I, Rodrigo Gerardo, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
**Docosahexaenoic acid status and blood lipids in overweight/obese pregnant women**

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**This work and its defense approved by:**

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Committee member: Graciela Falciglia, Ph.D.
Docosahexaenoic acid status and blood lipids in overweight/obese pregnant women

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science

In the Department of Nutritional Sciences of the College of Allied Health Sciences

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Rodrigo G. Gerardo, BS University of Cincinnati 2011

Committee Chair: Dr. Debra A. Krummel, PhD, RD
Abstract

Objective: The objective of this thesis was to investigate whether plasma erythrocyte DHA (EDHA) status was related to blood lipid concentrations measured at the end of the third trimester in healthy, overweight/obese pregnant women.

Design: Observational analysis of women grouped by EDHA status.

Sample: Pregnant women, 18 – 40 years of age, with a BMI $\geq 25$ were recruited from the regional areas of Cincinnati, Ohio and San Antonio, Texas.

Methods: Pregnant women were categorized by EDHA status determined at the 36th week of pregnancy. EDHA status was divided into two groups based on the reported concentration to achieve DHA equilibrium for the mother and the baby; high status was defined as an EDHA concentration $\geq 6\%$ and low status was <.599%. Fasting venous blood was analyzed total triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and total cholesterol (TC) concentrations. Each lipid was measured at the 26th gestational week (baseline, study visit 1 {SV1} and the 36th gestational week (study end, study visit 3 {SV3}). For this analysis, change in the lipid concentrations were defined as the absolute change (mg/dl) or the percentage of change (%) in TG, LDL-c, HDL-c, and TC from baseline to study end.

Results: EDHA% was significantly correlated with TG concentrations ($r = +0.338$, $p=0.001$), absolute change in LDL-c ($r = +0.238$, $p = 0.021$), TC concentrations ($r = +0.262$, $p = 0.01$), and change in TC ($r = +0.209$, $p = 0.045$). At SV3, subjects in the high EDHA status group had significantly higher mean TG concentration (low status: 200.65 mg/dL; high status: 245.55 mg/dL; $p=0.001$) and TC concentration (low status: 214.94 mg/dL; high status: 234.22 mg/dL; $p=0.039$).
**Conclusion:** EDHA% in overweight/obese pregnant women was significantly, positively correlated with TG concentration, absolute change in LDL-c, TC concentration, and absolute change in TC. Women in the high EDHA status group had significantly higher TG and TC when compared to women in the low EDHA status group. Further research is needed to determine if these observations are related to changes in outcomes for the mother and infant.
Acknowledgments

I would like to thank my committee chair, Dr. Debra A. Krummel for her outstanding support and guidance as an advisor. I would like to thank Dr. Grace Falciglia for serving on my committee and also for her assistance as a teacher. I would also like to thank the other members of Dr. Krummel’s lab for volunteering their time and effort to help me complete this thesis project.
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INTRODUCTION

The National Institutes of Health (NIH) defines body mass index (BMI) between 25 kg/m$^2$ and 29.9 kg/m$^2$ as overweight and $\geq 30$ kg/m$^2$ as obese. $^1$ Surveillance data from the Pregnancy Risk Assessment Monitoring System demonstrated that the rate of pre-gravid obesity in nine states rose by 69% between 1993-2003 with rates of overall prevalence of 13% in 1993-1994 and 22% in 2002-2003. $^2$ Pre-gravid obesity is associated with an increase in risk for several gestational complications that have underlying metabolic causes and therefore associations with dyslipidemia. $^3$ Hypertension in pregnancy has been linked to dyslipidemia as maternal plasma triglycerides (TG) are independently and positively associated with pregnancy-induced hypertension and preeclampsia. $^4$ A positive association between maternal TG and an increased risk for gestational diabetes mellitus (GDM) has also been reported. $^5$ Finally, an association between maternal TG and an increased risk for large-for-gestational age (LGA) infant has been found. $^6$

Irrespective of body size, lipid concentrations increase throughout gestation. When grouping women by BMI, differences have been observed between women of normal body weight versus those with pregravid overweight/obesity. Several studies support an association between pregravid overweight/obese BMI and higher TG, low-density lipoprotein cholesterol (LDL-c), and lower high-density lipoprotein cholesterol (HDL-c) concentrations. $^6$-$^9$ Interventions that can beneficially modify the intrauterine environment by altering lipid metabolism may help to decrease the rate of complications associated with dyslipidemia.

LITERATURE REVIEW
**Adiposity and pregnancy complications**

Excess maternal adiposity is associated with the development of problems during all stages of gestation. Short-term effects on the mother include gestational hypertension and GDM as hypertriglyceridemia (192.2 mg/dL-255.9 mg/dL) has been reported to be associated with a 3-fold increase risk for developing pre-eclampsia and a 2.8-fold increase in risk for developing GDM. Negative birthing outcomes associated with maternal adiposity that can affect the mother include elective/emergency Cesarean section delivery and postpartum endometritis. Delivery complications associated with maternal adiposity that can affect the baby include shoulder dystocia, polyhydraminos, fetal hypoglycemia, and low apgar score. Complications during pregnancy may also be detrimental to the health of the mother after delivery. Some long-term effects of pregnancy complications associated with maternal adiposity include glucose intolerance, hypertension, metabolic syndrome, and cardiovascular disease (CVD). Similarly, maternal adiposity during pregnancy is associated with negative effects on the long-term health of the baby including neural cord defects, macrosomia, childhood obesity, metabolic syndrome, and CVD.

**ADIPOSI**

Total triglycerides

In the fasting state, a clinical measurement of total TG includes triglyceride molecules that are transported on very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) particles in the blood. While all of these lipoproteins carry TG, the greatest percentage is carried on VLDL, a lipoprotein synthesized in the liver in response to endogenous needs for free fatty acids (FFA). TG in
Chylomicrons and VLDL particles are broken down by lipoprotein lipase (LpL) into FFA, an enzyme that hydrolyzes TGs into chylomicron remnants and intermediate-density lipoproteins (IDL). This is the transport mechanism used to deliver FFA to the fetus.

Maternal hypertriglyceridemia is observed in pregnant women of any size. Increased hepatic TG synthesis is essential for providing FFA to the developing fetus.

In normal, uncomplicated pregnancy, TG-rich VLDL increase from 10-35th week of gestation with median concentrations of large TG-rich VLDL increasing from an average of ~19mg/dL to 109 mg/dL. Pregnancy induced increases in TG are further augmented in overweight/obese pregnant women. Compared to normal-weight mothers, obese pregnant women have significantly higher mean TG concentrations during the third trimester. A study of 150 pregnant mothers found that normal-weight women had a mean TG concentration of 241 mg/dL while obese women had a mean TG concentration of 290.5 mg/dL. Dube et al. speculate that the increase in TG concentrations of obese women is due in part to a non-significant genetic upregulation of LpL and simultaneous inefficiencies of lipid transport in obese pregnant mothers. A Scandinavian study examined data collected from 2001-2008 on pregnant women with a BMI ranging from 17-39. Using univariate regression, TG was found to be positively and significantly associated with placental weight, suggesting that TG is an independent predictor for placental weight.

A positive association between TG and inflammation has been reported in pregnant women. McLachlan et al. reported a significant correlation between TG and TNF-alpha in overweight/obese pregnant women. Mean values for both TG and inflammatory cytokines interleukin-6 (IL-6) and C-reactive protein (CRP) were reported.
to be significantly higher in obese women (TG: 239mg/dL; CRP: 4.45 mg/mL; IL-6: 3.15 pg/mL) when compared to lean women (TG: 192mg/dL; CRP: 2.13 mg/mL; IL-6: 2.1 pg/mL). These differences in lipids and inflammation observed between BMI groups may be linked to differential rates of developing gestational complications, especially pre-eclampsia. Bodnar et al. speculated that a combined effect of increased inflammation and increased TG plays a mediating role in the association between pre-gravid obesity and pre-eclampsia.²²

Insulin has an anabolic role of promoting the uptake of FFA to be stored as TG in adipose tissue.²³ In adipocytes, insulin increases LpL mRNA transcription and therefore enzymatic activity. By doing so, more LpL molecules are able to hydrolyze circulating TG for greater transfer of FFA into adipocytes allowing for enhanced storage.²⁴ Hyperinsulinemia in overweight non-pregnant diabetic adults may lead to a significant decrease in total plasma LpL concentration,²⁵ thus leading to a dyslipidemic condition. If desensitized to insulin, the adipocyte releases FFA which are then combined into TG, creating a hypertriglyceridemic state in non-pregnant adults.

In pregnancy, GDM is associated with significantly increased TG concentrations throughout pregnancy in both normal weight and obese pregnant women.²⁶ Pregnant mothers diagnosed with GDM and with a BMI ranging from normal to obese have significantly higher mean TG concentration (186 mg/dL) when compared to similar weight mothers with uncomplicated pregnancy (150 mg/dL).²⁷ Therefore, regardless of BMI, a pregnant mother diagnosed with GDM may experience elevated TG concentration because of changes in insulin sensitivity.
In the third trimester, normal-weight women can have TG concentrations ranging from 175 mg/dL\textsuperscript{13} to as high as 248 mg/dL\textsuperscript{28}, while overweight women can have TG concentrations ranging from 168 mg/dL\textsuperscript{29} to 220 mg/dL\textsuperscript{8}. In a healthy woman, at any BMI, TG concentration can be as high as 245 mg/dL; however, it usually does not exceed 332 mg/dL in uncomplicated pregnancies.\textsuperscript{30} Overweight hypertensive women have TG concentration around 341 mg/dL in their third trimester of pregnancy.\textsuperscript{8}

Dysbetalipoproteinemia is caused by a mutation in the APOE gene that leads to impaired hepatic uptake of lipoproteins containing apoE. Patients with dysbetalipoproteinemia have TG concentration ranging from 300-1,000 mg/dL.\textsuperscript{31} The current recommendations for severe hypertriglyceridemia in pregnancy are to monitor pregnancies in which the fasting TG concentration exceeds 354 mg/dL and begin therapy when fasting TG concentration exceeds 885 mg/dL.\textsuperscript{32} Therapies for reducing severe hypertriglyceridemia include nutrition (low fat diet, nutritional supplements {such as omega-3 fatty acids (n-3)}), medication (fibrates, niacin based preparations), and infusions (heparin, insulin, and therapeutic exchange).\textsuperscript{32}

TG concentrations in women with pregravid obesity increases the risk for developing preeclampsia or GDM and for having a macrosomic infant.\textsuperscript{10,33} For example, obese pregnant women with TG concentration in the range of 192.2 mg/dL – 255.9 mg/dL were at a 3-fold increased risk for developing preeclampsia and a 2.8-fold increased risk for developing GDM.\textsuperscript{10} In a comprehensive review, Ray \textit{et al}. found that out of 22 studies looking at TG and preeclampsia in pregnant women, 14 reported a significant positive association between TG and risk for developing preeclampsia in pregnant mothers of varying levels of BMI.\textsuperscript{34} Obese pregnant women who gave birth to
macrosomic infants have significantly higher TG concentration (250 mg/dL) in their third trimester when compared to non-obese pregnant women who also gave birth to macrosomic babies (93.8 mg/dL).  

Both eicosapentanoic acid (EPA) and DHA supplementation have been found to reduce TG concentrations from baseline in adult men and women with normal to midly hypertriglyceridemic lipid profiles. DHA is slightly more effective at reducing TG compared to EPA as mean TG reduction by DHA was -22.4 ± 13.3% while the mean TG reduction by EPA was -15.6 ± 12.3%. If supplementation with n-3 provides the benefit of reducing TG concentrations in non-pregnant populations, it is possible that DHA could be used as an intervention to control the excessive dyslipidemia associated with gestational complications seen during obese pregnancy so long as supplementation does not add harm to the woman or baby. Maternal plasma TG are transported to the placenta in lipoproteins that are broken down by lipases on the placental membrane. LpL and other lipases hydrolyze TG in lipoproteins at the maternal placental surface to allow FFA to be taken up by the fetal circulation. Currently, the use of TG reducing therapy for pregnant mothers with fasting TG < 885 mg/dL is not advised.

N-3 PUFA are recommended as adjunctive therapy for hypertriglyceridemia non-pregnant adults. After 8 weeks of supplementation, a dose of 3.4 g/day of fish oil, which is a very concentrated source of EPA and DHA significantly lowered TG concentrations in a cross over study of overweight/obese adults. Even lower doses, 2 g/day algal DHA for longer periods (4.5 months) significantly decreased VLDL-TG (p = 0.009) and TG (p = 0.006) in a randomized, controlled trial in overweight and obese adults. Harris et al. speculate that DHA may reduce plasma TG by reducing hepatic
synthesis, promoting fatty acid degradation, and inducting TG clearance out of the body. If these positive effects are seen in non-pregnant obese adults with high TG, perhaps they can be replicated in pregnant obese adults with high TG concentration as well. Not all studies have found the TG lowering effect of fish oil.  

In the United States, the intake of EPA and DHA is suboptimal. Therefore, most epidemiological investigations of the association of EPA and DHA with lipids and disease biomarkers focus on lower erythrocyte eicosapentanoic acid (EEPA) and EDHA concentrations as a marker of higher risk. In contrast, Makhoul et al. examined higher EPA and DHA status in a sample of Yup’ik Eskimos with a traditional diet that is high in these n-3 PUFA. The upper quartile of EEPA and EDHA was 3.9% and 8.2%, respectively. At these concentrations, the association between obesity and plasma TG was attenuated. Therefore, examining TG concentrations as a function of DHA status in obese pregnant women may be beneficial.

**LDL-c**  
LDL serve as a vessel for hydrophobic cholesterol to be transported in the blood from the liver to other tissues in the body. During pregnancy, increased LDL-c is caused by the maternal metabolism responding to the nutritional demand of the fetus. The fetus relies on maternal FFA and cholesterol in LDL particles throughout gestation for maximal growth. Healthy non-obese pregnant women have significantly higher LDL-c concentrations when compared to their non-pregnant counterparts (an average difference of 34.03 mg/dL). Husain et al. explained that this difference is likely the result of differential hormone status during pregnancy. Normal weight pregnant mothers in their third trimester of pregnancy have LDL-c concentration around 142.15
mg/dL. Overweight and obese pregnant women in their third trimester of pregnancy have LDL-c concentration ranging from 119.8-181.7 mg/dL. The rate of change in LDL-c concentration may also be of importance as other studies have found contrary results. Overweight and obese pregnant women were reported to have significantly lower LDL-c concentrations (mean difference of 17.7 mg/dL) compared to normal weight counterparts.

The relationship between and inflammation during pregnancy is less cause-effect and more of a cycle in which both increased LDL-c and augmented inflammatory statuses are perpetuated. Pregnant women have higher concentration of oxidized LDL-c when compared to non-pregnant women. As LDL-c concentration increases during dyslipidemic pregnancy, the potential for oxidized LDL-c to accumulate becomes an inflammatory concern. Oxidized LDL-c plays a role in several pro-inflammatory pathways including toll-like receptor 4 (TLR4), part of the innate immune system involved in pathogen recognition.

The relationship between insulin and LDL-c is complex. It is difficult to say with certainty if insulin resistance is causing an increase in LDL-c concentrations or if pro-inflammatory pathways modified by LDL-c are changing insulin sensitivity. Until further evidence is provided, conclusions must be drawn from associations between the two factors. In non pregnant studies, the accumulation of small, dense LDL-c particles is characteristic of insulin resistance in both healthy subjects and those with type 2 diabetes. Insulin resistance was reported to be significantly correlated with LDL-c concentrations in a population of obese non-pregnant women (Spearman coefficient
r=+0.044 and p=0.043 between LDL-c and homeostasis model assessment-insulin resistance \{HOMA-IR\}.  

Normal weight pregnant women have LDL-c concentration of 142 mg/dL between the 24th-36th weeks of pregnancy. In healthy pregnancy, LDL-c concentration during the third trimester can reach up to 353 mg/dL in uncomplicated pregnancy. Obese pregnant mothers (mean prepregnancy BMI 31) have LDL-c concentrations of 150.8 mg/dL, not significantly different when compared to normal-weight, pregnant controls. In instances of familial hyperlipoproteinemia, LDL-c concentration can reach as high as 332 mg/dL during the 36th week of pregnancy.

Elevated LDL-c concentrations are linked to an increased risk in pregnancy complications. In nulliparous women, the development of preeclampsia was reported to be associated with higher LDL-c concentrations after adjusting for BMI as determined by an adjusted odds ratio of 3.74 (95% CI 3.65-3.84). Obese pregnant mothers who gave birth to macrosomic infants had a mean LDL-c concentration of 223.5 mg/dL during their third trimester, significantly different from 3 other groups in the study; obese mothers who gave birth to normal weight babies, normal weight mothers who gave birth to normal weight babies, and normal weight mothers who gave birth to macrosomic babies. Increased LDL-c concentration in normal weight mothers may not be as problematic. Normal weight pregnant mothers who experienced preterm labor had significantly lower LDL-c concentrations compare to controls with healthy pregnancies (mean LDL-c for preterm labor: 125 mg/dL, healthy controls: 142 mg/dL).

However, further investigation suggests that the increases in LDL-c brought on by increased fish oil intake may be more benign than previously thought. Algal DHA
supplementation of 2 g/day for 4.5 months increases not only the number but also the size of LDL particles when compared to a corn oil placebo group. Larger LDL particles are better substrates for LpL, thereby enhancing TG clearance and creating a more optimal lipid profile.  

**HDL-c**

Maternal HDL-c particles carry cholesterol to the developing fetus. Cholesterol from HDL is used for the development of adrenal glands, sexual organs and steroidogenesis. Liver development is dependent on endogenous cholesterol transported in HDL-c. Intrauterine fluctuations in HDL-c concentration coincide with growth patterns of the fetus. There is a decrease in placental HDL-c as the fetus approaches the 40th week of gestation because the cholesterol carried on the HDL particle is transferred to the fetus at high rates. In normal weight pregnant women, mean HDL-c concentration are around 68.32 mg/dL while overweight/obese pregnant women have mean HDL-c concentration around 61 mg/dL. Maternal HDL-c is negatively and significantly associated with placental weight because of HDL’s role in reverse cholesterol transport.

In animal models and non-pregnant populations, a link between HDL-c and insulin resistance is widely accepted. *In vitro* analysis of human and mouse blood has found that HDL-c has a protective effect on pancreatic beta-cells, inhibiting their apoptosis. Obese, non pregnant women with HDL-c concentrations lower than 50 mg/dL are at a higher risk for developing type 2 diabetes and decreased HDL particle size, suggesting a more atherogenic lipid profile with lower concentrations of HDL-c. In this study, the correlation between HDL particle diameter and concentration was
r=0.606, p<0.001. The mean HOMA-IR for subjects with HDL-c<50mg/dL was 4.5, significantly higher than the high HDL-c group which had a mean HOMA-IR of 3.2. Whether or not HDL-c concentrations during pregnancy are linked to impaired insulin resistance is still up for debate. Harville, et al. reported nearly no difference in the risk of developing GDM when comparing pregnant women in the bottom 10\textsuperscript{th} percentile for HDL-c concentrations to all other subjects, regardless of BMI. Of women diagnosed with GDM, the women who also had type 2 diabetes were found to have significantly lower HDL-c concentrations (mean HDL-c for type 2 diabetics with GDM: 36.9 mg/dL than pregnant mothers who otherwise have normal glucose metabolism (mean HDL-c for healthy controls: 44.8 mg/dL). Although it should be noted that the type 2 diabetic mothers had a mean BMI of 29 while the controls had a mean BMI of 24.

Normal weight pregnant mothers with uncomplicated pregnancies have HDL-c concentration around 68 mg/dL and some studies have found normal weight mothers with very low HDL-c concentrations of 58 mg/dL during the 25\textsuperscript{th} week of pregnancy or even 50 mg/dL during the 30\textsuperscript{th} week of pregnancy. Overweight women have HDL-c concentrations around 61 mg/dL during their third trimester of pregnancy. This value is strikingly similar to mean HDL-c concentrations of pregnant women with familial hyperlipoproteinemia (61.8 mg/dL).

Obese pregnant mothers who already have lower concentration of HDL-c before pregnancy are at risk of developing preeclampsia, macrosomia, and diabetes later in life. In pregnant women with a history of GDM, those with HDL-c lower than 50 mg/dL developed diabetes later in life at 3 times the rate at which higher HDL-c mothers developed the disease (hazard ratio 2.88;95% CI 1.27-6.67).
The use of DHA as an intervention to increase HDL-c concentrations has worked in both non-pregnant and pregnant populations. Non-pregnant, overweight adults supplemented with 2 g algal DHA/day for 4.5 months experienced increased serum HDL-c concentration higher than that achieved by a placebo group the same dose of corn oil, 43 mg/dL in the DHA group vs. 38 mg/dL in the placebo group. Furthermore, healthy pregnant women supplemented with 10 mL cod liver oil/day from their first trimester through delivery experienced higher concentrations of HDL-c when compared to a group supplemented with corn-oil, 73 mg/dL in the cod liver oil group vs. 65 mg/dL in the placebo group.

Purpose

The purpose of this thesis project is to investigate the effect of achieved DHA status on blood lipids overweight/obese pregnant mothers, 18-40 years of age.

Research Question

Does achieved DHA status affect plasma concentrations of blood lipids? Is there a relationship between EDHA% and lipid biomarkers?
Null Hypothesis

- There is no relationship between EDHA% and blood lipid measurements defined as TG, LDL-c, HDL-c, and TC.
- There is no relationship between EDHA% and change measurements in blood lipids defined as change in TG, percent change in TG, change in LDL-c, percent change in LDL-c, change in HDL-c, percent change in HDL-c, change in TC, and percent change in TC.
- There is no significant difference in mean blood lipid measurements between EDHA status groups. Mean blood lipid measurements are defined as TG, LDL-c, HDL-c, and TC.
- There is no significant difference in mean change of blood lipid measurements between EDHA status groups. Blood lipid change variables defined as change in TG, percent change in TG, change in LDL-c, percent change in LDL-c, change in HDL-c, percent change in HDL-c, change in TC, and percent change in TC.

METHODS

This project was a sub-study of the randomized, double-blinded, placebo-controlled trial, “DHA, Inflammation, and Insulin Sensitivity in Obese, Pregnant Women” (National Institute of Health [5R21HL093532, UL1RR026314-03], Mead Johnson Nutritionals, and DSM Nutritional Products. Debra Krummel, PhD, RD and Theresa Powell, PhD are co-principal investigators in Cincinnati and San Antonio, respectively. The study protocol was approved by the Institutional Review Boards at the
University of Cincinnati, Cincinnati Children’s Hospital Medical Center, and the University of Texas Health Sciences Center at San Antonio.

**Subjects**

Subjects of this sub-study were part of the larger study conducted in Cincinnati, Ohio and San Antonio, Texas. Healthy gravidas between the ages of 18–40 years, with a singleton pregnancy, a BMI $\geq 25 \text{ kg/m}^2$, and who had complete baby data, were included for analysis.

**Study Visits**

The study visits occurred at the General Clinical Research Center located at the Cincinnati Children’s Hospital Medical Center or the University of Texas Health Sciences Center at San Antonio. Three study visits (SV) were conducted in the third trimester; the first during the 26$^{\text{th}}$ week of pregnancy (SV1) and the third during the 36$^{\text{th}}$-37$^{\text{th}}$ week of gestation (SV3).

Before the initiation of the first study visit, participants completed informed consent and Health Insurance Portability and Accountability Act (HIPAA) forms. Thereafter, they were enrolled in the study. Randomization occurred in the pharmacy from which DHA or placebo capsules were dispensed. DHA (800 mg form odorless, flavorless, algal oil) or placebo (soybean blend with no DHA) capsules were identical in shape, color, and flavor (orange). Four capsules were to be consumed daily.

The GCRC staff completed height, weight, and venous blood collection using standardized protocols to obtain fasting cholesterol and EDHA concentrations. Participants received a 45-day supply of DHA or placebo capsules. Adherence to consumption was assessed by a (1) telephone call conducted by study staff at 2 weeks
after the study visit, (2) supplement tracking tool, and (3) measurement of EDHA. No adverse effects were reported.

**Statistical Analysis**

For this project, statistical significance was set as a p value of <.05. Values are given as mean ± standard deviation. The relationship between DHA status and lipid concentrations was examined and the lipid variables assessed at study visit 3 (SV3) were as follows: TG, LDL-c, HDL-c, and TC. Change variables we calculated as the measurement at SV3 minus the measurement at SV1. Percent change variables were also calculated as the change in the measurement from study visit 1 to 3 divided by the measurement at study visit 1 and then multiplied by 100. All variables were tested for normality. The following variables were square root transformed to obtain normal distribution: TG at SV3, change in TG, LDL-c at SV3, percent change in LDL-c, HDL-c at SV3.

When normality was violated, nonparametric tests were used. When normality was not violated, the following parametric tests were used. Pearson’s bivariate correlations were used to identify the strength and direction of the linear relationship between EDHA and the lipid measurements. Change and percent change variables were measured against EDHA at SV3 for all Pearson correlations. One-way analysis of variance (ANOVA) was used to determine if lipids varied by DHA status. Chi-square test between EDHA status groups vs. treatment group was used and significance <0.05.

Data analysis was performed through the use of the statistical software, Statistical Package for the Social Sciences (version 20.0, 2012, SPSS, Inc. Chicago, IL).
Demographic data (including age, education, race/ethnicity) were self-reported using a survey instrument.

**Laboratory methods**

**Erythrocyte DHA**

At each study visit, 2 mL of blood was collected into an EDTA-coated vacutainer tube and placed on ice. Samples were centrifuged for 20 min (3000 x g @ 4°C), the plasma was removed, and erythrocytes were washed three times in NaCl. Fatty acid compositions were determined using methylation and saponification methods. Capillary gas chromatograph Shimadzu-GC201 and helium carrier gas were used to perform this analysis. The identification of FFA was performed using the retention times of authenticated fatty acid standards.

**Blood lipids**

At the first and third study visits, the blood samples were also tested for TC, high-density lipoprotein, and TG using a Siemens Dimension RxL Max Chemistry Analyzer and standardized protocols. The concentration of LDL-c was then calculated using the Friedewald equation as follows (LDL-c: low-lipoprotein cholesterol, TC: total cholesterol, HDL-c: high-density lipoprotein cholesterol, TG: total triglycerides): \[ LDL-c \approx TC - HDL-c - \frac{TG}{5} \]
RESULTS

EDHA Status

In our study population, 62.36% of women were in the high status versus low status group, respectively women who were in the treatment group were more likely to have high EDHA status ($X^2 = 27.35, p < 0.05$). 72% of women with high EDHA status were in the DHA treatment group while 27% were in the placebo group. Conversely, 82% of women with low EDHA status were in the placebo group while 17.1% were in the DHA treatment group. Women in the high status group were older than those in the low status group ($p = 0.006$) but otherwise, there was no significant difference in characteristics at baseline between groups (Table 1). More Hispanic women were likely to be in the high status group ($X^2 = 6.00, p = 0.047$) with Hispanic women making up 54% of the high EDHA status group while African American and White were 27% and 18% respectively.

**Table 1.** Characteristics of women at baseline

<table>
<thead>
<tr>
<th></th>
<th>EDHA$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Status$^2$</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Pregravid BMI$^4$</td>
<td>35.82</td>
</tr>
<tr>
<td>Pregravid weight lbs.</td>
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</tr>
<tr>
<td>Age (years) **</td>
<td>26</td>
</tr>
<tr>
<td>Education</td>
<td>13.1</td>
</tr>
</tbody>
</table>
| Demographic data of women. $^1$EDHA status is defined as EDHA higher or lower than 6% at SV3. $^2$Low status is EDHA <6%. $^3$High status is EDHA $\geq$6% $^4$BMI calculated as mass (kg) divided by height (m) squared. $^*$Significantly
more Hispanic women in the high EDHA status group. Values significantly different between groups at *p<0.05,  
**p<0.01, ***p<0.001

Table 2. Mean values of plasma lipids grouped by EDHA status

<table>
<thead>
<tr>
<th></th>
<th>Low status^2</th>
<th>High Status^3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>TG SV3 (mg/dl)</td>
<td>183.51</td>
<td>68.29</td>
<td>232.10</td>
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<tr>
<td>TG change (mg/dL)</td>
<td>35.80</td>
<td>41.96</td>
<td>47.07</td>
</tr>
<tr>
<td>TG percent change</td>
<td>30.34</td>
<td>35.62</td>
<td>27.91</td>
</tr>
<tr>
<td>LDL-c SV3 (mg/dl)</td>
<td>119.09</td>
<td>38.18</td>
<td>127.69</td>
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<td>LDL- change (mg/dL)</td>
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<td>HDL-c SV3 (mg/dL)</td>
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<td>HDL- change (mg/dL)</td>
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<td>HDL-c percent change</td>
<td>-3.80</td>
<td>10.84</td>
<td>-6.35</td>
</tr>
<tr>
<td>TC SV3 (mg/dL)</td>
<td>215</td>
<td>46</td>
<td>234</td>
</tr>
<tr>
<td>TC change (mg/dL)</td>
<td>9.17</td>
<td>23.53</td>
<td>11.67</td>
</tr>
<tr>
<td>TC percent change</td>
<td>4.36</td>
<td>10.54</td>
<td>5.69</td>
</tr>
</tbody>
</table>

Mean values and standard deviations for plasma lipids and change measurements divided by EDHA status group.

^1EDHA status is defined as EDHA higher or lower than 6% at SV3. ^2Low status is EDHA < 5.99%. ^3High status is EDHA > 6%. NOTE; acronyms are as follows: triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and total cholesterol (TC). Change variables were calculated as SV3 – SV1 and percent change variables were calculated as (change variable/SV1)*100.
relationship between EDHA% and any of the TG change variables. There was a significant difference between mean TG at SV3 between DHA status groups. The mean value for the low status group was 13.33 ± 2.41 and the mean value for the high status group was 15.06 ± 2.32, p = 0.001 (Figure 2). TG change variables did not vary by EDHA status group.

Figure 1. Pearson correlation between EDHA (%) and TG concentrations measured at SV3. Correlation r = +0.338 and significance p = 0.001
Figure 2. Mean triglyceride concentrations of women divided by EDHA status group. EDHA status is defined as EDHA higher or lower than 6% at SV3. Low status is EDHA <6%. High status is EDHA ≥6%.

Error bars are 95% confidence interval. Values significantly different between groups at *p<0.05, **p<0.01, ***p<0.001.

**LDL-c**

There was a significant positive correlation between EDHA% and the change in LDL-c (Figure 3). The strength of the correlation is small to moderate (Table 3). There was no relationship between EDHA% and any of the other LDL-c measurements. LDL-c and LDL-c change variables did not vary by EDHA status group.
Figure 3. Pearson correlation between EDHA measured at SV3 and change in LDL-c calculated as SV3-SV1 (mg/dL). Correlation $r = +0.238$ and significance $p = 0.021$

**HDL-c**

There was no relationship between EDHA% and any of the HDL-c measurements. HDL-c concentration and HDL-c change variables did not vary by EDHA status group.

**Total Cholesterol**

There was a significant positive correlation between EDHA% and TC measured at SV3 (Figure 4). The strength of the correlation is defined as small to moderate (Table
There was also a significant positive correlation between EDHA% and the change in TC from SV1 to SV3 (Figure 5). The strength of the correlation is defined as small to moderate (Table 3). There was a significant difference in the mean TC at SV3 between EDHA status groups. The mean value in the low status group was 214.94 ± 45.98 mg/dL and the mean value in the high status group was 234.22 ± 41.08 mg/dL, p = 0.039 (Figure 6).

Figure 4. Pearson correlation between EDHA and TC at SV3. Correlation r = +0.262 and significance p = 0.011
**Figure 5.** Pearson correlation between EDHA and change in TC calculated as SV3-SV1 (mg/dL). Correlation $r = +2.09$ and significance $p = 0.045$
Figure 6. Mean TC concentrations (mg/dL) of women divided by status group. EDHA status is defined as EDHA higher or lower than 6% at SV3. Low status is EDHA <6%. High status is EDHA ≥6%. Error bars are 95% confidence interval. Values significantly different between groups at *p<0.05, **p<0.01, ***p<0.001
Table 3. Pearson correlations between EDHA and plasma lipids and change measurements

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG SV3 mg/dl</td>
<td>+0.338</td>
<td>0.001</td>
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<tr>
<td>TG change mg/dL</td>
<td>+0.021</td>
<td>0.838</td>
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<td>TG percent change</td>
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<td>0.237</td>
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<tr>
<td>LDL-c SV3 mg/dL</td>
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<tr>
<td>LDL- change mg/dL</td>
<td>+0.238</td>
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<td>LDL-c percent change</td>
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<td>HDL-c SV3 mg/dL</td>
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<td>HDL- change mg/dL</td>
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</tr>
<tr>
<td>HDL-c percent change</td>
<td>+0.044</td>
<td>0.678</td>
</tr>
<tr>
<td>TC SV3 mg/dL</td>
<td>+0.262</td>
<td>0.011</td>
</tr>
<tr>
<td>TC change mg/dL</td>
<td>+0.209</td>
<td>0.045</td>
</tr>
<tr>
<td>TC percent change</td>
<td>+0.195</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Correlations for EDHA% and plasma lipids. N=93. TG: total triglycerides, LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, TC: total cholesterol. Change variables were calculated as the difference in concentration between SV1 and SV3. Percent change variables were calculated as the change divided by the concentration at SV1 multiplied by 100.

DISCUSSION

In our study, women with higher EDHA levels were more likely to be in the supplementation group and women with lower EDHA levels were more likely to be in the low status group. The Pearson X² test verified that this is not an absolute assumption, justifying the division of subjects by EDHA status rather than treatment group.

We observed a positive, moderately strong, significant correlation between TG concentrations and EDHA%. To date, no other study examines EDHA% and TG concentrations in overweight/obese pregnant women. Both Browning et al. and Ottestad et al. have shown that increased EPA and DHA consumption will increase the
incorporation of n-3 FA into phospholipids and TG.\textsuperscript{56,57} Ottestad goes on to speculate that lipid remodeling brought on by increased EPA and DHA consumption may modify the functionality of phospholipids and TG, explain some of the beneficial effects of consuming fish oils.\textsuperscript{57} Further studies on the effects of lipid remodeling are needed but it is possible that our observed increase in TG with EDHA\% was accompanied by increased integration of DHA into phospholipids and TG molecules, allowing for enhanced signaling in lipid metabolism pathways.

Most previous studies look at supplementation vs. control group instead of EDHA status groups. A systematic review of randomized controlled trials performed by Lopez-Huertas \textit{et al.} found that studies providing fish oil supplements $>1$ g/day for over three months results in a significant decrease in plasma TG of non-pregnant adults diagnosed with metabolic syndrome.\textsuperscript{58}

The high DHA status group also had significantly higher mean TG concentrations when compared to the low status group. While this difference may seem detrimental, a study of Yup’ik Eskimos found that the association between obesity and TG is attenuated in populations experiencing higher EDHA concentrations and a lower risk of developing chronic disease.\textsuperscript{40} Fish oil supplementation (up to 8 g/day) is associated with increased DHA integration into TG molecules in adults and may also lead to beneficial modulation of lipid biomarkers and lipid metabolism.\textsuperscript{57} Contrary to these results, the Salmon in Pregnancy Study looked at the effect of increased fish-oil consumption (2 portions of salmon/week, 150 g/portion, average 2.3 g DHA/day for 20 weeks) on lipid biomarkers (TG, TC, HDL-c, LDL-c) in women from the Southampton, UK area (BMI: 25). While TG and LDL-c increased and HDL-c decreased through pregnancy, there was no effect of
fish consumption on lipids measured. Further studies on EDHA and TG concentrations in pregnancy are needed as the current literature is inconclusive.

There was a significant positive correlation between EDHA% and change in LDL-c from SV1 to SV3. A positive correlation between the change in LDL-c and EDHA% indicates that as a subject’s erythrocytes incorporate more DHA, they are likely to have a larger increase in LDL-c. Fish oil’s role in increasing LDL-c concentrations in unequivocally supported by the current literature.\textsuperscript{35, 58, 60} Higher doses, > 3 fish oil g/day may lead to significant increases in LDL-c similar to our findings.\textsuperscript{58} During pregnancy, excessive elevation of LDL-c may seem problematic considering the increased risks for gestational complications associated with increased LDL-c but recent studies have shown that the increase in LDL-c concentration associated to increased DHA is also accompanied by increases in LDL particle size resulting in larger LDL particles, thus improving a subject’s lipid profile by enhancing TG clearance as larger LDL particles are better substrates for LpL.\textsuperscript{37}

There were no significant correlations between EDHA% and HDL-c measurements, nor were there any significant differences between DHA status group and any of the HDL-c measurements. This data is in opposition to what is currently reported in the literature. Some studies have found that fish oil consumption is effective at increasing HDL-c in both pregnant and non-pregnant populations.\textsuperscript{37, 54}

There was a significant positive correlation between EDHA% and both TC at SV3 and change in TC. These correlations may be the products of non-significant positive correlations between EDHA% and both high and low-density lipoprotein concentrations. Non-significant increases in all lipoprotein particles collectively add up to a significant
increase in TC with increased EDHA integration. The high EDHA status group had significantly higher mean TC concentrations when compared to the low EDHA status group. The current literature is mixed on the effect of fish oil supplementation in adults as some studies have shown that fish oil will decrease the TC/HDL-c ratio and TC, 54 other studies have shown that fish oil will increase TC. 37 The higher TC concentration that we observed may lead to the development of gestational disease. Bartha et al. reported that a combined effect of low TC concentration and high TC/HDL-c ratio are associated with inflammation and subsequent spontaneous preterm labor, suggesting that higher TC concentrations without increases in HDL-c may be linked to the pathogenesis of this gestational complication. 13 Because of estrogen’s known effect of increasing HDL-c and LDL-c, 61, 62 the literature does not focus on fish oil supplementation and TC changes during pregnancy as estrogen may play a confounding role.

Our study was not without limitations. We did not examine lipoprotein particle diameter. As previous studies have shown, the increase in lipoprotein concentration may be less harmful if the size of LDL-c and VLDL particles are large. 37 Having particle size data would provide would have provided more information about how EDHA affects lipid metabolism but we did not have access to such techniques. We also had an issue in differential methodologies between the Cincinnati and San Antonio laboratories that may have lead to a higher number of Hispanic subjects in the higher EDHA status group.

In summary, EDHA% in overweight/obese pregnant women was significantly, positively correlated with TG concentration, absolute change in LDL-c, TC concentration, and absolute change in TC. Women in the high EDHA status group had significantly higher TG and TC when compared to women in the low EDHA status
group. Further research is needed to determine if these observations are related to changes in outcomes for the mother and infant.
REFERENCES


