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Article

Prenatal maternal docosahexaenoic acid (DHA) supplementation and newborn anthropometry in India: findings from DHANI

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Abstract: Long-chain omega-3 fatty acid status during pregnancy may influence newborn anthropometry and duration of gestation. Evidence from high-quality trials from LMICs is limited. We conducted a double-blind, randomized, placebo-controlled trial among 957 pregnant women (singleton gestation, 14-20 weeks' gestation at enrollment) in India to test the effectiveness of 400 mg/d algal docosahexaenoic acid (DHA) compared to placebo provided from enrollment through delivery. Among 3379 women who were screened, 1171 were found eligible; 957 enrolled and were randomized. The intervention was two microencapsulated algal DHA (200 X 2= 400 mg/d) or two microencapsulated soy and corn oil placebo tablets to be consumed daily from enrollment (≤20 weeks) through delivery. The primary outcome was newborn anthropometry (birth weight, length, head circumference). Secondary outcomes were gestational age and 1 and 5 min Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score. The groups (DHA; n=478 and placebo; n=479) were well balanced at baseline. There were 902 live births. Compliance with the intervention was similar across groups (DHA: 88.5%; placebo: 87.1%). There were no significant differences between DHA and placebo group for birth weight (2750.6 ± 421.5 vs. 2768.2 ± 436.6 g, p=0.54), length (47.3 ± 2.0 vs. 47.5 ±2.0 cm, p=0.13) or head circumference (33.7 ± 1.4 vs 33.8 ± 1.4 cm, p=0.15). The mean gestational age at delivery was similar between groups (DHA: 38.8 ± 1.7 placebo: 38.8 ± 1.7 wk, p= 0.54) as were APGAR scores at 1 and 5 min. Supplementing mothers through pregnancy with 400mg/d DHA did not impact the offspring birthweight, length or head circumference.

Keywords: Docosahexaenoic acid (DHA), long chain omega-3 fatty acids, maternal supplementation, pregnancy outcomes, anthropometry, birth weight, birth length, head circumference

1. Introduction

Birth weight is a key predictor of the health trajectory of a child [1]. In 2015, the global prevalence of Low birth weight (LBW) was recorded to be 14.6% and 91% of these were from low-and-middle income countries (LMIC), primarily southern Asia (48%) and sub-Saharan Africa (24%). [2]LBW and preterm birth are leading causes of neonatal death in LMIC [3]. In addition, LBW is associated with increased risk of numerous adverse health outcomes in childhood [4, 5] and adulthood [6, 7]. Women in deprived socio-economic conditions frequently have poor nutrition and consequently deliver infants with LBW [8]. Evidence from several studies, including from birth cohorts in Brazil,

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Guatemala, India, The Philippines, and South Africa [9] shows that poor foetal growth carries higher risk of chronic diseases related to nutrition later in adult life.

LBW can be the result of preterm birth (PTB) and/or intrauterine growth restriction (IUGR). The underlying causes of both PTB and IUGR are multi-factorial, however, the etiologies have a common pathway of insufficient uterine-placental perfusion and fetal nutrition [10]. Maternal nutritional status has been identified as one of the key determinants for LBW in India [11]. Current dietary recommendations for pregnant women emphasize protein, energy, vitamin, and mineral adequacy, but increasing attention is being given to dietary lipids, especially essential fatty acids (EFAs) [12]. Long-chain polyunsaturated fatty acid (LC-PUFA) intake during pregnancy influences both maternal and infant fatty acid status at birth [13], which in turn is associated with birth weight and gestational age at birth [14]. A substantial proportion of the Indian population are vegetarian (35%, ranging from 10-62% across regions) or observe religious dietary restrictions that can result in multiple nutrient deficiencies [15]. Since the main dietary source of DHA is oily fish, non-supplemented vegetarian diets contain little DHA and vegan diets contain virtually none. Indian women have low intakes of omega-3 fatty acids – median Alpha linolenic acid (ALA), Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA) levels are 560, 3 and 1.1 mg/d during pregnancy, respectively [16].

Growing evidence suggests that supplementation during pregnancy with omega-3 fatty acids, especially DHA may improve birth outcomes. In a prospective cohort study from southern India, women who did not eat fish during the third trimester had a significantly higher risk of LBW (OR: 2.49, P=0.019) when compared to women whose intake was above median that is, 9.33 g/d (interquartile range: 5.10–15.69) [17]. A review by Makrides and Best [18] suggested that N-3 LCPUFA supplementation during pregnancy increased the mean duration of gestation by 2 days; there was a also a 40-50% reduction in early preterm birth (<34 weeks' gestation) [18]. In the United States of America, DHA supplementation resulted in longer gestation duration (2.9 d; P = 0.041), and greater birth weight (172 g; P = 0.004), length (0.7 cm; P = 0.022), and head circumference (HC) (0.5 cm; P = 0.012) [19]. Among Mexican women randomized to 400 mg/day of algal DHA or placebo from 18-22 weeks of gestation through delivery, intent-to-treat analysis showed no differences between the placebo and DHA groups in newborn anthropometry, but offspring of supplemented primigravidae were 99.4 g heavier (95% CI, 5.5 to 193.4) and had 0.5 cm larger HC (diff=95% CI, 0.1 to 0.9) than controls [20]. In the DHA to Optimize Mother Infant Outcome [DOMInO] trial from Australia, women who received fish oil supplements had lower risk of very preterm birth (1.09% in the DHA group compared to 2.25% in the control group); mean birth weight was 68 g (95% CI, 23-114 g) heavier and fewer infants had LBW (3.41% vs 5.27%; 95% CI, 0.44-0.96) [21].

As results have been mixed, and little research on this question comes from LMIC contexts where the underlying nutritional status and etiology of LBW may differ, we assessed the impact of maternal DHA supplementation on newborn anthropometry, APGAR score, duration of gestation and low birth weight among Indian women.

2. Materials and Methods

2.1. Trial Design and Setting

DHANI (Effect of n-3 fatty acid (**DHA**) Supplementation during pregnancy on **N**ewborn birth weight and gestational age in **I**ndia) was established as a randomized, double-blinded, placebo-controlled trial, to assess the effect of 400 mg/day algal prenatal DHA consumption by healthy Indian women from ≤20 weeks of singleton gestation till delivery on their offspring's size (weight, length and head circumference) at birth.. The detailed trial protocol has been published elsewhere [38]. DHANI is registered on CTRI website as CTRI/2013/04/003540 and on clinical trials.gov as NCT01580345. Ethical clearance was obtained from institutional review boards (IRBs) of all participating institutions: Center for Chronic Disease Control (CCDC-IEC_04_2015), Public Health

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Foundation of India (PHFI) (TRC-IEC-261/15) and Jawaharlal Nehru Medical College (MDC/IECHSR/2016-17/A-85).

Participants and Trial Procedures

The study population was healthy pregnant women, aged 18–35 years with singleton pregnancy under ≤20 weeks of gestation, with no obstetric high risk conditions, medical complications or chronic diseases, attending the Department of Obstetrics and Gynecology at the Prabhakar Kore Hospital (PKH) in Belgavi, a largely rural district in Karnataka State, southwest India for antenatal care. Designated project staff approached women, and the consulting obstetrician on site, considering obstetric history and complications, affirmed final eligibility. Consenting eligible women were randomized by project staff to receive either 400 mg/day DHA or a placebo after providing written informed consent using a form in their preferred local language (Kannada, Marathi, or Hindi) and observed by a witness. Information on sociodemographic characteristics, obstetric and medical history, dietary intake (with a pre-piloted semi-quantitative food frequency questionnaire focusing on n-3 LC-PUFA-rich Indian foods), anthropometric measurements, a non-fasting blood draw, and vital signs were obtained at enrollment. The women were then given their first 15-day supply of supplements in the form of coded bottles matching the allotted code for the participant. Further supplements were either collected by the women from the study site or were delivered to the women's homes every fortnight by fieldworkers.

Research staff maintained contact with all women, especially during the last trimester, and visited the woman in the delivery ward within 24 hours of delivery to collect data on gestational age at delivery, type of delivery, complications (if any), pregnancy outcome, APGAR (Appearance, Pulse, Grimace, Activity, Respiration) score, newborn anthropometry (weight, length, and head circumference), and maternal and cord blood samples.

Randomization, Masking, and Intervention

The randomization list was generated using a permuted block design. The randomization code list was generated for 1200 women (randomly allocating 600 women to DHA or placebo) to allow for potential loss to follow up. The assignment code list was kept in a sealed envelope at the beginning of the study and in a secure location at PHFI by a staff member not involved in the trial. The assignment code list was used by this staff member to code the supplement bottles in the warehouse before the bottles were shipped to the study site. Study participants and research staff (including those at the study site) remained blinded to the treatment allocation throughout the duration of fieldwork. Full data analysis was carried out after the Data Safety Monitoring Board (DSMB) approved the blinded preliminary descriptive analyses. Unblinding of the treatment group was done only after generation of the primary tables.

The intervention comprised of capsules having either 200 mg/d algal DHA or a placebo (soy/corn oil in 50:50 ratio), which were given in the form of soft-gel capsules, identical in taste and appearance. These were donated by DSM Nutritional Supplements, Mumbai. The capsules had a shelf life of 24 months from the date of manufacture when stored at room temperature (25 ◦C) and sealed, and 90 days once the bottle was opened. The coded capsule bottles were stored in an on-site refrigerator to further slow oxidation. Each bottle contained a 15-day supply of capsules. The women were instructed to take two capsules daily, preferably at the same time each day. They were told to keep them in a cool, dry place. Supplements were provided for more than two weeks in cases where the woman shared plans to travel. Enrolled women received supplements from the date of randomization through 6 months postpartum; for the present analysis only supplement intake through delivery was considered.

Outcomes

The primary outcome for DHANI trial was newborn anthropometry (birth weight, birth length, head circumference). Secondary outcomes included gestational age, APGAR scores at 1 and 5 minutes; still births, LBW and preterm. All research staff at the study site were apprised of the data collection methods before the start of the trial and were provided regular refresher training every 6 months. Abstracted data included gestational age, pregnancy outcome (live birth, sex of baby, type of delivery), and APGAR score at 1 minute and 5 minutes. Gestational age at delivery was calculated

in weeks by noting the number of days from last menstrual period (LMP) until delivery. Preterm delivery was defined as delivery after 20 weeks and before 37 completed weeks. Anthropometric data were collected by a trained research assistant within 24 h of delivery. Birth weight was measured to the nearest 10 g by using a portable single-pan digital pediatric weighing scale. Low birth weight was defined as recorded birth weight less than 2500 g. Birth length and head circumference were measured by trained research staff to the nearest 1 mm using a portable anthropometer with a fixed headpiece and a non-stretchable measuring tape, respectively, according to standard procedures. Fetal losses during pregnancy – including miscarriages/abortions and still-births and the APGAR scores were obtained from the hospital records by study personnel on site or details were brought by field workers (in case mother went to any other hospital). Stillbirths were defined as fetuses delivered at 20 weeks of gestation or later with no signs of life and recorded as occurring before or during the onset of labor; neonatal deaths were defined as deaths among live-born infants occurring within 28 days after delivery.

Adherence and follow up

Subjects were asked to maintain a daily record of their supplement-consumption using a form provided by study staff. Weekly calls were made by the research staff to encourage compliance and inquire about general well-being. The used bottles were collected (for pill count) by the field-workers during the fortnightly home visits. The compliance was calculated as the total number of capsules actually consumed, expressed as a percentage of the total number expected to be consumed, which was assessed on the basis of a compliance form filled by the participant and verified by the research staff at all home visits. A sub-sample of venous blood samples collected from the mother at recruitment, delivery and 6 months postpartum and their infants at 12 months of age were analyzed for DHA levels.

Statistical analysis

Baseline maternal and offspring characteristics were summarized as means and standard deviations or medians and inter-quartile ranges as appropriate and categorical variables were summarized using proportions.

We used two-sample t test to compare the differences in mean birth weight, birth length, head circumference and APGAR score at 1 min and 5 min at delivery between the DHA and placebo group. We also calculated the z score for birth weight, length and head circumference using standards established by the (International Fetal and Newborn Growth Consortium for the 21st century (INTERGROWTH-21) Project [22] and compared the difference in z score between DHA and placebo group using two-sample t-tests. The differences in proportion for preterm birth and LBW between the DHA and placebo groups were compared using the two-proportion z-test. Analysis was done using the intent to treat (ITT) principle.

We conducted several pre-specified subgroup analyses to estimate treatment effects within categories of maternal age (18-20, 21-25, 26-30, 31-35 years), body mass index (BMI) at enrollment (< 18·5 kg/m^2; 18·5–23.0 kg/m^2; 23.0–27·5 kg/m^2; and 27·5 kg/m^2) as per Asian cut-offs [23], gravidity (multi-gravida, primi-gravida), gestational age at delivery (<37, ≥37 weeks), compliance (<80.0%, ≥80.0%), and vegetarian diet (yes, no), and child sex (male, female). The p-value for heterogeneity was calculated by including the interaction term between the characteristic of interest and treatment group in the linear regression model. The significance of within-subgroup treatment effects was adjusted for multiplicity for multiple subgroup analyses using Bonferroni criterion i.e. by dividing the overall significance level by total number of subgroup analyses performed. For sensitivity analysis, we compared the baseline characteristics between the final study sample and those who were lost to follow-up. P values <0.05 were considered to be statistically significant. All statistical analysis was done using STATA 16.0 version (College Station, Texas, USA) and R 3.6.2 software

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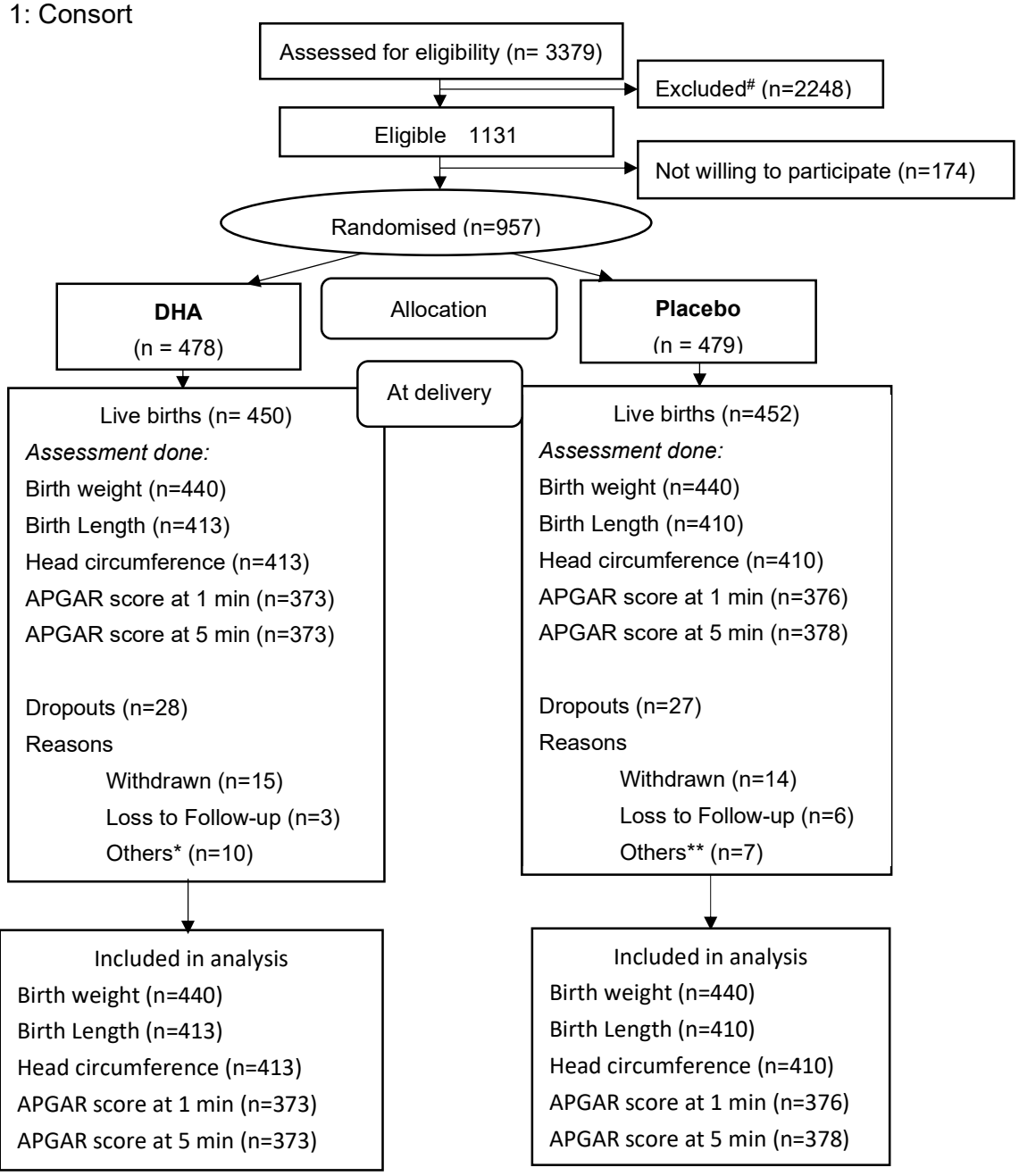
3. Results

Trial population

A total of 3379 women were screened and 1131 were found to be eligible. Among these, 957 mothers provided informed consent and were randomly assigned to DHA (n= 478) or placebo (n= 479) (Figure 1).

Figure 1: Consort



 # Reasons for exclusion: Gestational Diabetes (n=69); Hb<7gm% (n=46); Gestational age >20 weeks (n=673); High Risk Pregnancies (n=118); Chronic conditions (n=246); Under any other trial (n=4); Delivery plan other than PK (n=835); Missing/wrong contact information (n=257)

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**Others included: abortion (n= 1); abruptio placenta (n=1); fresh still birth (n=4); Macerated still birth (n= 3); neonatal death (n=1) in DHA group*

***Others included: fresh still birth (n=4); Macerated still birth (n= 2); medical termination (n= 1) in Placebo group*

Overall the mean (SD) age of the mothers was 23.5 (3.6) years and gestational age (median (Interquartile interval)) at enrolment was 15.0 (12.0, 18.0) weeks. 79% of the women had completed at least secondary school and 23% of the women were employed. About 12% of the women reported monthly household income more than Rs 20,000 (285 USD taking 1 USD =70 INR). Characteristics were similar between DHA and Placebo group (Table 1). There were no difference in baseline characteristics between those who were retained through delivery and those who did not (Table S1).

The two groups did not differ in estimated energy, macronutrient, or DHA-source foods at baseline (Table S2). The mean DHA levels at baseline also did not differ between the two groups (Table S3). The mean DHA levels at baseline and delivery by birth weght (<2500gm, ≥2500gm), length (<50 cm; ≥50cm) and head circumference(<34 cm; ≥34cm) are shown in Table S3.

Table 1: Maternal characteristics according to treatment group at randomization

Variable	DHA (N=478)	Placebo (N=479)
Maternal age (year), mean ±SD	23.5 ± 3.5	23.6 ± 3.7
Gestational age at enrollment (weeks), median (p25, p75)	15.0 (12.0, 18.0)	14.0 (12.0, 18.0)
Primigravida, n (%)	180 (37.7)	206 (43.0)
Education, n (%)		
College graduate and above	88 (18.4)	82 (17.1)
High school/Secondary	371 (77.6)	386 (80.6)
Employed, n (%)	119 (24.9)	104 (21.7)
Monthly household income (INR), n (%) *(Rs >20,000)*	65 (13.6)	47 (9.8)
Dietary Habits-Vegetarian, n (%)	73 (15.3)	87 (18.2)
Consuming Fish / sea food, n (%)	258 (53.9)	202 (57.8)
Weight (Kg), mean ± SD	48.9 ± 9.0	48.9 ± 8.5
Height (cm), mean ± SD	154.1 ± 5.6	153.9 ± 5.7
Body mass index (Kg/m^2), mean ± SD	20.5 ± 3.5	20.7 ± 3.6
MUAC (cm), mean ± SD	24.3 ± 3.0	24.3 ± 3.1
Hb (gm%), mean ± SD	11.1 ± 1.3	11.2 ± 1.3
DHA (moL% of fatty acid) *, mean ± SD	0.86 ± 0.78	0.88 ± 0.71

*BMI: Body mass index; DHA: Docosahexaenoic acid; MUAC: Mid upper arm circumference; Hb: Haemoglobin; DHA: Docosahexaenoic acid * n=258 (DHA); n=224 (Placebo);*

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There were 450 (94.1%) and 452 (94.3%) births in the DHA and placebo groups, respectively. Compliance was high in both groups (DHA: 88.5% and placebo: 87.1%). There were 234 (52.0%) and 243 (54.0%) male children in the DHA and placebo groups, respectively

Outcomes

Table 2 shows birth outcomes for all live births according to treatment group. There were no significant differences between DHA and placebo group for mean birth weight (2750.6 ± 421.5 vs. 2768.2 ± 436.6 g, p = 0.54), birth length (47.3 ± 2.0 vs. 47.5 ±2.0 cm, p = 0.13) or head circumference (33.7 ± 1.4 vs 33.8 ± 1.4 cm, p = 0.15). The APGAR scores at 1 min and 5 min were similar between the groups. We did not find any significant difference between DHA and placebo group in z scores for birth weight, length and head circumference.

Gestational age at delivery was similar between DHA and placebo group (DHA vs placebo: 38.8 ±1.7 vs 38.8 ± 1.7 wk, p = 0.54). The prevalence of preterm birth and low birth weight did not differ significantly between the groups.

Table 2: Birth outcomes for all live births according to treatment group

	DHA		Placebo		Mean difference§ (95% CI)	p-Value
	n	Mean ± SD / n (%)	n	Mean ± SD / n (%)		
Gestational age at delivery (weeks)	440	38.8 ± 1.7	440	38.8 ± 1.7	0.07 (-0.16,0.30)	0.54
Preterm birth (Gestation<37 week) †	440	28 (6.4%)	440	33 (7.5%)	0.01 (-0.02,0.04) ‡	0.52
Newborn anthropometry						
Birth weight (grams)	440	2750.6 ± 421.5	440	2768.2 ± 436.6	17.6 (-39.2,74.4)	0.54
Low Birth weight (<2500 g) †	440	105 (23.9%)	440	99 (22.5%)	-0.01 (-0.07,0.04) ‡	0.63
Birth Length (cm)	413	47.3 ± 2.0	410	47.5 ± 2.0	0.21 (-0.06,0.48)	0.13
Birth Head Circumference (cm)	413	33.7 ± 1.4	410	33.8 ± 1.4	0.14 (-0.05,0.34)	0.15
Apgar score at 1 min	372	6.9 ± 0.8	376	6.9 ± 0.8	0.01 (-0.11,0.13)	0.91
Apgar score at 5 min	373	8.0 ± 0.7	378	8.0 ± 0.7	0.03 (-0.07,0.12)	0.60
Size for gestational age and sex according to standardized measures¶						
Birth weight for gestational age z score	440	-0.97 ± 0.98	440	-0.95 ± 0.95	0.03 (-0.1,0.16)	0.67
Birth length for gestational age z score.	413	-0.84 ± 1.04	410	-0.73 ± 1.12	0.11 (-0.03,0.26)	0.13
Birth head circumference for gestational age z score.	413	0.09 ± 1.05	410	0.20 ± 0.97	0.11 (-0.03,0.25)	0.11
Small for gestational age*@	440	172 (39.1%)	440	172 (39.1%)	na	na

† n (%); ‡ Difference in proportions reported; § Difference= (Placebo-DHA); Difference in mean values reported using two-sample t-test. Difference in proportions reported using proportion test

¶ Standards are based on those established by the INTERGROWTH-21st (International Fetal and Newborn Growth Consortium for the 21st century) Project [22]

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@: Infants considered to be small for gestational age had a weight-for-age z score that was below the 10th percentile according to neonatal standards established by the INTERGROWTH-21st Project

Sub-group analysis

Figures 2, 3 and 4 show the results of sub-group analyses for birth weight, birth length and head circumference, respectively. The effect of DHA on the birth size (i.e. weight, length and head circumefernce) did not differ across any of the the subgroups examined (p= 0.007, p value adjusted for multiplicity using Bonferroni correction). Similarly, there was no evidence of differences by compliance, gender of the child or preterm status.

Figure 2: Sub-group analysis for Newborn anthropometry

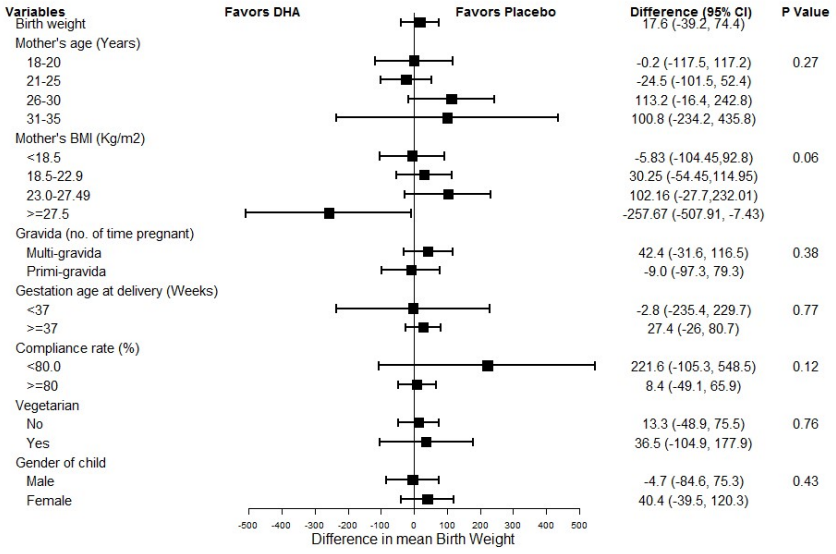

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Figure 3: Birth length

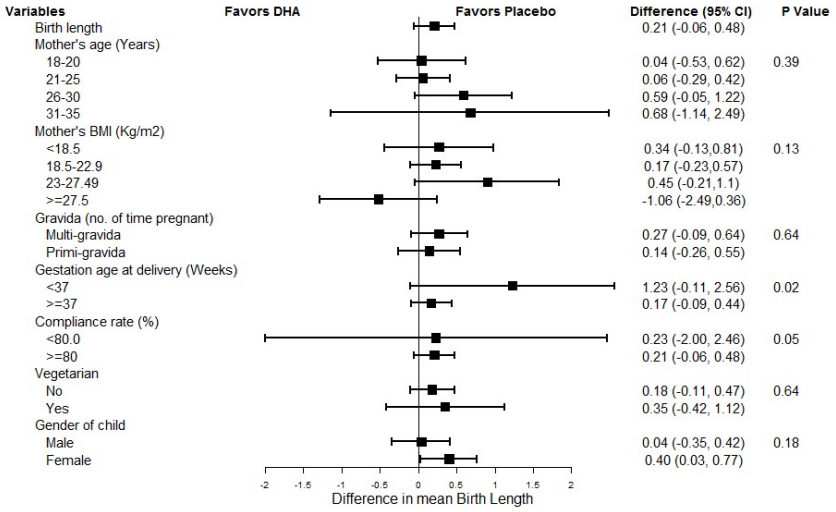
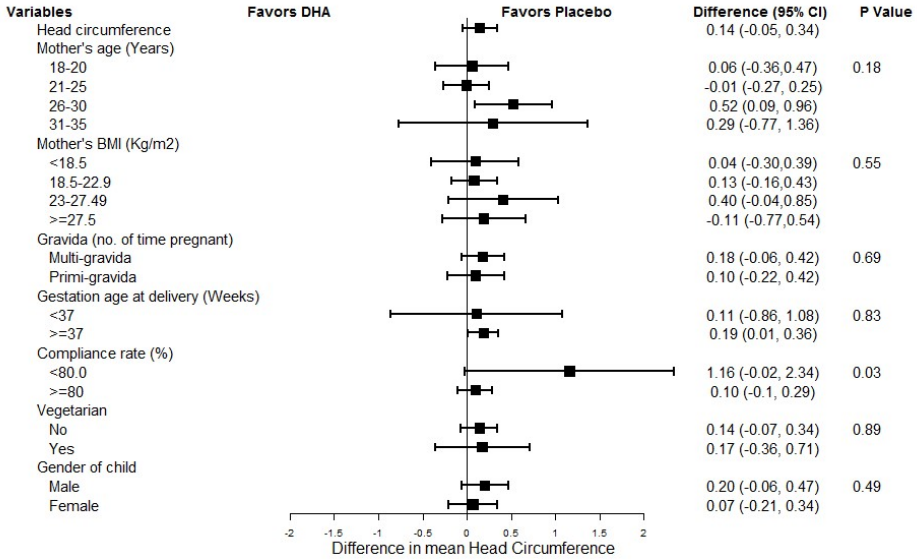


Figure 4: Head circumference



4. Discussion

In this study, maternal supplementation with 400mg/d DHA in the second half of pregnancy did not affect the weight, length or head circumference of the offspring at birth. While this is in contrast to findings from some high income settings [14], it concurs with other studies from relatively comparable settings [20].

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Although mechanistic pathways linking maternal polyunsaturated fatty acid (PUFA) especially DHA status with gestational length are poorly delineated, prenatal DHA supplementation has been shown to enhance the gestation duration in some studies [24]. This longer gestation duration with fish oil that contains EPA as well as DHA may be due to an alteration in the balance of prostaglandins derived from EPA and arachidonic acid [25]. A high proportion of omega-6 to omega-3 FAs can contribute to increased pro-inflammatory eicosanoids (i.e. prostaglandin E2 [PGE2] and prostaglandin F2 [PGF2]) production. These metabolites have been shown to be linked with the initiation of labor and premature labor. Including more EPA in the diet may guide to reduction in the production of pro-inflammatory eicosanoids and expanded production of prostacyclin (PGI2), which may promote myometrial relaxation. Omega-3 LC-PUFA especially DHA downregulates prostaglandins PGE2 and PGF2 production and may thus inhibit the process of parturition. This has been postulated to be associated with increased birth weight and the accretion of intrauterine LC-PUFA. Longer gestation also influences birth weight positively and thus DHA was shown to confer small benefits on newborn anthropometry too because of its impact on gestation duration. However, our trial did not find any such benefit.

A recent Cochrane review of high-quality evidence from 15 trials with 8449 participants concluded that there was a reduced risk LBW (15.6% versus 14%; RR 0.90, 95% CI 0.82 to 0.99) [26]. Bernardi et al noted that in several studies, increased birthweight due to prenatal DHA supplementation was observed in only primiparous women [27]. The authors suggest that since primiparous women were, on average, younger than multiparous women, their own body stores of DHA are not well established and available to the fetus and infant. Another study by Ramakrishnan et al showed that the offspring of primigravid women who received DHA were heavier at birth than the offspring of primigravid women who received placebo (difference, 99.4 g; 95% CI, 5.5 to 193.4) and had larger head circumferences (difference, 0.5 cm; 95% CI, 0.1 to 0.9 cm) [20]. In the current study, however, the woman's parity did not affect the effect of DHA on the newborn's birth weight, length or head circumference.

Key strengths of this study are the strong study design combined y with high retention rates and compliance (verified by the rise in erythrocyte DHA levels). However, the complexity of multiple other factors apart from DHA in affecting birth size need to be recognized. Factors like maternal diet at multiple time points during pregnancy, family support, stress levels [28], consumption of other important micronutrients like iron, and zinc that were not assessed may have influenced birth size [29]. Further, we do not have data on the single nucleotide polymorphisms (SNPs) in the fatty acid desaturase (FADS) gene that have been known to affect the activity of the enzymes that convert PUFAs into their long-chain active form and may determine who benefits from supplementation [30, 31]. Future large-scale trials taking into account all these factors are warranted.

5. Conclusions

In summary, no beneficial effects of prenatal supplementation of Indian women with DHA from mid pregnancy through delivery on newborn anthropometry were observed.

DATA SHARING STATEMENT

The request for accessing de-identified data plus data dictionary will be put forth for approval by the trial's mentoring and advisory committee (MAC). Interested researchers may submit a proposal with a valid reason or justification. Eg. Meta-analysis etc.

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Author Contributions: Dr Shweta Khandelwal had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. She also takes the final responsibility for the decision to submit for publication.

Conceptualization, Shweta Khandelwal, Dorairaj Prabhakaran, Nikhil Tandon, Usha Ramakrishnan and Aryeh Stein; Data curation, Dimple Kondal; Formal analysis, Dimple Kondal and Ruby Gupta; Funding acquisition, Shweta Khandelwal and Dorairaj Prabhakaran; Investigation, Shweta Khandelwal, Gangubai Pujeri, Swati Mane, Yashaswi Kudachi; Methodology, Shweta Khandelwal and Usha Ramakrishnan, ; Project administration, Shweta Khandelwal, Monica Chaudhry and Kamal Patil; Resources, Kamal Patil, Mallaiah K Swamy and Dorairaj Prabhakaran; Software, Dimple Kondal; Supervision, Kamal Patil, Mallaiah K Swamy, Nikhil Tandon and Aryeh Stein; Validation, Ruby Gupta; Writing – original draft, Shweta Khandelwal; Writing – review & editing, Dimple Kondal, Monica Chaudhry, Dorairaj Prabhakaran, Nikhil Tandon, Usha Ramakrishnan and Aryeh Stein.

All authors have read and agreed to the published version of the manuscript.

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Supplementary Tables

Table S1: Comparison of baseline characteristics comparing women who continued to participate in the study through delivery and those who did not

Variable(s)	Randomized and continued through delivery (N=880)	Randomized but did not continue through delivery (N=77)	p-Value
Maternal age (year), mean ± SD	23.5 ± 3.6	23.8 ± 3.8	0.50
Gestational age at enrolment (weeks), median (p25, p75)	15.0 (12.0, 18.0)	14.0 (11.0, 17.0)	0.37
Primigravida, n (%)	349 (39.7%)	37 (48.1%)	0.15
Education, n (%)			
College graduated and above	158 (18.0%)	12 (15.6%)	0.82
High school / Secondary	695 (79.0%)	62 (80.5%)	
Employed, n (%)	213 (24.2%)	10 (13.0%)	0.03
Household monthly income (INR), n (%) *(Rs >20,000)*	102 (11.6%)	10 (13.0%)	0.48

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Treatment assignment, n (%)			
DHA	440 (50.0%)	38 (49.4%)	
Placebo	440 (50.0%)	39 (50.6%)	0.91

Table S2-Dietary data on subsample at randomization (n=278)

	DHA (N=140)	Placebo (N=138)	p-Value
Energy (Kcal), mean ± SD	1358.2 ± 431.0	1391.4 ± 370.9	0.49
Energy (Kcal), median (p25, p75)	1231.6 (1066.3, 1682.2)	1347.3 (1088.4, 1629.6)	0.22
Protein (g), mean ± SD	48.9 ± 13.7	49.2 ± 12.7	0.84
Protein (g), median (p25, p75)	45.2 (39.7, 59.1)	48.7 (40.6, 56.8)	0.64
Fat (g), mean ± SD	38.8 ± 16.6	38.2 ± 13.1	0.73
Fat (g), median (p25, p75)	35.9 (28.2, 45.4)	36.3 (28.8, 45.0)	0.92
Carbohydrates (g), mean ± SD	202.3 ± 75.4	212.4 ± 71.4	0.25
Carbohydrates (g), median (p25, p75)	178.0 (156.2, 247.3)	198.1 (163.4, 254.3)	0.14

Kcal: Kilocalories; g: grams; Data are presented as mean ± standard deviation or median (p25, p75) ;(p25, p75): Interquartile interval. p-Value for difference in mean values calculated using two-sample t-test. p-Value for difference in median values calculated using Wilcoxon rank sum test.

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Table S3: Mean DHA (mol % of fatty acid) levels in RBC phospholipids.

	DHA	Placebo	mean difference* [95% CI]	p-Value
	n, Mean ± SD, median (p25, p75)	n, Mean ± SD, median (p25, p75)		
Birth weight <2500 grams				
DHA at baseline	n=63, 0.96 ± 0.89, 0.59 (0.39, 1.41)	n=47, 0.74 ± 0.69, 0.46 (0.37, 0.95)	-0.22 (-0.53,0.09)	0.170
DHA at delivery	n=67, 2.00 ± 1.81, 1.39 (0.63, 2.79)	n=50, 1.17 ± 0.80, 0.96 (0.48, 1.74)	-0.83 (-1.37, -0.29)	0.003
Birth weight ≥2500 grams				
DHA at baseline	n=193, 0.83 ± 0.75, 0.53 (0.3, 1.11)	n=177, 0.92 ± 0.71, 0.59 (0.37, 1.33)	0.09 (-0.06,0.24)	0.221
DHA at delivery	n=202, 2.04 ± 1.74, 1.43 (0.6, 3.17)	n=192, 1.10± 0.88, 0.78 (0.4, 1.7)	-0.94 (-1.22, -0.66)	<0.001
Gestation age <37 weeks				
DHA at baseline	n=18, 1.1 ± 0.74, 0.79 (0.61, 1.55)	n=19, 0.55 ± 0.37, 0.41 (0.37, 0.66)	-0.55 (-0.93, -0.16)	0.007
DHA at delivery	n=238, 0.84 ± 0.79, 0.53 (0.31, 1.14)	n=205, 0.91 ± 0.72, 0.59 (0.37, 1.3)	-1.25 (-2.16, -0.33)	0.009
Gestation age ≥37 weeks				
DHA at baseline	n=17, 2.24 ± 1.81, 1.72 (0.96, 2.97)	n=19, 0.99 ± 0.72, 0.62 (0.41, 1.72)	0.07 (-0.07,0.21)	0.318
DHA at delivery	n=252, 2.02 ± 1.76, 1.4 (0.59, 3.08)	n=223, 1.13 ± 0.87, 0.83 (0.42, 1.72)	-0.89 (-1.15, -0.64)	<0.001
Birth length <50 cm				
DHA at baseline	n=234, 0.89 ± 0.81, 0.58 (0.32, 1.33)	n=194, 0.85 ± 0.71, 0.52 (0.34, 1.23)	-0.04 (0.1, -0.33)	0.556
DHA at delivery	n=245, 2.04 ± 1.77, 1.39 (0.62, 3.17)	n=213, 1.1 ± 0.86, 0.78 (0.42, 1.72)	-0.94 (-0.68, -0.33)	<0.0001
Birth length ≥50 cm				
DHA at baseline	n=21, 0.51 ± 0.33, 0.44 (0.28, 0.59)	n=30, 1.11 ± 0.64, 1 (0.62, 1.47)	0.6 (0.91, -0.33)	0.0003
DHA at delivery	n=24, 1.97 ± 1.7, 1.72 (0.58, 2.45)	n=29, 1.28 ± 0.87, 1.14 (0.54, 1.73)	-0.69 (0.04, -0.33)	0.063
Head circumference <34 cm				
DHA at baseline	n=123, 0.95 ± 0.89, 0.59 (0.34, 1.36)	n=98, 0.82 ± 0.66, 0.53 (0.39, 1.23)	-0.12 (0.09, -0.33)	0.258
DHA at delivery	n=132, 2.16 ± 1.81, 1.49 (0.73, 2.98)	n=106, 1.16 ± 0.89, 0.93 (0.45, 1.74)	-1.00 (-0.62, -0.33)	<0.0001
Head circumference ≥ 34 cm				
DHA at baseline	n=132, 0.78 ± 0.67, 0.52 (0.29, 0.97)	n=126, 0.93 ± 0.74, 0.57 (0.34, 1.4)	0.15 (0.32, -0.33)	0.0916

	DHA	Placebo	mean difference* [95% CI]	p-Value
	n, Mean ± SD, median (p25, p75)	n, Mean ± SD, median (p25, p75)		
DHA at delivery	n=137,1.91 ± 1.7, 1.19 (0.55, 3.17)	n=136, 1.08 ± 0.84, 0.78 (0.41, 1.7)	-0.83 (-0.51, -0.33)	<0.0001

*Data are presented as mean ± standard deviation, median (p25, p75); DHA: Docosahexaenoic acid; p-Value calculated using unpaired t-test; * difference = Placebo minus DHA.*

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