Effect of Prenatal EPA and DHA on Maternal and Cord Blood Insulin Sensitivity: A secondary analysis of the mothers, omega 3, and mental health study

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Joey A. England
joey.a.england@uth.tmc.edu
ORCiD: https://orcid.org/0000-0001-6292-3220

Joses Jain
University of New Mexico

Bradley D. Holbrook
University of New Mexico

Ronald Schrader
University of New Mexico

Clifford Qualls
University of New Mexico

Ellen Mozurkewich
University of New Mexico Health Sciences Center

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Abstract

Background
We sought to determine whether prenatal supplementation with the omega-3 fatty acids eicosapentaenoic acid (EPA) or docosahexanoic acid (DHA) would increase markers of insulin sensitivity in maternal or cord blood compared with placebo supplementation and to evaluate the association of serum EPA and DHA fractions as well as plasma 25-OH vitamin D with adiponectin, leptin and the adiponectin:leptin ratio (ALR). We hypothesized that omega-3 fatty acid supplementation would increase markers of insulin sensitivity in maternal and umbilical cord plasma.

Methods
We analyzed stored plasma samples collected from a prior 3-arm prospective, double-blinded, randomized controlled trial in which 126 women with singleton pregnancies between 12- and 20-weeks gestation were randomized to receive: 1) an EPA-rich fish oil supplement, 2) a DHA-rich fish oil supplement, or 3) a soy oil placebo. Maternal venous blood samples were collected at 12-20 weeks gestation (before supplementation) and at 34-36 weeks gestation. At delivery, cord blood was collected. Samples were analyzed using sandwich enzyme-linked immunosorbent assay kits to quantify leptin and adiponectin levels which were utilized to calculate the ALR, a proxy measure for insulin sensitivity.

Results
We found no difference in adiponectin, leptin, and the ALR between the treatment and placebo groups at baseline, after supplementation, or in umbilical cord blood. In regression analyses, higher maternal serum DHA fraction was associated with increased ALR before (p = 0.01) and after (p = 0.04) DHA supplementation. Vitamin D at enrollment was also significantly associated with adiponectin (p < 0.05). Early pregnancy BMI was significantly associated with maternal leptin levels at baseline and in late pregnancy (p < 0.001) and was inversely associated with the ALR (p < 0.001). The ALR decreased significantly between the early and late pregnancy visits (p < 0.001). There was no association of EPA fraction with any measure of insulin sensitivity. Cord blood DHA fraction was significantly associated with cord plasma leptin (p = 0.02).
Conclusions
EPA- and DHA- rich fish oil supplementation had no effect on plasma markers of insulin sensitivity. However, maternal serum DHA fraction and plasma 25-OH vitamin D were significantly associated with markers of insulin sensitivity.

Introduction
Insulin resistance is associated with several pregnancy-associated morbidities including gestational diabetes, increased birth weight, and increased risk of hypertensive disease. Recent estimated prevalence of gestational diabetes mellitus was estimated as 6% among women who had a live birth in the United States between 2012 and 2016. Women with gestational diabetes have an increased risk of developing Type II diabetes in later life; a systematic review and meta-analysis involving 20 studies that included 675,455 women found that 12.5 percent of women with gestational diabetes developed Type II diabetes between 6 weeks after delivery and the end of the various follow-up periods. Risks to the neonate include: hypoglycemia, respiratory distress, cardiac dysfunction and birth injury due to macrosomia. Effects of maternal insulin resistance are not limited to the neonatal timeframe, but may also predispose the child to obesity and type 2 diabetes.

It is not known whether dietary factors during pregnancy may influence the risk for patients to develop insulin resistance and gestational diabetes mellitus. In rodent models, dietary supplementation with omega-3 fatty acid docosahexanoic acid (DHA) during pregnancy has been shown to be insulin-sensitizing. In several studies in rodents, the omega-3 fatty acids DHA and eicosapentaenoic acid (EPA), have been shown to facilitate formation of insulin sensitizing adipokines such as adiponectin and reduce adipokines associated with insulin resistance. However, it is not known if EPA- and DHA-rich fish oil supplementation would increase markers of insulin sensitivity in human pregnancy.

Other dietary factors may also influence insulin sensitivity. For example, Vitamin D supplementation has been reported to be insulin-sensitizing in non-pregnant individuals.

The gold standard test to measure insulin resistance and beta-cell function is the Homeostatic Model Assessment (HOMA-IR test). This was first described in 1985 by Matthews. The approximating
equation for insulin resistance, used a fasting plasma sample and was delivered by the insulin-glucose product divided by a constant. This model correlates well with estimates using the euglycemic clamp method. However this test requires an overnight fast.\textsuperscript{11} In situations in which the HOMA-IR test is impractical, the adipose secreted proteins, adiponectin and leptin may be used as proxy measures of insulin sensitivity and resistance during pregnancy.\textsuperscript{12} In particular, the adiponectin:leptin ratio (ALR) has been shown to be inversely related to the HOMA-IR test.\textsuperscript{12}

Our study objective was to determine whether maternal EPA- and DHA-rich fish oil supplementation would increase markers of insulin sensitivity in maternal and cord blood plasma compared with placebo supplementation. We hypothesized that maternal EPA- and DHA-rich fish oil supplementation would increase plasma adiponectin and increase the adiponectin:leptin ratio. We also sought to evaluate the association of DHA, EPA, and Vitamin D levels with adiponectin, leptin, and the ALR. We further hypothesized that maternal and umbilical cord DHA serum percentage and 25-OH vitamin D would be associated with insulin sensitivity in maternal and fetal cord plasma. Our null hypothesis was that maternal fish oil supplementation would lead to no difference in plasma levels of adiponectin and leptin in either maternal or fetal samples compared with controls.

Materials And Methods

We conducted this analysis using maternal plasma samples collected from a prior investigation, “The Mothers, Omega-3, and Mental Health Study,” a 3-armed prospective, double-blinded, randomized controlled trial designed to test whether EPA- or DHA-fish oil supplementation would prevent perinatal depressive symptoms among women at risk. The full details of the trial have been previously described.\textsuperscript{12,13} In brief, patients with singleton pregnancies between 12- and 20-weeks gestation were recruited. Subjects were selected based on an elevated risk for depression, defined as a past history of major depressive disorder, a history of postpartum depression, or an Edinburgh Postnatal Depression Scale (EPDS) score between 9 and 19. Exclusion criteria included age <18, current major depressive disorder diagnosis, bipolar disorder or schizophrenia, substance abuse disorder, history of bleeding disorder, or clotting disorder requiring anticoagulation.\textsuperscript{13,14}

Women who met eligibility criteria and who consented to participate were randomized to one of
three arms: 1) an EPA-rich fish oil supplement (1060 mg EPA plus 274 mg DHA), 2) a DHA-rich fish oil supplement (900 mg DHA plus 180 mg EPA), or 3) a soy oil placebo. Because the DHA- and EPA-rich fish oil capsules were not identical in appearance, all participants took some placebo capsules (double-dummy design). Full details of the randomization procedure and the supplements are described elsewhere. ¹³,¹⁴

Maternal venous blood samples were collected after a three hour fast; baseline samples were collected at the time of study enrollment at 12-20 weeks gestation (visit 1), and again between 34- and 36-weeks’ gestation (visit 3). After delivery, a sample of fetal blood was collected from the umbilical cord (visit 4). Samples were centrifuged within 12 hours of collection and plasma aliquots were stored at -70 degrees Celsius.

Stored maternal and umbilical cord plasma samples from participants of the study were analyzed using commercially-available sandwich enzyme-linked immunosorbent assay kits (EMD Millipore, St. Charles, MO) to quantify leptin and adiponectin levels according to the manufacturer’s protocols. ¹⁵,¹⁶ The only variation from the protocol was a dilution of fetal adiponectin samples to 1:1000 as opposed to 1:500 for maternal samples, based on higher fetal levels of adiponectin. Absorbance of both adiponectin and leptin were measured at 450 nm and 690 nm and the results compared. 25-hydroxyvitamin D levels, as well as DHA and EPA fractions in maternal and cord blood serum had been previously assayed. ¹⁴,¹⁷

ETHICS:

The procedures followed were approved and conducted in accordance with the institutional review boards of the University of Michigan Medical Center, Ann Arbor, MI, St. Joseph Mercy Hospital in Ypsilanti, MI, and The University of New Mexico Health Sciences Center Human Research Protections Office. All subjects gave written informed consent to participate in the study. The trial was registered on 7/7/2008 at clinicaltrials.gov: NCT00711971 under the title: “Does Fish Oil Prevent Depression in Pregnancy and Postpartum”. The secondary blood sample analyses described in this manuscript were judged exempt by the University of New Mexico Health Sciences Center Human Research Protections Office.
Statistics: Demographic variables were compared among three randomized groups (EPA-rich fish oil, DHA-rich fish oil, and placebo) as means and standard deviations if continuous or ordinal scale using 1-way ANOVA and as frequencies using Fisher’s exact test. We computed medians, and interquartile ranges for each outcome parameter, adiponectin, leptin and the adiponectin:leptin ratio (ALR). Using raw (unadjusted) data, in order to test whether these parameters changed over time or were different between groups we performed repeated measures ANOVA with group as grouping factor and visit as repeated factor. The analysis of the ALR was also performed using repeated measures ANOVA with time (enrollment before supplementation, and after supplementation) as a repeated factor and 3 groups as a grouping factor. This was an intent-to-treat analysis in that all available data was used. Adiponectin and leptin, constituents of ALR, were analyzed similarly. Because box plot analysis of the data revealed non-normality, we also analyzed these data after square root transformation.

Covariate adjustments were done for BMI, maternal weight gain, age, and ethnicity. Post hoc analyses of trend in our measures of insulin sensitivity in each group were done by regression. In addition, multivariable regression analyses exploring the relationships between measured serum DHA and EPA fractions and 25-OH vitamin D concentrations with measures of insulin sensitivity were done in the three groups and two maternal time points pooled unless otherwise specified; other predictors included BMI and age at enrollment. We calculated the effect of a one unit increase in each of the predictive variables on the ALR using the two-tailed inverse student's T distribution.

We calculated the medians and interquartile ranges for adiponectin, leptin and the ALR in umbilical cord blood. We used raw unadjusted data, as well as square root transformation to evaluate difference according to group assignment using ANOVA. Covariate adjustments were done for birth weight and length of gestation. The association of DHA and EPA fractions, as well as 25-OH vitamin D concentrations in cord blood with measures of insulin sensitivity were analyzed by multivariable linear regression. P-values ≤ 0.05 were considered statistically significant.

Results

There were 126 women who were randomized, of whom 118 completed the trial. For this secondary analysis, there were 113 plasma samples available at trial entry, 109 samples available
post-supplementation (34-36 weeks) as well as 98 cord blood samples. The flow of participants and samples is shown in Figure 1.

The baseline characteristics of the three randomized groups are shown in Table 1. There was no significant difference in the baseline characteristics between the three randomized groups.

**TABLE 1**  
*Demographics*

| Variable                        | EPA-rich fish oil group | DHA-rich fish oil group | Soy oil placebo group | Significance |
|---------------------------------|-------------------------|-------------------------|-----------------------|-------------|
| Maternal age at enrollment      | 29.9 +/- 5.2            | 30.7 +/- 4.4            | 30.4 +/- 5.9          | NS*         |
| White race                      | 32                      | 28                      | 34                    | NS‡         |
| Non-white                       | 4                       | 9                       | 6                     |             |
| BMI at enrollment               | 28.5 +/- 6.3            | 28.5 +/- 6.7            | 27.7 +/- 8.2          | NS*         |
| Gestational age at enrollment (weeks) | 15.9/2.7              | 16.9/2.3               | 16.0/2.3             | NS*         |
| Gravidity                       | 2.42/1.36               | 2.55/1.22              | 2.41/2.02            | NS‡         |
| Parity                          | 0.87/0.84               | 1.08/0.94              | 0.85/1.20            | NS‡         |

NS = not significant  
*1-way ANOVA  
‡ Fisher’s Exact test

Our primary intent-to-treat analysis using repeated-measures modeling showed no difference in adiponectin, leptin, and the ALR in maternal plasma between the treatment groups and placebo group at baseline or after supplementation. (Table 2). These results were unchanged after square root transformation of the data.

**TABLE 2**  
*Adipokine values before and after supplementation according to treatment group*
| Parameter       | Visit | EPA            | DHA            | Placebo        | RM ANOVA P values |
|-----------------|-------|----------------|----------------|----------------|-------------------|
|                 |       |                |                |                | Group  | Visit | Interaction |
| Adiponectin     | 1     | 24.15 (17.72,29.70) | 26.15 (14.94,38.04) | 25.29 (15.56,33.69) | 0.0.83 | <0.001 | 0.97        |
|                 | 3     | 18.69 (13.79,22.05) | 19.91 (12.95,27.90) | 18.92 (14.20,25.71) |        |        |             |
| Leptin          | 1     | 27.93 (17.38,35.77) | 22.04 (11.46,34.44) | 19.72 (15.04,28.34) | 0.13   | 0.32   | 0.86        |
|                 | 3     | 28.70 (19.69,47.33) | 24.38 (15.97,33.34) | 23.91 (16.97,32.72) |        |        |             |
| A:L Ratio       | 1     | 0.83 (0.41,1.63)  | 1.44 (0.44,2.32)  | 1.17 (0.52,1.97)  | 0.60   | <0.001 | 0.92        |
|                 | 3     | 0.54 (0.34,0.91)  | 0.77 (0.50,1.54)  | 0.75 (0.36,1.26)  |        |        |             |

Note. Cell formats are median (IQR).

Legend: Adipokine values are summarized as median and interquartile range. The last 3 columns list ANOVA P values. The ANOVA design is three groups times two visits.

Of interest, for the total cohort adiponectin significantly decreased between enrollment and 33-36 weeks gestation (p<0.001) while leptin significantly increased during the same time period (p=0.008). The ALR significantly decreased during this same time period (p<0.001) indicating increasing insulin resistance with advancing pregnancy. The magnitude of decrease in the ALR was 31%.

We performed regression analyses to explore the relationships between measured serum DHA, EPA and vitamin D concentrations with measures of insulin sensitivity. Variables included in the model included BMI at enrollment, DHA fraction, EPA fraction, vitamin D, and age at enrollment. After adjusting for BMI and maternal age, higher maternal serum DHA fraction was significantly associated with increased ALR before (p= 0.01) and after (p= 0.04) DHA supplementation. DHA fraction was significantly associated with adiponectin after supplementation (p= 0.03); there was a non-significant trend towards association of DHA with adiponectin at study enrollment. Vitamin D was also significantly associated with adiponectin (p<0.05) at enrollment but was not significantly associated with adiponectin after supplementation.

Early pregnancy BMI was significantly associated with maternal leptin levels at baseline and in late pregnancy (p< 0.001) and was inversely associated with the ALR (p< 0.001). ALR decreased
significantly between the early and late pregnancy visit (p< 0.001). There was no association of EPA with any measure of insulin sensitivity.

Maternal weight gain between the early pregnancy visit and late pregnancy visit was inversely associated with adiponectin and the ALR, even after adjusting for baseline BMI. The effect of a one unit increase in each of the variables of interest on the ALR is shown in Table 3.

**TABLE 3**

| Variables predictive of the change (delta) in the ALR |
|------------------------------------------------------|
| **Variable**                                           | **Estimate** | **95 % CI**        |
| *Pregnancy Weight Gain (1 kg)*                        | -0.04 (p<0.02) | [-0.07, -0.007]   |
| *Study Enrollment BMI*                                 | -0.03 (p<0.0001) | [-0.04, -0.02]   |
| *After Supplementation BMI*                            | -0.02 (p<0.001) | [-0.03, -0.01]   |
| *Study Enrollment DHA (1 unit)*                        | 0.2 (p<0.02)   | [0.02, 0.17]      |
| *After Supplementation DHA (1 unit)*                   | 0.1 (p<0.02)   | [0.003, 0.112]    |

Note. Two-tailed inverse student’s T distribution.

There were no differences in adiponectin, leptin, or the ALR in umbilical cord blood according to maternal treatment allocation group. (Table 4) Regression analysis was performed to evaluate the association of EPA fraction, DHA fraction, vitamin D concentration, birth weight, gestational age at delivery, and mode of delivery with adiponectin, leptin and the ALR in umbilical cord blood. There were no significant associations between the variables of interest on cord blood adiponectin or the ALR. However, birth weight and cord blood DHA fraction were significantly associated with serum leptin (p=0.02, both). The association of birth weight with cord blood leptin persisted when controlling for DHA fraction, and the association of DHA fraction with cord blood leptin persisted when controlling for birth weight.

**TABLE 4**

*Cord blood adipokine values according to treatment group*
| Parameter       | EPA                        | DHA                        | Placebo                      | Group |
|-----------------|----------------------------|----------------------------|-------------------------------|-------|
| Adiponectin     | 52.07 (38.99, 70.98)       | 55.67 (38.81, 68.34)       | 52.40 (43.42, 65.70)         | 0.93  |
| Leptin          | 9.25 (4.60, 13.55)         | 7.98 (4.26, 19.84)         | 7.10 (3.23, 13.71)           | 0.56  |
| A:L Ratio       | 5.85 (3.63, 11.19)         | 6.21 (2.47, 15.07)         | 8.69 (4.03, 19.36)           | 0.27  |

Note. Cell formats are median (IQR).

Legend: Adipokine values are summarized as median and interquartile range.

**Discussion**

The main finding of our study was that DHA- and EPA-rich fish oil supplementation had no significant effect on plasma markers of insulin sensitivity, compared to placebo. However, maternal serum DHA fraction and plasma 25-OH vitamin D were significantly associated with markers of insulin sensitivity. Of interest, we found that maternal BMI and weight gain negatively affect insulin sensitivity as measured by the ALR, increased maternal leptin and decreased maternal adiponectin levels.

The findings of our study were similar to that of the Haghiac trial, which randomly assigned 49 overweight pregnant women to omega-3 fatty acid supplementation conferring 2 grams DHA plus EPA daily; this study found no difference in adiponectin, leptin, or HOMA-IR between the randomized groups. Similarly, a pilot study of 3.36 grams of omega-3 fatty acid supplementation versus matched placebo among 62 hypertensive male adults found no effect of supplementation on serum adiponectin. Similar to our findings, in a Mexican cohort, Solis-Paredis, et al, found maternal leptin levels to be significantly associated with maternal pregnancy weight gain.

Our findings contrast with those of the ComparED study, which randomized 154 otherwise healthy obese non-pregnant adults to receive 2.7 grams of EPA or DHA versus corn oil placebo. In that population, the investigators found that DHA, but not EPA supplementation significantly increased serum adiponectin, compared with corn oil placebo.

Contrary to our expectation, we found serum DHA fraction to be significantly associated with higher cord blood leptin levels. In other investigations, cord blood leptin has been significantly
associated with higher birth weight.\textsuperscript{22,23} Elevated cord blood leptin may confer risk for childhood obesity, through developmental programming.\textsuperscript{22}

Our study had several strengths and limitations. Strengths of our study included the randomized design as well as the double blinded nature of the trial. Availability of both maternal blood and umbilical cord blood was also a strength. However, our study was limited in the respect that our subjects were selected based on predisposition to depression and may not be representative of the general population of pregnant women.

**Conclusions**

In conclusion, our findings suggest that there exists a relationship between maternal DHA and 25-OH vitamin D levels and insulin sensitivity in pregnancy. Maternal BMI and pregnancy weight gain are inversely related to insulin sensitivity during pregnancy.

Future studies may include a well powered randomized controlled trial of maternal DHA and/or vitamin D supplementation in women at risk for insulin resistance in order to augment insulin sensitivity.

**Abbreviations**

EPA = eicosapentaenoic acid  
DHA = docosahexaenoic acid  
ALR = adiponection/leptin ratio

**Declarations**

**Ethics approval and consent to participate**

The procedures followed in the original trial were approved and conducted in accordance with the institutional review boards of the University of Michigan Medical Center, Ann Arbor, MI, study number HUM00004684; St. Joseph Mercy Hospital in Ypsilanti, MI, and The University of New Mexico Health Sciences Center Human Research Protections Office. All subjects gave written, informed consent to participate in the study. The trial was registered at clinicaltrials.gov: NCT00711971 under the title: “Does Fish Oil Prevent Depression in Pregnancy and Postpartum”. The secondary blood sample
analysis described in this study was approved by the University of Michigan Medical Center Institutional Review Board, and was determined to be exempt by the University of New Mexico Health Sciences Center Human Research Protections Office

Consent for publication
Not applicable

Availability of data and material
The dataset from the original trial is the property of the University of Michigan, Ann Arbor, which was made available to the University of New Mexico under a formal data sharing agreement. The data generated through the secondary blood sample analysis described in this manuscript are the property of the University of New Mexico Health Sciences Center. The data will not be publically available. The plasma specimens described in this manuscript were made available to the investigators at the University of New Mexico under a material transfer agreement. They have been analyzed and destroyed, and as such will not be available for further analyses.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
EM, and JE designed the study.
JE, JJ, and BH performed the blood sample analyses described in the study.
RS and CQ analyzed the data.
JE, CQ and EM wrote the paper.
All authors read and approved the final manuscript.

This study was previously presented in poster format at the 62nd Annual Scientific Meeting for the Society for Reproductive Investigation, San Francisco, CA, March 25-28, 2015.

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University of New Mexico Clinical and Translational Science Center Laboratory

Authors’ information

Correspondence: Joey A. England, MD (joey.a.england@uth.tmc.edu), Department of Obstetrics, Gynecology and Reproductive Sciences, McGovern Medical School, University of Texas Health Science Center at Houston, 6431 Fannin, MSB 3.286 Houston TX 77030; office phone: 713-500-7780; cell phone: 210-373-1069

Authors’ email addresses:

Joey England, MD (joey.a.england@uth.tmc.edu)
Bradley Holbrook, MD (Bradholbrook@gmail.com)
Ronald Schrader, PhD (RSchrader@salud.unm.edu)
Clifford Qualls, PhD (CQualls@salud.unm.edu)
Ellen Mozurkewich, MD, MS (emozurkewich@salud.unm.edu)

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Figures
Figure 1. CONSORT Flow Diagram

**Enrollment**
- Assessed for eligibility (n=2657)
- Excluded (n=2531)
  - Not meeting initial inclusion criteria (n=2262)
  - Declined to participate (n=234)
  - Not eligible after randomization (n=35)

**Randomized (n=126)**

**Allocation**
- Allocated to EPA intervention (n=42)
  - Received intervention (n=42)
  - Did not receive allocated intervention (n=0)
- Allocated to DHA intervention (n=42)
  - Received intervention (n=42)
  - Did not receive allocated intervention (n=0)
- Allocated to placebo (n=42)
  - Received allocated intervention (n=42)
  - Did not receive allocated intervention (n=0)

**Follow-Up**
- Lost to follow-up (n=3)
  - Discontinued intervention (n=5)
  - Completed trial (N=39)
- Lost to follow-up (n=4)
  - Discontinued intervention (n=4)
  - Completed trial (N=38)
- Lost to follow-up (n=1)
  - Discontinued intervention (N=7)
  - Completed trial (N=41)

**Analysis**
- Analysed plasma samples
  - Visit 1 (n=36)
  - Visit 3 (n=34)
  - Visit 4 (n=32)
- Analysed plasma samples
  - Visit 1 (n=36)
  - Visit 3 (n=34)
  - Visit 4 (n=33)
- Analysed plasma samples
  - Visit 1 (n=41)
  - Visit 3 (n=41)
  - Visit 4 (n=33)