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Pregnancy and the relationship to age-related macular degeneration

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**PREGNANCY AND THE RELATIONSHIP TO AGE-RELATED
MACULAR DEGENERATION**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment for the
requirements for the degree of
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by

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ABSTRACT

The Macular Study was a case control study that evaluated if parity and other participant characteristics predicted the diagnosis of age-related macular degeneration (AMD). Women, compared to men, are at higher risk for AMD. AMD is one of the leading causes of blindness in the elderly population [1]. Docosahexaenoic acid (DHA, 22:6n-3) is a long-chain fatty acid that is essential for the structure and function of the eye. During pregnancy the growing fetus depletes the maternal stores of DHA through placental transfer. The fetus needs an ample supply of DHA for proper retinal and central nervous system development. To date there is no research evaluating the number of pregnancies and their effect on development of AMD. We posed the question: “Does the number of pregnancies have an effect on the development of AMD in women?” Degree of AMD was documented and evaluated by four different eye doctors in Baton Rouge for 501 women. The women in the study completed a health history form that included demographic information, information about past pregnancies, and general health.

Using analysis of variance (ANOVA), women with a higher number of births were more likely to be diagnosed with early, intermediate or advanced AMD versus those women never diagnosed (3.27 ± 0.19 , 3.64 ± 0.22 , 3.33 ± 0.24 versus 2.53 ± 0.15 , number of children $p < 0.0001$). Numerous risk factors were considered, along with parity, in subsequent analyses; these were age, race, eye color, smoking history, vitamin intake, fish oil intake, family history of AMD, history of hypertension, and body mass index (BMI). Using backwards-stepwise regression the most significant risk factors predicting the diagnosis of AMD were determined ($p \leq 0.01$) and entered into a logistic regression model. Age, parity, BMI, and BMI by parity significantly predicted the

diagnosis of AMD. As age, BMI and the number of pregnancies increased, the probability of being diagnosed with AMD also increased.

In conclusion, older women, with a higher BMI, who have had more pregnancies, were more likely to have AMD compared to younger women with a lower BMI and fewer pregnancies. It is important that future studies consider parity as a possible risk for AMD, especially as it relates to other participant characteristics. Such studies may provide insight as to why women are at greater risk for AMD.

CHAPTER 1

INTRODUCTION

Age related macular degeneration (AMD) is one of the leading causes of blindness in the elderly population [1]. AMD is the degeneration of the macula area in the eye. It leads to distorted central vision and can eventually lead to blindness in more advanced forms. AMD is currently untreatable and currently there are only recommendations that have not been completely proven to slow down the development of AMD. Women have been shown to be at higher risk for development of AMD and here we speculate that this high risk could be associated with pregnancy.

During pregnancy the fetus depends on the mother for all nutrients to develop. Docosahexaenoic acid (DHA, 22:6n-3), a long-chain n-3 fatty acid is an important nutrient that the fetus must obtain directly through placental transfer from the mother [2]. DHA is necessary for the fetus to have proper retinal and brain development [3]. It is found in high concentrations in the retinal photoreceptors where it is needed for photoreceptors to function properly. However, consumption of DHA in the average American woman's diet, especially pregnant women, is low [4] and this may cause harm to the mother's eye sight in the future, especially in women who have had multiple pregnancies. The relationship between number of pregnancies and retinal health was explored in the current study.

JUSTIFICATION

DHA's role in the eye is not completely understood. DHA is an important long-chain polyunsaturated fatty acid that is recognized for its role in proper brain and retinal development. A growing fetus obtains DHA through placental transfer from the mother.

This transfer depletes maternal DHA stores. After pregnancy it can be difficult for the mother to return her DHA stores back to pre-pregnancy levels [5].

In the retina, DHA protects the photoreceptors and offers neuroprotective properties [6]. Although little is known about how DHA protects the macular pigment, Johnson et al. [7] hypothesized that the mechanism involves DHA's role in the transport of lutein and zeaxanthin through the circulation via high-density lipoproteins (HDL) to the macula. The macular pigment (MP) is composed of the carotenoids lutein and zeaxanthin which help protect the macula by filtering blue light which causes damage to the photoreceptors [8].

ASSUMPTIONS

It is assumed that women who participate in the study will give correct information about their health and past pregnancies to the nurses recording the information. It is also assumed that the nurses and the doctors will document the correct information following the guidelines in the study.

RESEARCH HYPOTHESIS

Women who have had a higher number of pregnancies will have a higher risk of diagnosis with AMD.

OBJECTIVE

To evaluate parity and other known predictors of AMD among women diagnosed with AMD.

LIMITATIONS

1. Self-reported health history.
2. Observational study at one time point; not a longitudinal study.

CHAPTER 2

LITERATURE REVIEW

LIPIDS

Lipids are hydrophobic components comprised of carbon, hydrogen, and oxygen atoms [9]. Major classes of lipids include fatty acids, triacylglycerols, phospholipids, and sterols [9]. Lipids are important sources of energy, constituents of cells, membranes, hormones, and mediators of electron transport [10]. Fatty acids are the most abundant class of lipids that are essential to the function and structure of the eye.

Fatty acids contain a carboxylic acid group (-COOH) at one end and a methyl group (-CH₃) at the other end. The different groups at the opposite ends of the fatty acid allow fatty acids to have polar (-COOH) as well as non-polar, hydrophobic (-CH₃) characteristics [10]. Fatty acids classified as saturated fatty acids (SFA) are those that contain only carbon-carbon single bonds, while unsaturated fatty acids contain carbon-carbon double bonds. The double bonds imbue the structure with flexibility. Monounsaturated fatty acids (MUFA) contain one carbon-carbon double bond. Polyunsaturated fatty acids (PUFA) contain more than one carbon-carbon double bond [9].

Fatty acids are also further classified according to chain length. Fatty acids exceeding a 12-carbon chain are considered long chain fatty acids [9]. Longer chain fatty acids have higher melting and boiling points. The longer chain fatty acids are also more water insoluble, underlining the need for complex processes for digestion, absorption, transport, and circulation of long chain fatty acids [9].

Fatty acids exist in either the “cis” or “trans” configuration. The majority of naturally occurring fatty acids are found in the “cis” form, which means that the hydrogen atoms are positioned on the same side of the double bond [9]. If the hydrogen atoms are on opposite sides of the double bond, the fatty acid is in the “trans” configuration [9]. Dietary “trans” fatty acids usually result from the partial hydrogenation of fats and oils during processing [10].

The omega “ω” or “n” nomenclature is used in nutritional sciences to name fatty acids. The omega nomenclature categorizes fatty acids into groups based on where the first double bond is located relative to the methyl group [9]. For example, in the case of docosahexaenoic acid (DHA, 22:6n-3), the 22 indicates the total number of carbons, 6 represents the total number of double bonds, and the location of the first double bond from the methyl group is signaled by the number following n. A methylene group (-CH₂-) separates double bonds.

ESSENTIAL FATTY ACIDS AND THE FATTY ACID DERIVATIVES

Essential fatty acids (EFA) are fatty acids that are needed by the body for growth and development. The two EFAs linoleic acid (LA, 18:2n-6) and alpha linolenic acid (ALA, 18:3n-3) must be obtained from the diet because the body does not make them. Humans cannot make these fatty acids because humans lack specific enzymes to introduce additional double bonds to the carbon chain before the 9 position from the methyl group [10]. The longer chain polyunsaturated fatty acid (LCPUFA) arachidonic acid (AA, 20:4n-6) can either come from the shorter 18-carbon chain acid (18:2n-6) or can be obtained directly from the diet. The LCPUFA DHA comes primarily from the diet

because enzyme competition results in very little DHA being synthesized from the shorter 18-carbon chain acid (ALA).

The shorter chain fatty acids, LA and ALA, cannot be synthesized by humans but once consumed they can be desaturated and elongated to the LCPUFA's through a process taking place in the liver (Figure 1). Although ALA and LA are both needed in the human diet, studies of the average American diet indicate that people consume significantly more omega 6 than omega 3 fatty acids [11]. Additionally, as noted here, very little ALA is converted to the long chain derivatives.

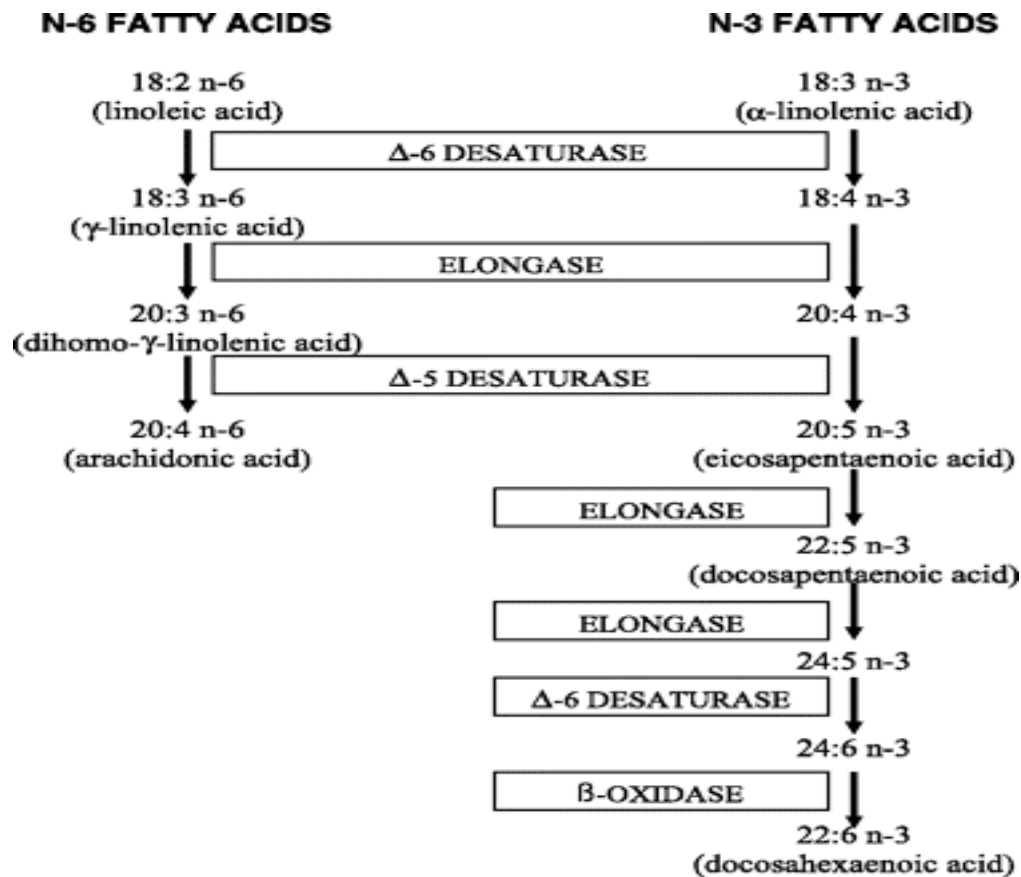


Figure 1: Pathways of LA and ALA and enzymes involved (Adopted from [12]).

LA is converted to longer chain fatty acids, including AA. AA is very abundant in the American diet because it is found in animal meats and processed foods made from animal fats. Arachidonic acid is involved in the cell signaling pathways and cell division [4]. It is an eicosanoid precursor that plays an important role in inflammation, and cyclooxygenase and lipoxygenase pathways, which modulate platelet aggregation, smooth muscle contraction, and vascular constriction [10]. LA is first converted to gamma- linolenic acid by n-6 desaturase and then elongated to dihomo-gamma-linolenic acid (DGLA) [11]. DGLA is converted to AA, which produces the pro-inflammatory prostaglandins.

ALA can be converted to the longer chain fatty acid including eicosapentaenoic acid (EPA, 20:5, n-3) and DHA. EPA is an eicosanoid precursor involved with anti-inflammatory prostaglandins and DHA is a central determinant of brain and visual system development [4]. ALA is converted to stearidonic acid by n-6 desaturase, which is then elongated to eicosatetraenoic acid [11]. Eicosatetraenoic acid is converted to EPA by n-5 desaturase [11]. EPA is elongated to docosapentaenoic acid, which is further elongated, desaturated, and β -oxidized to produce DHA [11]. However, the conversion of ALA to EPA and particularly DHA, lacks efficiency due to the rate limitation introduced by the n-6 desaturase during the process of long chain conversion of PUFA precursors [13]. Moreover, if a person has higher plasma levels of LA (typical in the diets of Americans), research has shown that there may be a decrease in the conversion of ALA to EPA and DHA, and an increase in the conversion of LA to AA because of the competition for the n-6 desaturase [14]. It is estimated that adults convert only about 0.2% to 9% of ALA to EPA [4]. Therefore, it is important that pregnant women obtain their nutritional

requirements for EPA and DHA directly from the diet as preformed acids to meet their needs, as well as the needs of the growing fetus.

Physiologically, EPA and AA are precursors to eicosanoids. Eicosanoids are hormone-like molecules that are necessary for numerous physiological processes in the human body, such as movement of calcium, muscle contraction and relaxation, control of fertility, cell division, and growth [15]. The eicosanoids for EPA and AA produce different subgroups of substances known as prostaglandins, leukotrienes, and thromboxanes (Figure 2) [15]. The eicosanoid subgroups produced by EPA tend to have an anti-inflammatory effect and the subgroups produced by AA tend to have more of an inflammatory response. However, the body requires the production of both types of eicosanoid subgroups because anti-inflammatory properties as well as pro-inflammatory properties are required by the body to function properly.

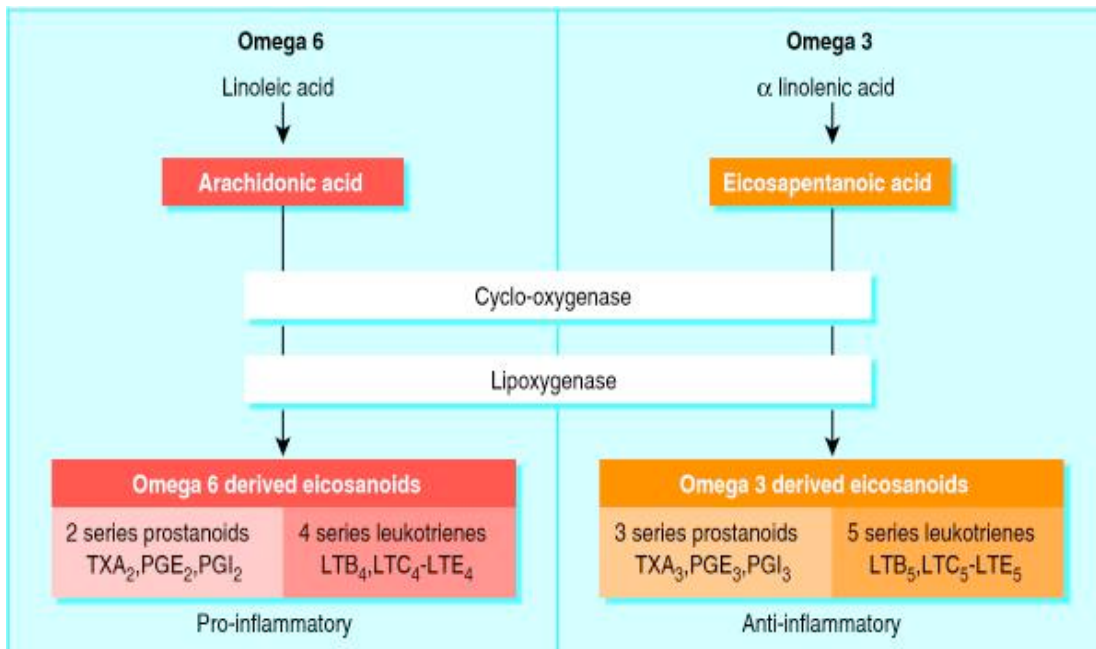


Figure 2: Eicosanoid pathways from n-3 and n-6 (Adopted from [16]).

Some sources of ALA are walnuts, firm tofu, and flaxseed oil (Table 1). DHA is found naturally in deep-water fish because they eat algae. Table 2 list different types of fish that are high in DHA such as salmon and tuna. Some sources of LA are pine nuts, corn oil, and sunflower oil (Table 3).

Table 1: Naturally Occurring Sources of Alpha-linolenic Acid [17]

| Some Food Sources of Alpha-linolenic Acid (18:3n-3) | | |
|--|----------------|---------------------------------|
| Food | Serving | Alpha-Linolenic acid (g) |
| Flaxseed oil | 1 tablespoon | 7.3 |
| Walnuts, English | 1 oz | 2.6 |
| Flaxseeds, ground | 1 tablespoon | 1.6 |
| Walnut oil | 1 tablespoon | 1.4 |
| Canola oil | 1 tablespoon | 1.3 |
| Soybean oil | 1 tablespoon | 0.9 |
| Mustard oil | 1 tablespoon | 0.8 |
| Tofu, firm | ½ cup | 0.7 |
| Walnuts, black | 1 oz | 0.6 |

Table 2: Naturally Occurring Sources of EPA and DHA [17]

| Some Food Sources of EPA (20:5n-3) and DHA (22:6n-3) | | | | |
|---|----------------|----------------|----------------|--|
| Food | Serving | EPA (g) | DHA (g) | Amount providing 1 g of EPA + DHA |
| Herring, Pacific | 3 oz* | 1.06 | 0.75 | 1.5 oz |
| Salmon, Chinook | 3 oz | 0.86 | 0.62 | 2 oz |
| Sardines, Pacific | 3 oz | 0.45 | 0.74 | 2.5 oz |
| Salmon, Atlantic | 3 oz | 0.28 | 0.95 | 2.5 oz |
| Oysters, Pacific | 3 oz | 0.75 | 0.43 | 2.5 oz |
| Salmon, sockeye | 3 oz | 0.45 | 0.60 | 3 oz |
| Trout, rainbow | 3 oz | 0.40 | 0.44 | 3.5 oz |
| Tuna, canned, white | 3 oz | 0.20 | 0.54 | 4 oz |
| Crab, Dungeness | 3 oz | 0.24 | 0.10 | 9 oz |
| Tuna, canned, light | 3 oz | 0.04 | 0.19 | 12 oz |

*A 3-oz serving of fish is about the size of a deck of cards.

Table 3: Naturally Occurring Sources of Linoleic Acid [17]

| Some Food Sources of Linoleic Acid (18:2n-6) | | |
|--|--------------|-------------------|
| Food | Serving | Linoleic Acid (g) |
| Safflower oil | 1 tablespoon | 10.1 |
| Sunflower seeds, oil roasted | 1 oz | 9.7 |
| Pine nuts | 1 oz | 9.4 |
| Sunflower oil | 1 tablespoon | 8.9 |
| Corn oil | 1 tablespoon | 7.3 |
| Soybean oil | 1 tablespoon | 6.9 |
| Pecans, oil roasted | 1 oz | 6.4 |
| Brazil nuts | 1 oz | 5.8 |
| Sesame oil | 1 tablespoon | 5.6 |

DHA located in the photoreceptors of the eye and is necessary for the photoreceptors to function properly (Figure 3). It is also involved in neural development and structural integrity of membranes (Figure 3).

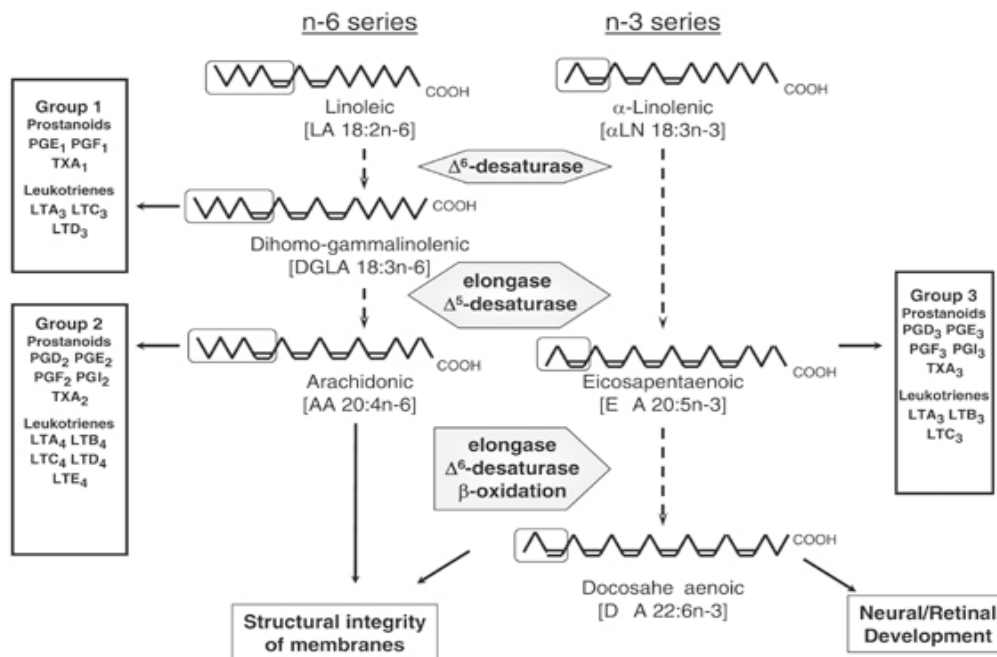


Figure 3: Omega 3 and Omega 6 pathways (Adopted from [18]).

DHA AND PREGNANCY

The developing fetus requires LCPUFAs, which are critical for fetal development, especially during the third trimester [2, 3]. The essential fatty acids, LA and ALA, are inefficiently transferred from the mother to the fetus; if transferred to the fetus, the fetus has limited ability to convert the shorter chain fatty acids into the LCPUFAs because of enzyme competition [3]. The fetus normally efficiently obtains DHA and AA directly from the mother via placenta transfer because the human placental tissue and the fetus lack the n-6 and n-5 desaturase enzymes necessary for the formation of longer chain fatty acids [2]. Therefore, a pregnant mother requires LCPUFAs such as AA and DHA not only for herself but also for her growing baby. DHA is required for the development of the central nervous system and the retina of the fetus. During the last trimester, it is estimated that the fetus needs about 12 g DHA per week for proper brain development [19]. During pregnancy the maternal stores of DHA decline because of the mobilization of DHA from the mother to the growing fetus [20]. Through placental transfer the fetus obtains the LCPUFAs, such as DHA, EPA, and AA. Therefore, the mother needs to consume enough LCPUFAs, especially DHA to ensure proper fetal growth and development. Since the fetus requires an ample supply of LCPUFAs, there is a concern that the majority of pregnant women are not getting enough DHA for themselves or their fetuses [4].

For placental transfer of fatty acids to occur the fatty acids cross the syncytiotrophoblast, which is the outer fetal component of the placenta that separates the maternal microvillus vasculature from the fetal basal membrane vasculature [21, 22]. The syncytiotrophoblast consist of syncytiotrophoblast cells that are responsible for nutrient

exchange, such as fatty acids [22]. The essential and nonessential free fatty acids cross the placenta by simple diffusion and/or fatty acid binding proteins (FABPs) [22]. The placental plasma membrane fatty acid proteins (p-FABP_{pm}) are located on the maternal microvillus membrane and allow preferential binding of the LCPUFAs and aid in the unidirectional flow to the fetus [2]. Research by Haggarty et al. [23] showed that when the placenta is presented with a mixture of fatty acids the order of uptake is AA>DHA>ALA>LA. However, they also determined that when the placenta is involved in transferring fatty acids to the fetus the placenta retains AA and the order of transferring is DHA>ALA>LA>AA.

Otto et al. [5] looked at the postpartum DHA plasma phospholipid profiles of lactating and non-lactating women. The plasma phospholipids profiles showed that after parturition DHA values in maternal plasma were decreased significantly in both lactating and non-lactating women [5]. When compared to non-pregnant women the decline in DHA levels was more significant for the lactating group than the non-lactating group [5]. Therefore, the DHA stores not only decrease during pregnancy but they remain decreased after parturition for at least 16 weeks and are decreased further if the mother breastfeeds.

To maintain DHA status during and after pregnancy, women who plan on becoming pregnant need to get DHA in their diet. Because of the high fetal demand for DHA, the mother's stores will be drained especially during the third trimester [24]. This decrease will continue after parturition and replenishing the DHA stores can take several months if the mother is lactating [5, 20]. If the mother does not replenish her DHA stores then she puts herself at risk for "DHA deficiency". N-3 fatty acid deficiency during pregnancy can increase the chances of preterm deliveries and postpartum depression [25].

High amounts of n-3 LCPUFAs are thought to possibly prolong gestation because they inhibit specific prostaglandins that are mediators of uterine contractions and cervical ripening [26].

Two studies have been conducted to determine the effects of n-3 LCPUFA supplementation and postpartum depression. Mozurkewich et al. [27] performed a double blind, placebo-controlled study using EPA and DHA. The study concluded that molecularly distilled n-3 fatty acid preparations provided a way for pregnant women to reduce their risk for depression [27]. Because n-3 fatty acids are critical to the nervous system a depletion in n-3 fatty acids could possibly affect certain neurotransmitters' biosynthesis, signaling transduction, and uptake of certain hormones resulting in depression [28]. However, further research is needed to determine if dietary supplementation of n-3 fatty acids reduces the prevalence of post-partum depression [28].

Currently, there is not a recommended dietary allowance (RDA) for DHA during pregnancy or lactation. However, much research has shown the need to increase DHA intake before, during, and after pregnancy. The typical American diet lacks sufficient amounts of DHA needed for pregnant women [29]. It is recommended that pregnant women get at least 200 mg of DHA a day [30]. Because the western diet is high in meats and processed foods that tend to be high in n-6 LCPUFAs, the ratio of dietary n-6 to n-3 is higher in the majority of people and including pregnant women when the fetus depletes the maternal stores [4].

Pregnant women should consume about 200 mg of DHA a day by a supplement or consumption of one to two servings of oily fish per week [30]. However, due to the high mercury content in some of the deep-water oily fish, pregnant women need to be careful

of the fish they choose to consume. Predatory fish that are larger tend to contain more mercury [31]. The predatory fish obtain the mercury from the ocean sediment that enters the fish through their gills as they swim and their digestive tracts as they feed and from the numerous smaller fish they consume [31, 32]. Some examples of predatory fish that are high in mercury are swordfish, shark, and orange roughy.

The fetus is most affected by mercury during the second trimester of pregnancy and the negative effect it can have on development, such as delayed walking and talking, may not be noticed until later in life [31]. Fish such as salmon and chunk light canned tuna are both good sources of DHA without high mercury toxicity. Pregnant women can also add a fish oil supplement to their daily routine to increase DHA intake.

THE RETINA

The retina is part of the central nervous system and the majority of humans sensory inputs transmit through the retina [33]. The retina is responsible for the transformation of light into a neural signal through the process of phototransduction, which is carried out by a number of different cells in the retina. The three main cells in the retina involved in the phototransduction process are the photoreceptors, the bipolar, and the ganglion cells (CAVS). There are two types of photoreceptors: rods and cones. The rods are larger, located more peripherally in the retina, and are important in dim light (scotopic vision). Humans have more rod photoreceptors than cones. The cones are smaller, located more centrally in the retina, are important for well-lit conditions, and are responsible for our central vision. The cone photoreceptors make up the macula and are used for central vision; these are the photoreceptors that are affected most in macular

degeneration. The rods and the cones contain photopigment that absorbs photons of light to aid in our vision [34].

The composition of the rods and cones is somewhat similar in that they both contain an outer segment consisting of a stalk, the cilium, the inner segment, and the outer fiber, cell body, and inner fiber, which terminate at the synaptic terminal [34]. The photoreceptor outer segments (POS) are made up of lipid filled membranous discs that contain visual pigment that converts light into a neural signal [34].

Light absorbed by the eye causes damage to the tips of the outer segments of the photoreceptors, thereby requiring them to be replaced on a daily basis [33]. The rod outer segments contain DHA and rhodopsin in their discs. The rod outer segment (ROS) tips are shed in the morning and the replacement proteins are synthesized in the inner segments, eventually making their way to the outer segments [33]. The cone outer segments contain three different types of visual pigments and not as much DHA as the ROS's [34]. The cone outer segments are shed in the evening, but little is known about the mechanism involved [33]. The regeneration of the outer segment tips is important for the survival of the photoreceptors because the buildup of light-damaged outer segment tips accumulate waste products such as lipofuscins, oxidized lipid end products that can lead to damage in the eye [33].

The retinal pigment epithelium (RPE) is the most anterior layer of the retina, located between the POS and Bruch's membrane. The cells in the RPE have many different functions that aid in the maintenance of the photoreceptors; they conduct the daily shedding, internalization, and degradation of the photoreceptors [6]. The RPE cells aid in the absorption of light and they transport nutrients as well as fatty acids to the

photoreceptors. They are responsible for the reisomerization of all-*trans*-retinal to 11-*cis*-retinal that is needed by the photoreceptors for the visual cycle [35]. They stabilize the ion composition in the sub-retinal space, which is also required for photoreceptors to function properly [35]. Another important function of the RPE cells is the phagocytosis of the shed POS. This is especially important because build up of POS can lead to waste that can affect vision. When the RPE cells phagocytose the POS, they send the essential components, such as retinal and DHA, back to the photoreceptors to be reused [35]. The DHA is redelivered to the photoreceptors as the fatty acid and the retinal is redelivered as 11-*cis*-retinal [35].

Finally, the RPE cells secrete specific growth factors that are required for photoreceptor functioning [35]. The loss of any of these functions can lead to retinal degeneration and eventually loss of vision [35].

Bruch's membrane located below the RPE and above the choroid, is the passageway for nutrients to the retina (Figure 4). The build up of basement membrane material in the Bruch's membrane, is referred to as "drusen" [34]. AMD involves the degeneration of the retina-choroid interface [34]. As a person ages, phospholipids accumulate, causing a barrier that prevents the passage of metabolites and water, and subsequently resulting in loss of nutrients to the retina [34]. If the retina is no longer receiving the nutrients it needs to function, there is neovascularization and retinal atrophy leading to AMD [34].

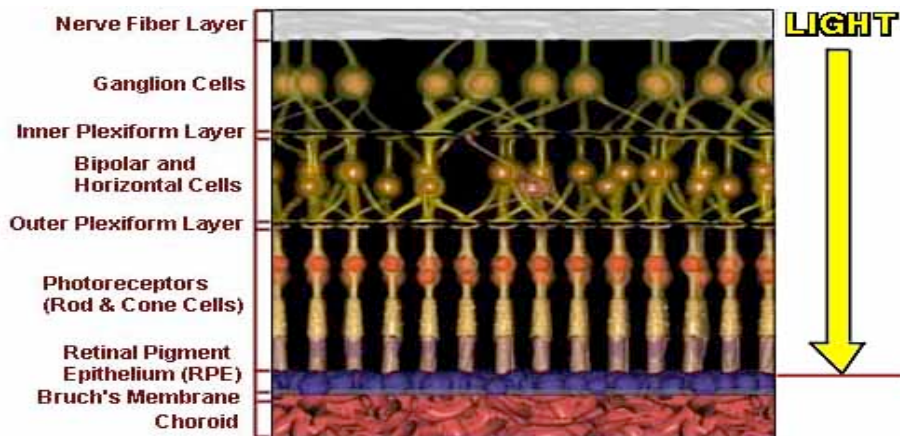


Figure 4: Layers and cells of the retina (Adopted from [36]).

ABSORPTION OF LIGHT AND AGE-RELATED MACULAR DEGENERATION

The lens, the photoreceptors, and the RPE absorb the majority of the light that enters the eye. The lens absorbs most of the ultraviolet light before the light reaches the photoreceptors and the RPE cells. The photoreceptors contain carotenoids that absorb harmful blue light [35]. The RPE cells have numerous pigments, such as melanin in melanosomes and lipofuscin, that aid in the absorption of light and protect the RPE cells. Lipofuscin is beneficial in younger people but as people age it becomes toxic [35]. The RPE cells are also protected by antioxidants and the cell's ability to repair damaged DNA [35].

AMD is one of the leading causes of blindness in the elderly [1]. It is more common in women than men and currently there is no cure for AMD. AMD is a retinal degenerative condition that usually affects people over the age of 50 [37]. Known risk factors for the development of AMD are age, gender, race, smoking history, obesity, family history of AMD, and history of HTN [38-45]. AMD damages the macula, the area of the retina responsible for central vision. The majority of people with AMD experience

central vision distortion and therefore have to rely on their peripheral vision. There are two types of AMD: dry AMD and wet AMD. Dry AMD is more common and less visually disabling. Dry AMD can be classified as early, intermediate, or advanced. Dry AMD is characterized by drusen, which is formed from the build up of basement membrane material in Bruch's membrane [35]. The classification of dry AMD depends on the size and amount of the drusen. In advanced cases, the individual can develop geographic atrophy and this causes more severe central vision loss. The other type of AMD is called wet or neovascular AMD. In wet AMD, the individual develops new vessel growth in the macular area that can lead to unwanted bleeding. Wet AMD is more visually devastating [37].

Although many theories exist to explain the pathogenesis of AMD, it remains a complex disease that is poorly understood, with no effective therapy or prevention currently available [45]. Recent research suggests that both genetic and exogenous factors contribute to the pathogenesis of AMD [45]. AMD is believed to develop from increased oxidative stress as a result of an increase in the number of reactive oxygen species in the RPE [35]. The increase in stress and reactive oxygen species leads to degeneration of the functioning of the RPE, causing vision loss with the macula most affected. Increased oxidative stress can result in the build-up of toxic aged pigment (lipofuscin) as a person ages or the reduction of melanosomes, which are needed for protection from oxidative stress [35]. One main effect of the buildup of lipofuscin is decreased ability of RPE to convert all-*trans*-retinal into 11-*cis*-retinal which is required for the visual cycle [35]. Many different theories exist on how the retinoid from lipofuscin, lipophilic cation *N*-retinyl-*N*-retinylidene ethanolamine (A2E), causes AMD,

with most studies finding that it increases the sensitivity of the RPE to blue light and it induces apoptosis in RPE cells [35, 45]. A2E accumulates in the mitochondria of the RPE cells leading to the release of different proapoptotic proteins that lead to further retinal degeneration [45]. The destruction of RPE cells leads to the formation of drusen, one of the most important symptoms of AMD [35]. Drusen can be formed between RPE in Bruch's membrane or within the Bruch's membrane. These deposits consist of metabolic products such as lipoproteins [35].

DHA (NUTRITION) AND MACULAR PROTECTION

DHA is found in very high concentrations in both the rod and cone photoreceptors in the retina. These concentrations of DHA are highest in the rods, due in part to the cones' heightened exposure to harmful light rays that can destroy DHA. DHA acts as an essential structural component in the retina with many protective functions [46]. DHA protects against inflammation, oxidative damage, and ischemia, all of which are possible pathogenic factors for the development of AMD [46]. The main dietary source of DHA is fish oil, which is especially high in tuna, salmon, and herring. These deep-water fish get DHA from the algae they consume. Our understanding of DHA's role in the retina remains limited but it appears to play a role in photoreceptor cell survival and function [6]. DHA is found in the lipid-filled discs in the POS and it is constantly recycled from the RPE to the POS [6]. Figure 5 shows the extracellular trafficking of DHA [6]. For dietary DHA to reach the photoreceptors it is first taken up by the liver, where it is esterified into phospholipids [6]. Circulating lipoproteins deliver DHA phospholipids to the RPE and the brain; this is considered the long loop [6]. The short loop recycles the

DHA from the RPE to the inner segment, where rebuilding of the photoreceptors takes place [6]. As the POS tips are phagocytized in the RPE the remaining DHA moves to the inner segment of the photoreceptors, via the short loop and then to the outer segment of the photoreceptors, eventually making its way back to the RPE [6]. Therefore, the DHA in RPE comes directly from the diet via the long loop or it is recycled, coming from the shedding of the POS tips via the short loop [6].

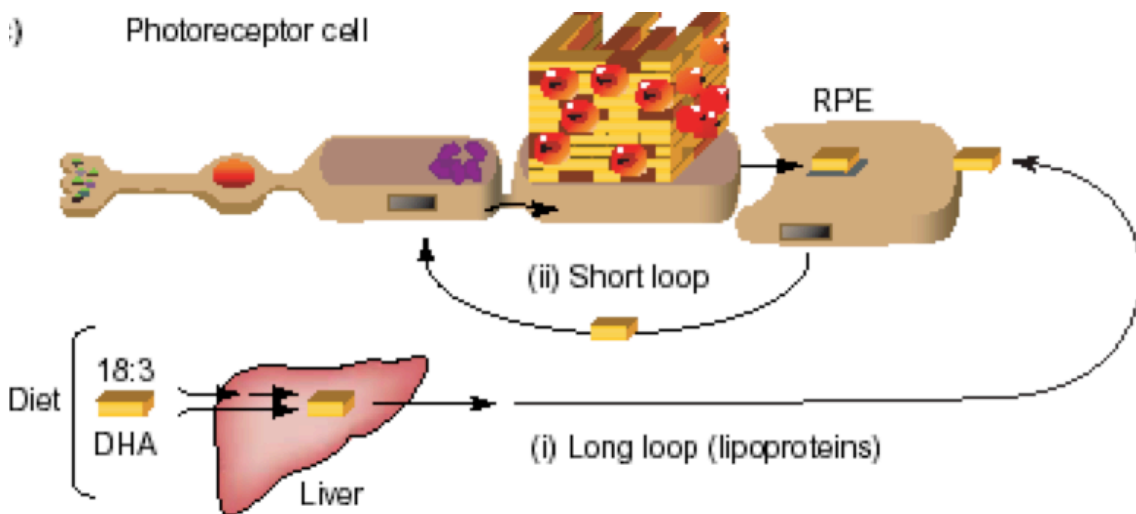


Figure 5: Transport of DHA from the diet into the photoreceptor cells (Adopted from [6]).

According to Bazan [6] DHA has neuroprotective properties because neuroprotectin D1 (NPD1), a neuroprotective mediator, is derived from DHA. NPD1 inhibits oxidative stress induced by apoptosis and inflammation. DHA also inhibits ceramide formation, a marker of cell death [6].

Chucair et al. [47] investigated if lutein (LUT), zeaxanthin (ZEA), and/or *B* – carotene (BC) protected photoreceptors from oxidative stress and whether this protection was synergistic with that of DHA. The experiments were in vitro and involved rat retinal neuron cultures that were challenged with oxidative stress to damage the photoreceptors.

The cultures were treated with BC, DHA, LUT, ZEA, or DHA combined with ZEA. The results indicated that BC, DHA, LUT, ZEA, and DHA combined with ZEA all reduced oxidative stress-induced apoptosis in photoreceptors, preserved the mitochondrial potential and prevented the release of cytochrome *c* [47]. These results point to a protective role for DHA in the retina.

DHA has also been shown to increase the macular pigment optical density (MPOD). MPOD is the measurement of the macular pigment, which represents a ratio of the different amounts of light absorbed in the retina. If a person has a high MPOD then they are less likely to develop AMD because they are more protected by the increase in macular pigment. An investigation by Johnson et al. [7] found that women, aged 60-80 years, supplemented with 800 mg per day of DHA had an increase in MPOD centrally in the retina therefore creating more protection for the macula. These authors also looked at DHA's effect on lipoproteins and found that DHA aided in the transport of carotenoids, such as lutein, which promoted the uptake of the protective carotenoids to the retina.

Chong et al. [46] performed a meta-analysis using numerous databases to determine the association between fish consumption and development of early and late AMD. The results of their investigation showed that a high dietary intake of n-3 fatty acids (ALA, DHA and EPA) with supplementation or fish was associated with a 38 percent reduction in the risk of late AMD and that consumption of fish rich in n-3 fatty acids twice or more a week was associated with decreased risk of development of early and late AMD [46]. Other studies that supported their results, including the Blue Mountains Eye Study [48] and the Nurses' Health Study and the Health Professional Follow-up Study [49] all showed a protective effect for increase n-3 fatty acid

consumption for early AMD [46]. The Blue Mountains Eye Study assessed the associations between dietary fatty acids and incidence of age-related maculopathy (ARM) [48]. Chua et al. [48] concluded that weekly intake of fish high in n-3 LCPUFAs offers protection against both early and late ARM. In the Nurses' Health Study and the Health Professional Follow-up Study the authors looked at intake of total and specific types of fat and their relationship to AMD [49]. Cho et al. [49] concluded that a high intake of fish possibly reduced the risk of AMD. Chong et al. [46] also reported from prospective cohort studies that fish intake of twice or more per week was associated with a 37 percent decrease in risk of early AMD compared to intake of fish less than once per month.

Augood et al. [50] showed that increased consumption of oily fish high in DHA and EPA at least once a week decreased the chances of development of neovascular AMD. These investigators conducted their study using fundus photography and food-frequency questionnaires. The fundus photos were graded by the International Classification System for Age Related Maculopathy [50]. The data from the questionnaires were converted into nutrient intakes with the use of food-composition tables for comparison [50]. These data revealed that individuals with higher weekly intakes of oily fish were significantly less likely to have neovascular AMD.

SanGiovanni et al. [51] performed a multicenter clinic-based prospective cohort study from the Age-Related Eye Disease Study participants [52, 53]. They compared fundus photos with nutritional intake data obtained from food frequency questionnaires. They determined that consumption of n-3 LCPUFA (DHA and/or EPA) reduced the risk of progression of bilateral drusen into central geographic atrophy, a precursor of AMD [51].

A more recent study by Christen et al. [54], examined the effects of n-3 fatty acids, particularly EPA and DHA, on incidence of AMD in women. All the women who entered the study were free of AMD ($n = 39,876$) and by the completion of the 10-year follow up study 235 of the original women were diagnosed with AMD, which could have been low because diagnosis of AMD was self-reported. The examiners used food frequency questionnaires to determine the amount of EPA and DHA the women consumed. The results of the study suggested that regular consumption of EPA and DHA significantly reduced the risk of incident AMD [54].

DHA has been shown in numerous studies to reduce the risk of development of different forms of AMD [7, 46, 48-51]. However, despite evidence that DHA plays a protective and preventative role in AMD, more research must be undertaken to advance concrete recommendations for DHA intake for AMD prevention. Further research involving large sample sizes to examine the relationship between DHA and AMD is needed.

CHAPTER 3

MATERIALS AND METHODS

PARTICIPANTS, RECRUITMENT, CONSENTING, AND PROCEDURE

Women in the Baton Rouge area diagnosed with AMD and without AMD who were 50 years or older, not incarcerated and were patients at Southern Eye Centers, Vitreoretinal Institute, or Baton Rouge Eye Physicians, all located in Baton Rouge, LA, were recruited to participate in this case controlled study. The doctors and nurses at these offices obtained all study information from the subjects during their regularly scheduled eye doctors' visits. The Institutional Review Board of Louisiana State University's Agricultural Center approved the protocol.

When the women checked in for their appointments at one of these offices they were given a "We Need You!" card that explained the study (Appendix A). Once inside the private examination room they were then given a verbal explanation of the study and the consent form (Appendix B). If the individual was interested in participating in the study, the nurse consented the individual and proceeded to the questionnaire (Appendix C). Participants were asked questions pertaining to their physical health, retinal health, and past pregnancies. Family history of AMD, vitamin supplementation, and fish oil supplementation were self-reported. Many subjects could not remember the exact brand of vitamins and/or fish oil they took nor could they remember the exact number of years they had been taking their supplements. The nurses at each of the offices followed the "Nurse script/directions" provided by the researcher to maintain consistency between offices (Appendix D). They were also provided demonstrations of the exact procedure to follow.

The doctors evaluated the health of the macula by using a direct ophthalmoscope, a binocular indirect ophthalmoscope, or a 90-diopter lens with a slit lamp. The degree of macular degeneration was recorded as ‘no AMD’, ‘early’, ‘intermediate’, or ‘advanced.’ These categories of AMD are based on the description in the Will Eye Manual [37]. Early AMD was defined as small or medium size drusen with positive or negative changes in pigment and no symptoms of vision loss [37]. Intermediate AMD was defined as medium size drusen or one or more large drusen, may have some blurry vision and a need for more light at near task [37]. Advanced AMD was defined as large drusen and geographic atrophy with blurred areas in the central vision [37].

Once all of the questions were answered and the doctor agreed with the degree of macular degeneration diagnosed, a label was put on the chart to ensure that the patients were not recruited again. The signed consent form and the completed questionnaire were stapled together and put in a locked file cabinet at the respective offices. The completed forms were picked up weekly and brought to LSU where they were kept in a locked file cabinet in Knapp Hall.

DATA ANALYSIS

Data were analyzed using SAS 9.2 (2011 SAS Institute, Cary NC). Analysis of variance (ANOVA) and χ^2 analyses were used to determine differences in participant characteristics. Participant characteristics considered independent variables: included age, parity, eye color, smoking history, family history of AMD, history of HTN, fish oil intake, vitamin intake, and BMI. Diagnosis of AMD, as having or not having AMD, was considered the dependent outcome variable. Using a backwards-stepwise regression,

non-significant variables were dropped from the model ($p \leq 0.01$). In the final binary logistic regression model, only the significant variables from the backwards-stepwise regression analysis were entered into the model ($p \leq 0.05$). The total effects of the independent variables on the predictor of the outcome variable were reported. The significance level was set to $p \leq 0.05$.

CHAPTER 4

RESULTS

Over a five-month period, 501 women were recruited and completed the study on pregnancy and the relationship to AMD. As shown in Table 4, 40% of the women had not been diagnosed with AMD and 60% were diagnosed with varying degrees of AMD. Of the 300 women who were diagnosed with AMD 24.8% were diagnosed with early stage AMD, while 19.4% were diagnosed with intermediate stage AMD and 15.8% were diagnosed with advanced AMD. Participants diagnosed with AMD were significantly older than those who had never been diagnosed (61.11 ± 0.71 versus 75.08 ± 0.58 years, $p < .0001$). The mean age of participants increased with more advanced forms of AMD (70.64 ± 0.85 , 75.04 ± 0.96 , 82.11 ± 1.07 , age $p < 0.001$) (Table 5).

The majority of the women in this study had brown (52%) or blue (22.6%) eyes and were predominately Caucasian (77.6%). Women with AMD were more likely to have currently smoked for more years compared to women without AMD (39.22 ± 3.01 , 28.92 ± 3.00 years, $p = 0.0205$). There was also a significant difference in past smoking history between women with AMD versus those without AMD (19.18 ± 14.88 , 12.98 ± 10.35 years past, $p = 0.0084$). Eye color, race, family history of AMD, and history of HTN were not significantly different between women diagnosed with AMD and those without AMD. There was no significant difference between vitamin and fish oil supplementation between women with AMD and those without AMD.

Table 4. Characteristics of Women With and Without Age-Related Macular Degeneration

| | Without AMD (n=201) | With AMD (n=300) |
|-----------------------------|--------------------------------|--------------------------------|
| Age | 61.11 \pm 0.71 ^A | 75.08 \pm 0.58 ^B |
| Parity | 2.53 \pm 0.15 ^A | 3.41 \pm 0.12 ^B |
| Eye Color [n (%)] | | |
| Blue | 34 (16.92) | 79 (26.33) |
| Brown | 117 (58.21) | 143 (47.67) |
| Green | 19 (9.45) | 32 (10.67) |
| Hazel | 31 (15.42) | 44 (14.67) |
| Black | 0 (0.00) | 2 (0.67) |
| Race [n (%)] | | |
| Asian | 2 (1.00) | 0 (0.00) |
| Blacks | 57 (28.36) | 52 (17.33) |
| Caucasian | 142 (70.65) | 246 (82.00) |
| Hispanic | 0 (0.00) | 2 (0.67) |
| Other | 0 (0.00) | 0 (0.00) |
| How long currently smoke | 28.92 \pm 3.00 ^A | 39.22 \pm 3.01 ^B |
| How long smoked in the past | 12.98 \pm 10.35 ^A | 19.18 \pm 14.88 ^B |
| Family Hx of MD | | |
| Yes | 28 (13.93) | 85 (28.33) |
| No | 173 (86.07) | 215 (71.67) |
| Hx of HTN | | |
| Yes | 105 (52.24) | 204 (68.00) |
| No | 96 (47.67) | 96 (32.00) |
| Supplement with a MV | | |
| Yes | 71 (35.32) | 141 (47.00) |
| No | 130 (64.68) | 159 (53.00) |
| Supplement with Fish oil | | |
| Yes | 34 (17.00) | 67 (22.48) |
| No | 166 (83.00) | 231 (77.52) |
| BMI (kg/m ²) | 30 \pm 0.51 ^A | 28 \pm 0.41 ^B |
| Degree if MD | | |
| No MD | 201 (40.0) | |
| Early MD | | 124 (24.8) |
| Intermediate MD | | 97 (19.4) |
| Advanced MD | | 79 (15.8) |

Abbreviations: MD= macular degeneration, MV= multivitamin, BMI= body mass index, Hx= history, HTN= hypertension, calculated kg/m², Mean \pm standard error. N (%), percentage based on number of participants analyzed. ^{AB} Groups with different letters are significantly different, $p \leq 0.05$.

Table 5. Characteristics of Women with Early, Intermediate, and Advanced Age-Related Macular Degeneration.

| | No AMD (n=201) | Early AMD (n=124) | Intermediate AMD (n= 97) | Advanced AMD (n= 79) |
|-----------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------------|
| Age | 61.11 \pm 0.67 ^A | 70.64 \pm 0.85 ^B | 75.04 \pm 0.96 ^C | 82.11 \pm 1.07 ^D |
| Parity | 2.53 \pm 0.15 ^A | 3.27 \pm 0.19 ^B | 3.64 \pm 0.22 ^B | 3.33 \pm 0.24 ^B |
| Eye color [n (%)] | | | | |
| Blue | 34 (16.92) | 34 (27.42) | 24 (24.74) | 21 (26.58) |
| Brown | 117 (58.21) | 54 (43.55) | 52 (53.61) | 37 (46.84) |
| Green | 19 (9.45) | 22 (17.74) | 7 (7.22) | 3 (3.80) |
| Hazel | 31 (15.42) | 14 (11.29) | 12 (12.37) | 18 (22.78) |
| Black | 0 (0.00) | 0 (0.00) | 2 (2.06) | 0 (0.00) |
| Race [n (%)] | | | | |
| Asian | 2 (1.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| Blacks | 57 (28.36) | 26 (20.97) | 18 (18.56) | 8 (10.13) |
| Caucasian | 142 (70.65) | 97 (78.23) | 78 (80.41) | 71 (89.87) |
| Hispanic | 0 (0.00) | 1 (0.81) | 1 (1.03) | 0 (0.00) |
| Other | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| How long currently smoke | 28.92 \pm 2.84 ^A | 30.38 \pm 4.91 ^A | 40.00 \pm 4.19 ^{AB} | 53.75 \pm 6.95 ^B |
| How long smoked in the past | 12.98 \pm 1.78 ^A | 16.95 \pm 2.34 ^{AB} | 19.11 \pm 2.76 ^{AB} | 22.11 \pm 2.64 ^B |
| Family Hx of MD | | | | |
| Yes | 28 (13.93) | 27 (21.77) | 25 (25.77) | 33 (41.77) |
| No | 173 (86.07) | 97 (78.23) | 72 (74.23) | 46 (58.23) |
| Hx of HTN | | | | |
| Yes | 105 (52.24) | 81 (65.32) | 69 (71.13) | 54 (68.35) |
| No | 96 (47.76) | 43 (34.68) | 28 (28.87) | 25 (31.65) |
| Supplement with MV | | | | |
| Yes | 71 (35.32) | 44 (35.48) | 51 (52.58) | 46 (58.23) |
| No | 130 (64.68) | 80 (64.52) | 46 (47.42) | 33 (41.77) |
| Supplement with fish oil | | | | |
| Yes | 34 (17.00) | 28 (22.76) | 22 (22.92) | 17 (21.52) |
| No | 166 (83.00) | 95 (77.24) | 74 (77.08) | 62 (78.48) |
| BMI (kg/m ²) | 29.54 \pm 0.51 ^A | 28.27 \pm 0.64 ^{AB} | 28.91 \pm 0.73 ^{AB} | 26.82 \pm 0.81 ^B |

Abbreviations: MD= macular degeneration, MV= multivitamin, BMI= body mass index, Hx= history, HTN= hypertension, calculated kg/m², Mean \pm standard error. N (%), percentage based on number of participants analyzed. ^{ABCD} Groups with different letters are significantly different, $p \leq 0.05$.

In this study, all women diagnosed with AMD birthed (only live births considered in data) more children than those never diagnosed (3.41 ± 0.12 versus 2.53 ± 0.15 children, $p < 0.0001$). As shown in Table 6, the most significant predictors of being diagnosed with AMD were age ($p < .0001$), parity ($p = 0.0161$), BMI ($p = 0.1319$), and BMI by parity (the interaction of BMI and parity together) ($p = 0.0059$).

Table 6. Logistic Regression Results for Significant Predictors of Age-Related Macular Degeneration.

| Parameter | DF | Standard Estimate | Wald Error | Wald Chi-Square | Pr > ChiSq |
|------------|----|-------------------|------------|-----------------|------------|
| Intercept | 1 | -7.3745 | 1.1399 | 41.8561 | < .0001 |
| Age | 1 | 0.1290 | 0.0126 | 104.5265 | < .0001 |
| Parity | 1 | -0.5367 | 0.2230 | 5.7910 | 0.0161 |
| BMI | 1 | -0.0401 | 0.0267 | 2.2695 | 0.1319 |
| Parity*BMI | 1 | 0.0211 | 0.00766 | 7.5736 | 0.0059 |

Abbreviations: DF= degrees of freedom, Pr= probability, ChiSq= Chi-Square, BMI= body mass index. Significance $p = 0.05$.

Age was highly significant predictor of AMD ($p < .0001$). The odds ratio for age was 1.138 with a 95% confidence limit indicating that for every year increase in age there is a 1.138 increase in risk of being diagnosed with AMD (Table 7). The model was significant based on a high concordance of 89.9% (table not shown).

As shown in Figure 6, the interaction between BMI and parity indicates that as a participant's BMI increases with the number of babies, the probability of being diagnosed with AMD is more likely. For example, as shown in Figure 6, if a 50-year-old women is pregnant with eight full term babies and is obese with a BMI of 45 kg/m^2 there is a 64.0% chance, according to the logistic regression model, that she will be diagnosed with AMD. Where as a 50-year-old women with two full term babies and a BMI of 45 kg/m^2 is predicted to have only an 8% chance of being diagnosed with AMD.

Table 7. Odd Ratio for Age and Diagnosis of Age-Related Macular Degeneration.

| Effect | Point Estimate | Confidence Interval |
|--------|----------------|---------------------|
| Age | 1.138 | 1.110 - 1.166 |

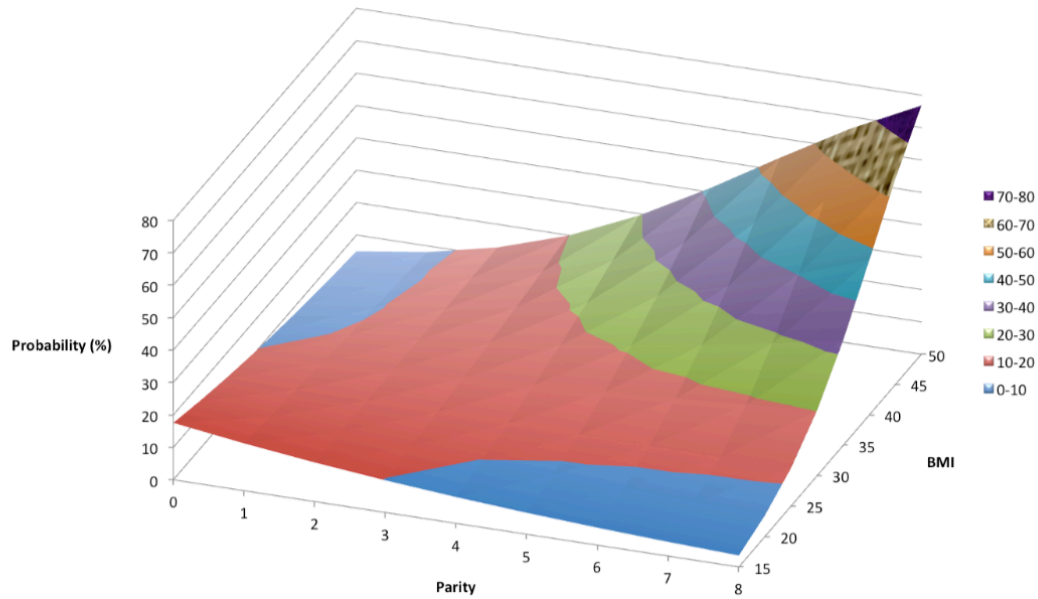


Figure 6: Probability being diagnosed with MD for 50-year-old women based on BMI and Parity. Based on the equation that probability = EXP (-7.3745+0.129*50-0.0401*BMI-0.5367*Parity+0.0211*BMI*Parity).

CHAPTER 5

DISCUSSION

To the best of our knowledge, this is the first study to examine the relationship between parity and diagnosis of AMD. Results from the current study showed that the women having more children were more likely to be diagnosed with early, intermediate, and advanced stage AMD. In addition to parity, other risk factors commonly associated with having AMD were considered, such as age eye color, race, family history of AMD, history of HTN, and BMI [38-45]. Like other studies [39, 40, 45] age was considered a significant predictor of AMD. However, this study uniquely found that BMI and parity interacted together as a predictor for diagnosis of AMD.

Increased age is a well-established risk factor for AMD [39, 40, 45]. According to Suter et al. [45] older individuals are at increased risk for developing AMD. Despite the fact that the pathogenesis of AMD is poorly understood, as an individual ages there is an increase in the accumulation of age pigment lipofuscin in lysosomes of the retinal pigment epithelium (RPE) and this is thought to contribute to the development of AMD lesions in the retina [45]. The lesions result from increased oxidative stress from the build-up of toxic age pigment (lipofuscin) as the person ages [45]. The retinoid produced from the lipofuscin, lipophilic cation *N*-retinyl-*N*-retinylidene ethanolamine (A2E), causes AMD, with most studies reporting that it increases the sensitivity of the RPE to blue light, inducing apoptosis in RPE cells [39, 45]. A2E accumulates in the mitochondria of the RPE cells leading to the release of different proapoptotic proteins that lead to further retinal degeneration [45]. The destruction of RPE cells from the lesions (retinal degeneration) leads to the formation of drusen, one of the most significant

symptoms of AMD [35]. Therefore, our finding that age is an important risk factor is supported by previous research.

Unlike most studies [43, 44], we did not find that BMI was a significant independent predictor of being diagnosed with AMD in women, it was only considered significant because of its interaction with parity. This may be explained in part because height and weight were self-reported. Other studies have considered BMI, waist circumference, and hip-to-waist ratios and it has been reported that increase in BMI is associated with an increase in developing AMD. Seddon et al. [44] found that a BMI greater than 30 kg/m² was associated with an increase in the progression to more advanced forms of AMD [44] in comparison to those individuals with a BMI less than 25 kg/m². Those investigators also found similar results with increased waist circumference and increased hip-to-waist ratio.

It is hypothesized that obesity (BMI > 30 kg/m²) is a risk factor for AMD because it is associated with increased diagnosis of vascular diseases, decreased intake of important nutrients, and increased inflammation, all of which can affect macular health [44]. Peeters et al. [43] found that a reduction in waist-hip ratio in obese individuals decreased the likelihood of having AMD. It is hypothesized that a decrease in waist-hip ratio leads to improved cardiovascular health, which affects macular health [43]. While the current exploratory study did not measure other physiological factors associated with BMI, future studies should.

In the current study, parity was a significant predictor of AMD. To date no other studies have evaluated the effects of pregnancy on the risk for developing AMD. Because women are at higher risk for AMD, it is possible pregnancy may impact macular

health and it should therefore be considered when evaluating the risk factors associated with being diagnosed with AMD. Herein we have provided evidence for the relationship between parity and AMD.

During pregnancy there is a transfer of DHA from the mother to the growing fetus [20]. DHA is a critical component found in high concentrations in both the rod and cone photoreceptors [20]. DHA acts as an essential structural component in the retina with many protective functions [46]. DHA protects against inflammation, oxidative damage, and ischemia, all of which are possible pathogenic factors for the development of AMD [46]. A concern regarding DHA and pregnancy is that the majority of women are not getting sufficient amounts of DHA during pregnancy because the typical American diet lacks DHA [29]. The fetus efficiently obtains DHA and AA directly from the mother through placental transfer, the fetus lacking the n-6 and n-5 desaturase enzymes necessary for the formation of longer chain fatty acids from the 18 carbon precursors [2]. Because of the transfer of DHA from the mother to the growing fetus and the important roles that DHA has in the human body, DHA had been added to many prenatal vitamin supplements in recent years. Therefore, the possible impact that pregnancy has on macular health should be considered when evaluating the risk factors associated with the diagnosis of AMD.

It is of interest to better understand how parity and other subject characteristics may function together to impact an individual's risk for AMD. In this study, we found that BMI and parity significantly interact with one another and predict the likelihood of a woman being diagnosed with AMD. We showed that the probability of being diagnosed with AMD dramatically increased with increased BMI and number of pregnancies.

These results indicate the combined effects of the increased in diagnosis of vascular diseases, decreased intake of carotenoids (research has shown that people with high BMI's tend to have a decrease in certain nutrients) [44], and increased in inflammation possibly due to a high BMI $>25 \text{ kg/m}^2$ in conjunction with a decrease in DHA supply due to pregnancy all together may significantly impact the diagnosis of AMD. While dietary information was not collected in this population, evidence indicates that an increase in consumption of DHA among women decreases the chance of being diagnosed with AMD [7, 38, 40-44]. Therefore, it may be possible to improve macular health and decrease the likelihood of being diagnosed with AMD in light of increased BMI and pregnancies by consuming foods high in DHA. It remains to be studied, but supplementation with DHA during pregnancy may be protective against future AMD. However, more research is needed to make this association.

CHAPTER 6

CONCLUSION

The purpose of this study was to determine if parity is a risk factor for AMD in a group of women over 50 years of age. This exploratory study has provided data that suggest that parity is an important risk factor for diagnosis of AMD. Other significant predictors were age and the interaction between BMI and parity. Age and BMI have previously been researched and shown to be significant risk factors for AMD in women. This current study is the first to describe parity as it relates to diagnosis of AMD. The significant interaction between BMI and parity is an important finding and warrants further investigation for a full understanding of the association with macular health and the high risk of AMD in women.

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APPENDIX A: “WE NEED YOU” CARD

The LSU Division of Human Nutrition and Food is conducting a study to evaluate eye health and its relationship to pregnancy. With your help, LSU will better understand eye health of women. If you are interested in participating in the study, you will be asked a few questions concerning your health and previous pregnancy history. If you choose to participate, the doctor or nurse will explain the study and answer any questions you have about the study.

APPENDIX B: CONSENT FORM

SUBJECT CONSENT FORM

Title of Research Study: Eye Health as it May Relate to Number of Pregnancies

Performance Sites: 202S Knapp Hall, Louisiana State University, Baton Rouge, LA

Contact: Ann Shaw, Graduate Student
Telephone: (225) 578-7160 or email at ashaw8@lsu.edu
202S Knapp Hall, Louisiana State University

Carol J. Lammi-Keefe, Ph.D., R.D. Professor
Principal Director
Telephone: 225 578-1518
297B Knapp Hall, Louisiana State University
Baton Rouge, LA 70803

Purpose of the Study: This study will assess if the number of pregnancies a woman has experienced is related to her eye health.

Subjects: Females, 50 years or older

Study Procedures: You will be asked a few health-related questions and your eye care doctor will provide some information about your current eye health.

Benefits: In the long term, you will help us establish if there is a relationship between pregnancy and eye health.

Risks/Discomforts: There are no major identifiable risks. You may be uncomfortable about questions involving your previous pregnancies.

If you wish to discuss any possible discomforts you might experience, you may contact the research assistant or the principal investigator listed on this form.

Right to Refuse: Your participation in this study is entirely voluntary. You may change your mind and withdraw from the study at any time without penalties or consequences.

Privacy: Your private information will be kept confidential as required by law. You will be assigned an ID code and all

data will be labeled only with the code number and not your name. All of your data will be kept in a locked file in 202S Knapp Hall, LSU.

The results from this study may be incorporated into research papers, presented at scientific meetings, or published in professional journals. Any information gathered will be protected from unauthorized access by the principal investigator. Data will be kept confidential unless release is legally required.

Removal:

The principal investigator reserves the right to remove a subject from the research if the subject fails to meet the requirements of the study protocol.

Questions:

If you have any questions about this study, we will be happy to answer these; you may contact the principal investigator, Carol Lammi-Keefe Ph.D., R.D. at (225) 578-1518. If you have questions about your rights as a subject or other concerns, you can contact Michael Keenan, Chairman, LSU AgCenter Institutional Review Board at (225) 578-1708.

Authorization:

I have read this form and decided that _____ will participate in the
(name of subject)
project described above. Its general purposes, procedures and risks have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Signature of Subject

Date

Name of Subject (print)

Signature of Person
Obtaining Consent

Date

Name of Person
Obtaining Consent (print)

APPENDIX C: QUESTIONNAIRE (HEALTH HISTORY FORM)

ID: _____

QUESTIONS NURSE ASKS DIRECTLY TO PARTICIPANT:

1. Age? _____
2. Eye color? Blue Brown Green Hazel Black
3. Race (please circle one)? Asian African American Caucasian Hispanic or Other _____
4. Do you currently smoke? YES or NO. If so how long?

5. Have you smoked in the past? YES or NO. If so how long?

6. Do you have a family history of macular degeneration? YES or NO
7. Do you have hypertension? YES or NO
8. Are you currently taking any vitamins or fish oil capsules? If yes, what are the name brands of the vitamins and/or fish oil capsules? And how long in years have you been taking them?

9. How much do you weigh (pounds)? _____
10. How tall are you (feet)? _____
11. How many babies have you had? _____
12. Were any of your babies premature? Or born early? If so how early?

QUESTIONS OBTAINED BY NURSE FROM THE EYE EXAM OR DOCTOR:

13. Degree of macular degeneration please circle one:
 - a. Early: Small or medium size drusen. Positive or negative pigment changes. No symptoms of vision loss.
 - b. Intermediate: Medium drusen or one or more large drusen. May experience some blurry vision and need more light for near task.

- c. Advance: Large drusen and geographic atrophy. Blurred areas in the central vision.

14. How long has the patient been diagnosed with macular degeneration?

APPENDIX D: “NURSES SCRIPT/DIRECTIONS”

- 1) Before entering the exam room, make sure you have three forms two copies of the consent forms and one questionnaire. The questionnaire will be coded with a number.
- 2) Nurse states to the patient, "LSU Division of Human Nutrition and Food, is conducting a study involving eye health and it's relationship to pregnancy. Your participation is completely voluntary and your name will not be associated with any of your answers to the questions. All the information that we obtain today will be kept completely confidential and you will not be contacted again after today."
- 3) Next, show the consent form to the patient and ask if there are any questions. If the patient agrees to participate in the study, ask her to sign the consent form. The patient needs to sign two of the same consent form -one for the patient's records and one will be kept for LSU's records. Then pick up the card that was handed to the patient at the front desk.
- 4) Once the signatures are obtained, then proceed to the questions. Some of the questions can be answered from the chart and other questions will need to be asked directly to the patient. Once the questions are filled out and the doctor agrees to the degree of macular degeneration, put a designated label on the chart so the patient is not recruited twice.

VITA

Ann Hardin Shaw is an optometrist who decided to extend her education by getting her master's at Louisiana State University in human nutrition in fall 2009. She did her under graduate at University of Georgia in Athens, Georgia. She got her under graduate degree in international business with a concentration in marketing. She also took all the pre-optometry courses at University of Georgia. In 2008, she graduated from Southern College of Optometry in Memphis, Tennessee. While in optometry school she worked at Bethesda Navy Hospital in Maryland and with Dr. Charles Shidlofsky in Dallas, Texas. In May of 2008, she joined her father, Dr. Roger F. Shaw at Southern Eye Centers. She currently works at both offices seeing a variety of patients. She hopes that her extended education in nutrition will better help her serve her patients because of the strong association between eye health and nutrition.