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ORIGINAL ARTICLE

A Randomized Trial of Prenatal n–3 Fatty Acid Supplementation and Preterm Delivery

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ABSTRACT

BACKGROUND

Previous studies have suggested that maternal supplementation with n–3 long-chain polyunsaturated fatty acids may reduce the incidence of preterm delivery but may also prolong gestation beyond term; however, more data are needed regarding the role of n–3 long-chain polyunsaturated fatty acids in pregnancy.

METHODS

We performed a multicenter, double-blind, randomized trial in which women who were pregnant with single or multiple fetuses were assigned to receive either fish-oil capsules that contained 900 mg of n–3 long-chain polyunsaturated fatty acids (n–3 group) or vegetable-oil capsules that contained trace n–3 long-chain polyunsaturated fatty acids (control group) daily, beginning before 20 weeks of gestation and continuing to 34 weeks of gestation or delivery, whichever occurred first. The primary outcome was early preterm delivery, defined as delivery before 34 completed weeks of gestation. Other pregnancy and neonatal outcomes were also assessed.

RESULTS

A total of 5544 pregnancies in 5517 women were randomly assigned at six centers in Australia; 5486 pregnancies were included in the primary analysis. Early preterm delivery occurred in the case of 61 of 2734 pregnancies (2.2%) in the n–3 group and 55 of 2752 pregnancies (2.0%) in the control group; the between-group difference was not significant (adjusted relative risk, 1.13; 95% confidence interval [CI], 0.79 to 1.63; P=0.50). There were no significant differences between the groups in the incidence of interventions in post-term (>41 weeks of gestation) deliveries, in adverse events, or in other pregnancy or neonatal outcomes, except that a higher percentage of infants born to women in the n–3 group than in the control group were very large for gestational age at birth (adjusted relative risk, 1.30; 95% CI, 1.02 to 1.65). Percentages of serious adverse events did not differ between the groups. Minor gastrointestinal disturbances were more commonly reported in the n–3 group than in the control group.

CONCLUSIONS

Supplementation with n–3 long-chain polyunsaturated fatty acids from early pregnancy (<20 weeks of gestation) until 34 weeks of gestation did not result in a lower incidence of early preterm delivery or a higher incidence of interventions in post-term deliveries than control. (Funded by the Australian National Health and Medical Research Council and the Thyne Reid Foundation; ORIP Australian New Zealand Clinical Trials Registry number, ACTRN12613001142729.)

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Preterm delivery (defined as delivery before 37 weeks of gestation) occurs in 15 million pregnancies each year and is the leading cause of early childhood complications and death[1]; early preterm deliveries (defined as delivery before 34 weeks of gestation) represent approximately 20% of all preterm deliveries but account for the largest burden of neonatal deaths and childhood disability.[2] The incidence of preterm deliveries is rising globally,[2,3] and although the drivers for this vary among populations, identifying an effective, broad-based prevention strategy is a priority.

Labor initiation in humans is a complex and incompletely understood interplay of maternal and fetal endocrine signaling. The balance between oxylipins derived from n–6 arachidonic acid and those derived from n–3 eicosapentaenoic acid and docosahexaenoic acid (DHA), which are also known as n–3 long-chain polyunsaturated fatty acids, plays an essential role, with proinflammatory 2-series prostaglandins such as PGE_2 and $PGF_{2\alpha}$ known to stimulate cervical ripening and uterine contractility.[4] Typical Western diets are relatively low in n–3 long-chain polyunsaturated fatty acids, which leads to a predominance of 2-series prostaglandin substrate in the fetoplacental unit and potentially confers a predisposition to preterm delivery. Epidemiologic studies have shown significant associations between lower fish consumption in pregnancy and increased rates of preterm delivery.[5-8] The World Health Organization recommends an intake of 300 mg of n–3 long-chain polyunsaturated fatty acids per day in pregnant women; however, the median intake among Australian and American women of childbearing age is less than one third of this.[9,10] Dietary insufficiency of n–3 long-chain polyunsaturated fatty acids may therefore play a role in the pathophysiology of preterm delivery and presents a potential target for intervention.

We previously conducted the DOMInO (DHA to Optimise Mother Infant Outcome) trial, which showed that DHA had no significant effect on postpartum depression or infant neurodevelopment (the primary outcomes). In that trial, the use of DHA resulted in a lower incidence of early preterm delivery than control, but a higher incidence of post-term (>41 weeks of gestation) deliveries involving obstetrical interventions.[11] Both of those outcomes were secondary and the analyses were not adjusted for multiplicity, which limits confidence in these results. Several systematic reviews have shown inconsistent findings regarding the effect of supplementation with n–3 long-chain polyunsaturated fatty acids during pregnancy on early preterm and preterm delivery.[12-15] We designed the Omega-3 to Reduce the Incidence of Preterm Birth (ORIP) trial to assess whether supplementation with n–3 long-chain polyunsaturated fatty acids, administered from early pregnancy (<20 weeks of gestation) until 34 weeks of gestation, would result in a lower incidence of early preterm delivery than control, without increasing the incidence of post-term obstetrical interventions, in a broad population of pregnant women.

Methods

Trial Design and Oversight

The ORIP trial was designed as a multicenter, double-blind, randomized, controlled clinical trial and was funded by the Australian National Health and Medical Research Council and the Thyne Reid Foundation. The trial capsules were donated by Croda UK and Efamol/Wassen UK; neither company had any other role in the trial. The funders were not involved in the design of the trial, the collection or analysis of the data, or the writing of the manuscript. The trial protocol (which has been published previously[16] and is available with the full text of this article at NEJM.org) was developed by the authors and was approved by the human research ethics committee at each trial center. The trial was conducted according to the 2007 National Statement on Ethical Conduct in Human Research of the National Health and Medical Research Council and the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95). An independent serious adverse events committee, whose members were unaware of the group assignments, evaluated adverse outcomes in mothers and babies. An independent data monitoring committee, whose members had access to unblinded data related to key trial outcomes (including the primary outcome), reviewed trial safety and progress; the committee also reviewed a planned interim analysis of early preterm delivery halfway through the trial and recommended that the trial continue. The authors analyzed the data, vouch for the accuracy and completeness of the data and for the fidelity of the trial

to the protocol, wrote or contributed to the writing of the manuscript, and made the decision to submit the manuscript for publication.

PARTICIPANTS

Women who were pregnant with a single fetus or multiple fetuses and who attended one of six centers in four states in Australia were recruited at their first antenatal appointment from November 1, 2013, to April 26, 2017. Women were excluded if they were taking daily supplements containing more than 150 mg of n–3 long-chain polyunsaturated fatty acids or if they were unwilling to discontinue daily supplements containing 150 mg or less of n–3 long-chain polyunsaturated fatty acids. Women were also excluded if they had coagulopathy, were receiving anticoagulants, had a known history of substance abuse, or if their fetus had a known congenital abnormality. Women could participate in the trial for successive pregnancies and underwent a separate randomization for each pregnancy. The last infant was born on November 26, 2017, and data collection was completed on January 8, 2018.

RANDOMIZATION AND MASKING

After written informed consent was obtained, women were randomly assigned, with the use of a Web-based randomization service, to receive either n–3 long-chain polyunsaturated fatty acid capsules (n–3 group) or vegetable-oil capsules (control group); the randomization schedule was prepared by an independent statistician, with balanced variable blocks and stratification according to trial center and previous use of n–3 long-chain polyunsaturated fatty acid supplements (yes or no). At randomization, women were provided with five bottles, each containing 90 capsules. Additional capsules were reissued as necessary. The bottles and capsules of n–3 fatty acid and control were identical in appearance, and the control capsules contained 5% tuna oil to aid in the masking of the group assignment. Participants, clinicians, and researchers remained unaware of the group assignments until the data analysis was complete.

TRIAL INTERVENTIONS

Women in the n–3 group received three 500-mg fish-oil capsules per day, which provided a total of approximately 900 mg of n–3 long-chain polyunsaturated fatty acid per day (approximately 800 mg of DHA and approximately 100 mg of eicosapentaenoic acid [Incromega DHA 500TG capsules]). Patients in the control group received three 500-mg capsules per day containing an isocaloric vegetable-oil blend that provided a total of approximately 15 mg of DHA and approximately 4 mg of eicosapentaenoic acid per day. The assigned capsules were to be taken orally from the time of trial entry (<20 weeks of gestation) until 34 weeks of gestation or until delivery, whichever occurred sooner. Control capsules were formulated to be consistent with the fatty acid composition of a typical Australian diet. The full compositions of the capsules are provided in Table S1 in the Supplementary Appendix, available at NEJM.org.

At the time of enrollment, baseline clinical and sociodemographic characteristics of the participants were assessed, and a dried blood spot for fatty acid analysis was collected on filter paper.[17,18] Adherence to the trial regimen and the occurrence of any adverse events were assessed by means of telephone contact 2 weeks after enrollment and at 28 weeks of gestation. Women returned for in-person visits at 34 weeks of gestation so that the number of unused capsules could be recorded and a dried blood spot could be obtained. Data were extracted from maternal and infant medical records 6 weeks after delivery by trained trial staff who were not members of the clinical care team.

OUTCOMES

The length of gestation at the time of delivery was determined on the basis of both the date of the last menstrual period and ultrasonographic data obtained in early pregnancy (the full algorithm is provided in Fig. S1 in the Supplementary Appendix). The primary outcome was early preterm delivery, defined as delivery before 34 completed weeks of gestation. Secondary outcomes included other pregnancy, delivery, and neonatal outcomes (Table S2 in the Supplementary Appendix).

STATISTICAL ANALYSIS

On the basis of the incidence of early preterm deliveries among singleton pregnancies in a similar population[11] and the higher rates of early preterm delivery that are expected among pregnancies with multiple fetuses than among singleton pregnancies, and assuming a 5% loss to

follow-up, we calculated that 5540 pregnancies (2770 per group) would be needed to provide the trial with 85% power to show a rate of early preterm delivery that was 1.16 percentage points lower in the n–3 group than in the control group (1.29% vs. 2.45%), at a two-sided alpha level of 0.049 at the final analysis. A single interim analysis of the primary outcome was prespecified when the first 2270 deliveries had occurred. An O'Brien–Fleming alpha spending function was used to control the type I error rate at 0.05.[19]

The primary analysis was performed on an intention-to-treat basis. The incidence of early preterm delivery was compared between the groups with the use of a log-binomial regression model. Adjustment was made for the randomization strata in the primary analysis; unadjusted analyses were also performed. Generalized estimating equations were used to account for clustering of pregnancies in women who participated in the trial for successive pregnancies.

Secondary outcomes were compared between the groups with the use of log-binomial, linear, and log-Poisson regression models for binary, continuous, and count outcomes, respectively. Generalized estimating equations were used in the assessment of maternal outcomes to account for clustering of pregnancies in women who participated in the trial for successive pregnancies and in the assessment of neonatal outcomes to account for clustering due to siblings (from the same pregnancy or successive pregnancies). Time-to-event outcomes were analyzed with the use of Fine and Gray regression models for competing risks, with a cluster-adjusted variance estimator.[20]

Multiple imputation with the use of chained equations was performed separately in each group to address missing data under a missing-at-random assumption.[21] Data on outcomes that were undefined because of miscarriage, termination of pregnancy, or stillbirth were not imputed. Adjusted analyses that used imputed data were considered to be the primary analyses.

Prespecified analyses of early preterm delivery and related secondary outcomes (27 outcomes total) were performed in subgroups defined according to pregnancy with single or multiple fetuses, history of preterm delivery (yes or no), and baseline DHA level (in quartiles). Four of the 81 planned interaction tests were expected to be significant (P<0.05) by chance.

No adjustment was made for multiple comparisons of outcomes, so P values are reported only for the primary outcome, serious adverse events, and interaction tests in subgroup analyses. The 95% confidence intervals reported for secondary outcomes and subgroup analyses have not been adjusted for multiplicity and therefore should not be used to infer treatment effects. All analyses were performed with the use of SAS software, version 9.4 (SAS Institute), and followed a prespecified statistical analysis plan (available at NEJM.org).

RESULTS

TRIAL PARTICIPANTS

A total of 5544 pregnancies in 5517 women were randomly assigned at the six trial centers; 27 women participated in the trial twice and underwent a separate randomization for each pregnancy (for 14 of these women, the group to which they were assigned the second time was different from the group to which they had been assigned the first time). A total of 2770 pregnancies in 2766 women were assigned to the n–3 group, and 2774 pregnancies in 2765 women to the control group (Fig. 1). After withdrawal of consent, loss to follow-up, miscarriages, and terminations of pregnancies, primary outcome data were available for 5431 pregnancies (98%). After multiple imputation for missing data, 5486 pregnancies were included in the primary analysis (2734 in the n–3 group and 2752 in the control group).

Baseline characteristics were balanced between the groups (Table 1, and Table S3 in the Supplementary Appendix). At the end of the intervention period, the mean DHA concentration in the dried blood spots obtained from women in the n–3 group was 32% higher than that among women in the control group (Table S7 in the Supplementary Appendix).

OUTCOMES

There was no significant between-group difference in the primary outcome of early preterm delivery. Early preterm delivery occurred in the case of 61 of 2734 pregnancies (2.2%) in the n–3 group and 55 of 2752 pregnancies (2.0%) in the control group (unadjusted and adjusted relative risk, 1.13; 95% confidence interval [CI], 0.79 to 1.63; P=0.50).

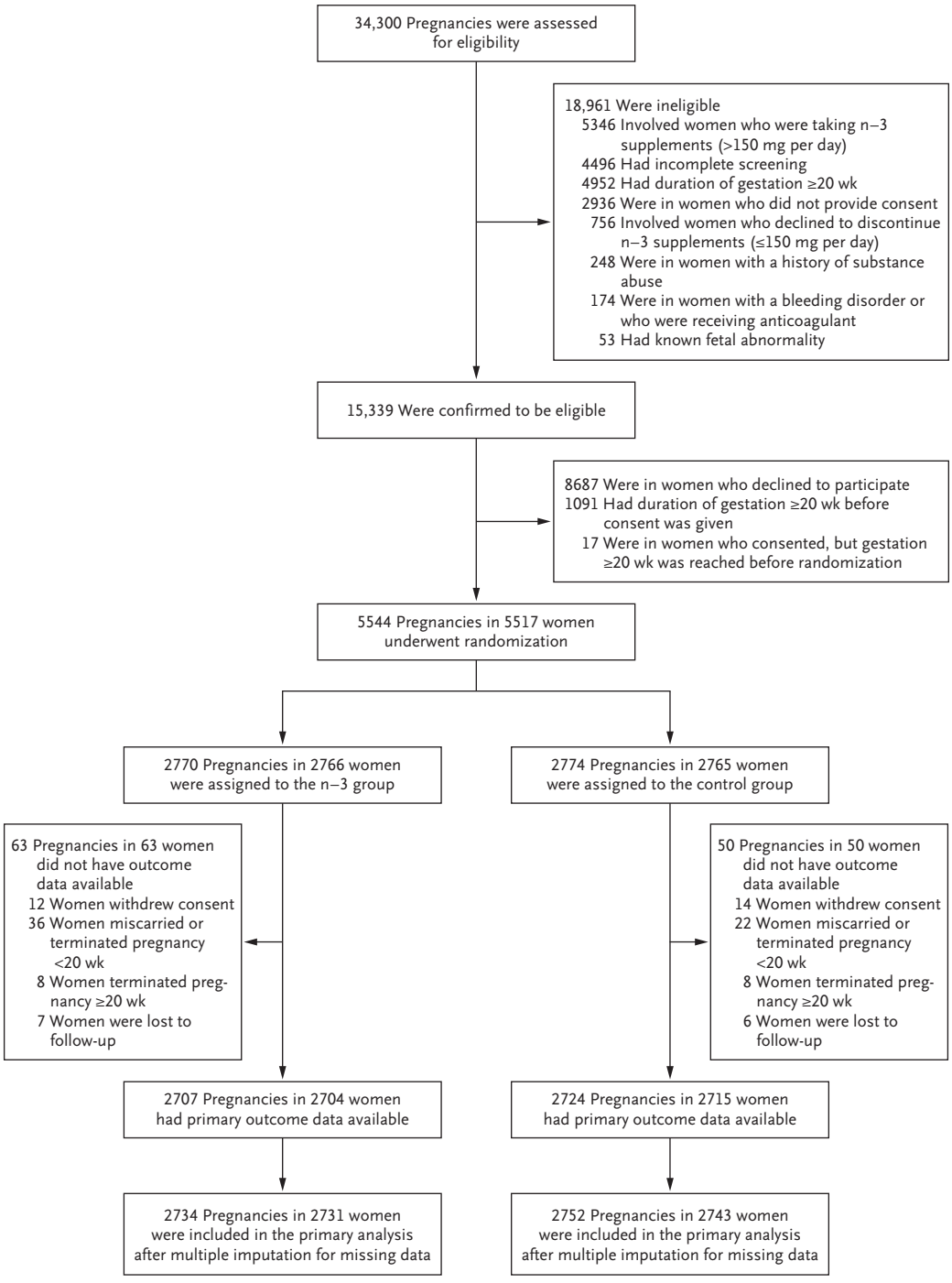

Figure 1. Screening, Randomization, and Follow-up.

A total of 27 women participated in the trial twice and underwent a separate randomization for each pregnancy; for 14 of these women, the group to which they were assigned the second time was different from the group to which they had been assigned the first time. One woman who was assigned to the n–3 group twice withdrew consent for one pregnancy but provided primary outcome data for the other. Multiple imputation was used for missing data in the primary analysis, with the exception of miscarriages and terminations before 20 weeks of gestation.

Table 1. Baseline Characteristics of the Pregnancies.*

Characteristic	n–3 Group (N=2770)	Control Group (N=2774)
Mother consumed dietary supplements containing n–3 long-chain polyunsaturated fatty acid in the previous 3 mo — no. (%)	374 (13.5)	368 (13.3)
Median maternal age (IQR) — yr†	30.0 (26.0–34.0)	30.0 (27.0–33.0)
Median gestation (IQR) — wk‡	14.1 (12.7–16.4)	14.1 (12.7–16.6)
Mother was primiparous — no./total no. (%)	1223/2754 (44.4)	1209/2765 (43.7)
Pregnancy with multiple fetuses — no./total no. (%)	52/2698 (1.9)	48/2721 (1.8)
Median maternal weight at trial entry (IQR) — kg§	69.7 (60.9–81.5)	69.0 (60.9–81.0)
Maternal white race — no./total no. (%)¶	2057/2764 (74.4)	2041/2767 (73.8)
Mother completed a high school education — no./total no. (%)	2246/2756 (81.5)	2194/2764 (79.4)
Mother smoked cigarettes at trial entry or leading up to pregnancy — no./total no. (%)	435/2755 (15.8)	430/2766 (15.5)
Mother drank alcohol at trial entry or leading up to pregnancy — no./total no. (%)	1513/2755 (54.9)	1562/2764 (56.5)
Mother had previous preterm delivery: <37 wk of gestation — no./total no. (%)	185/2754 (6.7)	184/2766 (6.7)
Median maternal DHA level (IQR) — % of total fatty acids‖	2.7 (2.3–3.1)	2.7 (2.3–3.1)
Male fetus — no./total no. of fetuses (%)**	1395/2758 (50.6)	1409/2770 (50.9)

* There were no significant differences between the groups in any baseline characteristics at a two-sided alpha level of 0.05 except for the number of mothers who completed a high school education (P=0.047). DHA denotes docosahexaenoic acid, and IQR interquartile range.
† Data were missing for 1 pregnancy in the control group.
‡ Data were missing for 15 pregnancies in the n–3 group and 7 pregnancies in the control group.
§ Data were missing for 78 pregnancies in the n–3 group and 64 pregnancies in the control group.
¶ Race was reported by the mothers.
‖ Data were missing for 135 pregnancies in the n–3 group and 133 pregnancies in the control group.
** The total number of fetuses was 2823 in the n–3 group and 2822 in the control group. The total number shown is the total number for which data were available.

The mean duration of gestation was 273 days in both groups. There were no significant between-group differences in key secondary outcomes, including maternal outcomes of preterm delivery, post-term interventions of induction of labor or cesarean section, and spontaneous early preterm delivery and neonatal outcomes of birth weight, low birth weight (<2500 g), and admission to the neonatal intensive care unit (Tables 2 and 3, and Tables S4 and S5 in the Supplementary Appendix).

Analysis of secondary neonatal outcomes showed that there was a greater incidence of infants born very large for gestational age (birth weight, >97th percentile for corresponding gestational age and sex[22]) in the n–3 group than in the control group (adjusted relative risk, 1.30; 95% CI, 1.02 to 1.65) but not a greater incidence of infants born large for gestational age (birth weight, >90th percentile for corresponding gestational age and sex) (Table 3).[22] The greater incidence of infants born very large for gestational age did not correspond to a higher incidence in the n–3 group of obstetrical interventions such as cesarean section or post-term induction of labor, nor to a higher incidence among neonates in that group of shoulder dystocia or complications related to shoulder dystocia (e.g., admission to the neonatal intensive care unit of infants born after 34 weeks of gestation, use of supplemental oxygen, or intraventricular hemorrhage) (see Table S5 in the Supplementary Appendix). Results of the prespecified subgroup analyses are reported in Tables S11 through S13 in the Supplementary Appendix.

SAFETY AND SIDE EFFECTS

No significant differences in serious adverse events were noted between the groups (Table 4). The percentage of miscarriages (loss of pregnancy before 20 weeks of gestation) was 1.1% in the n–3 group and 0.6% in the control group. Of the 32 women in the n–3 group who had a miscarriage, 11 women (34%) had not commenced

Table 2. Trial Outcomes.*

Outcome	n–3 Group (N=2734)	Control Group (N=2752)	Unadjusted Effect Size (95% CI)†	Adjusted Effect Size (95% CI)†
Primary outcome: early preterm delivery — no. (%)	61 (2.2)	55 (2.0)	1.13 (0.79 to 1.63)‡	1.13 (0.79 to 1.63)‡
Key secondary outcomes				
Preterm delivery — no. (%)	211 (7.7)	246 (8.9)	0.86 (0.72 to 1.03)	0.86 (0.72 to 1.03)
Early preterm spontaneous labor — no. (%)	28 (1.0)	20 (0.7)	1.44 (0.81 to 2.56)	1.44 (0.81 to 2.57)
Preterm spontaneous labor — no. (%)	77 (2.8)	95 (3.5)	0.82 (0.60 to 1.10)	0.82 (0.60 to 1.10)
Gestation at delivery — days	273.2±15.2	273.2±14.9	0.02 (–0.78 to 0.82)§	0.02 (–0.78 to 0.82)§
Time to spontaneous labor — days¶	274.8±14.9	275.2±13.2	0.97 (0.90 to 1.05)	0.97 (0.90 to 1.05)
Post-term induction — no. (%)	138 (5.0)	150 (5.5)	0.92 (0.74 to 1.16)	0.92 (0.74 to 1.16)
Clinical indication for post-term induction — no. (%)	208 (7.6)	191 (7.0)	1.10 (0.91 to 1.32)	1.10 (0.91 to 1.33)
Post-term, prelabor cesarean section — no. (%)	3 (0.1)	5 (0.2)	0.63 (0.15 to 2.58)	NA
Clinical indication for post-term, prelabor cesarean section — no. (%)‖	1 (<0.1)	0	NA	NA
Post-term induction or post-term, prelabor cesarean section — no. (%)	141 (5.2)	156 (5.7)	0.91 (0.73 to 1.14)	0.91 (0.73 to 1.14)
Clinical indication for post-term induction or post-term, prelabor cesarean section — no. (%)	209 (7.7)	192 (7.0)	1.10 (0.91 to 1.33)	1.10 (0.91 to 1.33)
Other secondary outcomes				
Prelabor, premature rupture of membranes — no. (%)	456 (16.7)	411 (15.0)	1.11 (0.99 to 1.26)	1.11 (0.99 to 1.26)
Prelabor, preterm, premature rupture of membranes — no. (%)	86 (3.1)	101 (3.7)	0.86 (0.64 to 1.14)	0.85 (0.64 to 1.14)
Prolonged gestation — no. (%)	12 (0.4)	12 (0.4)	1.01 (0.43 to 2.36)	NA
Cesarean section — no. (%)	926 (33.9)	932 (33.9)	1.00 (0.93 to 1.08)	1.00 (0.93 to 1.07)
Gestational diabetes — no. (%)**	439 (16.1)	406 (14.8)	1.09 (0.95 to 1.24)	1.09 (0.95 to 1.24)
Preeclampsia — no. (%)	96 (3.5)	91 (3.3)	1.07 (0.80 to 1.43)	1.07 (0.80 to 1.43)
Postpartum hemorrhage — no. (%)	398 (14.6)	398 (14.5)	1.01 (0.89 to 1.15)	1.01 (0.88 to 1.14)
Post hoc exploratory outcome: shoulder dystocia — no. (%)	69 (2.5)	57 (2.1)	1.21 (0.86 to 1.72)	1.21 (0.86 to 1.72)

* Plus–minus values are means ±SD. Missing data, with the exception of miscarriages and terminations before 20 weeks of gestation, were multiply imputed. Average numerators across the 100 imputed data sets were rounded to the nearest integer, and therefore reported percentages may differ from the expected values. Early preterm was defined as less than 34 weeks of gestation, preterm as less than 37 weeks of gestation, post-term as more than 41 weeks of gestation, and prolonged gestation as more than 42 weeks of gestation. All outcomes were prespecified unless otherwise indicated. NA denotes not applicable because of insufficient numbers of events.

† The effect sizes are relative risks (n–3 group vs. control group) unless otherwise indicated. The adjusted values were adjusted for randomization strata: recruitment hospital and consumption of dietary supplements containing n–3 long-chain polyunsaturated fatty acid in the previous 3 months (yes or no). Except in the case of the primary outcome, the 95% confidence intervals were not adjusted for multiplicity and therefore should not be used to infer treatment effects.

‡ P=0.50.

§ The effect size is the difference in means (n–3 group minus control group).

¶ Data are shown for 1238 pregnancies in the n–3 group and 1275 pregnancies in the control group in which spontaneous labor occurred. The effect sizes are the subdistribution hazard ratios of spontaneous labor, with induction and prelabor cesarean section treated as competing risks and with data on outcomes for women who withdrew from the trial censored at the time of withdrawal.

‖ No imputation of data was performed because of insufficient numbers of events. Data are shown for 2704 pregnancies in the n–3 group and 2721 pregnancies in the control group.

** Gestational diabetes was defined as a baseline blood glucose level of 5.1 mmol or more per liter (92 mg per deciliter), a 1-hour blood glucose level of 10.0 mmol or more per liter (180 mg per deciliter), or a 2-hour blood glucose level of 8.5 mmol or more per liter (153 mg per deciliter).

Table 3. Secondary Clinical Outcomes in Infants.*

Outcome	n–3 Group	Control Group	Unadjusted Effect Size (95% CI)†	Adjusted Effect Size (95% CI)†
Birth weight — g‡§	3351±628	3340±591	10.53 (–23.94 to 45.00)¶	10.56 (–23.87 to 44.99)¶
Birth-weight z score‡§	0.2±1.1	0.2±1.0	0.03 (–0.02 to 0.09)¶	0.03 (–0.02 to 0.09)¶
Low birth weight: <2500 g — no./total no. (%)‡§	204/2787 (7.3)	173/2800 (6.2)	1.18 (0.95 to 1.48)	1.18 (0.95 to 1.47)
Very low birth weight: <1500 g — no./total no. (%)§	43/2787 (1.6)	36/2800 (1.3)	1.21 (0.75 to 1.95)	1.21 (0.75 to 1.94)
Small for gestational age: <10th percentile — no./total no. (%)§	206/2787 (7.4)	196/2800 (7.0)	1.06 (0.87 to 1.28)	1.06 (0.87 to 1.28)
Very small for gestational age: <3rd percentile — no./total no. (%)§	39/2787 (1.4)	47/2800 (1.7)	0.83 (0.53 to 1.29)	0.83 (0.54 to 1.29)
High birth weight: >4000 g — no./total no. (%)§	310/2787 (11.1)	280/2800 (10.0)	1.11 (0.95 to 1.30)	1.11 (0.95 to 1.30)
Large for gestational age: >90th percentile — no./total no. (%)§	392/2787 (14.1)	355/2800 (12.7)	1.11 (0.97 to 1.27)	1.11 (0.97 to 1.27)
Very large for gestational age: >97th percentile — no./total no. (%)§	146/2787 (5.2)	113/2800 (4.0)	1.29 (1.02 to 1.65)	1.30 (1.02 to 1.65)
Birth length — cm§	50.1±3.5	50.2±3.3	–0.02 (–0.21 to 0.17)¶	–0.02 (–0.21 to 0.17)¶
Head circumference at birth — cm§	34.4±2.2	34.4±2.0	–0.03 (–0.15 to 0.08)¶	–0.03 (–0.15 to 0.08)¶
Admission to neonatal intensive care unit — no./total no. (%)‡‖	107/2763 (3.9)	86/2779 (3.1)	1.24 (0.92 to 1.68)	1.24 (0.92 to 1.68)
Intermittent positive-pressure ventilation — no./total no. (%)‖	257/2763 (9.3)	232/2779 (8.3)	1.11 (0.94 to 1.33)	1.11 (0.94 to 1.33)
Receipt of supplemental oxygen — no./total no. (%)‖	71/2763 (2.6)	56/2779 (2.0)	1.28 (0.87 to 1.87)	1.27 (0.87 to 1.86)
Neonatal convulsion — no./total no. (%)**	1/2735 (<0.1)	3/2758 (0.1)	0.34 (0.03 to 3.23)	NA
Any brain injury — no./total no. (%)**	9/2735 (0.3)	9/2758 (0.3)	1.01 (0.38 to 2.66)	NA
Surgery — no./total no. (%)**	13/2735 (0.5)	11/2758 (0.4)	1.19 (0.54 to 2.65)	1.19 (0.53 to 2.64)
Necrotizing enterocolitis — no./total no. (%)**	2/2735 (0.1)	0/2758	NA	NA
Sepsis — no./total no. (%)**	11/2735 (0.4)	6/2758 (0.2)	1.85 (0.68 to 4.99)	NA
Retinopathy of prematurity — no./total no. (%)**	11/2735 (0.4)	7/2758 (0.3)	1.58 (0.58 to 4.35)	NA

* Plus–minus values are means ±SD. All listed outcomes were prespecified. NA denotes not applicable because of insufficient numbers of events.

† The effect sizes are relative risks (n–3 group vs. control group) unless otherwise indicated. The adjusted effect values were adjusted for randomization strata: recruitment hospital and consumption of dietary supplements containing n–3 long-chain polyunsaturated fatty acid in the previous 3 months (yes or no). The 95% confidence intervals were not adjusted for multiplicity and therefore should not be used to infer treatment effects.

‡ The outcome is a key secondary outcome.

§ Missing data, with the exception of miscarriages and terminations before 20 weeks of gestation, were multiply imputed. Average numerators across the 100 imputed data sets were rounded to the nearest integer, and therefore reported percentages may differ from the expected values. Data are shown for 2787 infants in the n–3 group and 2800 infants in the control group.

¶ The effect size is the difference in means (n–3 group minus control group).

‖ Missing data, with the exception of miscarriages, terminations, and stillbirths, were multiply imputed. Average numerators across the 100 imputed data sets were rounded to the nearest integer.

** No imputation of data was performed because of insufficient numbers of events.

Table 4. Serious Adverse Events.*

Variable	n–3 Group	Control Group	Unadjusted Relative Risk (95% CI)†	P Value
Maternal outcomes‡				
No. of pregnancies	2734	2752		
Death — no. (%)	0	0		
Admission to the intensive care unit — no. (%)	14 (0.5)	10 (0.4)	1.41 (0.63–3.17)	0.41
Any serious adverse event — no. (%)	14 (0.5)	10 (0.4)	1.41 (0.63–3.17)	0.41
Neonatal or pregnancy outcomes				
No. of fetuses or infants	2823	2822		
Admission of infants born >34 wk of gestation to the neonatal intensive care unit — no. (%)	58 (2.1)	50 (1.8)	1.16 (0.79–1.70)	0.45
Perinatal death — no. (%)§	32 (1.1)	25 (0.9)	1.28 (0.76–2.17)	0.36
Stillbirth — no. (%)	16 (0.6)	13 (0.5)	1.23 (0.59–2.55)	0.58
Termination of pregnancy <20 wk of gestation — no. (%)	4 (0.1)	4 (0.1)	1.00 (0.25–3.99)	1.00
Termination of pregnancy ≥20 wk of gestation — no. (%)	8 (0.3)	8 (0.3)	1.00 (0.38–2.66)	1.00
Neonatal death — no. (%)	8 (0.3)	4 (0.1)	2.00 (0.60–6.63)	0.26
Major congenital abnormality — no. (%)	30 (1.1)	25 (0.9)	1.20 (0.71–2.03)	0.50
Miscarriage <20 wk of gestation — no. (%)	32 (1.1)	18 (0.6)	1.78 (1.00–3.16)	0.05
Any serious adverse event in an infant or related to the pregnancy — no. (%)	142 (5.0)	112 (4.0)	1.27 (0.99–1.62)	0.06

* All listed outcomes were prespecified secondary outcomes that were considered serious adverse events.
† The 95% confidence intervals were not adjusted for multiplicity and therefore should not be used to infer treatment effects.
‡ Pregnancies that resulted in miscarriage or termination before 20 weeks of gestation were excluded from the analysis of maternal outcomes.
§ The outcome is a key secondary outcome.

taking their capsules, and 21 (66%) had consumed capsules for a mean of 13 days before the miscarriage.

Differences between the groups were observed in side effects, particularly gastrointestinal side effects; women in the n–3 group were more likely to report burping than women in the control group — an effect that is commonly reported in fish-oil supplementation studies.[11] Our addition of a trace quantity of tuna oil to the control capsules was unsuccessful in masking group assignment, since more women in the n–3 group than in the control group were able to correctly guess their assigned intervention. Maternal reports of diarrhea at 28 weeks of gestation and constipation overall were slightly higher in the n–3 group than in the control group (Tables S6 and S7 in the Supplementary Appendix).

DISCUSSION

We found that women who received supplementation with n–3 long-chain polyunsaturated fatty acids from approximately 14 weeks of gestation until 34 weeks of gestation did not have a lower risk of early preterm delivery (delivery before 34 weeks of gestation) or preterm delivery (delivery before 37 weeks of gestation) than women in the control group. The incidences of post-term induction and cesarean section were not significantly higher in the n–3 group than in the control group. Overall, the duration of gestation was similar in the two groups. Maternal and neonatal outcomes did not differ between the groups except that more babies born to women in the n–3 group than in the control group were very large for gestational age at birth; this was one of multiple secondary outcomes assessed, and adjustment was not made for multiplicity.

This trial was designed with the primary aim of confirming the secondary observation in our earlier DOMInO trial that suggested that supplementation with n–3 long-chain polyunsaturated fatty acids during pregnancy reduced the risk of early preterm delivery.[11] We ceased supplementation at 34 weeks of gestation, since the results

of the DOMInO trial also suggested that supplementation with n–3 long-chain polyunsaturated fatty acids until delivery increased the risk of post-term obstetrical intervention.[11] Pregnancy outcomes in the DOMInO trial were secondary outcomes, and results were not adjusted for multiple comparisons and may have been chance findings. The results of the current ORIP trial do not support our earlier results in the DOMInO trial and also contrast with findings in other trials[23,24] and with the most recent Cochrane systematic review.[25] Many previous studies were designed to assess n–3 long-chain polyunsaturated fatty acid supplementation in specific pregnancy populations or were designed with childhood allergy or childhood neurodevelopment as primary outcomes. The ORIP trial was designed to assess duration of gestation, it had few exclusion criteria related to prematurity risk, and it started supplementation earlier in pregnancy than the previous studies.

The overall reported adherence among patients in the n–3 group in this trial in mid-to-late pregnancy was lower than that in the DOMInO trial.[11] Nevertheless, the overall increase in the percentage of whole-blood DHA and eicosapentaenoic acid as total fatty acids and the associated reduction in arachidonic acid were the expected biochemical responses to n–3 long-chain polyunsaturated fatty acid supplementation, and restricting the analysis to women who adhered to the protocol did not alter the conclusions.

The baseline level of n–3 long-chain polyunsaturated fatty acids in the women in this trial may have been higher than that in previous studies, and this may have contributed to the absence of appreciable effects of supplementation on outcomes. Approximately 80% of Australian women now consume perinatal supplements, many of which contain small doses of DHA.[26] Although we excluded women who were taking more than 150 mg of DHA per day, we enrolled more than 700 women who were known to have been regularly consuming a low dose of DHA (≤150 mg per day). This may have influenced the baseline level of DHA among the women included in the ORIP trial, which was about 20% higher than that observed in the Kansas University DHA Outcomes Study (KUDOS), in which a significantly lower rate of early preterm delivery was observed with n–3 supplementation than with placebo.[23] The results of subgroup analyses in one of the few other trials to assess baseline n–3 intake suggested that the prolongation of pregnancy with n–3 long-chain polyunsaturated fatty acid supplementation was most evident among women with the lowest intakes, although the interaction test did not reach significance.[27] It is possible that women with low n–3 status are the ones most likely to benefit from dietary supplementation strategies; however, we did not find evidence to support this hypothesis in the prespecified subgroup analysis we performed according to baseline DHA status. Further study is needed to determine whether there may be benefit in women who have low n–3 levels.

Our observation of a higher incidence in the n–3 group than in the control group of infants (primarily in singleton pregnancies) who were very large for gestational age adds to the debate of whether n–3 long-chain polyunsaturated fatty acid supplementation directly influences fetal growth.[24,25,28] In the absence of clear evidence of change in other measures of infant anthropometry, and given the large number of outcomes assessed (without adjustment for multiplicity), this result may be a chance finding.

In conclusion, in this large, randomized trial with broad inclusion criteria, n–3 supplementation in pregnancy did not result in a lower risk of early preterm delivery than control.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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REFERENCES

1. March of Dimes, The Partnership for Maternal, Newborn & Child Health, Save the Children, World Health Organization. Born too soon: the global action report on preterm births. Geneva: World Health Organization, 2012 (https://www.who.int/pmnch/media/news/2012/201204_borntoosoon-report.pdf).
2. Martin JA, Osterman MJK. Describing the increase in preterm births in the United States, 2014-2016. NCHS Data Brief 2018;312:1-8.
3. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379:2162-72.
4. Patel FA, Challis JRG. Prostaglandins and uterine activity. In: Smith R, ed. The endocrinology of parturition: basic science and clinical application. Basel, Switzerland: Karger, 2001:31-56.
5. Olsen SF, Hansen HS, Sommer S, et al. Gestational age in relation to marine n-3 fatty acids in maternal erythrocytes: a study of women in the Faroe Islands and Denmark. Am J Obstet Gynecol 1991;164:1203-9.
6. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. Am J Epidemiol 2004;160:774-83.
7. Leventakou V, Roumeliotaki T, Martinez D, et al. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. Am J Clin Nutr 2014;99:506-16.
8. Olsen SF, Halldorsson TI, Thorne-Lyman AL, et al. Plasma concentrations of long chain n-3 fatty acids in early and mid-pregnancy and risk of early preterm birth. EBioMedicine 2018;35:325-33.
9. Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PR. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids 2003;38:391-8.
10. Nordgren TM, Lyden E, Anderson-Berry A, Hanson C. Omega-3 fatty acid intake of pregnant women and women of childbearing age in the United States: potential for deficiency? Nutrients 2017;9(3):E197.
11. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA 2010;304:1675-83.
12. Imhoff-Kunsch B, Briggs V, Goldenberg T, Ramakrishnan U. Effect of n-3 long-chain polyunsaturated fatty acid intake during pregnancy on maternal, infant, and child health outcomes: a systematic review. Paediatr Perinat Epidemiol 2012;26:Suppl 1:91-107.
13. Kar S, Wong M, Rogozinska E, Thangaratinam S. Effects of omega-3 fatty acids in prevention of early preterm delivery: a systematic review and meta-analysis of randomized studies. Eur J Obstet Gynecol Reprod Biol 2016;198:40-6.
14. Newberry SJ, Chung M, Booth M, et al. Omega-3 fatty acids and maternal and child health: an updated systematic review. Evid Rep Technol Assess (Full Rep) 2016;224:1-826.
15. Saccone G, Saccone I, Berghella V. Omega-3 long-chain polyunsaturated fatty acids and fish oil supplementation during pregnancy: which evidence? J Matern Fetal Neonatal Med 2016;29:2389-97.
16. Zhou SJ, Best K, Gibson R, et al. Study protocol for a randomised controlled trial evaluating the effect of prenatal omega-3 LCPUFA supplementation to reduce the incidence of preterm birth: the ORIP trial. BMJ Open 2017;7(9):e018360.
17. Collins CT, Makrides M, McPhee AJ, et al. Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants. N Engl J Med 2017;376:1245-55.
18. Liu G, Mühlhäusler BS, Gibson RA. A method for long term stabilisation of long chain polyunsaturated fatty acids in dried blood spots and its clinical application. Prostaglandins Leukot Essent Fatty Acids 2014;91:251-60.
19. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35:549-56.
20. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-509.
21. Sullivan TR, White IR, Salter AB, Ryan P, Lee KJ. Should multiple imputation be the method of choice for handling missing data in randomized trials? Stat Methods Med Res 2018;27:2610-26.
22. Beeby PJ, Bhutap T, Taylor LK. New South Wales population-based birthweight percentile charts. J Paediatr Child Health 1996;32:512-8.
23. Carlson SE, Colombo J, Gajewski BJ, et al. DHA supplementation and pregnancy outcomes. Am J Clin Nutr 2013;97:808-15.
24. Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in high risk pregnancies. BJOG 2000;107:382-95.
25. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11:CD003402.
26. Zhou SJ, Condo D, Ryan P, et al. Association between maternal iodine intake in pregnancy and childhood neurodevelopment at age 18 months. Am J Epidemiol 2019;188:332-8.
27. Olsen SF, Østerdal ML, Salvig JD, Weber T, Tabor A, Secher NJ. Duration of pregnancy in relation to fish oil supplementation and habitual fish intake: a randomised clinical trial with fish oil. Eur J Clin Nutr 2007;61:976-85.
28. Zhou SJ, Yelland L, McPhee AJ, Quinlivan J, Gibson RA, Makrides M. Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia. Am J Clin Nutr 2012;95:1378-84.