# Docosahexaenoic acid supplementation and time at achievement of gross motor milestones in healthy infants: a randomized, prospective, double-blind, placebo-controlled trial[1–3]

*Carlo Agostoni, Gian Vincenzo Zuccotti, Giovanni Radaelli, Roberto Besana, Alberto Podestà, Andrea Sterpa, Amilcare Rottoli, Enrica Riva, and Marcello Giovannini*

**ABSTRACT**

**Background:** Docosahexaenoic acid (DHA) intake throughout the first year of life is associated with neurodevelopmental and neuropsychological benefits. Few studies have evaluated the role of DHA intakes on age at achievement of gross motor milestones.

**Objective:** The objective was to assess the effects of DHA supplementation throughout the first year of life on the achievement of four gross motor milestones in healthy infants.

**Design:** In this multicenter prospective, randomized, double-blind, placebo-controlled trial, 1160 healthy neonates were assigned to receive supplementation with either 20 mg liquid DHA ($n = 580$) or placebo ($n = 580$) orally once daily throughout the first year of life. The primary endpoint was the time at achievement of 4 gross motor milestones (sitting without support, hands-and-knees crawling, standing alone, and walking alone). All analyses were performed on an intention-to-treat basis.

**Results:** The time to achievement of sitting without support was shorter ($P < 0.001$) in infants who received DHA [median: 26 wk; interquartile range (IQR): 24–29 wk] than in those who received placebo (27 wk; 26–31 wk). No significant difference between infants who received DHA or placebo was found for hands-and-knees crawling [39 wk (34–44 wk) compared with 40 wk (35–44 wk), respectively], standing alone [49 wk (43–55 wk) compared with 49 wk (44–57 wk), respectively], and walking alone [55 wk (50–60 wk) compared with 56 wk (52–61 wk), respectively].

**Conclusions:** Despite the 1-wk advance in sitting without support associated with DHA supplementation, no demonstrable persistent effects of DHA supplementation on later motor development milestones were found. Thus, the long-term clinical significance of the 1-wk change in sitting without support, if any, remains unknown. This trial is registered at clinicaltrials.gov as NCT00610922. *Am J Clin Nutr* 2009;89:64–70.

## INTRODUCTION

Docosahexaenoic acid (DHA, 22:6n−3) is a major component of brain tissues (1). Its accretion rate is maximal in the fetus during the last weeks of gestation (2) and in the newborn during the first year of life (3), whereas its deposition may continue throughout an individual's lifetime (4, 5). Although genetic polymorphisms may influence an individual's ability to synthesize DHA (6), early dietary enrichment might further increase the concentrations of DHA in the body, as indicated by erythrocyte and brain fatty acid composition (7, 8). Human milk is a natural source of DHA (9), and higher mental ability in breastfed than in formula-fed infants is associated with early DHA intake from maternal milk (10). In addition, DHA supplementation in mothers during pregnancy or lactation and in infants throughout the first year of life may be associated with neurodevelopmental and neuropsychological benefits in both breastfed (11, 12) and formula-fed (13, 14) infants.

Functional effects of DHA supplementation have been assessed by using developmental scales or neurophysiologic tests (15, 16). Developmental scales include tests that investigate neural domains. Neurophysiologic tests can detect differences in sensory motor function whose relevance to daily activities and later outcomes may be unclear. The assessment of motor development milestones may be important in the evaluation of an infant's growth and development (17–19) and may represent a reasonable outcome measure that is readily understood by pediatricians and families everywhere. Although studies of the effects of long-chain polyunsaturated fatty acids on developmental or neurophysiologic performance have been conducted, no studies, to our knowledge, have tested the hypothesis of any effect of DHA or other specific nutrient on the gross motor milestones of infants in Western countries, at least during the past 30 y. However, one study examined the possible negative effect of environmental pollutants in a high-fish diet on infant development (20). This multicenter,

[1] From the Department of Pediatrics, San Paolo Hospital, University of Milan, Milan, Italy (CA, ER, and MG); the Department of Pediatrics, Luigi Sacco Hospital, University of Milan, Milan, Italy (GVZ); the Unit of Medical Statistics, San Paolo Hospital, University of Milan, Milan, Italy (GR); the Unit of Pediatrics and Neonatology, Presidio Ospedaliero di Desio, Desio, Italy (RB); the Department of Pediatrics and Neonatology, San Carlo Borromeo Hospital, Milan, Italy (AP); the Unit of Pediatrics and Neonatology, Presidio Ospedaliero di Carate Brianza, Carate Brianza, Italy (AS); and the Unit of Pediatrics and Neonatology, Presidio Ospedaliere di Cernusco sul Naviglio and Presidio Ospedaliero di Melzo, Cernusco sul Naviglio and Melzo, Italy (AR).

[2] Humana Italia SpA kindly supplied the intervention and control products free of charge.

[3] Address reprint requests and correspondence to C Agostoni, Department of Pediatrics, San Paolo Hospital, University of Milan, Via A Di Rudinì 8, I-20142 Milan, Italy. E-mail: carlo.agostoni@unimi.it.

Received June 25, 2008. Accepted for publication September 9, 2008.

First published online December 3, 2008; doi: 10.3945/ajcn.2008.26590.

 

prospective, randomized, double-blind, placebo-controlled trial assessed the effects of DHA supplementation on the achievement of gross motor developmental milestones in healthy infants.

## SUBJECTS AND METHODS

### Subjects

The study included 1160 neonates born consecutively throughout a 2-mo period in 7 care centers located in Milan and its surroundings in northern Italy. Enrollment occurred between 1 May 2005 and 30 June 2005, and data collection ended on 31 December 2006. Inclusion criteria were as follows: weight at birth ≥2500 g, gestational age between 37 and 42 completed weeks, single birth, absence of neonatal or birth abnormalities, Apgar score ≥7 at 5 min, and white parents. Exclusion criteria were as follows: presence of neonatal diseases requiring hospitalization for >7 d, involvement of neonate in another clinical study, unknown father, and parents unable to understand the protocol requirements, to fill out the infant's diary, or to understand and speak the Italian language adequately.

Gestational age was based on the date of the last menstrual period and confirmed by ultrasound examination performed within the 12th week of pregnancy. Neonates underwent clinical examination by an attending pediatrician within 24 h of birth and were weighed naked with an electronic scale (Sartorius AG, Gottingen, Germany) that was accurate to ±5 g.

The parents of eligible infants, or their legal guardians, received detailed explanations of the study protocol, and participants gave written informed consent. The study was performed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The review board of the coordinating center, Maternal-Child Department, San Paolo Hospital, University of Milan, approved the study protocol.

### Procedures

A computer-generated block sequence balanced by participating center and gestational age (<40 and ≥40 wk) randomly assigned subjects in blocks of 4. The investigator who generated the randomization sequence was independent of the research staff. The study was double-blinded.

At hospital discharge, neonates were randomly assigned to receive 1 mL orally of either a liquid study (intervention) preparation (400 IU vitamin $D_3$ + 20 mg DHA) or placebo (400 IU vitamin $D_3$) once daily throughout the first year of life starting within 24 h after discharge. The vitamin $D_3$ dosage was chosen in accordance with the Italian Recommended Dietary Allowances adjusted for age (21). Vitamin $D_3$ was included in the study preparations to improve maternal compliance with the prescribed supplementations. The daily amount of supplemented DHA was the minimum amount that would possibly be supplied by human milk to infants at the age of 1–3 mo (9). This amount would correspond to differences in brain composition between breastfed and formula-fed infants (22). Both the intervention and placebo preparations were packaged in identical opaque, coded bottles provided by Humana Italia SpA (Milan, Italy), each containing 10 mL product. Intervention and placebo preparations were identical in aroma, taste, and texture. Bottles were first distributed at hospital discharge and thereafter every 4 mo. At each 4-mo interval, bottles not consumed in the previous period were retrieved. Parents were advised to store the bottles in a dry and fresh environment. During the study period, consumption of any other commercially available product containing vitamin $D_3$ and/or DHA was not allowed by the infants. All individuals involved in the trial, including doctors, nurses, research staff, and parents of neonates, were unaware of the product administered during the entire study period. The randomization codes were revealed after completion of the data analysis, and results were presented to the review board of the coordinating center.

The study examined the following 4 gross motor developmental milestones, which corresponded to those currently suggested by the World Health Organization (WHO): sitting without support, hands-and-knees crawling, standing alone, and walking alone (17). In addition, the study examined 2 early fine motor milestones, reaches an object to touch (19) and brings toy to mouth (18, 19), and an early language milestone, saying the first comprehensible word composed of ≥2 syllables (excluding "dada" and "mama") (23).

The primary endpoint was the time at achievement of the 4 gross motor milestones (sitting without support, hands-and-knees crawling, standing alone, and walking alone). The secondary endpoints were time at achievement of the early fine motor milestones and language.

Baseline information was collected during admission by the local pediatrician (one per center) from parents (educational level, socioeconomic status, smoking during pregnancy, and prepregnancy body weight) and hospital records (gestational age, whether the infant has siblings, anthropometric measures of the infant, and breastfeeding during the hospital stay). Mothers also had their height measured at the hospital and their prepregnancy body mass index (BMI; $kg/m^2$) calculated. Education was categorized as low (≤8 y), medium (9–13 y), or high (≥13 y). Socioeconomic status was graded in 5 levels according to the Italian National Institute of Statistics, and the results were grouped as low (score: 1), intermediate (score: 2–4), or high (score: 5). Smoking during pregnancy was defined as the mother smoking ≥1 cigarette/d during at least the first 6 mo of pregnancy. Breastfeeding was defined according to the WHO as follows: exclusive breastfeeding (infants received only breast milk), predominant breastfeeding (infants received breast milk and allowed liquids), and breastfeeding (infants received breast milk and any other food) (24). Prepregnancy BMI was also dichotomized at the cutoff of ≥25, according to the current standard definitions of overweight and obesity used by the Centers for Disease Control and Prevention and the WHO.

During the postpartum hospital stay and at each successive examination, the pediatrician instructed parents on the criteria used in the correct assessment of milestones and showed them an example. At discharge, parents received a case record form containing instructions and pictures of the skills they had to observe; they used this form to record the first date the infant achieved the milestone, the date at which breastfeeding stopped, the date of introduction of any solid food, the date of introduction of fish or egg, the use of any other commercially available product containing vitamin $D_3$ or DHA, and any adverse event. They were advised to immediately inform an independent monitoring team in cases of serious adverse events. Infants were assessed by pediatricians at the care centers at the ages of 12, 32, and 52 wk ± 3 d to confirm upon examination the performance of each

milestone. Each examination was performed independently of previous assessments. The number of people present during examination was limited to the pediatrician, the infant and his or her mother, and another medical worker if needed. When after examination the achievement of a milestone was confirmed, the pediatrician recorded the date of achievement observed by the mother and that was reported on the case record form. If the infant was able to perform a milestone at the examination but the mother had not previously observed the achievement of this milestone, the pediatrician recorded the date of achievement observed at the actual examination. Last, if the infant was not able to perform the milestone during the examination, this was discussed in detail by the pediatrician and the mother. If the mother confirmed that all evaluation criteria had been met by the infant, the pediatrician recorded the date reported by the mother on the case record form. If the pediatrician judged that the evaluation criteria had not been met, the observation was considered missing. Parents of infants who had not achieved any milestone during the first year of life were contacted by phone monthly to provide an assessment of their infant and to record their assessment in the case report form.

### Statistical analysis

The sample size was calculated to detect a difference between intervention and placebo groups of $\geq$10% in the time to achievement of the primary 4 gross motor milestones. With an accepted 2-sided type I error level of 0.01, for a power of 95%, and an assumed reference mean of 23.6 wk and expected SD of 8.9 wk (for sitting without support) (18), 490 infants were needed in each group. To allow for a dropout rate of $\leq$15%, 580 infants per group were recruited. Sample sizes that were calculated with respect to any other gross motor milestone, and considering corresponding reference means and SDs, were lower.

A statistician blinded to randomization and allocation performed all the statistical analyses. All analyses were performed in accordance with a preestablished analysis plan. The results that are related to primary and secondary endpoints are presented as means $\pm$ SDs and medians with interquartile ranges (IQRs). For baseline continuous variables, differences between groups were tested with Student's *t* test for normally distributed data (based on the Kolmogorov-Smirnov test) or the Mann-Whitney *U* test for nonnormally distributed data. The Pearson chi-square test was used for proportions. Comparisons between times at achievement of each milestone were performed by the log-rank test. Stepwise multiple Cox regression models were fitted to assess the independent association of the time at achievement of each milestone with the intervention product and were adjusted for potential confounders. Significance of endpoints was further adjusted with Bonferroni correction.

All analyses were performed on an intention-to-treat basis. A 2-sided significance level $< 0.05$ was used to indicate statistical significance. SPSS software (version 15.0; SPSS Inc, Chicago, IL) was used for the statistical analyses.

## RESULTS

A total of 1519 consecutive neonates were registered prospectively during the enrollment period; 1160 were randomly assigned, and 1091 (94.0%) completed the study. The progress of participants throughout the study is detailed in **Figure 1**.

The baseline characteristics and feeding practices throughout the first year of life were comparable in the 2 groups (**Table 1**). Full (exclusive/predominant) breastfeeding was reported by 95.8% (843/1091) of mothers (intervention group: 96.3%; placebo group: 95.4%). Of them, cessation of full breastfeeding occurred before 1 mo, between 1 and 4 mo, or between 4 and 6 mo in, respectively, 35.5%, 21.9%, and 40.6% of mothers in the intervention group compared with 29.8%, 23.6%, and 45.2% in the placebo group ($P = 0.139$). Twelve mothers (5 in the intervention group and 7 in the placebo group) predominantly breastfed their infants >6 mo (maximum: 6.7 mo). Introduction of solid foods occurred before 4 mo in 12 (1.1%) infants (6 in the intervention group and 6 in the placebo group) and after 6 mo in 23 (2.1%) infants (9 in the intervention group and 14 in the placebo group). No significant differences between infants who completed or withdrew from the study were observed for any baseline characteristic.

The statistical descriptive measures of the endpoints in the intervention and placebo groups are shown in **Table 2**. The time to achievement of the milestone of sitting without support was shorter in the intervention group. The time to achievement of the other gross motor milestones did not significantly differ between groups. The time to achievement of the fine motor milestones or language was shorter in the intervention group.

Multiple Cox regression analysis showed an independent association of the intervention product with a shorter time for sitting without support ($P < 0.001$), reaching an object to touch ($P < 0.001$), bringing toy to mouth ($P < 0.001$), and saying the first comprehensible word ($P < 0.001$) (**Table 3**). These differences also remained significant after Bonferroni correction ($P < 0.01$). In addition, these analyses showed that a longer gestational age was independently associated with a shorter time at achievement of the 4 gross motor milestones ($P < 0.05$), whereas female sex was associated with a shorter time to saying the first comprehensible word ($P < 0.05$).

Both intervention and control preparations were well tolerated by all infants, and no adverse events were observed that were related to the intervention product or placebo. Compliance, which was estimated on the basis of the number of bottles dispensed and the number returned, was 97% and 95% in the intervention and control groups, respectively.

## DISCUSSION

To our knowledge, this is the first randomized, double-blind, placebo-controlled trial conducted to assess the effects of early DHA supplementation on the time of achievement of gross motor developmental milestones of infants. Primary endpoints were 4 of the milestones suggested by the WHO (17) and those in the literature (18, 25–28): sitting without support, hands-and-knees crawling, standing alone, and walking alone. These milestones are familiar to pediatricians and families everywhere. However, there are studies that examined the role of DHA on gross motor development (29–33), with differing results. In particular, Makrides et al (33), in a randomized controlled trial conducted in term infants, found that the Bayley Psychomotor Developmental Index did not differ between those supplemented with DHA during the first year of life and those who did not receive supplementation or breastfed control infants at 12 and 24

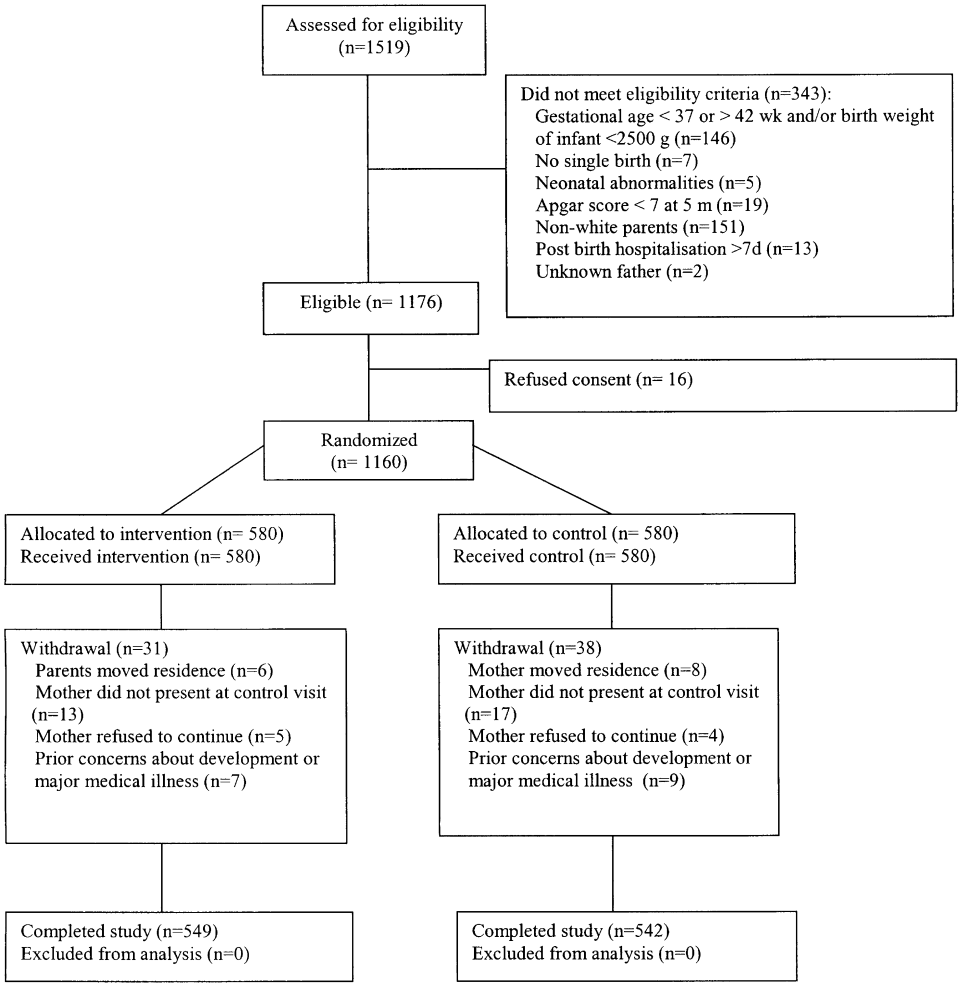


**FIGURE 1.** Flow diagram of subject progress throughout the study.

mo of age. Birch et al (29), in a randomized trial conducted in term formula-fed infants, found that dietary DHA supplied with formula until 17 wk of age failed to have a significant effect on Psychomotor Developmental Index scores at 18 mo. However, differences in the motor subscale of the Mental Developmental Index were found between dietary groups, ie, DHA- and DHA + arachidonic acid–supplemented groups performed better than did the group that received standard formula.

In the present study the times at achievement of milestones were in the range of previous observational studies (17–19, 23, 25–28). For example, median ages in months for sitting without support were 5.4 (18), 8.0 (25), 6.6 (26), 6.4 (27), and 5.9 (17, 28) rather than 6.7 mo as in the present trial.

Analysis of the data showed a small effect of DHA supplementation on the time at achievement of sitting without support, whereas no effect was found on the time at achievement of the other gross motor milestones. The adjusted hazard ratio for the association between DHA supplementation and shorter time at achievement of sitting without support was 1.3 (95% CI: 1.2, 1.5). Secondary endpoints included 2 fine motor milestones and language, as suggested in the literature (18, 19, 23). Supplemental DHA exerted a beneficial effect on these secondary endpoints, particularly language.

It should be noted that ≈98% of infants were breastfed, and that DHA-supplemented infants and control infants had similar breastfeeding profiles. Indeed, a relatively wide range of times to achievement of the milestones was observed in both groups, with CVs ranging from 12% to 24%, in accordance with previous large studies (17–20).

Recent studies suggested that DHA functions in membrane biogenesis and neurotransmission and protects against oxidative stress (34). In addition, autopsy studies in infants whose cause of death was not neurologically related showed that DHA accretion in the forebrain increases from ≈650 mg at birth to ≈2 g at 2 y of age, and that most of the accumulation takes place in the first 12 mo of life (3). DHA supplementation at a dose of 25 mg/d (the dose used in the present study, according to the estimated minimum supply of DHA received through human milk; 9) corresponds to a dose of >7 g/y. Because the capability to synthesize DHA from α-linolenic acid (18:3n−3) may dramatically decrease throughout the first 7 mo of life (35), dietary supplementation with DHA in the second 6 mo of life might result in higher net differences in tissue accretion. Three trials showed benefits on visual acuity after 12 mo of dietary enrichment with DHA, in increasing amounts, through supplementation in formula (36, 37) or eggs (38) during the complementary feeding period.

**TABLE 1**
Baseline characteristics and feeding practices of randomly assigned infants according to allocation

| Characteristic | Intervention ($n$ = 580) | Control ($n$ = 580) | $P$ value[1] |
|---|---|---|---|
| Mothers | | | |
| Age (y) | 31.9 ± 4.6[2] | 32.2 ± 4.3 | 0.133 |
| BMI (kg/m$^2$) | 23.2 ± 3.4 | 23.4 ± 3.5 | 0.324 |
| ≥25 | 69 (11.9) | 74 (12.8) | 0.721 |
| Educational level [$n$ (%)] | | | |
| Low (≤8 y) | 179 (30.9) | 169 (29.1) | |
| Medium (9–13 y) | 335 (57.7) | 340 (58.6) | 0.776 |
| High (≥13 y) | 66 (11.4) | 71 (12.3) | |
| Socioeconomic status[3] | | | |
| 1 (Low) | 78 (13.5) | 65 (11.2) | |
| 2 | 131 (22.6) | 121 (20.9) | |
| 3 | 169 (29.1) | 206 (35.5) | 0.210 |
| 4 | 155 (26.7) | 147 (25.3) | |
| 5 (High) | 47 (8.1) | 41 (7.1) | |
| Smoked during pregnancy [$n$ (%)] | 58 (10.0) | 52 (9.0) | 0.616 |
| Infants | | | |
| Female sex [$n$ (%)] | 261 (45.0) | 281 (48.4) | 0.264 |
| Gestational age (wk) | 39.4 ± 1.2 | 39.4 ± 1.2 | 0.946 |
| Birth weight (g) | 3310 ± 383 | 3292 ± 382 | 0.338 |
| Birth length (cm) | 49.9 ± 1.6 | 50.0 ± 1.6 | 0.189 |
| Birth head circumference (cm) | 34.1 ± 1.1 | 34.2 ± 1.1 | 0.120 |
| Has siblings [$n$ (%)] | 244 (42.1) | 262 (45.2) | 0.314 |
| Feeding | | | |
| Duration of breastfeeding [$n$ (%)][4] | | | |
| 0 mo (never breastfed) | 13 (2.4) | 12 (2.2) | |
| ≤4 mo | 227 (41.3) | 201 (37.2) | 0.421 |
| 4–6 mo | 54 (9.8) | 66 (12.2) | |
| >6 mo | 255 (46.4) | 261 (48.3) | |
| Age at introduction of solid foods (mo)[4] | 4.9 ± 0.8 | 5.0 ± 0.9 | 0.595 |
| Age at introduction of egg (mo)[5] | 10.2 ± 1.3 | 10.3 ± 1.3 | 0.602 |
| Age at introduction of fish (mo)[5] | 9.5 ± 1.5 | 9.5 ± 1.4 | 0.756 |

[1] Significance of difference between groups (Student $t$ test, Mann-Whitney $U$ test, or chi-square test).
[2] Mean ± SD (all such values).
[3] Rated according to the Italian National Institute of Statistics.
[4] $n$ = 549 and $n$ = 540 for the intervention and control groups, respectively.
[5] $n$ = 551 and $n$ = 542 for the intervention and control groups, respectively.

The major strengths of the present study were its strong design and practical outcomes. Moreover, the accuracy of parental evaluation of their infants' milestone achievements was validated in a previous study (39). Limitations of the study included the lack of direct measurements of blood DHA concentrations and tests of maternal mental ability. However, the use of randomization should have ensured that these characteristics were comparable in the supplemented and control infants.

**TABLE 2**
Age (wk) at achievement of motor development milestones and language, according to allocation[1]

| | Intervention | | | Control | | | |
|---|---|---|---|---|---|---|---|
| Developmental milestone | No. of subjects | Mean ± SD | Median (IQR) | No. of subjects | Mean ± SD | Median (IQR) | $P$ value[2] |
| Gross motor | | | | | | | |
| Sitting without support | 551 | 26.8 ± 4.2 | 26.1 (23.6–29.1) | 542 | 28.3 ± 4.2 | 27.2 (25.8–30.9) | <0.0001 |
| Hands-and-knees crawling | 482 | 38.9 ± 6.4 | 39.0 (34.2–43.8) | 476 | 39.4 ± 6.2 | 39.7 (35.3–44.1) | 0.090 |
| Standing alone | 549 | 49.2 ± 7.6 | 48.6 (43.5–55.4) | 542 | 50.1 ± 8.1 | 49.5 (44.0–57.2) | 0.075 |
| Walking alone | 549 | 54.9 ± 6.8 | 55.2 (50.0–60.3) | 542 | 55.8 ± 6.7 | 56.0 (52.4–61.3) | 0.060 |
| Fine motor | | | | | | | |
| Reaches an object to touch | 552 | 16.6 ± 3.0 | 16.9 (14.2–17.9) | 544 | 17.9 ± 3.2 | 18.0 (15.9–20.0) | <0.0001 |
| Brings toy to mouth | 551 | 17.5 ± 3.1 | 17.7 (15.2–20.4) | 544 | 18.9 ± 3.1 | 18.6 (17.1–21.6) | <0.0001 |
| Language | | | | | | | |
| Saying the first comprehensible word | 549 | 41.0 ± 9.8 | 39.3 (34.0–46.6) | 542 | 44.0 ± 9.3 | 43.9 (36.1–49.6) | <0.0001 |

[1] Sixty-four infants withdrew (intervention: $n$ = 31; control: $n$ = 31), 121 infants never exhibited hands-and-knees crawling (intervention: $n$ = 58; control: $n$ = 63), and 3 infants had missing data for some milestones. IQR, interquartile range.
[2] Significance of difference between groups (log-rank test).

**TABLE 3**
Adjusted hazard ratio (95% CI) of variables independently associated with shorter time to achievement of the developmental milestones[1]

| Variable | Sitting without support ($n$ = 1089)[2] | Hands-and-knees crawling ($n$ = 1089) | Standing alone ($n$ = 1087) | Walking alone ($n$ = 1086) | Reaches an object to touch ($n$ = 1089) | Brings toy to mouth ($n$ = 1089) | Saying the first comprehensible word ($n$ = 1086) |
|---|---|---|---|---|---|---|---|
| Treatment (intervention vs control) | 1.31 (1.16, 1.48)[3] | 1.09 (0.96, 1.24) | 1.07 (0.95, 1.21) | 1.11 (0.98, 1.26) | 1.39 (1.23, 1.57)[3] | 1.46 (1.30, 1.65)[3] | 1.37 (1.21, 1.54)[3] |
| Gestational age | 1.09 (1.03, 1.14)[4] | 1.07 (1.01, 1.12)[4] | 1.06 (1.01, 1.12)[4] | 1.06 (1.01, 1.12)[4] | —[5] | — | — |
| Sex (female vs male) | — | — | — | — | — | — | 1.13 (1.00, 1.28)[4] |

[1] Multiple Cox regression models were fitted for each of the milestones. The dependent variable was as follows: time to achievement of the developmental milestones. Covariates included: treatment (intervention or control), duration of breastfeeding (≤4 or >4 mo), maternal age, gestational age, maternal educational level (low, medium, or high), socioeconomic status (low, intermediate, or high), smoking during pregnancy (yes or no), prepregnancy BMI (in kg/m$^2$; <25 or ≥25), sex (female or male), and siblings (yes or no).
[2] $n$ = number of observations entered in the model (all such values).
[3] $P < 0.001$.
[4] $P < 0.05$.
[5] Dashes in cells indicate a nonsignificant effect of the confounder at the 0.05 level.

Overall, the results of the present trial suggest that an association may exist between early nutritional supplementation with DHA and earlier achievement of sitting without support in the first period of complementary feeding, when the endogenous synthesis of DHA may be low (35) because of a reduced DHA intake from human milk and a poor supply of DHA from solid foods. Fish and eggs (natural DHA sources) are generally introduced later to avoid their hypothesized contribution to allergic reactions (40). Yet, one can conclude that despite the observed 1-wk advance in sitting without support associated with DHA supplementation, no demonstrable persistent effects of DHA supplementation on later motor developmental milestones were observed. Thus, the long-term clinical significance of the 1-wk difference in sitting without support, if any, remains unknown.

We thank the medical and nursing staff of the participating centers for their assistance in enrolling and monitoring the infants in this study. In particular, we thank Tiziana Bonabitacola, Maria Elena Capra, Rosella Colli, Paolo Colombo, Maddalena Gibelli, Fabio Meneghin, Vincenzo Milone, Concetta Scalfaro, Serena Scarlino, Daniele Spiri, Margherita Urbano, and Tiziana Varisco, who participated in the collection of data.

The authors' responsibilities were as follows—CA, MG, and GVZ: designed the study; MG: coordinated and supervised the research team; RB, AP, AR, AS, and GVZ: coordinated the research team at local centers; GR: analyzed the data; GR and CA: drafted the manuscript; and MG and ER: revised the manuscript. All authors read and approved the final version of the manuscript. The authors declared that they had no conflict of interest.

## REFERENCES

1. Crawford MA, Bloom M, Broadhurst CL, et al. Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain. Lipids 1999;34(suppl):S39–47.
2. Green P, Yavin E. Mechanisms of docosahexaenoic acid accretion in the fetal brain. J Neurosci Res 1998;52:129–36.
3. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129–38.
4. Uauy R, Dangour AD. Nutrition in brain development and aging: role of essential fatty acids. Nutr Rev 2006;64(suppl):24–33.
5. Caspi A, Williams B, Kim-Cohen J, et al. Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. Proc Natl Acad Sci USA 2007;104:18860–5.
6. Schaeffer L, Gohlke H, Muller M, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 2006;15:1745–56.
7. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189–94.
8. Martinez M. Polyunsaturated fatty acids in the developing human brain, erythrocytes and plasma in peroxisomal disease: therapeutic implications. J Inherit Metab Dis 1995;18(suppl 1):61–75.
9. Marangoni F, Agostoni C, Lammardo AM, Giovannini M, Galli C, Riva E. Polyunsaturated fatty acid concentrations in human hindmilk are stable throughout 12-months of lactation and provide a sustained intake to the infant during exclusive breastfeeding: an Italian study. Br J Nutr 2000;84:103–9.
10. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. Am J Clin Nutr 1999;70:525–35.
11. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n–3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39–44.
12. Dunstan JA, Mitoulas LR, Dixon G, et al. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: a randomized controlled trial. Pediatr Res 2007;62:689–94.
13. Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463–8.
14. Willatts P, Forsyth JS, Di Modugno MK, Varma S, Colvin M. Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 1998;352:688–91.
15. Colombo J. Recent advances in infant cognition: implications for long-chain polyunsaturated fatty acid supplementation studies. Lipids 2001;36:919–26.
16. Cheatham CL, Colombo J, Carlson SE. n–3 Fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations. Am J Clin Nutr 2006;83(suppl):S1458–66.
17. WHO Multicentre Growth Reference Study Group. WHO Motor Development Study: windows of achievement for six gross motor development milestones. Acta Paediatr Suppl 2006;95(450):86–95.
18. Carruth BR, Skinner JD. Feeding behaviors and other motor development in healthy children (2–24 months). J Am Coll Nutr 2002;21:88–96.
19. Viholainen H, Ahonen T, Cantell M, Lyytinen P, Lyytinen H. Development of early motor skills and language in children at risk for familial dyslexia. Dev Med Child Neurol 2002;44:761–9.
20. Myers GJ, Davidson PW, Shamlaye CF, et al. Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. Neurotoxicology 1997;18:819–29.
21. Italian Society of Nutrition. LARN (Italian Recommended Dietary Allowances). 1996 Revision. Rome, Italy: National Institute of Nutrition, 1996.
22. Farquharson J, Cockburn F, Patrick WA, Jamieson EC, Logan RW. Infant cerebral cortex phospholipid fatty-acid composition and diet. Lancet 1992;340:810–3.
23. Coplan J, Gleason JR. Quantifying language development from birth to 3 years using the Early Language Milestone Scale. Pediatrics 1990;86:963–71.

24. World Health Organization. Indicators for assessing breastfeeding practices. Geneva, Switzerland: World Health Organization, 1991. (WHO Publication WHO/CDD/Ser/91.14.) Available from: http://www.who.int/child_adolescent_health/documents/cdd_ser_91_14/en/ (cited 8 August 2008).
25. Griffiths R. The abilities of babies. New York, NY: McGraw-Hill Book Co., 1954.
26. Bayley N. Manual of the Bayley Scales of Infant Development. San Antonio, TX: Psychological Corporation, 1969.
27. Neligan G, Prudham D. Norms for four standard developmental milestones by sex, social class and place in family. Dev Med Child Neurol 1969;11:413–22.
28. Frankenburg WK, Dodds J, Archer P, et al. The DENVER II training manual. Denver, CO: Denver Developmental Materials, 1992.
29. Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000;42:174–81.
30. Bouwstra H, Dijck-Brouwer DA, Wildeman JA, et al. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. Am J Clin Nutr 2003;78:313–8.
31. Jensen CL, Voigt RG, Prager TC, et al. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr 2005;82:125–32.
32. Bouwstra H, Dijck-Brouwer J, Decsi T, et al. Neurologic condition of healthy term infants at 18 months: positive association with venous umbilical DHA status and negative association with umbilical trans-fatty acids. Pediatr Res 2006;60:334–9.
33. Makrides M, Neumann MA, Simmer K, Gibson RA. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. Pediatrics 2000;105:32–8.
34. Innis SM. Dietary (n–3) fatty acids and brain development. J Nutr 2007;137:855–9.
35. Carnielli VP, Simonato M, Verlato G, et al. Synthesis of long-chain polyunsaturated fatty acids in preterm newborns fed formula with long-chain polyunsaturated fatty acids. Am J Clin Nutr 2007;86:1323–30.
36. Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD. A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. Am J Clin Nutr 2002;75:570–80.
37. Hoffman DR, Birch EE, Castaneda YS, et al. Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. J Pediatr 2003;142:669–77.
38. Hoffman DR, Theuer RC, Castaneda YS, et al. Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 2004;134:2307–13.
39. Knobloch H, Stevens R, Malone A, Ellison P, Risemberg H. The validity of parental reporting of infant development. Pediatrics 1979;63:872–8.
40. Fiocchi A, Assa'ad A, Bahna S. Food allergy and the introduction of solid foods to infants: a consensus document. Adverse Reactions to Foods Committee, American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 2006;97:10–20.