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Supplementation of Fish-oil and Soy-oil during Pregnancy and Psychomotor Development of Infants

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ABSTRACT

Supplementation of docosahexaenoic acid (DHA) in infancy improves neuro-developmental outcomes, but there is limited information about the impact of supplementing pregnant mothers with DHA on the development of their infants. In a follow-up of a randomized, double-blind controlled trial with 400 pregnant mothers, the effects of supplementation of fish-oil or soy-oil (4 g/day) during the last trimester of pregnancy on psychomotor development and behaviour of infants at 10 months of age (n=249) were assessed. The quality of psychosocial stimulation at home (HOME) and nutritional status of the subjects were also measured. There were no significant differences in the fish-oil group and soy-oil group in any of the developmental (mean±SD mental development index: 102.5±8.0 vs 101.5±7.8, psychomotor development index: 101.7±10.0 vs 100.5±10.1) or behavioural outcomes. It may, therefore, be concluded that supplementation of fish-oil during the last trimester of pregnancy does not have any added benefit over supplementation of soy-oil on the development or behaviour of infants in this population.

Key words: Fish-oil; Soy-oil; Psychomotor development; Infant development; Child development; Infants; Child; Bangladesh

INTRODUCTION

Docosahexaenoic acid (DHA) is a long-chain polyunsaturated fatty acid (LCPUFA), which plays an important role in the structural development of major membrane constituent of retinal photoreceptors, neurones, and synaptic membrane. It also influences important functions, such as ion transport, receptor activity, enzyme action, and membrane fluidity, thereby improving neurogenesis and synaptogenesis (1-5). Adequate supply of DHA, therefore, needs to be ensured from maternal circulation during embryonic life and from breastmilk/infant diet in early postnatal life (6-9). DHA is formed *denovo* by conversion of its parent essential fatty acid (EFA)—α-linolenic acid (LNA, 18:3n-3)—through a series of desaturation and subsequent chain-elongation reactions. Older children and adults consume the ready form of DHA from its animal source, particularly marine fish-oil, and young infants receive DHA through breastmilk (2,8,10).

In full-term infants, supplementation of DHA to infant formula results in improved mental development, comparable to breastfed infants (11-15). Pre-term low-birth-weight babies are most likely to suffer from DHA deficiency because of their lower body stores. Several studies have shown benefits of supplementation of DHA on the neuro-developmental measures in pre-term low-birth-weight infants (16-21). In the foetal brain, DHA is primarily accrued during the 'brain growth spurt', which begins in the last trimester of pregnancy and continues through the early postnatal period (22-23). A decline in maternal DHA status has also been observed in the later

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half of pregnancy (24-26). Another study has shown that supplementation of α-linolenic acid (LNA) failed to influence maternal DHA status (27), raising the question if mothers under prevailing dietary conditions are able to meet the high foetal requirements of DHA or they need additional supplementation for optimal development of foetal brain. Despite the importance of DHA, there is limited research to assess the impact of supplementation of DHA during pregnancy on development of infants. A recent study observed that more mature neonatal sleep-pattern, a measure of central nervous system (CNS) integrity, was associated with higher maternal DHA levels (28). In a randomized, double-blind controlled trial, pregnant mothers were supplemented with DHA (29) from 17-19 weeks gestation until three months after delivery. Although no difference was observed in cognitive development of infants as measured at six or nine months, a developmental benefit became apparent at the age of four years (30).

In developing countries, the impact of supplementation of DHA to pregnant mothers on the development of their infants remains largely unexplained. These mothers tend to have low hepatic and adipose tissue stores of EFA due to chronic maternal under-nutrition. We, therefore, hypothesized that supplementation of fish-oil (ready form of DHA) rather than soy-oil (precursor form of DHA), during the last trimester of pregnancy, will provide additional DHA to fulfil increased foetal requirement of DHA for developing the nervous system via placental circulation and breastmilk, which will have a positive effect on the mental and psychomotor development of infants.

MATERIALS AND METHODS

Study design and sampling

This study is the follow-up of an intervention study that aimed at improving pregnancy outcomes by supplementing women with DHA during the last trimester of their pregnancy. The study population was chosen from the community in Dhaka city, where illiteracy, poverty, overcrowding, poor housing, and poor hygiene are common.

In the main study, a house-to-house survey was conducted during January-March 2000, and 400 eligible pregnant women at 25 weeks of gestation (based on the first date of the last menstrual period) were enrolled and randomly assigned either to the treatment (fish-oil) or to the placebo group (soy-oil) after obtaining an informed consent. The treatment group received four fish-oil capsules (1 g in each) as a single daily dose, and the placebo group received an equal number of capsules containing an equal volume of soy-oil. The capsules were identical in appearance, and mothers were given clear instructions about the dosage. Total daily fish-oil supplement contained 1.2 g of DHA and 1.8 g of eicosapentaenoic acid (EPA) and soy-oil supplement contained 2.25 g of linoleic acid (LA) and 0.27 g of LNA. We chose soy-oil as a control because of its common use in this country. The intervention continued from 25 weeks gestation until delivery.

As part of the main study, a detailed baseline and socioeconomic history was collected on enrollment followed by weekly visits to assess compliance to the intervention.

Measurements

Developmental assessment: Developmental assessment was made at the age of 10 months (±15 days) using the Bayley Scales of Infant Development II (BSID-II), which included two sub-scales: mental development index (MDI) and psychomotor development index (PDI) (31). The children were brought to ICDDR,B: Centre for Health and Population Research and were tested in a quiet room in the presence of their mothers. Two female psychologists, unaware of the infant's group assignment, tested all infants. Sick infants were treated and tested after recovery. The inter-observer agreement between the testers and the trainer assessed on 30 infants before the study was high (intra-class correlation: r >0.98 for both MDI and PDI).

Infant's behaviour: Infant's behaviour was rated during the test using a modified five-scaled tool, designed by Wolke (32). Each of the scales had 9-point ratings and included infants' activity (very still=1 to over-active=9), emotional tone (unhappy=1 to extremely happy=9), responsiveness to examiner in the first 10 minutes (avoiding=1 to inviting=9), cooperation with the test procedure (resists all suggestions=1 to always complies=9), and vocalization (very quiet=1 to constantly vocal=9). The inter-observer reliability was r >0.92 for all five behaviour ratings.

Quality of stimulation at home: Quality of stimulation at home was assessed using Caldwell's Home Observation for Measurement of Environment (HOME) (33), which was modified for Bangladesh and was used in other studies by the same research team (34,35). Two female interviewers interviewed mothers at their homes in the presence of their babies. The trainer observed 30 interviews before initiation of the study, and agreements between the trainer and the interviewers were high (>90%).

Socioeconomic status: Socioeconomic status was measured using a detailed questionnaire at enrollment. Several indices were constructed by using relevant information as follows: housing index (structure of floor, roof, and wall), crowding index (number of family members divided by number of rooms), and utility index (water, electricity, and latrine). Occupation of father (stable/unstable), occupation of mother (house-wife/working woman) and education of parents (up to grade 5 or more than grade 5) were used as dichotomous variables. An asset score was constructed by giving judged arbitrary values to family possessions, for example, possession of a radio/cassette player was given a score of '2', whereas possession of a TV was scored as '6' taking into account that the price of a TV is three times more than that of a radio/cassette player. In the same way, we scored all the possessions of the family according to the market values and then added these up to construct the asset score.

Anthropometry: Anthropometry was performed within 72 hours of birth and then monthly up to six months using standard techniques (36) and also at the time of Bayley test. Z-scores for weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) were then calculated using NCHS reference data (36). Ponderal index (PI) was calculated using the formula: PI=(birth-weight in g/length cm^3)∗100.

Ethics

The research and ethical review committees of ICDDR,B approved the study, and informed written consents were obtained from all mothers prior to their enrollment in the study.

Statistics

Data were entered and analyzed using SPSS for Windows (version 10; SPSS Inc., Chicago). Distribution of each variable was checked for normality, and where necessary appropriate transformations were made. Bivariate correlations between developmental variables and socioeconomic measures were assessed.

To examine the treatment effect, series of multiple linear regression analyses were performed where each of the developmental variables was treated as a dependent variable. In the first block, we entered age and sex. In the second block, we offered socioeconomic and biological variables that were significantly different between the groups and between the lost and the tested sample (family income, education of father, age of mother, body mass index (BMI) of mother, pedal oedema, systolic blood pressure, pregnancy weeks at enrollment, and number of children). In the third block, we offered the birth-related variables, which were significantly different between the lost and the tested sample and between the groups (gestational age, birth-weight, birth-length, head size, resuscitation of child at delivery, problem of mother during delivery). In addition, we offered those variables, which were significantly associated with outcome variables (crowding, housing, and home environment). In the final block, we entered the treatment group.

RESULTS

Attrition

Of the 400 enrolled pregnant women, 76 were lost before delivery due to out-migration (fish-oil: 19 vs soy-oil: 16) and refusal to take capsules (22 vs 19). Therefore, 324 mothers were available at delivery. Only 301 liveborn singletons completed the original study because further losses occurred after delivery due to stillbirth (8 vs 6), early neonatal death (4 vs 5), subsequent out-migration (19 vs 26), and infant death (3 vs 4) in the fish-oil and soy-oil groups respectively. From this birth cohort, we could locate 249 infants for measurement of their development at 10 months of age.

We compared the characteristics of mothers and infants who were lost at different stages with the tested infants and their mothers (Flow-chart). The lost and tested infants and their mothers differed significantly on a number of variables. Before delivery, the mothers in the lost group had higher systolic blood pressure and gestational age at enrollment, more pedal oedema, and lower family income than the tested group. The lost infants had significantly lower birth-weight and birth-length and required more resuscitation time after birth than the tested infants.

Difference between the groups

The groups were similar in parental schooling, paternal occupation, breastfeeding and socioeconomic status, except that mothers in the fish-oil group were younger ($p<0.02$) and had fewer children ($p<0.008$) (Table 1). MDI, PDI, behaviour ratings, and HOME were also similar in the two groups (Table 2). There were also no significant group differences in any anthropometric measurements at birth or at 10 months (Tables 1 and 2).

Association with developmental variables

Bivariate correlations were determined between outcome variables and biological and social background variables. Both MDI and PDI significantly correlated with the HOME, asset, housing index, family income, maternal

Flow-chart of fish-oil study

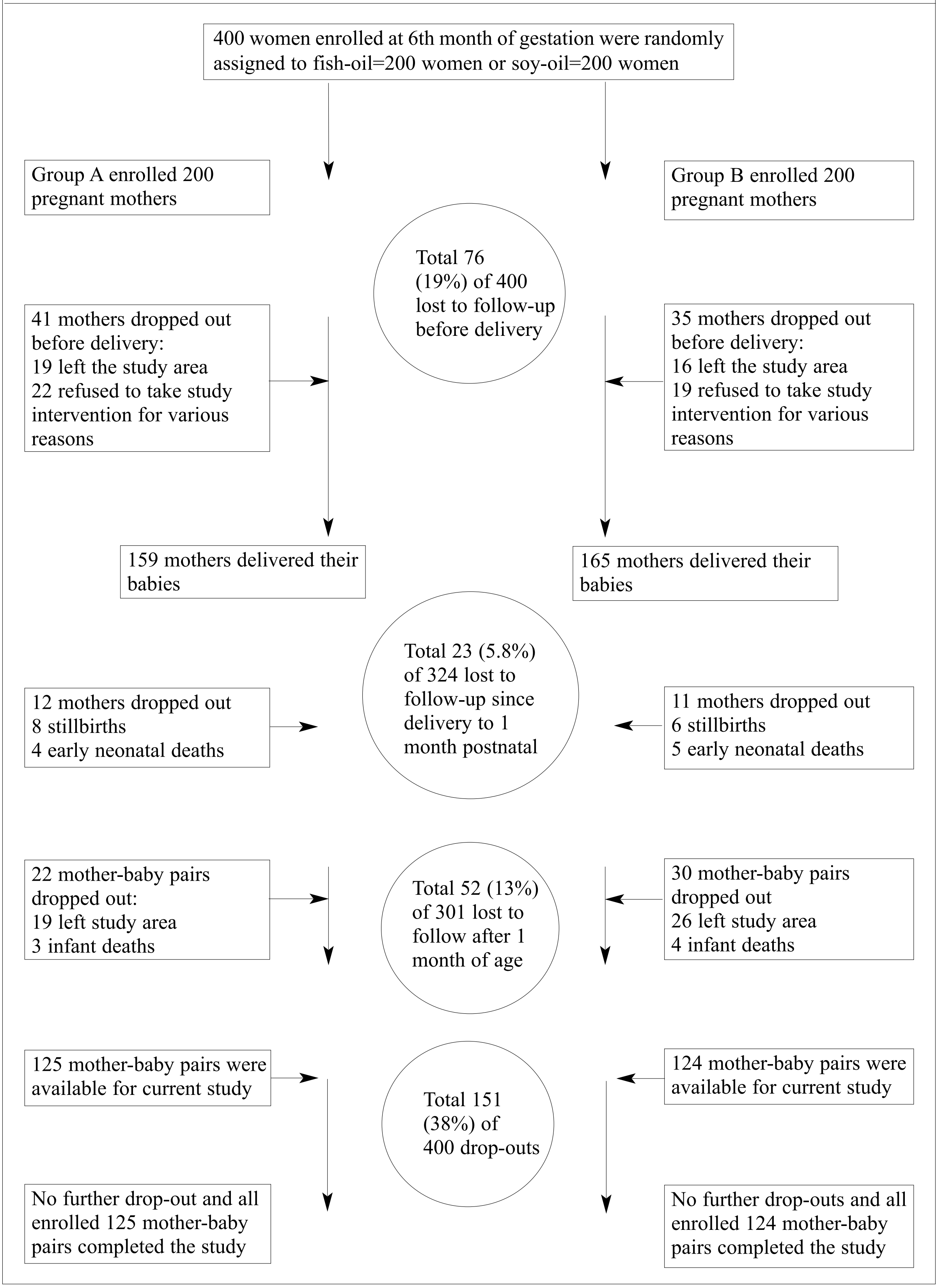

Table 1. Characteristics of families at enrollment

Characteristics	Fish-oil group (n=125)	Soy-oil group (n=124)	p value*
Maternal biological characteristics at enrollment			
Age (years) of mothers	22.1 (4.2)	23.4 (4.5)	0.02
Body mass index	20.3 (3.1)	20.4 (3.1)	0.8
Mid-upper arm circumference (cm)	23.6 (2.7)	23.8 (2.7)	0.6
Pregnancy duration (weeks) at enrollment	24.9 (1.2)	24.9 (1.1)	0.9
Women with >2 children (%)	16.8	31.5	<0.01
Compliance (mean capsule intake-days)	84.7 (10.3)	86.5 (7.4)	0.1
Compliance (% took capsule ≥90 days)	68	71.8	0.3
Socioeconomic factors			
Crowding index	3.9 (1.6)	4.2 (1.5)	0.09
Utility index	8.3 (0.8)	8.3 (0.9)	0.5
Housing index	3.5 (0.8)	3.5 (0.9)	0.7
Asset index	24.4 (1.4)	24.3 (1.3)	1.0
Family income (Taka/month)†	5,890 (2.1)	5,484 (2.0)	0.4
Parental characteristics			
Mothers with >5 years of schooling (%)	36.8	32.3	0.5
Working mothers (%)	16.0	12.1	0.5
Fathers with stable job, %	65.6	65.3	1.0
Fathers with >5 years schooling (%)	64.0	54.0	0.1
Factors relating to child at and after birth			
Male (%)	50.0	50.4	1.0
Gestational age (weeks)	38.7 (2.6)	39.2 (2.5)	0.2
Preterm birth (%)	24.0	21.8	0.4
Birth-weight (kg)	2.7 (0.4)	2.7 (0.4)	0.5
Birth-length (cm)	47.6 (2.1)	48.1 (2.0)	0.1
Birth-head circumference (cm)	32.3 (1.8)	32.4 (1.7)	0.7
Ponderal index	2.5 (0.2)	2.4 (0.2)	0.2
Duration of exclusive breastfeeding (months)	4.0 (1.6)	4.0 (1.8)	0.9
Age (days) of testing	300.7 (4.3)	300.7 (4.3)	1.0

*Independent sample *t*-test or χ^2-test
†1US$=Taka 59.00
Values are mean (SD) or %

Table 2. Developmental and nutritional variables and psychosocial stimulation (HOME) of infants at 10 months of age

Variable	Fish-oil group (n=125)	Soy-oil group (n=124)	95 % CI of difference between means
Mental developmental index	102.5 (8.0)	101.5 (7.8)	-0.98, 3.0
Psychomotor developmental index	101.7 (10.9)	100.5 (10.1)	-1.3, 3.8
Approach	6.3 (1.3)	6.4 (1.3)	-0.41, 0.23
Activity	5.7 (1.4)	5.8 (1.4)	-0.45, 0.25
Cooperation	5.7 (1.6)	5.7 (1.5)	-0.37, 0.39
Emotional tone	5.6 (1.6)	5.7 (1.5)	-0.50, 0.26
Vocalization	5.0 (1.7)	5.1 (1.7)	-0.47, 0.39
Head circumference (cm)	43.0 (1.4)	43.2 (1.4)	-0.47, 0.22
Weight-for-height z-score	- 0.6 (0.9)	-0.6 (0.8)	-0.23, 0.21
Weight-for-age z-score	-1.5 (1.1)	-1.5 (1.0)	-0.27, 0.24
Height-for-age z-score	-1.3 (1.0)	-1.3 (0.9)	-0.24, 0.22
HOME	89.4 (9.9)	90.8 (9.3)	-3.8, 1.0

Values are mean (SD)
CI=Confidence interval; HOME=Home observation for measurement of environment

BMI, parental education, and anthropometric measurements at birth and at 10 months. MDI and PDI negatively correlated with crowding index. Age and sex of infants significantly correlated with MDI, and utility index significantly correlated with PDI.

Treatment effect

There were no differences in multiple regression analysis, controlling for the possible confounders. The effect size (±SE) for MDI and PDI was -1.1±1.0 (95% CI -2.9,0.7), and -2.1±1.1 (-4.3,0.1) respectively. Age, HOME, and birth-weight were significant predictors of MDI, while sex, gestational age, BMI of mother, presence of utilities at home, birth-length, and HOME significantly predicted PDI.

DISCUSSION

Supplementation of fish-oil during the last trimester of pregnancy did not show any added benefit over soy-oil on the development of infants. Despite a substantial loss at 10-month follow-up, the calculated power of the study with the achieved sample size was >80%. Therefore, there was sufficient power to detect any significant differences. The women satisfactorily complied to the study, and almost 70% took capsules more than 90 days. We had trained the testers intensively and had achieved good inter-observer reliabilities before and during the tests.

There are several possibilities for lacking a benefit in the study. We had suffered a loss of 38% from the original randomized sample, and there were significant differences in the lost and tested subjects in both the groups. The lost infants were from poorer families, had lower birth-size, and required more resuscitation at birth, and their mothers had more clinical problems during pregnancy. They were definitely more at risk, and had they remained in the study, it is likely that they would have benefited more from the intervention.

The supplementation in our study was started at 25 weeks of gestation and was continued until delivery. The other study that showed improvement with supplementation of DHA at four years of age (30) started supplementation at 17-19 weeks of pregnancy and continued so until three months after delivery. It is possible that the duration and timing of our supplementation was not sufficient to demonstrate a significant effect of supplementation of fish-oil in this population. Moreover, the same study did not show any effect at six or nine months of age. It is possible that the developmental domains that require DHA for their maturation are not fully functional or expressed at an early age and are only manifested later in life. Improvements from supplementation of DHA on the development of these children may become apparent at a later age.

The other factor that may have contributed to the lack of any benefit demonstrated from supplementation of fish-oil is that DHA is available in breastmilk in abundance, and it is usually found that children who are exclusively breastfed have high levels of DHA (10). In our study, 82% of the children were predominantly breastfed and, therefore, have received sufficient amount of DHA from breastmilk.

In our study, we used the Bayley test to assess the development of children. The Bayley test has not been standardized for Bangladesh, and there has been no study of validity here. The test has, however, been used in many developing countries, including Bangladesh, and in many of our studies. It has been the instrument of choice for much nutrition and child-development research at this age and has often been sensitive to changes following interventions. The Bayley is the only test we know of for this age-group that has been used in research in Bangladesh, where sensible and predictable correlations were found between the scores and socio-economic conditions, education of parents, stimulation in the home, and nutritional status of children. As it is not a standardized test in this population, it may not have been sensitive to minor differences expected to occur between the two intervention groups.

In our study, we used soy-oil as control since it is usually consumed by people in this community. Soy-oil contains no DHA but α-linolenic acid, which is a precursor of DHA and can also influence fatty acid pattern in umbilical plasma phospholipid.

About 28% of mothers of this population suffered from maternal under-nutrition, taking pregnancy BMI cut-off of 19.8, and were likely to have low hepatic and adipose tissue reserves. Studies in adequately-nourished mothers have noted a gross fall in maternal DHA status during the last trimester of pregnancy (24,25), suggestive of rapid accretion of DHA by foetal tissues during this period. In a rat model, it has also been observed that accretion of DHA was most profound compared to other fatty acids, particularly in the last three prenatal days (37). This suggests a possible role of supplementation of DHA during the last weeks of pregnancy on the development of infants. During pregnancy, higher maternal intake of DHA results in increased transfer of DHA to the foetus via the placenta (29,38), and there is an indication of preferential placental transfer of maternal plasma DHA over α-lenolenic acid (39). Studies in infants observed better developmental impacts of supplementation of DHA (through breastmilk or supplemented formula) than supplementation of α-lenolenic acid during the crucial period of rapid development of the brain (40-43).

We are aware of only one study in which supplementation of DHA during pregnancy failed to show any benefit on infants at six or nine months of age as assessed by the Fagan test (29), but a significant benefit on IQ at four years of age was observed (30); however, loss from the study was considerable.

In our study, we did not follow up the infants for future developmental assessment because they were from a migrating population. Moreover, as we did not measure serum or RBC DHA levels in these infants, we were unable to correlate biochemical improvements with functional outcomes.

It may be concluded that supplementation of fish-oil during the last trimester of pregnancy may not have any added benefit over supplementation of soy-oil on the development or behaviour of infants in this population when assessed by the Bayley Scale of Infant Development. Further studies with a true placebo and, if possible, with more sensitive tests are recommended to ascertain the role of antenatal supplementation of fish-oil on the development of children. As development of the foetal brain begins as early as the third week after conception (44), supplementation throughout pregnancy may also be considered for subsequent child development. Follow-up of these subjects in childhood is important to determine if the effects appear at a later time.

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