4-2016

FAILURE TO ACHIEVE RECOMMENDED OMEGA-3 DHA INTAKE DURING PREGNANCY: NUTRITION EDUCATION NEEDED

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Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of the Upper Division Honors Program

April 2016

Louisiana State University
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Baton Rouge, Louisiana

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor, Dr. Carol Lammi-Keefe, for her dedication to this project and to me as a student. I could not have done this without her time, wisdom, and guidance. I would like to thank my lab mates, Adriana Gaitán and Merritt Drewery, for the countless hours they spent investing in and working with me. I would also like to thank my committee members, Dr. Patrick DiMario and Dr. Diane Sasser for their time and support.

Lastly, I would like to thank my family and friends who have supported me endlessly throughout my undergraduate years. This accomplishment would not have been possible without their love, support, and encouragement.

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ABSTRACT

Background: The omega-3 long chain polyunsaturated fatty acids (omega-3 LCPUFAs) are essential for pregnancy, as they promote optimal growth and development of the infant. Current data show that pregnant women in the United States are not meeting the recommendations set by the 2015-2020 Dietary Guidelines for Americans (DGAs) regarding the omega-3 LCPUFAs, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Objectives: The objectives of this study were to assess the consumption of DHA and EPA in overweight pregnant women and their status with respect to these fatty acids.

Design: This cross-sectional study examined the dietary intake of omega-3 LCPUFAs by overweight pregnant women using repeated twenty four-hour dietary recalls.

Participants/setting: All participants (n=10) were overweight women (pregravid BMI 25.0-29.9 kg/m^2), 17-20 weeks pregnant at time of study entrance, and treated by physicians in outpatient clinics at Woman's Hospital in Baton Rouge, Louisiana.

Methods: Data were collected using repeated (n=7) twenty four-hour dietary recalls and the University of Minnesota Nutrition Data System for Research (NDSR). Recalls were conducted beginning at 17-20 weeks of pregnancy until delivery. Red blood cell fatty acid analyses were performed using gas chromatography. Descriptive statistics, two-sample t-tests, and Pearson correlations were used to analyze data.

Results: Women did not meet the recommendation for DHA+EPA (164.5 ± 82.3 mg DHA/day and 37.4 ± 28.5 mg EPA/day). Women 25 years and older consumed more EPA (46.5 ± 24.2 mg/day) than did women younger than 25 years (17.2 ± 8.9 mg/day). Women who had previously been pregnant consumed more DHA (233.5 ± 36.2 mg/day) than

primiparous women (95.5 ± 45.0 mg/day). There was no relationship between dietary omega-3 LCPUFAs and status.

Conclusions: The current finding that pregnant women in the Greater Baton Rouge area do not consume adequate levels of omega-3 LCPUFAs during pregnancy is reinforced by previous reports. This finding points to the need for exploration of the nutrition education currently provided to pregnant women. Our data warrant a need for refined educational approaches to improve omega-3 LCPUFA consumption by pregnant women.

INTRODUCTION

It is well documented that what a mother eats during her pregnancy can affect infant outcomes. Thus, a mother's diet during pregnancy is of extreme importance for her own health and well-being, as well as that of the developing fetus. A woman's macronutrient and micronutrient needs increase during pregnancy, and it is important that she consume foods that will meet these increased requirements[1]. Two of the essential micronutrients for a pregnant woman to consume are the omega-3 LCPUFAs, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). These fatty acids cannot be produced efficiently by the body and therefore must be consumed in adequate amounts to promote optimal pregnancy outcomes[2,3]. They play an important role in regulation of inflammation in the mother, maturation of the fetus, and development of the infant.

Pregnancy is a natural inflammatory state, which is necessary for the transfer of nutrients across the placenta, but inflammation may become overly exaggerated in overweight or obese pregnant women. Prolonged inflammation may cause damage to host tissues and promote pathogenesis of inflammatory conditions, including hypertension and gestational diabetes[4-6]. The presence of these conditions increases maternal and fetal risks during pregnancy, including preterm birth and large-for-gestational age babies[5]. Consumption of the omega-3 LCPUFAs, DHA and EPA, has been shown to reduce production of inflammatory cytokines and thereby prevent the development of conditions characterized by an exaggerated inflammatory response[4,6].

Studies have shown that the omega-3 LCPUFAs are essential for the cognitive development of the infant[2,7]. Consumption of omega-3 LCPUFAs increase gestational

length and infant birth weight, while decreasing infant adiposity[7-9]. Consumption of omega-3 LCPUFAs may also lower the mother's risk of depression[1,10-12]. Multiple studies have shown that DHA plays a role in the maturation of the infant's visual system and neurodevelopment [2,7,13,14]. In these studies, mothers who supplemented with DHA during pregnancy gave birth to infants who had better problem-solving skills, better visual acuity, and better sleep organization or patterning[15-18].

The 2015-2020 Dietary Guidelines for Americans (DGAs) recommend pregnant women consume 200-250 mg DHA+EPA/day[19]. Additionally, the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation Experts recommend consumption of 200 mg DHA/day for pregnant or lactating women[10]. It is advised that pregnant women and women of child-bearing ages consume 8 – 12 ounces of seafood each week, with a focus on fish that contain high concentrations of omega-3 PUFAs, such as sardines, Atlantic and sockeye salmon, and rainbow trout[19]. According to the Food and Drug Administration and the Environmental Protection Agency, pregnant women should avoid consumption of shark, swordfish, king mackerel, and tilefish, as these fish varieties consume large amounts of methylmercury, which is toxic to the developing fetus[20].

The ratio of omega-6:omega-3 PUFAs consumed by a pregnant woman is important because these fatty acids have different functions, but compete for the same enzymes and cannot be interconverted[2,3]. Therefore, increased consumption of the omega-6 PUFA, linoleic acid (LA, 18:2n-6), will decrease availability of the elongation and desaturation enzymes necessary for conversion of the omega-3 precursor, alpha-linolenic acid (ALA, 18:3n-3), to the omega-3 LCPUFAs, DHA and EPA[3]. Exposure to excessive quantities of

omega-6 PUFAs during fat cell formation in utero may promote excess fat deposition in early life, increasing the infant's risk of childhood obesity[21]. The recommended consumption of omega-6:omega-3 PUFAs is between 4:1 and 2:1[6]. Current evidence suggests that the omega ratio in Western diets is much higher, due to an inadequate omega-3 intake and an excessive omega-6 intake[6]. A high omega ratio promotes pathogenesis of many diseases, including obesity, diabetes, and cardiovascular disease, whereas a low ratio may prevent these complications[6].

To assess maternal fatty acid status in the current study, the fatty acid content of red blood cell phospholipid membranes was analyzed[22]. During pregnancy the long-chain PUFAs are preferentially transferred across the placenta to the fetus over the shorter-chain parent fatty acids[17]. Because red blood cell phospholipid stores are indicative of longer-term status, these values may not immediately reflect changes in dietary intake[22].

Studies have shown that intake of DHA by pregnant women in various regions of the United States and Canada is significantly lower than the 200 mg DHA/day recommendation[10,23-26]. Other than a recent report by our laboratory[27], there is currently no published data on the intake of DHA+EPA in pregnant women living in Louisiana. Accordingly, we are conducting an ongoing study to evaluate dietary and supplemental intake of the omega-3 fatty LCPUFAs, DHA and EPA, by pregnant women in the Greater Baton Rouge area. The data presented represent only a glimpse of the current study population. Our aim is to assess DHA+EPA intake in this population of pregnant women against current recommendations for DHA+EPA intake during pregnancy as well as published findings in other populations of pregnant women and women of childbearing

ages. We also aim to assess fatty acid status of pregnant women by analyzing the fatty acid content of red blood cells using established methodology[22].

LITERATURE REVIEW

Polyunsaturated Fatty Acids: DHA+EPA

Fatty acids are structurally described as carboxylic acids attached to an aliphatic chain. The chain may have no double bonds (saturated fatty acids), one double bond (monounsaturated fatty acids), or up to six double bonds (polyunsaturated fatty acids, PUFAs)[28]. The more unsaturated fatty acids, which generally contain more than 18 carbons, are referred to as long chain polyunsaturated fatty acids (LCPUFAs)[28]. PUFAs include fatty acids with double bonds at the omega-3 or omega-6 positions. Unlike saturated and monounsaturated fatty acids, the omega-3 and omega-6 families of PUFAs cannot be synthesized endogenously and must be provided in the diet[10].

ALA and LA are considered parent fatty acids. Both the ALA and LA parent fatty acids are found in storage lipids, cell membrane phospholipids, intracellular cholesterol esters, and plasma lipids[28]. LCPUFAs synthesized from these precursors, including AA, EPA, and DHA, are found primarily in cell membrane phospholipids[28]. Once consumed, ALA and LA can be converted to longer chain, more unsaturated fatty acids by enzymatic chain elongation and desaturation. ALA and LA are converted to EPA and DHA, or AA, respectively, by a sequence of elongations and desaturations presented in Figure 1[10,29,30].

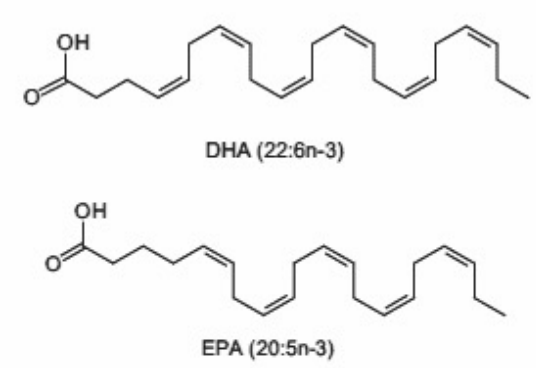
EPA can also be further converted into DHA by successive elongation of EPA (20:5n-3) to 24:5n-3, followed by desaturation to form 24:6n-3, and chain shortening to form DHA (22:6n-3)[29,30]. The structures of EPA and DHA are presented in Figure 2[31]. Omega-3 fatty

acids compete with omega-6 fatty acids for the same desaturation and elongation enzymes as well as incorporation into membrane phospholipids[7]. The relatively high concentration of LA compared to ALA in the Western diet results in greater production of omega-6 PUFAs than omega-3 PUFAs. Evidence suggests that high levels of LA interfere with the conversion of DHA and EPA from ALA due to competitive inhibition[2,3]. This decreases the efficiency of the ALA to EPA and DHA conversions. Humans are also considered inefficient at converting ALA to EPA and DHA perhaps due to evolutionary changes that occurred many eons ago. For these reasons, DHA and EPA are essential nutrients for humans to consume[2,3].

Figure 1. Metabolic pathways of omega-6 and omega-3 PUFAs in humans

Omega-6 PUFAs		Omega-3 PUFAs
18:2n-6		18:3n-3
↓	Δ6-Desaturase	↓
18:3n-6		18:4n-3
↓	Elongase	↓
20:3n-6		20:4n-3
↓	Δ5-Desaturase	↓
20:4n-6		20:5n-3
↓	Elongase	↓
22:4n-6		22:5n-3
↓	Elongase	↓
24:4n-6		24:5n-3
↓	Δ6-Desaturase	↓
24:5n-6		24:6n-3
↓	β-oxidation	↓
22:5n-6		22:6n-3

Figure 2. The structures of DHA and EPA[31]



Fetal Requirements for DHA+EPA

The developing fetus requires fatty acids as a source of energy, as well as to maintain the physical and chemical properties of membranes[15]. Seventy percent of the energy required for fetal growth is devoted to brain development. The brain is a structural, lipid-rich organ that uses highly unsaturated fatty acids, particularly AA and DHA, for structure and function[32]. Lipids comprise 50-60% of the structural matter of the brain[32]. Around 35% of the lipids in the brain are PUFAs, primarily LCPUFAs[15]. During pregnancy, small quantities of LA and ALA can be transferred across the placenta from the mother to the fetus, though the LCPUFAs (AA, DHA, and EPA) are preferentially transferred over the parent fatty acids[33]. In other words, there is a preferential uptake of preformed fatty acid substrates by the placenta compared with the biosynthetic route of metabolizing these LCPUFAs from their parent fatty acids[32]. After placental transfer, the LCPUFAs are compartmentalized into lipid fractions that prevent them from moving back across the placental barrier[17]. The brain and retina concentrate DHA against a concentration gradient and incorporate it at high levels into their membrane phospholipids[34]. Maternal dietary intake of LCPUFAs regulates the quantity available for transfer to the fetus[10]. Placental

supply of LCPUFAs, such as AA and DHA, is critical for the synthesis of structural lipids and normal fetal development[35,36].

DHA can be formed in the liver from the dietary essential fatty acid ALA[24]. However, only one to four percent of dietary ALA is converted to DHA due to a lack of necessary conversion enzymes in the placenta[17,25,35]. Studies show that dietary consumption of DHA results in higher levels of DHA in tissue phospholipids and greater fetal DHA accretion than does dietary consumption of its parent fatty acid, ALA. Higher intakes of ALA fail to increase plasma DHA in both infants and adults, providing evidence for the inefficiency of the ALA to DHA conversion[25]. Thus, the fetus relies on the placental ability to remove omega-3 LCPUFAs from maternal circulation and deliver them to the growing fetus[35,37].

Metabolic requirements for DHA increase during pregnancy due to fetal demand coupled with the mother's own metabolic requirements[1]. The fetus is supplied with DHA from maternal circulation and has significantly increased needs during the last trimester of pregnancy when brain development is most critical[1]. Holman et al. reported that pregnant women in North America have lower levels of plasma phospholipid PUFAs both at term and at delivery than non-pregnant women[38]. Likewise, DHA is higher and LA is lower in fetal than in maternal plasma[25]. This suggests a preferential transfer of the omega-3 LCPUFAs and omega-6 LCPUFAs from the mother to the fetus[29,38]. Studies show that the fetus obtains an average of 67 mg/day of omega-3 PUFAs, mainly DHA, from the mother during the third trimester of pregnancy[1,25]. The average whole-body accretion of DHA during this time is greater than 50 mg/kg of body weight per day, which is equivalent to a dietary DHA requirement of 1% of total fatty acids[39]. DHA supplementation by the mother during

pregnancy increases both maternal status and DHA incorporation into placental and cord blood lipids[40].

Evidence suggests that insulin sensitivity and adiposity also influence the ability of enzymes to regulate essential fatty acid metabolism[13]. According to the National Center for Health Statistics, two-thirds of adults in the United States are overweight or obese[41] and nearly half of the women that are of childbearing age are overweight or obese[42]. An overweight or obese body mass index (BMI) at the onset of pregnancy decreases enzymatic metabolism of essential fatty acids, promotes inflammation, and is associated with an increase in maternal and fetal risks[5,6]. These risks include pre-eclampsia, hypertension, gestational diabetes, preterm birth, and large-for-gestational age births[5]. However, consumption of omega-3 fatty acids have been found to reduce cardiovascular disease risk, treat disorders involving inflammation, and potentially reduce effects of preeclampsia[6].

Benefits of DHA During Pregnancy

The most intense period of human growth occurs from 28 weeks gestation to 18 months after birth, and DHA accretion in the brain peaks during the third trimester of pregnancy[43]. Premature infants who are born early in the third trimester of pregnancy receive less LCPUFAs prior to birth than infants born at term and may have a higher postnatal LCPUFA requirement[28]. Therefore, premature infants are particularly vulnerable to nutritional deficiency due to their limited adipose tissue mass and immaturity in various metabolic pathways at birth[29].

Supplementation with fish oil during the second half of pregnancy has been shown to result in increased length of gestation and reduced incidence of pre-term labor[1,7]. A high

concentration of omega-3 fatty acids regulates prostaglandin production involved in the initiation of labor and may therefore delay the onset of labor[7,44]. A longer period of gestation provides the developing fetus more time for central nervous system development[18]. Omega-3 supplementation during pregnancy is also associated with higher birth weight and greater head circumference[8]. Evidence suggests that maternal DHA supplementation during pregnancy may positively affect infant body composition. In a study conducted by Bergmann et al., infants born to mothers who supplemented with 200 mg DHA/day during pregnancy had a reduced body weight and BMI at 21 months of age, reducing their risk of childhood obesity[9].

Optimal nutritional status during the period of fetal development is crucial to the long-term health status of the individual[3]. DHA plays a vital role in fetal visual and neurological development, as it is the predominant structural fatty acid in the central nervous system and retina[7,13]. Fetal accumulation of DHA is significantly increased during the second half of gestation, when the grey matter of the brain grows rapidly[45]. Observational studies have associated higher maternal fish consumption during pregnancy and higher DHA concentrations in cord blood at birth with a better neurologic outcome[15]. Improved hand-eye coordination in children two and a half years old is directly correlated with omega-3 intake and inversely correlated with omega-6 intake[46]. Omega-3 LCPUFA supplementation is associated with a better performance on problem-solving tasks at nine months of age, as well as a higher intelligence quotient at four years of age[15,16].

Higher maternal fish consumption during pregnancy is also associated with improved visual recognition memory and language development[2,14]. DHA status of the child at two and twelve months of age is positively correlated with visual acuity. DHA

status of the child at fourteen months of age is positively associated with language production and comprehension during the first two years of life[47]. Beneficial effects have also been found regarding development of fine motor function and social behavior in children with mothers who consumed high quantities of seafood during the last trimester of pregnancy[2]. Infants with higher amounts of DHA demonstrated a more regulated sleep pattern based on time spent in each of five stages of sleep. These sleep patterns represented a more mature central nervous system and reinforced the theory that high levels of maternal DHA benefits infant cognitive development[18,33].

Mothers selectively transfer DHA to their fetuses to support optimal neurological development in utero. Without sufficient dietary intake, mothers may become depleted of DHA and may increase their risk of suffering from postpartum depression[11]. Evidence indicates that greater seafood consumption during pregnancy and higher levels of DHA in breast milk are associated with a lower incidence of postpartum depression[10]. Lower DHA levels, coupled with a high omega-6 to omega-3 ratio, have been reported in women with postpartum depression[12]. Hibbeln et al. reported that the mean prevalence rate of postpartum depression worldwide in 2002 was 12.4%[11]. He also reported that higher national seafood consumption and higher DHA content of the mothers' breast milk independently predicted lower prevalence rates of postpartum depression in a sample of 14,532 pregnant women in 23 countries[11]. In countries with the lowest omega-3 intake, the prevalence of postpartum depression is 50 times greater than in the country with the highest omega-3 intake[48]. These data support the hypothesis that dietary DHA deficiency may be associated with postpartum depression.

Recommendations for DHA Consumption During Pregnancy

The 2002 Dietary Reference Intakes (DRIs) established an Acceptable Macronutrient Distribution Range (AMDR) of 0.6-1.2% of energy from omega-3 PUFAs with up to 10% of the AMDR consumed as EPA and/or DHA[49]. Due to the widespread benefits of omega-3 DHA to fetal development, the 2015-2020 DGAs recommend pregnant or lactating women consume 8-12 ounces of seafood a week, corresponding to an average of 250 mg DHA+EPA/day[19]. Additionally, the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation Experts recommend consumption of 200 mg DHA/day for pregnant or lactating women to reduce the risk of preterm birth and support infant brain development[10,50]. This recommended intake of DHA can be met by consuming one to two portions of seafood a week, such as fatty fish, which are a good source of omega-3 fatty acids[10].

Prior to 1995, infant formulas worldwide were devoid of LCPUFAs[51]. Due to the growing body of evidence supporting the necessity of DHA intake in infants, the American Dietetic Association and Dietitians of Canada recommend that DHA comprises at least 0.2% of the fatty acids in infant formula[52]. Various studies have shown a beneficial effect on infant nervous system development and/or function using a relative weight percentage (wt%) of 0.12-0.36 DHA in formula[34]. Infants consuming unsupplemented formula must synthesize all LCPUFAs from precursors. Evidence shows that these infants have less DHA in the central nervous system, plasma, and red blood cells than infants who consume formula that contains DHA[41]. Thus, leaving DHA out of infant formula could result in suboptimal neurodevelopment in the infant[34].

Infant formulas in the United States have contained DHA since 2002, and around 80% of the infant formulas consumed in the US in 2006 contained DHA[51]. Lipid provides 60% of the infant's dietary energy in breast milk (10-12% of which is essential fatty acids), while protein provides only 6%[53]. Farquharson et al. reported that infants who were fed breast milk, which contains DHA, had significantly higher concentrations of DHA in the cerebral cortex than did infants who were fed formula without DHA[54]. The levels of DHA in breast milk may vary depending on maternal intake but are generally between 0.2-0.3 wt% of the total fatty acids[55]. A recent sampling of breast milk DHA content in nine countries indicated a range of 0.17 wt% in the United States and Canada to 0.99 wt% in Japan[34]. The low DHA content of breast milk in the United States and Canada is the result of low omega-3 LCPUFA intake by lactating women in these countries[7,34]. DHA supplementation by lactating women may increase breast milk DHA content and may be beneficial for both the mother and the nursing infant[7].

Seafood as a Source of DHA

The predominant source of omega-3 PUFAs, including DHA and EPA, is seafood. Seafood varieties such as salmon (1200-2400 mg DHA+EPA/4 oz serving), oysters (1150 mg DHA+EPA/4 oz serving), and trout (1000-1100 mg DHA+EPA/4 oz serving) are recommended for consumption during pregnancy[56]. However, consumption of certain fish types during pregnancy may increase the exposure of the mother and fetus to methylmercury contaminants[10]. Methylmercury concentrations in fish and shellfish range from 2 μg/4 oz serving of oysters, salmon, and tilapia and 7 μg/4 oz serving of catfish to 147 μg/4 oz serving of swordfish and 151 μg/4 oz serving of shark[56]. The 2010 DGAs

recommend avoidance of shark, tilefish, swordfish, and king mackerel for women who are pregnant or lactating due to their high methylmercury content, which may be toxic for the developing fetus[56]. Consumption of two servings of seafood per week from recommended varieties will generally not provide more than the tolerable intake of these contaminants[10].

According to data from the 2007-2010 National Health and Nutrition Examination Survey's (NHANES) What We Eat in America survey, average intakes of seafood are low for all age-sex groups[57]. Females 19 to 30 years old consumed an average of three of the recommended eight to ten 4 ounce servings of seafood a week, and females 31 to 50 years old consumed an average of four of the recommended eight to nine 4 ounce servings of seafood a week[57]. Females 20-39 years old consumed an average of 0.03 g (30 mg) of EPA and 0.05-0.06 g (50-60 mg) of DHA, which is significantly less than the recommendation of 250 mg DHA+EPA/day[57]. According to data from the 2005-2010 NHANES involving 15,407 adults, 75.2% of the women 19-30 years old and 83.6% of the women 31-50 years old reported consuming seafood in the past thirty days[58]. However, females 19-30 years old consumed 4.3 ± 0.4 ounces and females 31-50 years old consumed 5.0 ± 0.3 ounces of the recommended 8-10 ounces of seafood per week. Additionally, 82-92% of these two groups did not meet seafood recommendations for their age and sex[58].

Dietary Intake of DHA+EPA Among Various Populations

Data on omega-3 fatty acid intake by pregnant women is sparse for populations in the United States and Louisiana. The mean DHA intake in Western countries is 70-200 mg/day and the median intake is 30-50 mg/day[1]. This translates to an intake less than that required for the growing fetus during the last trimester of pregnancy and may potentially

lead to suboptimal DHA status for both the mother and infant[1]. Numerous studies have documented supplementation with 2.7 g DHA+EPA/day, which is 20 times greater than the average intake of pregnant women in Western countries[1]. This level of intake has not been associated with increased risk of adverse effects commonly associated with marine oil supplementation[1]. These adverse effects include increased risk of bleeding complications and blood loss at birth due to the anticoagulant properties of marine oil[1].

Lewis et al. reported that the mean daily consumption of omega-3 fatty acids by low-income pregnant women in the Midwestern United States was 1.060 ± 0.03 g/day[23]. DHA and EPA comprised 5% and 2% of this total, respectively. Almost one-half of the study population consumed less than 75% of the 1990 Canadian Recommended Nutrient Intakes (RNIs) for omega-3 fatty acids, which were in place at the time of this study[23]. The 1990 Canadian RNIs recommend consumption of 1.2 g of omega-3 fatty acids/day for women 25-49 years old[59]. This recommendation increases in the second and third trimesters of pregnancy to 1.36 g/day for women 19-24 years old and 1.26 g/day for women 25-49 years old[59]. The food sources of DHA and EPA consumed by Lewis et al.'s study population included fish, seafood, chicken, and eggs[23].

A similar study conducted by Judge et al. in Connecticut focused on DHA intake and separated the population according to ethnicity[24]. The investigators reported an intake of 11 ± 64 mg DHA/day in the Asian group, 113 ± 37 mg DHA/day in the African American group, 21 ± 26 mg DHA/day in the Caucasian group, and 64 ± 18 mg DHA/day in the Latino group[24]. The DHA intake by each of these ethnic populations is substantially less than the recommendation of 200 mg DHA/day[10,48].

Innis et al. reported that a population of 55 pregnant women in British Columbia had a mean daily intake of 160 ± 20 mg DHA and 78 ± 2 mg EPA according to two food frequency questionnaires administered at 28 and 35 weeks of gestation[25]. These researchers reported that seafood accounted for 80% of dietary DHA consumption and 65% of EPA consumption by this population[25]. Another Canadian study, conducted by Denomme et al. in 2005, analyzed the food consumed by participants for omega-3 PUFA content using lipid extraction and gas-liquid chromatography[26]. Total PUFA intake accounted for 21.2 ± 1.1% of fat consumption, with considerably more omega-6 PUFAs than omega-3. Omega-3 PUFA intake was 1.45 ± 0.18 g/day and omega-6 intake was 8.35 ± 0.77 g/day. The ratio of omega-6 to omega-3 PUFAs was 6.3 ± 0.4. DHA intake was 82 ± 33 mg/day and EPA intake was 35 ± 19 mg/day. Only 50% of the population met the Canadian RNI of 0.5% of energy as omega-3 PUFAs[26,59]. Additionally, only 35% of the population met the lower limit of the AMDR. The AMDR is the range of adequate intakes for a nutrient that is associated with a reduced risk of chronic disease. The AMDR for omega-3 PUFAs was set at 0.6-1.2% of energy at the time of this study[26,49].

Although there are very little data on DHA intake by pregnant women who are overweight, Drouillet et al. conducted a study in France involving seafood consumption before pregnancy and birth outcomes[60]. A higher intake of seafood before pregnancy may lead to variations in the fatty acid content of the woman's adipose tissue. Researchers reported higher fetal growth measures in overweight women who consumed seafood more than nine times a month as opposed to overweight women who consumed seafood less than five times a month. In this study, fetal growth measures included birth weight, birth length, head circumference, arm circumference, and wrist circumference. These results

may be explained in part by the fact that overweight women have an enhanced ability to release fatty acids from adipose tissue, which serves as a reserve of fatty acids for the developing fetus[60].

In a recent survey conducted in Baton Rouge, Louisiana involving 221 pregnant women, researchers found that 87.1% of the population consumed at least one type of fish during pregnancy[61]. Catfish and tilapia, fish low in DHA, were the most widely consumed fish. Catfish (116 mg DHA/3 oz serving) was consumed by 59.0% of the population and tilapia (97 mg DHA/3 oz serving) was consumed by 46.8% of the population. Researchers found that of the fish varieties included in the survey, the population rarely consumed those highest in DHA (bass, herring, and salmon). According to the survey, 98% of the population never consumed swordfish, which contain harmful methylmercury contaminants and are not recommended for consumption by pregnant women[19,61].

The Omega-6:Omega-3 Ratio

As previously noted, omega-6 fatty acids compete with omega-3 fatty acids for the same enzymes but cannot be interconverted[2,3]. Therefore, the ratio of omega-6 to omega-3 fatty acids is important. Studies show that Western diets are deficient in omega-3 fatty acids and excessive in omega-6, leading to a high omega-6:omega-3 ratio[6]. It has been estimated that the Western diet has an omega-6:omega-3 ratio of 15-20:1[6]. A high omega-6:omega-3 ratio promotes the development of many diseases including cardiovascular disease, cancer, and inflammatory diseases, whereas a low ratio decreases risk for these diseases[6]. The recommended ratio of omega-6:omega-3 for a healthy pregnancy and

avoidance of disease varies from 4:1 to 2:1[6]. The best source for omega-3 LCPUFAs is seafood, whereas omega-6 LCPUFAs are found in seed oils, eggs, and poultry[62].

It is feasible to think that the study population consumes fish and seafood more frequently than women in other geographical areas, as Louisiana is a southern state found along the coast of the Gulf of Mexico. This may lead to a greater intake of omega-3 fatty acids than previously reported in other regions of the United States and Canada. A high consumption of omega-3 fatty acids translates to a lower omega-6:omega-3 ratio, resulting in beneficial pregnancy outcomes. However, based on data reported in other regions of the country, we hypothesize that the seafood and omega-3 intakes by this population of overweight pregnant women is actually suboptimal. If so, this population's omega-3 fatty acid intake may be inadequate to promote beneficial pregnancy outcomes, as both the mother and infant may be at risk for suboptimal omega-3 LCPUFA status.

MATERIALS AND METHODS

Subjects

Pregnant overweight women with a pregravid BMI of 25.0-29.9 kg/m^2 (n=10), who were less than 20 weeks pregnant, between 18 and 35 years of age, and who had passed the oral glucose tolerance test for diabetes were recruited from outpatient clinics at Woman's Hospital in Baton Rouge, Louisiana. Subjects were pre-screened to determine if they qualified. Pre-screening questions included age, height and weight, weeks pregnant, if they were being cared for by a physician at Woman's Hospital, and if they planned to deliver at Woman's Hospital. Subjects who passed the pre-screening were asked additional questions to determine eligibility. Exclusion criteria included: having more than five

children; a history of high blood pressure, high blood lipids, kidney disease, liver disease, or high blood sugar; having polycystic ovarian syndrome; having uncontrolled thyroid disorder; having multiple fetuses; having smoked in the past six months; having been pregnant or breastfeeding in the past six months; testing positive for Human Immunodeficiency Virus (HIV), syphilis, sepsis, group B streptococcus, or Hepatitis B; and planning a Cesarean delivery. In order to qualify, subjects must also plan to deliver at Woman's Hospital in Baton Rouge, Louisiana. Eligible participants entered the study 17-20 weeks into their pregnancy and were followed until delivery.

Red Blood Cell Analyses

Participants underwent a 10-milliliter venous blood draw by a trained phlebotomist in the Outpatient Laboratory in the Physician Office Building at Woman's Hospital. Samples were collected at time of study entrance (17-20 weeks of pregnancy). Participants' blood was drawn into EDTA-containing tubes that were pre-labeled with the participant's ID. The tubes were then placed into a biohazard bag and transported to the Pathology Laboratory at Woman's Hospital for processing of blood. Red blood cells (RBC) were separated from plasma, portioned into aliquots, and stored in the freezer at -80 °C. RBC were later transported to Pennington Biomedical Research Center in Baton Rouge, Louisiana to be stored at -80°C until analyses.

RBC were prepared for analysis of fatty acids using a direct methylation procedure. Heptadecanoic acid, or C17, was added as an internal standard to calculate a relative weight percentage (wt%) of each fatty acid based on the total fatty acids quantitated in the sample. Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography equipped

with a flame ionization detector (FID). In gas chromatography, the sample of RBC is vaporized and transported through a column by the flow of a gaseous, mobile phase[22]. The column also contains a liquid, stationary phase. Each component of the sample goes back and forth from the gas phase to the liquid phase, allowing separation into individual fatty acids. Each fatty acid in the RBC sample exits the column at a different time, referred to as the retention time. The retention time depends both on the length and unsaturation of the fatty acid chain. When the pure fatty acid exits the column, it is combusted by a flame and broken up into ionized fragments, which are quantitatively detected. As the sample is analyzed, a chromatogram is produced that contains peaks representing the time at which each fatty acid exited the column as well as the relative amount of each fatty acid in the sample. These peaks are used to calculate the relative weight percentage of each fatty acid identified the red blood cell sample. The weight percentage of each fatty acid in a RBC sample was determined using the following formula:

$$FA\ wt\% = \frac{FA\ area\ \% * 100}{\Sigma\ FA\ area\% - iSTD\ area\%}$$

where FA area % is the area percentage for a specific fatty acid, 100 is the factor used to determine a relative weight percentage, Σ FA area % is the area percentage of all fatty acids identified and quantitated in the sample, and iSTD% is the area percentage of the internal standard added to the RBC sample. Using the relative weight percentage, we calculated the fatty acid content of the red blood cell sample taken from each participant.

Estimation of Dietary Intake

Women were interviewed for twenty four-hour dietary recalls at 17-20, 22, 24, 26, 30, 32, and 36 weeks of pregnancy for a total of seven recalls. Interviews were conducted by a trained researcher either in person or via telephone. A twenty four-hour dietary recall is a method of gathering dietary data in which the participant lists all food and drink items consumed in the past twenty four hours, as well as amounts of each food and the time consumed[63]. The participant also lists any supplements taken and amounts of each. To-scale depictions of food and food containers including measuring cups, drinking glasses, and serving sizes of various food items were used to assist subjects with estimating quantities consumed. The interview was conducted using the University of Minnesota Nutrition Data System for Research (NDSR)[64]. The NDSR is a dietary analysis software application that is widely used for the collection and analysis of twenty four-hour dietary recalls. This software provides a very proscribed method of interview while using the multiple pass method. The multiple pass method serves as a tool to help assure the validity of the information gathered by asking the participant about the information provided multiple times and in multiple ways. The interview begins by asking for a broad description of the foods consumed and the times at which they were consumed. Next, the information is repeated to the participant and corrected if necessary. The interviewer then asks the participant specific questions about each food item, including the brand of food if applicable, preparation methods, and the amount consumed. At the conclusion of the interview, the participant's dietary intake is repeated again for confirmation. The same process is then repeated for any vitamins or supplements the participant may have consumed. Questions regarding the type of supplement, brand name, number of times

consumed that day, and how many capsules were consumed each time are asked. The interviews normally require one to one and a half hours to complete. The NDSR provides the amounts of both macronutrients and micronutrients, including calories, carbohydrates, protein, fat, and various other nutrients including fatty acids, such as DHA and EPA. Dietary information from the repeated (n=7) twenty four-hour dietary recalls was averaged for each participant to calculate daily dietary intake. The NDSR also considers supplementation of nutrients, providing researchers with a total intake of specified nutrients. This information was later compared to the 2015-2020 DGAs[19] to assess the intake of the study population.

Data Analyses

Descriptive statistics, including mean, standard deviation, and range, were used to characterize the study population's anthropometrics and dietary intake. The population was separated into various groups based on categories of age, ethnicity, socioeconomic status (SES), and parity. Two-tailed t-tests were conducted to determine significant differences in DHA, EPA, and DHA+EPA intake between the groups. Each category consisted of two groups of five women and *P* values less than 0.05 were considered significant. Participants were separated by age into a group of women younger than 25 years old (n=5) and a group of women equal to or older than 25 years old (n=5). The ethnicity category consisted of a group of Caucasian women (n=5) and a group of African American (AA) women (n=5). The SES category was based on the participant's type of health insurance and contained a group of women with medium/high SES (n=5) and a

group of women with low SES (n=5). The parity category consisted of primiparous women (n=5) and women who had at least one previous pregnancy (n=5).

The omega-6:omega-3 ratio was determined by dividing the participant's total omega-6 consumption by her total omega-3 consumption. Omega-6 fatty acids included LA and AA, while omega-3 fatty acids included ALA, EPA, and DHA.

A Pearson correlation was used to determine the linear relationship between RBC fatty acid status and dietary intake of omega-3 fatty acids.

RESULTS

Subject Description

The characteristics of the study population of women (n=10) are shown in Table 1. All participants were overweight women (pregravid BMI 25.0-29.9 kg/m^2), 20-34 years, who entered the study at 17-20 weeks of pregnancy. Ethnicity, socioeconomic status, and parity are also presented in Table 1. Eighty percent of African Americans in this population (n=4) were of low socioeconomic status, and eighty percent of Caucasians (n=4) were of medium/high socioeconomic status according to their health insurance.

Dietary Intake

The dietary intake of this population of overweight pregnant women based on seven repeated twenty four-hour dietary recalls is presented in Table 2. The average intake of total fat and saturated fat exceeded recommendations set forth by the 2015-2020 DGAs[19]. These guidelines state that total fat intake should comprise 20-35% of the total kilocalories

consumed and saturated fat intake should comprise no more than 10% of the total kilocalories consumed[19].

Table 1. Characteristics of study population at time of study entrance: overweight pregnant women in the Greater Baton Rouge area (n=10)

	Mean ± SD	Min-Max
Age	(25.6 ± 4.0)	20-34
<25 (y)	n=5	
≥25 (y)	n=5	
Ethnicity		
Caucasian	n=5	
African American (AA)	n=5	
Pregravid BMI[a] (kg/m^2)	(27.5 ± 1.4)	25.3-29.1
Gestational Age (weeks)	(18.7 ± 1.3)	17-20
Socioeconomic Status (SES)[b]		
Low	n=5	
Med/high	n=5	
Parity		
0	n=5	
≥1	n=5	

[a]BMI= body mass index, [b]SES determined using type of health insurance

Omega-3 LCPUFA Intake

On average, the study population did not meet the recommendations for DHA or EPA set forth by the 2015-2020 DGAs[19]. All women (n=10) consumed a prenatal supplement. Nine women consumed a prenatal supplement that contained DHA, and four women consumed a prenatal supplement that contained EPA. None of the women met the recommendation of 200 mg DHA/day before supplementation and all ten of the women consumed less than 50% of the recommendation[10]. After supplementation, four of the

women met the recommendation for DHA consumption during pregnancy, though two women had an intake less than 50% of the recommendation.

Table 2. Mean dietary intakes (before supplementation) by overweight pregnant women in the Greater Baton Rouge area (n=10)

	Mean ± SD	Min-Max
Energy (kcal)	2213.8 ± 213.7	1788.0-2497.2
Fat (g)	88.7 ± 11.0	71.8-113.5
Saturated Fat (g)	30.1 ± 3.9	25.1-38.2
Polyunsaturated Fat (g)	21.5 ± 6.8	15.9-39.4
Omega-3[a] (g)	2.1 ± 0.7	1.5-3.8
α-linolenic acid (ALA) (g)	2.0 ± 0.7	1.4-3.7
Eicosapentaenoic acid (EPA) (mg)	31.8 ± 23.1	7.0-75.1
Docosahexaenoic acid (DHA) (mg)	48.1 ± 23.9	17.7-95.0
Omega-6[b] (g)	19.3 ± 6.3	14.4-35.8
Linoleic acid (LA) (g)	19.1 ± 6.3	14.3-35.6
Arachidonic acid (AA) (mg)	177.7 ± 55.3	101.8-257.9
Omega-6:omega-3[c]	9.4 ± 1.4	7.6-12.6

[a]Omega-3= α-linolenic acid (ALA) + eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)
[b]Omega-6= linoleic acid (LA)+ arachidonic acid (AA)
[c]Omega-6:omega-3=(LA + AA)/(ALA + EPA + DHA)

There were also no women who met the recommendation of 250 mg DHA+EPA/day during pregnancy when assessing intake before supplementation[19]. Eight women consumed less than 50% of the recommendation. After supplementation, 20% of this population (n=2) met this recommendation for DHA+EPA, while two participants consumed less than 50% of the DHA+EPA recommendation during pregnancy[19].

One recall from one of the subjects included consumption of 6.94 servings of crawfish. This recall was considered an outlier due to the subject's intake of 299 mg

DHA/day and 1,009 mg DHA+EPA/day compared to the average intake of the other subjects in the study (48.1 ± 2.4 mg DHA/day, 80.0 ± 4.6 mg DHA+EPA/day). This recall was excluded from the analyses because this subject's average intake according to her six additional recalls is more representative of her usual diet (45.2 mg DHA/day, 65.7 mg DHA+EPA/day). The study population's consumption of DHA, EPA, and DHA+EPA without the outlying recall is summarized in Table 3.

Table 3. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and DHA+EPA intake from dietary and supplemental sources by overweight pregnant women in the Greater Baton Rouge area

	DHA	EPA	DHA+EPA
Recommendation	≥200 mg/day[10]		≥250 mg/day[19]
From Diet (mg)	48.1 ± 23.9[a] 17.7-95.0[b]	31.8 ± 23.1[a] 7.0-75.1[b]	80.0 ± 45.6[a] 24.7-170.1[b]
Women Meeting Recommendation from Dietary Intake	n=0	----	n=0
From Supplements (mg)	116.4 ± 74.2[a] 0.0-200.0[b]	5.5 ± 8.2[a] 0.0-24.0[b]	121.9 ± 76.4[a] 0.0-212.0[b]
Dietary + Supplemental Intake (mg)	164.5 ± 82.3[a] 34.0-269.1[b]	37.4 ± 28.5[a] 7.0-99.1[b]	201.9 ± 94.4[a] 55.6-365.6[b]
Women Meeting Recommendation After Supplementation	n=4	----	n=2

[a]Mean ± SD, [b]Min-max

Fat Intake

When assessing dietary information from 69 recalls, fifty percent of the population (n=5) had an adequate fat intake of 20-35% of their total kilocalories[19]. The remaining fifty percent of the population (n=5) consumed more than 35% of their total kilocalories from fat. Only one woman had an adequate saturated fat intake of less than 10% of her total kilocalories from fat, with the remaining women (n=9) having consumed more than 10% of their kilocalories as saturated fat[19]. Polyunsaturated fats, which included LA, ALA, stearidonic acid (18:4n-3), AA, EPA, docosapentaenoic acid (22:5n-6), and DHA accounted for 23.8 ± 4.7% of the population's total fat intake. The fat intake of this population is summarized in Table 4.

Table 4. Fat intake by overweight pregnant women in the Greater Baton Rouge area (n=10) compared to recommendations by the 2015-2020 Dietary Guidelines for Americans[1]

	Intake (g)	kcals from fat (%)	DGA Recommendation[19]	Women Meeting Recommendation
Total fat	88 ± 11[a]	36 ± 4[a]	20-35% total kcals[19]	n=5
Saturated Fat	30 ± 4[a]	12 ± 2[a]	<10% total kcals[19]	n=1
Polyunsaturated Fat (PUFA)[b]	21 ± 7[a]	8.7 ± 2.2[a]	----	----

[a]Mean ± SD, [b]PUFA= 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6, 20:5n-3, 22:5n-6, 22:6n-3

Seafood Consumption

Eighty percent of this population consumed seafood at least once (n=8). The majority of seafood consumed was shellfish (73%), including crawfish, shrimp, and crab. Lean fish, consumed as catfish, comprised 22% of seafood consumed, while fried fish,

consumed as whitefish and catfish, comprised 6%. Two of the ten women consumed lean fish, two consumed fried fish, and seven consumed shellfish on at least one occasion. The varieties of seafood consumed were crawfish, shrimp, crab, catfish, and whitefish. The seafood intake of this population is presented in Table 5.

Table 5. Consumption of various seafood varieties by overweight pregnant women in the Greater Baton Rouge area (n=10)

Women who consumed fish at least once	n=8
Meals fish were consumed	21/209
Days fish were consumed	17/69
Total servings consumed	20.3
Average servings consumed per day	0.29 ± 0.32[a]

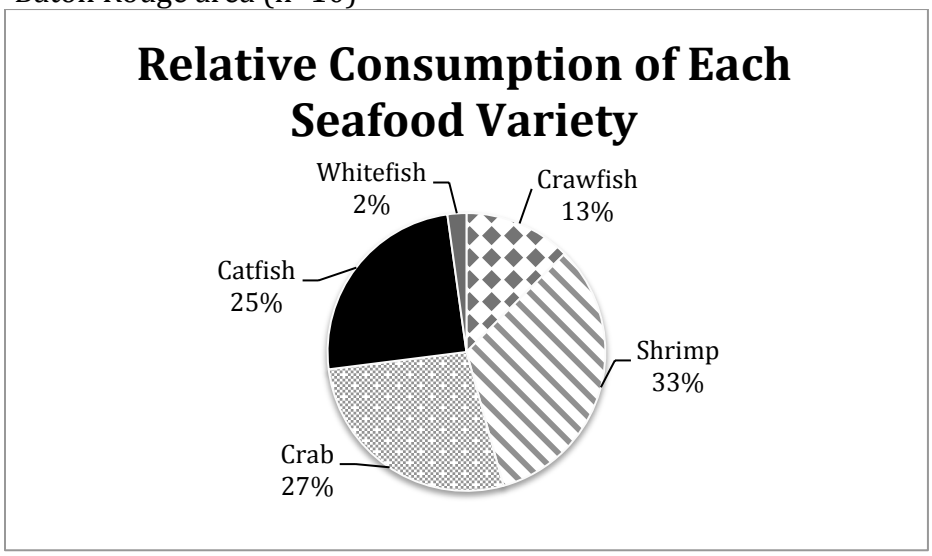
[a]Mean ± SD

All varieties of seafood consumed by the study population had a relatively low DHA content. These fish included shrimp, crawfish, crab, catfish, and whitefish. The DHA content of seafood varieties consumed by the population is presented in Table 6. The amount of each seafood variety that was consumed by the population is presented in Figure 3. The top sources of dietary omega-3 DHA consumed by this population were crawfish, chicken, shrimp, eggs, and crab.

Table 6. DHA content of seafood varieties consumed by overweight pregnant women in the Greater Baton Rouge area (n=10)

Type of fish	DHA Content[62]
Shrimp	17 mg/4 oz serving
Crawfish	53 mg/4 oz serving
Crab	76 mg/4 oz serving
Catfish	
Farmed	78 mg/4 oz serving
Wild	155 mg/4 oz serving
Whitefish	177 mg/4 oz serving

Figure 3. Percentage of total seafood consumed as each seafood variety by overweight pregnant women in the Greater Baton Rouge area (n=10)



Further Examination into Omega-3 LCPUFA Intake

There were no significant differences in dietary consumption of DHA for age, ethnicity, SES, or parity ($P \geq 0.09$). However, age was a significant factor for dietary EPA intake ($P \leq 0.05$). Women ≥25 years consumed significantly higher EPA from dietary

sources than women <25 years. Ethnicity, SES, and parity did not affect EPA dietary intake. Age was also the only factor that affected DHA+EPA dietary intake ($P \leq 0.05$). Again, the group of women ≥25 years consumed significantly more DHA+EPA than women <25 years. Ethnicity, SES, and parity did not affect dietary intake of DHA+EPA ($P \geq 0.5$).

When supplementation was considered, there was a significant difference between women who had no previous pregnancies and those who had at least one previous pregnancy in terms of DHA intake and supplementation. Women with at least one previous pregnancy had significantly higher levels of DHA intake from diet and supplements than did primiparous women ($P \leq 0.05$). Women who had at least one previous pregnancy had a dietary and supplemental DHA intake of 233.5 ± 36.2 mg/day and those women who had not been previously pregnant had an average intake of 95.5 ± 45.0 mg/day. There were no significant differences in DHA dietary and supplement intake in terms of age, ethnicity, or SES ($P \geq 0.4$). There were also no significant differences in EPA dietary and supplement intake in terms of ethnicity, SES, or parity ($P \geq 0.4$). Age remained a factor for EPA consumption ($P \leq 0.05$). Women ≥25 years consumed significantly more EPA from diet and supplements than women <25 years. There were no significant differences in age, ethnicity, SES, or parity when DHA+EPA consumption from the diet and supplements were combined ($P \geq 0.06$). These results are summarized in Table 7.

Red Blood Cell Fatty Acid Status

The average relative weight percentage (wt%) of omega-3 and omega-6 fatty acids, including DHA and EPA, in the participants' RBC are presented in Table 8.

Further Examination into Red Blood Cell Fatty Acid Status

There were no significant differences in DHA status by age, ethnicity, or SES ($P \geq 0.2$). There were also no significant differences in EPA status of the RBC by age, ethnicity, SES, or previous pregnancies ($P \geq 0.2$). No significant differences were found between the groups when DHA+EPA statuses of the RBC were combined ($P \geq 0.2$). Fatty acid concentrations of the participant's RBC by group are shown in Table 9.

There were no correlations between dietary DHA intake and DHA status (r=-0.06), between dietary EPA intake and EPA status (r=-0.30), or between dietary DHA+EPA intake and DHA+EPA status (r=-0.16).

There were also no correlations between dietary and supplemental intake of DHA and DHA status (r=0.25), dietary and supplemental intake of EPA and EPA status (r=0.1), and dietary and supplemental intake of DHA+EPA and DHA+EPA status (r=0.04).

Table 7. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and DHA+EPA dietary intake and dietary + supplemental intake by overweight pregnant women in the Greater Baton Rouge area

	Dietary Intake						Dietary + Supplemental Intake					
	DHA Mean ± SD (mg)	*p* value	EPA Mean ± SD (mg)	*p* value	DHA+EPA Mean ± SD (mg)	*p* value	DHA Mean ± SD (mg)	*p* value	EPA Mean ± SD (mg)	*p* value	DHA+EPA Mean ± SD (mg)	*p* value
Age												
<25 (y)	35.4±12.0	NS	17.2±8.9	$p \leq 0.05$	52.6±20.1	$p \leq 0.05$	149.5±81.0	NS	19.0±10.6	$p \leq 0.05$	168.9±74.2	NS
≥25 (y)	60.9±27.0		46.5±24.2		107.4±48.9		179.5±90.1		55.7±29.6		176.9±125.9	
Ethnicity												
Caucasian	45.3±19.5	NS	25.3±12.3	NS	70.6±30.4	NS	181.1±76.7	NS	29.7±16.3	NS	152.6±85.1	NS
AA	51.0±29.6		38.3±30.7		89.3±59.4		147.9±93.1		45.0±37.6		192.9±114.6	
SES												
Low	50.6±29.9	NS	35.8±32.9	NS	86.4±61.7	NS	187.5±72.3	NS	42.5±39.9	NS	230.0±85.4	NS
Med/high	45.6±19.3		27.9±8.9		73.5±27.5		141.5±93.2		32.3±12.9		115.4±76.9	

NS, non-significant

Table 8. Fatty acid status of overweight pregnant women in the Greater Baton Rouge area (n=10) at 17-20 weeks of pregnancy

	Mean ± SD (wt%)	Min-Max (wt%)
Omega-3[a]	9.52 ± 1.31	7.60-11.34
α-linolenic acid (ALA)	0.13 ± 0.04	0.08-0.23
Eicosapentaenoic acid (EPA)	0.14 ± 0.03	0.1-0.22
Docosahexaenoic acid (DHA)	9.25 ± 1.31	7.33-11.05
DHA+EPA	9.40 ± 1.32	2.25-3.99
Omega-6[b]	28.27 ± 1.82	25.24-30.63
Linoleic acid (LA)	11.28 ± 1.72	8.42-15.0
Arachidonic acid (AA)	17.00 ± 1.29	14.70-19.64
Omega-6:Omega-3[c]	3.04 ± 0.61	2.25-3.99

[a]Omega-3= α-linolenic acid (ALA) + eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)
[b]Omega-6= linoleic acid (LA)+ arachidonic acid (AA)
[c]Omega-6:omega-3=(LA + AA)/(ALA + EPA + DHA)

Table 9. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and DHA+EPA red blood cell status of overweight pregnant women in the Greater Baton Rouge area

	DHA Mean ± SD (wt%)	*p* value	EPA Mean ± SD (wt%)	*p* value	DHA+EPA Mean ± SD (wt%)	*p* value
Age						
<25 (y)	9.6±1.3	NS	0.2±0.1	NS	9.8±1.3	NS
≥25 (y)	8.9±1.4		0.1±0.0		9.0±1.4	
Ethnicity						
Caucasian	9.9±1.5	NS	0.1±0.1	NS	10.0±1.5	NS
AA	8.6±0.8		0.1±0.1		8.8±0.8	
SES						
Low	9.2±1.3	NS	0.1±0.0	NS	9.3±1.3	NS
Med/high	9.3±1.5		0.2±0.0		9.5±1.5	

NS, non-significant

DISCUSSION

The findings of this study support the hypothesis that overweight women in the Greater Baton Rouge area are not consuming adequate levels of omega-3 LCPUFAs during pregnancy, a reflection of the fact that they are not consuming enough seafood to meet the dietary recommended intakes of DHA+EPA[19]. These data are consistent with findings reported in other regions of the United States[23,24] and Canada[25,26] and demonstrate a need for increased prenatal education for this population.

As shown in Table 2, this population of overweight pregnant women consumed excess fat, saturated fat, and PUFAs, but failed to meet the recommendations for the omega-3 LCPUFAs, DHA and EPA. This population consumed an average of 2.1 ± 0.7 g/day omega-3 PUFAs (ALA, DHA, and EPA). The omega-3 PUFA intake by the current study population is almost double that consumed by Midwestern pregnant women in a study conducted by Lewis et al (1.1 ± 0.3 g/day omega-3 PUFAs)[23], but is similar to that reported by De Vriese et al. (1.8 ± 0.5 g/day omega-3 PUFAs)[66]. The current population consumed an average of 19.3 ± 6.3 g/day omega-6 PUFAs (LA and AA), which corresponded to an omega-6:omega-3 ratio of 9.4 ± 1.4. The omega-6 PUFA intake by this population is greater than that reported in other studies, including the of consumption of 13.1 ± 0.5 g/day omega-6 PUFAs reported by De Vriese et al[66]. The dietary omega-6:omega-3 ratio of the current study population is greater than that reported by Denomme et al. in Canadian pregnant women (6.3 ± 0.4)[26], but comparable to that reported by Gaitán et al. in Louisiana pregnant women (9 ± 1)[27]. A high dietary omega-6:omega-omega:3 ratio, as reported in many other studies of the Western population, increases the risk for developing various chronic diseases, including

cardiovascular disease, diabetes, and cancer[6]. A low ratio is more desirable and is recommended, as it reduces the risk of disease.

Previous studies have reported low intakes of the omega-3 LCPUFAS, DHA and EPA, by pregnant women. No women in the current study met the recommendation for DHA or DHA+EPA intake during pregnancy from dietary sources alone. All ten women consumed less than 50% of the DHA recommendation from dietary intake and eight women consumed less than 50% of the DHA+EPA recommendation. The average intake of DHA by this population was 48.1 ± 28.9 mg/day and the average intake of EPA was 31.8 ± 23.1 mg/day. These findings are consistent with the report of Lewis et al., who reported that pregnant women consumed 48 ± 81 mg DHA/day and 23 ± 60 mg EPA/day[23]. However, these quantities are lower than a recent report by our laboratory, in which overweight pregnant women consumed an average of 72 ± 63 mg DHA/day and 104 ± 158 mg EPA/day[27], as well as a report by Innis et al., in which pregnant women consumed 160 ± 20 mg DHA/day and 78 ± 2 mg EPA/day[25]. The differences in intake may be due to different methods for collecting data on nutrient intake, as some studies used twenty four-hour recalls[23,27] and others used food frequency questionnaires[25], or preferential eating behaviors based on geographical location.

All women in the current study consumed a prenatal supplement and 90% of the population consumed supplements that contained DHA. These data point to a higher percentage of women in the current study taking supplements as opposed to Gaitán et al.'s study population, in which only 57% consumed supplements that contained DHA[27]. On average, supplements provided over half of the current study population's recommended DHA intake (58%) and just under half of the recommended DHA+EPA intake (49%). After

supplementation, four women met the recommendation for DHA and two met the recommendation for DHA+EPA. Additionally, only two women in the current study population consumed less than 50% of the recommended DHA and DHA+EPA intakes after supplementation. These data emphasize the importance of prenatal supplementation during pregnancy to facilitate adequate intake of omega-3 LCPUFAs.

Fifty percent of the study population over consumed fat, while ninety percent over consumed saturated fat. On average, the current population consumed 88 ± 11 g of fat/day and 30 ± 4 g of saturated fat/day. These results are consistent with a previous Belgian report by De Vriese et al.[66], in which pregnant women consumed 85 ± 25 g of fat/day and 33 ± 11 g of saturated fat/day; as well as Gaitán et al.'s study in the Greater Baton Rogue area, in which pregnant women consumed 87 ± 24 g of fat/day and 29 ± 9 g of saturated fat/day[27]. Consumption of excess saturated fat may lead to adverse health effects, especially during pregnancy, as it increases inflammation. The current population also had a lower PUFA intake (8.7% of fat) than was reported by Denomme et al. in pregnant Canadian women (21% of fat)[26], by De Vriese et al. in Belgian pregnant women (17.9% of fat)[66], and by Gaitán et al. in American pregnant women (25.3% of fat)[27]. PUFA intake during pregnancy has been reported to influence neurodevelopmental and visual outcomes, as well as gestational length and weight. The 2015-2020 DGAs recommend that the majority of fat intake come from PUFAs, as they may provide antioxidants and help reduce HDL cholesterol, therefore lowering the risk of cardiovascular disease and stroke[19].

Eighty percent of this population consumed seafood at least once. These data are consistent with data reported by Drewery et al. regarding seafood consumption by pregnant women in Baton Rouge, Louisiana[61]. Of the pregnant women surveyed by

Drewery et al., 87.1% consumed at least one type of fish[61]. In the current study, the average number of servings of seafood consumed per day was only 0.29 servings, with all seafood sources containing a relatively low DHA content (17-177 mg DHA/4 oz serving). These data correspond to Drewery et al.'s findings in which tilapia and catfish, both low in DHA, were the most commonly consumed fish varieties[61]. One-third of all seafood consumed in the current study was shrimp, while 25% was consumed as crab and 25% as catfish. None of the current study population consumed fish high in methylmercury, decreasing the risk of methylmercury poisoning in this population[61].

The main contributors of DHA to the diet of the current population were crawfish, chicken, shrimp, eggs, and crab. In the United States, chicken is eaten more often than fish and therefore poultry may be a major dietary source of DHA+EPA in the diets of women who seldom eat fish[23,61]. Similarly, Lewis et al. reported that fish and seafood comprised only 2% of the omega-3 PUFA intake, but comprised 65% of the DHA consumed and 83% of the EPA consumed. Other foods that provided DHA+EPA in Lewis' study were chicken and eggs[23].

Women who were 25 years or older consumed significantly more EPA than women younger than 25 years. Although both groups of women consumed the same EPA-containing foods, women 25 years or older consumed these food varieties foods more often. Jovicic reported that knowledge about healthy eating increases with age[67], which may explain why older women consumed more EPA during pregnancy than younger women. Consequently, this difference in EPA intake increased the 25 years and older group of women's combined DHA+EPA intake. Women 25 years or older consumed significantly more DHA+EPA than did women younger than 25 years. Additionally, women

25 years or older consumed more DHA, while also consuming higher levels of DHA and EPA supplements than women younger than 25 years.

As shown in Table 7, African Americans in the current study consumed more DHA and EPA than Caucasians. However, Caucasians consumed more DHA from supplements, while African Americans consumed more EPA from supplements. Additionally, the low SES women consistently consumed more DHA and EPA than the medium/high SES women, and also consumed more DHA and EPA from supplements than did the medium/high SES women.

Women who had at least one previous pregnancy consumed significantly more DHA from dietary and supplemental sources combined than primiparous women. Women who had been previously pregnant also consumed more EPA than women who had not. Supplements consumed by women who had at least one previous pregnancy provided an average of 233.5 ± 36.2 mg DHA/day, while those consumed by primiparous women provided an average of 95.5 ± 45.0 mg DHA/day. Women who had at least one previous pregnancy also consumed more EPA from supplements than primiparous women.

The average DHA status, expressed as a wt%[22], of our population was 9.25 ± 1.31. This finding is in contrast to previous studies, which have reported lower values in pregnant women. In 2009, Courville et al. reported a DHA wt% of 4.75 ± 0.24 in pregnant women from Connecticut[22]. Additionally, De Vriese reported a DHA wt% of 4.8 ± 1.3 in Belgian pregnant women[66]. These differences may be due to geographical location and differences in food preferences. Alternatively, the higher DHA wt% reported in the current study may be due to the excess adipose tissue present in overweight women. Excess adiposity may increase the efficiency of enzymes to convert LA to EPA and DHA, resulting

in a higher DHA wt% in overweight women[13]. This finding may also be attributed to release of DHA from maternal adipose stores in an effort to compensate the loss of DHA that has been transferred to the fetus[13].

There was no correlation between omega-3 LCPUFA intake and status in this population. This may be explained by the preferential transfer of certain fatty acids (DHA, EPA, and AA) across the placenta to the fetus to promote adequate growth and development of the fetus. Intake of these fatty acids is not directly related to status, since the fatty acids consumed are preferentially delivered to the infant and do not remain in the maternal phospholipid stores. If adequate intakes of these nutrients are not consumed, both the mother and infant may be at risk for developing adverse pregnancy outcomes[32,33].

A major limitation of this study is the small sample size. Subsequent studies may aim to increase the sample population size across multiple geographical locations to assess omega-3 LCPUFA intake by pregnant women across the United States.

In conclusion, intakes of the omega-3 LCPUFAs, DHA and EPA, by overweight pregnant women in Baton Rouge, Louisiana are inadequate. These findings are consistent with those reported in other regions of the United States and Canada. Suboptimal intake and status of these essential fatty acids may contribute to adverse health outcomes for both the mother and the fetus during pregnancy. Further exploration of the currently available nutrition education for this population and approaches to improve education is warranted.

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