Effect of Docosahexaenoic Acid Supplementation on Glucose Tolerance and Markers of Inflammation in Overweight/Obese Pregnant Women: A Double-blind, Randomized, Controlled Trial

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Introduction

The prevalence of pregravid obesity is a common problem that only recently has stopped increasing in the United States [1]. Women with pregravid overweight or obesity are more likely to exhibit insulin resistance beyond what is observed in normal-weight, pregnant women including gestational impaired glucose tolerance (GIGT) and diabetes mellitus (GDM) [2,3]. Both of these aberrations in glucose tolerance have been associated with higher neonatal fat mass and increased risk of newborn macrosomia [4-6]. In turn, higher maternal insulin sensitivity is associated with lower adiposity in one-year old infants [7]. From early childhood onward, children born to obese mothers exhibit higher risk for metabolic syndrome, obesity, and type 2 diabetes and later premature death [8-10]. Despite the excess risk conferred to both mother and infant, many women fail to achieve a normal body weight prior to conception and often retain adipose tissue gained in pregnancy. Thus, a cycle of obesity, and related sequelae, for both the mother and child begins [11]. Interventions that may impact glucose metabolism related to subsequent obesity, diabetes, and CVD are needed [12].

Subclinical inflammation is one underlying mechanism for impaired glucose metabolism. Elevated serum TNF-α is associated with high levels of C-peptide and insulin resistance and impaired compensation by the β cells in pregnant women of normal body weight [13-16]. In obese women, maternal TNF-α and IL-6 are also positively correlated with fetal adiposity and/or birth size [17,18].

The n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have many anti-inflammatory effects and could potentially improve glucose metabolism. In cell culture, DHA lessened secretion of TNF-α and IL-6 and maintained insulin sensitivity [19,20]. And, in animal studies, DHA/EPA lessened insulin resistance (HOMA-IR) by 60% [21]. Similarly, endothelial cells from the umbilical cord of pregnant women, who consumed two salmon meals per week from 20 weeks gestation until parturition, lowered IL-6 and TNF-α by decreasing gene expression of related genes in the placenta and adipose tissue [22].

DHA supplementation in pregnancy has also been shown in some studies to affect adiposity in the offspring. At birth, infants born to mothers who consumed a functional food fortified with ~ 200 g DHA/d had lower insulin concentrations in umbilical cord blood and
a lower Ponderal Index [24]. Both body weight and body mass index were lower in toddlers whose mothers consumed 200 mg DHA/d from fish oil from mid-pregnancy to parturition [25]. At age 5 years, supplementation with 400 mg DHA/d from ~21 weeks gestation to term was not associated with growth in a cohort of Mexican children [26].

Given the observed putative effects of n-3 LCPUFA, the fetal needs for DHA, and the low intake in pregnant women (75 mg/d, Krummel unpublished data), DHA supplementation could be a potential intervention strategy to improve the metabolic milieu in obese, pregnant women.

The purpose of this randomized, placebo-controlled trial was to determine the effectiveness of 10 weeks of DHA supplementation on glucose tolerance and markers of inflammation measured at the end of the third trimester in overweight/obese pregnant women.

Materials and Method

Study design and subjects

This was a double-blinded, placebo-controlled, randomized clinical trial registered at clinicaltrials.gov (Identifier: NCT00865683). Ninety-one pregnant women (~26 weeks gestation), 18-40 years of age, with a pregravid BMI ≥ 25, and a singleton pregnancy were recruited from the greater Cincinnati, Ohio, USA, metropolitan area for participation. The exclusion criteria included diseases affecting study outcomes (e.g., gestational or other diabetes mellitus, hypertension, or concurrent inflammatory, vascular or metabolic disease); high unusual intake of DHA (more than 1 fish meal per week, use of DHA-fortified foods or supplements); current or previous use of tobacco, illicit drugs, or medications such as corticosteroids that affect inflammatory markers; and inability to travel to the research center for study visits.

Screening of potential subjects was conducted over the telephone using a standardized script and screening form. To determine eligibility for overweight status, self-reported height and body weight were used to calculate pregravid BMI. Individuals meeting all the screening criteria were invited to participate in the study, and scheduled for the first study visit at 26 weeks gestation.

The study was conducted in the General Clinical Research Center (GCRC) which is funded by the National Institutes of Health. Our GCRC is a collaborative entity between the University of Cincinnati (UC) and Cincinnati Children’s Hospital Medical Center (CHMMC), located in Cincinnati, Ohio, USA. All study personnel completed IRB (Institutional Review Board) and University of Cincinnati Academic Health Center, Heath Insurance Portability and Accountability Act (HIPAA) training prior to the start of the study. The study procedures were approved by the IRBs at UC and CHMMC. Also, the study coordinators completed the yearly CITI (Collaborative Institutional Training Initiative) training prior to the beginning of the study.

The study visits (SV) occurred at approximately 26 weeks (SV1), 30 weeks (SV2), and 36 weeks of pregnancy (SV3). After an overnight fast, subjects reported to the GCRC for the study visits for SV1 and SV3. Since only EDHA was measured at SV2, subjects were not in a fasted state at this visit. All participants signed informed consent at SV1. The study participants were randomized to either placebo or DHA group by the pharmacy department at CHMMC prior to dispensing of the gelcap capsules. The randomization scheme was prepared prior to start of the trial by the study pharmacist using a tested system utilizing a random-number generator. Each study subject was assigned to study group on a consecutive basis. Study staff, subjects, and investigators were blinded to the group assignment. At the end of the study, pharmacy staff mailed the study group assignment to the subject and investigators. The gelcaps were identical in size, appearance, taste, and smell (orange flavored). The placebo capsules contained a corn/soy oil blend (11% stearate, 25% oleate, 52% linoleate, no DHA) and the DHA capsules contained algal oil (800 mg DHA)(DSM Nutritional Products ( Parsippany, New Jersey, USA); Martek Biosciences Corporation). Both groups took four small gelcaps per day until parturition. Acceptance was assessed by completion of a tracking form and measurement of erythrocyte DHA (EDHA).

Total gestational weight gain (GWG) was self-reported at the last study visit. Therefore, it is weight gain up to 36 weeks gestation. Height and weight were measured at the GCRC using a standardized protocol.

Measurement of Glucose tolerance and inflammatory markers

At the last study visit, i.e., 36 weeks gestation, subjects completed an oral glucose tolerance test, using a standardized breakfast consisting of 75 grams of carbohydrate and 500 kcal with a macronutrient distribution of ~60% carbohydrate, 18% protein, and 22% fat. All food 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 for measurement of blood glucose (bedside glucometer, enzymatic) and plasma insulin (radioimmunoassay, Linco Research, St. Charles, MO). Using the online homeostasis model of assessment (HOMA) program (University of Oxford, Oxford England, version 2.2), insulin resistance (HOMA-IR), β-cell function (HOMA-B %), and insulin sensitivity (HOMA-S %) were calculated. None of the women exceeded the acceptable range for use of the calculator. Use of HOMA-IR has been validated in pregnant women [27].

As a valid biomarker of DHA intake, EDHA content was measured at the three study visits [28]. Fasting venous blood was drawn into an EDTA-coated vacutainer tube and then placed on ice to prevent hemolysis. After centrifugation 20 min (3000 x g @4), the plasma was removed and the red blood cells (RBCs) were washed three times in saline solution (0.9%). The RBCs were placed in another fresh tube of pre-filled NaCl and spun again for 4 minutes, discarding the supernatant at the end. This RBC pellet was frozen at ~80°C and sent to the UC Metabolic Diseases Institute for analysis. Fatty acid composition of the RBC membrane was 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. A 500-threshold analysis was performed which enables the detection of omega-3 fatty acids found in concentrations of less than 1% in the RBC membrane.

A1C was measured using a modification of high-performance-liquid-chromatography (HPLC). In the Hemoglobinopathy Lab, an Alliance 2690/2695 HPLC (Waters Corporation, Milford, MA) and a PolyCAT A™ (PolyLC, Inc., Columbia, MD) column were used to separate the hemoglobin fractions by cation-exchange chromatography. A1C was then quantified using a dual wavelength detector (Model 2487, Waters Corp.) and Empower Software (Waters Corp.). Plasma IL-6 and TNF-α were measured using an enzyme-linked immunosorbsent assay (ELISA) method (Milliplex, Merck Millipore, Billerica, MA).

Statistical analyses

Power estimates were calculated based on differences in insulin
sensitivity using the reported effects of n-3 LCPUFA in overweight, hyperinsulinemic women [29]. In the latter study, insulin sensitivity (as measured by area under the curve over 2 hours following a glucose load of 75 g) in the intervention group decreased by 30% from baseline to post-intervention, whereas in the control group there was over a 10% increase. We calculated the sample based on this decrease of 30% in the intervention group using the reported means and associated standard deviations with no change in the placebo group and within subject correlation of 0.5 using 80% power and a 2-sided test with type 1 error of 0.05 to be 30 per group. SAS® PROC POWER was used for estimating sample size. No studies in pregnant women were available to calculate power. Variables were checked for normality of the distribution and were log transformed before further analysis when necessary. An independent sample t-test or $X^2$-test was used to determine the difference in variables between the placebo or DHA group at baseline (SV1), SV3, and change from SV1 to SV3; mean ± standard deviation or n (%) are reported. Treatment effects were analyzed using the independent two sample t-test, one-way ANOVA or ANCOVA (controlling for baseline level). Sensitivity analyses involved using general linear models invoking generalized estimating equations (GEE) to account for the within-subject correlation between visits. SAS® PROC GENMOD was used for this analysis. The treatment by group interaction was of interest and is reported. In addition, as EDHA was measured at three time points (~26 weeks, 30 weeks, and 36 weeks gestation) this same methodology was used to assess treatment effect. Means and standard deviations or least square means and associated standard errors are reported, as appropriate.

A two-way ANCOVA test was used to assess the impact of supplementation and pregravid BMI category as an interaction effect or main effect on markers of glucose metabolism and inflammation. Pregravid BMI was categorized as (a) BMI 25–29.99 (overweight), (b) BMI 30–39.99 (obese class I and II), or (c) BMI ≥ 40 (obese class III) [30]. Because mean body weight was significantly higher in the placebo group, it was used as the covariate. When the interaction was significant, a one-way ANOVA was used to explore the impact of pregravid BMI on the outcome variables in the treatment group. Post-hoc comparisons were made using the Least Significant Difference test.

The effect size for the intervention was estimated using the effect size statistic eta squared, $\eta^2$, which shows the percentage of variance in the dependent variable explained by the independent variable. When more than one independent variable is used, then the effect size was calculated as $\eta^2_{\text{partial}}$. For both statistics, Cohen specifies these interpretive guidelines for the strength of the association: (a) a small effect size is 0.01, (b) a medium effect size is 0.06, and (c) a large effect size is 0.14 [31].

Intention-to-treatment analysis was performed for EDHA. Since the change in EDHA was negligible between the second and third study visit, SV2 was carried forward for any missing EDHA for SV3. Outcome variables were assessed as the differences in the absolute measurement (values) between baseline and study-end measurements. $P<0.05$ was used as the level of statistical significance. Statistical analyses were performed using the Statistical Packages for Social Sciences (SPSS, IBM, Chicago, IL, USA, version 22, 2013) and SAS, version 9.3 (SAS Institute, Cary, NC).

### Results

#### Participants’ characteristics

Ninety-one women were randomized and 60 women completed the study. There was no significant difference in the amount of gestational weight gain between groups. Using the American College of Obstetrics and Gynecology’s criteria, none of the women who completed the study were diagnosed with GDM at baseline [32]. At 36 weeks gestation, three women met these criteria for GDM. There were no adverse effects reported in either group. At baseline, there were no significant differences between the groups except for pregravid BMI and current body weight (Table 1). However, the proportion of women classified as overweight or obese was not significantly different between groups.

#### EDHA

Baseline EDHA levels are reflective of low dietary intake in this sample, average 75 mg/day (Krummel, data unpublished). Indicative of adherence to the supplementation protocol, there was a significant interaction between treatment group and time on EDHA concentrations ($P<0.006, \eta^2_{\text{partial}}=0.33$), a large effect size (Cohen=0.14). Thus, 33% of the variance in EDHA could be explained by the randomization group and the time on the supplement or placebo. At study end, EDHA was 14% lower in the placebo group and 26% higher in the DHA group compared to baseline.

##### Primary outcome: effect of DHA supplementation during the last trimester on glucose metabolism

None of the markers of glucose metabolism were significantly different between study groups after supplementation, without adjustment of covariates (Table 2). Because pregravid BMI is associated with most of our measures of glucose metabolism, we next looked at whether DHA and pregravid BMI affected the outcomes while controlling for body weight at baseline and baseline value of the parameter. Pregravid BMI was categorized as: (a) overweight - pregravid BMI $\geq 30$, (b) obesity class I and II - pregravid BMI 30–39.99, or (c) obesity class III - pregravid BMI $> 40$.

| Placebo (n=28) | DHA (n=32) | p-value |
|---------------|------------|--------|
| Age (y)       | 26.3 ± 5.0 | 27.9 ± 4.6 | 0.19 |
| Education (y) | 14.9 ± 3.4 | 14.8 ± 2.1 | 0.92 |
| Marital status| 0.73       |        |      |
| Single (%)    | 9 (42.9%)  | 12 (48.0%) | 0.21 |
| Married (%)   | 12 (57.1%) | 13 (52.0%) |      |
| Race          |            |        |      |
| African American (%) | 15 (53.6%) | 12 (37.5%) |      |
| White (%)     | 13 (46.4%) | 20 (62.5%) |      |
| Pregravid BMI (kg/m²) | 36.88 ± 9.22 | 32.11 ± 6.27 | 0.03 |
| Pregravid BMI (% of sample) | 0.30     |        |      |
| 25–29.99 (%)  | 9 (32.1%)  | 13 (40.6%) |      |
| 30–39.99 (%)  | 11 (39.3%) | 15 (46.9%) |      |
| ≥ 40 (%)      | 8 (28.6%)  | 4 (12.5%)  |      |
| Body weight (kg) | 107.40 ± 27.11 | 92.10 ± 17.52 | 0.01 |
| Erythrocyte DHA (%) | 4.70 ± 0.32 | 4.72 ± 0.26 | 0.95 |
| Glucose (mmol/L) | 4.68 ± 0.47 | 4.73 ± 0.48 | 0.68 |
| Insulin (μU/mL) | 22.73 ± 1.53 | 25.51 ± 2.43 | 0.35 |
| IL-6 (pg/mL)  | 8.26 ± 1.58 | 9.99 ± 2.30 | 0.55 |
| TNF-α (pg/mL) | 5.45 ± 0.33 | 4.74 ± 0.47 | 0.24 |

Calculations:

| HOMA-IR      | 2.81 ± 0.19 | 3.13 ± 0.29 | 0.36 |
| HOMA-B%      | 224.78 ± 9.66 | 235.11 ± 16.26 | 0.60 |
| HOMA-S%      | 40.15 ± 2.82 | 39.88 ± 3.21 | 0.95 |

Table 1: Characteristics of women at baseline.
BMI 25.00 to 29.99; (b) obese - pregravid BMI 30.00 to 39.99; and (c) morbidly obese - pregravid BMI ≥ 40.

**Glucose:** There was no significant interaction of group and pregravid BMI on blood glucose level (P=0.78) (Table 3). Neither pregravid BMI (p=0.19) nor DHA group (0.57) had an independent effect on blood glucose at 36 weeks.

**Insulin:** There was no significant interaction of group and pregravid BMI on insulin (P=0.84) (Table 3). DHA group (0.56) and BMI > 30 (0.56) had an independent effect on insulin at 36 weeks.

### Table 2: Effect of DHA supplementation on markers of glucose metabolism and inflammation by treatment group.

|                      | Placebo N=9 | DHA N=13 | P for treatment effect | P for treatment by time interaction* |
|----------------------|-------------|----------|------------------------|---------------------------------------|
| **EDHA**             |             |          |                        |                                       |
| **SV<sub>i</sub> (%)** |             |          |                        |                                       |
| Glucose (mmol/L)     | 4.8 ± 0.7   | 4.73 ± 0.48 | 0.68                   |                                       |
| **SV<sub>i</sub> (mmol/L)** | 4.85 ± 0.48 | 4.81 ± 0.60 | 0.76                   |                                       |
| **Absolute Δ in glucose** | 0.17 ± 0.52 | 0.08 ± 0.48 | 0.47                   | 0.46                                  |
| **Insulin (uU/mL)**   | 22.73 ± 8.07 | 25.51 ± 13.73 | 0.35                   |                                       |
| **SV<sub>i</sub> (%)** | 3.18 ± 1.04 | 3.71 ± 2.08 | 0.21                   |                                       |
| **Absolute Δ in insulin** | 2.92 ± 8.01 | 3.87 ± 12.42 | 0.72                   | 0.46                                  |
| **HOMA-IR**          | 224.78 ± 51.12 | 235.11 ± 92.00 | 0.60                   |                                       |
| **SV<sub>i</sub> (%)** | 4.01 ± 14.93 | 39.88 ± 18.15 | 0.95                   |                                       |
| **SV<sub>i</sub> (%)** | 35.28 ± 13.99 | 36.66 ± 20.59 | 0.76                   |                                       |
| **Absolute Δ in HOMA-S%** | -4.87 ± 11.93 | -3.22 ± 12.81 | 0.61                   | 0.60                                  |
| **IL-6 (pg/mL)**     | 8.26 ± 3.85 | 9.99 ± 13.01 | 0.55                   |                                       |
| **SV<sub>i</sub> (%)** | 9.56 ± 9.90 | 8.02 ± 5.14 | 0.45                   |                                       |
| **Absolute Δ in IL-6** | 1.29 ± 4.11 | -1.57 ± 10.83 | 0.13                   | 0.10                                  |
| **TNF-α**            | 5.45 ± 1.77 | 4.74 ± 2.66 | 0.24                   |                                       |
| **SV<sub>i</sub> (%)** | 6.52 ± 2.75 | 4.96 ± 3.02 | 0.04                   |                                       |
| **Absolute Δ in TNF-α** | 1.07 ± 1.80 | 0.22 ± 1.12 | 0.03                   | 0.03                                  |
| **Weight**           | 107.40 ± 27.11 | 92.10 ± 17.52 | 0.01                   |                                       |
| **SV<sub>i</sub> (%)** | 111.23 ± 25.94 | 96.86 ± 18.06 | 0.02                   |                                       |
| **Absolute Δ in weight** | 3.83 ± 3.71 | 4.90 ± 3.88 | 0.28                   | 0.33                                  |

Independent t-test performed; results are expressed as mean ± standard deviation; EDHA–Erythrocyte Docosahexaenoic Acid; SV1–Study Visit 1 at 26 Weeks Gestation, Baseline; SV2–Study Visit 2 at 30 Weeks Gestation, Baseline; SV3–Study Visit 3 at 36 Weeks Gestation, Study End; HOMA-IR–Homeostasis Model of Assessment, Insulin Resistance; HOMA-B%–Homeostasis Model of Assessment, β-cell Function; HOMA-S%–Homeostasis Model of Assessment, Insulin Sensitivity: Δ Variables Calculated as SV3-SV1.

**Table 3:** Markers of glucose metabolism and inflammation by pregravid BMI and study group at 36 weeks gestation.

|                      | BMI<29.99 | BMI 30-39.99 | BMI ≥ 40 | P                      |
|----------------------|-----------|--------------|----------|------------------------|
| **Glucose (mmol/L)** | 4.9 ± 0.2 | 4.8 ± 0.2    | 4.9 ± 0.1 | 4.9 ± 0.1              |
| **Insulin (uU/mL)**  | 28.8 ± 3.8 | 27.2 ± 3.4  | 25.1 ± 3.1 | 28.3 ± 3.0            |
| **HOMA-IR**          | 3.6 ± 0.4 | 3.4 ± 0.4    | 3.1 ± 0.4 | 3.5 ± 0.3              |
| **HOMA-B%**          | 235 ± 22.3 | 236 ± 21.6  | 218 ± 18.5 | 241 ± 17.1            |
| **HOMA-5%**          | 30.0 ± 4.6 | 37.7 ± 4.4  | 38.5 ± 3.7 | 35.6 ± 3.5            |
| **IL-6 (pg/mL)**     | 10.4 ± 2.2 | 7.2 ± 2.2   | 10.1 ± 1.8 | 8.1 ± 1.6             |
| **TNF-α (pg/mL)**    | 7.0 ± 0.54 | 5.4 ± 0.53  | 5.4 ± 0.45 | 5.4 ± 0.40            |

DHA–Docosahexaenoic Acid; HOMA-IR–Homeostasis Model of Assessment-Insulin Resistance; IL-6–Interleukin-6; TNF-α–Tumor Necrosis Factor- α. TWO-WAY ANCOVA with BMI and group as factors and initial value at 26 weeks gestation (baseline) and pregravid weight as covariates. Results are presented as least square mean ± SE.
BMI on plasma insulin level (P=0.48) (Table 3). Neither pregravid BMI (p=0.84) nor DHA group (0.56) had an independent effect on plasma insulin level at 36 weeks.

**HOMA-IR:** There was no significant interaction of group and pregravid BMI on HOMA-IR (P=0.54) (Table 3). Neither pregravid BMI (p=0.91) nor DHA group (0.61) had an independent effect on HOMA-IR at 36 weeks.

**HOMA-B%:** After adjusting for baseline weight and initial HOMA-B%, there was no significant interaction effect of group and pregravid BMI (P=0.54) on HOMA-B%. Neither the main effect of pregravid BMI (p=0.41) nor DHA group (p=0.28) was significant.

**HOMA-S%:** After adjusting for baseline weight and initial HOMA-S%, there was no significant interaction effect of DHA group and pregravid BMI (P=0.32) on HOMA-S%. Neither the main effect of pregravid BMI (p=0.82) nor DHA group (p=0.67) was significant.

**Secondary outcomes: effects of DHA supplementation in the third trimester on markers of inflammation**

There was no effect of DHA supplementation on IL-6. Mean concentrations of TNF-α for the placebo and DHA groups were significantly different at study end (P=0.04) (Table 2). After adjusting for baseline TNF-α, the DHA group had significantly lower TNF-α levels (M=5.32 pg/ml, SE=0.26) than placebo (M=6.10 pg/ml, SE=0.28), (η² = 0.07) (P=0.05). Seven percent of the variation in TNF-α at study end was explained by being in the DHA group. The magnitude of the difference in the means 1.56 (95% CI 0.06 to 3.05), was between a medium to a large effect (η²=0.07).

Within subjects, there was a significant difference in change of TNF-α between SV1 and SV3 (η² =0.08) (P=0.03). In the placebo group, the increase was 1.1 pg/ml while in the DHA group the increase was 0.2 pg/ml. Thus, DHA supplementation blunted the rise seen in TNF-α between 26 weeks to 36 weeks gestation. These results were corroborated by the GEE models examining the group by time interaction (p=0.03).

When examining the effect of BMI categories defined above and DHA supplementation on SV3 levels, controlling for pregravid weight and baseline parameter value, neither interaction (randomization group by pregravid BMI) nor main effects for group or for pregravid BMI was significant for TNF-α or IL-6.

**Discussion**

This RCT conducted in an urban sample of overweight/obese pregnant women is the first known to us to report the effects of DHA supplementation from 26-36 weeks gestation on EDHA status, glucose tolerance, and inflammation. As expected, baseline EDHA concentrations were consistent with other reports on pregnant women whose habitual diet is low in DHA [33]. Indicative of adherence, women in the DHA group had significantly higher EDHA (26% increase) than the placebo group (15% decrease) with a large effect size as a result of supplementation.

Using criteria from the American College of Obstetrics and Gynecology, no one in our sample of overweight and obese women with a median pregravid BMI of 32 was diagnosed with GDM [32]. Despite this, the HOMA-IR observed was similar to the fourth quartile reported in a sample of overweight women with GDM measured at – the same gestational week [34]. Accordingly, these participants would be classified as having high insulin resistance. While HOMA-IR can be used to estimate insulin resistance in the latter half of pregnancy, it has inadequate sensitivity for diagnosis [35]. We observed lower HOMA-5% than previously reported in “obese” women [27]. The latter study only reported BMI measured during the second trimester of pregnancy, which cannot be used in the classification of obesity due to weight associated with the state of pregnancy. Indicative of impaired β-cell function, participants had a mean HOMA-8% (230) lower than previously observed in women with a normal BMI and normal glucose tolerance (269) or GDM (274) measured between 24 to 28 gestational weeks [36].

We hypothesized that DHA would lessen insulin resistance and improve insulin sensitivity after 10 weeks of supplementation in overweight/obese pregnant women. When comparing treatment groups, we found no statistically significant effect of DHA on markers of glucose metabolism. To further investigate the potential effect of pregravid status we created three BMI categories (<29.99, 30-39.99 and ≥ 40) to examine the potential interaction with DHA supplementation, while controlling for both baseline weight and initial value of the parameter under investigation. However we found neither significant interaction nor significant main effects for insulin resistance and insulin sensitivity.

No studies addressing the effects of DHA supplementation on glucose tolerance in pregnant women are available. Comparisons to other studies is challenging as the form, amount, purity and time period of supplementation with DHA varies widely as does the type of study. Using a placebo cross-over design, a daily supplemental dose of 230 mg EPA and 154 mg DHA decreased insulin sensitivity as determined by the Matsuda index in overweight men [37]. A very large dose of n-3PUFA (1.8 g EPA, 3.0 g DHA) consumed for 9 weeks lessened insulin sensitivity in subjects with type 2 diabetes [38]. Others did not observe a beneficial effect of DHA or n-3 PUFA on glucose metabolism in subjects with [39,40], or without type 2 diabetes [41]. Nor did supplementation with fish oil from ~ 21 weeks gestation onward lessen the risk of developing gestational diabetes mellitus [42].

Compared to studies that used the same method for analysis and measured plasma IL-6 concentrations at the same gestational week in obese, pregnant women, [43-45] our IL-6 concentrations were higher; this may be a function of the distribution of pregravid BMI (35% overweight, 47% class 1 and 2 obesity, and 18% class 3 obesity) in our study. Consumption of farmed salmon (~ 162 mg/d EPA, ~ 330 mg/d DHA) in pregnant women, with an unknown BMI failed to alter plasma IL-6 [46]. In agreement, we found no treatment effect of 800 mg DHA on plasma IL-6. A much higher dose of n-3 PUFA (800 mg DHA and 1200 mg EPA) lowered IL-6 and TNF-α in overweight/obese pregnant women [23].

Blood concentrations of TNF-α have been reported to be higher in obese, pregnant women compared to leaner women [13,44,47]. In the present study, TNF-α concentrations were higher than three studies of overweight/obese, pregnant women [13,44,48], similar to one study [49], and lower than another [43]. One explanation could be the differences in the extent of pregravid obesity. Farah et al. reported the highest concentration of TNF-α in the literature (>~7 pg/ml) for a sample of Irish women with weight status ranging from normal to class III obesity, yet without gestational diabetes or hypertension [43]. Consistent with their findings, TNF-α concentrations, measured at SV1, were not significantly correlated with pregravid BMI in our sample, yet, a trend was noted (r=+0.23, P=0.08) Despite the women in the current sample having normal glucose tolerance tests, the concentrations of TNF-α were closer to what has been observed in...
women with impaired glucose tolerance or gestational diabetes in the third trimester [50].

In our study, with 800 mg DHA/d, a medium treatment effect of DHA supplementation on TNF-α occurred. Since TNF-α concentrations rise throughout pregnancy and may be associated with adverse outcomes [13,43,48], we hypothesized that DHA may improve the inflammatory milieu of overweight/obese pregnant women. Accordingly, we observed an attenuation of the rise in TNF-α concentration from 26 to 36 weeks gestation after 10 weeks of supplementation. In agreement, Haghiac et al. found that supplementation with 2 grams of n-3 PUFA (800 mg DHA) from the first trimester until birth lowered IL-6 and TNF-α in overweight/obese pregnant women [23]. Elevated TNF-α concentrations promote insulin resistance in obese, pregnant women [13,15,50], and in women with GDM, elevated TNF-α expression in skeletal muscle persisted until one-year postpartum [51]. Despite these observations, the stabilization in TNF-α that we observed in the supplemented groups was not associated with an improvement in insulin sensitivity.

Strengths of the study include the randomized, double-blinded, placebo-controlled design and the stringent inclusion/exclusion criteria. Also, we had adequate power to detect differences in glucose and these inflammatory markers after supplementation. The start of the supplement in the second trimester is a limitation as earlier exposure has now been recommended [52].

In conclusion, we failed to find any benefit of DHA supplementation on measures of glucose metabolism even after accounting for pregravid BMI. We did observe reduced TNF-α in overweight/obese pregnant women after 10 weeks of supplementation. However, this change in inflammation was unrelated to change in glucose metabolism. The long-term implications of our findings for the mother and infant are unknown and should be investigated in future studies.

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