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Omega-3 Fatty Acid Status in Overweight Pregnant Women of Louisiana

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OMEGA-3 FATTY ACID STATUS IN OVERWEIGHT PREGNANT WOMEN OF
LOUISIANA

A Thesis

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Louisiana State University and
Agricultural and Mechanical College
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by
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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ALA	Linolenic Acid
ARA	Arachidonic Acid
CDC	Centers for Disease Control and Prevention
CVD	Cardiovascular Disease
DHA	Docosahexaenoic Acid
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FADS	Fatty Acid Desaturase
FAME	Fatty Acid Methyl Ester
GC	Gas Chromatography
IOM	Institute of Medicine
IRB	Institutional Review Board
LA	Linoleic Acid
LCPUFA	Long Chain Polyunsaturated Fatty Acid
NDSR	Nutrition Data System for Research
PUFA	Polyunsaturated Fatty Acid
RBC	Red Blood Cell

ABSTRACT

Omega-3 fatty acids are well known to benefit pregnant women and infant outcomes. More women are entering pregnancy with excess weight which puts them at higher risk for complications such as gestational diabetes, preeclampsia, and preterm delivery, amongst others. In the present study we assessed the omega-3 fatty acid (docosahexaenoic acid, DHA) status in overweight (BMI = 25.0 – 29.9 kg/m^2) pregnant women of Louisiana. Age, ethnicity, weeks of pregnancy, weight, and length were recorded for each participant. Dietary intakes were assessed by repeated 24-h dietary recalls using the University of Minnesota Nutrient Data System for Research. Blood samples were collected at 16.5 – 20 weeks of pregnancy to evaluate red blood cells (RBC) fatty acids by gas chromatography. Pregnant women (n = 21) were 19 – 34 years of age; 62% were African Americans, 29% Caucasians, and 9% Hispanics; and 62% of the population had low socioeconomic status. On average, pregnant women consumed 72 ± 63 mg DHA/day; therefore, pregnant women were not meeting the recommended intake of at least 200 mg of DHA/day ($p \leq 0.05$). When including supplementation, only 38% met the recommended intake ($p \leq 0.05$). Using logistic regression analysis it was determined that age, ethnicity, and socioeconomic status ($p > 0.05$) did not affect the probability of achieving the recommended DHA dietary intake for pregnant women. RBC DHA was 8.48 ± 1.39 wt%. African Americans had lower RBC DHA (7.98 ± 0.94 wt%) compared to Caucasians + Hispanics (9.29 ± 1.68 wt%) ($p \leq 0.05$). Multi-source regression analysis revealed that only ethnicity affected the RBC DHA wt% ($p \leq 0.05$); whereas age, intake (diet + supplement), and SES did not ($p > 0.05$). Our data point to a need for nutrition education regarding the benefits of consuming DHA during pregnancy for pregnant women and women of childbearing ages.

CHAPTER 1
INTRODUCTION

Nutrition during the perinatal period plays a key role in fetal development. Every nutrient, whether received or not in adequate quantities, will determine in part the long-term health for the infant. Louisiana is reported to be one of the leading states in the Unites States for obesity. More specifically, a report from the Gallup-Healthways Well-Being Index in May 2015 revealed that Louisiana ranks as the number 3 state for obesity in the nation; Baton Rouge is the community with the highest incidence of obesity with more than one third (35.9%) of its residents classified as obese (1).

Overweight and obesity can lead to a host of different metabolic diseases. Diabetes, high blood pressure, high cholesterol, and cardiovascular problems are just a few of the diseases that can develop or be complicated when weight gain is excessive. Weight gain is also linked to quality of life and well-being. Medical cost is also increased. The Institute of Medicine (IOM) in 2012 reported an estimated annual cost of $190.2 billion for obesity-related illnesses (2).

Gestation is viewed as a critical period for the development of obesity. It is thought that either over- or undernutrition during the perinatal period influence the development of obesity later in life (3). The risk for obesity in children at seven years of age has been associated with obesity in the mother or both parents (4).

Clearly, the environment in early life plays a significant role for infants' development of overweight or obesity. Fetal programming can influence the development of certain diseases later in life. For instance, the *in utero* environment is considered a critical period in which the long term regulation of energy balance may be programmed (4). Given that intrauterine life is a critical period of development, alterations in fetal nutrition and endocrine status will modify fetal

physiology and metabolism, predisposing the infant to a host of diseases later in life. This has come to be known as the 'fetal origins' hypothesis (5).

Justification

Nowadays, more females are entering pregnancy with overweight or obesity and this extra weight puts them at higher risk for complications that can be detrimental to their own health, their babies', or both. According to the Centers for Disease Control and Prevention (CDC), almost 60% of U.S. women enter pregnancy with overweight and more than 30% of pregnant women gain weight during pregnancy in excess of what is recommended by the IOM (6): 15-25 pounds for women who enter pregnancy with overweight and 11-20 pounds for women who enter pregnancy with obesity (7).

It is well established that DHA during pregnancy can benefit the child later in life. For example, Courville et al. (2011) conducted a study that demonstrated the influence of DHA during pregnancy on infant adiposity. Infants born to mothers supplemented with 300 mg of DHA from 24 weeks of pregnancy until delivery had lower ponderal index and lower umbilical cord insulin concentrations than infants born to mothers consuming a placebo (8).

The Dietary Guidelines for Americans 2010 recommends that pregnant women eat at least 8 ounces per week of seafood that provide an average of 250 mg per day of DHA and EPA (9). This could be attained by eating a variety of seafood and selecting choices that are high in DHA and EPA but low in methylmercury. Recent research conducted in our laboratory has demonstrated that females of reproductive age are consuming low amounts of DHA and EPA (46-66 mg/day) (10, 11). In late pregnancy the fetal demand for nutrients is higher than in early pregnancy, as it is a time of significant growth for the fetus (5, 12). Therefore, our interest is

focused on evaluating the omega-3 long chain polyunsaturated fatty acid (LCPUFA) status of overweight pregnant women at mid-pregnancy.

By investigating the factors that might reduce the risk of obesity later in life, health professionals could focus on preventive actions rather than treatment. Simultaneously, by generating more evidence for the benefits of omega-3 LCPUFAs (DHA) during pregnancy, nutrition education can be better focused on teaching women of childbearing ages.

Research Hypothesis

Overweight pregnant women from Louisiana are not meeting the recommended intake of omega-3 long chain polyunsaturated fatty acids for pregnant women.

Specific Aim

The aim of this research is to assess the status of omega-3 LCPUFA (DHA) of pregnant women by measuring the fatty acids of red blood cells in overweight pregnant women of the greater Baton Rouge area and estimating their dietary intake at 16.5 – 20 weeks of pregnancy.

Objectives

1. Estimate the omega-3 LCPUFA (DHA) dietary intake.

2. Compare the omega-3 LCPUFA (DHA) dietary intake of Baton Rouge overweight pregnant women against the current recommendation for DHA for pregnant women.

3. Assess if overweight pregnant women from Baton Rouge are consuming supplements that include DHA.

4. Determine the status of omega-3 LCPUFA (DHA) in overweight pregnant women.

Limitations

1. Not being able to recruit enough participants for the study. Despite the fact that 234 women were invited to participate, only 21 were interested in enrolling in the study.

2. Dietary intakes were assessed with repeated 24-h dietary recalls, which relied on the memory of the participants. However, repeated dietary recalls represent the best estimates of food consumed.

CHAPTER 2
REVIEW OF LITERARURE

Lipids

Lipids, commonly called fats, are organic molecules found in all types of living organisms. Compared to proteins and carbohydrates, fats are characterized for being insoluble in water but soluble in organic solvents (12). Therefore, fats are capable of interacting with other molecules to be utilized in digestion, absorption, transport, and storage (13). Triglycerides, phospholipids and, sterols are the three types of lipids found in the human body. Triglycerides are the major form of lipid in the food we consume and it is how fat is stored in our bodies. Fats are the major source of fuel energy for the body and they also help in the absorption of fat-soluble vitamins (14).

Triglycerides (also known as triacylglycerols) consist of three fatty acids (FA) attached to a three-carbon glycerol backbone (12). A fatty acid is formed by a chain of carbon atoms bound to each other as well as to hydrogen atoms with a carboxylic acid group (COOH) at one end and a methyl group (CH_3) at the other. Therefore, fats have a polar, hydrophilic end (-COOH) and a nonpolar, hydrophobic end (-CH_3) (14) (Figure 1).
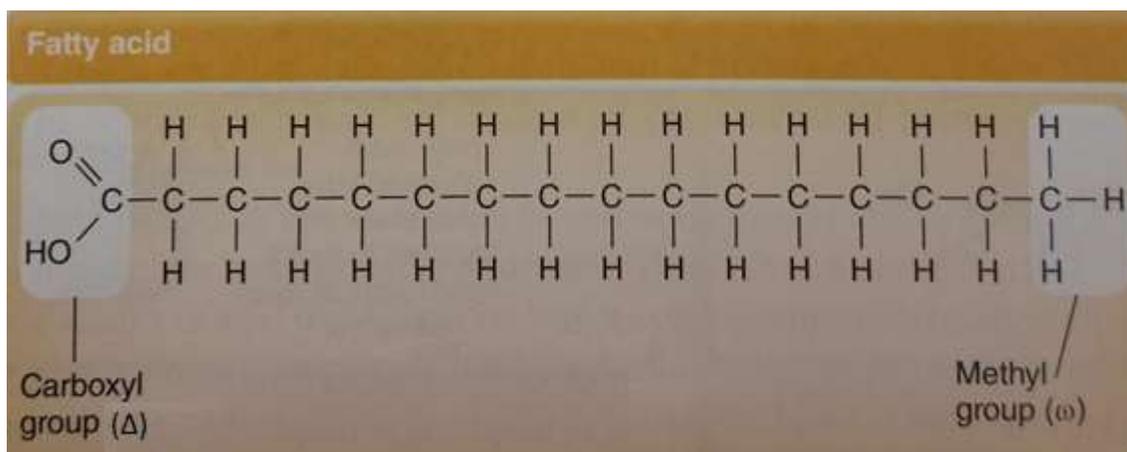

Figure 1. Fatty acid structure (12).

Based on the length of the carbon chain, FAs are classified as: short chain, 4-7 carbons; medium chain, 8-12 carbons; and long chain, more than 12 carbons. FAs are also classified based on the degree of saturation. The carbon atom is capable of forming four bonds to attach them to four other atoms. Carbons attached to hydrogen or to another carbon will dictate the degree of saturation of a FA. Thus, a saturated FA has each carbon within the chain bound to two hydrogens. That means each carbon atom is saturated with hydrogen. If carbons within the chain are not saturated with hydrogen (carbon bound only to one hydrogen) they form carbon-carbon double bonds making the FA monounsaturated. A polyunsaturated FA contains more than one double bond in its carbon chain (15) (Figure 2). Further, unsaturated FAs can occur in either cis or trans configuration. A cis FA has both hydrogen atoms attached on the same side of the double carbon bond, whereas a trans FA has both hydrogen atoms attached on diagonally opposite sides of the double carbon bond (12). The length of the carbon chain and the type and location of the bonds between the carbon atoms determine the physical properties of the fatty acid and the influence it can have on health.
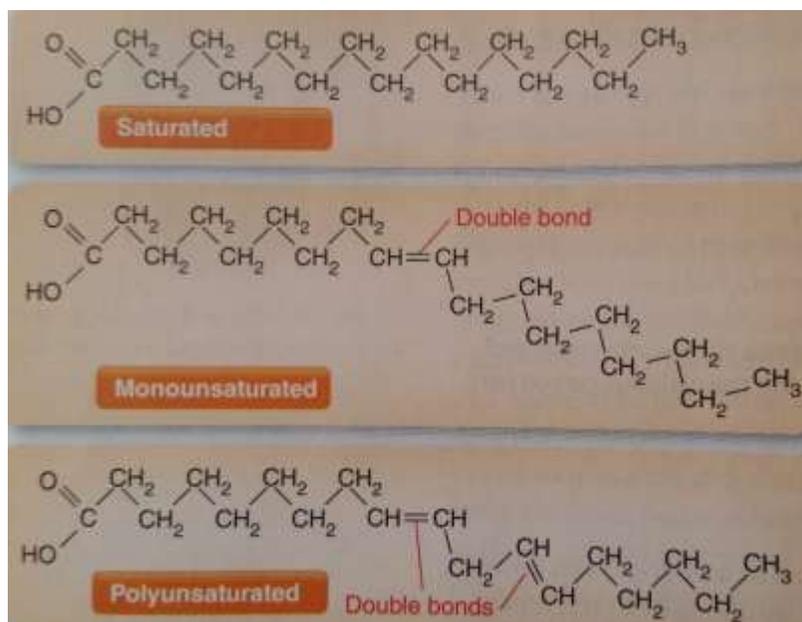

Figure 2. Levels of saturation among fatty acids (12).

A commonly used system to name a FA is based on the -CH$_3$ end of the FA chain. The position of double bonds between carbons is counted from the CH$_3$ end, also called omega ("ω" or "n"), in the carbon chain. For instance, DHA under this system will be named as 22:6n-3; this indicates that the carbon chain is formed by 22 carbon atoms, 6 double bonds, and the first double bond is located in the 3rd carbon atom, counting from the -CH$_3$ of the molecule.

Essential Fatty Acids and their Metabolism

Essential fatty acids (EFA) are lipids that cannot be synthesized within the body, or cannot be synthesized in sufficient quantities to meet the body's needs; consequently they must be consumed in the diet or from supplements. Humans are not capable of synthesizing double bonds in the omega-6 and omega-3 positions (15). For that reason, linoleic acid (LA, 18:2n6) and alpha-linolenic acid (ALA, 18:3n3) are considered EFA. After ingestion, they are distributed between adipose triglycerides, other tissue stores, tissue structural lipids, and some of them are used to provide energy (13). EFA are required for physiologic functions such as normal growth, oxygen transport, energy storage, and regulation of inflammation (14-16).

Human FA synthesis occurs predominantly in the liver. Omega-6 and omega-3 LCPUFA (i.e. C20:3n6, C20:4n6, C20:5n-3, and C22:6n-3) are derived from linoleic acid (LA, 18:2n6) and alpha-linolenic acid (ALA, 18:3n3), respectively (13). During this process, desaturases that incorporate double bonds, and elongases that add two carbon atoms at the carboxylic end of the chain, react with FAs until the 22 carbon chain FAs are formed (13, 14) (Figure 3).

DHA and EPA are synthesized in limited amounts from alpha-linolenic acid (ALA) (17). Barceló-Coblijn and Murphy, and Brenna et al. (18, 19), have summarized the findings pointing to a very limited conversion of ALA to DHA in the human body. The LCPUFAs (20- and 22-carbon chain length) are more rapidly incorporated into the developing brain than the 18-carbon

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chain length FAs, approximately 10 times more efficiently (13). DHA and EPA are most

prevalent in membrane phospholipids. DHA is the most abundant omega-3 fatty acid in cell

membranes of the brain and the retina.
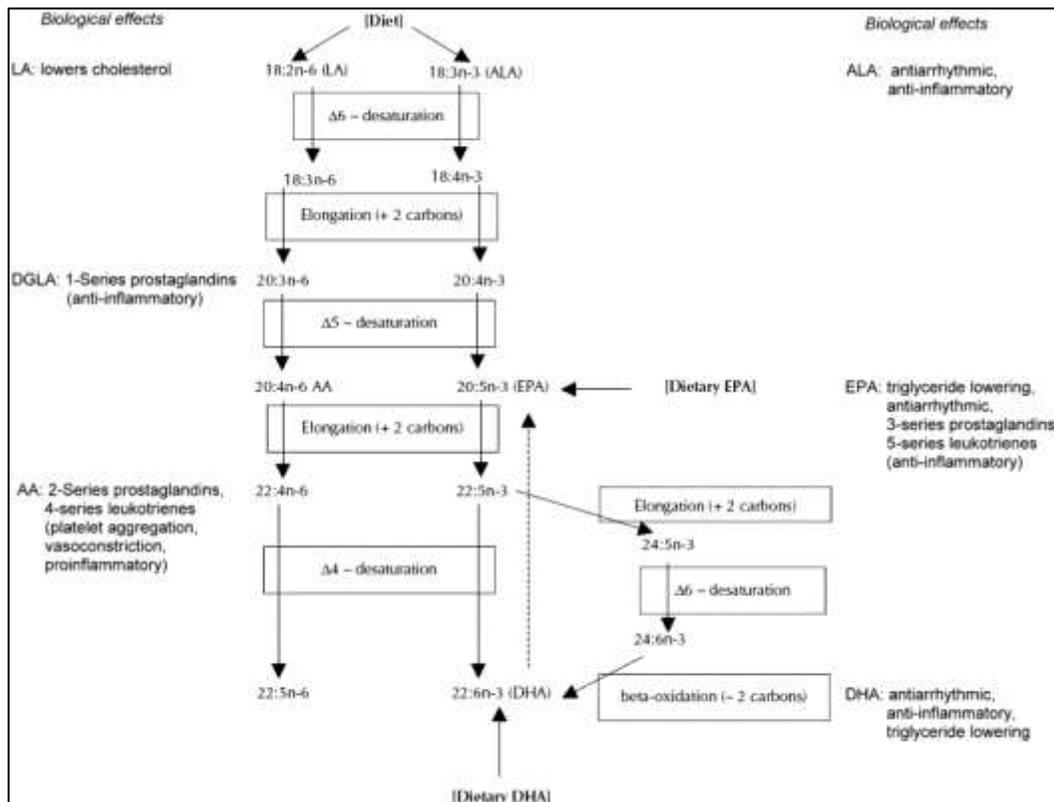
Figure 3. Metabolism of Omega-6 and Omega-3 Fatty Acids (20).

Benefits of Fatty Acids during Pregnancy

More than 35 years ago investigation on the role of omega-3 and omega-6 LCPUFA on

fetal development started. Different authors evaluated fetal tissues at different time points during

pregnancy and the conclusion was the same: fatty acid accretion increases with gestation,

reaching the maximum level during the last trimester. Arachidonic Acid (AA, 20:4n6) and DHA

are the most abundant LCPUFAs in fetal brain as they are used for structure and function (Figure

4) (21-24). Hence the nutrient needs, especially for LCPUFAs, are increased during pregnancy

with the purpose of providing the fetus with sufficient LCPUFAs for his/her adequate

development.

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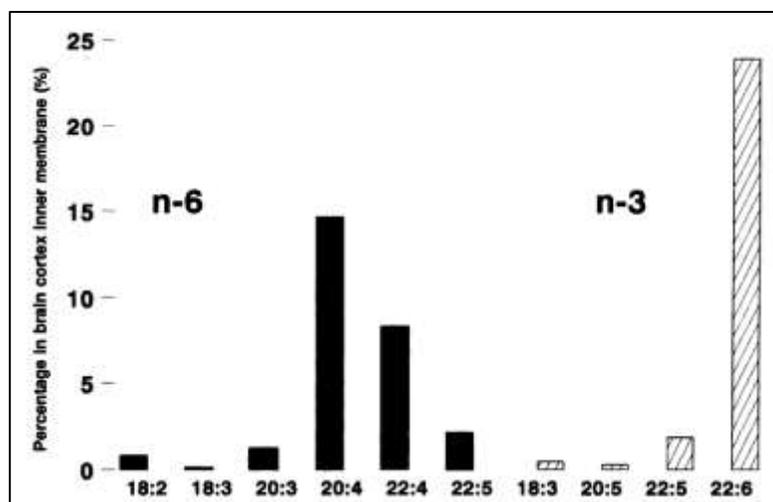
Figure 4. Fatty acid composition in fetal brain (22).

During the last three decades evidence has accumulated to support the thesis that a supply of EFA and LCPUFAs are needed for normal development of the fetus (25). Fetal FA concentrations will be driven by maternal diet as they are transferred from the mother to the fetus through the placenta, which concentrates the LCPUFAs in favor of the fetus (25, 26).

During the last trimester of pregnancy lipid deposition in the fetus increases around 7 g/day with the baby's brain growing at a rate of 1 mg/min (27). The brain is 60% structural lipids, thus high amounts of AA and DHA are found in it as the incorporation of these LCPUFAs in the fetal brain has preference over other types of FAs (22, 26). Rapid accretion of the omega-3 LCPUFAs in the central nervous system occurs in the last weeks of pregnancy. The fetus accumulates LCPUFA in adipose tissue during the last trimester for use of the deposits in the first days after birth (27).

Studies have repeatedly demonstrated that DHA plays a key role in fetal brain and retina development; it may also reduce the prevalence of intrauterine growth retardation and preterm birth by increasing the length of gestation (16). Therefore, the requirement for omega-3 LCPUFAs can be considered critical during the *in utero* period (28). Below some trials that provide evidence of the importance of LCPUFAs during pregnancy:

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<u>Gestational length</u>

In a randomized, double-blinded, controlled, clinical trial conducted by Smuts et al. (29), 291 pregnant women at 24-28 weeks of pregnancy consumed 4.6 – 5.6 DHA-enriched eggs (133 mg DHA/egg) or regular eggs (33 mg DHA/egg) per study week until delivery. It was found that gestational age was significantly increased by 6 ± 2.3 days in the group consuming DHA-enriched eggs. Also, in the DHA-enriched eggs group there was a positive tendency in an increment of infant's birth weight, length, and head circumference. In another randomized, double-blinded, controlled trial by Carlson et al. (30), 350 pregnant women consumed a placebo or DHA capsules (600 mg/day from marine algae oil) from less than 20 weeks of gestation until delivery. Gestation was increased by 2.9 days, the infants had higher birth weight, and head circumference in the group with DHA supplementation.

<u>Infant development</u>

Most of the human trials have focused on the infant's central nervous system and visual development. In a randomized, longitudinal, double-blinded, and placebo-controlled trial evaluating the effects of a DHA-functional food (cereal bar containing 300 mg of DHA fish oil – an average of 5 bars/week was consumed) versus a placebo in pregnant women at 24 weeks of gestation until delivery, a series of studies were conducted to evaluate different aspects in infant development:

1. In a study by Judge et al. (31) with 30 pregnant women, infant visual acuity was assessed at four and six months of age. Babies born to mothers in the DHA-functional food group had higher visual acuity scores at four months. The results indicated better visual development in infants whose mothers had consumed DHA when pregnant.

2. In another study by Judge et al. (32) 29 pregnant women participated. Infants at 9 months of age were presented problem-solving and recognition memory tests. Infants born to mothers in the DHA-functional food indicated better problem-solving but not recognition memory abilities.

3. A study by Judge et al. (33) with 48 participants, found that babies born to mothers consuming the DHA-functional food had better neurobehavioral development (assessed by infant sleep pattering) when compared to the group consuming the placebo bar.

4. Courville et al. (8), evaluated the prenatal impact of the DHA-functional food on infant intrauterine growth in 47 pregnant women. Infants 21 months-old whose mothers consumed the DHA-functional food had lower infant ponderal index (an indicator of infant body fatness) than infants whose mothers consumed the placebo. The venous cord blood plasma insulin concentrations in infants born to mothers consuming the DHA-functional food were lower compared to infants born from mothers consuming the placebo.

Thus, intervention studies with different amounts of DHA have laid the groundwork to demonstrate that DHA during pregnancy provides benefits for infant neural and visual development, and this may influence function throughout the rest of life.

Overweight Pregnancy and its Related Complications

More females are entering pregnancy with high body mass index (BMI) and are also gaining more weight than is recommended (7). High BMI, overweight and obesity, have been linked to complications during pregnancy: preeclampsia, hypertension, higher rates of cesarean delivery, increased risk for gestational diabetes mellitus (GDM), preterm delivery (13, 34, 35), and other related complications predisposing the infant to higher risks of developing chronic

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diseases (obesity, diabetes, metabolic syndrome, etc.) later in life. A meta-analysis conducted by

Chu et al. (36) in 2007 revealed that high maternal weight was associated with higher risk of

GDM and the risk was increased with weight gain. Obese women are also at higher risk of

postpartum weight retention and are less likely to initiate and sustain breastfeeding (37).

Obesity during pregnancy is common in the U.S. and it has been associated with

increased use of health services. A study conducted by the CDC in collaboration with Kaiser

Permanente Northwest (38) (a health care provider) identified that high BMI (overweight and

obesity) in pregnant women was associated with more prenatal fetal tests, obstetrical ultrasound

examinations, medications, telephone calls to the department of obstetrics and gynecology, and

prenatal visits with physicians compared to normal BMI. The CDC identified that severe

maternal morbidity, a term that includes the most severe complications of pregnancy, affect more

than 50,000 women in the U.S. every year (39). Elevated pre-pregnancy BMI is one of the

factors that is considered to increase severe maternal morbidity.

The American College of Obstetricians and Gynecologists established that obese women

are more likely to give birth to an infant with congenital anomalies, for example neural tube

defects; and excessive weight gain during pregnancy is associated with large-for-gestational-age

infants, who are at higher risk of developing childhood obesity (37) and their birth presents

delivery risk for both the infant and the mother.

Role of Omega-3 LCPUFA in Decreasing Inflammation in Pregnancy Complicated by Overweight

A healthy pregnancy involves a physiological maternal inflammatory response (34) that

is aggravated with weight gain as obesity is considered a metabolic inflammatory disease (13)

and this excessive weight gain triggers an abnormal inflammatory response during pregnancy.

Overweight and obese people have higher C-reactive protein levels (an important biomarker of

the inflammatory response) than normal weight people (40), suggesting that higher BMI is associated with higher inflammation. Challier et al. (41) in 2008 demonstrated that obesity in pregnancy is associated with peripheral inflammation. Thus, overweight pregnant women are more sensitive to exaggerated inflammation which exposes them and their babies to adverse consequences.

EFA are precursors for eicosanoids, important biological compounds that regulate cellular functions of significance in human health and disease, such as reproductive function, blood clotting, blood pressure, immune function, and inflammation, among others (12, 42). Different biological compounds derived from LCPUFAs are involved in the inflammatory response. The three major classes of eicosanoids are prostaglandins, thromboxanes, and leukotrienes (42). Some eicosanoids are derived from AA, which is an omega-6 LCPUFA. EPA (omega-3 LCPUFA) is a precursor for eicosanoids, resolvins and docosanoids; and DHA (omega-3 LCPUFA) is a precursor for resolvins and protectins. Compounds derived from omega-6 LCPUFAs (i.e. AA) are pro-inflammatory mediators, whereas compounds derived from omega-3 LCPUFAs (i.e., DHA and EPA) have anti-inflammatory properties (43) (Figure 5). Thus, eicosanoids act as mediators and regulators of inflammation (13).
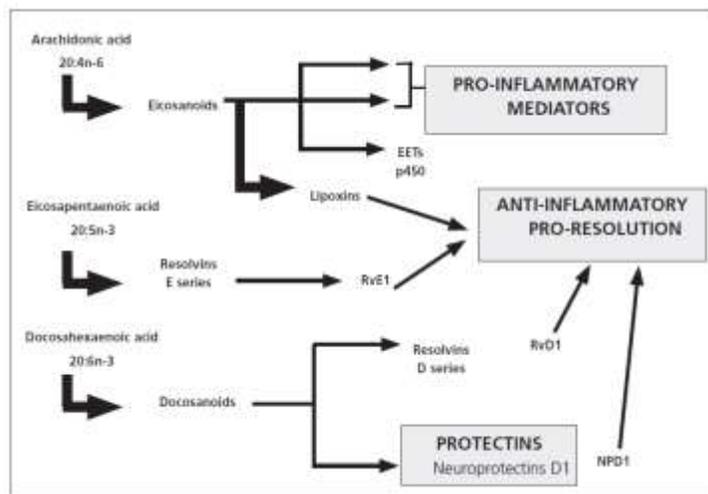

Figure 5. Mediators derived from LCPUFA (43).

By increasing the amounts of omega-3 LCPUFAs less synthesis of pro-inflammatory eicosanoids will occur. Thus, the mechanism of action for DHA is antagonism of production of pro-inflammatory eicosanoids.

Dietary DHA Recommendations during Pregnancy

Dietary reference intakes (DRI) for carbohydrates, protein, vitamins, and minerals during pregnancy are well established. But the case for fat is different because there are no set values for total fat intake due to insufficient data to determine a level at which inadequacy or prevention of chronic disease occurs. Yet, an acceptable macronutrient distribution range (AMDR) for total fat has been estimated at 20-35% of energy (44).

For the LCPUFAs there is neither EAR (estimated average requirement) nor RDA (recommended dietary allowance) because no potential adverse effects have been identified in the limited information available. However, an AI (adequate intake) has been established based on the median intake of LA and ALA in the U.S. For pregnant women the IOM of the National Academies has set an AI for LA of 13 g/day, and for ALA 1.4 g/day. While an AMDR for ALA has been set at 0.6 – 1.2 % of energy, from which 10% can be consumed as EPA and DHA (44, 45).

Different organizations recommend eating seafood with the purpose of consuming omega-3 FAs:

- Dietary Guidelines for Americans, 2010: "Consume 8 – 12 ounces of seafood per week from a variety of seafood types" that will provide an average of 250 mg per day of EPA and DHA (9)

- MyPlate: No specific quantity of fish is recommended, but it is suggested to eat seafood twice a week including seafood high in omega-3s and lower in mercury (46)

- American Heart Association Guidelines: "Eat a variety of fish at least twice a week, especially fish containing omega-3 fatty acids" (47)

All of these recommendations (9, 46, 47) have been established based on the benefits of omega-3 LCPUFAs for the prevention and treatment of chronic diseases, principally to reduce the risk for cardiovascular disease (CVD).

Additionally, experts recommend that pregnant and lactating women should achieve an average daily intake of at least 200 mg of DHA per day (48). Adequate amounts of DHA during pregnancy are essential because of its important role in fetal brain and retina development.

DHA Dietary Sources

Plant-based foods as flaxseeds, walnuts, their derived oils, canola oil, and olive oil are sources of ALA. DHA is found mainly in fish. Some types of seafood are known to be a rich sources of omega-3 FAs. Salmon, white tuna (bluefin and albacore), sea bass, trout, and oysters are good sources of DHA that contain low amounts of methylmercury, therefore they are allowed to be consumed during pregnancy. On the other side, large predatory fish such as shark, swordfish, tilefish, or king mackerel should be avoided because they are more likely to bioaccumulate methylmercury since they eat smaller organisms that contain methylmercury as well (17). Main sources of omega-6 PUFA are vegetable oils such as soybean oil, safflower oil, sunflower oil, and corn oil (20, 49).

DHA-enriched eggs are another source of omega-3 LCPUFAs. Smuts et al. (50) conducted a study observing that women who consume low amounts of fish were more likely to consume eggs as a source of DHA. The trial compared a group of pregnant women between 24 and 28 weeks of pregnancy consuming a dozen of DHA-enriched eggs with 135 mg DHA/egg with another group consuming ordinary eggs with 18 mg DHA/egg until delivery. It was found

that eggs with high amounts of DHA (135 mg DHA/egg) could increase DHA intake among

pregnant women. DHA-enriched eggs available in the market may provide up to 150 mg

DHA/egg (51).

DHA Supplementation

Different types of supplements are a convenient alternative to achieve recommended

intakes, especially during pregnancy when requirements for the mother and the baby need to be

reached. Some supplements contain ALA, but it still needs to be converted to EPA and DHA in

the body to provide physiological benefits. However, it is known that this conversion is not

efficient in humans (13). EPA and DHA supplementation can be an alternative option for vegans

and for individuals who do not like or are allergic to fish (45). DHA-rich microalgae

supplementation or fish oil supplementation are the main ingredients in any supplement product.

The fetus depends on the maternal supply of omega-3 LCPUFAs. Consequently,

supplementation with omega-3 LCPUFAs during the last trimester of pregnancy has been

advised (25), mainly because brain growth is quite remarkable during this time (12). Every day,

more and more prenatal manufacturers are including omega-3 LCPUFAs in their products giving

the consumer a variety of options according to their possibilities and needs.

Omega-6/omega-3 Ratio

The 18-carbon chain FAs compete with each other during the desaturation and elongation

steps. Omega-6 and omega-3 FAs can interfere with the metabolism of the other. Therefore, an

excess of omega-6 FAs might reduce the metabolism of ALA leading to a deficit of EPA and

DHA (13, 28). For this reason, the ratio of omega-6 (LA):omega-3 (ALA) in the diet is critical

for health. Desaturases are the rate-limiting step, especially the Δ6 desaturase (see Figure 1). *In*

vitro studies point to the Δ6 desaturase enzyme having higher affinity for the most unsaturated

substrates; the order of preference being ALA (omega-3 FA) over LA (omega-6 FA). However, the conversion rate efficiency from the 18-carbon chain to the 20 – 22 carbon chain FAs is very limited in humans (13, 44). Thus, what we see on a laboratory level does not hold in human life.

An optimal ratio of omega-3 and omega-6 FAs is required in the diet to provide a healthy balance. A recommendation varies from 5:1 to 10:1 omega-6:omega-3 ratio (15, 52). However, an optimal ratio has not been established and it could vary according to the stage of life (13, 44). According to Russo (49), a 'very high omega-6:omega-3 ratio is considered detrimental for human health'. A high omega-6:omega-3 ratio (excessive amount of omega-6) promotes the pathogenesis of CVD, cancer, and inflammatory diseases (i.e. inflammatory bowel disease, rheumatoid arthritis, and asthma); conversely a low omega-6:omega-3 ratio reduces the risks or suppresses the symptoms for these diseases (53).

CHAPTER 3
EXPERIMENTAL DESIGN AND METHODS

The study described in this document is an ancillary study to LAMBS, the LA Moms and Babies Study for Nutrition and Growth. LAMBS is a randomized, double-blinded, placebo-controlled intervention trial recruiting overweight pregnant women who are less than 20 weeks in their pregnancy. Subjects considered in the present study have not started the intervention. Therefore, this proposed study is focused on assessing the baseline status of the omega-3 LCPUFA of the LAMBS participants, a population which may be considered typical of pregnant women in the greater Baton Rouge area.

LAMBS is a collaborative study between Louisiana State University, LSU AgCenter, Woman's Hospital, and Pennington Biomedical Research Center. The Institutional Review Board (IRB) from the different institutions approved the study.

Subject Recruitment

Overweight (BMI = 25.0 – 29.9 kg/m^2) pregnant women at 16.5 – 20 weeks of pregnancy were recruited from the community of Baton Rouge, mainly through the Associates in Women's Health and other outpatient clinics at Woman's Hospital, Baton Rouge; community outreach recruitment was also approached by posting flyers (see Appendix A) at shops, libraries, coffee shops, and participation in Baby Grand (a special event for pregnant women at Woman's Hospital, Baton Rouge). Pregnant women were pre-screened to determine if they qualified for the study. The pre-screening included questions about age, pre-pregnancy body weight and height to calculate BMI, weeks of pregnancy, if they were planning for vaginal delivery, if they were being cared for by a physician affiliated at Woman's Hospital, and if they were planning to deliver at Woman's Hospital (see Appendix B).

If they qualified, another screening was done to determine eligibility based on the following criteria:

- Eligibility criteria:

    o Pregnant women between 18 to 35 years of age

    o Pass the glucose tolerance test for diabetes

    o Pre-pregnancy BMI of 25.0 – 29.9 kg/m^2 (overweight classification)

- Exclusion criteria:

    Women were excluded if they:

    o Have more than five children

    o Have a history of high blood pressure, high blood lipids, kidney or liver disease

    o Have inability to handle blood sugar normally

    o Have polycystic ovary syndrome

    o Have thyroid disorder

    o Have multiple fetuses, pregnancy related complications (preterm labor, gestational diabetes, high blood pressure, premature rupture of the membranes)

    o Have smoked in the past 6 months

    o Have been pregnant or lactating (breastfeeding) in the past year

    o Deliver at a hospital other than Woman's Hospital

    o Test positive for human immunodeficiency virus (HIV), syphilis, sepsis, group B streptococcus, and hepatitis B

    o Do not follow study procedures

Study Design

A cross-sectional study was conducted with overweight pregnant women. Eligible participants were asked to meet at Woman's Hospital, Baton Rouge to consent and to have their blood drawn. Blood samples were drawn at 16.5 to 20 weeks of pregnancy. Women completed a health history form that included questions about their date of birth, age, height, weight, ethnicity, and history of previous pregnancies (see Appendix C). Using the University of Minnesota Nutrition Data System for Research program (NDSR), between one and seven 24-h dietary recalls, either in person or via the telephone, were completed by each participant at different time points in their pregnancy (16.5 – 20, 22, 24, 26, 30, 32 and 36 weeks of pregnancy).

NDSR is a dietary analysis program designed for the collection and analyses of 24-h dietary recalls, food records, menus, and recipes. To perform a 24-h dietary recall individuals are asked to recount all food, beverages and nutritional supplements consumed the day before the interview. Individuals are provided a food amounts booklet to help them calculate the amount of food and beverages they have consumed. The period being recalled consists of 24 hours which go from midnight to midnight from the previous day. NDSR also offers calculation of nutrients providing data per ingredient, food, meal, and day in report and analysis file formats.

Sample Collections

Blood samples were collected at 16.5 to 20 weeks of pregnancy by a trained phlebotomist in the Outpatient Lab, Physician Office Building at Woman's Hospital. Participants' blood was drawn into 4 ml EDTA vacutainer tubes that were pre-labeled with participants' ID. Immediately, the tubes were placed into a biohazard bag and transported to the Woman's Hospital Pathology Laboratory for processing of the blood. Aliquot tubes were labeled with the

study name (LAMBS), analyte, participant ID, date and blood draw collection number. Samples were centrifuged using a Thermo Scientific Sorvall™ ST 16 centrifuge at 3000 rpm for 10 minutes. Plasma was separated from the red blood cells (RBCs). The RBCs were washed with an AirLife® Modulose® 0.9% sodium chloride solution and re-spun at 3000 rpm for another 10 minutes. The saline solution was discarded and the RBCs were aliquoted into 2 ml tubes. Aliquots were placed into cardboard cryoboxes and stored in the freezer at -80 °C until the time of analysis.

Sample Analysis

Samples were analyzed in one batch. Samples were removed from the freezer and thawed at room temperature. RBC samples were prepared for analysis of FAs using the following established procedure:

1. To process the samples, 100 µl of RBCs were placed into glass tubes with Teflon lined caps.

2. Two ml of methanol:benzene (4:1, v/v) containing 40 µg/ml of internal standard (heptadecaenoic acid, C17:0) were added to each tube.

3. Under the fume hood, 200 µl of acetyl chloride were slowly added over one minute to each tube. The tubes were capped tightly and manually mixed thoroughly.

4. The mixture was heated at 100 °C for one hour using a VWR analog heat block under a fume hood.

5. Samples were removed from the heat block and cooled down at room temperature. When cool, 5 ml of 6% (w/v) potassium carbonate were added to neutralize the mixture and to stop the chemical reaction.

6. Samples were spun at 3500 rpm for 10 minutes at 4 °C in an Allegra™ 6R Centrifuge from Beckman Coulter.

7. Fatty acid methyl esters (FAMEs) at the top of each tube were removed and placed into labeled amber vials with rubber caps.

8. Vials were placed into a Hewlett-Packard 6890 series gas chromatograph (GC) with a flame ionization detector (FID) to analyze the samples.

For the GC analysis, an external standard (FIM-FAME from Matreya, LLC) reconstituted with dichloromethane (1:60, v/v) was also injected in the GC with the samples to ensure proper operation of the equipment and to compare and identify the FAMEs found in the samples by comparing the peak retention times. The GC was equipped with a Supelco Omegawax™ 250 Fused Silica Capillary Column 30m X 0.25 mm X 0.25 μm. The inlet was set at a split ratio of 10:1 and maintained at 180 °C. Helium was used as the carrier gas and it was maintained at 1.2 ml/min. The oven was programmed with an initial temperature of 180 °C and increased to 210 °C at a rate of 2 °C/min. The FID was set at 280 °C.

The heptadecaenoic acid (C17:0) added as internal standard was used to calculate a relative weight percentage (wt%) by using the formula:

$$\text{Fatty Acid wt\%} = \frac{(\text{FA area \%} * 100)}{\text{Total FAs area \%} - \text{iSTD area \%})}$$

Where:

FA area % = area percentage of an individual fatty acid identified in the RBC sample

100 = factor to get a relative weight percentage

Total FAs area % = area percentage of all the fatty acids identified in the RBC sample

iSTD area % = area percentage of the internal standard added to the RBC sample

Statistical Analysis

  Statistical analysis was performed using Statistical Analysis Software (SAS, version 9.4). Descriptive statistics (mean, standard deviation, and range) were used to characterize our population. One-sample *t* test was used to assess if pregnant women were meeting the DHA dietary recommendation. Two-sample *t* test was used to determine differences in dietary intake between ethnic groups; and also to determine differences between pregnant women who were meeting the DHA recommendation and those who were not. Logistic regression analysis was used to determine if age, ethnicity, and socioeconomic status impacted achieving the dietary DHA recommendation of 200 mg DHA/day. Multi-source regression analysis was used to evaluate the effect of dietary DHA and categorical variables such as age, ethnicity, and socioeconomic status against the RBC DHA wt%. Level of significance was set at $p \leq 0.05$.

CHAPTER 4
RESULTS

Twenty-one overweight (BMI = 25.0 – 29.9 kg/m^2) pregnant women between 19 and 34 years of age who were between 16.5 and 20 weeks of pregnancy were recruited to participate in the study. Pre-gravid BMI ranged from 25.18 to 29.35 kg/m^2. Most of the pregnant women participating in the study were African Americans (62%), almost a third were Caucasians (29%), and two were Hispanics (9%) (Table 1). By evaluating the type of health insurance that participants had at enrollment, it was determined that 62% were of low socioeconomic status (SES). Sixty-two percent of the low income SES were comprised of 48% African Americans, 9% Caucasians, and 5% Hispanics.

Table 1. Characteristics of Study Subjects

Characteristic	Overweight pregnant women (n = 21)
Ethnicity (n)	
African American	13
Caucasian	6
Hispanic	2
Age (y)	25 ± 4.38[*]
Gestational age (weeks)	19 ± 1.19[*]
Pregravid BMI[**] (kg/m^2)	27.23 ± 1.60[*]

[*]Mean ± SD. [**]BMI, body mass index.

In table 2 the dietary intake of subjects is presented as mean ± standard deviation. On average, overweight pregnant women were consuming 2,239 ± 371 kcal/day from which 22 ± 7 g/day were coming from polyunsaturated fats (LA, ALA, AA, EPA, and DHA). Dietary intake of DHA was 72 ± 63 mg/day and EPA was 104 ± 158 mg/day; the combined DHA + EPA was 176 ± 219 mg/day. ALA intake was 1.95 ± 0.73 g/day which is eleven times the combined DHA + EPA. The most dominant FA in the diet was LA (19.26 ± 6.64 g/day). The calculated omega-6:omega-3 ratio was 9:1. Using a one-sample *t* test it was determined that the total sample (n =

21) was not meeting the recommended dietary intake for pregnant women of at least 200 mg DHA/day (48) ($p \leq 0.05$). On Appendix E a table with daily dietary intake separated by ethnic groups is presented.

Table 2. Daily dietary intake of overweight pregnant women

Nutrient	Average daily intake (mean ± SD)	Range (Min – Max)
Energy (kcal)	2,239 ± 371	1,564 – 3,215
Protein (g)	100 ± 26	59 – 154
Carbohydrate (g)	268 ± 54	155 – 381
Total fat (g)	87 ± 24	32 – 159
Saturated fat (g)	29 ± 9	11 – 57
Polyunsaturated fat (g)	22 ± 7	8 – 39
Linoleic acid (g) – LA	19.26 ± 6.64	6.73 – 35.23
α-Linolenic acid (g) – ALA	1.95 ± 0.73	1.04 – 4.10
Arachidonic acid (mg) – AA	195 ± 94	58 – 413
Eicosapentaenoic acid (mg) – EPA	104 ± 158	3 – 537
Docosahexaenoic acid (mg) – DHA	72 ± 63	9 – 237
omega-6/omega-3[*]	9.14 ± 1.23	6.47 – 11.27

[*]omega-6/omega-3 = (LA + AA) / (ALA + EPA + DHA).

Based on the total of one hundred and twelve 24-h dietary recalls for the 21 pregnant women, the main types of seafood consumed were shrimp, crawfish, crab, catfish, tilapia, and oysters. The top five sources of dietary DHA were crawfish, chicken, shrimp, eggs, and crab.

Based on dietary intakes, only 5% (n = 1) pregnant women in this population were meeting the recommended intake of at least 200 mg of DHA/day (48). When considering supplemental DHA, the percentage of pregnant women meeting the recommended intake increased to 38% (n = 8) ($p \leq 0.05$). [Mean ± SD and p-value were calculated based on 7 subjects because 1 subject was considered an outlier due to the high level of supplementation (1,000 mg/day) compared to the other subjects in the study (246 ± 27 mg/day) (Table 3)]. The mean intake for pregnant women not meeting the recommended intake was 64 ± 52 mg of DHA/day.

For pregnant women who were consuming a supplement with DHA, although without meeting the recommended intake, the mean was 77 ± 59 mg of DHA/day.

Table 3. Consumption of DHA by overweight pregnant women

	< 200 mg DHA/day (mean ± SD)	≥ 200 mg DHA/day (mean ± SD)	P value
Dietary DHA (mg/day)	$64 \pm 52 \ (20)^*$ $p \leq 0.05$	$237^{**} \ (1)^*$	$p \leq 0.05$
Dietary + Supplemental DHA (mg/day)	$77 \pm 59 \ (13)^*$ $p \leq 0.05$	$246 \pm 27 \ (8)^*$ $p \leq 0.05$	$p \leq 0.05$

*Number of subjects. **Unique value. P values in a column represent a comparison of the mean ± SD against 200 mg DHA/day; P values in a row indicate difference between pregnant women who were meeting the DHA recommendation and those who were not.

At 16.5 – 20 weeks of pregnancy, 57% (n = 12) overweight pregnant women were supplementing with DHA. At this time point in their pregnancies, 43% (n = 9) overweight pregnant women did not report taking a supplement containing DHA (Table 4).

Table 4. Consumption of supplemental DHA at 16.5 – 20 weeks of pregnancy

DHA/day	Overweight pregnant women (n = 21)
< 100 mg	5 (24%)
100 – 200 mg	6 (28%)
> 200 mg	1 (5%)
No supplementation	9 (43%)

Table 5 shows the RBC fatty acid content as a relative weight percent of total fatty acids identified and quantitated (wt%). The average omega-6 and omega-3 RBC FA content of overweight pregnant women at 16.5 – 20 weeks of pregnancy was 11.06 ± 1.43 wt% of LA, 17.62 ± 1.34 wt% of AA, 0.11 ± 0.04 wt% of ALA, 0.13 ± 0.04 wt% of EPA, and 8.48 ± 1.39 wt% of DHA. The average omega-6/omega-3 ratio was 2.25 ± 6.92.

Table 6 provides the omega-6 and omega-3 RBC FAs by ethnic groups. African Americans had significantly lower RBC DHA (7.98 ± 0.94 wt%) than Caucasians + Hispanics (9.29 ± 1.68 wt%) ($p \leq 0.05$). There were no differences among ethnic groups for LA, AA, ALA,

and EPA (p > 0.05). The AA to DHA ratio was calculated; African Americans had a higher

AA/DHA ratio (2.25 ± 6.95) compared to Caucasians + Hispanics (1.86 ± 5.67).

Table 5. RBC fatty acids of overweight pregnant women

Fatty acid	RBC fatty acid wt% (mean ± SD)
C18:2n-6 – LA	11.06 ± 1.43
C20:2n-6 – EA	0.33 ± 0.07
C20:3n-6 – DGLA	1.44 ± 0.49
C20:4n-6 – AA	17.62 ± 1.34
Total LCPUFA n-6	19.39 ± 9.68
C18:3n3 – ALA	0.11 ± 0.04
C20:5n-3 – EPA	0.13 ± 0.04
C22:6n-3 – DHA	8.48 ± 1.39
Total LCPUFA n-3	8.61 ± 5.90
n-6/n-3	2.32 ± 0.48

EA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; n-6, omega-6; n-3, omega-3; n-6/n-3 = total LCPUFA n-6 / total LCPUFA n-3.

Table 6. Omega-6 and omega-3 RBC fatty acids (wt%) of overweight pregnant women among ethnic groups

Fatty acid	African Americans (mean ± SD)	Caucasians + Hispanics (mean ± SD)	P value
C18:2n-6 – LA	11.55 ± 1.42	10.26 ± 1.10	NS
C20:4n-6 – AA	17.82 ± 1.37	17.30 ± 1.31	NS
Total n-6	29.37 ± 4.43	27.56 ± 4.98	---
C18:3n3 – ALA	0.11 ± 0.04	0.11 ± 0.03	NS
C20:5n-3 – EPA	0.12 ± 0.04	0.14 ± 0.05	NS
C22:6n-3 – DHA	7.98 ± 0.94	9.29 ± 1.68	p ≤ 0.05
Total n-3	8.21 ± 4.54	9.54 ± 5.29	---
AA/DHA	2.27 ± 0.39	1.95 ± 0.53	NS

n-6, omega-6; n-3, omega-3; NS, non significant.

Using logistic regression analysis it was determined that neither age, ethnicity, nor SES

(p > 0.05) affected the probability for women in this study of achieving the recommended dietary

intake for pregnant women of 200 mg of DHA per day (48). Multi-source regression analysis

revealed that only ethnicity affected the RBC DHA wt% (p ≤ 0.05). African Americans had an

estimated predicted RBC DHA wt% lower value (-1.6236) compared to Caucasians + Hispanics.

The regression analysis corroborates results of the *t* test for ethnicity where African Americans had lower RBC DHA wt% compared to Caucasians + Hispanics (7.98 ± 0.94 versus 9.29 ± 1.68, respectively). In this relatively small population, age, intake (diet + supplement) and SES did not significantly affect the RBC DHA wt% ($p > 0.05$).

CHAPTER 5
DISCUSSION

To our knowledge, this is the first study reporting on the fatty acid status, and specifically omega-3 LCPUFA status of overweight pregnant women in the greater Baton Rouge area. Other studies have reported FA status and omega-3 FA dietary intakes in pregnant women from different populations (54-61) but none of them have been focused on pregnant women with pregravid overweight.

In the current study, dietary FA intake is in line with reports from our laboratory (10) where it was previously demonstrated that women of childbearing ages from Louisiana were not consuming enough DHA and EPA in their diets (mean $\pm$ SE, 66 ± 17 mg/day) and were not supplementing with omega-3 LCPUFAs. In the current study, at $16.5 - 20$ weeks of pregnancy, 57% (n = 12) pregnant women were supplementing with DHA. While not documented, it is likely that the reason for supplementation is because they are pregnant and a health care provider may have recommended taking a prenatal with DHA during their pregnancy.

Previous studies have reported a low intake of DHA in pregnant women at mid-pregnancy. Loosemore et al. (61) evaluated pregnant women with and without GDM who consumed 67 and 34 mg of DHA/day, respectively. Stark et al. (59) reported a consumption of 81 mg of DHA per day for African American pregnant women, which is similar to our finding of 72 mg of DHA per day, in our population who was 62% African Americans. On the other hand, some studies have reported high DHA intakes that are more in line with recommendations. Innis and Elias (55) reported 160 mg/day in pregnant Canadian women and De Vriese et al. (54) reported 300 mg/day in pregnant Belgian women. These higher intakes may have been influenced by the diet in those countries, which is different from the typical American diet that is

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characteristically high in the amounts of saturated fats and low in the polyunsaturated fats (with higher intake of omega-6 than omega-3 PUFAs) consumed (62).

With respect to ALA, the EFA that is a precursor for DHA, other researchers (55, 57-59) provide results similar to what we are reporting here: 1.95 ± 0.73 g/day. This amount is considerably higher than the recommended AI of ALA for pregnant women of 1.4 g/day (44). In the case for LA, compared to the other studies (55, 57-59, 61), our population was consuming more (19.26 ± 6.64 g/day). Additionally, the amount is above the recommended AI for pregnant women of 13 g/day (44).

Although an optimal omega-6/omega-3 ratio has not been established, recommendations oscillate around 5:1 to 10:1 (15, 52). The omega-6/omega-3 ratio of 9:1 for our population of overweight pregnant women is in line with the recommendations, but hovers around the extreme upper limit. However, it is necessary to emphasize that pregnant women should be consuming more omega-3 FAs in their diet to supply the fetus for optimal growth and development (8, 25, 31). Consuming more omega-3 FAs would reduce the 9:1 omega-6/omega-3 ratio. Donahue et al. (58) reported an 11:1 omega-6/omega-3 ratio in a prospective observational cohort study in 2009 with 1,666 pregnant women from Massachusetts. The omega-6/omega-3 ratios mentioned here could give us an estimate about FA intake patterns of pregnant women from the U.S., where the omega-6/omega-3 ratio is at the limit of what has been recommended to date (5:1 to 10:1).

Because seafood is the main dietary source of DHA, we explored the top five sources of DHA consumed by our population of overweight pregnant women in the current study. It was noticeable that crawfish was the main source of DHA in this Louisiana population. However, consumption of crawfish appeared to have been influenced by the crawfish season in Louisiana, and this observation suggests that pregnant women usually consume less DHA than what is

reported here. This needs to be further explored. Although it is well known that seafood is a good source of DHA and different institutions recommend its weekly intake (9, 46, 47), pregnant women in this study were consuming very little seafood other than crawfish. It is surprising that women from Louisiana were not consuming more seafood with the locale's proximity to the coast and the availability of seafood in Louisiana. Additionally, many local dishes include seafood. Reasons for this warrants further exploration. The question is posed: are pregnant women afraid of consuming fish because of warnings of methylmercury in fish that can harm the baby? Pregnant women might not be aware that there are many types of seafood that can be consumed during pregnancy as good sources of DHA with low methylmercury levels (salmon, white tuna, sea bass, trout, oysters, for example) (17). The question is posed: are health care providers addressing this issue during prenatal visits?

The present study reveals that 95% (n = 20) of overweight pregnant women were not meeting the recommended dietary intake for pregnant women of at least 200 mg of DHA per day (48). The percentage was reduced to 62% (n = 13) when amounts provided by supplementation were included. It is alarming that although it is well documented that DHA is beneficial for pregnant women and their offspring, very few women in this limited population were consuming DHA in the amount recommended for pregnancy.

Having determined that age, ethnicity, and SES did not affect the probability of achieving the recommended dietary intake for pregnant women of at least 200 mg of DHA per day, we suggest that pregnant women of the greater Baton Rouge area in general have very limited knowledge regarding the recommendation for inclusion of DHA during pregnancy. Thus, we advocate for nutrition education for women of childbearing ages with the intention that these

women will be prepared to supply their own needs, and most importantly fetal needs, at the time of conception.

Women in our population had a RBC DHA wt% of 8.48. Other studies have reported lower values. Courville et al. (60) reported 4.75 wt% of RBC DHA in healthy pregnant women. Similarly, De Vriese et al. (54) and Donahue et al. (58) reported 4.8 and 4.74 wt% of RBC DHA, respectively. We speculate that the difference in RBC DHA wt% might have been influenced by supplementation. Those earlier studies (54, 58, 60) were conducted approximately around 6 to 13 years ago when supplementation with DHA during pregnancy was not a common practice. Excess body weight could have also affected the FA metabolism in our population as we were evaluating overweight pregnant women. A possible explanation could be that mobilization of DHA from adipose tissue has occurred providing DHA for transfer to the fetus (63). Also, excess adipose tissue might have influenced enzyme activity during the elongation and desaturation process (64, 65), altering FA metabolism, resulting in higher DHA.

Our results point to the role of ethnicity in the RBC DHA content. African Americans had lower RBC DHA (7.98 ± 0.94 wt%) compared to Caucasians + Hispanics (9.29 ± 1.68 wt%). A study conducted by Mathias et al. (66) revealed that African Americans have higher circulating levels of plasma AA than European Americans. The difference was accredited to genetic differences in the fatty acid desaturase (FADS) family of genes that increase the rate of conversion of LA to AA (omega-6 FAs) during the elongation and desaturation process. In our population, although the RBC ALA content was the same among ethnic groups [0.11 ± 0.04 wt% for African Americans and 0.11 ± 0.03 wt% for Caucasian + Hispanics ($p > 0.05$)] the RBC DHA content was lower in African Americans. This can be better understood by evaluating the AA/DHA ratio. African Americans had a higher AA/DHA ratio in RBC (2.25 ± 6.95) compared

to Caucasians + Hispanics (1.86 ± 5.67). Therefore, the results presented by Mathias et al. and our observations, allow us to hypothesize that FA metabolism could be different among ethnic groups, perhaps due to polymorphisms in the FADS that encode the Δ6 and Δ5 desaturase enzymes. Even though African Americans appear to have higher conversion rates for the omega-6 FAs (LA to AA) during the desaturation and elongation process, it might be possible that genetic and physiological adaptations during the years have altered the efficiency for converting the omega-3 FAs (ALA to DHA). Thus, the efficiency rate during the elongation and desaturation of ALA to DHA might be less efficient in African Americans compared to Caucasians + Hispanics.

A limitation of this study was the small population available (n = 21; 13 African Americans, 6 Caucasians, and 2 Hispanics). There was a wide range in dietary intakes reported for this limited population. Consequently, results should be interpreted with some caution. Future research will include a larger study population.

CHAPTER 6
CONCLUSION

Nutrition during the perinatal period plays a key role for the infant. With the higher rates of obesity in the U.S., more women are entering pregnancy with excess weight which increases the risk for complications. It is well recognized that DHA is beneficial for the mother and the infant as it is necessary for optimal fetal growth and development. Experts recommended that pregnant women should consume at least 200 mg of DHA per day. Therefore, the aim of this study was to establish the DHA status in overweight pregnant women of Louisiana by determining the FAs of RBCs and estimating dietary intakes. Our results suggest that pregnant women at mid-pregnancy are not consuming enough DHA from their diet to meet the recommended intake; further, not all pregnant women are supplementing with DHA. Ethnicity might play a role in how DHA is metabolized in the body. Our results suggest that Caucasians + Hispanics are more efficient in converting the 18-carbon omega-3 FA, ALA, to DHA than African Americans.

In conclusion, there is a need for nutrition education for pregnant women regarding the benefits of DHA during pregnancy. The aim is for DHA to be included in the diet on a routine basis or to be consumed as a supplement in order to meet the recommended intake of at least 200 mg of DHA per day. Because fish is the main dietary source of DHA, pregnant women should also be encouraged to consume fish with high amounts of DHA.

Further research will include a larger population of study to enrich the data presented here. Additionally, a population of normal weight pregnant women could be explored for future comparisons with the data from the current study.

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To learn more about this study, please contact:

(225) 578-7160

or

LAMBSstudy@gmail.com

Are you pregnant?

If you answered yes, you may be eligible to participate in the LA Moms and Babies Study for nutrition & growth!

Purpose of the study:
- LAMBS will evaluate how diet during pregnancy can benefit pregnancy and infant outcomes, such as infant weight
- LAMBS will help us make the best nutrition and diet recommendations to pregnant women and women of child-bearing ages

LAMBS

LA Moms and Babies Study for nutrition & growth

You may be eligible to participate if you are:
- Pregnant
- Overweight
- 18 – 35 years old

Qualified participants will receive:
- General nutrition guidelines
- Summary of infant's growth in the first year of life
- Compensation up to $575 throughout study

B. SCREENING FORM

LAMBS SCREENING FORM
Name:
Phone Number:
Weeks Pregnant:
AGE (18-35 years):
Pre-pregnancy weight:
Height:
BMI:

	YES	no
Have you had an oral glucose tolerance test during this pregnancy?		
Have you been told you have gestational diabetes?		
Have you had 5 or more children?		
Do you have a history of high blood pressure, high blood lipids, kidney or liver disease?		
Do you have high blood sugar?		
Do you have polycystic ovary syndrome?		
Do you have a thyroid disorder?		
Pregnant with more than one baby?		
Do you have preeclampsia?		
First degree relative with diabetes?		
Previously given birth to a baby who was considered large for gestational age?		
Any preterm labor?		
Have you smoked in the past 6 months?		
Have you been pregnant or lactating in the past year?		
Delivering at a hospital other than Woman's?		
Been told you are positive for HIV?		
Been told you are positive for syphilis, sepsis, group B strep, or Hep B?		
Planning to bank cord blood or are you unwilling to donate your cord blood to the study?		
Are you planning to have a c-section?		

C. HEALTH HISTORY FORM

LAMBS
Participant Health History form

Participant name:_____

DOB:_____ Age:_____

Height (ft):_____ Current weight (lbs):_____

Pre-pregnancy weight (lbs):_____Pregnancy weight gain (lbs):_____

Ethnicity: American Indian or Alaska Native

                  Asian

                  Black or African American

                  Native Hawaiian or Other Pacific Islander

                  White

                  Hispanic or Latino

Previous pregnancies

No. of previous pregnancies:_____No. of live births:_____

Date of last delivery:_____Have you ever breast-fed?   Y / N

If yes, how long has it been since you last breast-fed?_____

Pregnancy complications:_____

_____

Pregnancy and birth information

Date of birth:_____Sex: Male   Female

Pregnancy duration: Full term (38 – 40 wks)   Premature (_____ wks)

Mode of delivery: Vaginal   Caesarean

Medications taken during pregnancy:_____

1

Complications during pregnancy, if applicable:_____

Birth weight:_____lbs_____oz   Infant length:_____cm

Head circumference:_____cm   Size for age:_____

Ponderal index:_____

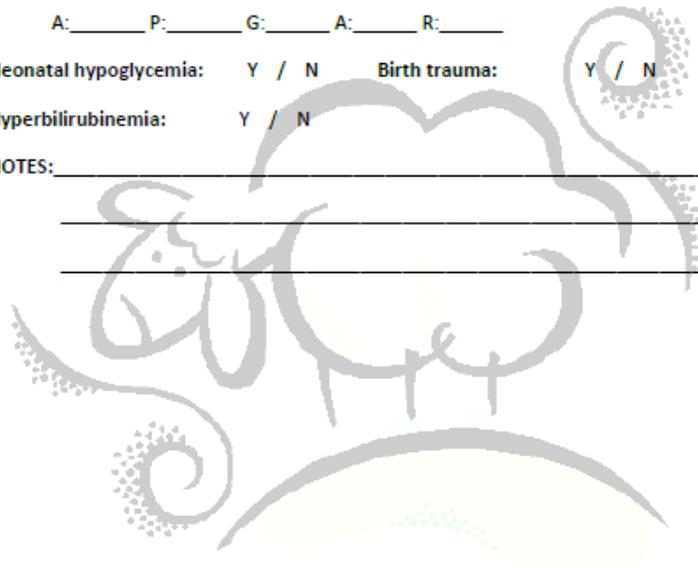Maturity rating:_____Classification:_____

APGAR Score (1 – 10): _____

     A:_____ P:_____ G:_____ A:_____ R:_____
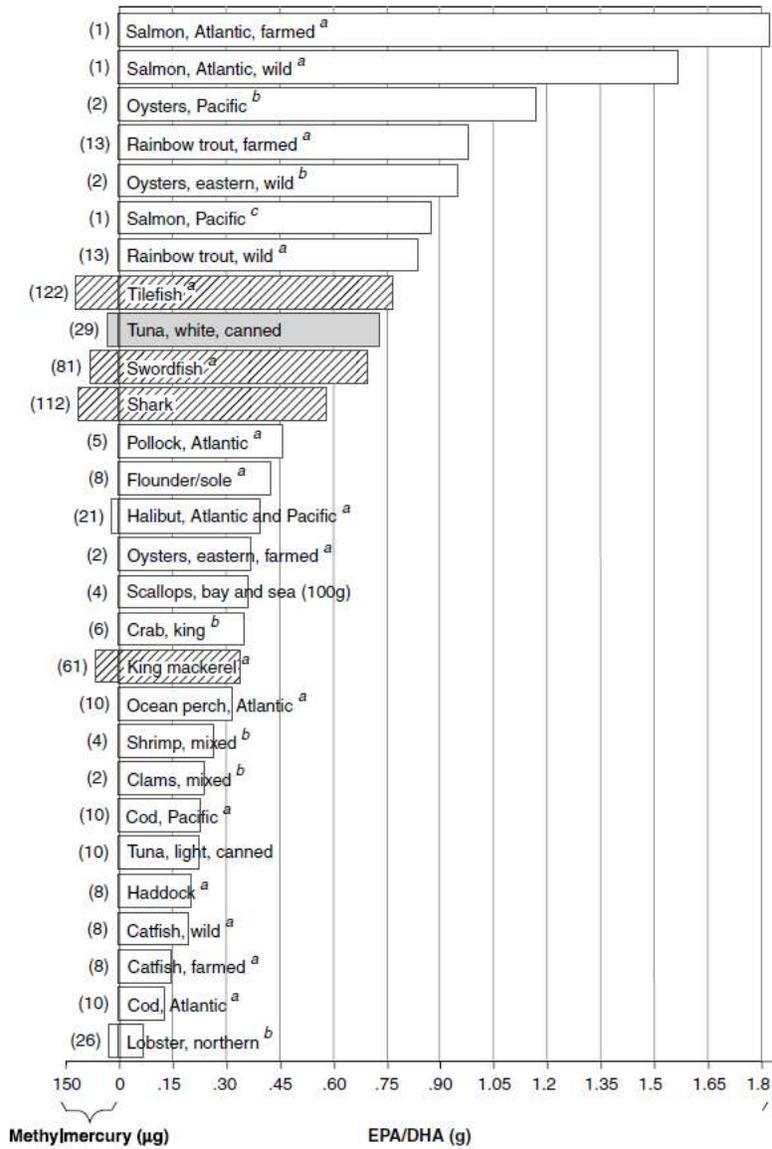
Neonatal hypoglycemia: Y / N   Birth trauma: Y / N

Hyperbilirubinemia: Y / N

NOTES:_____

     _____

     _____

2

D. SEEAFOOD SOURCES OF EPA + DHA

Source: Seafood Choices: Balancing Benefits and Risks. Figure 7-6a. Example of estimated EPA/DHA (grams [g]) and methylmercury (microgram [μg]) amounts in one 3-ounce portion of seafood (17).

E. DAILY DIETARY INTAKE OF OVERWEIGHT PREGNANT WOMEN AMONG ETHNIC GROUPS

Nutrient	African Americans (mean ± SD)	Caucasians + Hispanics (mean ± SD)	P value
Energy (kcal)	2,334 ± 362	2,086 ± 355	NS
Protein (g)	109 ± 28	86 ± 13	$p \leq 0.05$
Carbohydrate (g)	267 ± 56	269 ± 56	NS
Total fat (g)	93 ± 23	76 ± 22	NS
Saturated fat (g)	29 ± 9	28 ± 9	NS
Polyunsaturated fat (g)	25 ± 7	17 ± 5	$p \leq 0.05$
Linoleic acid (g) – LA	21.83 ± 6.60	15.09 ± 4.39	$p \leq 0.05$
α-Linolenic acid (g) – ALA	2.14 ± 0.83	1.64 ± 0.43	NS
Arachidonic acid (mg) – AA	227 ± 98	144 ± 61	$p \leq 0.05$
Eicosapentaenoic acid (mg) – EPA	155 ± 185	21 ± 13	NS
Docosahexaenoic acid (mg) – DHA	92 ± 73	40 ± 24	NS
omega-6/omega-3[*]	9.32 ± 1.22	8.86 ± 1.29	NS

[*]omega-6/omega-3 = (LA + AA) / (ALA + EPA + DHA); NS, non significant.

F. IRB APPROVAL FORMS

Version Date: 4/08/2008

Institutional Review Board (IRB) Authorization Agreement

Name of Institution or Organization Providing IRB Review (Institution/Organization A):
Pennington Biomedical Research Center (PBRC)

IRB Registration #: IRB00000708   Federal Wide (FWA) #: FWA00006218

Name of Institution Relying on the Designated IRB (Institution B):
Louisiana State University Agricultural Center Baton Rouge

IRB Registration #: IRB00001053   Federalwide Assurance (FWA)#: FWA00009344

The Officials signing below agree that Institution B may rely on the designated IRB of Institution A as the IRB of record for review and continuing oversight of its human subjects research described below: *(check one)*

(____) This agreement applies to all human subjects research covered by Institution B's FWA.

(X) This agreement is limited to the following specific protocol(s):

Name of Research Project: **PBRC IRB # 11031 LAMBS; LA Moms and Babies Study (LAMBS) for Nutrition and Growth**
Name of Principal Investigator: Carol Lammi-Keefe, Ph.D.
Sponsor or Funding Agency: __USDA__   Award Number, if any: _____

(____) Other *(describe)*: _____

The review performed by the designated IRB will meet the human subject protection requirements of Institution B's OHRP-approved FWA. The IRB at Institution/Organization A will follow written procedures for reporting its findings and actions to appropriate officials at Institution B. Relevant minutes of IRB meetings will be made available to Institution B upon request. Institution B remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

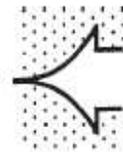Signature of Signatory Official (Institution A):

_____Will Cefalu_____   Date: ___11-1-10___

Print Full Name: William Cefalu, M.D. Institutional Title: Associate Executive Director for Clinical Research

Signature of Signatory Official (Institution/Organization B):

_____Michael Keenan_____   Date: _1-13-2013_

Print Full Name: Michael Keenan   Institutional Title: IRB Chair
LSU AgCenter

Peggy Dean, RPh, MBA, Chair   (225) 231-5359

Carol Lammi- Keefe, PhD
Louisiana State University
297B Knapp Hall
Baton Rouge, LA 70803

Dear Dr. Lammi-Keefe:

This letter is to inform you that at the Woman's Hospital Foundation Institutional Review Board meeting of November 10, 2014, the protocol, informed consent form, authorization forms, screening form, case report forms, physician letter, food amounts booklet, and advertisements for **RP-11-007, LA Moms and Babies Study (LAMBS) for Nutrition and Growth,** were reviewed for continuing review.

Approval has been granted for one year. The study is next subject to continuing review on or before November 10, 2015. We recommend that it be presented two months prior to this date to avoid a delay in enrollment in the case of unforeseen circumstances.

Attached are the informed consent form and authorization forms with the IRB stamp of approval in the lower right hand corners. Please note that these documents are the official ones and copies for future participants must be reproduced from these originals.

Changes to the study must be promptly reported and approved. Contact Ericka Seidemann, Human Protections Administrator, at (225) 231-5296 if you have any questions or require further information.

Sincerely,

Peggy Dean, RPh., MBA
IRB Chair

VITA

Adriana Virginia Gaitán was born in Guatemala City, Guatemala. She received her Bachelor of Science degree in Food Science in 2007 from the Escuela Agrícola Panamericana, Zamorano in Honduras. Adriana was working in the food science industry during five years prior to begin her Master's degree with a concentration in human nutrition at Louisiana State University in 2013. Adriana is continuing her education towards a PhD in human nutrition at Louisiana State University. She is a board member of the Zamorano Agricultural Society at LSU and a student member of the American Oil Chemists' Society (AOCS).