University of Cincinnati

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I, Loren P Brook, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
The effect of DHA supplementation on inflammatory biomarkers in overweight/obese pregnant women of different ethnic groups

Student's name: Lorenzo P. Brook

This work and its defense approved by:

Committee chair: Debra Ann Krummel, PhD

Committee member: Graciela Falciglia, PhD

University of Cincinnati
The effect of DHA supplementation on inflammatory biomarkers in overweight/obese pregnant women of different ethnic groups

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

7/10/2012

Loren P. Brook, BS The Ohio State University 2008

Committee Chair: Debra A. Krummel, PhD, RD
ABSTRACT

Objective: To examine the relationship between erythrocyte docosahexaenoic acid (DHA) and inflammatory biomarkers in the 35th-37th week of gestation in healthy overweight/obese pregnant women 18-40 years of age of different races/ethnicities (African American, Hispanic, White), following 10 weeks of supplementation with or 530 mg corn/soybean oil blend.

Design: Randomized, double-blinded, placebo-controlled trial.

Subjects: 107 healthy gravidas between the ages of 18-40 years with a singleton pregnancy, body mass index (BMI) ≥25, and who completed all study visits.

Methods: Pregnant women were randomized into either the DHA or control group. Subjects were given either 800 mg purified algae docosahexaenoic or 530 mg corn/soybean oil blend beginning at the 26th week until the 35th-37th week of their pregnancy. Venous blood was collected during three study visits and analyzed for erythrocyte DHA, adiponectin, IL-6 and TNF-α. Outcome variables were assessed as the differences in the absolute measurement (values) and percent change (%) between baseline and study end measurements. Mean values are reported for normally distributed data and median values with interquartile ratios were reported for skewed data. One-way ANOVA was used to compare means by study group. The Kruska-Wallis Test was used as a non-parametric alternative to a one-way ANOVA. Two-way ANOVA was used to
identify effects between inflammatory biomarkers (TNF-\(\alpha\), IL-6, adiponectin), and ethnicity between study groups.

Results: Women supplemented with DHA had significantly higher erythrocyte DHA as compared to the control group. There was a significant interaction between the study group and ethnic background on change in erythrocyte DHA, and blood levels of TNF-\(\alpha\) (p<0.03, p<0.05 respectively). Hispanic and White women had an increase and African-American women a decrease in erythrocyte DHA following supplementation. In the control group, the African-American and White women had a slight increase in TNF-\(\alpha\) (0.92 pg/ml, 1.2 pg/ml, respectively) and the Hispanic women had a slight decrease (-0.36). In the DHA group, all women had less than a 0.25 pg/ml increase in TNF-\(\alpha\). When experimental groups were divided based on race/ethnicity, the median level of absolute change in blood levels of adiponectin, IL-6, and TNF-\(\alpha\) were different between the three groups (p<0.05, p<0.01, p<0.001 respectively).

Conclusion: DHA supplementation has varying effects on inflammatory biomarkers in healthy overweight/obese pregnant women of different races/ethnicities. Ethnicity and DHA supplementation have interacting effects on both erythrocyte DHA and blood TNF-\(\alpha\) levels. Modifying DHA supplementation based on race/ethnicity may lower inflammatory status in pregnancy and improve negative fetal outcomes.
Acknowledgements

I would like to thank my committee chair, Dr. Debra Krummel for all her help and time. She is a wonderful mentor and teacher. I would like to thank Dr. Couch and Falciglia for serving on my committee. I would also like to thank the members of my lab for giving up their time to work with me and help me complete this project.
## Contents

Abstract .................................................................................................................. ii

Acknowledgements ................................................................................................. v

List of Tables .......................................................................................................... vii

List of Figures .......................................................................................................... vii

Introduction .............................................................................................................. 1

Literature Review .................................................................................................... 3

Purpose ..................................................................................................................... 9

Null Hypotheses ...................................................................................................... 10

Methods .................................................................................................................... 11

Statistical Analyses ................................................................................................. 13

Results ...................................................................................................................... 14

Discussion/Conclusion ............................................................................................ 21

References ............................................................................................................... 26
List of Tables

1. Baseline demographic characteristics of women
2. Baseline demographic characteristics of women by race/ethnicity
3. Erythrocyte DHA level before and after 800 mg DHA supplementation
4. Effect of DHA supplementation on erythrocyte DHA
5. Change in levels of inflammatory markers between baseline and study end
6. Effect of DHA supplementation on adiponectin
7. Effect of DHA supplementation on IL-6
8. Effect of DHA supplementation on TNF-α

List of Figures

1. Means of the change in absolute erythrocyte DHA levels
2. Mean change in erythrocyte DHA level by ethnicity
3. Means of the change in absolute in blood TNF-α levels
4. Mean change in TNF-α blood levels
Introduction

The worldwide prevalence of obesity has reached epidemic levels. In the United States, 33% of the total population is considered overweight/obese and women of reproductive age are similarly affected. Early research done in the Pregnancy Risk Assessment Monitoring System (PRAMS) between 1993-2003 demonstrated that rate of obesity, defined as a body mass index (BMI) ≥29.0 kg/m², rose by a total 69%, with rates of 13% in 1993-1994 and 22% in 2002-2003.¹ Later data collected in the National Health and Nutrition Examination Survey (NHANES 2000-2004), demonstrated that the number of women who are overweight or obese has been increasing for the decade. While the incidence has leveled off in recent years, 36% of women were categorized as overweight/obese in 2010. ²

As women increase their girth, they also are at increased risk for developing metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease; all of which have an underlying inflammatory process. These negative outcomes affect women who are overweight or obese before, during, and after they become pregnant. Pregravid obesity can result in serious consequences for both the mother and her developing fetus. These problems include, but are not limited to: gestational diabetes, hyperinsulinemia, cesarean delivery, gestational hypertension, pre-eclampsia in the mother and macrosomia, stillbirth and congenital anomalies in the infant. It is imperative to find interventions that can prevent these consequences for the health and survival of both the mother and child. ³-⁵

Evidence has been accumulating regarding the role of n-3 fatty acids in the prevention and management of cardiovascular disease (CVD) that is associated with
long-term obesity. In a recent review, it was found that n-3 supplementation of around 1 g/day can reduce all cause mortality, cardiac and sudden death, and stroke. This data suggests that n-3 supplementation is preventive for heart health and one mechanism to reducing the level of inflammation throughout the human body.

In non-pregnant women, obesity is described as a low-grade inflammatory condition associated with an increased production of pro-inflammatory cytokines that are released from the macrophages residing in adipose tissue. Macrophages play a significant role in inflammation during pregnancy as well. Recent evidence has demonstrated the positive effects of DHA on decreasing inflammation in both overweight/obese non-pregnant, and pregnant females. With evidence accumulating in both animal models and in obese non-pregnant humans, DHA supplementation is a prime candidate to counteract inflammation in obese/overweight pregnant women. It may also prevent negative birth outcomes this population.

The purpose of this thesis was to determine if supplementing overweight/obese pregnant women with DHA will affect inflammatory biomarkers and if there are racial/ethnic differences in response.
Literature Review

Overweight/obesity and its effects on pregnancy-related complications. While it is important to gain the appropriate amount of weight during pregnancy, most pre-pregnant overweight/obese women increase their girth and develop weight-related pregnancy complications throughout gestation. In women who have a BMI considered underweight (<18.5), the main risk is small-for-gestational age (growth restricted) fetuses. When considering pregnant women with pre-gravid obesity, they are more likely to develop gestational diabetes and hypertension during pregnancy, along with fetal complications such as large-for-gestational age infants. Recent data showed that as waist circumference (an alternate method for measuring subcutaneous/visceral body fat) increases, so does the risk of developing gestational diabetes, preeclampsia, and fetal macrosomia.\textsuperscript{3-5,10-15} Hincz et al. examined the effect of maternal obesity on and demonstrated that obesity was positively associated with hypertension during pregnancy, either nonproteinuric or preeclampsia. Obese women had significantly higher blood glucose during pregnancy (gestational diabetes) and emergency and elective C-section delivery.\textsuperscript{3}

Steinfeld et al. looked at pregnancy complications (chronic hypertension, preeclampsia, pre-gestational diabetes, gestational diabetes, fetal macrosomia, C-section delivery, operative delivery, endometritis) by race/ethnicity (Caucasian, African American, and Hispanic) Hispanic women had a significantly increased risk of gestational diabetes and fetal macrosomia, with trend towards decrease incidence of preeclampsia.\textsuperscript{16} Singh et al. compared fetal outcomes between Caucasians and African Americans.\textsuperscript{17} They found that African-American women's neonates had significantly lower birth weights (LBW) and
less lean body mass, but no difference in fat mass as compared to matched Caucasians. The higher risk of LBW infants from African-American mothers has been reported previously.\textsuperscript{15} Anglo and Hispanic women have similar rates of LBW deliveries.\textsuperscript{17}

\textit{Pregnancy, maternal obesity and inflammation.} There are multiple theories about the effects that pregnancy has on inflammation in the human body. The prevailing hypothesis, describes pregnancy as a “TH-2 state,” suggesting that it is an anti-inflammatory condition.\textsuperscript{18} Recent evidence suggests that there are three distinct inflammatory phases.\textsuperscript{18} Early pregnancy is pro-inflammatory with high levels of cytokines, “comparable to an open wound.” The second state, during rapid fetal growth and development, is categorized as anti-inflammatory. In the last state, late pregnancy, inflammation once again predominates. This late pro-inflammatory environment promotes parturition, contraction of the uterus, and delivery of the baby and placenta.

Evidence has been accumulating for years that an increase in obesity in non-pregnant women results in increasing levels of adiposity and as a result, higher levels of inflammation. Adipose tissue can recruit macrophages which produce high levels of pro-inflammatory cytokines, including, but not limited to: tumor necrosis factor (TNF)-a, interleukin (IL)-6, transforming growth factor (TGF)-b, and pro-coagulant proteins such as plasminogen activator inhibitor type 1, tissue factor and factor VII. When adipose tissue is excessive, these pro-inflammatory cytokines can have negative affects throughout the body. Within the placenta, there is an infiltration of these pro-inflammatory macrophages, which express the same pro-inflammatory cytokines, along
with IL-1. Madan et al. reported an increase in both leptin and C-reactive protein with increasing pre-pregnant BMI, but did not find any relationship between TNF-alpha and BMI.\textsuperscript{18} This is in contradiction to Winkler et al. and Gao et al. who reported a positive correlation between TNF-a and BMI in pregnancy.\textsuperscript{18}

*IL-6 and TNF-alpha are major inflammatory biomarkers.* Interleukin 6 (IL-6) is a 26 kDa protein secreted by T-lymphocytes, macrophages, endothelial cells, subcutaneous adipose tissue, and the placenta to stimulate a cascade of immune responses. It increases the synthesis of many, if not all, of the acute phase reactants: C-reactive protein, serum amyloid A, fibrinogen, α1-chymotyrpsin, and haptoglobin. IL-6 levels are directly correlated with BMI and percent body fat in overweight/obese pregnant women. This is not surprising given the finding that adipose tissue releases a significant amount of IL-6 along with C-reactive protein.\textsuperscript{6} Genetic studies done by Woods et al. showed that patients with cardiovascular disease might have a single base mutation in the flanking region of IL-6 that lends them to have higher levels of basal inflammation.\textsuperscript{19} In pregnancy, high levels of IL-6 are associated with gestational diabetes in both healthy and overweight pregnant women. Pre-pregnant obesity is also correlated with a greater expression of placental pro-inflammatory cytokines including IL-6.\textsuperscript{20}

Tumor necrosis factor- alpha (TNF-α) is also involved in the acute phase reactant inflammatory pathway. Discovered in the late 1960’s, TNF-α is a 51kDa trimeric protein, named after its ability to kill mouse fibrosarcoma cells.\textsuperscript{21} It was later discovered to be one of the main cytokines released by activated macrophages. Its role in inflammation is
based on its ability to induce a strong inflammatory response in human innate immunity. Just like IL-6, TNF-α up-regulates B and T lymphocytes, and activates endothelial cells. It also acts as a pyrogen, induces apoptosis in combination with IL-6, induces cachexia, and inhibits tumorgenesis and viral replication. The inflammatory response by TNF-α, along with IL-6, is beneficial when combating infection and deleterious during autoimmune diseases. Studies have demonstrated that TNF-α expression is elevated in obesity and insulin-resistant states in both human and mouse models, due to the direct effects on adipocyte metabolism, especially in regards to CV health (lipid levels) and glucose homeostasis. The chronic inflammatory condition of pre-gravid obesity strongly affects the growing fetus increasing levels of macrophage recruitment leading to and levels of pro-inflammatory cytokine expression (TNF-α and IL-6). While TNF-α is known to be produced in the placenta, in women with pre-gravid obesity, levels are exaggerated. Kirwan et al. reported a correlation between fat mass measured in late pregnancy and TNF-α levels.

*Adiponectin is an adipokine with anti-inflammatory properties in pregnancy.* Adipose tissue is not an static organ. It is an endocrine organ known to produce hormones and cytokines (adipokines) such as leptin, resistin, TNF-α, IL-6 and adiponectin. All of these adipokines, except adiponectin, have been associated with insulin resistance, hyperlipidemia, obesity, inflammation, and atherosclerosis, along with metabolic syndrome. Adiponectin levels are lower in obese individuals than non-obese matched controls, and when obese subjects lose weight, the plasma concentrations of adiponectin increase. This finding has led to the hypothesis that adipose tissue may have a negative
feedback mechanism with adiponectin production or secretion. Since adiponectin concentrations are low in obese, chronically ill patients, researchers have been investigating its properties on normal subjects. There is now evidence that adiponectin may have insulin sensitizing effects, as well as anti-atherogenic, anti-inflammatory, and anti-angiogenic properties. 25

Pregnancy is a state of insulin resistance originally brought about by placental hormones. However, increasing evidence shows that low levels of adiponectin may also play a role. Studies have shown an association between gestation diabetes, preeclampsia associated with insulin resistance and low levels of adiponectin. 26 A recent study done by Nien et al., demonstrated that in all three trimesters women with pre-gravid obesity have lower levels of circulating adiponectin than normal weight pregnant women. This team also concluded that increasing weight during pregnancy proportional decreased the plasma concentration of adiponectin. 26

DHA may have an effect on markers of inflammation. DHA can influence inflammation through a variety of ways from the membrane to the nucleus. DHAs anti-inflammatory affects are thought to be a result of decreases in leukotriene B4, platelet activating factor, and the reduction in creation of prostaglandins, and thromboxanes. 27 These factors, which halt platelet aggregation, are also related to DHA’s cardio-protective effects. In a model of rheumatoid arthritis demonstrate that DHA may also slow down T-cell proliferation through the modulation of the cytokine IL-2. 28 Current in-vitro data suggests that DHA leads to decreases in inflammatory biomarkers, including IL-6 and
These fatty acids mostly act through cell surface and intracellular receptors that control inflammatory cell signaling and gene expression. Adjustments of inflammatory cell membrane fatty acid composition, such as supplementing with DHA, may alter cell signaling leading to changes in gene expression that can alter the pattern of lipid and peptide production. Oliver et al. demonstrated that DHA supplementation promotes a decrease in IL-6 and TNF-α, while at the same time increasing IL-10 in pretreated macrophages. The proposed mechanism behind this is that dietary DHA alters plasma membrane lipid composition, thereby directly influencing protein signaling that regulate immune responses and inflammation. DHA also may inhibit post-translational protein signaling, which subsequently may alter lipid raft targeting and protein function, and thereby suppressing inflammatory mediators in humans. DHA has also been shown to increase plasma levels of adiponectin by directly increasing adiponectin synthesis through a PPARY-related mechanism. Johnson et al. demonstrated a direct relationship in which DHA up-regulated membrane-associated high-density adiponectin in rodents.

Differences in multiple inflammatory markers may exist between ethnic groups. Pro- and anti-inflammatory cytokines that regulate the magnitude of pro-inflammatory immune activity are polymorphic in nature. Distinct alleles of polymorphic genes can be attributed to individual and ethnic differences in the rate and extent of production of individual proteins. This difference in protein translation can be attributed to a difference in disease frequency and/or severity. Nguyen et al. investigated this hypothesis in pregnant women of different racial/ethnic groups (Caucasian, African American, and Hispanic). They
found significant differences in the IL-1 and IL-4 genes among the groups.

TNF-α levels vary by race/ethnicity as well. Waisberg et al. demonstrated that TNF-α levels were significantly higher in the African group, and visceral fat levels were significantly lower than Indians. CRP and IL-6 levels do not vary by group. Similarly, Chambers et al. found that concentrations of C-reactive protein were higher in healthy Indian-Asians than in Europeans. This finding may be related to fat distribution as central obesity and insulin resistance is higher in Indian-Asians. In a study done by Velez et al., there were significant differences between concentrations of cytokines, both pro- and anti-inflammatory depending if the subject was African American or Caucasian. Picklesimer et al. looked at racial differences in C-reactive protein levels during normal pregnancy. African American women had higher median levels of CRP, even after controlling for confounders such as smoking and maternal weight.

To our knowledge, no one has yet to look at the differences in inflammatory in obese pregnant women who have been supplemented with DHA during pregnancy.

**Purpose**

The purpose of this thesis project was to investigate the effect of supplemental DHA on inflammatory biomarkers in healthy overweight/obese pregnant women, 18-40 years of age, of different races/ethnicities (White, African American, Hispanic) following 10 weeks of supplementation with docosahexaenoic acid or placebo.
Research Question

After supplementing obese/overweight pregnant women with DHA will there be a change in inflammatory biomarkers? Will the response vary in women by race/ethnicity? Can ethnicity account for changes in inflammation due to DHA supplementation? Does race/ethnic group affect the response of inflammatory biomarkers to DHA supplementation in overweight/obese pregnant women?

Null Hypothesis ($H_0$)

1. In overweight/obese pregnant women 18-40 years old, race/ethnicity will not effect erythrocyte DHA.
   a. There is no main effect of ethnicity on erythrocyte DHA. There will be no interaction between ethnicity and DHA supplementation on erythrocyte DHA levels.
   b. There is no difference in the population means between the DHA vs. control group when examining the change in erythrocyte DHA levels.

2. In overweight/obese pregnant women 18-40 years old, race/ethnicity will not effect markers of inflammation
   a. There is no main effect of ethnicity on inflammatory markers (adiponectin, IL-6, and TNF-α). There is no interaction between ethnicity and DHA supplementation on inflammatory markers (adiponectin, IL-6, and TNF-α).
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 Institutes of Health [5R21HL093532, UL1RR026314-03], Mead Johnson Nutritionals, and Martek Corporation). 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 Science 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 ≥25 kg/m², and who had complete data, were included for analysis.

Study Visits

The study visits occurred at the General Clinical Research Center located at Cincinnati Children’s Hospital Medical Center (CCHMC) or the University of Texas Health Sciences Center at San Antonio. Three study visits were conducted in the third trimester; the first during the 26th week of pregnancy, the second during the 29th-32nd week, and the third during the 35th-37th week of gestation.
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) and 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. 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 erythrocyte DHA. No adverse effects were reported.

Survey

Demographic data (age, education, race/ethnicity) were self-reported on a survey instrument.

Laboratory Methods

Erythrocyte DHA

Blood (2 ml) was collected at each study visit. Venous whole blood was collected into EDTA-coated vacutainer tubes and then placed on ice. After being centrifuged for 20 min (3000 x g @4), the plasma was removed and stored for future analysis. The erythrocytes were washed three times in a NaCl solution. 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 fatty acids was performed using the retention times of authenticated fatty acid standards.

Inflammatory Markers

Adiponectin, IL-6, TNF-alpha were analyzed in plasma samples using enzyme-linked, immunosorbent assays with a Milliplex™ Multiplex kit (Millipore, Billerica, MA). Using a 96-well plate, 40 samples were run in duplicate and the plates were then read using luminex technology on the Bio-Plex™ (Bio-Rad, Hercules, CA). Standard curves using recombinant proteins were used to calculate the concentrations.

Statistical Analysis

Data were explored to determine the normality of the distributions. When normality was violated, nonparametric tests were used. Baseline data were compared between the experimental and control groups using an independent samples t-test. Nominal data were compared using a chi-squared test.

Outcome variables (adiponectin, IL-6, and TNF-α) were assessed as the differences in the absolute measurement (values) and percent change (%) between baseline and study end measurements. Percent change was calculated as [(study end – baseline) / study end] *100. Mean values are reported for normally distributed data and median values with interquartile ratios for skewed data.

One-way analysis of variance (ANOVA) was used to compare means of the outcome variables by study group. The Kruska-Wallis Test was used as a non-parametric
alternative to a one-way ANOVA, allowing the comparison of medians on a continuous variable for three or more groups.

Two-way analysis of variance (ANOVA) was used to identify relationships between inflammatory biomarkers (TNF-α, IL-6, adiponectin) and group and ethnicity. When a main effect was observed, multiple post-hoc analyses were done to determine least significant differences in means between ethnic groups. ANCOVA was with baseline DHA as a covariate.

The variable, change in erythrocyte DHA intention-to-treat, was chosen as assessing change in DHA expresses true compliance. Using intention-to-treat analysis allows compensation for any missing data that occurs throughout the course of the study as participants drop out. In this study, intention-to-treat was used for participants who completed the study but were missing DHA data at study visit three. It was determined that the change in erythrocyte DHA is negligible between the second and third study visit, thus erythrocyte DHA data available from the second study visit was carried forward.

For this analysis, statistical significance was set as a p value of <0.05 with a power greater than 80% for the models. Data analysis was performed though the use of the statistical software Statistical Package for the Social Sciences (version 18.0, 2010, SPSS, Inc, Chicago. IL).

**Results**

Randomization was effective as an equal number of women of the different racial/ethnic groups were present in the DHA and control groups (p>0.05). There were no
significant differences in baseline demographic characteristics of subjects when the study group was compared to controls (Table 1). However, when baseline demographic data was divided by racial/ethnic group, African-American women were younger and more educated than Hispanic women (p<0.01). White women were more educated than both African American and Hispanic women (p<0.04, p<0.001 respectively) (Table 2). Since both “age at trial entry” and “years of education” were not correlated with outcome variables, they were not controlled for in the ANCOVA.

**Table 1**
Baseline demographic characteristics of women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DHA (n=56)</th>
<th>Control (n=51)</th>
<th>Total (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at trial entry (y)</td>
<td>28 ± 5.00</td>
<td>27 ± 5.00</td>
<td>28 ± 5.00</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.80 (30.1-)</td>
<td>34.1 (30.70-)</td>
<td>33 (30.20-)</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>13 (23)</td>
<td>18 (35)</td>
<td>31 (29)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>23 (41)</td>
<td>21 (41)</td>
<td>44 (41)</td>
</tr>
<tr>
<td>White</td>
<td>20 (35)</td>
<td>12 (23)</td>
<td>32 (29)</td>
</tr>
<tr>
<td>Years of education (y)</td>
<td>12.4 ± (3.40)</td>
<td>12.9 ±(3.70)</td>
<td>12.6 ± 3.5</td>
</tr>
</tbody>
</table>

¹The baseline characteristics were not significantly different between groups.
²Mean ± SD (all such values)
³Median: IQR in parentheses (all such values)
Table 2
Baseline demographic characteristics of women by race/ethnicity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>African American (n=31)</th>
<th>Hispanic (n=44)</th>
<th>White (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at trial entry (y)</td>
<td>25.97 ± 4.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.02 ± 4.95&lt;sup&gt;b, **&lt;/sup&gt;</td>
<td>28.00±4.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>35.77 ±8.60</td>
<td>34.231 ± 3.76</td>
<td>33.02 ±7.01</td>
</tr>
<tr>
<td>Years of education (y)</td>
<td>13.73 ±2.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>±2.95&lt;sup&gt;b, ***&lt;/sup&gt;</td>
<td>±2.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD (all such values)

Note: Values in the same row and subtable not sharing the same subscript are significantly different at *p<0.05, **p<0.01, ***p<0.001.

Erythrocyte DHA

Women supplemented with DHA had significantly higher percent erythrocyte DHA as compared to control group (Table 3). The model, including study group and ethnicity as factors, with baseline DHA as a covariate explained 50% of the variability in DHA change between baseline and study end (F=16.78, p<0.0005). There was a significant interaction between the study group and ethnic background on change in erythrocyte DHA (p<0.03)(Figure 1). In the control group, the African-American and White women had a slight decrease and the Hispanic women a slight increase in erythrocyte DHA following supplementation (Figure 2). In the DHA group, Hispanic and White women had an increase and African-American women a decrease in erythrocyte DHA. The effect size was .12, a moderate to large effect as defined by Cohen (1998, p. 22).
Table 3
Erythrocyte DHA level before and after 800 mg DHA supplementation

<table>
<thead>
<tr>
<th></th>
<th>DHA (n=56)</th>
<th>Control (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte DHA at baseline (%)</td>
<td>4.78 ± 1.73$^I$</td>
<td>5.02 ± 1.57</td>
</tr>
<tr>
<td>Erythrocyte DHA at study visit 3</td>
<td>7.70 ±</td>
<td>5.222 ±</td>
</tr>
<tr>
<td></td>
<td>3.15$^b$***</td>
<td>2.09$^a$</td>
</tr>
<tr>
<td>Δ in erythrocyte DHA$^2$</td>
<td>2.28 ±</td>
<td>-0.11 ±</td>
</tr>
<tr>
<td></td>
<td>3.03$^a$***</td>
<td>2.04$^a$</td>
</tr>
</tbody>
</table>

$^I$ Mean ± SD (all such values)
$^2$ Calculated as (Baseline (SV1) - study end(SV3))

Note: Values in the same row and subtable not sharing the same subscript are significantly different at *p<0.05, **p<0.01, ***p<0.001.

Table 4
Effect of DHA supplementation on Erythrocyte DHA (%)

<table>
<thead>
<tr>
<th></th>
<th>Study Visit 1</th>
<th>Study Visit 3</th>
<th>Absolute Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHA Control</td>
<td>DHA Control</td>
<td>DHA Control</td>
<td>DHA Control</td>
</tr>
<tr>
<td>African American</td>
<td>5.275 ± 1.994$^I$</td>
<td>4.956 ± 1.461</td>
<td>4.000 ± 2.420</td>
<td>4.846 ± 2.404</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5.817 ± 1.992</td>
<td>5.335 ± 1.872</td>
<td>9.961 ± 2.348</td>
<td>6.150 ± 1.241</td>
</tr>
<tr>
<td>White</td>
<td>5.081 ± 0.849</td>
<td>4.956 ± 1.461</td>
<td>6.932 ± 2.376</td>
<td>4.163 ± 2.080</td>
</tr>
</tbody>
</table>

$^I$ Mean ± SD (all such values)

Note: Values in the same row and subtable not sharing the same subscript are significantly different at *p<0.05, **p<0.01, ***p<0.001.

Figure 1. Estimated marginal means of the change in absolute erythrocyte DHA levels
Inflammatory Biomarkers

Table 5
Δ in levels of inflammatory markers between baseline and study end

<table>
<thead>
<tr>
<th></th>
<th>DHA (n=56)</th>
<th>Control (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Δ adiponectin (ug/ml)²</td>
<td>-0.24 ± 1.98¹</td>
<td>-0.62 ± 2.07</td>
</tr>
<tr>
<td>Percent Δ adiponectin³</td>
<td>-24.45 ± 80.65</td>
<td>-30.67 ± 77.07</td>
</tr>
<tr>
<td>Absolute Δ IL-6 (pg/ml)</td>
<td>-0.81 ± 8.17</td>
<td>1.23 ± 3.36</td>
</tr>
<tr>
<td>Percent Δ IL-6</td>
<td>-5.567 ± 77.95</td>
<td>10.678 ± 64.17</td>
</tr>
<tr>
<td>Absolute Δ TNF-α (pg/ml)</td>
<td>0.19 ± 1.17</td>
<td>0.45 ± 1.62</td>
</tr>
<tr>
<td>Percent Δ TNF-α</td>
<td>-3.12 ± 42.24</td>
<td>-14.75 ± 86.92</td>
</tr>
</tbody>
</table>

¹ Mean ± SD (all such values)
²Calculated as (Baseline (SV1) - study end(SV3))
³Calculated as ((SV3-SV1/SV3)*100))

Adiponectin

There was not a significant difference in the mean absolute change of adiponectin between the DHA and control groups (Table 5). When experimental groups were divided
based on race/ethnicity, the median level of absolute change in adiponectin was different between the three groups (Table 6) (p<0.05).

IL-6

There was not a significant difference in the mean absolute change of IL-6 between the DHA and control groups (Table 5). When experimental groups were divided based on race/ethnicity, the median level of absolute change in IL-6 was different between the three groups (Table 7) (p<0.01).

TNF-α

Ethnicity and study group explained 12% of the variance in TNF-α (F=2.82, p<0.02). There was a significant interaction between the study group and ethnic background on absolute change in blood TNF-α levels (p<0.05)(Figure 3). In the control group, the African-American and White women had a slight increase in TNF-α (0.92 pg/ml, 1.2 pg/ml, respectively) and the Hispanic women had a slight decrease (-0.36). In the DHA group, all women had less than a 0.25 pg/ml increase in TNF-α (Figure 4). The effect size was 6%, or a medium size, as defined by Cohen (1998, p.22).

There was not a significant difference in the mean percent change of TNF-α between the DHA and control groups (Table 4). When experimental groups were divided based on race/ethnicity, the median level of percent change in TNF-α was different between the three groups (Table 7) (p<0.001).
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<tr>
<td>Percent Δ adiponectin(^3)</td>
<td>-24.45 ± 80.65</td>
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\(^1\) Mean ± SD (all such values)
\(^2\)Calculated as (Baseline (SV1) - study end(SV3))
\(^3\)Calculated as ((SV3-SV1/SV3)*100))

Table 6
Effect of DHA supplementation on Adiponectin (ug/ml)

<table>
<thead>
<tr>
<th></th>
<th>Study Visit 1</th>
<th>Study Visit 3</th>
<th>Absolute Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DHA</td>
<td>Control</td>
<td>DHA</td>
</tr>
<tr>
<td>African American</td>
<td>2.87 (2.54-4.46)</td>
<td>5.27 (3.83-6.16)</td>
<td>3.16 (2.11-4.21)</td>
<td>6.47 (4.44-8.52)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5.65 (4.30-8.99)</td>
<td>6.91 (4.67-7.20)</td>
<td>5.66 (4.00-10.92)</td>
<td>7.75 (4.53-11.11)</td>
</tr>
<tr>
<td>White</td>
<td>6.05 (3.69-6.02)</td>
<td>7.23 (3.70-5.83)</td>
<td>6.48 (2.65-8.83)</td>
<td>7.65 (3.24-11.55)</td>
</tr>
</tbody>
</table>

Table 7
Effect of DHA supplementation on IL-6 (pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>Study Visit 1</th>
<th>Study Visit 3</th>
<th>Absolute Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DHA</td>
<td>Control</td>
<td>DHA</td>
</tr>
<tr>
<td>African American</td>
<td>3.10 (2.84-5.04)</td>
<td>4.35 (3.39-6.74)</td>
<td>2.52 (2.51-4.92)</td>
<td>4.72 (3.93-5.73)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.51 (1.15-3.12)</td>
<td>1.91 (1.35-4.62)</td>
<td>1.87 (1.07-3.63)</td>
<td>2.78 (1.08-8.32)</td>
</tr>
<tr>
<td>White</td>
<td>4.99 (3.41-5.96)</td>
<td>6.32 (4.68-6.13)</td>
<td>5.07 (3.26-5.80)</td>
<td>6.27 (4.93-8.10)</td>
</tr>
</tbody>
</table>

Table 8
Effect of DHA supplementation on TNF-alpha (pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>Study Visit 1</th>
<th>Study Visit 3</th>
<th>Absolute Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DHA</td>
<td>Control</td>
<td>DHA</td>
</tr>
<tr>
<td>African American</td>
<td>4.58 (3.01-7.81)</td>
<td>6.45 (2.07-12.36)</td>
<td>2.59 (1.48-4.13)</td>
<td>7.27 (3.40-9.08)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.49 (0.87-2.33)</td>
<td>1.46 (0.90-1.71)</td>
<td>2.01 (1.11-3.99)</td>
<td>2.80 (1.40-4.09)</td>
</tr>
<tr>
<td>White</td>
<td>6.21 (4.25-8.94)</td>
<td>6.66 (5.00-7.08)</td>
<td>4.83 (3.52-11.09)</td>
<td>6.73 (4.25-12.36)</td>
</tr>
</tbody>
</table>

\(^4\)Median. IQR in parentheses (all such values)
\(^5\)Note: Values in the same row and not sharing the same subscript are significantly different at \(^*\)p<0.05, \(^**\)p<0.01, \(***\)p<0.001.

Figure 3. Estimated marginal means of the absolute change in blood TNF-alpha levels

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20
Discussion

To our knowledge, this study is the first of its kind to examine ethnic effects of DHA supplementation on inflammatory markers in overweight/obese pregnant women during the 3rd trimester. As this is a pilot study, it is impossible to compare our results to others. Nguyen et al did a study most related to ours. His team examined genetic differences related to ethnicity on inflammation in healthy weight pregnant women. His team used gene polymorphisms to determine immune related gene regulation as a marker for inflammatory status. The team concluded that ethnic variation could factor into negative pregnancy outcomes. Other studies indicate that CRP levels during normal pregnancy are different depending on ethnicity. No study to date has looked at overweight/obesity, inflammation and genetics, nor has a study looked at these factors before and after supplementation with DHA.

Our results indicate that ethnicity impacts inflammation, and this, may in-turn may be differently responsive to DHA supplementation. We found that changes in erythrocyte DHA levels were significantly different among members of different
ethnicities. Hispanics had significantly higher DHA levels at baseline, and Hispanics pregnant women that were supplemented with DHA had significantly higher changes in DHA levels at the third study visit. This is line with a recent study done among female college students (a group whose age was similar to ours) demonstrated that Hispanic women had significantly higher mean percent intake of arachidonic acid and ratio of linoleic acid to alpha-linolenic acid than non-Hispanic white females, and Hispanic males had significantly higher mean percent intake of EPA and [EPA plus docosahexaenoic acid (DHA)] than non-Hispanic white male. However, there have been multiple studies that show low DHA levels among the Hispanic ethnicity, one study done with pregnant women of multiple ethnicities showed no differences in DHA levels across any race. Clearly, more research needs to be done in this area. We did not see a change in DHA levels in our African American subjects after supplementation. Many reasons could explain why this happened. First, compliance is always an issue. We relied on self-reported data to determine if our subjects were taking the treatment. They could have easily falsified these reports. There may also be genetic differences among different races/ethnicities, making African American subjects more resistant to changes in blood DHA levels than other groups.

Our results indicate that ethnicity had an effect on median adiponectin levels in overweight/obese pregnant women in the third trimester of pregnancy. While our study represents the first attempt to look directly at adiponectin blood levels in multiple ethnic groups, research indicates that gene polymorphisms encoding adiponectin do exists. In fact, when stratified by ethnicity, certain polymorphisms of adiponectin may increase an individual’s chance of developing type II diabetes. What is surprising about our results
is the fact that we did not a significant main effect between DHA supplementation and alterations in blood adiponectin levels. When we divided our experimental and study groups by race/ethnicity, the DHA supplemented group our DHA groups had significantly different levels of blood adiponectin. Fasshauer et al, reported that healthy normal weight pregnant women have significant increases in adiponectin by the second trimester of pregnancy. It is interesting to note that Fasshauer subjects had similar blood levels of adiponectin during their second trimester as compared to our baseline data. Their data also exhibited had higher levels of adiponectin during their subjects third trimester than ours. This may indicate that DHA is having some small effect of the rise of adiponectin. In fact, our data, representing women in the third trimester is similar to his in the second trimester. Our study did not look at the changes occurring in adiponectin throughout all trimesters of pregnancy. Both of our study visits were during the third trimester; we cannot estimate the effect that adiponectin would have if given earlier in pregnancy.

Just as with adiponectin, absolute blood IL-6 levels after DHA supplementation was different when divided by race/ethnicity and compared. This is in opposition to what is reported in the literature. The Salmon in Pregnancy Study looked at healthy pregnant women throughout all trimesters of pregnancy. After supplementing them with Atlantic salmon 150g twice a week from 20 weeks of pregnancy on, they found no change in the levels of IL-6 or TNF-alpha. Hawkes et al supplemented 600 mg of DHA and the examined breast milk of pregnant mothers 4 weeks postpartum. They found no significant difference in cytokine profiles between DHA supplementation and placebo. As mentioned previously, Waisberg et al. examined IL-6 in non-pregnant overweight
African women as compared to Indian. Their results were also different similar to ours. While they did not look at pregnancy (which inherently increases inflammation), they examined that effect of ethnic differences on IL-6 levels in obese non-pregnant women. They did not find a significant difference between IL-6 levels these two groups of women.32

Ethnicity had a significant main effect on the absolute change in TNF-alpha levels. After splitting the data based on ethnicity, Caucasian pregnant women had a significantly smaller change in TNF-alpha levels after supplementation compared to controls. Neither Hispanics nor African Americans had significant differences in means. This data is in agreement with both Waisberg and Olson. Waisberg found a significant different level of TNF-alpha based on ethnicity.32 Olson et al found that, after adjusting for age, sex and BMI, TNF-alpha differed by ethnicity, with Hispanics having the highest levels and African-Americans having the lowest.42 We did not find a main effect of DHA supplementation on changes in TNF-alpha levels, however we did show that DHA supplementation is affected by race/ethnicity. Again, this is at odds with previously reported data. Studies of non-pregnant population found that TNF-alpha levels are not changed by DHA supplementation.43

To our knowledge this is the first time that any study has looked at an interaction between DHA and ethnicity in overweight/obese pregnant women in the third trimester of pregnancy. With conflicting results across a wide range of inflammatory cytokines, its is imperative that more in-depth follow-up studies by done to reach strong specific conclusions.
In conclusion, DHA supplementation has varying effects on inflammatory biomarkers in healthy overweight/obese pregnant women of different races/ethnicities. Ethnicity and DHA supplementation have interacting effects on both erythrocyte DHA and blood TNF-alpha levels. Modifying DHA supplementation based on race/ethnicity may lower inflammatory status in pregnancy and improve fetal outcomes.

Our study was not without limitations. We only followed subjects during the third trimester of pregnancy. We did not analyze the effects of starting supplementation at conception until birth. We also did not look at a dose dependent response of DHA. Future studies will include a longer supplementation period to see if DHA has significant affect on inflammatory markers that may be modified early in pregnancy.
References


differences of polymorphisms in cytokine and innate immune system genes in pregnant

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