Fish oil supplemental dose needed to reach 1 g% DHA + EPA in mature milk

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Abstract

Introduction: Erythrocyte (RBC) DHA + EPA is considered optimal at 8 g%. Mothers with lifetime high fish intakes exhibiting this status produce milk with about 1 g% DHA + EPA. We established DHA + EPA supplemental dosages needed to augment RBC DHA + EPA to 8 g% and milk DHA + EPA to 1 g%.

Materials and methods: Pregnant women were randomly allocated to DHA + EPA dosages of: 225 + 90 (n = 9), 450 + 180 (n = 9), 675 + 270 (n = 11) and 900 + 360 (n = 7) mg/day. Samples were collected at 20 and 36 gestational weeks and 4 weeks postpartum.

Results: Linear regression revealed needed dosages rounded at 750 mg/day to reach 8 g% RBC DHA + EPA and 1000 mg/day for 1 g% milk DHA + EPA. RBC DHA + EPA increment depended on baseline values. There was no effect on milk AA, but milk EPA/AA ratio increased.

Conclusion: Women with an RBC DHA + EPA status of 5.5 g% need 750 and 1000 mg DHA + EPA/day to reach 8 g% RBC DHA + EPA at the pregnancy end and 1 g% mature milk DHA + EPA, respectively.

1. Introduction

The health benefits of breastfeeding are widely acknowledged. Long chain polyunsaturated fatty acids (LCPs), notably eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (AA) acids, in milk are important for infant (neuro)development. Meta analyses of randomized controlled trials with prenatal and/or postnatal LCP supplementation are inconclusive [1–5]. Failure to demonstrate the importance of LCP in cognitive and behavioral outcomes may derive from many causes, including, dose, duration and infant heterogeneity (including different baseline status and variety of post weaning foods) [6,7], while nutrient interactions are usually ignored. Small differences may, however, result in subtle effects that are difficult to detect but could nevertheless be relevant [7].

Breast milk DHA content varies from 0.13 to 0.37 g per 100 g fatty acids (g%) in the Netherlands [8] to medians of 0.73 g% (Chole, Tanzania) [9], 0.96 g% (Ukerewe, Tanzania) [10], up to 1.4 g% (Inuit, Canada) [11]. Breast milk AA exhibits less variation, ranging from 0.26 to 0.60 g% in the Netherlands [8] to medians of 0.50 g% (Chole, Tanzania) [9], 0.55 g% (Ukerewe, Tanzania) [10], and 0.60 g% (Inuit, Canada) [11]. The estimated worldwide biological variation of breast milk DHA is among the highest of all fatty acids (68%), while that of AA is among the lowest (28%) [12]. This observation is in line with the dependence of milk DHA on maternal DHA intake and status, and the independence of milk AA on maternal linoleic acid or AA intakes and status [10,13]. Hsieh et al. [14,15], showed that, in neonatal baboons, DHA in most tissues, including the brain, is more sensitive to dietary intake than AA. Taken together, these observations, plead for a sufficient DHA intake to reach an optimal status in both mother and child.

The Institute of Medicine (IOM) did not define adequate intakes (AI) for EPA and DHA for 0–6 months infants [16]. The current view is that during the first months of life, term infants should receive 100 mg DHA/day and 140 mg AA/day, and hence infant formulas should provide at least 0.3 g% DHA [17]. Although it has been argued that DHA should be provided along with similar or higher levels of AA [17–19], the European Food Safety Authority (EFSA) stated that there is no necessity to add AA to infant formula even in the presence of DHA [20]. A recent study with delta-6 desaturase (FADS2) knockout mice showed that, postnatally, both AA and DHA intakes are important for (brain) growth and motor development [21]. Breastfeeding mothers in the Netherlands are advised to achieve a minimum average daily intake of 200 mg DHA, which translates to one portion of (oily) fish per week [22]. The advice is expected to reach 0.3–0.4 g% DHA in milk [13]. Recently, the Global Organization for EPA and DHA Omega-3S (GOED) recommended an intake of 700 mg DHA + EPA/day for pregnant and lactating women [23].

Hibbeln et al. [24] showed that a daily intake of 1 g DHA + EPA from seafood during pregnancy associates with lowest offspring risk of low verbal IQ at 8 years. Their studies [25] also indicated that a milk DHA reaching 1 g% is associated to the lowest risk of postpartum (PP)
depression. Other investigators showed that an RBC DHA + EPA of about 8 g% at adult age associates with lowest risk of cardiovascular disease [26], lowest risk of depression [27] and optimal balance between DHA and AA status [28]. Taken together there is evidence that an RBC DHA + EPA content of 8 g% in adults should be considered optimal. We argue that mothers with adequate DHA + EPA status are likely to produce milk with adequate DHA + EPA contents. Data from our group showed that mothers with lifetime high fish intakes and an RBC DHA status of 8 g% at delivery, give birth to infants with an RBC DHA of 7–8 g%. After 3 months exclusive breastfeeding, maternal RBC DHA was 7–8 g%, while infant RBC DHA had increased to 8 g%. The corresponding breast milk DHA content at 3 months PP was 1 g% [10,29].

In the present study we investigated what DHA + EPA supplemental dose, provided from 20 gestational weeks (GW), augments RBC DHA + EPA to 8 g% and breast milk DHA + EPA to 1 g%. In the FRISO MUM intervention study, conducted earlier by our group, a daily dose of 220 mg DHA increased RBC DHA from a median of 4.2 g% at 16 GW to a median of 5.5 g% at 36 GW [Van Goor, unpublished]. The median milk DHA contents were 0.60 g% and 0.39 g% at 2 and 12 weeks PP, respectively [13]. From their dose-response study, Flock et al. [30] concluded that healthy adults with low RBC DHA + EPA contents, also named omega 3 index, of about 4.3 g% need intakes of 1 g DHA + EPA/ day for 5 months to reach an RBC DHA + EPA of 8 g%. Based on these studies we choose 4 daily supplemental dosages, ranging from 225+90 to 900+360 mg DHA + EPA. In view of nutrient interaction we also supplemented the women with a multivitamin and vitamin D. It has e.g. been shown that the DHA status in rats is dependent on methylation capacity (folate, and vitamins B6 and B12 status) [31,32], while EPA, DHA and vitamin D may interact in serotonin biology [33]. In addition, we investigated whether the DHA + EPA supplements had adverse effects on the AA status.

2. Methods and materials

This is a randomized controlled trial (ZOOG MUM) that was conducted in Groningen, The Netherlands. It is part of the ZOOG (‘Zonder Ontsteking Oud en Gezond’) project. The study was approved by the Ethics Committee of the University Medical Center Groningen (UMCG) (METc number 2014.263) and was registered in The Netherlands National Trial Register (Trial ID NTR4959). All women provided us with written informed consent. The study was in agreement with the Helsinki declaration of 1975, as revised in 2013.

2.1. Subjects, supplements, sample collection, storages and analyses

Forty-three apparently healthy and well-nourished Dutch women in their first trimester of a singleton pregnancy were invited to participate in the study. All expressed their intention to exclusively breastfeed after birth. They were randomly allocated, by use of block randomization, to four groups (Fig. 1). Women with hyperemesis gravidarum, or a vegetarian or vegan diet were excluded. Pregnancy- or neonatal complications and premature delivery were criteria for termination of participation. Table 1 depicts the various supplements, their daily dosages and percentages of the RDA/AI for pregnant and lactating women. Each participant received a multivitamin (Omega Pharma; Rotterdam, The Netherlands) that provided 12–125% of the Dutch RDA/AI for vitamins and minerals for pregnant and lactating women. They also received increasing dosages of DHA-rich fish oil and vitamin D supplements (both from Bonusan; Numansdorp, The Netherlands). The total dosages were: 225 + 90 mg DHA + EPA and 10 µg vitamin D in group A; 450 + 180 mg DHA + EPA and 35 µg vitamin D in group B; 675 + 270 mg DHA + EPA and 60 µg vitamin D in group C; and 900 + 360 mg DHA + EPA and 85 µg vitamin D in group D.

Data on anthropometrics, socioeconomic status and average fish intake were collected via questionnaires at the study beginning and/or end. Maternal EDTA-anticoagulated venous blood samples were taken in the non-fasting state at the study start and at 36 GW and 4 weeks PP. A milk sample was collected at 4 weeks PP. Blood samples were processed to plasma by centrifugation. RBC were isolated as described by Luxwolda et al. [10]. A 200 µL aliquot of the washed 50% hematocrit blood was transferred to a telofn sealable Sovirel tube containing methanol/hydrochloric acid (5:1 v/v), butylated hydroxytoluene (antioxidant) and 50 µg 17:0 (internal standard). These samples were stored at 4 °C until analysis. Breast milk samples from a single completely emptied breast were collected around noon (10:00–14:00) at the day prior to blood sampling. Milk was collected manually or by breast milk pump. Following careful swirling it was divided into two portions. The participants stored the milk samples in their private freezers. They took the samples with them in a Cool transport container for frozen specimens (Sarstedt; mailing containers) on the following day of blood sampling in the UMCG. The milk samples were subsequently stored at −20 °C until analysis. Fatty acids in RBC and milk were measured by capillary gas chromatography with flame ionization detection [34,35]. RBC DHA + EPA and milk RBC DHA + EPA were expressed in g/100 g fatty acids (g%).

2.2. Data analysis and Statistics

The IBM PASW Statistics 22 software was employed. Since not all data were Gaussian distributed we report medians and ranges. Between-group differences were analyzed with the Kruskal Wallis test for continuous data. Chi-square test was used for nominal data. A p value < 0.050 was considered significant. Between-time points differences were analyzed by Wilcoxon Signed Rank Test. A p value < 0.0167 was considered significant (Bonferroni correction for 3 time points). The best fits for the analysis of the dose-response curves were found in this order: cubic (R2 = 0.496), quadratic (R2 = 0.478) and linear regression (R2 = 0.472). For practical purposes and small differences in fit, we choose the linear regression curves to estimate the dosages needed to reach an RBC DHA + EPA of 8 g% and a milk DHA + EPA of 1 g%.

3. Results

Median (range) GW (expressed in weeks + days) at the study entry was 20.6+3 (16.0–21.4). Due to their stay abroad at 20 GW, two women planned their first visit at GW 16 and 18, respectively. They were instructed to start taking the supplements at 20 GW. Of the 43 women randomly assigned to groups A-D, 36 completed the study (Fig. 1). Three discontinued voluntarily, two were excluded before 36 GW (pregnancy bleeding and use of anticoagulants) and two were excluded because of late pregnancy complications (pregnancy induced hypertension, gestational diabetes). All 36 included women reported compliance with the study protocol. Breast milk samples were available from only 33 mothers, since three discontinued breastfeeding prior to 1 month PP. One mother provided a milk sample at 4 weeks PP, but did not report for blood sampling.

3.1. Characteristics of mothers and their infants

Table 2 shows the characteristics of the 36 included mothers and their infants. The median (range) maternal age at the study start was 31 (21–38) years. Their pre-pregnancy BMIs were 24 (18–29 kg/m2). They delivered at term at a median (range) of 41 (37–42) GW. Eighty percent completed college or university and 42% had an annual household income of €50,000 or more. Median (range) fish consumption at the study start was 0.8 (0–2) portions (a portion fish equals 100–150 g fish [36]) per week, of which 0.4 (0–2) consisted of oily fish. Seventy-five percent of the women took a vitamin supplement before the study start, of which 25% contained low dose fish oil (200 mg DHA). Of the 36 infants, 17 were boys and 19 were girls. Their median (range) birth weights were 3790 (2440–5020) g. The milk

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**Table 1**

<table>
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<tr>
<th>Supplement</th>
<th>Dose</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Multivitamin</td>
<td>125%</td>
<td>Vitamin A, C, D, E, B6, B12, folate</td>
</tr>
<tr>
<td>DHA+EPA</td>
<td>450+180 mg</td>
<td>Omega Pharma; Rotterdam, The Netherlands</td>
</tr>
<tr>
<td>Folate</td>
<td>35 µg</td>
<td>For improved DHA status</td>
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**Table 2**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (Range)</th>
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<tr>
<td>Maternal Age</td>
<td>31 (21–38) yrs</td>
</tr>
<tr>
<td>BMI</td>
<td>24 (18–29) kg/m²</td>
</tr>
<tr>
<td>Household Income</td>
<td>€50,000 or more</td>
</tr>
<tr>
<td>Fish Consumption</td>
<td>0.8 (0–2) portions</td>
</tr>
</tbody>
</table>

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E. Stoutjesdijk et al.  
samples were collected at 4.4 (3.5–5.3) weeks PP. There were no significant differences between the characteristics of the various supplemental groups, other than the collection of milk samples, which we consider not to be clinically relevant.

3.2. Dose needed to reach RBC DHA+EPA of 8 g% at 36 weeks GA

Fig. 2 shows the relations between the DHA+EPA dosages and RBC DHA+EPA at the study start, at 36 GW and at 4 weeks PP. Groups A-D did not exhibit differences between RBC DHA+EPA contents at the start ($p=0.267$). The median (range) RBC DHA+EPA content for the whole group at the study start was 5.5 (3.3–8.5) g%. At 36 GW the RBC DHA+EPA medians (ranges) had increased to 6.5 (5.5–8.6) g% for group A, 7.4 (6.2–9.3) g% for group B, 8.7 (8.1–10.4) g% for group C and 9.5 (6.0–11.3) g% for group D ($p < 0.01$ for between group differences). The linear relation between the DHA+EPA dosages and RBC DHA+EPA at 36 GW was $y = 5.8 + 2.9 \times 10^{-3}x$, where $x$ is the DHA+EPA dosage (mg) and $y$ is the RBC DHA+EPA content (g%). Employing this relation we found that the DHA+EPA dose needed to reach the 8 g% RBC DHA+EPA target at 36 GW was 759 mg/day. At 4 weeks PP the medians (ranges) of the RBC DHA+EPA contents were 6.5 (6.0–7.6) g% for group A, 7.4 (6.5–8.9) g% for group B, 8.6 (6.8–9.9) g% for group C and 9.4 (6.7–11.3) g% for group D. Supplemental Table 1 presents the data of RBC DHA+EPA, DHA, EPA and AA, and the EPA/DHA and EPA/AA ratios for groups A-D at 20 GW, 36 GW and 4 weeks PP.

3.3. Dose needed to reach milk DHA+EPA of 1 g% at 4 weeks PP

Fig. 3 panels A-C show, for each of the groups A-D at 4 weeks PP, the dose-response curves for milk DHA+EPA (panel A), DHA (panel B) and EPA (panel C), respectively. Supplemental Table 2 presents the corresponding hard data. The medians (ranges) of milk DHA+EPA at 4 weeks PP were 0.36 (0.27–0.75) g% for group A, 0.81 (0.56–1.06) g% for group B, 1.01 (0.71–1.31) g% for group C, and 1.08 (0.68–1.68) g% for group D. The linear relation between the DHA+EPA dosage and milk DHA+EPA was $y = 0.21 + 8.3 \times 10^{-3}x$, where $x$ is the DHA+EPA dosage (mg) and $y$ is the milk DHA+EPA content (g%). The calculated DHA+EPA dose needed to reach the 1 g% milk DHA+EPA target at 4 weeks PP was 952 mg/day. The milk DHA medians (ranges) at 4 weeks PP (panel B) were 0.29 (0.22–0.60) g% for group A, 0.63 (0.42–0.83) g% for group B, 0.83 (0.55–1.07) g% for group C, and 0.85 (0.57–1.40) g% for group D. The milk EPA medians (ranges) at 4 weeks PP (panel C) were 0.08 (0.04–0.16) g% for group A, 0.19 (0.11–0.23) g% for group B, 0.18 (0.04–0.26) g% for group C, and 0.25 (0.11–0.30) g% for group D.

3.4. Dependence of RBC DHA+EPA increments on baseline status and dose

Fig. 4 shows, for each of the supplemented groups A-D, the relation between the baseline RBC DHA+EPA content (at 20 GW) and the increment of the RBC DHA+EPA content ($\Delta$ DHA+EPA in g%) from 20 to 36 GW. The RBC DHA+EPA increments were found to be dependent on the baseline DHA+EPA status: there were higher RBC DHA+EPA increments at low baseline status. The increments also gradually diminished with dose, suggested the reach of RBC DHA+EPA saturation at high dose. Although all participants reported compliance with the protocol, one woman in group D showed no increment of RBC DHA+EPA (triangle in Fig. 4). RBC DHA+EPA saturation was estimated to occur at about 10 g%, as suggested by extrapolation of the curves to $y=0$.

3.5. Effects of the supplements on milk AA and the milk EPA/DHA and EPA/AA ratios

Fig. 3 panels D-F show the relations between the DHA+EPA dosages and milk AA content (panel D), EPA/DHA ratio (panel E), and EPA/AA ratio (panel F) for groups A-D at 4 weeks PP. Supplemental Table 2 presents the corresponding hard data. There were no relations between the DHA+EPA dosage and milk AA (panel D; $p=0.159$) and milk EPA/DHA ratio (panel E; $p=0.195$). The median (range) milk AA content was 0.36 (0.23–0.63) g% and the median (range) milk EPA/DHA ratio was 0.25 (0.05–0.37) g%. There was, however, a significant dose-dependent increase of the milk EPA/AA ratio (panel F; $p < 0.01$).
Panel D, no increase of the milk EPA/DHA ratio (Fig. 3, panel E), but increases of the milk EPA content (Fig. 3, panel C) and milk EPA/AA ratio (Fig. 3, panel F).

4.1. Dose needed to reach the 8 g% RBC DHA + EPA target at 36 gestational weeks

We found that 750 mg DHA + EPA/day is sufficient to increase the RBC DHA + EPA to 8 g% at the pregnancy end for women with a median (range) baseline RBC DHA + EPA of 5.5 g% (3.3–8.5). It was previously shown that RBC DHA contents of 7.3 [37] and 7.2 g% [38] at the pregnancy end were reached by supplementing 600 mg DHA for 25 weeks and 1200 mg for 17 weeks, respectively. Consistent with dependence on baseline, these pregnant women had somewhat lower baseline RBC DHA of 4.3 and 4.6 g%.

The maternal RBC DHA + EPA contents did not change from 36 GW to 4 weeks PP. However, within this period, RBC EPA increased, whereas RBC DHA tended to decrease (Supplemental Table 1). Decreasing postpartum maternal RBC DHA and increasing RBC EPA were previously demonstrated in supplemented-[38] and unsupplemented-[10] lactating women. The observed trend of decreasing RBC DHA from 36 GW to 4 weeks PP might indicate deteriorating maternal DHA status due to transplacental transfer and losses via the milk.

4.2. Dose needed to reach milk DHA + EPA of 1 g% at 4 weeks PP

A daily dose of 1000 mg DHA + EPA was needed to reach a milk DHA + EPA of 1 g% at 4 weeks PP. In a previous study, daily DHA dosages of 0, 200, 400, 900 and 1200 mg, provided to lactating women 5 days PP resulted in milk DHA contents of 0.21, 0.35, 0.46, 0.86 and 1.13 g% at 12 weeks PP [39]. This regimen, solely aimed at lactation, produced similar or even higher milk DHA as compared with the present (median milk DHA of 0.29, 0.63, 0.83 and 0.85 g%, following 225+90, 450+180, 675+270 and 900+360 mg DHA + EPA/day, respectively, from 20 GW to 4 weeks PP). The INFAT study [40] showed a higher mean milk DHA of 1.34 g% at 6 weeks PP in women receiving 1,020+180 mg DHA + EPA/day from 15 GW, as compared to the median 0.85 g% (range 0.57–1.40) milk DHA in our group D (900+360 mg DHA + EPA/day) at 4 weeks PP. Taken together, the currently available information from DHA or DHA + EPA supplementation studies indicate differences in the milk DHA contents that are reached. These may at least in part be explained by differences in baseline status (see also 4.5), maternal body size and composition (notably fat percentage), supplement composition (DHA + EPA vs. DHA only), dose, intervention duration and intervention period (i.e. pregnancy + lactation vs. lactation only). In addition, long chain fatty acids in milk may derive notably from adipose tissue. It has e.g. been estimated that 70% of linoleic acid (LA) in milk derives from stores [41], while selective mobilization of polyunsaturated fatty acids from adipose tissue has also been suggested [42]. The influence of maternal weight gain and losses during pregnancy and lactation may therefore also be important.

4.3. Discrepancy between dose needed to reach RBC DHA + EPA of 8 g% and milk DHA + EPA of 1 g%
Table 2
Characteristics of the 36 included mothers and their infants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dimensions</th>
<th>All (n = 36)</th>
<th>A (n = 9)</th>
<th>B (n = 9)</th>
<th>C (n = 11)</th>
<th>D (n = 7)</th>
<th>p-value</th>
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<td>Dose DHA + EPA (mg)</td>
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<td>225 + 90</td>
<td>450 + 180</td>
<td>675 + 270</td>
<td>900 + 450</td>
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<td>Age (years)</td>
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<td>32 (27–37)</td>
<td>31 (26–36)</td>
<td>32 (25–36)</td>
<td>30 (21–38)</td>
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<td>24 (19–29)</td>
<td>24 (18–28)</td>
<td>22 (19 –27)</td>
<td>24 (21–26)</td>
<td>0.896</td>
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<td>Para n (0 – 2)</td>
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<td>1 (0 – 2)</td>
<td>1 (0 – 2)</td>
<td>1 (0 – 2)</td>
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<td>41 (40 –42)</td>
<td>40 (38 –42)</td>
<td>40 (37 –41)</td>
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<td>Married/living together n (%)</td>
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<td>36 (100)</td>
<td>9 (100)</td>
<td>9 (100)</td>
<td>11 (100)</td>
<td>7 (100)</td>
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<td>3 (2–4)</td>
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<td>Annual household income €10,000 –€30,000 n (%)</td>
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<td>28 (80)</td>
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<td>7 (78)</td>
<td>7 (64)</td>
<td>6 (86)</td>
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<td>€30,000 –€50,000 n (%)</td>
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<td>15 (42)</td>
<td>4 (44)</td>
<td>4 (44)</td>
<td>5 (45)</td>
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<td>€50,000 or more n (%)</td>
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<td>14 (39)</td>
<td>4 (44)</td>
<td>4 (44)</td>
<td>5 (45)</td>
<td>5 (45)</td>
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<td>Weekly fish intake portion</td>
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<td>0.8 (0 –2.0)</td>
<td>0.5 (0 –1.5)</td>
<td>1.5 (0 –2)</td>
<td>1 (0 –2)</td>
<td>0.5 (0 –1)</td>
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</tr>
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<td>Weekly oil fish intake portion</td>
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<td>0.4 (0 –2.0)</td>
<td>0.5 (0 –1)</td>
<td>1 (0 –1.5)</td>
<td>0 (0 –2)</td>
<td>0.5 (0 –1)</td>
<td>0.501</td>
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<tr>
<td>Vitamin supplements before start study of which contained fish oil n (%)</td>
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<td>27 (75%)</td>
<td>8 (89%)</td>
<td>6 (67%)</td>
<td>8 (75%)</td>
<td>5 (71%)</td>
<td>0.476</td>
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<td>Infant characteristics</td>
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<td>Length (cm)</td>
<td></td>
<td>52.5 (45 –59)</td>
<td>51.5 (48 –55.5)</td>
<td>55 (45 –59)</td>
<td>51 (48 –55)</td>
<td>54.5 (49.5 –58)</td>
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<td>4.5 (3.6 –5.7)</td>
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<td>4.7 (3.9 –5.7)</td>
<td>4.4 (4.0 –5.2)</td>
<td>5.2 (3.6 –5.6)</td>
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<td>Birth weight (g)</td>
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<td>3790 (2440)</td>
<td>3790 (2870)</td>
<td>3965 (2440)</td>
<td>3750 (3070)</td>
<td>3870 (3440)</td>
<td>0.860</td>
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<tr>
<td>Gender (male)</td>
<td></td>
<td>17 (47%)</td>
<td>3 (33%)</td>
<td>4 (44%)</td>
<td>7 (64%)</td>
<td>3 (43%)</td>
<td>0.579</td>
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<td>Lactation duration (weeks)</td>
<td></td>
<td>4.4 (3.5 –5.3)</td>
<td>4.4 (3.5 –4.7)</td>
<td>4.4 (3.7 –5.3)</td>
<td>4 (3.6 –4.9)</td>
<td>4.6 (4 –5.1)</td>
<td>0.033</td>
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</tbody>
</table>

Data represent medians (ranges) or n (%). Groups A-D received supplements from 20 gestational weeks (GW) to 4 weeks postpartum (PP). The nutrients and their dosages are given in Table 1. A p-value < 0.050 is considered significant.

4.4. Comparison of needed dose with existing recommendations

The need of 750 and 1000 mg DHA + EPA/day to reach current RBC and milk DHA + EPA targets is in agreement with the recommendation of 1 g DHA + EPA by Flock et al. and the 700 mg DHA + EPA recommendation for pregnant and lactating women by GOED [23]. Not surprisingly, this dosage is also in agreement with optimal secondary prevention from cardiovascular disease by the American Heart Association [45] and for the treatment of affective disorders by the American Psychiatric Association [27,46].

4.5. Dependence of RBC DHA + EPA increments on baseline status and ceiling effect

The RBC DHA + EPA increment did not only depend on dose, but also on baseline RBC DHA + EPA (Fig. 4). This finding is in agreement with Flock et al. [30], who showed that participants with lower baseline status had more profound responses upon supplementation than participants with higher baseline status. Our data also suggested that a maximum RBC DHA + EPA content may be reached at 10 g%, which is consistent with our earlier studies reporting on RBC DHA contents in populations with life-long high fish intakes. In these studies, we
observed that the RBC DHA content plateaus at about 9 g%, with an upper maximum of about 12 g% [10].

4.6. Effects on milk AA content and milk EPA/DHA and EPA/AA ratios

The synthesis of AA from LA, and of EPA and DHA from ALA, makes use of the same desaturases and chain elongases. Each of these LCP, endogenously synthesized or from the diet, may subsequently compete for incorporation into (phospho)lipids [47]. The outcome of the latter has functional consequences, since the lipid mediators from the LCPω3 and -ω6 series, like eicosanoids, resolvins and (neuro)protectins, often have opposing action. For instance, in contrast to those from AA, those from EPA lower blood clotting, inflammation and cell growth, while they lower vascular resistance by vasodilatation [48]. A low EPA/AA

![Diagram](image_url)
important roles in brain development [63], but also interact others [61], jointly named brain selective nutrients [62]. They play
while a high EPA/AA ratio in (very low birthweight) infants has been
ratio in adults is strongly related to a pro-inflammatory state [49],
prevailing at 03/06 balance, the 2008 guidelines for LCP in infant formulas and baby foods recom-
recommend that EPA should not exceed DHA and that DHA should not
exceed AA [51]. Like others [39,52,53], we found that the increases of
milk DHA and EPA (Fig. 3, panels B and C) did not negatively affect
milk AA (Fig. 3, panel D), but we did notice a trend. Although EPA
never exceeded DHA (Fig. 3, panel E), there was an about 3-fold in-
crease of milk EPA/AA ratio when the lowest dose was compared with the
highest (panel F, 0.23 vs. 0.74 g/g). The supplement-induced higher
EPA/AA ratio is unlikely to be potentially deleterious. These ratios are
well within the physiological range of 0.17–1.08 g/g (unpublished data
calculated from [10]) of mothers with life-long high fish intakes.
The current milk EPA/AA ratio is also more favorable compared with the
seminal study of Carlson et al. [54] in which infants exhibited retarded
growth upon feeding with marine oil-supplemented infant formula, as
compared with control formulas. The former contained 0.3 g% EPA,
0.2 g% DHA and no AA, whereas the control held no LCP. It should also
be noted that their study was with very low birth weight infants
weighing 748–1390 g at delivery [54]. It seems, on the contrary, pos-
sible that, like in adults [49], a higher milk EPA/AA in infants also
contributes to an advantageous, less pro-inflammatory state.

4.7. Implications for future studies

The outcome of this study may have implications for future inter-
vention trials. Contemporary societies are dealing with a conflict be-
tween a man-made environment and our only slowly evolving genome
[55]. The current Western diet is low in fish intake, as compared to our
ancestors who have evolved for a great part in the land-water ecosystem
[56–59]. Various randomized controlled trials with DHA/DHA + EPA or
fish oil have been performed during pregnancy and/or lactation, with
subtle outcomes at most [1–5,7,60]. Apart from the above mentioned
shortcomings of these studies (see 4.2), fish also contains relevant
amounts of vitamins B12, A and D and iodine and selenium, among
others [61], jointly named brain selective nutrients [62]. They play
important roles in brain development [63], but also interact
[31,33,64–67]. By providing DHA and EPA, together with vitamin D
and a multivitamin with 12–125% of the RDA/AI, we tried to avoid
suboptimal vitamin, mineral and trace element status. Suboptimal mi-
cronutrient status may e.g. affect fatty acid metabolism [68] and
incorporation into lipids [31,32].

4.8. Limitations

A limitation of this study is the small number of participants. As
previously mentioned in 4.2 the current dose might not be fully re-
produced by others, because of the many factors involved, one of these
being the interaction with other nutrients.

4.9. Conclusions

Women with a RBC DHA + EPA of about 5.5 g% need about 750 mg
DHA + EPA/day to reach an RBC DHA + EPA of 8 g% at the pregnancy
end, and about 1000 mg DHA + EPA/day to reach a milk DHA + EPA of
1 g% at 4 weeks PP. The RBC DHA + EPA increment depends on base-
line values. The supplement had no potentially adverse effects on milk
AA. The milk EPA/AA ratio increased but remained within the phy-
siological range. A daily 1000 mg DHA + EPA supplement in pregnancy
may optimize both mother and child. This dosage is in excellent
agreement with those recommended for secondary prevention of car-
diovascular disease and affective disorders.

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Conflict of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the
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