

## OBSTETRICS

# Developmental programming for allergy: a secondary analysis of the Mothers, Omega-3, and Mental Health Study

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**OBJECTIVE:** Fetal dysregulation of T helper cell pathways may predispose to allergy, as high cord blood T helper 2/T helper 1 ratios have been shown to precede development of allergic diseases. We aimed to determine whether prenatal eicosapentaenoic acid and docosahexaenoic acid supplementation reduces T helper 2 to T helper 1-associated chemokine ratios. We also explored the effect of mode of delivery on T helper 2/T helper 1 ratios.

**STUDY DESIGN:** We conducted a secondary analysis of a randomized placebo controlled trial initially performed to assess the effects of docosahexaenoic acid or eicosapentaenoic acid supplementation on pregnancy-related depressive symptoms among 126 participants. Cord plasma specimens from 98 newborns were assayed for chemokines associated with T helper 2 (thymus and activation-regulated chemokine [CCL17], macrophage-derived chemokine [CCL22], eotaxin [CCL11]) and T helper 1 (interferon-inducible protein-10 [CXCL10]) by enzyme-linked immunosorbent assay and Multiplex immunoassays. Ratios of log-transformed chemokines macrophage-derived chemokine/interferon-inducible protein-10 and thymus and activation-regulated chemokine/interferon-inducible protein-10 were compared between groups by analyses of variance. Multiple linear regression was performed to examine associations between treatments and chemokine ratios, adjusting for covariates.

**RESULTS:** After adjusting for gestational age at delivery, birthweight, and mode of delivery, both omega-3 supplementation groups were associated with lower macrophage-derived chemokine/interferon-inducible protein-10 ratios than placebo (eicosapentaenoic acid: coefficient  $-1.8$ ; 95% confidence interval [CI],  $-3.6$  to  $-0.05$ ;  $P = .04$ ; docosahexaenoic acid:  $-2.0$ ; 95% CI,  $-3.9$  to  $-0.07$ ;  $P = .04$ ). Similar associations were found for thymus and activation-regulated chemokine/interferon-inducible protein-10 (eicosapentaenoic acid:  $-1.5$ ; 95% CI,  $-3.0$  to  $0.06$ ;  $P = .06$ ; docosahexaenoic acid  $-2.2$ ; 95% CI,  $-3.8$  to  $-0.52$ ;  $P = .01$ ). Cesarean delivery was associated with higher macrophage-derived chemokine/interferon-inducible protein-10 ( $1.6$ ; 95% CI,  $0.01$ – $3.3$ ;  $P = .049$ ) and thymus and activation-regulated chemokine/interferon-inducible protein-10 ( $1.5$ ; 95% CI,  $0.1$ – $2.9$ ;  $P = .042$ ) ratios than vaginal delivery.

**CONCLUSION:** Prenatal supplementation with eicosapentaenoic acid and docosahexaenoic acid resulted in decreased cord blood T helper 2/T helper 1 chemokine ratios. Cesarean delivery was associated with a pronounced T helper 2 deviation at birth.

**Key words:** allergy, chemokines, fetal, omega-3, programming

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Allergic diseases have become an important cause of childhood morbidity in the developed world.<sup>1</sup> Because allergic diseases are often diagnosed in early childhood, it has been suggested that etiologic factors may take place in utero or during the neonatal period.<sup>2-5</sup> Prenatal factors that have been suggested to predispose to allergic diseases include

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TABLE 1

**Maternal baseline characteristics among participants included in secondary analysis (n = 117)**

| Characteristic                            | Treatment arm              |                            |                  | P value |
|-------------------------------------------|----------------------------|----------------------------|------------------|---------|
|                                           | EPA-rich fish oil (n = 40) | DHA-rich fish oil (n = 37) | Placebo (n = 40) |         |
| Age, y                                    | 30.0 ± 4.9                 | 30.2 ± 4.7                 | 30.7 ± 5.8       | NS      |
| Gestational age at enrollment, wks        | 16.1 ± 2.6                 | 16.8 ± 2.3                 | 16.2 ± 2.4       | NS      |
| Gravidity                                 | 2.4 ± 1.3                  | 2.5 ± 1.2                  | 2.6 ± 2.06       | NS      |
| Race/ethnicity                            |                            |                            |                  | NS      |
| White                                     | 33 (82.5)                  | 28 (75.7)                  | 34 (85.0)        |         |
| African American                          | 4 (10.0)                   | 5 (13.5)                   | 1 (2.5)          |         |
| Hispanic/Latina                           | 1 (2.5)                    | 3 (8.1)                    | 2 (5.0)          |         |
| Asian                                     | 1 (3)                      | 1 (3)                      | 1 (2)            |         |
| American Indian or Alaska Native          | 0 (0)                      | 0 (0)                      | 1 (2)            |         |
| Native Hawaiian or other Pacific Islander | 1 (2.5)                    | 0 (0)                      | 0 (0)            |         |
| History of allergy (self-reported)        | 8 (21)                     | 9 (24)                     | 9 (22)           | NS      |

Data are expressed as mean ± SD or frequency (%).

DHA, docosahexaenoic acid-rich fish oil group; EPA, eicosapentaenoic acid-rich fish oil group; NS, not significant; SD, standard deviation.

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a maternal diet relatively deficient in omega-3 fatty acids (the Western diet) and cesarean delivery.<sup>6,7</sup> One mechanism whereby developmental programming for allergic diseases may take place is through an imbalance of the T helper cell 1 and 2 (Th1/Th2) pathways during fetal life.<sup>8-10</sup> A plausible mechanism that explains how omega-3 fatty acids alter the T helper balance is through the suppression of interleukin 13 (IL-13) cytokine production, which induces immunoglobulin E (Ig E) synthesis in B cells as well as Th2 differentiation in T cells.<sup>11</sup> Chemokines have a crucial role in establishing the Th1/Th2 balance and are considered useful markers of immunity. Higher levels of Th2-associated chemokines as well as higher ratios of Th2- to Th1-associated chemokines in umbilical cord blood have been reported to precede allergy development.<sup>12-14</sup>

Recent research has focused on prenatal dietary supplements as potential primary preventive interventions that might decrease risk for allergic diseases. A recent metaanalysis of trials of prenatal supplementation suggested that prenatal

supplementation with the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) might reduce risk for asthma and positive skin prick test to egg.<sup>15</sup> Likewise, a large randomized controlled trial (n = 706) of prenatal DHA supplementation with 800 mg daily found that prenatal supplementation significantly reduced eczema and egg sensitization in the first year of life, although there was no overall effect on Ig E-associated allergic diseases.<sup>16</sup> One potential mechanism whereby maternal omega-3 fatty acid supplementation may induce these protective effects in the immune system is by altered expression of umbilical cord chemokines. However, no previous studies have evaluated whether dietary supplementation with omega 3-fatty acids during pregnancy has an impact on the Th1/Th2 balance of circulating chemokines at birth.

We performed this study to evaluate whether Th2- to Th1-associated chemokine ratios (macrophage-derived chemokine [MDC]/interferon-inducible protein-10 [IP10]) and (thymus and activation-regulated chemokine [TARC/IP10]) are de-

creased in neonates born to mothers who received prenatal supplementation with EPA-rich fish oil and DHA-rich fish oil compared with those who received soy oil placebo. We likewise sought to evaluate whether levels of Th2-associated chemokines TARC (CCL17), eotaxin CCL11, and MDC (CCL22) are decreased in neonates born to supplemented mothers. Our secondary aims were to examine the effect of mode of delivery on these same chemokine ratios and to evaluate the effect of supplementation on Th2 and Th1-associated chemokines and their ratios in maternal blood.

## MATERIALS AND METHODS

This research study was a secondary analysis of the Mothers, Omega-3 and Mental Health Study. The parent study was a 3-arm, prospective, double blind, placebo-controlled, randomized controlled trial designed to assess whether omega-3 fatty acid supplementation during pregnancy may prevent antenatal and postpartum depressive symptoms among pregnant women selected based on risk for depression. Our analysis used stored samples collected from the parent study. Details of the overall study design have been published previously.<sup>17</sup>

### Study population

In the parent study, we enrolled 126 pregnant women from prenatal clinics at the University of Michigan Hospital and Health Centers and St. Joseph's Mercy Hospital/Integrated Health Associates and followed them prospectively through pregnancy and up to 6 weeks postpartum. To qualify for the study, women needed to be at risk for depression, based on (1) a history of major depressive disorder, (2) a history of postpartum depression, or (3) an Edinburgh Postnatal Depression Score (EPDS) score between 9 and 19. Participants were enrolled between 12-20 weeks' gestation and were randomized to 1 of 3 groups: (a) EPA-rich fish oil supplement (1060 mg EPA plus 274 mg DHA; contained an approximate 4:1 ratio of EPA to DHA) (intervention 1); (b) DHA-rich fish oil supplement (900 mg DHA plus 180 mg EPA; contained an approximate of 4:1 ratio of DHA to EPA) (intervention 2); and

(c) a placebo (control arm; contained 98% soybean oil and 1% each lemon and fish oil). The aims and primary outcomes of the study have been reported elsewhere.<sup>17</sup>

The institutional review boards of the University of Michigan Hospital (Ann Arbor, MI) and St. Joseph Mercy Hospital (Ypsilanti, MI) approved the study and written informed consent was obtained from all women.

Baseline clinical and demographic characteristics were collected at enrollment. Maternal allergy history was abstracted by chart review. Maternal allergy was defined by any history of asthma or wheezing, eczema, skin allergy or atopic dermatitis, and allergic rhinitis. Maternal family history of allergy was also abstracted.

Chemokine analysis was performed on maternal venous blood samples drawn at enrollment between 12 and 20 weeks' gestation, ( $n = 117$ ), and after supplementation at 34–36 weeks' gestation ( $n = 112$ ). Participants were allowed to eat a low fat breakfast on the morning of these study visits; blood was drawn after a 4-hour fast. Umbilical cord blood was collected at delivery using aseptic technique from 98 neonates whose mothers participated in the trial. Blood samples were processed within 12 hours and aliquots of plasma were frozen at  $-70^{\circ}\text{C}$  temperatures under argon until analysis. Blood sample analyses from the parent study were performed for baseline, postsupplementation, and cord blood levels of omega-3 fatty acids.

### Chemokine analysis

We performed a secondary analysis of stored samples for the chemokines of interest in the following manner. The chemokines TARC (CCL17) and MDC (CCL22) were measured using commercially available Multiplex immunoassays kits (pg/mL; Invitrogen Corporation, Carlsbad, CA; detection threshold 23.46 and 68.3 pg/mL, respectively). Determination of eotaxin (CCL 11) and IP-10 (CXCL 10) were estimated using enzyme-linked immunoabsorbent assay (ELISA) kits (pg/mL; R&D systems, Minneapolis, MN; detection thresholds 19.2 and 3.84 pg/mL respectively) according to the manufacture's protocols.

TABLE 2

Birth outcomes among neonates with cord blood specimens analyzed in this study ( $n = 98$ )

| Variable                     | Treatment arm                  |                                |                      | P value |
|------------------------------|--------------------------------|--------------------------------|----------------------|---------|
|                              | EPA-rich fish oil ( $n = 32$ ) | DHA-rich fish oil ( $n = 32$ ) | Placebo ( $n = 34$ ) |         |
| Gestational age at delivery  | $39.2 \pm 1.3$                 | $40.1 \pm 1.4$                 | $39.1 \pm 1.5$       | $< .01$ |
| Birthweight, gm              | $3480 \pm 536$                 | $3749 \pm 469$                 | $3289 \pm 569$       | $< .01$ |
| Mode of delivery             |                                |                                |                      | NS      |
| Cesarean section             | 8 (25.0)                       | 11 (34.4)                      | 11 (32.4)            |         |
| Spontaneous vaginal delivery | 23 (71.9)                      | 21 (65.6)                      | 22 (64.7)            |         |
| Operative vaginal            | 1 (3.1)                        | 0 (0)                          | 1 (2.9)              |         |

Data are expressed as mean  $\pm$  SD or frequency (%).

DHA, docosahexaenoic acid-rich fish oil group; EPA, eicosapentaenoic acid-rich fish oil group; NS, not significant; SD, standard deviation.

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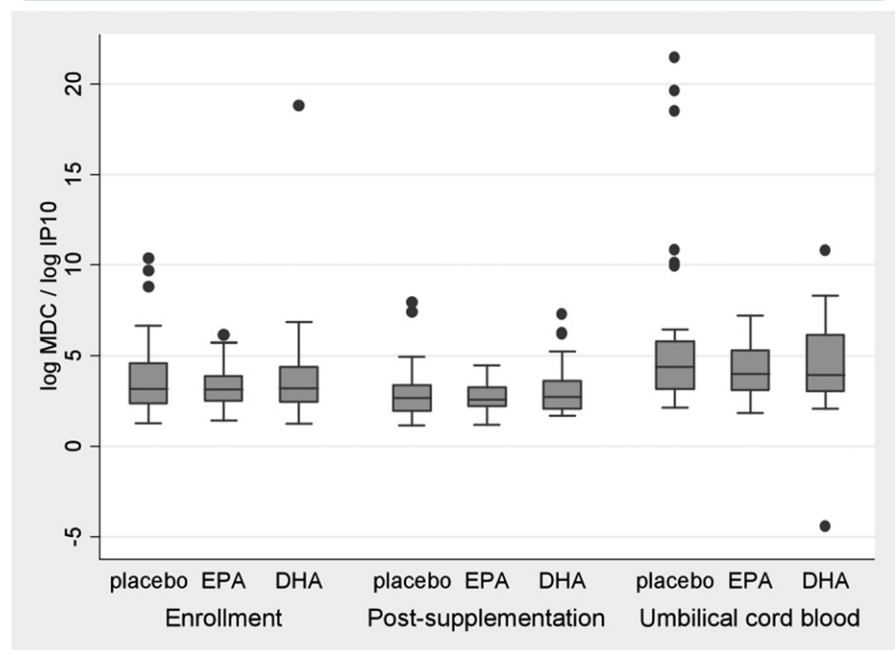
### Statistical analysis

Descriptive statistics were computed according to treatment arm, and appropriate transformations were made (eg, log transformation) for variables not follow-

ing a normal distribution. For comparison of continuous variables between groups within each study visit, analyses of variance (ANOVA) or 2-sample  $t$  tests for independent samples were used; for

FIGURE 1

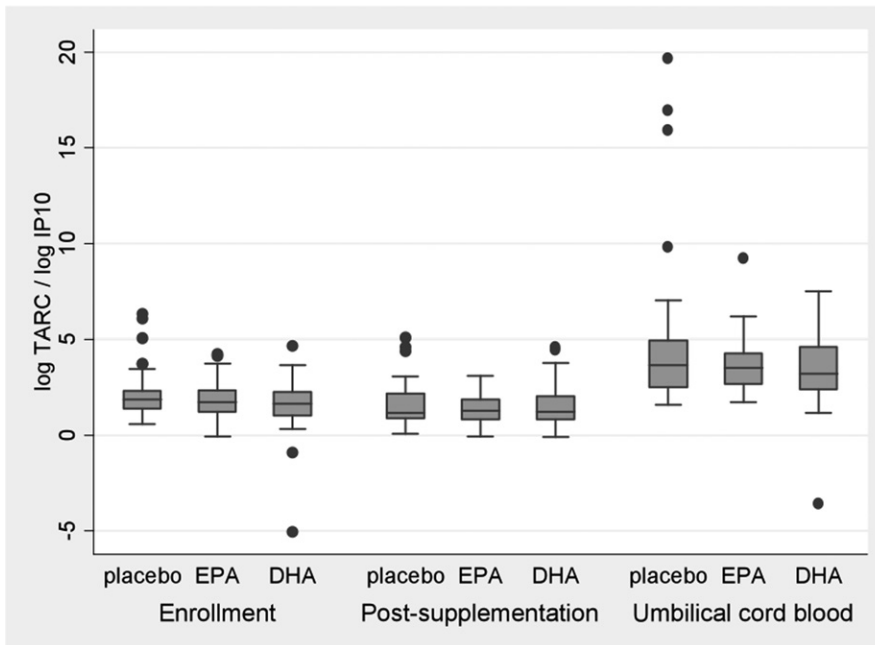
Ratios MDC/IP10 chemokines



This figure presents the unadjusted macrophage derived chemokine (MDC)/interferon inducible protein 10 (IP 10) ratios according to treatment group, ie, eicosapentaenoic acid-rich fish oil (EPA), docosahexaenoic acid-rich fish oil (DHA), or placebo. Ratios are presented from maternal plasma before and after supplementation and from umbilical cord blood.

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**FIGURE 2**  
**Ratios of TARC/IP10 chemokines**



This figure presents the unadjusted thymus and activation-regulated chemokine (TARC)/interferon inducible protein 10 (IP 10) ratios according to treatment group, ie, eicosapentaenoic acid-rich fish oil (EPA), docosahexaenoic acid-rich fish oil (DHA), or placebo. Ratios are presented from maternal plasma before and after supplementation and from umbilical cord blood.

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categorical variables Fisher exact test was used. Intention-to-treat analysis was performed. Multiple linear regression was used to examine the association between treatment arm and chemokine levels, although adjusting for covariates of a priori judgment to be relevant, including gestational age, birthweight, and mode of delivery. Gestational age (GA) of delivery was considered a potential confounder because pregnancy appears to have 3 distinct immunologic phases characterized by distinct biologic processes. There is a unique inflammatory or antiinflammatory environment characterizing each stage of pregnancy. It has been described that first and third trimesters are proinflammatory (Th1), whereas the second trimester represents an antiinflammatory phase also known as Th2 environment.<sup>18</sup> Mode of delivery and birthweight were also included as covariates, as both have been reported to impact immune function.<sup>19,20</sup> A probability level of  $< .05$  was considered to be statistically significant. Statistical analy-

ses were performed using Stata v12 (StataCorp, College Station, TX).

### Sample size considerations

This work is a secondary analysis of a randomized controlled trial of 126 subjects that was designed to detect a 50% reduction in depression score between the intervention and control groups. As such, it was not designed to detect possible differences in allergic diseases. Consequently, our evaluation of chemokines and allergic symptoms was exploratory.

## RESULTS

### Demographics data

Of the 126 women who enrolled in the parent study, 117 completed the trial and had available specimens for this ancillary study. The baseline characteristics of enrolled the participants are detailed in this analysis are detailed in Table 1. No differences in maternal age, ethnicity, and mode of delivery were encountered among the 3 groups. Prenatal supplementation with DHA was associated

with statistically significant increase in GA at delivery with a 1-week prolongation of pregnancy when compared with the EPA and placebo groups (Table 2); birthweight was also significantly higher in the DHA group. There were no differences in baseline maternal DHA and EPA levels or in the proportion of participants who reported a personal history of allergic diseases.

### Maternal chemokines and chemokine ratios

No differences in the levels of chemokines (IP-10, MDC, TARC, and eotaxin) were detected at baseline or after the intervention. There was no significant difference in the chemokine levels between the placebo, EPA, and DHA-treated groups.

There were no differences detected in the unadjusted mean maternal chemokine ratios MDC/IP10 and TARC/IP10 between the DHA, EPA and placebo groups at each visit (Figures 1 and 2).

### Umbilical cord plasma chemokines and chemokine ratios

Chemokines were assayed from the cord blood of 98 infants. We found no differences in cord plasma IP-10, MDC, TARC and eotaxin between the treatment groups. Ratios of log MDC/log IP10 were lower in the EPA ( $4.3 \pm 1.5$ ) and DHA ( $4.5 \pm 2.7$ ) arms compared with placebo ( $5.9 \pm 4.9$ ), a trend that bordered significance for EPA vs placebo ( $P = .08$ ). Similarly, ratios of log TARC/log IP10 were lower in the EPA ( $3.7 \pm 1.6$ ) and DHA ( $3.4 \pm 2.1$ ) groups compared with placebo ( $4.9 \pm 4.4$ ), bordering on significance for DHA vs placebo ( $P = .08$ ).

After adjusting for gestational age at delivery, birthweight, and mode of delivery, both EPA and DHA arms were associated with significant reductions in ratios of log MDC/log IP-10 compared with placebo (EPA:  $\beta = -1.8$ ; 95% confidence interval [CI],  $-3.6$  to  $-0.05$ ;  $P = .04$ ; DHA:  $-2.0$ ; 95% CI,  $-3.9$  to  $-0.07$ ;  $P = .04$ ). Further, cesarean delivery was independently associated with increased log MDC/log IP-10 compared with spontaneous vaginal delivery, in the multivariable model ( $\beta = 1.6$ ; 95% CI,  $0.01$ – $3.3$ ;  $P = .049$ ).

TABLE 3

**Ratios of Th2 to Th1 associated chemokines in umbilical cord blood after adjusting for gestational age of delivery, birthweight, and mode of delivery**

| Th2/Th1 associated chemokines ratios | Intervention | $\beta$ | 95% CI        | P value |
|--------------------------------------|--------------|---------|---------------|---------|
| log MDC/log IP10                     | EPA          | −1.8    | −3.6 to −0.05 | .04     |
|                                      | DHA          | −2.0    | −3.9 to −0.07 | .04     |
|                                      | Cesarean     | 1.6     | 0.01–3.3      | .04     |
| log TARC/log IP10                    | EPA          | −1.5    | −3.0 to −0.06 | .06     |
|                                      | DHA          | −2.2    | −3.8 to −0.52 | .01     |
|                                      | Cesarean     | 1.5     | 0.05–2.9      | .04     |

Data expressed as mean  $\pm$  SD.

CI, confidence interval; DHA, docosahexaenoic acid-rich fish oil group; EPA, eicosapentaenoic acid-rich fish oil group; IP10 (CXCL10), interferon-inducible protein-10; MDC (CCL22), macrophage-derived chemokine; NS, not significant; SD, standard deviation; TARC (CCL17), thymus and activation-regulated chemokine; Th1, T helper 1; Th2, T helper 2.

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Similar associations were found for TARC/IP-10 ratios in umbilical cord blood. After adjusting for GA at delivery, birthweight, and mode of delivery, the logTARC/logIP-10 ratios in the DHA group were significantly lower than placebo ( $-2.2$ ; 95% CI,  $-3.8$  to  $-0.52$ ;  $P = .01$ ) and trended toward significance for EPA ( $-1.5$ ; 95% CI,  $-3.0$  to  $0.06$ ;  $P = .06$ ). Again, cesarean delivery compared with spontaneous vaginal delivery was significantly associated with higher ratios ( $1.5$ ; 95% CI,  $0.05$  to  $2.9$ ;  $P = .042$ ) (Table 3).

### Umbilical cord levels of DHA and EPA

In infants born to mothers who had received DHA-rich fish oil, umbilical cord serum concentrations of DHA (expressed as percent of total serum fatty acids) were significantly higher ( $7.4 \pm 2.8$ ) compared with those from infants born to mothers who received EPA-rich fish oil ( $5.9 \pm 2.0$ ) and placebo ( $5.2 \pm 2.1$  arms  $P = .007$ ). Umbilical cord serum concentrations of EPA were higher in the EPA-rich fish oil group ( $0.61 \pm 0.5$ ) than in the DHA ( $0.56 \pm 0.4$ ) and placebo ( $0.55 \pm 0.5$ ) arms, though these differences did not reach statistical significance.

### COMMENT

The principal finding of this study was that prenatal EPA-and DHA-rich fish oil supplementation modulates fetal the Th2/Th1 balance in cord blood. Similarly, cesarean delivery also has a significant effect on Th2/Th1 ratios at birth.

These findings may help to explain the results of prior epidemiologic studies that found that maternal diet and mode of delivery may significantly increase risk for allergic disease.<sup>6–7</sup>

Conversely, our study found no significant effect of supplementation on maternal chemokine profiles. This finding is consistent with the results of a recent systematic review of the effect of dietary omega-3 fatty acid supplementation on inflammatory biomarkers including chemokines CCL2, CCL3, CCL5, and CCL11.<sup>21</sup> The authors found that supplementation has no effect on these markers in healthy volunteers.<sup>21</sup>

A strength of this study was its randomized, double blind study design, as well as its analysis by intent to treat. On the basis of the randomized design, there were no significant differences between subjects in the 3 groups in past history of allergic diseases. The chemokine outcome measure, although a proxy for risk for allergic disease, is also free of risk for recall bias.

A weakness of our study is that it is a secondary analysis and our subjects were not selected based on predisposition to allergic disease. However, our subjects were selected based on predisposition to perinatal depressive symptoms, which other investigators have shown to be associated with aberrant activation of the inflammatory response.<sup>22</sup> In nonpregnant individuals, Grass-Oliveri et al<sup>23</sup> have shown that major depressive disorder

and suicidal ideation are associated with increased levels of the TH2-associated chemokine, eotaxin. Finally, Mattes et al<sup>24</sup> have shown that mild to moderate maternal depressive symptoms in mid-pregnancy (BDI >10) are associated with a generalized activation of the fetal (cord blood) immune response. However, whether maternal depressive symptoms predispose the offspring to allergic diseases is currently unknown.

At present the mechanism whereby maternal omega-3 fatty acid supplementation modulates the fetal immune response is not known. One potential explanation of how omega-3 fatty acids may exert the observed effect is through competitive inhibition with omega-6 fatty acids in the formation of eicosanoids.<sup>25</sup> In particular, the omega-6 fatty acid, arachidonic acid, is the metabolic precursor of prostaglandin E2, an eicosanoid that reduces production of Th1-associated cytokines and increases production of Th2-associated cytokines.<sup>25</sup> By contrast, EPA and DHA are the metabolic precursors of antiinflammatory eicosanoids.<sup>25</sup> EPA and DHA are also the metabolic precursors of resolvins and protectins, specialized proresolving lipid mediators that have been demonstrated to reduce proinflammatory cytokines in animal models of allergic airway disease.<sup>25</sup> However, the effect of resolvins and protectins on Th2- and Th1-associated chemokines is unknown at present.

No data have been reported on influence of mode of delivery on the chemokines ratios. Cytokines and chemokines, in particular those with proinflammatory properties (Th1), play an important role during labor and full or preterm delivery that may alter the immunity of the fetus/neonate through regulation of many biologic functions. The mode of delivery has been thought to be crucial for establishing the infant's intestinal microflora. Cesarean delivery may alter the immunity of the fetus/neonate because of lack of intestinal flora leading to sustained Th2 responsiveness supporting the B cell Ig E production.<sup>26, 27</sup>

Because our study does not have data regarding clinical outcomes of allergic diseases in children born to mothers who participated in our study, more research is necessary before prenatal omega-3 fatty acid supplementation can be recommended for primary prevention of allergic diseases. More research is also needed to explore the molecular mechanisms that underlie the observed modulation of the fetal immune response. ■

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