Essential n−3 fatty acids in pregnant women and early visual acuity maturation in term infants1−3
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ABSTRACT
Background: Docosahexaenoic acid (DHA) is important to neural development. Whether DHA intakes are low enough in some pregnant women to impair infant development is uncertain.
Objectives: We sought to determine whether DHA deficiency occurs in pregnant women and contributes to poor infant development.
Design: Biochemical cutoffs, dietary intakes, or developmental scores indicative of DHA deficiency are not defined. Infant development has a distribution in which an individual’s potential development is unknown. This was a randomized intervention to establish a distribution of developmental scores for infants of women with DHA intakes considered to be above requirements against which to compare the development of infants of mothers consuming their usual diet. DHA (400 mg/d; n = 67) or a placebo (n = 68) was consumed by the women from 16 wk gestation until delivery. We determined maternal red blood cell ethanolamine phosphoglyceride fatty acids, dietary intakes at 16 and 36 wk gestation, and infant visual acuity at 60 d of age.
Results: We described an approach to identify DHA deficiency when biochemical and functional markers of deficiency are unknown. In multivariate analyses, infant visual acuity was related to sex (β = 0.660, SE = 0.93, and odds ratio = 1.93) and maternal DHA intervention (β = 1.215, SE = 1.64, and odds ratio = 3.37). More infant girls in the placebo than in the DHA intervention group had a visual acuity below average (P = 0.048). Maternal red blood cell ethanolamine phosphoglyceride docosatetraenoic fatty acids, dietary intakes at 16 and 36 wk gestation, and infant visual acuity at 60 d of age.
Conclusions: These studies suggest that some pregnant women in our study population were DHA-deficient. Am J Clin Nutr 2008;87:548–57.

KEY WORDS Docosahexaenoic acid, n−3 fatty acids, pregnancy, infant development, brain

INTRODUCTION
The importance of the n−3 fatty acid docosahexaenoic acid (DHA) is one of the most intensely studied areas relating nutrition to central nervous system (CNS) development and is also emerging as important to several neurologic problems in adults (1–10). Inadequate DHA during early development decreases DHA in the brain and retina, impairs neurogenesis and visual function, and results in long-term deficits in neurotransmitter metabolism and visual function in animals (1, 11–14). Intervention studies to show that dietary DHA increases visual, mental, and motor skill development in some preterm and term infants fed formula provide evidence that DHA is also important in early human development (reviewed in references 2–5). More recently, it has become clear that maternal to fetal transfer of DHA during gestation is influenced by the maternal circulating and dietary intake of DHA (15–19). Thus, attention has turned to consider whether low DHA intakes in pregnant women may contribute to poor infant CNS development (20–27). However, the extent of DHA deficiency, if present, biochemical cutoffs for circulating DHA, dietary intakes, or infant visual or other developmental scores indicative of inadequate maternal DHA status to support optimal infant development are not known.
Because n−3 fatty acids are essential nutrients, all of the DHA accumulated by the fetus must be derived by placental transfer and must originate from the maternal diet, either as DHA or as a precursor n−3 fatty acids (1, 15). α-Linolenic acid (ALA, 18:3n−3), not DHA, is currently considered the essential dietary n−3 fatty acid because humans lack Δ15-desaturase but can desaturate ALA via eicosapentaenoic acid (EPA, 20:5n−3) to DHA (1). However, stable-isotope tracer studies have shown that conversion of ALA to DHA is low in humans (28–30), and interventions to increase ALA intake during pregnancy do not increase DHA in either maternal or fetal circulating lipids (31). On the other hand, observational and intervention studies provide consistent evidence that maternal dietary and circulating DHA is an important determinant of fetal blood concentrations of DHA (14–18). Although the estimated average intakes of DHA in pregnant women in North America is 40–120 mg/d (32–34), individual intakes vary widely from 20 to >500 mg/d among women, with the exclusion of those following vegan diets (32).
We seek to determine whether poor DHA status sufficient to decrease infant CNS development occurs among pregnant women. However, although circulating concentrations of DHA increase with increasing DHA intake (35, 36), enhanced DHA intake is not expected to have benefit in individuals with a DHA status above their physiologic need (Figure 1). Neither

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2 Supported by the Canadian Institute of Health Research, Martek Biosciences Corp, Columbia, MD, provided all of the supplements.
3 Reprints not available. Address correspondence to SM Innis, Nutrition Research Program, 950 W 28th Avenue, Vancouver, BC, Canada V5Z 4H4. E-mail: sinnis@interchange.ubc.ca. Received July 27, 2007. Accepted for publication October 11, 2007.

Subjects and design

This is a prospective study designed to determine whether DHA status is so low among some pregnant women as to pose risk of poor infant CNS development. This study was not designed to demonstrate efficacy of DHA supplements in increasing infant development, which requires a different design. Healthy pregnant women were randomly assigned to 400 mg/d DHA or a soybean and corn oil placebo from 16 wk of gestation until delivery of their infant. The reason for using a placebo group and randomization was to minimize group bias. In this study, as in some studies with infant formulas, the supplements were not labeled with individual codes corresponding to subject codes, but with a product code. To avoid randomization to only 2 groups known by those conducting randomization to differ, we used 4 groups: 2 identical DHA and 2 identical placebo groups. On enrollment, each subject was assigned a unique, computer-generated, random code held in sealed opaque envelopes. Individual subject codes, not supplement codes, were used on data sheets and blood samples and were analyzed blinded. The research staff that conducted the biochemical analyses, compiled the data, and conducted the statistical analyses had no contact with the study subjects (double-double blind).

Eligible participants were at 14–16 wk gestation, were not taking any lipid supplement, had no complications likely to affect maternal or fetal metabolism or fetal development, and were expected to deliver one full-term infant. Maternal blood samples and dietary intake information were collected at 16 and 36 wk of gestation, and infant visual acuity was determined at 60 d of age. Because our purpose was to relate biochemical indexes of maternal DHA status to infant development, subjects who did not complete all assessments or whose infants were premature, had low birth weight, or had any complications likely to interfere with growth or development were not included in this study. All procedures were reviewed and approved by the Committee for Ethical Review of Research Involving Human Subjects at the University of British Columbia and the British Columbia Children’s and Women’s Hospital. All subjects provided written informed consent before participating in this study.

Supplements

The supplements were provided as capsules (2500 mg/d), each providing ~200 mg/g DHA from the single cell organism Crypthecodinium cohnii or a corn–soybean oil blend providing 265 mg linoleic acid (LA, 18:2n–6) and 40 mg ALA, both containing an orange flavor to assist in further blinding (Martek Biosciences, Columbia, MD). Each subject was asked to take 2 capsules/d with meals. The capsules provided ~9 kcal/d and negligible LA and ALA compared with that in the usual diet. No dietary instructions were given, except not to take any lipid or fatty acid supplement other than that provided as part of this study. We chose a supplement of 400 mg/d DHA (equivalent to ~400 g/wk fatty fish such as salmon) because available estimates suggest that the fetus accumulates ~70 mg/d DHA during the last trimester of gestation (37), and the International Society for the Study of Lipids and Fatty Acids has suggested that pregnant women consume 300 mg/d DHA (38). Thus, we considered that a DHA supplement of 400 mg/d in addition to the usual diet should result in a low risk of DHA deficiency.

Red blood cell fatty acids

Maternal venous blood was collected from each subject at 16 and 36 wk gestation in the outpatient laboratory of the British Columbia Children’s Hospital, labeled with the subject code, and immediately transferred to our Nutrition Research laboratory in the adjacent Child and Family Research Institute. The red blood cells (RBCs) were separated from plasma and then stored at −70 °C as described previously (18, 39, 40). We chose the RBC ethanolamine phosphoglyceride (EPG) fatty acids as a measure...
of maternal DHA status to avoid problems with the analysis of fatty acids in plasma total lipids or phospholipids that arise because of inter- and intraindividual differences in plasma lipoproteins, which are known to differ in fatty acid composition with stage of gestation and with short-term changes in dietary fat intake (41, 42). The RBC EPG fatty acids were analyzed because EPG is preferentially distributed on the inner surface of the RBC membrane bilayer and contains higher concentrations DHA and the n–6 fatty acids docosatetraenoic acid (DTA, 22:4n–6) and docosapentaenoic acid (DPA, 22:5n–6) than phosphatidylcholine, which is predominately on outer membrane bilayer of the RBC and exchanges with plasma phosphatidylcholine (43, 44). The RBC lipids were extracted, EPG was separated from other lipids, and then the fatty acid composition analyzed by gas–liquid chromatography as described in detail by us (18, 39, 40, 44).

Sociodemographic, dietary, and anthropometric data collection

Sociodemographic data including the mother’s highest level of education attained, family income, ethnicity, age, height, pregnancy weight gain, and obstetric history were recorded. The Toni III, a test of nonverbal intelligence (3rd edition; Pro-Ed, Austin, TX), was given to each woman as a measure of intelligence quotient. Dietary intake for the preceding 4 wk was recorded at 16 and 36 wk gestation by using an interview-administered food-frequency questionnaire validated for estimation of fatty acid intakes (31). We collected specific information on all sources of fat, including product brand names and types of fish and seafood. Infant weight and length were measured at birth and at 30 and 60 d of age, and the type of milk feeding was recorded (39, 40, 44).

Infant visual acuity assessments

Visual acuity was assessed by using the Teller Acuity Card Procedure (Vistech Inc, Dayton, OH) at 60 ± 3 d of age by using a test distance of 38 cm (40, 45). Looking acuity was determined as the finest grating to which the infant showed a reliable and consistent fixation response, based on the infant’s looking behavior. Visual acuity was determined at 60 d after birth because a meta-analyses of studies with preterm and term infants have shown that the behavioral tests of visual acuity used in the present study acuity is a sensitive and robust measure of differences in visual acuity due to differences in DHA status when given at 60 d after birth (46, 47).

Statistical analysis

Baseline differences in subject characteristics between the groups were compared using Student’s t test or means for categorical variables. Visual acuity scores were converted to cycles/degree, and a log_{10} transformation was applied to the data for statistical analyses. The results for visual acuity are presented as means (cycles/degree) and SD (octaves), where one cycle/degree is equivalent to 0.301 octaves, as is convention (40, 45). Changes in maternal fatty acid status due to stage of gestation (16 or 36 wk) or DHA intervention (DHA or placebo group) were determined by using 2-factor analysis of variance, with paired t tests and Fisher’s least-significant-difference test to detect significant effects of stage of gestation or DHA intervention, respectively, where appropriate. Visual acuity assessed with the acuity card procedure is a discontinuous variable (pass/fail), and visual acuity is higher in infant girls than boys (48). Therefore, distribution curves were constructed for the infant boys and infant girls separately to examine the frequency with which infants in the 2 groups achieved a visual acuity score of ≤1.6, 2.4, 3.2, or ≥4.8 cycles/degree. In multiple logistic regression, maternal DHA intervention, infant sex, birth weight, birth length, gestation length, breastfeeding duration, and maternal ethnicity accounted for 86% of the variability in infant visual acuity scores, but, of these variables, only maternal DHA intervention and infant sex were significant. Fisher’s exact test was used to determine whether infants in the placebo group were more likely than those in the DHA group to have a visual acuity score below the mean, and the results were analyzed for each sex. Spearman rank correlation coefficients were calculated to determine the relations between maternal RBC EPG concentrations of the fatty acids DHA, DTA, and DPA and visual acuity in the infant girls and boys. The maternal dietary fatty acid intakes in the placebo and DHA intervention group were compared by using the Mann-Whitney U test. All analyses were done with SPSS for WINDOWS (version 15; SPSS Inc, Chicago, IL).

RESULTS

Subject characteristics

A total of 135 women (n = 67 in the placebo and n = 68 in the DHA intervention group) completed this study to 60 d after birth. There were no significant differences in maternal age, ethnicity, education, family income, or any other sociodemographic variable between women assigned to the DHA and placebo groups (Table 1), although our study population was predominately white (73% of the subjects), well-educated (76% had attended university) mature women with a mean age of 33.2 y at enrollment. All the infants were born after term gestation (37–42 wk gestation), as defined by the inclusion criteria. There were no statistically significant differences in infant birth weight or length between the 2 groups (P > 0.05). At 60 d of age, 50 of 67 infants in the placebo group and 42 of 68 infants in the DHA intervention group were exclusively breastfed, an advantage potentially favoring the placebo group. All the infants fed with formula were given a term infant formula containing DHA and the n–6 fatty acid arachidonic acid (AA, 20:4n–6). Randomization in the present study was at 16 wk gestation, without knowledge of infant sex. By chance, 50% of the infants in the placebo and 30% of the infants in the DHA group were boys.

Maternal dietary n–6 and n–3 fatty acid intakes

We found no statistically significant differences in the maternal dietary fatty acid intakes at 16 and 36 wk gestation (data not shown). In Table 2 we report the mean, interquartile ranges, and ranges of saturated, monounsaturated, n–6, and n–3 fatty acid intakes at 36 wk gestation. The median intake of LA was 5.0% of total energy and ALA represented 0.66% of total energy (n = 134). The median intakes of AA, EPA, and DHA were 90, 70, and 110 mg/d, respectively. However, we draw attention to the large differences in fatty acid intake among individuals, which included a range of 10 to 760 mg/d DHA. There were no statistically significant differences in the intakes of total fat, saturated fat, monounsaturated fat, LA, ALA, EPA, or DHA between the placebo and DHA intervention group. However, the DHA group
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 67)</th>
<th>DHA intervention (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>33.6 ± 0.40</td>
<td>32.9 ± 0.49</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
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<td>31</td>
</tr>
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<td>&gt;2</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td>76</td>
<td>69</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Middle or higher income (%)</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>Vegetarian (n)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Smoker (n)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Education level (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>73</td>
<td>78</td>
</tr>
<tr>
<td>Moderate</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Infant weight (g)</td>
<td>3562 ± 59.7</td>
<td>3472 ± 47.1</td>
</tr>
<tr>
<td>Infant length (cm)</td>
<td>52.0 ± 0.27</td>
<td>51.7 ± 0.29</td>
</tr>
<tr>
<td>Infant diet at 60 d (%)</td>
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<td></td>
</tr>
<tr>
<td>Breastfed</td>
<td>75</td>
<td>61</td>
</tr>
<tr>
<td>Mixed</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Formula</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

1 There were no statistically significant differences between groups, P < 0.05. DHA, docosahexaenoic acid.
2 ± SEM (all such values).
3 Included mostly Chinese and other Asians (n = 26); n = 10 subjects of other ethnic backgrounds.
4 Defined as ≥$50 000/y.
5 None of the women followed a vegan diet.
6 None of the women smoked >10 cigarettes/d.
7 We classified university, college, and ≤high school as high, moderate, and low, respectively.
8 A nonverbal intelligence test.
9 For practical purposes, infants consuming <2 × 250 mL formula/wk or with <2 breastfeedings/wk were considered breastfed or formula-fed, respectively; all other infants were considered “mixed.”

Biochemical measures of fatty acid status

Although dietary assessments can provide useful information on nutrient intakes of groups, they have limited precision for calculating the intakes of individuals. For this reason, we used biochemical measures of the RBC EPG fatty acids to assess maternal DHA status. In Figure 2 we report the changes in RBC EPG n-6 and n-3 fatty acids between 16 and 36 wk gestation and the effect of intervention on enhancement of maternal DHA intake. There were no statistically significant differences in the RBC EPG concentrations of n-6 or n-3 fatty acids between women randomly assigned to the DHA and placebo groups at 16 wk gestation, except for ALA, which was lower in the DHA group than in the placebo group at both 16 and 36 wk gestation (P < 0.05). The maternal RBC EPG concentrations of DHA increased significantly, and EPA and AA decreased between 16 and 36 wk gestation in both groups (P < 0.05). However, whereas the RBC EPG concentrations of EPA were not different between the placebo and DHA intervention groups at 36 wk gestation, the concentrations of DHA were 32% higher in the DHA intervention group than in the placebo group (P < 0.05, Table 2). Women in the DHA intervention group, but not women in the placebo group, also had a significantly lower RBC EPG n-6 DTA, n-6 DPA, and n-3 DPA (22:5n-3) concentration at 36 wk gestation than at 16 wk gestation (Figure 2). In contrast, women in the placebo group had a significantly higher median intake of AA than did the placebo group (P < 0.05, Table 2).

Infant visual acuity

In the absence of n-3 fatty acid deficiency, intervention to enhance maternal DHA status can have no effect on the distribution of infant visual acuity scores. We report the frequency of visual acuity scores of ≥1.6, 2.4, 3.2, or ≥4.6 cycles/degree for infant boys and girls in the placebo and DHA intervention groups in Figure 3. The mean (±SD) visual acuity of the infants (n =

TABLE 2
Dietary fat and fatty acid intakes of pregnant women randomly assigned to placebo or a docosahexaenoic acid (DHA) supplement

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 135)</th>
<th>Placebo (n = 67)</th>
<th>DHA (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Interquartile range</td>
<td>Median</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>85.6</td>
<td>32.4–221</td>
<td>86.1</td>
</tr>
<tr>
<td>Saturates (g)</td>
<td>27.3</td>
<td>13.6–78.3</td>
<td>27.4</td>
</tr>
<tr>
<td>Monounsaturates (g)</td>
<td>32.4</td>
<td>15.2–90.6</td>
<td>33.3</td>
</tr>
<tr>
<td>Linoleic acid (g)</td>
<td>13.5</td>
<td>7.4–43</td>
<td>12.1</td>
</tr>
<tr>
<td>α-Linolenic acid (g)</td>
<td>1.48</td>
<td>0.95–9.2</td>
<td>1.60</td>
</tr>
<tr>
<td>Arachidonic acid (mg)</td>
<td>90</td>
<td>50–360</td>
<td>80</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (mg)</td>
<td>70</td>
<td>80–280</td>
<td>60</td>
</tr>
<tr>
<td>Docosahexaenoic acid (mg)</td>
<td>110</td>
<td>190–760</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Intakes exclude supplements at 36 wk gestation.
2 The intake of arachidonic acid in the DHA intervention group was significantly different from placebo, P = 0.01 (Mann Whitney U test).
was 2.51 cycles/degree (octaves) in the girls (n = 74) and 2.35 ± 0.63 cycles/degree in the boys (n = 60) (P = 0.08). The visual acuity of infants in the placebo group (n = 67) was 2.42 ± 0.50 and of infants in the DHA intervention group (n = 68) was 2.60 ± 0.63 cycles/degree (P = 0.30). The visual acuity of girls in the placebo (n = 33) and DHA intervention (n = 42) groups was 2.46 ± 0.39 and 2.81 ± 0.57 cycles/degree (octaves) (P = 0.10) and 2.39 ± 0.6 and 2.30 ± 0.68 cycles/degree (octaves) for boys in the placebo (n = 34) and DHA intervention (n = 26) groups, respectively (P < 0.73). In multivariate analysis, only infant sex (girls > boys: β = 0.660, SE = 0.93, and odds ratio = 1.93) and study group (DHA > placebo: β = 1.215, SE = 1.642, and odds ratio = 3.37) were related to infant visual acuity. Maternal smoking and alcohol were not important variables related to infant visual acuity in the present study. All of the infants were born at term gestation and were breastfed or fed an infant formula containing DHA and AA at 60 d of age. Neither birth characteristics

FIGURE 2. Mean, 25th–75th percentile, and minimum and maximum of values for maternal red blood cell (RBC) ethanolamine phosphoglyceride (EPG) n–3 and n–6 fatty acids at 16 and 36 wk gestation in women in the placebo group (□) and the docosahexaenoic acid (DHA, 22:6n–3) intervention group (■). #Significantly different from 16 wk gestation, P < 0.05. *Significantly different from the placebo group at the same time point, P < 0.05 (ANOVA). 22:5n–3, docosapentaenoic acid; 20:5n–3, eicosapentaenoic acid; 18:3n–3, α-linoleic acid; 22:5n–6, docosapentaenoic acid; 22:4n–6, docosatetraenoic acid; 20:4n–6, arachidonic acid; 18:2n–6, linoleic acid.
but not for boys (placebo and DHA intervention groups achieved visual acuity in adequate DHA. The frequency with which girls and boys in the distribution of test scores for infants of mothers following their usual diet. The present report describes a novel approach using multivariate analysis, infant sex (girls > boys: $\beta = 0.660$, SE = 0.930, odds ratio = 1.93) and study group (DHA intervention > placebo: $\beta = 1.215$, SE = 1.642, odds ratio = 3.369). A greater proportion of girls in the DHA intervention than in the placebo group achieved visual acuity scores above the mean acuity for girls, $P = 0.048$ (Fisher’s exact test).

Important to the present study, infant visual acuity has a distribution in which the potential visual acuity of individuals is unknown. As illustration of such, a theoretical distribution of visual acuities for 150 infants could be as follows (in cycles/degree): 1.6 ($n = 30$), 2.4 ($n = 500$), 3.2 ($n = 50$), and 4.8 ($n = 20$) to give a mean ($\pm$SD) of 2.67 $\pm$ 0.48 cycles/degrees (octaves). Assuming that DHA deficiency does not limit development in infants with a visual acuity of 4.8 cycles/degree, an increase by one acuity level in 10% of infants at all other acuity levels would change the distribution as follows (cycles/degree): 1.6 ($n = 27$), 2.4 ($n = 48$), 3.2 ($n = 50$), and 4.8 ($n = 25$) to give a mean ($\pm$SD) of 2.75 $\pm$ 0.50 cycles/degree (octaves) ($P = 0.433$). Regardless of the improved outcome in 8.7% of infants, which would have important consequences at a population level, statistically significant differences in visual acuity were not apparent after a comparison of the group means. However, if DHA status is so low among some pregnant women as to limit infant development, then deficiency will be apparent by comparison of the distribution of test scores for infants of mothers following their usual diet with that of infants born to mothers at low risk of inadequate DHA. The frequency with which girls and boys in the placebo and DHA intervention groups achieved visual acuity thresholds of $\leq 1.6$, 2.4, 3.2, or $\geq 4.8$ cycles/degree (Figure 3) shows that infants in the placebo group were more likely to have a lower visual acuity than were infants in the DHA group (odds ratio = 3.37, from the multivariate analysis). The proportion of infants with acuity scores above the mean for their sex was higher in the DHA group than in the placebo group for girls ($P = 0.048$), but not for boys ($P = 0.322$).

Next, we used Spearman rank correlation analyses to determine the strength of the relations between the maternal RBC EPG concentrations of DHA, DTA, and n-6 DPA and visual acuity for infant girls and boys (Figure 4). Again, we did not combine results for the placebo and DHA intervention groups because no functional benefit is expected at intakes above need (Figure 1). The results for boys and girls were analyzed separately because sex influences visual acuity (odds ratio = 1.93, from the multivariate analyses). The maternal RBC EPG concentrations of n-6 DTA were inversely related to infant visual acuity at 60 d of age in girls ($\rho = -0.37$, $P < 0.05$) and boys ($\rho = -0.48$, $P < 0.01$). The inverse relation between the maternal RBC EPG concentrations of n-6 DPA and infant visual acuity was not statistically significant in either girls ($\rho = -0.24$) or boys ($\rho = -0.21$). The maternal RBC EPG DHA concentration was not statistically significant in relation to infant visual acuity in girls ($\rho = 0.10$) or boys ($\rho = 0.07$).

**DISCUSSION**

Although it is well accepted that DHA is critically important in the CNS, human requirements for n-3 fatty acids remain uncertain. Central to current questions about n-3 fatty acid nutrition is whether DHA is so low among some individuals as to impair neural development in infants and children or as to increase the risk of neurologic problems in adults (1–10). However, neither biochemical nor dietary indexes of inadequate DHA to support CNS functions are defined. Furthermore, CNS development and function is influenced by many variables other than DHA. To add complexity, infant development is a distribution in which an individual’s potential is unknown. Thus, infants with low developmental potential and high DHA or high developmental potential and low DHA cannot be distinguished by currently used tests (Figure 1). The present report describes a novel approach using a randomized intervention to establish a group of infants for whom maternal DHA deficiency during gestation is considered unlikely against which to compare the distribution of development of infants of mothers following their usual diet. The presence of inadequate DHA in our population was suggested by comparison of the distribution of visual acuities, which shows that more infants of women following their diet had lower visual acuities than did infants of mothers in the DHA intervention group (odds ratio = 3.37, $\beta = 0.60$, and SE = 1.93).

Several studies have considered the effects of supplementation of pregnant or lactating women with DHA from fish oils, DHA-enriched foods, or triacylglycerols (20–26). In their study, Helland et al (20) supplemented pregnant women with 1183 mg EPA plus 803 mg DHA daily in gestation, but found no significant difference in electroencephalogram test results of infant neural development or novelty preference when compared with a placebo group using Student’s $t$ test or means tests, although infants with more mature electroencephalogram patterns had high plasma DHA concentrations at birth. Similarly, Malcolm et
al (22) found no difference in visual evoked potentials between infants of mothers assigned to 200 mg/d DHA or a placebo during gestation, but visual evoked potential peak latencies were significantly related to infant RBC DHA concentrations at birth. We also found no significant difference in the mean visual acuity between the group of infants of mothers assigned to 400 mg/d DHA and the group of infants of mothers in the placebo group, regardless of a 32% higher maternal RBC EPG concentration of DHA at 36 wk gestation in the DHA intervention group than in the placebo group. However, we illustrate that mean scores for functional tests among groups are not expected to reveal the presence of deficiency because CNS development is a distribution,
individual potential is unknown, and the study population is not limited to individuals known to be deficient before the intervention. Overlap between the groups, as illustrated in Figure 4, was expected in the present study because not all individuals in the placebo group were likely to be deficient, and the DHA status of individuals in the DHA intervention group with a low dietary DHA intake can overlap with that of individuals in the placebo group with a high dietary DHA intake. In contrast with the present study, which addressed the potential risk of inadequate n-3 fatty acid nutrition among pregnant women, studies such as those of Makrides et al (49) compared visual or other aspects of neurodevelopment between breastfed infants and infants fed formula without DHA involved a comparison of infants separated by exposure or no exposure to a dietary source of DHA.

We also considered information on DHA accretion in fetal tissues to provide supporting evidence that dietary n-3 fatty acid intakes are limiting in our population. Autopsy analyses have suggested that $\approx 70$ mg/d n-3 fatty acids, mostly DHA, accumulates in fetal tissue during the last trimester of gestation (37). At term gestation, the fetus represents $\approx 25\%$ of the total weight gain in pregnancy, but data on DHA accretion in the placenta or maternal pregnancy-associated tissues is not available. Women in the present study had a median intake of 110 mg/d DHA, and 40% of the women consumed $<70$ mg/d DHA. ALA and EPA can be desaturated and elongated to DHA, but stable-isotope tracer and dietary intervention studies have shown that conversion of ALA to DHA is limited, particularly at the level of EPA to DHA (28–31, 50). The median intakes of ALA and EPA in our study were 1480 and 70 mg/d, respectively, which, assuming 0.5% conversion to DHA (28, 29), could provide a potential mean total DHA from dietary n-3 fatty acids of 118 mg/d. On the basis of a value of 9% conversion of dietary ALA to DHA in pregnant women (51), the estimated potential median DHA derived from dietary n-3 fatty acids for the women in the present study could be 250 mg/d. Regardless of the limitations of the latter approach, it is clear that many women in our study consumed less than the recommended 300 mg/d DHA (38), from which we suggest that risk of insufficient n-3 fatty acid nutrition seems likely. Consistent with this, recent epidemiologic studies have shown a significant positive relation between seafood consumption in pregnant women and verbal communication skills in their infants at 6 and 18 mo of age (27).

The extent to which the n-3 fatty acid intakes of women in our study are representative of a broader population is important. Our study involved predominantly well-educated, mature white women, which had the advantage of reducing the effects of environment on infant development. In previous studies we estimated dietary intakes of 150 mg/d DHA (range: 20–520 mg/d) among pregnant women (32). Studies in central Canada, the United States, the Netherlands, and Norway have reported mean intakes of 82, 81, 140, and 200 mg/d DHA, respectively (20, 33, 34, 52). Pregnant women in the present study had a median intake of 110 mg/d DHA, which suggests that the dietary patterns with respect to sources of n-3 fatty acids are similar to those of other groups of pregnant women.

Although an increase in the desaturation of n-6 fatty acids leading to increases in n-6 DPA (22:5n-6) is a characteristic finding in animals fed an n-3 fatty acid–deficient diet (53), metabolism of fatty acids beyond the $\Delta^5$-desaturase is limited in humans (28–31, 50, 51). In the present study, the mean maternal RBC EPG concentrations of n-6 DPA and DHA were both higher at 36 wk gestation than at 16 wk gestation for the group of women in placebo group. However, the interindividual differences in n-6 DPA and DHA were wide, and 30% of the women in the placebo group showed no increase in their RBC EPG DHA between 16 and 36 wk gestation. Interestingly, even though we found no significant difference in the mean RBC EPG DTA concentration at 16 wk gestation than at 36 wk gestation in the placebo group, the maternal RBC EPG n-6 DTA concentration was significantly inversely related to visual acuity in the infant girls ($p = 0.37$, $P < 0.05$) and boys ($p = 0.48$, $P < 0.01$). Previous studies by us also reported a positive, rather than an inverse, relation between DHA and n-6 DPA in RBC lipids of preschool children, even though their intakes of n-3 fatty acids were very low (54). More specific studies will be needed to assess whether n-6 DTA in RBC or plasma lipids can provide a sensitive indicator of DHA deficiency in humans.

In summary, the present study used a novel approach to address whether poor DHA status sufficient to delay infant development occurs among pregnant women in our population. We showed an increased risk of low visual acuity among 60-d-old infants of mothers following their usual diet when compared with infants of women considered at low risk of inadequate DHA due to DHA supplementation. The low dietary intakes of the n-3 fatty acid DHA together with the increased blood concentration of n-6 DPA provide supporting evidence that current dietary practices place women at risk of inadequate DHA during pregnancy. However, the present study does not address the issue of n-3 fatty acid requirements, which requires identification of the asymptote in functions above which no further benefit occurs, regardless of increasing DHA status. Whether subtle delays in early visual acuity maturation can be recovered later on is also not addressed. However, studies in animals subjected to n-3 fatty acid deficiency during intrauterine development have found persistent deficits in retina electroretinogram responses and brain monoamine neurotransmission, regardless of restitution of an n-3 fatty acid–adequate diet (11, 14), although some behavioral functions can be restored (55). Some evidence of a positive relation between maternal DHA intake during gestation and mental skills in young children has also been published (21, 25, 27). We suggest the need for further studies to address dietary n-3 fatty acid requirements for pregnancy and lactation—specific stages of the life cycle that involve anabolism and transfer of nutrients to the developing infant.

RA Milner assisted with the study design and statistical analyses, JD King provided laboratory assistance, and C Neufeld, R Esdaile, and M George assisted with subject enrollment and assessments.

The authors’ responsibilities were as follows—SMI (Principal Investigator): conceived, designed, and implemented this study; and RWF: contributed throughout the entire study in compiling and analyzing the data and at all stages of the manuscript preparation and interpreted the data. None of the authors had any conflicts of interest.

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