Macular pigment optical density during pregnancy and its relationship to the diet

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MACULAR PIGMENT OPTICAL DENSITY DURING PREGNANCY AND ITS RELATIONSHIP TO THE DIET

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 Human Ecology

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# TABLE OF CONTENTS

ACKNOWLEDGMENTS ................................................................................. ii

ABSTRACT ................................................................................................. v

CHAPTER
1 INTRODUCTION ..................................................................................... 1
   Justification .......................................................................................... 2
   Assumptions ....................................................................................... 2
   Research Hypothesis .......................................................................... 2
   Objectives .......................................................................................... 2
   Limitations ......................................................................................... 3

2 REVIEW OF LITERATURE ........................................................................ 4
   Lipids .................................................................................................. 4
   Essential Fatty Acids ......................................................................... 5
   DHA during Pregnancy ....................................................................... 7
   Maternal Dietary DHA ....................................................................... 7
   The Retina: Anatomy and Physiology ................................................. 8
   Macular Pigment Optical Density (MPOD) ........................................ 11
   Lutein and Zeaxanthin ..................................................................... 13
   DHA in the Retina ............................................................................... 13
   DHA Offers Protection to the Retina .................................................. 15
   Importance of DHA during Infant Development ............................. 16
   DHA May Modulate MPOD ............................................................... 18
   Age-Related Macular Degeneration and DHA ................................. 19

3 EXPERIMENTAL DESIGN AND METHODS ........................................... 22
   Subject Recruitment ........................................................................... 22
   Study Design ..................................................................................... 22
   Measurement of MPOD ..................................................................... 25
   Assessment of MPOD Using the Macular Metrics Densitometer Machine ........................................... 26
   Data Analysis ..................................................................................... 27

4 MACULAR PIGMENT OPTICAL DENSITY IS ASSOCIATED WITH FISH
   CONSUMPTION .................................................................................. 28
   Introduction ....................................................................................... 28
   Methods ............................................................................................ 28
   Results ............................................................................................... 31
   Discussion ......................................................................................... 35

5 CONCLUSION ......................................................................................... 39

LITERATURE CITED ................................................................................... 40
ABSTRACT

Women, compared to men, are at higher risk for age-related macular degeneration (AMD), the leading cause of blindness in the elderly. The accumulation of dietary carotenoids, lutein (L) and zeaxanthin (Z) within the macula make up macular pigment and provide important protection to the retina. Macular pigment optical density (MPOD) gives a measure of macula pigment density. MPOD has been shown to be influenced by fruit and vegetable L and Z and docosahexanoic acid (DHA, 22:6n-3), cold water marine fish being the dietary source. A high MPOD may protect against AMD. Intake of DHA by women is low (40-120 mg/d) and transfer of DHA during pregnancy to the fetus tends to deplete their stores. To date, MPOD for pregnant women has not been evaluated. We posed the question: “Is MPOD decreased during pregnancy?” MPOD was measured for 22 women using a macularmetrics densitometer at 18-23, 24-26, 30, and 36-38 weeks of pregnancy. Dietary information was collected using repeated food frequency questionnaires and 24-hour dietary recalls.

Women consuming a prenatal supplement containing DHA/fish oil during the third trimester tended to have a higher MPOD than those not supplementing (0.41 vs. 0.28). While MPOD did not decrease from late second to third trimester in this small sample (MPOD, LSM ± SE: 0.34± 0.03, 0.34± 0.03, 0.35± 0.04, 0.31±0.04), our data provided the opportunity to calculate that with a power of 80% and a significance of 0.05 a sample size of 121 participants is required to assess difference over pregnancy. MPOD was positively correlated with L and Z, the consumption of fruits and vegetables high in L and Z (p-value<0.02) and weekly seafood intake (p-value<0.01). In conclusion, seafood as a source of DHA and dietary L and Z were associated
with increased MPOD during pregnancy and prenatal supplements with DHA/fish oil may be important in maintaining MPOD during pregnancy.
CHAPTER 1
INTRODUCTION

Age related macular degeneration (AMD) is the leading cause of blindness amongst elderly individuals aged 60 years and older [1]. Degeneration of the retina, in particular the macula, causes blind spots and eliminates use of central vision. These consequences are untreatable yet the causes for AMD are inconclusive. Further, women have a higher prevalence of AMD than do men for reasons that are also unknown [2]. Herein it is speculated that life choices, such as pregnancy, may explain the higher risk for women.

During pregnancy, the rate at which some nutrients are transferred from the mother to the developing fetus via the placenta is up-regulated during the third trimester when exponential growth of many organs occurs [3]. One of the nutrients which is preferentially transferred is docosohexaenoic acid (DHA, 22:6n-3) [4]. DHA is an important long chain polyunsaturated fatty acid (LCPUFA) that is abundant in the brain and the retina of the eye where it is concentrated in particular in the membranes and rod photoreceptors. DHA is believed to provide proper maintenance and functionality for the retina especially as it interacts with two dietary components, the carotenoids lutein (L) and zeaxanthin (Z) [5]. However, the consumption of DHA during pregnancy is documented to be extremely low [6-8] and this may compromise the eye health of the mother. L and Z from dietary sources provide protection for the retina as they accumulate within the macula [9], functioning to assemble macular pigment (MP). They also provide antioxidant functions by absorbing and filtering light [10]. High levels of MPOD are thought to provide a protective effect against AMD [11-13]. It is not known if MPOD modulation occurs during pregnancy when DHA is preferentially transferred from the maternal circulation to the developing fetus. This relationship between MPOD and pregnancy as it relates to the diet was explored in the current study.
Justification

DHA’s specific role in the eye is not completely understood. However, Judge et. al [14] showed DHA during pregnancy to be important to infant visual acuity. Infants born to women who were supplemented with DHA beginning in the second trimester of pregnancy had higher visual acuity scores at four months than infants of women consuming a placebo. Evidence for DHA’s effects on MPOD is very limited, and there is no research on DHA’s effect on the mother’s MPOD during pregnancy. However, some studies show promise for benefit of DHA in preventing AMD. In the Nurses’ Health Study and Health Professionals Follow up study from Cho et al. [15], researchers showed that individuals who consumed fish, a source of DHA, greater than 4 times per week were at decreased risk for AMD compared to individuals who consumed fish less than 3 times per month.

With respect to L and Z, Johnson et al.[5] and Kopsell et al [16], have shown that by increasing L and Z in the diet, MPOD increases. Others documented that dietary supplementation of L and Z increased serum and plasma response levels of L and Z [17-18]. Studies on how these nutrients affect MPOD during pregnancy have not been conducted. Based on the foregoing, it is hypothesized that MPOD will decrease during late pregnancy.

Assumptions

It is assumed that participants will use the macular metrics machine correctly and all MPOD measurements from the studies will be taken and recorded correctly.

Research Hypothesis

MPOD will decrease in women from 18-23 to 36-38 weeks of pregnancy.

Objectives

1. To evaluate MPOD longitudinally during pregnancy.
2. To evaluate the relationship between diet and MPOD of pregnant women.

Limitations

1. Dietary intakes will be assessed using a 24 hour recall. Repeated recalls and probing during interviews will be used to increase validity.

2. Limited sample size of pregnant women.
CHAPTER 2
REVIEW OF LITERATURE

Lipids

Lipids are a group of compounds that are insoluble in water and soluble in fat, organic or hydrophobic solvents [19]. Lipids also have subcategories, ranging from extremely hydrophobic triglycerides, to saturated and unsaturated fats, essential fatty acids, steroids and the more water soluble phospholipids. These compounds are extremely variable in size and polarity, all serving as components of cell membranes by regulating the transfer of nutrients into and out of cells, and the fatty acids (FA) especially provide a concentrated source of energy.

Fatty acids are biological lipids that are structurally composed of chains of unbranched hydrocarbon tails with a carboxylic acid group at one end and a methyl group at the other end [20]. This structure allows for one end of the FA to be polar or hydrophilic and the alternate end to be non-polar or hydrophobic making this end insoluble in water. The hydrocarbon tails have diverse chain lengths usually of even numbered carbon atoms ranging from four to 24 carbons in length. FA differ from one another in the number and arrangement of double bonds along the hydrocarbon chain [20]. Structure is very important in identifying the FA. The position of the first carbon of a double bond with respect to the methyl terminus allows for identification. The symbols “n” or “ω,” also known as ‘omega’, identify the FA by designating the distance of the first double bond from the methyl end of the hydrocarbon chain [20]. In particular, the LCPUFA docosohexaenoic acid (22:6n-3), also known as DHA, has a chain length of 22 carbons with 6 double bonds within the hydrocarbon tail and with the first double bond starting at the third carbon with respect to the methyl terminus. The FAs are also classified as saturated or unsaturated according to the presence of double bonds within the hydrocarbon chain; the
maximum number of double bonds that can be present in a chain is six. For example, FAs that have no double bonds on the hydrocarbon chain are designated ‘saturated’, one double bond is ‘monounsaturated’, and two or more double bonds, ‘polyunsaturated.’ The preferential fatty acid types are found as cis conformations (both hydrogens are on same side of carbon chain) and are the fatty acids mainly present in the foods we eat.

**Essential Fatty Acids**

Lipids in our diet include the essential fatty acids (EFA) for the maintenance of functionality amongst tissue membranes [20]. There are two EFA, alpha linolenic acid (ALA, 18:3n-3), an n-3 FA, and linoleic acid (LA, 18:3n-6), an omega 6 FA. Both of these EFA are necessary dietary components because they are required for growth and development as well as other physiological effects, but cannot be synthesized in the body. Burr and Burr developed the term EFA for “those fatty acids not synthesized in mammals and for which deficiencies could be reversed by dietary addition” [20]. After ingestion, EFAs are distributed amongst different types of lipids to supply energy and some will be metabolized into LCPUFA to be later integrated into structural lipids. The liver is the main site for synthesis of EFA. In the liver the n-3 and n-6 FAs undergo the same process of desaturation and elongation by enzymes to synthesize the LCPUFA. For example, the LCPUFA DHA and eicosapentanoic acid (EPA, C20:5n-3) can be endogenously derived from their EFA precursor ALA, and arachadonic acid (ARA) can be derived from its EFA, LA through a process of desaturation and elongation steps [20]. Dietary components of LCPUFA such as DHA are particularly important for humans, because of the extremely low endogenous biosynthetic conversion rate of the EFA to the LCPUFA. This low conversion rate may be insufficient to meet the high demands of the CNS of the developing fetus. Endogenously, humans are only able to insert double bonds at the n-9 position or higher.
from the methyl end, thus n-3 and n-6 fatty acids are not synthesized [20]. The rate of ALA converted to DHA in infants is less than 1% [21-23]. Therefore, maternal intake of DHA needs to be sufficient to support the high demands of the fetus.

Figure 1 shows the conversion of the EFA precursors to their respective LCPUFA. Because both ALA and LA share the same enzymatic pathway, they compete with each other for the enzymes that will further metabolize them into the longer chains such as the derivates DHA, EPA, and ARA. Thus, a higher intake of omega-6 fatty acids, which is more commonly consumed within the American diet, limits the synthesis of ALA to DHA.

Figure 1. Metabolism of Omega-6 and Omega-3 Fatty Acids. Source: DHA/EPA Omega-3 Institute [24].
**DHA during Pregnancy**

During the third trimester of pregnancy, the fetus undergoes an exponential rate of growth and development and will require large amounts of ARA for growth and DHA for development of the central nervous system and retina [14, 25-26]. Thus, DHA and ARA are preferentially transferred from the mother to the fetus via the placenta. The syncytiotrophoblast of the placenta is a barrier site that separates the mother’s microvillus membranous vasculature from the fetal basal membranous vasculature and it is at this site that nutrients can be exchanged between the mother and the fetus [27]. The placenta acquires free fatty acids such as DHA via passive diffusion along with the help of fatty acid-binding proteins (FABPs) that lie on the maternal side of the placenta and that transport fatty acids of chain lengths of 20 C or more, meeting fetal needs [22, 28].

Because the fetal basal placenta membrane binds more fatty acids than the maternal membrane, greater amounts of DHA are transferred to the developing fetus [14]. This continuous transfer across the placenta in effect drains the mother’s stores of DHA and these stores may be difficult to replenish even after birth [29], jeopardizing maternal DHA stores and status [2, 30]. The mother must consume ‘adequate’ amounts of DHA through exogenous sources instead of relying on the limited endogenous production to ensure that the developing fetus receives the required amounts for optimal development.

**Maternal Dietary DHA**

Currently, there is no recommended daily intake (RDI) for DHA in the United States [31]. However, for both pregnant and lactating women, experts suggest that approximately 200-300 mg/d will support optimal fetal brain and eye development [32]. However, pregnant women throughout the US have dietary intakes of DHA that are significantly below the optimal level [23].
On average, the typical pregnant woman in the US consumes only about 40–120 mg of DHA per day [33]. DHA consumption may be low because fatty cold water fish are not a part of the typical American diet [34]. One reason for the low consumption of fish may be the concern for the potential risk of mercury toxicity during pregnancy [31]. The main dietary sources of DHA are primarily oily fish such as salmon, tuna, sardines, mackerel, trout, bass, and halibut to name a few. Flaxseed oil and different types of nuts can contribute high amounts of dietary omega 3 fatty acids of the 18 carbon species but relatively low amounts of DHA.

**The Retina: Anatomy and Physiology**

The retina is a highly vascularized tissue derived from the development of the neural plate during embryogenesis and is thus part of the central nervous system (CNS) [35]. The retina is responsible for the transformation of light energy into neural signals. In order to complete this transformation, it is comprised of many different cell and neuronal layers that allow the human brain to interpret light signals. For the purpose of this research, the focus will be on a few of these cells and layers.

![Figure 2. The layers and cells of the retina](image-url)
The cells that line the retina are called photoreceptors. A photoreceptor’s main function is to absorb photons of light and thus initiate the visual cycle. The photons of light provide external information and help to create electrical changes that signal the brain via a process called phototransduction [35]. There are two different types of photoreceptors: rods and cones. Rods are large in number and are responsible for vision in dim light [35]. They are found mainly in the peripheral region of the retina [37] rather than its most central part known as the fovea. In contrast, cones which are smaller in number, are scarce in the peripheral region and are more abundant within the fovea [35]. Cones predominate in day light vision and are sensitive to color details because they are able to adjust quickly to various light levels [38].

Photoreceptors have both an inner and outer segment. It is the outer segments that capture photons and generate electrical signals [35]. Photoreceptor outer segment (POS) tips are the most vulnerable to everyday light energy. Rod outer segments (ROS) are comprised of lipid filled discs that pile amongst each other. The membranes in each disc concentrate both DHA and rhodopsin, a visual pigmented protein that absorbs photons [35]. The absorption of light is extremely damaging to ROS as shown by their high metabolic activity and short lifespan [39]. Light-damaged ROS tips accumulate waste products such as oxidized lipids, light-damaged proteins, and free radicals [39]. ROS must shed these tips to maintain excitability [39] and to rapidly regenerate after they have absorbed light [10, 39-40]. Therefore, the tips of the ROS are shed and phagocytized within the retinal pigment epithelium layer (RPE) [35].

The RPE is a layer of pigmented cells that has a variety of functions, however, for the purpose of this research, two functions that will be highlighted are its role in the phagocytosis of shed ROS discs and the absorption of scattered light [38]. As seen in Figure 2, the RPE’s apical membrane faces the extracellular space around POS which allow the RPE to work succinctly
with photoreceptors while its basal membrane faces the choroid [35, 38]. The RPE contains phagosomes, small compartments that digest damaged ROS which are released from the photoreceptor and accumulate for life within the Bruch’s membrane [35]. As the basal side of the POS becomes phagocytized, the apical side of the photoreceptors rebuild themselves and the RPE will conserve and recycle important nutrients such as DHA and retinal back to the photoreceptors. This process allows for restored excitability and the maintenance of vision [39]. This is important to note because if DHA is most commonly found in the rod outer segments, which are constantly being regenerated, in addition to some recycling of DHA from the RPE back to the photoreceptors, DHA intake during pregnancy may need to be increased to ensure proper functioning of the retina.

The Bruch’s membrane is located just above the choroid capillaries. This membrane allows for the exchange of nutrients from the choroid and catabolites from the retina [38]. The choroid, although not part of the retina, helps to absorb any additional scattered light, in particular the light that diffuses through the RPE. As currently understood, the photoreceptors, RPE, Bruch’s membrane and choroid each play an intricate role in the development of AMD.

As an individual ages, destructed lipids from the ROS accumulate in the Bruch’s membrane impeding for the exchange of metabolites, in particular water and catabolites, from both the retina and choroid [38]. As a result, water begins to accumulate between the RPE and Bruch’s membrane causing detachment of the retina [38]. Thus, nutrients from the choroid will not be delivered to the retina and as a result the RPE and photoreceptors deteriorate resulting in visual loss [38]. Additionally, the loss of nutrients causes the Bruch’s membrane to develop new vessels in its search for alternative nutrients [38]. Unfortunately, this event is extremely fruitless as these vessels easily hemorrhage into the retinal tissue and this contributes to visual
loss [38]. However, to date, the initiating event in AMD development remains elusive and undefined.

**Macular Pigment Optical Density (MPOD)**

Macular pigment optical density (MPOD) is the measure of retinal pigment, in particular the accumulation of lutein (L) and zeaxanthin (Z) within the macula. Macular pigment (MP) is comprised of oxygenated xanthophylls L and Z that function as non pro-vitamin A precursors. L and Z accumulate within photoreceptor axons [9] and act as free radical scavengers. MP also absorbs and appreciable amount of light thus preventing large amounts of light from reaching sensitive parts of the retina, such as the RPE [11, 41], by absorbing and filtering light before it reaches photoreceptors [10, 42].

Snodderly et al. [43], showed that L and Z are concentrated in the inner layers of the central retina, the macula, in particular the fovea. The further the distance from the fovea, the more dispersed L and Z become [9]. The peripheral region of the retina contains limited L and Z, while ROS which contain large amounts of DHA, are comprised of about 25% L and Z [9]. L and Z absorb a maximum of 445 nm light through their polyphenol chain structure. Light waves of 445 nm, known as blue light is considered the most energetic portion of the visible light spectrum and can cause the most damage to the retina [44].

Therefore, MPOD increases have been reported to mirror increased consumption of L and Z. Thus, MPOD has a significant and positive relationship with L and Z concentration amounts [9]. Berendschot et al. [42], demonstrated this relationship through supplementation with L. In that study, eight men were given 10 mg supplements of L for 12 weeks. There were significant increases in MPOD as early as week 4 of supplementation. Continued supplementation maintained a high MPOD until supplementation was discontinued. Thus, not
only did individuals show a significant increase in MPOD with supplementation, they also showed a decrease in MPOD four weeks after supplementation was terminated and MPOD returned to baseline values.

Supplementation was also evaluated in a study conducted by Kopsell et al [16]. They supplemented two groups of 10 males and females with spinach with a low concentration of L and Z or spinach yielding a high concentration of L and Z. MPOD was evaluated at baseline and subjects were asked to consume his/her spinach meal 5 times per week for 12 weeks. MPOD was measured for the second time at the twelfth week. MPOD for consumers of the spinach with high L and Z concentrations were higher during the twelfth week compared to baseline, while MPOD for subjects who consumed spinach with a low concentration of L and Z were not different at week 12 compared to baseline. Thus Kopsell et al. showed that MPOD can be increased by consumption of food high in L and Z.

In another study Landrum et al [45] showed that MPOD was influenced by the supplementation of 30 mg of L. In that study two male subjects, aged 41 and 52, were given a 30 mg dose of L esters each day for 140 days and MPOD was measured before, during and post supplementation. Subjects also had blood serum L levels measured before, during and after supplementation. There was a dramatic rise in MPOD 20-40 days after supplementation and MPOD continued to rise 40 to 50 days after supplementation was discontinued. The continued rise after supplementation was interpreted to be due to the high dose of L and the retina’s capacity to store L. Landrum et al suggested a potential benefit of increased MPOD with L supplementation for the protection of sensitive cells of the retina.
Lutein and Zeaxanthin

There are many different stereoisomers of the xanthophylls L and Z. However, within the retina, the stereoisomer that is most pronounced is lutein, (3R,3′R,6′R) Beta,E-carotene-3,3′-diol [41]. Z has three different stereoisomer forms [41]. Z is abundantly found in all three of its forms which include 3R, 3′R and 3R, 3′S [41]. Therefore, dietary practices may be influenced by noting the significance between L and Z and MPOD.

L and Z are derived from dietary components and are found most abundantly protein bound within chromoplasts and petals of various plant sources [9]. Sources of high levels of L and Z are colorful yellow, orange, and green plants that include, but are not limited to, kale, spinach, corn, orange peppers and honeydew [9, 41]. Within plants, L and Z also function as antioxidants and are believed to act as antioxidants for the retina’s rich light and oxygenated environment [11, 41, 46]. In addition, egg yolks, although lower in L than spinach, provide a high bioavailable source of L which rapidly increase L concentration in the blood [9].

There is no recommended daily allowance (RDA) for L, however, positive dietary effects of L on MPOD have been shown at levels of 6-10 mg/d [46]. On average, individuals consume about 0.8 to 4 mg of both L and Z together daily [9]. L and Z are fat soluble substances and when consumed they are transported via lipoproteins [9, 41] and accumulate in adipose tissue [9]. High density lipoproteins (HDL) are the main transporters of L and Z [9]. Research suggests that HDL preferentially transports L and Z to the retina because the retina favors HDL uptake [9]. L and Z are deposited in the retina via HDL at levels of 0.1 to 1 mM [9, 41].

DHA in the Retina

DHA is a key nutrient found within retinal membranes and in particular the rod outer segmented photoreceptors of the retina where it acts as an essential structural component [47].
DHA is selectively transported to photoreceptor membranes and these membranes accumulate the highest concentration of DHA of any cells in the human body [23]. Although DHA’s role within the retina is not completely understood, what we do know is that DHA helps to promote photoreceptor survival through the renewal process. DHA is highly accumulated within lipid filled discs of the ROS from the photoreceptors. Figure 3 [48], shows the transport of DHA into the photoreceptor cell [48]. For DHA to get to the photoreceptors it must first be ingested and esterfied into phospholipids packaged from the liver. DHA filled phospholipids will then be transported through the blood stream via lipoproteins that pass through the choroid layer to be deposited into the RPE. A short loop shunts the DHA from the RPE into the photoreceptor inner segment, a site of rebuilding for the photoreceptors. As ROS tips become phagocytized, DHA moves from the inner segments of the photoreceptors into the ROS via the connecting cilium between the inner and outer segments [48]. DHA may also be taken up from the shedded ROS that accumulate in the RPE and can be transported back to the inner segments via a short loop; thus, DHA has the ability to be both conserved and recycled within the retina [49]. Therefore, the RPE supports photoreceptors by supplying them with DHA, a vital relationship in the function and survival of photoreceptors [50]. Apoptosis or extensive damage to the RPE may impair photoreceptor survival and contribute to AMD.

Figure 3. The transport of DHA from the diet into the photoreceptor cell. Reproduced from [48].
DHA Offers Protection to the Retina

Every cell in the human body requires signals for survival. These signals are referred to as trophic factors (TF). In the absence of TF, cells undergo programmed cell death via interactions between pro-apoptotic and anti-apoptotic proteins. The mechanisms by which DHA protects the retina remains unknown. It may not be DHA per se, but rather, TF effects of DHA and neuroprotectin derivatives of DHA from both the RPE and photoreceptor cells that may respond to oxidative stress [48]. DHA has been demonstrated to impede photoreceptor apoptosis by acting as a TF [51]. Cell death occurs when the outer mitochondrial membrane becomes penetrated [52]. Politi et al [53] showed that addition of DHA and glial derived neurotrophic factor (GDNF) either simultaneously or individually preserved the mitochondria, decreasing photoreceptor apoptosis. GDNF and DHA also act as a TF [53]. Specifically, GDNF protects rat retinal photoreceptors from apoptosis progression in vitro by 70% as compared to controls. The addition of both DHA and GDNF on photoreceptor cells was dramatic. The combination of the two enhanced photoreceptor cell survival more than either DHA or GDNF alone.

Bazan et al [48] demonstrated that neuroprotectin D1 (NPD1), a DHA derived mediator, maintained the survival of the RPE. During a time of oxidative stress, DHA is a target of lipid peroxidation. However, NPD1 helps to protect retinal layers from oxidative damage. NPD1 acts within the RPE to protect photoreceptors by inhibiting cell death and oxidative stress mediated proinflammatory genes [48]. NPD1 may do this by up-regulating protective Bcl-2 proteins. Bcl-2 is the prototype for a family of genes and proteins that govern mitochondrial outer membrane permeabilization. There are two types of Bcl-2 proteins, pro-apoptotic Bcl-2 proteins such as Bax and Bad and anti-apoptotic proteins Bcl-2 proper and Bcl-w to name a few. Oxidative stress activates pro-apoptotic Bcl-2 proteins, however, these activators are suppressed by up-regulated
anti-apoptotic Bcl-2 proteins via NPD1. Thus, stress factors become unavailable to the outer mitochondrial outer membrane [52].

Rotstein et al [51], also showed the protective effects of DHA on oxidative stress [54]. These investigators conducted a cell culture study in which oxidative stress in the form of paraquat was presented to rat retinal neurons with or without DHA for 3 days [54]. In neurons without DHA, there was penetration of oxidative stress to the mitochondrial membrane; thus, oxidative stress was not prevented and neuronal apoptosis occurred. However, neurons exposed to paraquat and treated with DHA had an up-regulation of Bcl-2 molecules and protected cells against Bax or pro-apoptotic expression preserving the mitochondrial membrane. Apoptosis of photoreceptor cells was prevented by the addition of DHA. It was demonstrated that DHA helped to protect penetration of the mitochondrial layer by expression of mechanisms such as anti-apoptotic Bcl-2 proteins, protecting photoreceptor cells from apoptosis. Thus, although the mechanism(s) of DHA in the protection for the retina is not completely understood, both DHA derivatives and the up-regulation of Bcl-2 neurons may offer this protection. In turn, the maintenance of a healthy retina is dependent on the survival and longevity of photoreceptors in conjunction with the RPE.

**Importance of DHA during Infant Development**

The benefits of DHA within the retina are most commonly noticed in infant studies. Research suggests that if DHA status is insufficient, retinal function of the fetus may endure damage [29] and may become vulnerable to visual and neural abnormalities [55]. DHA aids in fetal eye development and approximately 60% of DHA derived from the mother during pregnancy will accumulate within the retina of the fetus [56]. In a randomized, longitudinal, double-blinded placebo-controlled trial conducted by Judge et al. [14] pregnant mothers received
either a placebo cereal bar or a DHA supplemented cereal bar and were asked to consume the bar three, five, or seven times per week, during 24 weeks of pregnancy to delivery. Infants of mothers who were supplemented with the cereal bars containing DHA had higher acuity card procedure visual acuity scores at four months of age compared to controls [23], validating a deviation from subnormal sensory maturation experienced by infants who lacked DHA within the photoreceptors of the membrane.

Carlson et al. [25] showed that development of the retina via DHA largely affects visual acuity. Carlson et al. supplemented preterm infants with marine-oil resulting in an improved infant DHA status and visual acuity [25]. Preterm infants at approximately 3 weeks of age were divided into four different groups which consisted of: 1. a commercial preterm formula, 2. a commercial term formula, 3. a marine oil-supplemented preterm formula or, 4. a marine oil-supplemented term formula. There were significant positive effects on visual acuity of the preterm infants fed either the marine oil supplemented preterm or term formulas at 2 months and four months of age [25]. Makrides et al. [57] showed that infants fed breast milk (which contains DHA) scored better on visual and developmental tests than non-DHA containing formula fed infants. Together the study results show that with increased DHA consumption either during pregnancy or breastfeeding, infant retinal physiology and visual acuity improves [23, 25, 57].

The impact of DHA depletion during pregnancy [4] on a mother’s retinal health has yet to be evaluated. Maternal stores of DHA are drained to provide an adequate supply of DHA to the fetus. This transfer is crucial during the last trimester when fetal development of the brain and retina is exacerbated [58]. Further, this elevated fetal need may pull DHA from sources such as the mother’s retina and possibly impact her own eye health. Therefore, based upon a current
review of literature, pregnancy may be related to a decreased MPOD. DHA interacts with photoreceptors which have been documented to play a large role in the maintenance of retinal health. Low DHA status may be inefficient in repairing damaged photoreceptors and may contribute to increased oxidation and damage to the retina.

**DHA May Modulate MPOD**

The cells and layers of the retina work together intricately. Rapp et al [59], showed that not only do L and Z accumulate within the macula, they also exist within ROS membranes and potentially offer antioxidant protection. The protection that L and Z may provide to the ROS is likely extremely essential to physiological maintenance, underscored by the fact that POS are targets for oxidative stress because of their high LCPUFA content [60]. These inter-relationships are found throughout the retina. DHA may influence blood and macula accumulation of L [5]. Sanders et al [47], showed that supplementation of 20 g of DHA plus EPA can increase HDL levels [47]. HDL is a major transporter for L and Z. It is hypothesized that by increasing DHA, the delivery of dietary L and Z to the macula may also be increased and therefore potentially influence MPOD. However, Huang et al [61] found that the addition of n-3 LCPUFA to oral supplementation of L/Z in elderly subjects with AMD did not change the serum levels of L and Z. This could have been due to L concentrations and the L formulation of the supplementation.

It is hypothesized that a higher content of DHA will help to alleviate large amounts of oxidative stress on the eye secondary to one’s environment possibly resulting in a higher MPOD. As mentioned earlier, pregnant mothers preferentially supply the fetus with DHA for adequate development of the retina and brain. Because the fetus receives DHA from the mother, mothers who do not get enough DHA from the diet or via DHA supplementation may lack DHA for themselves. This possible DHA deficiency may result in a lower MPOD for the mother.
Therefore, it is hypothesized that the ingestion of DHA may provide protection against oxidative stress. Thus, the larger burden the pregnant mother will endure with less DHA will negatively affect her MPOD. As a result, a prediction is that less DHA will result in a lower MPOD. This prediction is similar to Johnson et al [5] that showed an increase in central MPOD in elderly women with DHA supplementation alone.

**Age-Related Macular Degeneration and DHA**

Maximizing MPOD alleviates the amount of blue light reaching the retina and offers a reduction in the amount of free oxygen radicals within the retina. L and Z’s antioxidant abilities are instrumental in this protection [1]. Thus, L and Z probably protect against the development of eye diseases such as AMD [11].

In the United States (U.S), AMD is the leading cause of eye disease amongst senior citizens (60+) [1]. With damage to the macula there is visual disability or even vision loss [1, 15]. Usually, vision is not completely lost, but central vision may become depleted leaving only peripheral vision [62]. There are two types of AMD: Wet AMD and Dry AMD. The dry form of AMD is most common and is usually characterized by the presence of a soft drusen between the RPE and Bruch’s membrane [63]. The wet form however, represents the formation of new blood vessels from beneath the retina in search of the choroidal layer [63]. Both types of AMD cause central vision loss.

Currently, there is no cure or preventative method for AMD for it has no known source of onset. Key pathogenic processes of AMD are oxygenic, inflammatory, age and sex related factors (the risk of AMD increases with age and is more prevalent among elderly women than men [2]) and retinal damage [64]. Thus, DHA has the potential to influence factors responsible for the progression of AMD potentially through its neuroprotective role in the retina. In a
systematic review Chong et al [65] showed that consumption of fish two or more times per week and rich sources of omega-3 fatty acids were associated with a reduced risk for AMD. The results of Cho et al [15] were consistent with this finding. In this prospective cohort follow up study from the Nurses’ Health Study and the Health Professional, Cho et al. reported that fish intake was inversely related to the risk of AMD and that individuals 50 years and older who ate fish greater than four times per week, had a lower risk of AMD than individuals who ate fish less than 3 times per month. Similarly, Augood et al [66] reported that oily fish consumption at least once per week compared with less than once per week was associated with halving the odds ratio (OR) for AMD. Participants aged 65 years and older had their fundus images graded and classified into stages of AMD (late vs. early) and fish intake and risk factors were assessed by using a food frequency and risk factor questionnaires. Augood et al found a significant association between oily fish consumption and/or fish with higher amounts of DHA and EPA than non oily fish in decreasing the OR of AMD [66].

Currently, no large randomized clinical controlled trials that evaluate n-3 fatty acids and fish intake in the primary prevention of AMD exist [65]. A prospective cohort study conducted by Seddon et al [67] found no effect in the progression of AMD when subjects had an increased fish intake. In that trial, only individuals who were in the below median consumption group for linolenic acid , showed protective effects against the progression of AMD.

Thus, because the onset and treatment of AMD is still unknown, health precautions such as consuming recommended amounts of 2 to 3 servings of fish weekly, wearing sun glasses, eating a diet high in L and Z, and avoiding cigarette smoke, should be practiced and may help prevent the detrimental disabling effects of AMD. In sum, there are limited observational and clinical trials on LCPUFAs that give conclusive evidence concerning the relationship DHA has
with the progression of AMD and its effects on MPOD. There are no known studies noted evaluating the eye health in pregnant women.
CHAPTER 3
EXPERIMENTAL DESIGN AND METHODS

Subject Recruitment

Upon the approval of the protocol by the Institutional Review Board of Louisiana State University’s (LSU) Agricultural Center (AgCenter) adult pregnant women (n=24) who were residents, students, faculty and staff of LSU, East Baton Rouge Parish or surrounding parishes and 18-40 years old were recruited to participate in this study. Additionally, these women were determined to be appropriate participants because they did not smoke or drink alcohol, as well as reported no diagnosis of eye disease. In order to recruit participants, flyers were distributed on the LSU campus and recruitment occurred through the AgCenter website. Public announcements were also made at Women Infant and Children (WIC) clinics and birthing classes at community centers for women and at hospitals. Women agreed to visit the Maternal and Infant Nutrition Laboratory, Knapp Hall at LSU four times during their pregnancy to participate in the measurement of macular pigment. During each visit they also completed a health history form questionnaire and a 24-hour dietary recall (NDSR) to assess the foods they had eaten.

Study Design

Consenting women were required to make four visits during pregnancy at 18-23 weeks, 24-26 weeks, 30 weeks, and 36-38 weeks to the LSU Human Nutrition Assessment Laboratory, Knapp Hall. These weeks encompass the period in which DHA transfer from the mother to the developing fetus is most dramatic. The women completed a dietary survey health history form at each visit (see appendix). Health History Form #1 (Appendix A) was used at each participant’s first visit to document height, weight, BMI, ethnicity, eye color, diagnosis of eye disease, family
history of eye disease, presence of high blood pressure or cholesterol, nutrient supplementation, location where most foods were eaten, the most commonly eaten fruits and vegetables, the frequency for consumption of fruits and vegetables, amount and type of seafood consumed, and barriers for not consuming some fruits or vegetables and fish. Subjects were interviewed with Health History Form#2 (Appendix A) at the 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} visits to document new diagnoses during pregnancy, such as gestational diabetes mellitus.

First Visit:

- Height was measured using a stadiometer. Weight and BMI were determined using a SECA scale.

- The Macularmetrics instructional video was viewed by the subject for instruction on the method for recording null flicker matches. For example, participants were taught how to rotate the subject’s knob at a standard speed, find the point at which the flicker stops and select that point by pressing a red button. All questions about the use of the machine were answered before the subject began her trial.

- The Eye guard was sanitized before each participant used the machine. The participant then completed 10 trials in two stimuli (2 and 5). Stimuli 2 and 5 are the degrees of eccentricity that test the direct measurement of the amount of MPOD in the eye.

- Dietary intake via a 24-hour recall was completed either in person or via the telephone, depending on the participant’s personal schedule. The University of Minnesota 2008-2009 Nutrition Data System for Research program (NDSR) was used to quantify the recall.
The next appointment during the 24-26th week of pregnancy was scheduled and a reminder e-mail was sent out.

Visits 2, 3 and 4:

- Participants were reminded in advance via telephone call or email about the appointment day and time.
- Participants came to the lab, completed Health History Form #2 and had their weight measured and BMI calculated.
- The women were asked if they needed to be reminded about proper Macularmetrics machine use.
- The eye guard was sanitized and the participant performed 10 trials in each stimuli.
- Assessment of usual nutrient intake was assessed by conducting a 24-hour dietary recall either in person or over the phone. Once again, this assessment format was dependent upon the participant’s personal preference and the University of Minnesota 2009-NDSR Nutrition Data System for Research program was used.
- Appropriate appointment time was scheduled in advance for the follow up visit(s).
- After the second visit, a dietary assessment based on two dietary recalls was provided to participants for personal use to guide in the selection of foods for optimal health during pregnancy. Macronutrients as well as micronutrients from recalls were compared to recommendations and this information was shared with the individuals. LSU AgCenter brochures with information on adequate weight gain and good food sources for pregnant
women were also provided to each participant. Women were instructed on how to navigate MyPyramid.gov for pregnant women as well. DHA was not mentioned in the individual’s reports in order to avoid dietary changes.

**Measurement of MPOD**

- The Macularmetrics Densitometer machine was first calibrated in a dimly lit room to adjust LED blue and green light according to the manufacturer’s instructions.

- Measurement of MPOD was taken for the right eye of each participant.

- Subjects were instructed to find the optimal flicker frequency (OFF) while the evaluator increased or decreased the frequency in increments of 1 Hz until the null flicker was perceived. ‘OFF’ was adjusted for each participant during each visit to control the flicker rate using the participant’s right eye.

- Participants increased the frequency by turning the subject knob until the flicker stopped. Measurements were documented by the evaluator and the subject knob was then readjusted so that an area of “null” flicker was no longer perceived.

- This process was repeated for a total of ten trials in stimulus #2 and stimulus #5. Values were recorded. Any outliers were deleted and the participant was asked to complete additional readings (± 500). Note: Each subject was allowed to move away from the machine and blink eyes in order to readjust vision.
Assessment of MPOD Using the Macular Metrics Densitometer Machine

The instrument that was employed to evaluate MPOD eccentricity is the ‘Macular Metrics Densitometer.’ MPOD was measured in the right eye only by heterochromatic flicker photometry. The machine was calibrated at each visit. Such calibration allowed participants to see a visual target with an even mixture of both green and blue light and was adjusted so that no change in brightness of the target is encountered [68]. The luminance of the green light is 550 nm and blue light is 460 nm and was in the yoked position, meaning as one color increases, the other decreases and vice versa [68]. Blue light is the only light absorbed by the MP and the participant will also see a flicker based on the variation of blue light [10]. Blue light is necessary because it allows for only long (L) and medium (M) cones to be active and for rods and sensitive (S) cones to become inactive. Light needs to fall on the fovea to allow for distinct vision. Therefore, sensitive S-cones need to be saturated with the blue light before any flickering may be observed [10]. Variations between readings were controlled for by adjusting the flicker rate until a rate of no flicker was observed by the participant [68]. Testing then began. The densitometer used a reference location at 7 degrees (stimulus #5) in which the participant had to fixate on a red light that helped to map MP spatial profile using a parafoveal target [10, 68]. A 0.5 degrees retinal loci (stimulus #2) that accounted for a 1 degree diameter was used because this is the location at which the fovea may be measured where high concentrations of L and Z are present. Thus, at 0.5 degrees, a representation of MP may be measured [68], and gave the most direct measurement of MP. The women were asked to adjust the amount of blue light by rotating the subject’s knob in order to observe a point at which there was no flicker. The amount of blue light needed to achieve this goal was the measure of MPOD [10].
Data Analysis

Data were analyzed using JMP 8.0 (SAS institute, Cary, NC). The relationship between MPOD and DHA and lutein and zeaxanthin were evaluated using Pearson correlations. Information pertaining to DHA and lutein and zeaxanthin were obtained from dietary survey health history forms given to participants at each visit (Appendix A) in which women estimated weekly, monthly or yearly fruits and vegetables and seafood intake.

Differences at weeks 18-23, 24, 30, 36-38 during pregnancy were determined using the least significance mean (LSM). Analysis of variance (ANOVA) was used to evaluate group differences in MPOD, dietary, supplementation and total (diet + supplement) DHA between mothers who supplement with a DHA containing prenatal supplement (± DHA) or other DHA/fish oil supplements and mothers who did not supplement with DHA to determine the difference between the groups (T-test).

Repeated-measures analysis of covariance (ANCOVA) was used to evaluate MPOD while controlling for covariates that have been shown to impact MPOD (lutein and zeaxanthin, dietary and supplement DHA). Level of significance was set as p≤0.05.
CHAPTER 4  
MACULAR PIGMENT OPTICAL DENSITY IS ASSOCIATED WITH FISH CONSUMPTION

Introduction

Long chain polyunsaturated fatty acid, docosahexaenoic acid (DHA, 22:6n-3), accretion is particularly important for the developing fetus to ensure proper retinal and brain development and functional activity [69]. Thus, DHA from maternal stores is transferred across the placenta and becomes indispensable in the last trimester of pregnancy [58]. As a result, maternal DHA stores become drained and may leave a mother at risk for insufficient DHA for her own retinal health.

As women age, risk for age-related macular degeneration (AMD) increases. The reason why women are at a higher risk than men is unknown. However, research shows that dietary carotenoids lutein and zeaxanthin [12-13] (which accumulate and provide a foundation for macular pigment optical density, MPOD) and fish consumption [15, 65-66] are associated with a lower risk of AMD. Johnson et al also showed that DHA supplementation may increase central MPOD in elderly women [5]. Studies to evaluate DHA status during pregnancy and its relationship with MPOD have not been reported. Therefore, the purpose of the current study was to evaluate if MPOD decreases in the last half of pregnancy.

Methods

Subjects and Study Design

Twenty-four pregnant women (18-24 weeks) of gestation, 18-40 years of age and who did not drink or smoke were recruited from Louisiana State University (LSU) campus through a local newspaper ad and from local clinics by public announcement, word of mouth, and by website announcements on the LSU AgCenter homepage and at natural birthing group classes.
Each woman that expressed interest by responding to the request for study participants was sent an informational brochure via e-mail or was contacted by telephone. Women who had eye disease or history of disease such as blindness were excluded from the study. Women who met the inclusion criteria and agreed to participate signed an informed consent form which had been approved by the Louisiana State University AgCenter Institutional Review Board (IRB). By consenting women agreed to come into the Maternal & Infant Nutrition Laboratory at LSU and have their MPOD measured using a macularmetrics densitometer machine at weeks 18-23, 24-26, 30, 36-38 during pregnancy, complete a dietary questionnaire at each visit, and participate in a 24-hour dietary recall.

Respondents visited the Maternal & Infant Nutrition Laboratory, Knapp Hall at LSU during set appointments at time of recruitment for weeks 18-23, 24-26, 30, 36-38 weeks of pregnancy. Appointments were made in advance and participants were reminded via email or telephone a day before each visit about their selected appointment time. The first visit for each woman was the longest and ranged from an hour to an hour and a half. The subsequent visits ranged from thirty minutes to an hour to complete all requirements. Information pertaining to lutein and zeaxanthin and DHA were obtained from dietary survey health history forms given to participants at each visit (Appendix A) in which women estimated weekly, monthly or yearly fruits and vegetables and seafood intake. If women gave a range for intakes, the two numbers were averaged and the mean value used in analyses. To distinguish fruits and vegetables high in lutein and zeaxanthin and seafood high in DHA, documents listing fruits and vegetables high in lutein and zeaxanthin and high in DHA were used (Appendix B). After densitometer trials were completed 24-hour recalls were conducted using The University of Minnesota 2009 Nutrition Data System for Research (NDSR).
**Determination of MPOD**

Ten trials in each stimuli (2 and 5) were achieved if the participant’s luminance values varied no more than ± 500 log. If luminance values varied for a trial the trial was repeated and the new value was recorded. Values from each stimulus were recorded manually and at the end of each visit were recorded electronically. From this electronic data base, stimuli values were used to calculate MPOD.

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

**Statistical Analyses**

Data were analyzed using JMP 8.0 (SAS Institute, Cary, NC). The relationship between MPOD and dietary DHA and dietary lutein and zeaxanthin were evaluated using Pearson correlations.

Differences at weeks 18-23, 24, 30, 36-38 during pregnancy were determined using the least significance mean (LSM). Analysis of variance (ANOVA) was used to determine group differences in MPOD and dietary, supplementation and total (dietary intake + supplement) DHA intake between mothers who supplement with a DHA or fish oil containing prenatal supplement (± DHA) or other DHA/fish oil supplements and mothers who did not consume prenatal supplements with DHA or fish oil. A woman was considered to be supplementing if she consumed prenatal vitamins with DHA or fish oil before her initial visit (18-23 weeks) and continued to supplement at least up to her second visit (24-26 weeks). ANOVA was used to evaluate group differences in MPOD, dietary DHA intake, prenatal DHA/fish oil supplementation and total DHA (dietary intake and prenatal with DHA/fish oil) between mothers.
who met expert group DHA recommendations of 300 mg or more of DHA [32] and those who did not meet that recommendation.

Repeated measures analysis of covariance (ANCOVA) was used to evaluate MPOD while controlling for covariates. Covariates were those characteristics that can be associated with change in MPOD: lutein and zeaxanthin, dietary, supplement and total DHA [5, 16]. Level of significance was set at $p \leq 0.05$.

**Results**

**Subjects**

A total of 24 participants were recruited for assessment of MPOD [18-23 weeks (n=20), 24-26 weeks (n=22), 30 weeks (n=21), 36-38 weeks (n=18)] during pregnancy. Of the 24 participants recruited one participant withdrew citing permanent bed rest required by her physician. A second participant’s data were excluded from analysis because MPOD values indicated a probable technical misunderstanding of equipment use. Thus, data presented in this study are for 22 women. Women were on average 29 years of age, predominately white, and delivered one child previously (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Ethnicity (%)</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Parity</td>
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</tbody>
</table>

Mean ± standard error. Percentage based on number of participants analyzed.
While all women were consented and scheduled for MPOD assessments at four time points, there were four subjects with missing data for reasons due to: late recruitment (n=2), move to out-of-state (n=1), and premature delivery (n=1). One participant was diagnosed with gestational diabetes mellitus (GDM) while another participant began the study with type 2 diabetes. Participants with GDM and type 2 diabetes remain in the final data analysis because MPOD was not significantly different from other participants. Currently, two women have yet to complete assessments.

**Diet**

There were no differences between time points (18-23 to 36-38 weeks) for the following: dietary lutein + zeaxanthin; energy; dietary, prenatal and total DHA (dietary + supplement) (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>18-23 weeks (n=20)</th>
<th>24-26 weeks (n=22)</th>
<th>30 weeks (n=21)</th>
<th>36-38 weeks (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2247±610</td>
<td>2357±551</td>
<td>2389±716</td>
<td>2204±611</td>
</tr>
<tr>
<td>Total DHA (dietary + supplement mg)</td>
<td>296 ± 443</td>
<td>445 ± 802</td>
<td>186 ± 173</td>
<td>175 ± 186</td>
</tr>
<tr>
<td>Dietary DHA (mg)</td>
<td>146 ± 399</td>
<td>299 ± 802</td>
<td>58 ± 61</td>
<td>72 ± 73</td>
</tr>
<tr>
<td>Prenatal DHA (mg)</td>
<td>165 ± 167</td>
<td>146 ± 169</td>
<td>129 ± 148</td>
<td>102 ± 142</td>
</tr>
<tr>
<td>Lutein + Zeaxanthin (mcg)</td>
<td>1797 ± 1963</td>
<td>1371 ± 1916</td>
<td>1719 ± 1328</td>
<td>1453 ± 1340</td>
</tr>
</tbody>
</table>

Mean ± standard error.
Supplementing vs. Not Supplementing with a Prenatal Containing DHA/Fish Oil

In total, 11 women supplemented with a prenatal containing DHA or fish oil and 11 women supplemented with a prenatal not containing DHA or fish oil. Two women were consuming prenatals with DHA or fish oil during their first visit but discontinued supplementation for the remaining three visits and thus were categorized as not supplementing with prenatals. Of the 11 women supplementing with prenatals containing DHA or fish oil consumption on average was 255 ± 76 mg of DHA/day from prenatals. Women consuming prenatals containing DHA or fish oil versus women not consuming prenatals with DHA or fish oil consumed the same amount of dietary DHA (165 ± 220; 114 ± 225 mg DHA/day, p=0.60). Women supplementing with prenatals containing DHA or fish oil consumed 421 ± 208 mg DHA/day from total DHA (dietary + supplement), whereas those not supplementing with a prenatal containing DHA or fish oil consumed significantly less (114 ± 225, p≤0.01).

MPOD

There was no change in MPOD over the course of pregnancy when controlling for lutein + zeaxanthin and total DHA (dietary + supplement) (Means ± SD: 0.34± 0.03, 0.34± 0.03, 0.35± 0.03, 0.31±0.04) (Figure 4).

Figure 4. Macular Pigment Optical Density (MPOD) over time
Means ± SD. MPOD values over time. Superscript above each time indicates no significant difference in MPOD, p-value=0.64, R²=0.72.
MPOD, Dietary and Supplemental Intake

There were no differences in MPOD values during the second trimester between women supplementing with a DHA/fish oil containing prenatal (0.36 ± 0.18) versus those not supplementing with a DHA/fish oil prenatal (0.31 ± 0.16, \(p=0.54\)). However, there was a small trend \((p=0.13)\) for MPOD of women who supplemented with a prenatal with DHA/fish oil (0.41 ± 0.17) compared to MPOD of women who did not supplement with a prenatal with DHA/fish oil (0.28 ± 0.20) to decrease in the third trimester.

MPOD across pregnancy was positively correlated with dietary lutein + zeaxanthin (figure 5), number of fruits and vegetables high in lutein + zeaxanthin (figure 6) and seafood servings per week (figure 7): lutein + zeaxanthin, \((R^2=0.15, p\leq 0.01)\), number of fruits and vegetables high in lutein + zeaxanthin \((R^2=0.05, p\leq 0.03)\), seafood servings per week \((R^2=0.17, p\leq 0.01)\). MPOD was significantly correlated with seafood servings per week at 18-23 weeks \((R^2=0.33, p\leq 0.02)\) and at 36-38 weeks \((R^2=0.34, p\leq 0.02)\), but not at weeks 24-26 \((R^2=0.13=p\leq 0.15)\) and week 30 \((R^2=0.11 p\leq 0.20)\).

![Figure 5. Positive relationship between MPOD and lutein (L) + zeaxanthin (Z)](image-url)
Figure 6. Positive relationship between MPOD and fruits (f) and vegetables (v) high in lutein (L) + zeaxanthin (Z)

Figure 7. Positive relationship between MPOD and seafood servings per week

Discussion

The uniqueness of this study lies in the fact that MPOD was assessed across the late second and third trimester of pregnancy. No other data on MPOD in pregnancy have been published to the best of our knowledge. Results from the current study show that a woman’s diet does not change over the course of pregnancy and therefore, dietary lutein + zeaxanthin, and
DHA intakes either remain consistently high or low during pregnancy. This finding supports the previous finding of others [70].

Expert groups recommend 300 mg/d of DHA for pregnant and lactating women [32]. This recommendation is based on research studies [14, 25-26] indicating the positive benefits of supplemental DHA to promote brain and retina development of the fetus. In this study, average dietary DHA consumption for pregnant women was below expert group recommendations [32]. This finding is surprising considering women participating in this study live in coastal Louisiana, where the incorporation of seafood is generally typical in the local cuisine. However, evidence from this study indicates that average dietary DHA intakes (148 mg/d ±47mg) were similar to DHA intakes reported for women in both the U.S and Canada [6, 8, 71]. This finding has importance because maternal DHA intake and status is important to fetal retinal and brain development. DHA oil is currently in many prenatal vitamins, either as DHA or fish oil and women in this study consuming these were therefore consuming on average 255 mg of DHA/fish oil/day via their supplements. Thus, because dietary DHA intake was low for many women, our data lead us to suggest that pregnant women should be supplementing with a DHA prenatal to attain the recommended intake of DHA.

Results from the current study of a very small population of pregnant women showed that MPOD did not significantly decrease over the last trimester of pregnancy. However, our data suggest that during the third trimester, women who supplemented with DHA/fish oil prenatals tended to have higher MPOD compared to women not supplementing. This finding leads us to suggest a prenatal supplement with DHA/fish oil during the third trimester is important to maintaining MPOD; a larger population needs to be studied to get a true picture of MPOD during pregnancy. Our current data provided for a power calculation. In order to detect a change over
time with a power of 80%, an alpha of 0.05, mean values of MPOD (0.36, 0.34, 0.36, 0.29) and a standard deviation of 0.19 at each time point, a larger sample size is required (n=121).

This study did not capture pre-pregnancy lifestyle habits or the first to early second trimester of pregnancy. The course of pregnancy measured in the current study (18-23 to 36-38 weeks), may not have captured a large enough window in pregnancy to detect a change in MPOD. A study of women from the beginning of pregnancy may shed new light on what is occurring. It could be that DHA from the RPE were recycled back to the photoreceptors to help maintain the integrity of the retina and therefore MPOD. The retina acts as a conservatory for DHA [49]. Photoreceptor outer segments (POS), which store DHA filled discs, are shed daily and accumulate in the retinal pigment epithelium (RPE). This is in essence a conservation of DHA [49]. This mechanism may be particularly important during periods of low DHA intake and during pregnancy when DHA is being preferentially transported across the placenta to the developing fetus. Thus, recycling of DHA may conserve the macula and MPOD.

Additionally, MPOD was associated by dietary lutein + zeaxanthin, the number of fruits and vegetables eaten high in lutein + zeaxanthin and seafood servings per week. Dietary lutein + zeaxanthin are found in high amounts in foods such as, but not limited to: spinach, kale, corn, oranges, melons and egg yolks [41]. In our current study, pregnant women who consumed higher amounts of lutein + zeaxanthin had higher MPOD. This result is underscored by findings previously reported for both males and females [5, 16]. Johnson et al [5], showed that elderly women supplementing with 12 mg/d of lutein for four months had an increased MPOD. Likewise, Kopsell et al [16] showed that MPOD was higher among both males and females consuming spinach high in lutein compared to individuals consuming spinach lower in lutein.
Cho et al [15] showed that weekly fish consumption by elderly subjects (> 4 x per week) resulted in lower rates of age related macular degeneration compared to study participants who consumed fish less than 3 times per month. Thus, the current study in pregnant women extends previous findings, documenting that dietary sources of lutein + zeaxanthin and DHA are important to eye health and specifically to macula health.

This exploratory study provided useful data that can guide us in future studies of MPOD and diet during pregnancy. Further studies can focus on the following: (i) evaluating MPOD during pregnancy with a larger sample size (n=121); (ii) Studying MPOD during the course of the entire pregnancy; and (iii) exploring the benefits of DHA/fish oil intervention on MPOD during pregnancy.
CHAPTER 5
CONCLUSION

The purpose of this study was to explore MPOD during the course of pregnancy. These exploratory data suggest that women consuming a prenatal supplement containing DHA/fish oil, during the third trimester tended to have a higher MPOD than those not supplementing and DHA intake among pregnant women is low in Louisiana. Based on the data, a larger number of pregnant women are needed to determine if pregnancy and DHA/fish oil/fish intake modulate MPOD.

The U.S. Food and Drug Administration (FDA) recommend for women to consume 2-3 servings of fish weekly encompassing an expert group recommendation of 300 mg/d of DHA during pregnancy. Women are encouraged to eat fish weekly and to eat food high in lutein and zeaxanthin to protect macula health. Pregnant women should be encouraged to increase their intake of dietary DHA to ensure an adequate DHA supply for both the mother and the infant.

Furthermore, because dietary consumption of DHA was low for participants in this study, it is concluded that a prenatal supplement consisting of at least 200 mg of DHA should be supported and/or prescribed to ensure women are consuming the amount recommended to support the growth and development of the fetus.
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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. How many pregnancies have you had (including this one)
6. Do you have high cholesterol or high blood pressure? ______ Yes ______ No

7. 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: ____________________________________________

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

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

10. Do you use any nutritional supplements? ______ Yes ______ No
    If yes, which ones? (Include brand name if known) ________________________________

11. Do you eat most of your meals away from home? ______ Yes ______ No
    If yes, where do you usually eat? ____________________________________________

12. How many times do you eat fruits and vegetables?
   _____ per week
   (or) _____ per month
   (or) _____ per year

13. List the fruits and vegetables most commonly eaten
   1. ________________
   2. ________________
14. 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

15. How many times do you eat seafood (fresh, frozen or canned)?
   _____ per week
   (or) _____ per month
   (or) _____ per year

16. List the type of seafood most commonly eaten (if fish, what kind, example: salmon or catfish?)
   1.  
   2.  
   3.  
   4.  

17. If you don’t consume fresh fruits and vegetables frequently what are the main reasons?
   □ Too expensive
18. 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:___________________

- [ ] Other:___________________

**Health History Form #2**

Name: _______________________

Code #: ________

Date: ________________________

Age: ________

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

BMI: _________ (to be completed by investigator)

19. Have you been diagnosed with eye disease? ______ Yes ______ No

If yes, name disease __________________________________________

20. Do you have high cholesterol or high blood pressure? ______ Yes ______ No
21. Have you been diagnosed with gestational diabetes mellitus? _____Yes _____ No

22. 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:_____________________________________________

23. Do you smoke cigarettes, cigars or pipes? _____ Yes _____ No

        If yes, how many per day _______ per week _______ per month _______

24. Do you drink alcoholic beverages? _____ Yes _____ No

        If yes, how many per day _______ per week _______ per month _______

25. Do you use any nutritional supplements? _____ Yes _____ No

        If yes, which ones? (Include brand name if known)________________________

26. Do you eat most of your meals away from home? _____ Yes _____ No

        If yes, where do you usually eat? _______________________________________

27. How many times do you eat fruits and vegetables?

        _____ per week

        (or) _____ per month

        (or) _____ per year

28. List the fruits and vegetables most commonly eaten
29. 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

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

_____ per week
(or) _____ per month
(or) _____ per year

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

1.__________________
2.__________________
3.__________________
4.__________________
32. 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:___________________

33. 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 B: FOOD SOURCES HIGH IN L&Z AND DHA


<table>
<thead>
<tr>
<th>Food</th>
<th>Micrograms/ cup</th>
<th>Micrograms/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale</td>
<td>23720</td>
<td>18246</td>
</tr>
<tr>
<td>Spinach</td>
<td>20354</td>
<td>11308</td>
</tr>
<tr>
<td>Turnip greens</td>
<td>12154</td>
<td>8440</td>
</tr>
<tr>
<td>Collards</td>
<td>14619</td>
<td>7694</td>
</tr>
<tr>
<td>Mustard greens</td>
<td>8347</td>
<td>5962</td>
</tr>
<tr>
<td>Parsley, raw</td>
<td>556</td>
<td>5560</td>
</tr>
<tr>
<td>Dandelion greens</td>
<td>4944</td>
<td>4709</td>
</tr>
<tr>
<td>Peas, green, frozen</td>
<td>3840</td>
<td>2400</td>
</tr>
<tr>
<td>Lettuce, romaine, raw</td>
<td>1295</td>
<td>2313</td>
</tr>
<tr>
<td>Summer squash</td>
<td>4048</td>
<td>2249</td>
</tr>
<tr>
<td>Beet greens</td>
<td>2619</td>
<td>1819</td>
</tr>
<tr>
<td>Lettuce, green leaf, raw</td>
<td>969</td>
<td>1730</td>
</tr>
<tr>
<td>Broccoli</td>
<td>2367</td>
<td>1517</td>
</tr>
<tr>
<td>Squash, winter</td>
<td>2901</td>
<td>1415</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>2012</td>
<td>1290</td>
</tr>
<tr>
<td>Onions, spring or scallions, raw</td>
<td>1137</td>
<td>1137</td>
</tr>
<tr>
<td>Corn, sweet, yellow, canned</td>
<td>2195</td>
<td>1045</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>2484</td>
<td>1014</td>
</tr>
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</table>

### Fish High in DHA

<table>
<thead>
<tr>
<th>Fish Species and Description</th>
<th>DHA per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crustaceans, crab, blue, cooked, moist heat</td>
<td>0.231</td>
</tr>
<tr>
<td>Crustaceans, crab, queen, cooked, moist heat</td>
<td>0.145</td>
</tr>
<tr>
<td>Fish, anchovy, European, raw</td>
<td>0.911</td>
</tr>
<tr>
<td>Fish, anchovy, European, canned in oil, drained solids</td>
<td>1.292</td>
</tr>
<tr>
<td>Fish, bass, freshwater, mixed species, cooked, dry heat</td>
<td>0.458</td>
</tr>
<tr>
<td>Fish Species and Description</td>
<td>DHA per 100 g</td>
</tr>
<tr>
<td>------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Fish, bass, striped, cooked, dry heat</td>
<td>0.750</td>
</tr>
<tr>
<td>Fish, bluefish, cooked, dry heat</td>
<td>0.665</td>
</tr>
<tr>
<td>Fish, catfish, channel, farmed, cooked, dry heat</td>
<td>0.128</td>
</tr>
<tr>
<td>Fish, catfish, channel, wild, cooked, dry heat</td>
<td>0.137</td>
</tr>
<tr>
<td>Fish, caviar, black and red, granular</td>
<td>3.800</td>
</tr>
<tr>
<td>Fish, cod, Atlantic, cooked, dry heat</td>
<td>0.154</td>
</tr>
<tr>
<td>Fish, cod, Pacific, cooked, dry heat</td>
<td>0.173</td>
</tr>
<tr>
<td>Fish, drum, freshwater, cooked, dry heat</td>
<td>0.368</td>
</tr>
<tr>
<td>Fish, flatfish (flounder and sole species), cooked, dry heat</td>
<td>0.258</td>
</tr>
<tr>
<td>Fish, grouper, mixed species, cooked, dry heat</td>
<td>0.213</td>
</tr>
<tr>
<td>Fish, halibut, Atlantic and Pacific, cooked, dry heat</td>
<td>0.374</td>
</tr>
<tr>
<td>Fish, halibut, Greenland, cooked, dry heat</td>
<td>0.504</td>
</tr>
<tr>
<td>Fish, herring, Atlantic, cooked, dry heat</td>
<td>1.105</td>
</tr>
<tr>
<td>Fish Species and Description</td>
<td>DHA per 100 g</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Fish, herring, Atlantic, kippered</td>
<td>1.179</td>
</tr>
<tr>
<td>Fish, herring, Pacific, cooked, dry heat</td>
<td>0.883</td>
</tr>
<tr>
<td>Fish, lingcod, cooked, dry heat</td>
<td>0.130</td>
</tr>
<tr>
<td>Fish, mackerel, Atlantic, cooked, dry heat</td>
<td>0.699</td>
</tr>
<tr>
<td>Fish, mackerel, king, cooked, dry heat</td>
<td>0.227</td>
</tr>
<tr>
<td>Fish, mackerel, Pacific and jack, mixed species, cooked, dry heat</td>
<td>1.195</td>
</tr>
<tr>
<td>Fish, mackerel, Spanish, cooked, dry heat</td>
<td>0.952</td>
</tr>
<tr>
<td>Fish, mullet, striped, cooked, dry heat</td>
<td>0.148</td>
</tr>
<tr>
<td>Fish, ocean perch, Atlantic, cooked, dry heat</td>
<td>0.271</td>
</tr>
<tr>
<td>Fish, perch, mixed species, cooked, dry heat</td>
<td>0.223</td>
</tr>
<tr>
<td>Fish, pike, northern, cooked, dry heat</td>
<td>0.095</td>
</tr>
<tr>
<td>Fish, pike, walleye, cooked, dry heat</td>
<td>0.288</td>
</tr>
<tr>
<td>Fish, pollock, Atlantic, cooked, dry heat</td>
<td>0.451</td>
</tr>
</tbody>
</table>

Table continued
<table>
<thead>
<tr>
<th>Fish Species and Description</th>
<th>DHA per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish, rockfish, Pacific, mixed species, cooked, dry heat</td>
<td>0.262</td>
</tr>
<tr>
<td>Fish, roe, mixed species, cooked, dry heat</td>
<td>1.747</td>
</tr>
<tr>
<td>Fish, roe, mixed species, raw</td>
<td>1.363</td>
</tr>
<tr>
<td>Fish, sablefish, cooked, dry heat</td>
<td>0.920</td>
</tr>
<tr>
<td>Fish, sablefish, smoked</td>
<td>0.945</td>
</tr>
<tr>
<td>Fish, salmon, Atlantic, farmed, cooked, dry heat</td>
<td>1.457</td>
</tr>
<tr>
<td>Fish, salmon, Atlantic, wild, cooked, dry heat</td>
<td>1.429</td>
</tr>
<tr>
<td>Fish, salmon, Chinook, cooked, dry heat</td>
<td>0.727</td>
</tr>
<tr>
<td>Fish, salmon, chum, cooked, dry heat</td>
<td>0.505</td>
</tr>
<tr>
<td>Fish, salmon, chum, drained solids with bone</td>
<td>0.702</td>
</tr>
<tr>
<td>Fish, salmon, coho, farmed, cooked, dry heat</td>
<td>0.871</td>
</tr>
<tr>
<td>Fish, salmon, coho, wild, cooked, dry heat</td>
<td>0.658</td>
</tr>
<tr>
<td>Fish, salmon, pink, cooked, dry heat</td>
<td>0.751</td>
</tr>
<tr>
<td>Fish Species and Description</td>
<td>DHA per 100 g</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Fish, salmon, sockeye, cooked, dry heat</td>
<td>0.700</td>
</tr>
<tr>
<td>Fish, sardine, Atlantic, canned in oil, drained solids with bone</td>
<td>0.509</td>
</tr>
<tr>
<td>Fish, sea bass, mixed species, cooked, dry heat</td>
<td>0.556</td>
</tr>
<tr>
<td>Fish, sea trout, mixed species, cooked, dry heat</td>
<td>0.265</td>
</tr>
<tr>
<td>Fish, shad, American, raw</td>
<td>1.321</td>
</tr>
<tr>
<td>Fish, shark, mixed species, raw</td>
<td>0.527</td>
</tr>
<tr>
<td>Fish, smelt, rainbow, cooked, dry heat</td>
<td>0.536</td>
</tr>
<tr>
<td>Fish, snapper, mixed species, cooked, dry heat</td>
<td>0.273</td>
</tr>
<tr>
<td>Fish, spot, cooked, dry heat</td>
<td>0.526</td>
</tr>
<tr>
<td>Fish, sucker, white, cooked, dry heat</td>
<td>0.371</td>
</tr>
<tr>
<td>Fish, swordfish, cooked, dry heat</td>
<td>0.681</td>
</tr>
<tr>
<td>Fish, tilefish, cooked, dry heat</td>
<td>0.733</td>
</tr>
<tr>
<td>Fish, trout, mixed species, cooked, dry heat</td>
<td>0.677</td>
</tr>
</tbody>
</table>

Table continued
<table>
<thead>
<tr>
<th>Fish Species and Description</th>
<th>DHA per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish, trout, rainbow, farmed, cooked, dry heat</td>
<td>0.820</td>
</tr>
<tr>
<td>Fish, trout, rainbow, wild, cooked, dry heat</td>
<td>0.520</td>
</tr>
<tr>
<td>Fish, tuna, fresh, bluefin, cooked, dry heat</td>
<td>1.141</td>
</tr>
<tr>
<td>Fish, tuna, light, canned in oil, drained solids</td>
<td>0.101</td>
</tr>
<tr>
<td>Fish, tuna, light, canned in water, drained solids</td>
<td>0.223</td>
</tr>
<tr>
<td>Fish, tuna, skipjack, fresh, cooked, dry heat</td>
<td>0.237</td>
</tr>
<tr>
<td>Fish, tuna, white, canned in water, drained solids</td>
<td>0.629</td>
</tr>
<tr>
<td>Fish, tuna, yellowfin, fresh, cooked, dry heat</td>
<td>0.232</td>
</tr>
<tr>
<td>Fish, whitefish, mixed species, cooked, dry heat</td>
<td>1.206</td>
</tr>
<tr>
<td>Fish, whiting, mixed species, cooked, dry heat</td>
<td>0.235</td>
</tr>
<tr>
<td>Fish, wolffish, Atlantic, cooked, dry heat</td>
<td>0.405</td>
</tr>
<tr>
<td>Mollusks, mussel, blue, cooked, moist heat</td>
<td>0.506</td>
</tr>
<tr>
<td>Mollusks, oyster, eastern, farmed, cooked, dry heat</td>
<td>0.211</td>
</tr>
<tr>
<td>Fish Species and Description</td>
<td>DHA per 100 g</td>
</tr>
<tr>
<td>------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Mollusks, oyster, eastern, wild, cooked, dry heat</td>
<td>0.291</td>
</tr>
<tr>
<td>Mollusks, oyster, Pacific, cooked, moist heat</td>
<td>0.500</td>
</tr>
</tbody>
</table>

Table continued

VITA

Alicia Janaye Page was born in Houston, Texas. She received her Bachelor of Science degree in nutrition on May, 2008, from the University of Georgia in Athens, Georgia. She began her master’s degree program in fall 2008 at Louisiana State University School of Human Ecology with a concentration in human nutrition and food. She is a student member of the American Dietetic Association and American Oil Chemists Society.