td>
</tr>
<tr>
<td>Tilapia</td>
<td>113</td>
<td>[cooked, dry heat]</td>
</tr>
<tr>
<td>Flatfish (Flounder and sole species)</td>
<td>112</td>
<td>[cooked, dry heat]</td>
</tr>
<tr>
<td>Crab</td>
<td>96</td>
<td>[crustaceans, crab, dungeness, cooked, moist heat]</td>
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<tr>
<td>Scallop</td>
<td>88</td>
<td>[mollusks, scallop, (bay and sea), cooked, steamed]</td>
</tr>
<tr>
<td>Food Description</td>
<td>Common Measure</td>
<td>Lutein + zeaxanthin (μg)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Kale</td>
<td>1 cup</td>
<td>23720</td>
</tr>
<tr>
<td>Turnip Greens</td>
<td>1 cup</td>
<td>12154</td>
</tr>
<tr>
<td>Collards</td>
<td>1 cup</td>
<td>11774</td>
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<tr>
<td>Spinach</td>
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<tr>
<td>Peas</td>
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<tr>
<td>Brussels sprouts</td>
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<tr>
<td>Broccoli</td>
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<td>1685</td>
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<tr>
<td>Corn</td>
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<td>1350</td>
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<tr>
<td>Romaine Lettuce</td>
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<tr>
<td>Carrots</td>
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<td>Green Beans</td>
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<tr>
<td>Okra</td>
<td>1 cup (slices)</td>
<td>624</td>
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<tr>
<td>Celery</td>
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<tr>
<td>Tangerines and tangerine juice</td>
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<tr>
<td>Orange</td>
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<tr>
<td>Tomatoes</td>
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<td>Peaches</td>
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<td>Papayas</td>
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<td>Melons</td>
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Génesis Gisselle Guerra Gaitán was born in David, Chiriquí, Panama. She did her Bachelor of Science in Escuela Agrícola Panamericana, El Zamorano in Valle del Yegüare, Honduras. She got her degree in food sciences and technology in December, 2014. In 2015 she began an internship in the School of Nutrition and Food Sciences at Louisiana State University working in human nutrition research. She began her master’s degree program at Louisiana State University School of Nutrition and Food Sciences in Spring 2016 with a concentration in sciences in human nutrition. She is a student member of the American Society of Nutrition.