Dietary Counseling to Improve Fat Quality during Pregnancy Alters Maternal Fat Intake and Infant Essential Fatty Acid Status\textsuperscript{1–4}

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Abstract

To explore the effect of maternal dietary intervention on infant essential fatty acid (FA) status, we conducted a prospective, single-blind, randomized nutrition intervention study. At the first trimester of pregnancy, 90 women from families with a history of allergy were randomized either to receive intensive dietary counseling to modify dietary intake according to current recommendations or as controls. Infants' cord and 1-mo isolated serum phospholipid FA were identified and quantified by GC. Detectable levels of eicosatrienoic acid (ETA, 20:3\textit{n}-9) were taken as a biochemical marker for essential FA deficiency, and the DHA sufficiency index [22:6\textit{n}-3]:22:5\textit{n}-6] and the DHA deficiency index [22:5\textit{n}-6]:22:4\textit{n}-6] were taken as markers for DHA [22:6\textit{n}-3] status. The concentration of ETA was lower in cord blood in the intervention (I) group [median 0.64 (IQR 0.40–0.78) mg/L; 2.09 (1.31–2.54) \mu mol/L] than in the control (C) group [0.92 (0.54–1.20) mg/L; 3.00 (1.76–3.92) \mu mol/L] (\textit{P} = 0.048). The proportion of ETA in total FA in the I group [0.73\% (0.48–0.85\%)] was lower than in the C group [0.93\% (0.78–1.22\%)] (\textit{P} = 0.003). A higher DHA sufficiency index and lower DHA deficiency index were detected in cord blood in the I group than in the C group, although the groups did not differ in the DHA concentration or proportion of the total FA. There were no differences among groups at 1 mo for any of the variables measured. Our findings suggest a better supply of essential FA, particularly important during the period of rapid development, in infants whose mothers received dietary counseling. The results thus highlight the importance of maternal diet for child health, calling for dietary counseling for pregnant women in primary health care. J. Nutr. 141: 1281–1285, 2011.


Introduction

An adequate supply of essential fatty acids (EFA) and their subsequent metabolic derivatives is critical for infant growth and development. Research suggests that DHA [22:6\textit{n}-3] particularly and arachidonic acid [20:4\textit{n}-6] affect visual and cognitive functions (1), and the long-chain PUFA in general may exert permanent effects on the immature immune system (2). Their importance may culminate during the last trimester of pregnancy and the first months of life, as exemplified by the accumulation of DHA in brain and retina (3) during the period when the mother is the sole source of nutrients for the fetus via the placenta and the infant via breast milk (4). The maternal diet during pregnancy and breastfeeding would thus be of utmost importance.

In a typical Western diet, the average intakes of long-chain PUFA are lower and those of SFA higher than recommended (5). An unbalanced diet may be particularly accentuated in families with a history of allergy, where elimination diets have been practiced for decades in an attempt to reduce the risk of allergy in the child. Of note, such diets are frequently devoid of fish products and eggs. From an immunological point of view, this approach has been challenged, because oral tolerance requires exposure to the respective antigens (6), whereas the nutritional risks of elimination diets have been well characterized.

We hypothesized that a balanced diet can also be achieved in families with a history of allergy. With this in mind, we arranged intensive dietary counseling, with special attention to the quality and quantity of dietary fat, for pregnant mothers from the first trimester until exclusive breastfeeding ended. In the present study, we focused on possible means of modifying infant EFA supply by maternal dietary counseling without the previously adopted approach using supplements.
Methods

Study population. In an ongoing prospective mother and infant nutrition and probiotic study (7), a total of 236 pregnant women were recruited during their first visit to maternal welfare clinics from April 2002 to November 2004 in the city of Turku and neighboring areas in southwest Finland. Women from families with a history of allergy (mother, father, or sibling of the unborn child with allergic disease) with no other chronic condition than allergy were eligible if they were at $<17$ wk of gestation. Upon enrolment, a statistician not involved in the recruitment or study visits randomly assigned the women to receive dietary counseling with or without probiotics or to serve as controls according to computer-generated block randomization of 6 women, as described in detail elsewhere (7), and the women received a personal study number. For the purpose of the present study, 45 mother-child pairs from the dietary counseling [intervention (I)] group without probiotics and 45 mother-child pairs from the control (C) group, i.e., mothers either receiving dietary counseling or not, were included (Supplemental Fig. 1). The mothers were taken to the study in consecutive order of recruitment according to a predefined number sequence to avoid selection bias. The number of participants was based on previous fatty acid (FA) supplementation studies (8,9) and practical resources. This sample of 90 women is representative of the whole study population with respect to baseline characteristics, e.g., BMI and allergy status. There were no differences between included and excluded women for any of the variables in Table 1 (data not shown). Written informed consent was obtained from the mothers, and the Ethical Committee of the Hospital District of Southwest Finland approved the study. The study complies with the Declaration of Helsinki as revised in 2000.

Study design. Information on whether the participant would receive dietary counseling was enclosed in sealed envelopes to be opened by the study nurse and nutritionist at the first study visit in the presence of each study participant in order of recruitment. The first visit served as baseline and background information was collected by interview. The study visits took place 3 times during pregnancy at each trimester and at 1-mo postpartum. All women received standard dietary counseling in municipal well-women clinics, but beyond this, the I groups received detailed dietary counseling by a nutritionist during each study visit, each session lasting for $\sim30$ min. Maternal dietary intake was determined by 3-d food diaries, including 1 weekend day, collected at each visit to assess the baseline level and the impact of nutrition counseling on food and nutrient intake. The counseling aimed at a diet complying with that recommended for pregnant women at the time of initiation of the study (10), as previously described in detail (11). Briefly, special attention was paid to the quality and quantity of dietary fat, with the intention of increasing the intake of unsaturated FA and reducing that of SFA. Practical dietary advice, which was adjusted to the women’s current dietary habits and food diary analysis, was given. The recommended amounts of foods were planned to result in MUFA contributing 10–15% of total daily energy intake (E%), PUFAs contributing 5–10 E%, and SFA contributing $\leq10$ E%. Total intake of fat was aimed for 30 E %, carbohydrates 55–60 E%, and protein 10–15 E%. To enhance compliance with the recommended diet, readily available food products with favorable fat compositions (e.g., low-erucic acid rapeseed oil-based spreads and salad dressings) were provided for use at home. The products were provided until exclusive breastfeeding ended, a maximum of 6 mo, whereas dietary counseling continued. Daily intakes of energy and nutrients were calculated using a computerized program (Micro-Nutrica version 2.5; Research Centre of the Social Insurance Institution).

Infant EFA status was defined as follows. Detectable levels of eicosatrienoic acid [ETA, 20:3(n-9)] were taken as a marker for EFA deficiency (12), because ETA is synthesized by de novo lipogenesis when (n-3) and (n-6) FA stores are depleted. The ETA deficiency index, the Holman index, was calculated as the triene:diene ratio [20:3 (n-9):20:4(n-6)] with a cutoff point of 0.2, with greater values being taken to indicate ETA deficiency (13). Markers for functional DHA status, the DHA sufficiency index [22:6(n-3):22:5(n-6)] and the DHA deficiency index [22:5(n-6):22:4(n-6)], as well as a DHA deficiency marker, docosapentaenoic acid [22:5(n-6)] (14), were calculated.

FA analysis of serum lipids. FA were analyzed from infants’ cord blood (a mixture of venous and arterial blood) and 1-mo blood samples, and EFA deficiency indexes were calculated. At 1 mo of age, blood was drawn from the antecubital vein after topical lidocain anesthesia by a trained nursing staff. Cord blood samples were available from 31 neonates in the I group and 28 in the C group, and blood samples from 33 infants in the I and 31 in the C group at 1 mo of age. At least 1 sample was available from 81 infants (42 in the I and 39 in the C group) and both samples were available from 42 infants (22 in the I and 20 in the C group). The FA concentration and composition (proportion of total FA) of serum phospholipids were analyzed; the researchers were not aware of the randomization order.

The blood samples for FA analysis (5 mL) were collected into plain serum tubes and centrifuged after 30–120 min of cooling at 2500 $\times g$ for 10 min at room temperature. The serum samples were first stored at $-20^\circ C$ for 1 wk, then at $-70^\circ C$ until analyzed. Total lipids in the samples were extracted with HPLC-grade chloroform:methanol (2:1, v:v) (15). Phospholipids were separated by solid phase extraction with silica Sep-Pak Vac 1cc columns (Waters) (16). FAME were identified based on FAME reference mixtures and literature data. Quantification of FA in the serum phospholipid fraction was based on internal standard dinonadecanoylphosphatidylcholine (Sigma-Aldrich), which was added to the serum sample prior to analysis. Simultaneous addition of trieneicosanoic (Larodan) and heptadecanoylcholesterol (Sigma-Aldrich) was used to ensure adequate separation of phospholipids from TG and cholesteryl esters. The adequate separation efficiency of the FAME of the used GC system was confirmed by analyzing FAME mixture 68B from Nu-Chek-Prep several times during the study period.

FAME in the solid phase extraction-separated serum phospholipids were prepared with BF$_3$ in methanol procedure (17). FAME were analyzed with an Agilent HP 6890-series gas chromatograph (Agilent Technologies) equipped with an autosampler, a split/splitless injector, a DB-23 capillary column coated with (50%-Cyanopropyl)-methylpolysiloxane phase (60-m $\times$ 0.25-mm i.d. with a 0.25-$\mu$m film, Agilent J&W Scientific), a flame ionization detector, and ChemStation software for data processing. The oven temperature program was the following: from 50°C (hold 1 min) to 130°C with 20°C/min, to 170°C (hold 5 min) with 6.5°C/min, to 215°C (hold 5 min) with 4°C/min, and to 230°C with 30°C/min (hold 40 min). An aliquot of each sample (1 mL) was injected in splitless mode keeping the split valve closed for 1 min after injection. The temperature of the injector was 270°C and that of the detector 280°C. Helium (purity 99.999%) was used as carrier gas in constant pressure (207 kPa) mode.

Statistical analysis. The distributions of variables were checked using tests for normality (Shapiro-Wilk) and graphical plots. In variables describing the characteristics of the women and infants, departures from

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics of women and their infants in the dietary I and C groups$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>30.8 $\pm$ 5.4</td>
</tr>
<tr>
<td>College or university degree</td>
<td>31 (68.9)</td>
</tr>
<tr>
<td>Primigravida</td>
<td>21 (46.7)</td>
</tr>
<tr>
<td>Allergic disease</td>
<td>36 (80.0)</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m$^2$</td>
<td>24.2 $\pm$ 4.2</td>
</tr>
<tr>
<td>Weight gain during pregnancy, kg</td>
<td>15.2 $\pm$ 5.0</td>
</tr>
<tr>
<td>Infant characteristics</td>
<td></td>
</tr>
<tr>
<td>Birth at weeks of gestation</td>
<td>39.7 $\pm$ 2.1</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3610 $\pm$ 480</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>513 $\pm$ 1.7</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>35.2 $\pm$ 1.3</td>
</tr>
<tr>
<td>Exclusively breastfed at 1 mo</td>
<td>29 (74.4)</td>
</tr>
</tbody>
</table>

$^1$ Values are mean $\pm$ SD, n = 45 or n (%).
normal distribution were not marked and were thus analyzed using parametric methods. However, FA variables were skewed and for consistency all were analyzed using nonparametric methods.

Continuous baseline variables were presented as means ± SD and FA variables as medians and IQR. Two-group comparisons were made using the independent-samples t test or the Mann-Whitney U test, respectively. The chi-squared test was used for dichotomous variables. For internal validity, associations between ETA and other FA markers were assessed by Spearman’s rank correlation test. Intakes of energy and energy-yielding nutrients at the 3rd trimester and at 1 mo postpartum were baseline-adjusted and 2-group comparisons were performed using ANCOVA for repeated measures. The interactions between time and intervention were nonsignificant (P > 0.10) for all nutrient variables and thus any post hoc tests were not conducted. The assumptions of the tests were confirmed using the Shapiro-Wilk test for normality and Levene’s test for homogeneity of variances. P < 0.05 was considered significant for a 2-sided test. SPSS version 16.0 was used for all statistical analyses.

Results

Clinical characteristics. The pregnancies were uncomplicated and the children were born healthy and full term except for 3 preterm deliveries (range 30.3–33.6 wk). The infants’ mean (range) gestational age was 39.9 (30.3–42.1) wk, length was 51.0 (44.0–55.0) cm, and weight was 3600 (1630–4660) g at birth. The study groups did not differ in maternal or infant characteristics (Table 1).

Nutrient intake during pregnancy. At baseline, dietary intakes of nutrients were similar between the groups (data not shown). Dietary counseling resulted in higher intakes of PUFA (P < 0.001) and MUFA (P = 0.029) in the I group than in the C group and lower intakes of SFA in the I group than in the C group (P < 0.001) as a proportion of total daily energy intake (Table 2). Total energy intake (kJ) and intakes of total fat, protein, and carbohydrate as a percent of energy did not differ between the dietary counseling and the C group (Table 2).

Impact of intervention on infant EFA status markers. The proportion of ETA in total serum phospholipid FA was lower in cord blood in the I group than in the C group (P < 0.05) (Table 3). This result was supported by the lower concentration of ETA in the I group [median 0.64 (IQR 0.40–0.78) mg/L; 2.09 (1.31–2.54) μmol/L] than in the C group [0.92 (0.54–1.20) mg/L; 3.00 (1.76–3.92) μmol/L] (P = 0.048).

When evaluating the ETA status indexes in cord blood (Table 2), the Holman index was lower in the I group than in the C group (P < 0.05). The intervention was also reflected in the cord blood markers of functional DHA status: a higher DHA sufficiency index and lower DHA deficiency index as well as lower amounts of docosapentaenoic acid [22:5(n-6)] were detected in the I group than in the C group (P < 0.05). The differences between groups were also significant after exclusion of the preterm infants from the analysis (data not shown). However, the groups did not significantly differ in 22:6(n-3) (DHA), 20:4(n-6) (arachidonic acid), or 22:4(n-6) in the cord blood (used as components of the above-mentioned indexes). There were no differences among groups at 1 mo for any of the variables measured.

ETA correlated positively with docosapentaenoic acid (r = 0.57; P < 0.001) and the DHA deficiency index (r = 0.58; P < 0.001) and negatively with the DHA sufficiency index (r = −0.59; P < 0.001).

Discussion

In this population of metabolically healthy mothers and infants from families with a history of allergy, we showed that dietary counseling resulted in a significant improvement in the mother’s diet and was reflected as better infant ETA status compared with infants of mothers receiving standard health and diet advice in well-women clinics. It is of note that the counseling, which aimed to increase intakes of unsaturated FA using food products with favorable fat composition, resulted in an elevated functional DHA status in infants in the I group. Our observations confirm that the typical weaknesses of the Western diet (5), a high proportion of SFA and a low proportion of long-chain PUFA, are modifiable. Improved maternal dietary fat quality was shown here to be transferred to the infants, with the potential for a long-term impact on health (1,2), constituting one further justification for dietary counseling in this population.
Overlooking the importance of a wholesome diet (18), previous studies have focused mainly on selectively supplementing maternal diets with capsules containing fish oil rich in EPA and DHA and have demonstrated an enhanced neonatal DHA status (8,9,19,20). During this critical and sensitive period of development, safety data are called for, because the experience from some earlier studies with selective supplementation has yielded controversial results (21). It was thus encouraging to note that dietary counseling, without the use of extra supplements, achieved beneficial results in modifying infant FA status.

**Limitations of study.** We observed a clear difference between the groups in the indexes, but not in DHA. The indexes, however, are thought to be more sensitive and perhaps more important markers of FA status than the mere absolute amounts of the FA (12), because they reflect the relative proportions between the different classes of FA. The study population comprised healthy mothers and their infants representing mostly well-educated families from health clinics, and therefore all Holman index values were below the diagnostic limit for EFA deficiency (0.2) as expected (13). Thus, the effects of dietary modification on EFA status and its clinical impacts remain to be shown, particularly in at-risk populations, e.g. in preterm babies.

Some 80% of the mothers in this study had an allergic disease (asthma, allergic rhinitis or conjunctivitis, or atopic dermatitis), with potential effects on the FA composition in their infants, although not all studies have shown the effect (22). The proportion of allergic mothers in both study groups was the same and unlikely to affect the results obtained here. Due to practical reasons, FA were analyzed from a subsample of the study population, but the number of participants was considered sufficient based on earlier studies (8,9). Selection bias is unlikely to occur, because participants were included in consecutive order of recruitment, which did not affect randomization.

The use of ETA as an index of fetal FA status and its responsiveness to changes in FA supply has been questioned (23,24), although results may have been hampered by the study settings. Supplementation of the long-chain derivatives of EFA, DHA and EPA, has been compared with “placebo” oils, which actually contain the EFA linoleic acid [18:2(n-6)] and α-linolenic acid [18:3(n-3)] (23). Clinical evidence for the use of ETA derive from studies with infants born large for gestational age, when the EFA status index and the DHA sufficiency index were lower and ETA higher (25), and with infants born preterm with low birth weight, when linoleic acid decreased rapidly during fat-free alimentation concomitant with a rise in ETA and an elevation in the Holman index (26). In the present study, in addition to the differences in ETA detected between the groups, positive correlations of ETA with docosapentaenoic acid or the DHA deficiency index and a negative correlation with the DHA sufficiency index suggest the synthesis of ETA when EFA are available in insufficient amounts.

Because blood sampling of small infants is challenging and repeated attempts considered unethical, this was reflected in missing samples; thus, larger and repeated studies would be needed to evaluate the persistence and importance of this finding in the long term. A further constraint is encountered in the analysis of ETA, because its concentrations are particularly low. The elution of ETA with impurities and contaminated separation in GC is possible; this would, however, represent a systematic error. This would result in overestimation of ETA in both groups without altering the difference of ETA concentrations or proportions between the groups.

In conclusion, the results here suggest that dietary counseling during pregnancy and lactation provides a safe, noninvasive, clinically applicable tool for public health approaches to ensure the supply of EFA to the fetus and neonate, promising long-term health benefits with a programming effect (27). To the best of our knowledge, there are to date no other prospective, randomized, controlled dietary intervention studies demonstrating the effects of a change in maternal diet on the infant’s EFA status in a well-nourished population.

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**Literature Cited**


