Plasma long-chain omega-3 fatty acid status and risk of recurrent early spontaneous preterm birth: a prospective observational study

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Abstract

Introduction: A 2018 Cochrane review found that omega-3 supplementation in pregnancy was associated with a risk reduction of early preterm birth of 0.58; prompting calls for universal supplementation. Recent analysis suggests the benefit may be confined to women with a low baseline omega-3 fatty acid status. However, the contemporary omega-3 fatty acid status of pregnant women in the UK is largely unknown. This is particularly pertinent for women with a previous preterm birth, in whom a small relative risk reduction would have a larger reduction of absolute risk. This study aimed to assess the omega-3 fatty acid status of a UK pregnant population and determine the association between the long-chain omega-3 fatty acids and recurrent spontaneous early preterm birth.

Material and methods: A total of 283 high-risk women with previous early preterm birth were recruited to the prospective observational study in Liverpool, UK. Additionally, 96 pregnant women with previous term births and birth ≥39+0 weeks in the index pregnancy provided a low-risk population sample. Within the high-risk group we assessed the odds ratio of recurrent early preterm birth compared with birth at ≥37+0 weeks of gestation according to plasma eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) at 15–22 weeks of gestation.

Results: Our participants had low EPA+DHA; 62% (143/229) of women with previous preterm birth and 69% (68/96) of the population sample had levels within the lowest two quintiles of a previously published pregnancy cohort. We found no association between long-chain omega-3 status and recurrent early preterm birth (n = 51). The crude odds ratio of a recurrent event was 0.91 (95% CI 0.38–2.15, p = 0.83) for women in the lowest, compared with the highest three quintiles of EPA+DHA.

Conclusions: In the majority of our participants, levels of long-chain omega-3 were low; within the range that may benefit from supplementation. However, levels showed
1 | INTRODUCTION

Globally, preterm birth is the leading cause of death in children under 5 years old. Previous preterm birth is the strongest risk factor for subsequent preterm delivery. A 2018 Cochrane review concluded that omega-3 supplementation was an effective strategy to prevent preterm birth, with a 42% risk reduction (from 46 to 27 per 1000 births; 95% CI, 23–56) for preterm birth less than 34 weeks. A subsequent randomized controlled trial with secondary analysis suggested that the benefit may be confined to women with a low baseline total long-chain omega-3 fatty acid level. Worryingly, within the secondary analysis, supplementing women with higher total long-chain omega-3 fatty acid status was associated with increased rates of early preterm birth.

The fatty acid components with the strongest evidence of preterm birth prevention are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are collectively referred to as long-chain omega-3 fatty acids. These nutrients are predominantly obtained from oily fish and seafood and are associated with a more affluent diet. The long-chain omega-3 intake in pregnancy in the UK has been estimated from food frequency questionnaires as low, or adequate in three studies between 1991 and 2007.

Liverpool Women’s Hospital has a tertiary referral preterm birth prevention clinic that serves the fourth most deprived local authority area in England (out of 343). Based on the Cochrane review findings, we offered omega-3 supplementation to these high-risk women from February 2019. However, we were unsure whether this would offer benefit because of the unknown baseline long-chain omega-3 status in our population. Plasma levels of omega-3 in the UK pregnant population have not been assessed to our knowledge. The Danish National Birth Cohort showed that women in the lowest quintile of plasma EPA+DHA (<1.42% of total fatty acids), in the second trimester, had a 2.13 times increased risk (95% CI 1.18–3.79) of spontaneous preterm birth (sPTB) before 34 weeks of gestation compared with women in quintiles 3–5. The association between omega-3 and preterm birth was not present with levels in the third quintile and above. This is consistent with Simmonds et al and suggests that the main benefit of supplementation is in pregnancies with a lower baseline long-chain omega-3 status.

Importantly there has been a recent corrigendum to the original research within the Danish National Birth cohort based on the effect of thawing of stored samples before analysis of long-chain omega-3 fatty acids; this was therefore addressed within our analysis too.

We had two objectives. First, to determine the expected distribution of long-chain omega-3 fatty acids within “healthy pregnancies” in our locality; low-risk pregnant women who delivered at ≥39 weeks without preterm prelabor rupture of membranes (PPROM). Second, to assess the relationship between long-chain omega-3 status and recurrent early (under 34 weeks) sPTB and PPROM in our region.

2 | MATERIAL AND METHODS

Women with singleton pregnancies were enrolled at Liverpool Women’s Hospital from April 1, 2012 until December 31, 2017 as part of the “Development of novel biomarkers for prediction of preterm labor in a high-risk population” study. Participants were invited to two visits at approximately 16 (15–18 weeks) and 20 (19–23 weeks) of gestation. For the purposes of this analysis, the first sample available was used (single sample per participant).

A flowchart of selection entry from two different obstetric populations is shown in Figure 1. A “high-risk” population consisted of women with a history of sPTB or PPROM at 16–33 weeks of gestation. Low-risk women were parous women with all previous births at ≥37 weeks of gestation. Full details of the recruitment process, inclusion criteria, and careful pregnancy outcome classification criteria are given in the Supporting Information Appendix S1. Participants were excluded from the statistical analysis if omega-3 supplements had been used in pregnancy.

To describe the expected distribution of omega-3 fatty acids in our population, low-risk women that delivered at ≥39 weeks were selected (low-risk population sample).

Recurrent early sPTB/PPROM was defined as high-risk participants who had a late miscarriage, PPROM, or sPTB at 16–33 weeks of gestation. High-risk women who gave birth at ≥37 weeks of gestation without PPROM were allocated to the high-risk term birth group.
2.1 Omega-3 fatty acid analysis

Maternal blood samples were taken in 10 mL BD vacutainer® tubes (Becton Dickinson) containing dipotassium ethylenediaminetetraacetic acid, placed on ice immediately and processed within 1 hour of sampling. Tubes were centrifuged at 1200 × g for 10 minutes at 4°C. Plasma was aspirated and stored in cryovials at −80°C. A total of 30 µL was transferred to blood spot cards that were coated in antioxidants and chelating agents so as to minimize oxidation of polyunsaturated fatty acids. The dried blood spot cards were transported by post to the South Australian Health and Medical Research Institute where the plasma spots were transesterified and distributions of fatty acids were determined by capillary gas chromatography. The laboratory team was blinded to the pregnancy status of the samples.

2.2 Statistical analyses

Statistical analysis was performed in Stata version 15.1. (StataCorp.). The distributions of long-chain omega-3 fatty acids within the low-risk population sample were calculated and used to define quintiles of total omega-3, DHA, and EPA for our population.

Histograms were used to show the distribution of long-chain omega-3 fatty acids within the high-risk group according to whether the participant did, or did not, have recurrent early sPTB/PPROM. Two-term fractional polynomials were then used to visualize the expected non-linear association between long-chain omega-3 fatty acids levels and risk of recurrent early sPTB/PPROM within the high-risk group. High-risk participants in the early sPTB/PPROM and high-risk term birth groups were assigned to the quintiles based on the distribution of total omega-3, DHA, and EPA within the low-risk population sample and to the quintiles described by Olsen et al. Biomedical logistic regression was used to calculate the odds ratios of early sPTB/PPROM compared with term birth per quintile. Quintiles 3–5 were combined and used as the reference group based on previous work. Analysis was performed unadjusted and adjusted for covariates that were selected based on biological plausibility. The chosen covariates were: maternal age at study participation; maternal body mass index; maternal smoking at time of study visit (binary outcome of yes/no); index of multiple deprivation (IMD). Age and body mass index were converted to quadratic terms because of the bimodal relationships between these variables and risk of preterm birth. IMD was obtained using the woman’s home postcode on the UK government website. The IMD ranks every neighborhood in England from 1 (most deprived) to 32844...
The IMD is a collective score summarizing income deprivation, employment deprivation, health deprivation and disability, education skills and training deprivation, barriers to housing and services, living environment deprivation, and crime. IMD scores were used as continuous variables within the logistic regression.

Adjusted odds ratios for early sPTB/PPROM are presented both for the participants with all covariates available, and for all participants using imputation for missing covariates. Multiple imputation using chain equations was used to account for missing data as this allows for binary covariates (such as smoking). The proportion of total sampling variance due to missing data for IMD was 48%, therefore, as recommended, 50 imputations were performed. The variables used in the imputation model were all of the covariates described above as well as: pregnancy outcome (birth at term or early sPTB/PPROM); quintile of total omega-3, EPA, DHA, and DHA plus EPA; and quintile according to Olsen et al.

During the course of this work concern was raised that previous thawing may alter plasma long-chain omega-3 fatty acid analysis. We therefore undertook three further pieces of statistical analysis. First, assessment was performed of the long-chain omega-3 fatty acid status by number of previous freeze–thaw cycles of the sample. Second, the number of freeze-thaw cycles was included as a covariate in the logistic regression described above. Finally, the binomial logistic regression was repeated using only samples that had undergone previous freeze-thaw cycles, and those without.

### 2.3 Ethical Approval

The study was approved by the North West Research Ethics Committee, Liverpool Central, reference 11/NW/0720 on November 4, 2011. All participants provided written informed consent.

### 3 Results

We recruited 296 high-risk participants and 271 low-risk participants. Of 283 high-risk women with data suitable for analysis, 51 (18%) had a recurrent early sPTB or PPROM and 178 (63%) had term births (≥37 weeks) without PPROM (Figure 1). Of 271 low-risk participants, 188 gave birth at ≥39 weeks without PPROM, and had samples suitable for analysis. We selected the first 100 of these participants to send samples for laboratory analysis. Four of these participants were subsequently noted to have used omega-3 supplements, and so the remaining 96 participants formed the low-risk population sample.

The baseline characteristics and pregnancy outcomes are broadly similar across the pregnancy groups (Table 1), except for known risk factors for sPTB/PPROM. Compared with the low-risk population sample, more of the high-risk participants smoked (9.7% of low-risk versus 24.0% of high-risk group), and the high-risk participants had slightly lower IMD scores (more social deprivation). Preterm birth prevention treatment was offered in accordance with UK national guidelines. None of the low-risk women required an intervention but 32.8% (93/283) of the high-risk women did.

The low-risk population sample was used to define the expected distribution of omega-3 fatty acid levels in our population (Table 2).

Levels of total omega-3, DHA, and EPA within the high-risk group show similar distributions in women who have an early sPTB/PPROM, and those who do not (Figure 2A–D). The risks of recurrent early sPTB by total omega-3, DHA, and EPA levels are visualized in Figure 2E–H. Visually, it appears that there could be a weak relationship between higher levels of EPA, DHA, and total omega-3 and preterm birth, but the wide confidence intervals are also consistent with no correlation.

The high-risk group was then split into three groups according to total omega-3, DHA, and EPA quintiles obtained from the low-risk population sample: quintiles 1, 2, and 3–5 (reference group) (Table 3). When pregnancy outcomes were compared between quintile groups, the early sPTB/PPROM rate was lower in quintiles 1 and 2 for total omega-3 (crude odds ratio 0.65, 95% CI 0.23–1.84 and 0.52, 95% CI 0.23–1.15, respectively), EPA plus DHA, DHA and EPA, although none of these differences reached conventional statistical significance (p < 0.05) (Table 3).

We performed the same analysis adjusting for covariates of smoking, maternal age, body mass index, and IMD, both restricting the analysis to participants with all variables available and using multiple imputation to account for missing variables (Table 3). These results also showed no association between long-chain omega-3 fatty acids and early sPTB/PPROM, and the non-significant trend towards higher risk of preterm birth with higher levels.

Omega-3 fatty acid levels were universally lower in our population than in the Danish National Birth Cohort (Table 4). Of our low-risk population sample, 66% (63/96) had plasma DHA+EPA levels within the lowest two quintiles of the Danish cohort (compared with the expected 40%). Levels within the lowest two quintiles of the Danish cohort were also found in 51% (26/51) of high-risk women who had recurrent early sPTB/PPROM and 56% (100/178) of high-risk women who had term births. Unadjusted and adjusted analyses also showed no association between EPA plus DHA levels and early sPTB/PPROM using the Olsen et al. 11,12 quintiles (Table 4).

Before our analysis, samples from 17% (16/96) of our low-risk population sample, 9.0% (16/178) of our high-risk term birth group, and 57% (29/51) of our high-risk early sPTB/PPROM groups had undergone three freeze-thaw cycles (Table S1). The remainder of samples had undergone no previous freeze–thaw cycles. We found no statistically significant difference in DHA, EPA, or DHA+EPA levels when comparing samples with and without previous freeze–thaw cycles, but within the high-risk reference group there was a trend for slightly lower omega-3 fatty acid levels in samples that had undergone previous freeze–thaw cycles. When the logistic regression described in Table 3 was repeated in samples both without previous freeze–thaw cycles (Table S2) and with previous freeze–thaw cycles (Table S3) there remained a non-significant trend towards a reduced
### TABLE 1 Demographic details of the study population

| Purpose | High-risk (previous sPTB or PPROM 16–33+6 weeks) | Low-risk (previous term births) | Birth ≥37 weeks | sPTB/PPROM <34 weeks | p value HR term vs HR preterm | p value LR vs HR participants of nested case–control study |
|---------|--------------------------------------------------|---------------------------------|-----------------|-----------------------|-------------------------------|------------------------------------------------------------|
|         | Whole cohort | Birth ≥37 weeks (term) | Nested case–control study | Birth ≥39 weeks | n | SD/IQR/% | n | SD/IQR/% | n | SD/IQR/% | n | SD/IQR/% |
| N       | 283 | 178 | 51 | 96 | | | | | | | | |
| Age (mean, SD) | 30.4 | 5.1 | 30.6 | 5.1 | 30.8 | 5 | 31.2 | 4.2 | 0.829 | 0.375 | |
| BMI (median, IQR) | 24.6 | 21.8–28.7 | 25.0 | 21.8–28.9 | 25.6 | 23.1–32.5 | 23.6 | 21.9–27.6 | 0.208 | 0.209 | |
| Smoking (%) | 68 | 24.0 | 37 | 20.9 | 13.0 | 26.5 | 9 | 9.7 | 0.438 | 0.011 | |
| IMD quintile (%) | | | | | | | | | | | |
| 1 (most deprived) | 127 | 67.2 | 82 | 67.2 | 21 | 75.0 | 47 | 49.4 | 0.757 | 0.011 | |
| 2                  | 18 | 9.5 | 9 | 7.3 | 3 | 10.7 | 15 | 15.8 | |
| 3                  | 19 | 10.1 | 11 | 9.0 | 2 | 7.1 | 19 | 20.0 | |
| 4                  | 17 | 9.0 | 13 | 10.7 | 2 | 7.1 | 11 | 11.6 | |
| 5 (least deprived) | 8 | 4.2 | 7 | 5.7 | 0 | 0 | 3 | 3.2 | |
| Number of participants included in IMD data | 189 | 66.8 | 122 | 68.5 | 28 | 54.9 | 95 | 99.0 | |
| Total number of previous sPTB or PPROM (%) | | | | | | | | | | | |
| 0                  | 0 | 0 | 0 | 0 | 0 | 96 | 100 | | | |
| 1                  | 142 | 85.5 | 163 | 91.6 | 37 | 72.5 | | | | |
| ≥ 2                | 41 | 14.5 | 15 | 8.4 | 14 | 27.4 | | | | |
| Previous cervical surgery | | | | | | | | | | |
| None              | 254 | 89.8 | 167 | 93.8 | 42 | 82.4 | 88 | 91.7 | 0.094 | 0.395 | |
| ≤1× LLETZ <10 mm | 19 | 6.7 | 9 | 5.1 | 6 | 11.8 | 8 | 8.3 | |
| 1× LLETZ ≥10 mm/≥2 LLETZ/knife cone biopsy | 10 | 3.5 | 2 | 1.1 | 3 | 5.9 | | | | |
| Preterm birth prevention treatment used (%) | | | | | | | | | | |
| No                | 190 | 67.1 | 128 | 71.9 | 27 | 52.9 | 96 | 100 | 0.017 | not applicable | |
| Yes               | 93 | 32.9 | 50 | 28.1 | 24 | 47.1 | | | | |
| Gestational age at birth in weeks (median, IQR) | | | | | | | | | | |
| 37<sup>⁵</sup> | 35<sup>⁵</sup>–39<sup>⁵</sup> | 38<sup>⁶</sup> | 38<sup>⁶</sup>–39<sup>⁶</sup> | 31<sup>²</sup> | 26<sup>³</sup>–33<sup>²</sup> | 40<sup>²</sup> | 39<sup>³</sup>–41<sup>⁰</sup> | not applicable | |
| Birthweight in grams (mean, SD) | 2800 | 809 | 3238 | 477 | 1576 | 703 | 3614 | 439 | |
| PPROM <34 weeks (n, %) | 21 | 7.42 | 0 | 0 | 21 | 46 | 0 | 0 | |

Note: p value calculated by ANOVA for age, Kruskal–Wallis test for BMI and Fisher’s exact test for remainder of analysis. There were three missing values for BMI in the high-risk sPTB or PPROM <34 weeks group and three missing values in the high-risk birth ≥37 weeks term group. There was one missing value for smoking in the high-risk sPTB or PPROM group and three missing values in the birth ≥37 weeks group. The high percentage of missing data for IMD was because postcode was not recorded for the high-risk group at the start of the research study.

Abbreviations: BMI, body mass index; HR, high risk; IMD, index of multiple deprivation; IQR, interquartile range; LLETZ, large loop excision of the transformation zone; LR, low risk; OR, odds ratio.; PPROM, preterm prelabor rupture of membranes; sPTB, spontaneous preterm birth; SD, standard deviation.
chance of sPTB/PPROM with lower omega-3 fatty acid levels in both analyses.

4 | DISCUSSION

Contrary to previous findings, we did not demonstrate a relationship between long-chain omega-3 levels and spontaneous preterm birth. This was despite comparing plasma total omega-3, DHA, and EPA levels with both “healthy” pregnancies in our population, and to levels in Danish pregnant women that have previously been associated with preterm birth.11,12 In our population, both women at high and low risk of preterm birth had lower levels of plasma DHA plus EPA than those described in the Danish population.11,12

The plasma long-chain omega-3 levels within our population could have been so low that we did not have enough “replete” participants to show the benefit in preterm birth reduction with adequate levels. However, our results show a non-significant trend in the opposite direction to previous literature (ie, a higher risk of preterm birth with a higher level of omega-3, DHA, and EPA), and no biological gradient.

Women with a previous preterm birth are often highly motivated to avoid recurrence, and could have become aware of evidence to support increased omega-3 intake17,18 during their pregnancy. Omega-3 fatty acids may have a rapid effect on risk of preterm birth.19,20 If a substantial number of the women in our study actually did increase their omega-3 fatty acid intake during pregnancy, this may have confused the relationship between omega-3 measured in early second trimester and subsequent risk of early preterm birth.

To our knowledge this is the fourth analysis relating blood DHA and EPA levels in the second trimester to preterm birth risk. Previous studies include the analysis by Olsen et al.,21 and the secondary analysis of the ORIP trial,5 both demonstrating lower long-chain omega-3 levels in association with preterm birth under 34 weeks, in Danish and Australian populations, respectively. The third study is a secondary analysis of a trial of omega-3 supplements to prevent recurrent preterm birth before 37 weeks of gestation in the USA.21 Klebanoff et al did find that participants in the lowest quartile of DHA+EPA had a higher rate of preterm birth (83/176, 47.2%) compared with the highest quartile (63/175, 36%); however, their results also did not reach statistical significance.21

A strength of this study is that our preterm group included only recurrent sPTB, or PPROM, before 34 weeks of gestation. We aimed to achieve as pure a “phenotype” of spontaneous preterm birth as possible. Previous studies into the association between omega-3 levels and preterm birth have included all births under 34 weeks,5 or 37 weeks,21 or only excluded cases of preeclampsia before 34 weeks.3 It is possible that the benefit of omega-3 to prevent preterm birth is confined to preterm births that are medically indicated by conditions such as preeclampsia and growth restriction.22 However, the most recent Cochrane review shows no impact of omega-3 supplementation upon these conditions.3 In keeping with this, our initial visualization of the relationship between long-chain omega-3 status and early preterm birth in the whole high-risk group, including those with late medically indicated preterm births (Figure 2), did not show an association between long-chain omega-3 levels and all preterm births. Alternatively the impact of omega-3 upon preterm birth prevention may be within the low-risk population that was not assessed for preterm birth risk in this study.

A limitation of this study is that 56% of sPTB/PPROM samples had undergone previous freeze–thaw cycles, compared with only 9% of the high-risk reference group. However, we found higher than expected levels of omega-3 fatty acids in the sPTB/PPROM group, and freeze–thaw cycles might be expected to lower the expected levels of omega-3 fatty acids.12 We therefore do not feel that this has materially impacted our findings.
FIGURE 2  Visualization of the relationship between long-chain omega-3 fatty acid levels and pregnancy outcome in women with a previous spontaneous preterm birth/preterm prelabor rupture of membranes (sPTB/PPROM) <34.00 weeks of gestation. Total n = 283, of whom 51 had recurrent sPTB/PPROM, and the remainder (n = 232) delivered at ≥34.00 weeks without PPROM. (A–D) Histograms showing long-chain omega-3 fatty acid levels by pregnancy outcome. (E–H) Risk of recurrent early sPTB/PPROM <34.00 weeks by baseline fatty acid status in women with previous early sPTB or PPROM (n = 283). The pale gray lines represent the 95% CI for the risk. p values are for the association between long-chain omega-3 status and risk of early preterm birth using fractional polynomial logistic regression. Graphs show the unadjusted data. Adjusted p values include the covariates of age, body mass index (BMI), smoking, and Index of Multiple Deprivation (IMD). Percentages are of the total plasma fatty acids.
TABLE 3  Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group.

| Quintiles based on low-risk population sample | Quintile | Level (%) | Total number of high-risk participants | Birth ≥37 weeks | OR of early sPTB/PPROM (95% CI) | p value |
|---------------------------------------------|----------|-----------|----------------------------------------|----------------|---------------------------------|---------|
| total Omega-3                                | 1        | 1.69–2.18 | 27                                     | 5 9.8          | 12.4                            | 0.65   |
|                                             | 2        | 2.18–2.51 | 59                                     | 9 17.6         | 28.1                            | 0.52   |
|                                             | 3–5      | 2.51–5.29 | 143                                    | 37 72.5        | 59.6                            | 1 1     |
| EPA plus DHA                                | 1        | 0.88–1.36 | 37                                     | 8 15.7         | 16.3                            | 0.91   |
|                                             | 2        | 1.36–1.51 | 29                                     | 5 9.8          | 13.5                            | 0.69   |
|                                             | 3–5      | 1.51–4.1  | 163                                    | 38 74.5        | 70.2                            | 1 1     |
| DHA                                         | 1        | 0.72–1.1  | 32                                     | 7 13.7         | 14.0                            | 0.93   |
|                                             | 2        | 1.10–1.22 | 29                                     | 5 9.8          | 13.5                            | 0.69   |
|                                             | 3–5      | 1.22–3.10 | 168                                    | 39 76.5        | 72.5                            | 1 1     |
| EPA                                         | 1        | 0.07–0.22 | 53                                     | 8 15.7         | 25.3                            | 0.53   |
|                                             | 2        | 0.22–0.27 | 53                                     | 12 23.5        | 23.0                            | 0.87   |
|                                             | 3–5      | 0.27–1.18 | 123                                    | 31 60.8        | 51.7                            | 1 1     |
| total total                                  |          |           |                                        | 51 100         | 178                             | 100     |

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; sPTB, spontaneous preterm birth.

*Adjusted for age, BMI, and smoking (actual data only).
*Adjusted for age, BMI, smoking, and index of multiple deprivation (actual data only).
*Adjusted for age, BMI, smoking, and index of multiple deprivation including imputed data for missing data.

TABLE 4  Relationship between quintile of fatty acids (as defined by Olsen et al11) and pregnancy outcome.

| EPA plus DHA as per Olsen 2018 | Low risk (previous term births) | High risk (previous sPTB or PPROM 16–33 weeks) | Crude OR of PTB compared with quintile 3–5 within high risk (n = 229) |
|--------------------------------|--------------------------------|-----------------------------------------------|--------------------------------------------------|
|                                | Birth ≥39 weeks | Recurrent early sPTB/PPROM | Birth ≥37 weeks | OR of early sPTB/PPROM (95% CI) | p value |
| Quintile Value | n | % | n | % | n | % | (95% CI) | p value |
| 1 0.47–1.42     | 25 | 26.0 | 8 | 15.7 | 40 | 22.5 | 0.62 | 0.26–1.51 | 0.30 |
| 2 1.43–1.74     | 38 | 39.6 | 18 | 35.3 | 60 | 33.7 | 0.94 | 0.47–1.87 | 0.852 |
| 3–5 1.74–4.95   | 33 | 34.4 | 25 | 49.0 | 78 | 43.8 | 1 | 1 | – |
| total total     | 96 | 100 | 51 | 100 | 178 | 100 | – | – | – |

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; sPTB, spontaneous preterm birth.

*Adjusted for age, BMI, and smoking (actual data only).
*Adjusted for age, BMI, smoking, and index of multiple deprivation (actual data only).
*Adjusted for age, BMI, smoking, and index of multiple deprivation including imputed data for missing data.

We acknowledge that plasma levels of omega-3, DHA, and EPA were measured on samples from participants that were not fasted; however, the previous study to find an association between plasma levels of DHA and EPA and preterm birth used samples taken by GPs at routine visits and no mention is made of fasting in the description.11,23

This was a pragmatic study based on a biomarker study that had finished recruiting at the time of study inception. As such, no formal power calculation has been performed, and we did not have a predefined a priori level at which we are able to accept/reject our null hypothesis of no association between long-chain omega-3 levels and recurrent spontaneous early preterm birth. Nevertheless, we feel that knowledge of the low baseline levels of long-chain omega-3 fatty acids within pregnant women in the UK, and also no indication of an association between long-chain omega-3 fatty acids
and recurrent preterm birth in our high-risk group, are important to inform the discussion about omega-3 supplementation for preterm birth prevention.

It is possible that preterm birth prevention therapy averted preterm birth in some high-risk participants, attenuating an association between omega-3 and early sPTB/PPROM. Analysis limited to participants without preterm birth prevention treatment showed similar findings (data not shown). Any intervention involving omega-3 is likely to be applied in combination with current treatments, and so we felt it was optimal to assess the situation within current clinical practice.

Preterm birth is a multifactorial disease and the contribution of a single factor (such as omega-3 levels) is likely to only be modest. It is possible that other factors, genetic or environmental, leading to
recurrent preterm births are able to “overpower” any contribution of long-chain omega-3 status. We suggest that future research should include baseline long-chain omega-3 fatty acids testing on a large scale, and evaluate the influence of these levels on other risk factors of preterm birth. This would be relevant to both women with, and without, identifiable risk factors for preterm birth, and may be achieved by an individual patient data meta-analysis of already conducted work.

5 | CONCLUSION

We found low plasma omega-3, DHA, and EPA levels in the second trimester in women at high and low risk of preterm birth. The previously described association between low DHA and EPA and preterm birth was not replicated. We suggest that either plasma long-chain omega-3 fatty acids were so low in this population we did not have enough “replete” participants to show a benefit, or there are alternative mechanisms for recurrent early preterm birth in this setting.

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CONFLICT OF INTEREST

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REFERENCES

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the sustainable development goals. Lancet. 2016;388:3027-3035.

2. Ferrero DM, Larson J, Jacobsson B, et al. Cross-country individual participant analysis of 4.1 million singleton births in 5 countries with very high human development index confirms known associations but provides no biologic explanation for 2/3 of all preterm births. PLoS One. 2016;11:e0162506.

3. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev. 2018;11:CD003402.

4. Makrides M, Best K, Yelland L, et al. A randomized trial of prenatal n-3 fatty acid supplementation and preterm delivery. N Engl J Med. 2019;381:1035-1045.

5. Simmonds LA, Sullivan TR, Skubisz M, et al. Omega-3 fatty acid supplementation in pregnancy – baseline Omega-3 status and early preterm birth: exploratory analysis of a randomised controlled trial. BJOG. 2020;127:975-981.

6. Nykjær C, Higgs C, Greenwood DC, Simpson NAB, Cade JE, Alwan NA. Maternal fatty fish intake prior to and during pregnancy and risks of adverse birth outcomes: Findings from a British cohort. Nutrients. 2019;11:1-14.

7. Rogers I, Emmett P, Ness A, Golding J. Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. J Epidemiol Community Health. 2004;58:486-492.

8. Leventakou V, Roumeliotaki T, Martinez D, et al. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. Am J Clin Nutr. 2014;99:506-516.

9. Department for Communities and Local Government. The English index of multiple deprivation (IMD) 2015- guidance. 2015. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/464430/English_Index_of_Multiple_Deprivation_2015_guidance.pdf

10. Omega 3 for women attending the preterm birth prevention clinic. https://www.liverpoolwomens.nhs.uk/media/2884/omega-3-for-women-attending-the-preterm-birth-prevention-clinic_mat-2019-227-v1.pdf

11. Olsen SF, Halldorsson TI, Thorne-Lyman AL, et al. Plasma concentrations of long chain N-3 fatty acids in early and mid-pregnancy and risk of early preterm birth. EbioMedicine. 2018;35:325-333.

12. Olsen SF, Halldorsson TI, Thorne-Lyman AL, et al. Corrigendum to ‘Plasma concentrations of long chain N-3 fatty acids in early and mid-pregnancy and risk of early preterm birth’. EbioMedicine. 2020;51:102619.

13. Liu G, Muhlhausler BS, Gibson RA. A method for long term stabilisation of long chain polyunsaturated fatty acids in dried blood spots and its clinical application. Prostaglandins Leukot Essent Fatty Acids. 2014;91:251-260.

14. Ministry of Housing Communities & Local Government. English indices of deprivation 2015. 2015. http://imd-by-postcode.opendatacommunities.org/imd/2015

15. Institute for Digital Research and Education. Multiple Imputation in Stata. 2020. https://stats.idre.ucla.edu/stata/seminars/mi_in_stata_pt1_new/

16. National Institute for Health and Care Excellence. Antenatal care for uncomplicated pregnancies. Clin Guidel. 2008;1:55.

17. Olsen SF, Secher NJ, Tabor A, Weber T, Walker JJ, Gluud C. Randomised clinical trials of fish oil supplementation in pregnancy and risk of preterm delivery. BJOG. 2000;107:382-395.

18. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. Br Med J. 2002;324:1-5.

19. Olsen SF, Secher NJ, Bjørnssoen S, Weber T, Atke A. The potential benefits of using fish oil in relation to preterm labor: The case for a randomized controlled trial? Acta Obstet Gynecol Scand. 2003;82:978-982.

20. Olsen SF, Halldorsson TI, Li M, et al. Examining the effect of fish oil supplementation in Chinese pregnant women on gestation duration and risk of preterm delivery. J Nutr. 2019;149:1942-1951.

21. Klebanoff MA, Harper M, Lai Y, et al. Fish consumption, erythrocyte fatty acids, and preterm birth for the Eunice Kennedy Shriver national institute of child health and human development (NICHD) maternal-fetal medicine units network (MFMU) *. Obstet Gynecol. 2011;117:1071-1077.

22. Saccone G, Berghella V, Maruotti GM, Sarno L, Martinelli P. Omega-3 supplementation during pregnancy to prevent recurrent intrauterine growth restriction: systematic review and meta-analysis of randomized controlled trials. Ultrasound Obstet Gynecol. 2015;46:659-664.
23. Olsen J, Melbye M, Olsen SF, et al. The Danish national birth cohort - its background, structure and aim. *Scand J Public Health*. 2001;29:300-307.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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