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

It is entitled:
The association between erythrocyte docosahexaenoic acid (EDHA) status and insulin sensitivity in overweight/obese pregnant women of different racial/ethnic groups

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The association between erythrocyte docosahexaenoic acid (EDHA) status and insulin sensitivity in overweight/obese pregnant women of different racial/ethnic groups

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ABSTRACT

Objective: To investigate the relationship between erythrocyte docosahexaenoic acid (EDHA) status and insulin sensitivity in Non-Hispanic Black, Hispanic, or Non-Hispanic White overweight/obese pregnant women.

Design and methods: This is a secondary analysis of data collected in a parallel, double-blind, placebo-controlled study investigating the effects of 10 weeks of docosahexaenoic acid (DHA) supplementation (800 mg DHA in algal oil from 26 to 36 weeks gestation) on insulin sensitivity and metabolic markers in overweight/obese pregnant women. Insulin sensitivity was measured after a 2-hour meal challenge at 36 weeks of pregnancy. The homeostasis model assessment of insulin resistance (HOMA-IR), muscle insulin sensitivity index (MISI), and the product of insulin$_{1-30}$ [AUC]*glucose$_{1-30}$ [AUC], measured during the meal challenge, were calculated. Fasting plasma glucose, insulin, and adiponectin were measured at 36 weeks.

Subjects: Healthy, English-speaking pregnant women (N=106), between the ages of 18-40 years, with a singleton pregnancy, a BMI $\geq$25 and $\leq$ 60 kg/m$^2$, and who had complete data, were included for this analysis. Subjects were recruited from the greater Cincinnati, OH region (Non-Hispanic Black and White) and San Antonio, TX (Hispanic White).

Results: Hispanic women had significantly higher mean erythrocyte DHA (EDHA) concentrations than Non-Hispanic Black and White counterparts (p<0.001). There was no relationship between EDHA status, measured at 36 weeks gestation, and insulin sensitivity or resistance in any of the racial/ethnic groups of overweight/obese, pregnant women. However, there were racial/ethnic differences in measures of glucose metabolism. Being in the Non-Hispanic Black group explained 7.7% of the
variance in fasting glucose concentration (p=0.005). Being in the Hispanic group explained 20.5% of the variance in fasting glucose concentration (p=0.000); 5.3% of the variance in fasting insulin concentration (p=0.022); and 9% of the variance in HOMA-IR (p=0.037). Being in the Non-Hispanic White group explained 4.2% of the variance in fasting insulin concentration (p=0.005) and 5.3% of the variance in HOMA-IR (p=0.022). Adiponectin was significantly, negatively correlated with HOMA-IR, fasting insulin concentration, and insulin$_{1-30}$[AUC]*glucose$_{1-30}$[AUC] and explained 10.30% of the variance in HOMA-IR, 4.75% of the variance in fasting insulin concentration, and 4.97% of the variance in insulin$_{1-30}$[AUC]*glucose$_{1-30}$[AUC]. Adiponectin was significantly, positively correlated with MISI and it explained 3.84% of the variance in MISI.

**Conclusion:** There was no relationship between EDHA status and insulin sensitivity in overweight/obese, pregnant women of different racial/ethnic groups (Non-Hispanic Black, Hispanic, and Non-Hispanic White). However, there were racial/ethnic differences in measures of glucose metabolism. Compared to Non-Hispanic Blacks and Whites, Hispanic women had significantly lower concentration of fasting glucose, fasting insulin, and HOMA-IR and significantly higher concentration of EDHA than the other 2 groups. Adiponectin was a predictor of insulin sensitivity estimated by HOMA-IR, fasting insulin, insulin$_{1-30}$ [AUC]*glucose$_{1-30}$ [AUC], and MISI.
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INTRODUCTION

Healthy pregnancies can be characterized by a state of insulin resistance due to changes in carbohydrate metabolism. A change in insulin sensitivity is normal because, in order to provide consistent energy and nutrients to the fetus, concentrations of glucose and free fatty acids should increase. ¹ Pregnant women have lower fasting blood glucose and higher blood glucose after meals and a higher concentration of insulin in the blood compared to their non-pregnant counterparts. ² But if insulin resistance becomes abnormally severe, it will lead to gestational diabetes mellitus (GDM). There are many factors related to the incidence of GDM, but the strongest predictor is excess adiposity. Pre-gravid obesity, as measured by body mass index (BMI) is associated with excess insulin resistance, which in the final stage results in GDM.³

Obese women are more insulin resistant compared to normal weight women. ⁴ GDM and obesity are independently associated with adverse pregnancy outcomes, such as gestational hypertension, higher odds of Cesarean delivery, preeclampsia, and risk of perinatal mortality. ⁵ If a pregnant woman has a higher BMI and has GDM at the same time, the effects will be cumulative and have greater impact than either one alone.⁶

According to the Centers for Disease Control and Prevention (CDC), compared to Non-Hispanics, Hispanic Americans have a higher prevalence of diabetes and Non-Hispanic Blacks are more likely to have diabetes than Non-Hispanic Whites. Non-Hispanic Black subjects have significantly lower concentrations of adiponectin concentrations compared to Whites; adiponectin concentration is inversely associated with visceral adipose tissue (VAT).⁷ Compared to waist-hip ratio (WHR) and waist circumference, visceral fat at L2-3 was a
significantly better predictor of diabetes.\textsuperscript{8} Other studies show ethnicity plays an important role in VAT/ body fat distribution.\textsuperscript{9-11} Obese Hispanic people have more pancreatic fat accumulation than obese Non-Hispanic Blacks and this difference becomes larger with increasing age; pro-inflammatory cytokines TNF-\(\alpha\) is related to VAT inflammation.\textsuperscript{12}

Experiments on rats and mice indicate DHA could improve insulin sensitivity;\textsuperscript{13,14} but one experiment on pigs did not agree.\textsuperscript{15} One study showed dietary supplementation with omega-3 fatty acids improved insulin sensitivity in patients with type-2 diabetes.\textsuperscript{16} The purpose of this study was to investigate the relationship between status of EDHA and status of insulin sensitivity for Hispanic, Non-Hispanic Black and Non-Hispanic White overweight/obese, pregnant women.
LITERATURE REVIEW

The Problem of Pre-Gravid Obesity

In the Pregnancy Risk Assessment Monitoring System data, pre-gravid obesity has steadily increased over time. In the most recent survey of Ohio (2007), only 50% of women with a live-born baby are normal weight, and approximately two out of five women would be classified as overweight or obese based on the WHO BMI categories: normal weight (BMI of 18.5-24.9 kg/m²), overweight (BMI of 25.0-29.9 kg/m²) and obese (BMI>30 kg/m²). A recent survey on the prevalence of obesity between 1999 and 2010 shows no significant increase among women overall (odds ratio [AOR], 1.01; 95% CI, 1.00-1.03; \( P=0.07 \)); however, there was a significant increase among Non-Hispanic Black women (\( p=0.04 \)) and Mexican- American women (\( p=0.046 \)).

Pregravid Obesity And Adverse Outcomes Related to Glucose Metabolism

Pregravid obesity is associated with adverse pregnancy outcomes, such as increased gestational hypertension; pregnancy induced hypertension, higher odds for caesarean delivery, preeclampsia, gestational diabetes mellitus (GDM) in the mother and increased birth weight (macrosomia), increased neonatal fat mass, and congenital malformation in the baby.\(^3,5,18,19\) Recently, researchers reported that hyperglycemia below the threshold for GDM also increased the risk of perinatal mortality in a large sample of pregnant women who delivered after 34 weeks gestation.\(^20\) In the short term, GDM is independently associated with adverse pregnancy outcomes in both mother and infant.\(^6\) In the long term, GDM is associated with developing type 2 diabetes, and/or metabolic syndrome postpartum.\(^21,22\)
Why Might There Be Differences between Groups of People

Despite the observation that Non-Hispanic Black women are more likely to be obese compared to Hispanic women, their prevalence of diabetes is lower.\textsuperscript{24} Hispanic women have significantly greater insulin resistance than Non-Hispanic Black women and Non-Hispanic White women (p<0.001). However, Non-Hispanic Black women have lower whole-body insulin sensitivity than White women, independent of adiposity, diet, and physical activity.\textsuperscript{24, 25} According to Kieffer et al., the prevalence of GDM was 2.5 times higher among Hispanic women aged 14 to 47 compared to their Non-Hispanic Black counterparts.\textsuperscript{26}

Adiposity Derived Cytokines and Insulin Resistance in Abnormal Adipocytes

Insulin is an important hormone produced by pancreatic β-cells, participating in carbohydrate and lipid metabolism. Excess adipose tissue will lead to obesity, which not only plays a role in storing energy, but it also functions as endocrine glands and release a variety of adipokines, hormones such as leptin and adiponectin; cytokines like tumor necrosis factor-α (TNF-α), and substrates like free fatty acids.\textsuperscript{27} Based on recent studies of adipokines and inflammation, those adipokines might have the ability to interfere with intracellular insulin signaling on glucose transport in skeletal muscle or adipocytes, and with lipoprotein and with glucose metabolism in the liver.\textsuperscript{7} When adipocyte concentrations are abnormal (either absent or excessive), they will lead to complex processing of adipokines therefore damaging insulin sensitivity. But all sites of adiposity do not have the same effect on insulin sensitivity because intra-abdominal fat and visceral adipose tissue (VAT) are more strongly correlated to insulin resistance, T2DM, and cardiovascular disease than peripheral/ subcutaneous
TNF-α and Insulin Sensitivity Possible Mechanisms

TNF-α has an ability to bind to and activate a p55TNF receptor (TNFR), form exogenous sphingomyelinase and ceramides, inhibit IR and IRS-1 tyrosine phosphorylation, and convert IRS-1 into an inhibitor of the insulin receptor tyrosine kinase, which leads to a decrease in insulin receptor signaling. In ob/ob mice devoid of TNF receptors, there was a significant improvement in insulin sensitivity. GLUT4 protein, which is the only glucose transporter protein regulated by insulin, and exists in muscle, heart, brown and white adipocytes, increased significantly in muscle tissue (p<0.05). IR phosphorylation increased significantly in adipose tissue and muscle tissue (p<0.05); similar results were confirmed by Cheung et al.. that after TNF-α neutralization, there is a 2.5-fold increase in tyrosine phosphorylation of IR in skeletal muscle. An experiment on 3T3-L1 cells in adipocytes also confirmed this result. This might be caused by the different signaling pathways in muscle and liver. TNF-α receptor-deficient obese mice had lower concentrations of circulating free fatty acids (FFAs) (p<0.05). However, Lopez-Soriano found no significant changes in blood glucose, plasma triacylglycerol, or insulin in the Zucker rat with 4-day anti-TNF treatment. To test a thiazolidinedione drug, which is used broadly to treat diabetes, Souza et al. did an experiment on cells, and found that cells pretreated with TNF-α experienced an approximately 44-fold release of FFA compared with untreated cells. PPARγ is a key transcription factor for the adiponectin gene. Treatment of 3T3-L1 adipocytes with TNF-α resulted in a time- and concentration-dependent decrease in PPARγ mRNA expression, which is necessary for maintenance of the adipocyte phenotype, indicating that PPARγ expression is sufficient to
attenuate TNF-α-mediated effects on the adipocyte phenotype.\textsuperscript{37}

In obese animal models and human studies, overexpression of TNF-α, increased TNF-α messengers and increased circulating of TNF-α was observed.\textsuperscript{38} A study to examine the expression of TNF-α mRNA in adipose tissues in 19 obese women and 18 normal weight premenopausal women demonstrated that the adipose tissue from obese subjects secreted higher amounts of TNF-α protein compared to the lean controls, and TNF-α mRNA are 2.5 times as likely to express in fat tissue from obese women compared to lean women (p<0.001).\textsuperscript{39} TNF-α expression was strongly correlated with fasting plasma insulin concentrations (r=0.83,p<0.001); BMI (r=0.70,p<0.001); and fasting plasma triglyceride concentrations (r=0.04,p=0.02).\textsuperscript{39} After weight loss in obese subjects, there was a significant decrease in TNF-α mRNA expression (p<0.001).\textsuperscript{39}

TNF-α was significantly increased in both 18- and 20-day pregnant rats compared with virgin animals. Significant inverse correlations were observed for adipose tissue TNF-α vs. both QUICKI and log (FGIR).\textsuperscript{40} A case-control study indicates that TNF-α is significantly higher in the third trimester compared with the matched healthy control in first and second trimester, and a significant positive linear correlation was found between TNF-α and BMI (p<0.01).\textsuperscript{41} A study of 5 GDM women and 10 NGT women showed that TNF-α was inversely correlated with insulin sensitivity before (r=-0.54,p<0.03), early, (r=-0.68p<0.003) and late (r=-0.69,p<0.006) pregnancy. From pregravid to late pregnancy, the change in TNF-α was a significant predictor of the change in insulin sensitivity (r= -0.60,p<0.02). TNF-α was the most significant independent predictor of insulin sensitivity (p= -0.67, p<0.0001). Plasma TNF-α was correlated with fat mass (r=0.68,p<0.01)\textsuperscript{42}
Adiponectin and Insulin Resistance

As mentioned above, PPARγ is a key transcription factor for the adiponectin gene, which relates adiponectin with TNF-α, indicating that adiponectin and TNF-α should be inversely correlated with each other. Adiponectin could increase insulin’s ability to stimulate glucose uptake by increased GLUT4 gene expression and increased GLUT4 recruitment to the plasma membrane.43

Bruun et al. conducted a study to compare adiponectin concentrations in obese subjects before and after weight loss. He found that weight loss resulted in a 51% increase in plasma adiponectin.44 Plasma adiponectin was 53% higher in lean men versus obese men and was negatively correlated with adiposity and TNF-α (p<0.05). In obese subjects at baseline, the plasma concentration of adiponectin was inversely correlated with HOMA-IR (p<0.05), fasting insulin (p<0.05), BMI (p<0.05) and waist circumference (p<0.05); BMI was found to be a good predictor of circulating adiponectin concentrations (p<0.01).44

There are significant positive correlations between plasma adiponectin with subcutaneous adipose tissue (SAT), insulin sensitivity (Si), female gender, and HDL concentration, and negative correlations with glucose and visceral adipose tissue (VAT) according to a study of non-diabetic Hispanics and African Americans.7 As mentioned above, adipocyte-derived adiponectin is associated with whole body insulin sensitivity and therefore, is a good predictor of ameliorated insulin resistance.45

During pregnancy, two studies indicate significant but small decreases in adiponectin concentrations with advancing gestation.46,47 In the postpartum period, Mazaki-Tovi et al. found that adiponectin concentrations were significantly lower than concentrations measured during all three trimesters.48 A significant negative
relationship was found between serum adiponectin with BMI, VAT, and waist circumference.

**Other Factors Relating Pregnancy to Insulin Resistance**

A change in insulin sensitivity is considered normal, especially during late pregnancy. In order to provide consistent energy and nutrients to the growing fetus, concentrations of glucose and free fatty acids increase due to insulin resistance. The mechanism of insulin resistance in pregnancy is not fully understood. Catalano speculates that there are “post-receptor defects in the intracellular insulin signaling pathway.”

During pregnancy, one of the placental derived hormones, human placental growth hormone (hPGH) increases six-to eight-fold. hPGH has an effect on increasing the expression of the p85α subunit, while p85α competes with p110 subunit of phosphatidylinositol (PI) 3-kinase, which is located in skeletal muscle, resulting in inhibited PI3-kinase activity and therefore preventing insulin signaling downstream.

Compared with non-pregnant control subjects, maximal insulin-stimulated IRS-1 tyrosine phosphorylation was significantly lower by 59±24% (p<0.05) in pregnant control. This was reflected by a 23% (p<0.05) reduction in IRS-1 protein concentrations in muscle from pregnant control. The concentration of IRS-1 was significantly reduced by 16-28% and there is a 1.5-2 fold increase in concentrations of p85α regulatory subunit of phosphatidylinositol (PI) 3-kinase (p<0.05). Insulin resistance to glucose transport during pregnancy is uniquely associated with a decrease in IRS-1 tyrosine phosphorylation, primarily due to decreased expression of IRS-1 protein in muscles in pregnant women compared to non-pregnant women.
**Possible Mechanisms for Variability in Diabetes by Race/Ethnicity**

The prevalence of diagnosed diabetes for Ohio adults by ethnicity are 13.2%, 12.7%, and 9.9% for Hispanic, African-American, and Non-Hispanic White respectively.\(^5^6\) Non-Hispanic Blacks are 1.4-2.2 times more likely to have diabetes than Whites; Hispanic Americans have a higher prevalence of diabetes than Non-Hispanic.\(^5^7\)

In Hauth et al.’s study to determine insulin resistance of Hispanic woman and African-American women, they used glucose\(\geq 75^{th}\), insulin\(\geq 75^{th}\), HOMA-IR\(\geq 75^{th}\) and QUICKI\(< 25^{th}\) as cutoff points to define insulin resistance. The results showed obese woman had a higher percentage of all 4 criteria than normal weight women; while Hispanic women were higher than Non-Hispanic Black and White women.\(^2^5\) In contrast, Mather et al. did not find an influence of race on insulin sensitivity derived from clamp and proxy indices.\(^5^8\)

Waisberg et al. conducted a study on Indian and African non-pregnant women showing TNF-\(\alpha\) concentrations were significantly higher in the African group compared to Indian women, indicating African women are more insulin resistant, and ethnicity could be a factor on insulin sensitivity/resistance, which is correlated to TNF-\(\alpha\) as well as adiponectin.\(^5^9\) Duncan et al. showed mean values of adiponectin in African-American men and women was 22% lower than that of Whites (p<0.001).\(^6^0\)

A study done in Japan showed greater VAT is associated with an increase in future (10-11 years later) insulin resistance.\(^6^1\) A study of 442 overweight children in France showed VAT was influenced by sex and ethnicity, and Caucasians had more VAT than Non-Caucasians.\(^9\) A study of 1,636 Non-diabetic Hispanic and African American subjects indicates ethnicity was not independently correlated to adiponectin (p=0.27),
but they found a strong negative association of VAT with adiponectin in African Americans compared with Hispanics indicating that African Americans are more easily and affected by VAT. In other words, VAT has a stronger effect on African American population than Hispanics, with the same amount of VAT resulting in less adiponectin in African Americans as compared to Hispanics. A study in African American and White children indicates that there was a significant negative relationship between serum adiponectin and BMI. African American boys had significantly lower (37% less) serum adiponectin compared to White boys. A study by Bacha et al. on children showed us similar results that that Black children have about 35% lower adiponectin than their White peers. Another study showed Africa American women had significantly lower adiponectin than that of White women, even after accounting for adiposity. Lee et al. found that adiponectin was inversely associated with VAT and was positively related to insulin sensitivity in both African American and White youth, and the racial difference in adiponectin is significant. Ethnicity differences in body fat distribution are clearly evidenced in women and obese adolescents where lower VAT is found in Blacks compared with Whites. In this study, Hispanics were more likely to have VAT. Obese Hispanic adults have higher pancreatic fat than obese African Americans, which is negatively correlated with insulin secretion in people with impaired glucose tolerance, and as age increases, this difference becomes greater. Visceral fat (at both L2-3 and L4-5) and BMI predicted diabetes among women. Visceral fat at L2-3 was a significantly better predictor of diabetes than was waist-hip-ratio, and waist circumference. African American women had less visceral fat at L2-3 than did Hispanic or White women. These two studies could explain why African Americans have a higher BMI but a lower VAT. There are several studies indicate that ethnicity differences play a major
role in both hepatic insulin sensitivity and peripheral insulin sensitivity estimated by some indexes.

Osei & Schuster compared insulin sensitivity between Black and White healthy subjects. The mean SI (insulin sensitivity index calculated using the MINIMOD method described by Bergman et al.) was significantly (p<0.02) lower in the Blacks (4.93±0.46) than the Whites (7.17±0.88). A study compared three populations of West African ancestry and White Americans in terms of peripheral insulin concentrations, hepatic insulin extraction (HIE), insulin clearance (IC) and β-cell secretion. Mean basal and postprandial HIE and IC were significantly (p<0.05 to 0.01) reduced (25% to 52%) in the three populations of West African ancestry compared with the white Americans, indicating ethnicity may play a major role in HIE or IC in humans. Ellis et al. compared glucose disposal, hepatic insulin sensitivity, and endogenous glucose production among non-diabetic, African American and European American women. Both hepatic insulin action and glucose disposal were lower for African Americans (p=0.009 for both) compared to European Americans, and become more significant after adjustment of body composition.

Effects of DHA on Insulin Resistance

As mentioned above, skeletal muscle is an important tissue for whole-body energy metabolism, including insulin-stimulated glucose uptake. A study done by Clore et al. suggests that the fatty acid composition of skeletal muscle phospholipids has been related to peripheral insulin sensitivity in normal humans. An animal experiment showed improvement in glucose uptake after ingestion of PUFA into membrane by increased residency time of glucose transporters type 1 and 4 (GLUT 1 and GLUT 4), which leads to an expansion of the intracellular pool of glucose-6-
phosphate and to increased skeletal muscle glycogen synthesis. If that is the case, theoretically, DHA might have an effect on attenuating the damage of TNF-α on GLUT4 on skeletal muscle. DHA also enhanced (p<0.05) PPARγ and adiponectin mRNA expression compared with control. DHA increased adiponectin concentration to a greater extent (40% more, p< 0.05) compared with EPA, emphasizing the need to consider the independent action of EPA and DHA in adipocytes. Both EPA and DHA increase adiponectin concentration in human adipocytes, in vitro, and that EPA elicits the effect only partly via PPARγ, whereas DHA elicits this effect exclusively via PPARγ. Pan et al. conducted a study showing skeletal muscle insulin sensitivity in humans is also strongly influenced by the local supplies of triglycerides. Browning’s study shows improvement in insulin sensitivity after weight-reducing diet plus n-3 PUFA supplementation (1.3g EPA+2.9gDHA) on overweight and obese premenopausal non-diabetic women.

Indices to Measure the Metabolism of Glucose

Alvarez et al. assessed insulin sensitivity in Non-Hispanic Black and White overweight premenopausal women. Insulin sensitivity was tested before and after a weight-loss intervention. Stronger correlation between sensitivity of insulin and proxy indices were found in overweight women than weight-reduced women, and Non-Hispanic Blacks than White women. It means indices reflect insulin sensitivity better among Non-Hispanic Blacks, so it is more accurate to compare insulin resistance generated by the same indices among people of the same ethnicity.

There are several ways to estimate hepatic insulin sensitivity, muscle insulin sensitivity or whole body insulin sensitivity. Since most of the endogenous glucose production (EGP) occurs in the liver, the higher the EGP and fasting plasma insulin
(FGI), the more insulin resistance in liver; so hepatic insulin resistance is demonstrated by EGP×FPI. The greater the muscle insulin resistance and the lower the plasma insulin concentration, the slower the decline in plasma glucose concentration. Thus, skeletal muscle insulin sensitivity can be calculated using the rate of decline in plasma glucose concentration divided by plasma insulin concentration as follows: muscle insulin sensitivity index (MISI) = dG/dt ÷ mean plasma insulin concentration (I) during oral glucose tolerance test (OGTT). Results of this study show that the product of total area under curve (AUC) for glucose and insulin during the first 30 minutes of the OGTT (glucose0-30[AUC]×insulin0-30[AUC]) strongly correlated with the hepatic insulin resistance index (r=0.64, p<0.0001), and the MISI strongly correlated with muscle insulin sensitivity measured with the insulin sensitivity derived from the euglycemic hyperinsulinemic clamp (EHC) (p=0.78, p<0.0001).

Homeostasis model assessment (HOMA-IR)/ ISHOMA is also used to estimate hepatic insulin resistance calculated by fasting insulin (µU/ml)× fasting glucose (mmol/L)/22.5. A longitudinal study of six obese women to compare ISHOMA and glucose utilization rates during HEC (GRI) indicates that HOMA estimation of insulin resistance is accurate for use during both second and third trimesters of pregnancy in obese women with normal glucose tolerance.

ISHOMA and ISQUICKI also correlated with insulin sensitivity from the gold standard: euglycemic hyperinsulinemic clamp, and during late pregnancy, ISHOMA is a better indice than ISQUICKI.

PURPOSE

The purpose of this secondary analysis is to investigate the relationship
between DHA status and insulin sensitivity estimated by fasting insulin, fasting glucose, HOMA-IR, MISI and insulin_{0-30}[AUC]*glucose_{0-30}[AUC] in overweight/obese pregnant women of three racial/ethnic groups (Non-Hispanic Black, Mexican American, or Non-Hispanic White).

**RESEARCH QUESTIONS**

(1) Is there a relationship between DHA status and insulin sensitivity estimated by fasting glucose, fasting insulin, HOMA-IR, MISI and insulin_{0-30}[AUC]*glucose_{0-30}[AUC] in overweight/obese women of different racial groups?

(2) Does ethnicity (being an Non-Hispanic Black or not) influence the effects of DHA status on insulin sensitivity estimated by fasting glucose, fasting insulin, HOMA-IR, MISI, and insulin_{0-30}[AUC]*glucose_{0-30}[AUC]?

(3) Does ethnicity (being a Hispanic or not) influence the effects of DHA status on insulin sensitivity estimated by fasting glucose, fasting insulin, HOMA-IR, MISI, and insulin_{0-30}[AUC]*glucose_{0-30}[AUC]?

(4) How well do ethnicity, DHA status, adiponectin, and TNF-a predict the concentration of insulin sensitivity as estimated by fasting insulin SV3, fasting glucose SV3, and these indices measured during or after the meal challenge (HOMA-IR, MISI and insulin_{0-30}[AUC]*glucose_{0-30}[AUC])?

a. Of these variables, which is the best predictor of insulin sensitivity?

b. If we control for pre-gravid BMI, is this set of variables still able to predict a significant amount of the variance in insulin sensitivity

Null Hypotheses (H_0)

1) There is no relationship between DHA status and insulin sensitivity in Non-Hispanic Black overweight/obese women.
2) There is no relationship between DHA status and insulin sensitivity in Hispanic overweight/obese, pregnant women.

3) There is no relationship between DHA status and insulin sensitivity in white overweight/obese pregnant women.

4) There is no difference of DHA status and insulin sensitivity between Non-Hispanic Black and other overweight/obese women.

5) There is no difference of DHA status and insulin sensitivity between Hispanic and Non-Hispanic Black, White overweight/obese women.

METHODS

This thesis study was a secondary analysis of data collected in a randomized, double-blind, placebo-controlled trial, “DHA, Inflammation, and Insulin Sensitivity in Obese, Pregnant Women,” (National Institutes of Health [5R21HL093532, UL1RR026314-03]. The Institutional Review Boards at the University of Cincinnati, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio and the University of Texas Health Science Center, San Antonio, TX approved the study protocol.

Women were recruited from Cincinnati, Ohio and San Antonio, Texas. Of 142 healthy pregnant women who enrolled in the study, 106 had complete data and were included for analysis. The inclusion criteria were that subjects should be between the ages of 18-40 years, with a singleton pregnancy, and a BMI ≥25 and ≤ 60 kg/m².

The study visits occurred at the General Clinical Research Center located at Cincinnati Children’s Hospital Medical Center for African-American and White subjects and at the University of Texas Health Sciences Center at San Antonio for Hispanic subjects. Women were randomized to the DHA group (800 mg DHA from algae) or placebo group (identical capsule, no DHA) and consumed the capsules for ~
Pre-gravid BMI was calculated from self-reported height and pre-gravid weight. Race and ethnic group was also self-reported using one of the following categories: Asian or Pacific Islander; Black, not Hispanic; Chicano, Latino, or Hispanic; Native American, Native Alaskan, or Indian; White, not Hispanic; or other.

Laboratory Methods

The fasting blood measures used in this secondary analysis were obtained after 10 weeks of DHA supplementation. Fasting blood glucose was analyzed using a glucometer. Fasting plasma insulin concentrations were analyzed using a radioimmunoassay method. Adiponectin was measured by using enzyme linked immunosorbent assays (ELISA) (Millipore, Billerica, MA). RBC (Erythrocyte) DHA was determined by gas chromatography.

To calculate insulin sensitivity, a standardized oral glucose tolerance test, in the form of meal was performed at the end of the study. The meal, 500 kilocalories and 75 grams of carbohydrate, was consumed within 15 minutes. Venous blood samples were drawn by the nursing staff at baseline, 30, 60, 90 and 120 minutes after ingestion of the meal. Glucose measurements were obtained using a bedside glucometer while insulin was determined through venous blood samples.

Insulin sensitivity was estimated by fasting glucose, fasting insulin, and several indices calculated from the meal challenge: Homeostasis model assessment-Insulin resistance (HOMA-IR); Muscle Insulin Sensitivity Index (MISI) and it was calculated as the rate of decline in plasma glucose concentration divided by plasma insulin concentration; the product of total area under curve (AUC) for glucose and
insulin during the first 30 minutes of the OGTT (Insulin$_{0-30}$[AUC]×Glucose$_{0-30}$[AUC]).

Fasting glucose – fasting glucose was supposed to be lower in pregnant women compared to non-pregnant women, the normal range of fasting glucose during pregnancy was less than 95 mg/dL.

Fasting insulin – fasting insulin was supposed to be higher in normal pregnancy compared to non-pregnant women, especially during late pregnancy; this could be due to the increased need of glucose uptake of the fetus.\(^1\)

Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as (glucose × insulin)/22.5 assuming mmol/L units. It was indicated to be accurate for use during both second and third trimesters of pregnancy in obese women with normal glucose tolerance because the metabolic parameters of insulin sensitivity from HOMA-IR were significantly correlated with the rate of glucose disappearance.\(^77\)

Muscle insulin sensitivity index (MISI) was calculated as the rate of decline in plasma glucose concentration divided by plasma insulin concentration.

\[
\text{MISI} = \frac{\text{dG/dt}}{\text{mean plasma insulin concentration (I) during oral glucose tolerance test (OGTT)}}
\]

MISI was strongly correlated with muscle insulin sensitivity measured with the insulin sensitivity derived from the euglycemic hyperinsulinemic clamp (EHC) from a study of 155 Hispanic subjects with normal glucose tolerance and impaired glucose tolerance in both gender (p=0.78, p<0.0001).\(^75\)

The product of total area under curve for glucose and insulin during the first 30 minutes of the OGTT was calculated by glucose$_{0-30}$[AUC]×insulin$_{0-30}$[AUC] and it was strongly correlated with the hepatic insulin resistance index from the same study of 155 Hispanic subjects.\(^75\) (r=0.64, p<0.0001).
STATISTICAL ANALYSES

The statistical significance was set at a p value of <0.05. Demographic characteristics, RBC DHA, and pre-gravid BMI of overweight/obese women grouped by race/ethnicity and by DHA status were compared using the independent samples t-test. To determine differences in insulin sensitivity between groups, the independent variables were race/ethnicity (Non-Hispanic Black, Hispanic or Non-Hispanic White) and DHA status (high/low) and the dependent variables were fasting glucose, fasting insulin, HOMA-IR, MISI and insulin$_{0-30}$[AUC]*glucose$_{0-30}$[AUC]. For each racial or ethnic group, a two-way ANOVA was used to compare the mean concentrations of insulin sensitivity with DHA status as a predictor and pre-gravid BMI as a covariate (ANCOVA), if it was a significant contributor to the model. All the dependent variables were log-transformed to correct for skewed distributions. Multiple regression analyses were used to assess how much of the variance in insulin sensitivity could be explained by the independent variables ethnicity, DHA status, TNF-α, and adiponectin. The assumptions of normality, linearity, homoscedasticity, and independence of residuals were not violated. All analyses were performed using SPSS (version 21, 2012).

RESULTS

Of the 106 women who completed the study, 30 % were African-American, 41% were Hispanic, and 29% were White. At baseline, there was no significant difference in age, education concentration and pre-gravid BMI between the groups. There was a significant difference in red blood cell DHA (RBC DHA) at the end of the study between the women from the three racial/ethnic groups; Hispanic women had
significantly higher concentrations of RBC DHA (mean=8.20 % of total fatty acids) compared to Non-Hispanic Black women (mean=5.91%) and White women (mean=5.48%) (p<0.001).

Table 1
Characteristics of overweight/obese women grouped by race/ethnicity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Hispanic Black</th>
<th>Hispanic</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry (yrs.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age at entry (yrs.)</td>
<td>33</td>
<td>28.27</td>
<td>4.75</td>
</tr>
<tr>
<td>Education (yrs.)</td>
<td>26</td>
<td>12.23</td>
<td>2.89</td>
</tr>
<tr>
<td>RBC DHA (%)</td>
<td>32</td>
<td>5.91**</td>
<td>2.50</td>
</tr>
<tr>
<td>Pre-gravid BMI kg/m2</td>
<td>50</td>
<td>33.09</td>
<td>6.94</td>
</tr>
</tbody>
</table>

** p<0.001

There was a significant difference in the demographic characteristics of overweight/obese women between women grouped by DHA status (Table 2). RBC DHA in Hispanic overweight/obese pregnant women was significantly higher than that in their Non-Hispanic Black, or White counterparts (p<0.001). As expected, the low-DHA group had less RBC DHA than the high DHA group, 4.60% vs. 8.82%, respectively (p<0.001).

Table 2
Characteristics of overweight/obese women by DHA status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RBC DHA Status</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 6.3615%</td>
<td>&gt; 6.3616%</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>Age in study entry (years)</td>
<td>53</td>
<td>28.20</td>
<td>4.83</td>
<td>53</td>
</tr>
<tr>
<td>Education (years)</td>
<td>40</td>
<td>13.22</td>
<td>4.83</td>
<td>39</td>
</tr>
<tr>
<td>RBC DHA (%)</td>
<td>53</td>
<td>4.60**</td>
<td>1.36</td>
<td>53</td>
</tr>
</tbody>
</table>
In Non-Hispanic Black women, insulin sensitivity measures were not significantly different between women at high or low DHA status (Table 3). In ANCOVA, all means of the dependent variables from low-DHA status group are not significantly different from means of the other group. The means of those data are presented in Table 3. ANCOVA was used adjusting for pre-pregnancy BMI, there was no significant interaction effect found for all 5 dependent variables, neither of the main effects were statistically significant for fasting insulin, HOMA-IR, MISI and insulin$_{0-30}$[AUC]*glucose$_{0-30}$[AUC].

There was a relationship between being Non-Hispanic black and fasting glucose concentration, indicated by a partial eta squared value of 0.077, p=0.005.

Table 4

<table>
<thead>
<tr>
<th>RBC DHA Status</th>
<th>&lt; 6.3615</th>
<th>&gt; 6.3616</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>19</td>
<td>84.84</td>
<td>12.08</td>
</tr>
<tr>
<td>Insulin (uU/ml)</td>
<td>19</td>
<td>23.53</td>
<td>11.62</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>19</td>
<td>5.05</td>
<td>3.01</td>
</tr>
<tr>
<td>MISI</td>
<td>19</td>
<td>.29</td>
<td>.21</td>
</tr>
<tr>
<td>Insulin$<em>{0-30}$[AUC]*glucose$</em>{0-30}$[AUC]</td>
<td>16</td>
<td>2436</td>
<td>1162</td>
</tr>
</tbody>
</table>
For Hispanic women, Spearman’s rho correlation was run, and there was no relationship between all the dependent variables and pre-gravid BMI. Data explore was run to examine the normality of the data, it turns out data of fasting glucose and insulin0-30[AUC]*glucose0-30[AUC], log transformed fasting insulin, HOMA-IR and MISI were normally distributed. T-test was used to investigate the relationship of the means of those 5 variables in different DHA status groups. All means of the dependent variables from low-DHA status group are not significantly different from means of the other group. The means of those data are presented in Table 4. For Hispanic women, after adjusting for pre-pregnancy BMI, there was no significant interaction effect found for all five dependent variables, and neither of the main effects were statistically significant for MISI and insulin1-30[AUC]*glucose1-30[AUC]. There was a relationship between being Hispanic (not White and Non-Hispanic Black) and fasting glucose, and 20.5% of the variance in fasting glucose could be explained by being in the Hispanic ethnic group, p=0.000. There was a relationship between being Hispanic and fasting insulin concentration, indicated by a partial eta squared value of 0.053, p=0.022. There was a relationship between being Hispanic and HOMA-IR, as indicated by a partial eta squared value of 0.090, and p=0.003.
### Table 5

**Insulin sensitivity in white overweight/obese pregnant women grouped by DHA status**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RBC DHA Status</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 6.3615</td>
<td>&gt; 6.3616</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>21</td>
<td>86.62</td>
<td>7.93</td>
<td>10</td>
<td>85.01</td>
</tr>
<tr>
<td>Insulin uU/ml</td>
<td>21</td>
<td>29.71</td>
<td>11.19</td>
<td>10</td>
<td>27.04</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>21</td>
<td>6.43</td>
<td>2.68</td>
<td>10</td>
<td>5.87</td>
</tr>
<tr>
<td>MISI</td>
<td>21</td>
<td>.26</td>
<td>.16</td>
<td>10</td>
<td>.30</td>
</tr>
<tr>
<td>Insulin0-30 [AUC]*</td>
<td>21</td>
<td>3427</td>
<td>1490</td>
<td>10</td>
<td>3160</td>
</tr>
<tr>
<td>Glucose0-30 [AUC]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For White women, Spearman’s rho correlation was run, and pre-gravid BMI is related to fasting insulin (r=0.519, p=0.002) and HOMA-IR (r=0.512, p=0.003). Data explore was run to examine the normality of the data, resulting in a normal distribution of the data except for insulin0-30 [AUC]*glucose0-30 [AUC], so insulin0-30 [AUC]*glucose0-30 [AUC] was log transformed and reached normal distribution. T-test was used to investigate the relationship of the means of fasting glucose, MISI and insulin0-30 [AUC]*glucose0-30 [AUC] (log transformed) in different DHA status groups. There was no significant difference found. Means of fasting insulin and HOMA-IR were investigated using on-way ANCOVA with pre-gravid BMI as a covariate. Again, there was no significant difference. The means of the independent variables of White pregnant women was presented in Table 5. For White women, after adjusting for pre-pregnancy BMI, there was no significant interaction effect found for all five dependent variables, and neither of the main effects were statistically significant for fasting insulin, MISI and insulin0-30 [AUC]*glucose0-30 [AUC]. There was a relationship between being White (not Hispanic or Non-Hispanic Black) and HOMA-IR, as indicated by a partial eta squared value of 0.053, p=0.022. There was a relationship between being White and fasting insulin concentrations, but
only 4.2% of the variance in fasting insulin could be explained by being in the White group, \(p=0.037\).

### Table 6

**Insulin sensitivity of Non-Hispanic Black women compared to Hispanic and Non-Hispanic White women**

<table>
<thead>
<tr>
<th>Measures of insulin sensitivity</th>
<th>Race/Ethnicity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hispanic and Non-Hispanic White</td>
<td>Non-Hispanic Black</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>81</td>
<td>87(^a)</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>25.14</td>
<td>24.82</td>
<td></td>
</tr>
<tr>
<td>HOMAIR</td>
<td>5.18</td>
<td>5.46</td>
<td></td>
</tr>
<tr>
<td>MISI</td>
<td>.30</td>
<td>.30</td>
<td></td>
</tr>
<tr>
<td>Insulin(_{0,30}) [AUC]*</td>
<td></td>
<td>3065.62</td>
<td>2517.37</td>
</tr>
<tr>
<td>Glucose(_{0,30}) [AUC]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a. \ p=0.005\)

Compared to White and Hispanic women, Non-Hispanic Black women’s fasting glucose concentration (86.61mg/dL) was significantly higher (80.95mg/dL). Being Non-Hispanic Black accounted for 7.7% of the variance in fasting glucose.

### Table 7

**Insulin sensitivity of Hispanic women compared to Non-Hispanic Black and Non-Hispanic White women**

<table>
<thead>
<tr>
<th>Measures of insulin sensitivity</th>
<th>Race/Ethnicity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Hispanic Black and White</td>
<td>Hispanic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>87</td>
<td>77(^a)</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>28.05</td>
<td>20.74(^b)</td>
<td></td>
</tr>
<tr>
<td>HOMAIR</td>
<td>6.01</td>
<td>4.16(^c)</td>
<td></td>
</tr>
<tr>
<td>MISI</td>
<td>.28</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>Insulin(_{0,30}) [AUC]*</td>
<td></td>
<td>3020.43</td>
<td>2744.06</td>
</tr>
<tr>
<td>Glucose(_{0,30}) [AUC]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a. \ p=0.000\)

\(b. \ p=0.022\)

\(c. \ p=0.003\)

Compared to Non-Hispanic Blacks and Whites, Hispanic women’s fasting
glucose concentration (76.68 mg/dL) is significantly lower than that (86.46 mg/dL) of both groups. Being Hispanic accounted for 20.5% of the variance in fasting glucose.

Compared to African Americans and Whites, Hispanics’ fasting insulin concentration (20.80 uU/ml) is significantly lower (28.41 uU/ml). Being Hispanic accounted for 5.3% of the variance in fasting glucose.

Compared to Non-Hispanic Blacks and Whites, Hispanics’ HOMA-IR concentration (4.15) is significantly lower (5.91). While Hispanic women had significantly higher concentrations of RBC DHA compared to Non-Hispanic black and white women (Table 1).

Being Hispanic accounted 9% of the variance in fasting glucose.

We do not have the ability at this time to look at insulin sensitivity measures in Whites compared to African Americans and Hispanics.

**Regression test**

Even after transformation, the distribution of fasting glucose was skewed; therefore, a Kruskal-Wallis test was performed to compare high and low DHA status groups of varying racial ethnic groups. The median fasting glucose for low DHA status compared to the high DHA status was 84.84 and 87.23, respectively. (p = .033)

**Table 8 : Correlations between adiponectin, TNF-a and fasting insulin**

<table>
<thead>
<tr>
<th>Adiponectin</th>
<th>TNF-α</th>
<th>Fasting insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td>1</td>
<td>-.244*</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>.011</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Pearson Correlation</td>
<td>-.244*</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>.011</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>Pearson Correlation</td>
<td>-.324**</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>.001</td>
</tr>
</tbody>
</table>
Adiponectin was negatively associated with fasting insulin (r=-0.324, p=0.001), TNF-α (r=-0.244, p=0.011) and being White (r=-0.368, p=0.000).

The model containing DHA status, African American, Hispanic, White, TNF-α, and adiponectin explained 7% of the variance in fasting insulin in the last trimester. Of all these variables, only adiponectin made a significant contribution to the model when the variance explained by the other independent variables was controlled for.

**Table 9: Correlations between HOMA-IR and TNF-α, adiponectin**

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>Pearson Correlation</td>
<td>-.449**</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>.000</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 concentration (2-tailed).
**Correlation is significant at the 0.01 concentration (2-tailed).

HOMA-IR was negatively correlated with adiponectin (r=-.449, p=.000)

In this model containing (DHA status, Hispanic, Non-Hispanic White, Non-Hispanic Black, Adiponectin, TNF-α and pre-gravid BMI) accounted for 23.8% of the variance in HOMA-IR (p<0.0001); of these variables, only adiponectin and pre-gravid BMI made a significant contribution to the model, when the other independent variables were controlled for. Adiponectin uniquely explained 12.1% of the variance in HOMA-IR and pre-gravid BMI uniquely explained 7.3% of the variance in HOMA-IR.
MISI is positively related with adiponectin ($r=0.260$, $p=0.009$).

In this model containing DHA status, Hispanic, White, Non-Hispanic Black, Adiponectin, TNF-α and pre-gravid BMI it could not significantly predict MISI ($p=0.118$), but of these variables, adiponectin made a significant contribution to the model ($p=0.045$), when the other independent variables were controlled for, adiponectin uniquely explained 5.38 % of the variance in MISI.

### Table 10: Correlations between MISI and TNF-α, adiponectin

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>MISI</td>
<td>Pearson Correlation</td>
<td>.260**</td>
</tr>
<tr>
<td>p</td>
<td>.009</td>
<td>.093</td>
</tr>
</tbody>
</table>

### Table 11: Correlations between Insulin$_{0-30}$ [ACU]*glucose$_{0-30}$[AUC] and TNF-α, adiponectin

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin$<em>{0-30}$ [ACU]*glucose$</em>{0-30}$[AUC]</td>
<td>Pearson Correlation</td>
<td>-.245**</td>
</tr>
<tr>
<td>p</td>
<td>.011</td>
<td>.269</td>
</tr>
</tbody>
</table>

Insulin$_{0-30}$[ACU]*glucose$_{0-30}$[AUC] is negatively correlated to adiponectin ($r=-0.245$, $p=0.011$). In this model containing DHA status, Hispanic, White, Non-Hispanic Black, Adiponectin, TNF-α and pre-gravid BMI, it could not significantly predict Insulin$_{0-30}$[ACU]*glucose$_{0-30}$[AUC], $p=0.724$.
For all the overweight/obese women, the variance in fasting insulin could be represented by the model as a whole as 14.6%, $F(7,93)=2.264$, $p=0.036$. The variance in HOMA-IR could be explained by the model as a whole as 22.2%, $F(6,94)=4.473$, $p=0.001$. All the possible predictors/independent variables did not predict fasting glucose, MISI and insulin$_{1-30}$ [AUC]*glucose$_{1-30}$ [AUC]. No independent variable could predict the value of fasting glucose.

Adiponectin has a negative correlation with HOMA-IR, fasting insulin, and insulin$_{1-30}$ [AUC]*glucose$_{1-30}$ [AUC]; it represented 10.30% of the variance in HOMA-IR, 4.75% of the variance in fasting insulin, and 4.97% of the variance in insulin$_{1-30}$ [AUC]*glucose$_{1-30}$ [AUC]. Adiponectin has a positive correlation with MISI, and it explained 3.84% of the variance in MISI.

**DISCUSSION**

To our knowledge, no study was done investigating DHA status and insulin...
sensitivity estimated by fasting glucose, fasting insulin, HOMA-IR, MISI and insulin$_{0-30}$[AUC]*glucose$_{0-30}$[AUC] values, but there were several studies investigating the effect of DHA on insulin sensitivity. Some of the studies did not test or provide the DHA status before and after study. $^{79,80}$ Two studies on humans tested the DHA concentration. One investigated supplementation of DHA (mixed with EPA), 2.4g/d for 8 weeks were given to 35 patients with chronic renal failure on maintenance hemodialysis. After the study, DHA concentration increased significantly in the red blood cells and HOMA-IR significantly decreased (p=0.001) $^{81}$ The other study conducted by Richard J Woodman found that after supplementation of 4g DHA /d for 6 weeks, senior men and women (age 61.2+/−1.2ys) had a significant (p=0.002) increase of fasting glucose. $^{82}$ In this study, the researchers used the change of DHA concentration (positive or negative for the direction). A higher concentration of DHA (an increased concentration after treatment) is related to a higher concentration of fasting glucose (baseline =149mg/dL, study end=158mg/dL), indicating an increase in severity of insulin resistance with higher concentration DHA, while the insulin sensitivity of the subjects were already impaired. In our study mean fasting glucose in high-DHA status was compared to low-DHA status (Non-Hispanic Black: mean=85.81, higher in normal range; Hispanic: 77.42, lower; White: 86.10, lower). Even though fasting glucose did not significantly differ for each race/ethnic group, in Non-Hispanic Blacks, there was a relationship between being in this ethnic group and fasting glucose concentration, indicated by a partial eta squared value of 0.077, p=0.005. 20.5% of the variance in fasting glucose could be explained by being Hispanic, p=0.000. Higher DHA in FFAs increase the fluidity of the cell membrane, hence the increase in the residency time of glucose transporters type 1 and 4. $^{83}$ GLUT 4 is the only glucose transporter protein that could be regulated by insulin, which
exists in muscle, heart, brown and white adipocytes. It is possible that the increased residency time of glucose transporter protein allowed more insulin to bind to, and insulin acted on an increased amount of GLUT4s and increased skeletal muscle glycogen synthesis, so fasting glucose was expected to be lower in subjects with normal glucose metabolic function.

This might also explain the correlation of DHA status, MISI, and insulin0-30 [AUC]*glucose0-30 [AUC], which is representative of hepatic insulin resistance, because GLUT4 exists throughout the body. According to Clore et al., fatty acid composition of skeletal muscle phospholipids was related to peripheral insulin sensitivity in humans, while MISI is an accurate index strongly correlated with muscle insulin sensitivity measured using the gold standard, euglycemic hyperinsulinemic clamp method. D.A. Pan’s study also confirmed this conclusion.

In most of the studies conducted to investigate insulin sensitivity, VAT, and adiponectin concentrations between Hispanic, African American and Whites, Hispanic overweight/obese women had a relatively higher concentration of adiponectin and lower TNF-α concentration. BMI from the survey did not show any difference between these ethnic groups. It is reasonable to conclude that insulin resistance in different ethnic groups could be related to different concentrations of adiponectin and TNF-α. From Table 1, DHA status in Hispanic women was significantly higher than Africa Americans and whites (p<0.001), indicating that DHA could be an factor affecting TNF-α concentration and hence the adiponectin concentration, or independently act on both of them. Studies of pregnant and non-pregnant women suggest that there was a decrease in adiponectin concentration with advancing gestation.
In Lee et al.’s study on healthy Non-Hispanic Black and White youth, adiponectin was a strong independent predictor of insulin sensitivity and represented 27% of the variance in insulin sensitivity measured by a 3-h hyperinsulinemic euglycemic clamp. In our study, adiponectin represented 10.30% of the variance in HOMA-IR, 4.75% of variance in fasting insulin, 4.97% of variance in insulin$_{1,30}$[AUC]*glucose$_{1,30}$[AUC], and 3.84% of the variance in MISI.

CONCLUSION

There is no relationship between DHA status and insulin sensitivity in overweight/obese and pregnant women from each of the three-race/two-ethnicity groups (Non-Hispanic Black, Hispanic, and Non-Hispanic White).

Compared to Non-Hispanic Blacks and Whites, Hispanic women has significantly lower concentration of fasting glucose, fasting insulin, and HOMA-IR, while Hispanic women had significantly higher concentration of DHA than the other 2 groups.

Adiponectin can predict insulin sensitivity estimated by HOMA-IR, fasting insulin, insulin$_{1,30}$[AUC]*glucose$_{1,30}$[AUC], and MISI.
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71. Tishinsky JM, Ma DW, Robinson LE. Eicosapentaenoic acid and rosiglitazone increase adiponectin in an additive and PPARgamma-dependent manner in human adipocytes. *Obesity (Silver Spring).* 2011;19:262-268.


