A Randomized Trial of Docosahexaenoic Acid Supplementation During the Third Trimester of Pregnancy

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OBJECTIVE: **To hypothesize that higher intake of docosahexaenoic acid, an n-3 long chain polyunsaturated fatty acid, would increase duration of gestation and birth weight in US women.**

METHODS: **This was a randomized, double-blind, controlled, clinical trial. Subjects were enrolled in an ambulatory clinic where they received prenatal care. This was a population-based sample. Most subjects received government assistance for medical care and most were black (73%). Subjects were enrolled between the 24th and 28th week of pregnancy and consumed docosahexaenoic acid (33 or 133 mg) from eggs until parturition. Gestational age and birth weight were the main study outcomes. Infant length and head circumference, preterm birth, and low birth weight were secondary outcomes.**

RESULTS: **Eighty-three percent of subjects completed the study (291 of 350 enrolled). No subject was discontinued for an adverse event. After controlling for important predefined risk factors and confounding variables, gestation increased by 6.0 ± 2.3 days ($P = .009$) in the higher docosahexaenoic acid group. Birth weight, length, and head circumference increased, but did not reach statistical significance ($P = .06–.18$), although the increases could be clinically important indications of enhanced intrauterine growth. No safety concerns were raised by the study.**

CONCLUSION: **Duration of gestation increased significantly when docosahexaenoic acid intake was increased during the last trimester of pregnancy. The increase in gestation was similar to that reported for interventions with much larger amounts of n-3 long chain polyunsaturated fatty acids.** **(Obstet Gynecol 2003;101:469–79. © 2003 by The American College of Obstetricians and Gynecologists.)**

Cold-water fish are the highest dietary source of docosahexaenoic acid and eicosapentaenoic acid, two n-3 long chain polyunsaturated fatty acids.[1] Olsen et al suggested that higher docosahexaenoic acid and eicosapentaenoic acid intake from fish by Faroe Islanders compared with Danes was the reason for longer gestation in Faroe Islanders.[2] The authors subsequently demonstrated increases in gestation of 4 and 8.5 days, respectively, in randomized clinical trials that provided 2.7 g per day of docosahexaenoic acid and eicosapentaenoic acid to a group of healthy pregnant women[3] and to healthy pregnant women with a previous preterm delivery.[4]

In contrast to women in Denmark, women in the United States consume little n-3 long chain polyunsaturated fatty acids, including less than 100 mg per day from docosahexaenoic acid.[5] Evidence of lower maternal docosahexaenoic acid intake is reflected in the docosahexaenoic acid content of human milk in the United States, which is reported to be among the lowest in the world.[6] Earlier, we compared pregnancy outcomes of American women randomly assigned to consume 12 ordinary eggs or 12 high-docosahexaenoic acid eggs in a small pilot study. Results of our pilot study suggested that even an increase in docosahexaenoic acid intake of approximately 100 mg per day may increase length of

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gestation and infant birth weight in women who reported low prestudy docosahexaenoic acid intakes of approximately 50 mg per day (Borod E, Atkinson R, Barclay WR, Carlson SE. Effects of third trimester consumption of eggs high in DHA on DHA status and pregnancy [abstract]. Lipids 1999;34:S231).

In the present study, we hypothesized that women randomly assigned to consume high-docosahexaenoic acid eggs compared with ordinary eggs daily would have an increase in length of gestation and birth weight of their infants. Efforts to increase gestation and birth weight could have medical and economic significance as well as significance for infant development. It is well known that low birth weight (less than 2500 g) and preterm birth (less than 37 weeks' gestation) contribute disproportionately to poor developmental outcomes in infants.[7] However, even among normal weight, term infants[8–11] and moderately preterm infants,[12] higher birth weight has been shown to be an independent predictor of infant development. If an increase in docosahexaenoic acid intake during pregnancy could increase gestational age at delivery and birth weight, significant benefits could accrue to the offspring.

MATERIALS AND METHODS

This study was a randomized, double-blind, controlled, clinical trial to determine the effects of increasing docosahexaenoic acid intake during the third trimester of pregnancy on pregnancy and birth outcomes. Subjects were supplied with docosahexaenoic acid-enriched eggs (mean of 133 mg of docosahexaenoic acid per egg) or ordinary eggs (mean of 33 mg of docosahexaenoic acid per egg). The primary outcome variables of interest were gestational age and birth weight. Secondary outcomes included infant length and head circumference at birth. Additional outcomes of interest were the presence or absence of low birth weight (less than 2500 g), preterm delivery (less than 37 weeks' gestation), meconium staining of the infant, maternal gestational diabetes, preeclampsia/eclampsia, and cesarean delivery.

Data on the following were collected as variables that could affect study outcomes and potentially need to be included in regression analysis: egg group, study egg intake (total number consumed and average number consumed per week), maternal race (black/nonblack), number of prior pregnancies, previous preterm birth experience, prepregnancy smoking (yes/no and pack years), smoking during pregnancy (yes/no and number of cigarettes smoked per day), maternal body mass index (BMI) at enrollment, gestational age at enrollment, maternal fasting serum glucose at enrollment, maternal age at enrollment, alcohol intake before and during pregnancy (yes/no and number of drinks per day), maternal plasma folate and B12 concentrations at enrollment, third-trimester weight gain, infant gender, maternal red blood cell (RBC) phospholipid docosahexaenoic acid at enrollment, and maternal and infant RBC phospholipid docosahexaenoic acid at the time of birth.

A prestudy power analysis specified that 285 women be enrolled into the study to detect an increase in gestation of 7 days with high-docosahexaenoic acid eggs. Because there were no published data for low-level docosahexaenoic acid supplementation on which to base a power analysis, a blinded review of the data was conducted after the first 100 subjects delivered their infants to obtain a better estimate of study parameters and to refine the power analysis if needed. The blinded review detected mean gestational ages for the two egg groups of 39.26 weeks (274.8 days) and 38.5 weeks (269.5 days). The sample size was increased to 350 based on an expected dropout rate of 25% to yield the 130 subjects per group needed to reject the null hypothesis for an increase in gestation of 5.25 days (from 269.5 to 274.8 days), assuming a standard deviation of 1.99 weeks with 90% power and an $\alpha = 0.05$ based on a one-tailed *t* test. The final results are presented as the more conservative and conventional two-tailed *t* test.

Subjects were enrolled between July 1998 and July 2001 at Truman Medical Center in Kansas City, Missouri. Gestational age in weeks was determined by ultrasound. With few exceptions, the ultrasound examination occurred between 15 and 20 weeks' gestation. From the gestational age determined on the day of ultrasound, the expected date of delivery was calculated using Naegele's rule.[13] The ultrasound estimation of gestational age was done before randomization, and that gestational age was "fixed" for the study. Gestational age was fixed before enrollment to eliminate any possibility of bias such as could be introduced by changing gestational age after the outcomes of a pregnancy were known. The ultrasound estimation of gestational age was used to determine a woman's individual eligibility for enrollment in the study and to determine group mean gestational ages at enrollment and delivery using Naegele's rule.

All forms of gestational age assessment have an inherent variability for an individual woman. The assumption made by the study was that errors in assessing gestational age at enrollment would be similar between the groups. Despite the variability in assessing individual pregnancies, the true mean gestational age will be approximated by the average of a large group. In this case, the group means for gestational age at enrollment and delivery were compared.

Women who were between 24 and 28 weeks' gestation and who met inclusion and exclusion criteria (Table

Table 1. Study Inclusion and Exclusion Criteria

Inclusion criteria
- Pregnant women 16–36 y of age
- 24–28 wk of gestation at enrollment
- Able and willing to consume eggs
- Access to refrigeration
- Plan to deliver at Truman Medical Center
- Singleton gestation

Exclusion criteria
- <16 or >36 y of age
- Weight >240 lb at baseline
- Serious illness such as cancer, lupus, hepatitis
- Known to have any untreated serious, infectious disease
- Diabetes or gestational diabetes at baseline
- Elevated blood pressure attributed to any cause

1) were approached in prenatal clinic to obtain informed consent according to a protocol approved by the University of Missouri-Kansas City, Kansas City, Missouri (Adult Health Sciences Institutional Review Board) and the University of Kansas Medical Center, Kansas City, Kansas (Human Subjects Committee, protocol #8031-00). Most subjects received government assistance for medical care and most were black (73%). After consent was obtained, patients were assigned to an egg group using a randomization schedule created with PROC PLAN procedure in SAS 6.12 (SAS Institute Inc., Cary, NC). Patients were stratified into four age categories (16–20.99, 21–25.99, 26–30.99, and 31–35.99 years). Within each age category, patients were randomized in blocks of four, with an equal number of patients randomized to the ordinary and high-docosahexaenoic acid egg groups.

Blood samples from an antecubital vein were collected on ice with ethylenediaminetetra-acetic acid from each subject at enrollment and, in most cases, at approximately 5 AM the morning after the day a woman delivered, and from the cord umbilical vein at birth. After the plasma was removed, the RBCs were washed three times with 0.15 M NaCl in 1 mM of ethylenediaminetetra-acetic acid, resuspended in the saline ethylenediaminetetra-acetic acid solution, and stored at −80C under nitrogen until analysis. After the birth, the infant and placenta were weighed, the infant length and head circumference were measured, and maternal blood loss was estimated.

The study was conducted in accordance with Good Clinical Practices as published in the US Federal Register. Maternal and infant adverse events and serious adverse events were classified for each pregnancy in accordance with International Conference on Harmonization guidelines.[14] An adverse event was defined by the protocol as any reaction, side effect, or other undesirable event that occurred in conjunction with the use of the test product, whether or not the event was considered related to the test product. A serious adverse event was defined as any adverse event that resulted in the following: death, a life-threatening event, inpatient hospitalization or prolonging of an existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Adverse events (including serious adverse events) were reviewed quarterly during monitoring visits and assessed via a planned, interim blinded safety analysis. The obstetrician (DM) determined if a serious adverse event was related to study egg intake. His attribution was based on the temporal relation between study egg intake and the event and his judgment about the frequency of the event in relation to the study population. In the final analysis, adverse events and serious adverse events were compared by treatment to determine if there was a statistical difference in incidence.

To produce the eggs provided in this study, hens were fed a nutritionally modified feed with 1% docosahexaenoic acid-rich marine microalgae (high-docosahexaenoic acid eggs; Gold Circle Farms, Boulder, CO) or feed without microalgae (ordinary eggs).[15] The marine microalgae was previously determined to be "generally recognized as safe" for laying hen feed by a panel of independent scientific experts[16] and is marketed under the fat product, feed grade definition of Animal Feed Control Officials.[17] During the years of the study, the high-docosahexaenoic acid eggs were available commercially in a number of regions of the United States under the name Gold Circle Farms, but they were not available commercially in the Kansas City metropolitan area. Eggs were shipped to Kansas City via refrigerated truck as needed, and the two kinds of eggs had different color cartons.

Each lot of high-docosahexaenoic acid eggs and ordinary eggs provided during the 3 years of the study was analyzed for docosahexaenoic acid by the study sponsor, and the results averaged by egg type when the study was complete. The high-docosahexaenoic acid eggs contained a mean of 133 (±15) mg of docosahexaenoic acid per egg (range 108–165 mg), and the ordinary eggs contained a mean of 33 (±11) mg of docosahexaenoic acid per egg (range 22–51 mg). Subjects were given 12 eggs per study week from enrollment until they gave birth, and they were encouraged to consume as many of these as possible. Subjects were interviewed biweekly to determine how many eggs they had eaten in the previous interval. Eggs were maintained at refrigeration temperatures less than 5C at all times before being dispensed to subjects. Subjects were instructed to refrigerate the eggs and to cook the eggs before eating.

To determine total egg docosahexaenoic acid content, the lipids in eggs yolk were extracted with chloroform methanol and transesterified with 4% sulfuric acid in methanol. The resulting fatty acid methyl esters were extracted with hexane and separated, identified, and quantified by gas liquid chromatography as reported previously.[15]

Red blood cell and plasma lipids were extracted by a modified procedure of Dodge and Phillips[18] using chloroform and methanol. The methanol contained 50 mg/L of butylated hydroxytoluene as an antioxidant. An internal standard (diheptadecanoyl phosphatidylcholine; Sigma Chemical Co., St. Louis, MO) was coextracted with the RBC lipids. The lipid extracts in organic solvents were washed with 0.15 M of potassium chloride according to Folch et al,[19] and the organic solvents that remained were vaporized under nitrogen. Phospholipids were isolated from other lipid classes by thin layer chromatography (Silica gel G plates, 10 × 20 cm; Analtech Inc., Newark, DE) in hexane:diethyl ether:acetic acid (80:20:1, v:v:v).[20] The band containing phospholipids was removed completely from the plate and transmethylated with boron trifluoride-methanol (Sigma Chemical Co.) to yield fatty acid methyl esters.[21]

Individual fatty acid methyl esters were separated on a Varian 3300 gas chromatograph with an SP-2560 capillary column (100 m, Supelco Inc., Belfonte, PA). The instrument was programmed for column, injector, and detector temperatures of 175, 225, and 275C, respectively. Helium was used as a carrier gas at a flow rate of 21 cm per second. Individual peaks were integrated with a programmable Varian 4290 recorder/integrator. The peaks were identified by comparison to authentic standards. Standard mixtures with known concentrations were analyzed periodically to verify column integrity (NHI-C, NHI-F, NHI-C, and NHI-D, Supelco Inc.), and biologic mixtures of fatty acids were analyzed to validate separation characteristics (PUFA 1 and 2, Supelco Inc.).

All subjects who gave informed consent, consumed at least one egg, and had delivery data available were included in the analyses. A dropout was defined as an enrolled subject who did not consume at least one egg or for whom delivery information was not available.

To identify the effect of docosahexaenoic acid supplementation on the gestational age, weight, length, and head circumference, simple means and standard deviations were calculated for each group, overall and by important risk factors, race, and smoking during pregnancy. Simple two-tailed *t* tests were then conducted to compare the groups with respect to mean gestational age and mean birth weight. Tests were conducted both overall (ignoring these noted risk factors) and separately by racial category and smoking status. Categoric outcomes were assessed using χ^2 methods.

In addition to race and tobacco use, pregnancies vary with regard to many factors that have the potential to influence the primary and secondary outcomes of the study (potentially influential variables). The following variables were evaluated to determine if there was a significant relationship to any primary or secondary outcome of the study: egg group, study egg intake (total number consumed and average number consumed per study week), maternal race (black/nonblack), number of prior pregnancies, previous preterm birth experience, prepregnancy smoking (yes/no and pack years), smoking during pregnancy (yes/no and number of cigarettes smoked per day), maternal BMI at enrollment, gestational age at enrollment, maternal fasting serum glucose at enrollment, maternal age at enrollment, alcohol intake before and during pregnancy (yes/no and number of drinks per day), maternal plasma folate and vitamin B_{12} concentrations at enrollment, third-trimester weight gain, infant gender, maternal RBC phospholipid docosahexaenoic acid at enrollment, and maternal and infant RBC phospholipid docosahexaenoic acid at the time of birth.

If a variable was significantly related to a planned study outcome, it was included in the final regression model to examine the effect of docosahexaenoic acid supplementation on that outcome. For each outcome, group-specific intercepts and slopes with respect to egg intake were forced into the model. Then, a step-wise backward strategy was used to identify other important predictor variables. Diagnostics were performed on the final models to ensure that all statistical assumptions were met. The blinded review of the data after the first 100 deliveries did not constitute a formal interim analysis, as findings could not be used as a basis for early study termination. Consequently, no α adjustment for multiple comparisons was necessary. Results presented are for two-tailed *t* tests.

To assess safety, maternal and infant adverse events and serious adverse events were recorded, tabulated, and categorized for all subjects who consumed at least one egg. The proportion of women and infants in each group who experienced adverse events was compared by χ^2 tests.

RESULTS

A total of 347 women participated in this study. However, information was collected on 350 pregnancies, as three women were pregnant twice during the enrollment period. In two of these cases, both pregnancies were completed. To maintain independence among the study data, the second pregnancy was excluded from the analysis. An additional 57 pregnancies were not included in

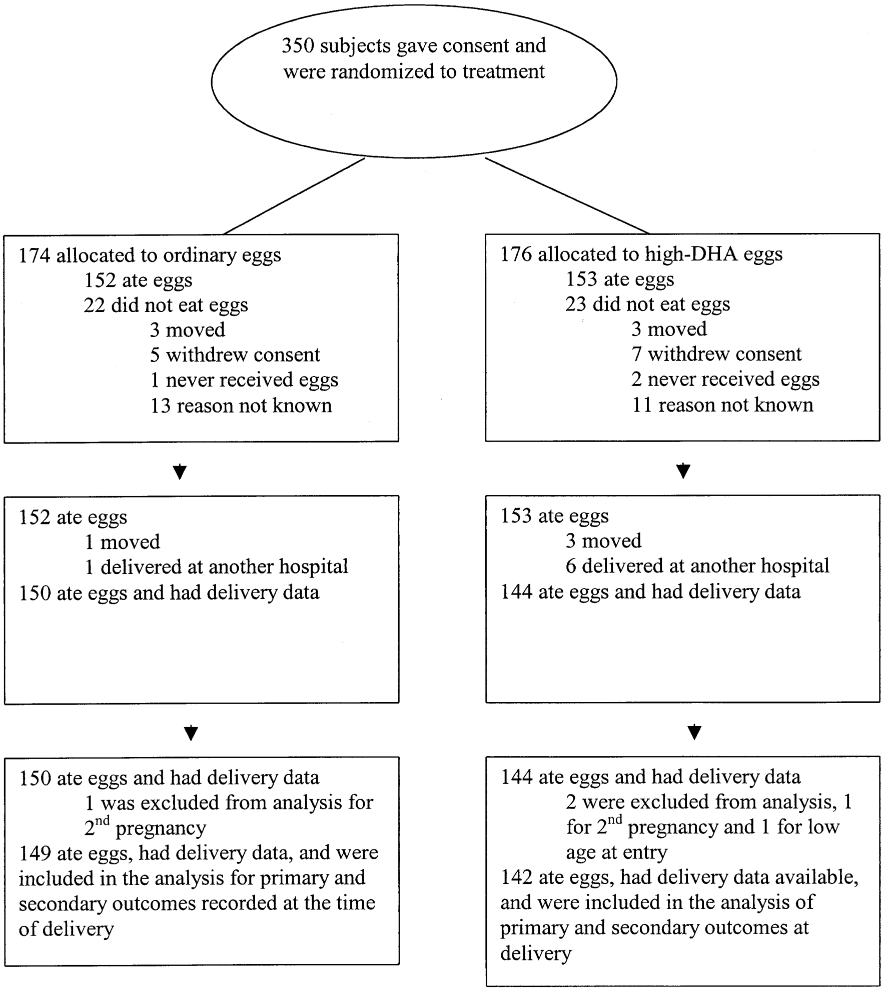

Figure 1. Disposition of study subjects. DHA = docosahexaenoic acid.

Smuts. DHA and Pregnancy Outcomes. Obstet Gynecol 2003.

the analysis because the subjects were study dropouts (Figure 1). In sum, 291 subjects completed the study and were evaluated for the planned study outcomes. The study dropout rate was 17%.

The safety monitoring included all subjects and infants of subjects who consumed eggs whether or not delivery information was available as is the standard practice for safety monitoring. A total of 305 pregnancies in 303 women and the 305 infants born to these pregnancies were assessed for safety.

Table 2 presents baseline subject characteristics by group for blood pressure at enrollment and a number of variables that were tested as potential predictors of study outcomes. The proportion of women assigned to each group was similar across the age categories. The study groups did not differ with respect to any potentially important variables in Table 2 ($P > .11$ for all tests).

Study outcomes are shown in Table 3. Egg intake and maternal and infant RBC phospholipid docosahexaenoic acid at delivery are included in Table 3 because they are not only study outcomes but also potentially related to the planned study outcomes of gestational age, birth weight, length, and head circumference. Average egg intake was the same in each group. Infant but not maternal RBC phospholipid docosahexaenoic acid increased significantly in the high-docosahexaenoic acid egg compared with the ordinary egg group.

The simple group means for gestational age are presented in Table 3. Before controlling for statistically significant predictors, the high-docosahexaenoic acid egg group had an average increase in gestation of 2.6 ± 1.7 days ($P = .135$). Among women who did not smoke, the high-docosahexaenoic acid egg group had an increase in gestation of 3.7 ± 1.9 days compared with the ordinary egg group ($P = .045$) (Table 3). After controlling for two statistically influential variables, maternal BMI at enrollment and number of prior pregnancies (Table 4), the high-docosahexaenoic acid egg group had a significant

Table 2. Baseline Characteristics of Subjects*

	Ordinary eggs (n = 149)	High-DHA eggs (n = 142)
Age (y)	21.6 (4.2)	21.7 (4.3)
16–20.99 (%)	49.7	53.5
21–25.99 (%)	36.2	30.3
26–30.99 (%)	10.1	11.3
31–35.99 (%)	4.0	4.9
Gestation (wk)	26.1 (1.4)	26.0 (1.5)
Descent (African/other)	109/40	104/38
BMI[†]	28.6 (5.4)	29.4 (5.9)
Systolic blood pressure (mm Hg)	111 (11)	110 (12)
Diastolic blood pressure (mm Hg)	64 (7)	64 (8)
Fasting serum glucose (mg/dL)	102 (23)	104 (22)
Nulliparous before study pregnancy (%)	58	68
Previous preterm delivery (%)	6	8
Smoked before pregnancy (%)	38.2	46.8
Smoked during pregnancy (%)	21.5	27.0
Alcohol during pregnancy (%)	4.7	1.4
Prenatal vitamin use (%)	83.2	84.6
Egg intake (number per wk)	6.8 (4.6)	7.7 (5.6)
RBC phospholipid DHA (g per 100 g of fatty acids)	5.49 (1.2)	5.45 (1.0)

DHA = docosahexaenoic acid; BMI = body mass index; RBC = red blood cell.

[*] Mean (standard deviation) or %.

[†] Determining BMI from weight at 24–28 weeks' gestation would be expected to overestimate BMI. However, reliable prepregnancy weights were not available.

Table 3. Birth Outcomes Overall and by Race and Smoking Category Uncontrolled for Influential Variables*

	Ordinary eggs (n = 149)	High-DHA eggs (n = 142)
Gestational age (d)		
All subjects	271.6 (15.6)	274.1 (13.5)
African descent	271.6 (15.1)	273.4 (14.1)
Non-African descent	271.6 (16.8)	276.2 (11.7)
Did not smoke during pregnancy	271.5 (15.3)	275.2 (11.8)[†]
Smoked during pregnancy	271.9 (16.7)	271.4 (17.4)
Birth weight (g)		
All subjects	3106 (551)	3209 (533)
African descent	3045 (524)	3166 (556)
Non-African descent	3274 (594)	3327 (451)
Did not smoke during pregnancy	3126 (547)	3232 (499)
Smoked during pregnancy	3033 (566)	3145 (626)
Birth length (cm)		
All subjects	49.6 (3.0)	50.3 (3.0)[†]
African descent	49.4 (3.0)	50.0 (2.8)
Non-African descent	50.1 (3.0)	51.0 (3.3)
Did not smoke during pregnancy	49.8 (2.9)	50.5 (2.5)
Smoked during pregnancy	48.7 (3.3)	49.7 (4.1)
Birth head circumference (cm)		
All subjects	33.4 (1.8)	33.8 (1.7)
African descent	33.3 (1.8)	33.7 (1.8)
Non-African descent	33.6 (2.0)	34.0 (1.1)
Did not smoke during pregnancy	33.5 (1.8)	33.9 (1.5)
Smoked during pregnancy	33.3 (2.0)	33.5 (2.1)
Placenta weight (g)	640 (158)	650 (157)
Study egg intake (number per wk)	7.3 (3.4)	7.2 (3.4)
Final RBC DHA (g per 100 g of fatty acids)	5.35 (1.2)	5.53 (1.0)
Infant RBC DHA (g per 100 g of fatty acids)	7.18 (1.4)	7.61 (1.3)[†]
Preterm delivery	17 (11)	14 (10)
Low birth weight	16 (11)	13 (9)
Preeclampsia/eclampsia	10 (6.6)	5 (3.3)
Gestational diabetes	3 (2)	4 (3)
Cesarean delivery	21 (14)	18 (13)
Meconium in amniotic fluid	28 (19)	26 (19)
Infant to intensive care	21 (14)	21 (15)

Abbreviations as in Table 2.

[*] Results are mean (standard deviation) for continuous outcomes and number (% incidence) for dichotomous outcomes.

[†] Groups were statistically different, $P < .05$. Results were available for over 90% of subjects except for placenta weight, estimated blood loss, maternal DHA at delivery, and neonatal DHA at delivery, which had data for 65%, 86%, 83%, and 67.7% of subjects, respectively.

increase in duration of gestation of 6.0 ± 2.3 days compared with the ordinary egg group (276.5 days versus 270.5 days, $P = .009$).

The simple group mean for birth weight of infants in the high-docosahexaenoic acid egg group was 103 ± 64 g higher than for infants whose mothers consumed ordinary eggs ($P = .108$) (Table 3). After controlling for maternal race and BMI at enrollment, variables that significantly influenced birth weight, infants in the high-docosahexaenoic acid egg group were on average 83 ± 62 g heavier at birth than infants in the ordinary egg group (3145 g versus 3063 g, $P = .184$) (Table 4).

The simple means by group for infant length and head circumference at delivery are presented in Table 3. Infants in the supplemented group were significantly longer at delivery ($P = .048$) and had a trend toward larger head circumference ($P = .081$). Prior preterm delivery and maternal BMI at enrollment were signifi-

Table 4. Regression Results for Primary and Secondary Continuous Outcomes After Controlling for the Significant Predictors Noted for Each Outcome*

Outcome	Difference ($\text{mean}_{\text{high DHA}}-\text{mean}_{\text{ordinary}}$)	P	Significant predictors
Gestational age (d)	6.0 ± 2.3	.009	Maternal BMI at enrollment Prior pregnancies (number)
Birth weight (g)	83 ± 62	.184	Maternal BMI at enrollment Maternal race
Infant length (cm)	0.66 ± 0.35	.061	Maternal BMI at enrollment Prior preterm delivery
Infant head circumference (cm)	0.36 ± 0.20	.081	Maternal BMI at enrollment Smoking before pregnancy

Abbreviations as in Table 2.
* Results are for two-tailed test.

cantly related to length at birth. After controlling for these variables, the increase in birth length with docosahexaenoic acid supplementation (0.66 ± 0.35 cm) was only statistically suggestive ($P = .061$) (Table 4). After controlling for smoking before pregnancy and change in maternal BMI during the study, the average difference in head circumference at birth for infants in the high-docosahexaenoic acid egg and ordinary egg groups was similar ($P = .081$) (Table 4).

The incidence of preterm delivery, low birth weight, and other dichotomous outcomes of interest did not differ significantly by group (Table 3).

Total study egg intake was significantly correlated with maternal RBC phospholipid docosahexaenoic acid at delivery in both groups of women and with infant RBC phospholipid docosahexaenoic acid in the ordinary egg group (Table 5). However, maternal RBC phospholipid docosahexaenoic acid at the time a woman was enrolled was a much more important predictor of maternal RBC phospholipid docosahexaenoic acid at delivery than was docosahexaenoic acid intake from eggs. The former accounted for 42–56% of the variance (r^2) (Table 5) in maternal RBC phospholipid docosahexaenoic acid at delivery and the latter for 4–6% of the variance.

Maternal RBC phospholipid docosahexaenoic acid at the time of delivery was not significantly correlated with gestational age in either group, but there was a statistically significant relationship between infant docosahexaenoic acid at delivery and gestation in the supplemented group ($r = 0.227$, $P = .022$) (Table 5). Including infants from both groups, each additional increase in the percent of docosahexaenoic acid in infant cord RBC phospholipid was associated with an increase in gestation of approximately 2 days ($P = .011$).

Study dropouts ($n = 57$) and completed subjects ($n = 291$) did not differ with regard to any variable evaluated as a potentially influential variable (data not shown).

A summary of maternal and neonatal adverse events and serious adverse events is shown in Tables 6 and 7, respectively. The proportion of mothers in the regular egg group who experienced one or more adverse events was significantly higher than the supplemented group, 38% versus 25% ($P = .01$). Gynecologic adverse events in general, and labor-related adverse events in particular, were more common among unsupplemented subjects than among those in the supplemented group (Table 6). Because patients who experience one adverse event often have an increased chance of experiencing other adverse events, we also looked at the number of subjects in the high-docosahexaenoic acid egg and ordinary egg groups with any adverse event related to labor or other gynecologic factors. A total of 25 subjects in the high-docosahexaenoic acid egg group (16%) and 44 subjects in the ordinary egg group (29%) had these kinds of adverse events, whereas the incidence of nongynecologic adverse events was similar between the treatment groups ($n = 14$, 9%, and $n = 13$, 9%, for the ordinary egg and high-docosahexaenoic acid egg groups, respectively).

No serious adverse events occurred in the high-docosahexaenoic acid egg group at a higher rate than expected for the population, and each group had the same number of total serious adverse events. Consequently, serious adverse events were not linked to docosahexaenoic acid supplementation.

The supplemented and unsupplemented groups of neonates did not differ in adverse event or serious adverse event incidence (Table 7). In addition, the two groups of neonates had similar incidences of preterm delivery, low birth weight, and intrauterine growth retardation (Table 3).

DISCUSSION

The current study found a 6-day longer period of gestation when docosahexaenoic acid intake was increased during the last trimester of pregnancy. The mechanism of action is not known, but it is plausible that dietary docosahexaenoic acid could increase gestational age by

Table 5. Correlation of Gestational Age, Birth Weight, Egg Intake, and Maternal RBC DHA at Enrollment With Maternal and Infant RBC DHA Levels at Delivery by Group

	Maternal RBC DHA at delivery	Infant RBC DHA at delivery
Gestational age		
Ordinary egg	$r = 0.072$ ($P = .429$)	$r = 0.110$ ($P = .272$)
High-DHA egg	$r = -0.017$ ($P = .847$)	$r = 0.227$ ($P = .022$)
Birth weight		
Ordinary egg	$r = 0.052$ ($P = .501$)	$r = 0.141$ ($P = .161$)
High-DHA egg	$r = -0.065$ ($P = .476$)	$r = 0.038$ ($P = .703$)
Total study egg intake		
Ordinary egg	$r = 0.208$ ($P = .022$)	$r = 0.226$ ($P = .023$)
High-DHA egg	$r = 0.240$ ($P = .007$)	$r = 0.130$ ($P = .193$)
Average study egg intake per wk		
Ordinary egg	$r = 0.120$ ($P = .189$)	$r = 0.145$ ($P = .148$)
High-DHA egg	$r = 0.196$ ($P = .029$)	$r = 0.068$ ($P = .495$)
Maternal RBC DHA at enrollment		
Ordinary egg	$r = 0.749$ ($P = .001$)	$r = 0.439$ ($P = .001$)
High-DHA egg	$r = 0.6545$ ($P = .001$)	$r = 0.515$ ($P = .001$)
Maternal RBC DHA at delivery		
Ordinary egg		$r = 0.637$ ($P = .001$)
High-DHA egg		$r = 0.641$ ($P = .001$)

Abbreviations as in Table 2.

several mechanisms. Prostaglandins E_2 and $F_{2\alpha}$ are required for labor and delivery.[22,23] These two-series prostaglandins are derived from the n-6 long chain polyunsaturated fatty acid, arachidonic acid, whereas the less potent three-series prostaglandins are derived from the n-3 long chain polyunsaturated fatty acid, eicosapentaenoic acid. Dietary docosahexaenoic acid could alter the balance of prostaglandins by retroconversion to eicosapentaenoic acid[24,25] or could reduce the production of two-series prostaglandins from arachidonic acid by displacing arachidonic acid in membrane phospholipids.[24,25] In an ovine model, the infusion of n-3 long chain polyunsaturated fatty acids (both docosahexaenoic acid and eicosapentaenoic acid) reversed labor induced by betamethasone[26] and inhibited prostaglandin synthesis.[27]

Other mechanisms of action may exist, however, and several mechanisms may be operative at the same time. For example, both eicosapentaenoic acid and docosahexaenoic acid have been found to impact heart muscle function.[28–30] The muscle effects of these n-3 long chain polyunsaturated fatty acids include modification of the eicosanoid system, a direct effect of nonesterified eicosapentaenoic acid and docosahexaenoic acid on muscle membranes, an effect on the inositol lipid cycle and cell

Table 6. Maternal Adverse Events, Most Common Types of Adverse Events,* and Serious Adverse Events, by Egg Group

	Ordinary eggs *n* (%)	High-DHA eggs *n* (%)
AE (including SAE)	58 (38)	38 (25)[†]
Gynecologic infections	20	16
Labor related	30	14
Premature rupture of membranes	9	3
Urinary tract infection	7	2
SAE	6 (4)	6 (4)

DHA = docosahexaenoic acid; AE = adverse event; SAE = serious adverse event.

[*] Types of AEs evaluated included cardiovascular, digestive, endocrine, genitourinary, general gynecology, gynecologic infections, hematologic and lymphatic, labor related, nervous, and respiratory. The total number of all types of AE was greater than the number of subjects with an AE (58 and 38, respectively) because some women had more than one AE.

[†] Groups were statistically different, $P < .01$.

Table 7. Neonatal Adverse Events, Most Common Types of Adverse Events,* and Serious Adverse Events, by Egg Group

	Ordinary eggs *n* (%)	High-DHA eggs *n* (%)
AE (including SAE)	94(62)	87(57)
Cardiovascular	42	48
Respiratory	32	31
Caused by pregnancy/delivery	34	33
SAE	31(20)	24(16)

Abbreviations as in Table 6.

[*] Types of adverse events evaluated included body general, cardiovascular, congenital and other chromosomal anomalies, related to delivery, digestive, hematologic and lymphatic, metabolic and nutritional, nervous, pregnancy related, respiratory, skin, special senses, and urogenital. The total number of all types of AEs was greater than the number of subjects with an AE (94 and 87, respectively) because some infants had more than one AE.

signaling, and an effect on calcium channels, enzymes, or receptors.[31] By counteracting stimuli that increase uterine muscle contractility, docosahexaenoic acid might increase the duration of gestation by any of these proposed mechanisms.

The increase in duration of gestation noted here is similar to the increases that have been found in two studies of women with already high background intakes of docosahexaenoic acid and eicosapentaenoic acid assigned to consume an additional 2.7 g of n-3 long chain polyunsaturated fatty acids per day. Danish women who consumed docosahexaenoic acid and eicosapentaenoic acid from the 30th week of pregnancy until parturition had a longer gestation (4.0 days, $P = .006$), and their infants tended to have a higher birth weight ($P = .07$) and length ($P = .1$) compared with the placebo control group.[3] Another group of Danish women, who had a prior preterm delivery, consumed 2.7 g per day of docosahexaenoic acid and eicosapentaenoic acid from the 20th week of pregnancy to parturition.[4] Compared with the placebo control group, they had a significantly lower recurrence of preterm delivery, an 8.5-day increase in duration of gestation, and a 209-g increase in mean birth weight.

In contrast, a third large randomized, clinical trial with fish oil docosahexaenoic acid and eicosapentaenoic acid was conducted in Norwegian women, who also have a high antecedent intake of docosahexaenoic acid and eicosapentaenoic acid from fish compared with US women.[32] In that study, neither infant birth weight nor gestation increased when women were supplemented with 2 g per day of n-3 long chain polyunsaturated fatty acids for the last half of pregnancy. One possible explanation for the absence of an effect on gestation in this study was that birth weight and gestation in the control group were already high: more than 3600 g and more than 279 days, respectively.[32]

If there were a threshold effect of docosahexaenoic acid intake on gestation, one might more easily reconcile the similar increases in gestation observed in our study and studies in Denmark, where usual intakes of n-3 long chain polyunsaturated fatty acids are much higher than in the United States. Indeed, a threshold effect of n-3 long chain polyunsaturated fatty acid intake for prevention of low birth weight and preterm birth is suggested by data from a recent analysis of 8729 pregnant Danish women.[33] The authors calculated that the major effect of fish intake on these outcomes occurred in the range between 0 and 150 mg of n-3 long chain polyunsaturated fatty acids per day.

Spontaneous preterm delivery and low birth weight are associated with risk factors that are prevalent in our study population, including black race, poor socioeconomic status, and cigarette smoking.[34,35] Despite this and the fact that women in our study consumed a mean of 137 mg of docosahexaenoic acid per day from high-docosahexaenoic acid eggs (133 mg per egg times 1.03 eggs per day), we did not observe any obvious reduction in preterm or low birth weight deliveries in the present study. Rather, our data suggest a general increase in duration of gestation with higher docosahexaenoic acid intake.

The present study was not powered to detect an increase in birth weight in response to maternal docosahexaenoic acid supplementation. Nevertheless, results for this outcome and other growth-related outcomes of the study (length and head circumference) suggest a positive impact of higher docosahexaenoic acid intake on fetal growth. Both the statistically significant increase in gestational age and the nonsignificant trend toward higher fetal growth could be of clinical importance because relatively higher birth weight among normal birth weight term infants is associated with higher infant development.[8–11]

In a separate, but possibly related, area of investigation, randomized, controlled trials have shown enhanced visual and cognitive development of term[36–38] and preterm[38–44] infants whose postnatal formulas included docosahexaenoic acid from sources such as fish oil, marine algae oil, and egg lipids. In the only randomized study to address development of children whose mothers consumed n-3 long chain polyunsaturated fatty acids during pregnancy (and the first 4 months of lactation), 4-year-old children who were exposed to supplementation early tested significantly higher on the Mental Processing Composite of the Kaufman Assessment Battery for Children than those who were not.[45,46]

A potential limitation of the study was that we had to rely on maternal report of egg intake to estimate docosahexaenoic acid intake. To assess the effects of reported egg intake, we measured RBC phospholipid docosahexaenoic acid, commonly used as an indicator of individual docosahexaenoic acid status. We found a high and significant correlation between maternal RBC phospholipid docosahexaenoic acid at enrollment and at delivery in both groups of women, evidence favoring this measure as an indicator of docosahexaenoic acid status. Moreover, total study egg intake (docosahexaenoic acid intake from eggs) was positively and significantly correlated with maternal RBC phospholipid docosahexaenoic acid at delivery in both groups of women, and maternal docosahexaenoic acid levels at delivery were positively and significantly correlated with infant RBC phospholipid docosahexaenoic acid levels at delivery in both groups. These observations show that eggs are an effective source of dietary docosahexaenoic acid, our measure of egg intake was fairly accurate, and higher maternal docosahexaenoic acid levels lead to higher infant docosahexaenoic acid levels. Other raw correlations are included between gestational age and maternal/infant docosahexaenoic acid levels for completeness. For example, there was a significant correlation between infant RBC phospholipid docosahexaenoic acid and gestation in infants of mothers who consumed the high-docosahexaenoic acid eggs. However, the correlations are not adjusted for other important factors that influence the length of gestation, and it should be kept in mind that the regression model gives a much more accurate picture of the relationship between docosahexaenoic acid intake and gestational age.

As mentioned earlier, cold-water fish are the highest food source of docosahexaenoic acid in the diet.[1] However, many fish carry advisories against consumption by pregnant and lactating women because of mercury and other environmental contaminants that have the potential to damage the developing fetus.[47,48] High-docosahexaenoic acid eggs could be an alternative source of dietary docosahexaenoic acid from a food that is more affordable and more broadly consumed than fish.

In conclusion, relatively modest amounts of dietary docosahexaenoic acid during pregnancy appear to extend gestational age and may lead to enhanced fetal growth. Further work is needed to understand the mechanism by which prenatal docosahexaenoic acid increases gestation and to determine whether this increase in gestation has an impact on developmental outcomes.

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