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Omega-3 fatty acid supplementation in pregnancy – baseline omega-3 status and early preterm birth: exploratory analysis of a randomised controlled trial

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Objective To identify a polyunsaturated fatty acid (PUFA) biomarker able to detect which women with singleton pregnancies are most likely to benefit from omega-3 supplementation to reduce their risk of early preterm birth.

Design Exploratory analysis of a randomised controlled trial.

Setting Six Australian hospitals.

Population Women with a singleton pregnancy enrolled in the ORIP trial.

Methods Using maternal capillary whole blood collected ~14 weeks' gestation, the fatty acids in total blood lipids were quantified using gas chromatography. Interaction tests examined whether baseline PUFA status modified the effect of omega-3 supplementation on birth outcomes.

Main outcome measure Early preterm birth (<34 weeks' gestation).

Results A low total omega-3 PUFA status in early pregnancy was associated with a higher risk of early preterm birth. Among women with a total omega-3 status ≤4.1% of total fatty acids, omega-3 supplementation substantially reduced the risk of early preterm birth compared with control (0.73 versus 3.16%; relative risk = 0.23, 95% confidence interval [CI] 0.07–0.79). Conversely, women with higher total omega-3 status in early pregnancy were at lower risk of early preterm birth. Supplementing women with a baseline status above 4.9% increased early preterm birth (2.20 versus 0.97%; relative risk = 2.27, 95% CI 1.13–4.58).

Conclusions Women with singleton pregnancies and low total omega-3 PUFA status early in pregnancy have an increased risk of early preterm birth and are most likely to benefit from omega-3 supplementation to reduce this risk. Women with higher total omega-3 status are at lower risk and additional omega-3 supplementation may increase their risk.

Keywords Biomarker, docosahexaenoic acid, omega-3 fatty acids, preterm birth.

Tweetable abstract Low total omega-3 fat status helps identify which women benefit from extra omega-3 to reduce early prematurity.

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Introduction

Our recently published Omega-3 to Reduce the Incidence of Prematurity (ORIP) randomised controlled trial (RCT) of 5544 pregnancies was designed to assess whether omega-3 polyunsaturated fatty acid (PUFA) supplementation, largely as docosahexaenoic acid (DHA), reduced the risk of early preterm birth (<34 weeks' gestation).[1] ORIP

investigated a broad supplementation strategy by including women with multiple pregnancies and those who were already taking low-dose omega-3 supplements. Even though omega-3 PUFA concentrations in the intervention group at 34 weeks were elevated relative to the control group, omega-3 supplementation did not reduce the overall risk of either early preterm birth or preterm birth (<37 weeks' gestation).

The ORIP trial findings do not appear consistent with the most recent Cochrane review, which showed that omega-3 supplementation until birth reduced the risk of early preterm birth by 42%.[2] The RCTs in the Cochrane review largely included singleton pregnancies, with many women likely to have low omega-3 fatty acid status. Drivers of preterm birth can differ for multiple pregnancies and may increase the risk of a range of obstetric complications necessitating iatrogenic preterm delivery.[4] Additionally, the demand for omega-3 fatty acids by the maternal-fetal unit is likely to be higher in multiple rather than singleton pregnancies.[3] Furthermore, recent epidemiological data have shown that low omega-3 PUFA concentrations in early pregnancy are strongly associated with an increased risk of early preterm birth in singleton pregnancies.[4]

Therefore, using fatty acid status data from blood collected at trial entry in singleton pregnancies in ORIP, our objective was to determine whether PUFA candidate biomarkers can distinguish, early in pregnancy, which women with singleton pregnancies are most likely to benefit from omega-3 supplementation to reduce their risk of early preterm birth. In addition to early preterm birth–the primary outcome of the ORIP trial–we considered preterm birth as a clinically important secondary outcome. As metabolism of precursor fatty acids to DHA is thought to be more efficient during pregnancy,[3] key fatty acids capable of influencing the omega-3 to omega-6 tissue balance were examined as candidate biomarkers.

Methods

Women were recruited to the ORIP trial, with few exclusions, prior to 20 weeks' gestation, including those who had been regularly consuming a low-dose DHA supplement (≤150 mg/day) and were willing to stop. They were randomised to receive either ≈900 mg omega-3 PUFA (3 × DHA enriched fish oil/day [≈800 mg DHA and ≈100 mg eicosapentaenoic acid (EPA)/day]) or isocaloric vegetable oil control capsules with trace fish oil for masking (≈15 mg DHA and ≈4 mg EPA/day) until 34 weeks of gestation. Gestational length at the time of birth was determined on the basis of both the date of the last menstrual period and ultrasound data obtained in early pregnancy; inclusion and exclusion criteria, methodology, treatment adherence and the statistical analysis plan are published elsewhere.[1]

In the present study, we restricted analyses to singleton pregnancies (excluding 100 multiple pregnancies in ORIP). Using data collected as part of the ORIP trial, we measured percentages of omega-3 fatty acids (alpha linolenic acid [ALA], EPA, DHA, EPA + DHA and total omega-3 PUFA [the sum of ALA, EPA, docosapentaenoic acid (DPA) and DHA) and omega-6 fatty acids (linoleic acid [LA] and arachidonic acid [AA]) in maternal capillary whole blood, collected at enrolment (~14 weeks; IQR 12.7–16.3 weeks) on chemically treated filter paper as dried blood spots.[5] The dried blood spot was added to 2 ml of 1% (v/v) H_2SO_4 (18M, AR grade, BDH, Sussex, UK) in anhydrous methanol (Merck, Darmstadt, Germany) in a 6-ml sealed vial (Wheaton, Millville, NJ, USA) and heated at 70°C for 3 hours. The resultant fatty acid methyl esters (FAME) were extracted into heptane (Merck, Darmstadt, Germany). FAME were separated and quantified using an Agilent 7890 Gas Chromatograph (Agilent, Santa Clara, CA, USA) equipped with a BPX70 capillary column 30 m × 0.25 mm, film thickness 0.25 µm (Trajan Pty Ltd., Victoria, Australia), programmed temperature vaporization injector (at 250°C) and a flame ionisation detector (at 300°C). A programmed temperature ramp (140–240°C) was used. Helium gas was utilised as a carrier at a flow rate of 1 ml/min in the column and the inlet split ratio was set at 20:1. The FAME were identified and quantified by comparing the retention times and peak area values of unknown samples with those of commercial lipid standards (Nu-Chek Prep Inc., Elysian, MN, USA) using the Agilent Chemstation data system.

Statistical analysis

Log binomial regression models were used to analyse the data. Initial analyses estimated the effect of omega-3 supplementation on the risk of early preterm and preterm birth among women who had a baseline dried blood spot available, and the effect of baseline omega-3 and omega-6 status among women in the control group. Interaction models were then used to test whether the effect of omega-3 supplementation on the risk of early preterm and preterm birth varied according to baseline omega-3 and omega-6 status. Global interaction tests were carried out using likelihood ratio tests. Following recommendations in Royston and Sauerbrei[6] we treated PUFA status as a continuous variable and used two-term fractional polynomials to model associations. All models were adjusted for variables used to stratify the randomisation (enrolment centre, recent omega-3 supplementation) and used robust variance estimation to account for the clustering of pregnancies, as some women (n = 22) participated in the trial more than once. Treatment effect plots were used to demonstrate graphically how the relative risk due to omega-3 supplementation varied by baseline omega-3 and omega-6 PUFA

status. No *P*-value adjustment was made for the number of subgroup analyses performed. Analyses were performed using STATA version 15.0 (StataCorp LP: College Station, TX, USA).

This study was supported by the National Health and Medical Research Council (NHMRC) Centre of Research Excellence in Targeted Nutrition to Improve Maternal and Child Health Outcomes (1135155). However, the NHMRC played no role in conducting the research or writing the paper. The full set of core outcomes for preterm birth were not included as this study is an exploratory analysis of specific outcomes. Patients (women) were not involved in development of this exploratory study.

Results

A total of 5070 singleton pregnancies had a baseline dried blood spot and birth outcome available (2525 omega-3 supplemented and 2545 controls; 5% missing data; Figure 1). Omega-3 supplementation in these singleton pregnancies did not have a large impact on the risk of early preterm birth (1.86 versus 1.65%, adjusted relative risk [RR] = 1.13, 95% confidence interval [CI] 0.75–1.70) or preterm birth (6.46 versus 7.82%, adjusted RR = 0.83, 95% CI 0.68–1.01), which is consistent with results for all singleton pregnancies reported in ORIP.[1] A descriptive summary of baseline omega-3 and omega-6 PUFA status is provided in supporting Information Table S1 and Figure S1; no clear baseline differences were seen between groups.

Baseline fatty acid status and birth outcome (control group only)

Within the control group, baseline total omega-3 ($P = 0.009$), EPA + DHA ($P = 0.02$) and DHA ($P = 0.02$) status were found to be associated with the risk of early preterm birth. As displayed in Figure 2, a low status of these PUFAs was associated with a higher risk of early preterm birth, whereas a high status was associated with a lower risk. In contrast, baseline EPA levels (and LA, AA, ALA – not shown) were not predictive of early preterm birth. No clear associations were observed between the omega-3 or omega-6 PUFAs tested

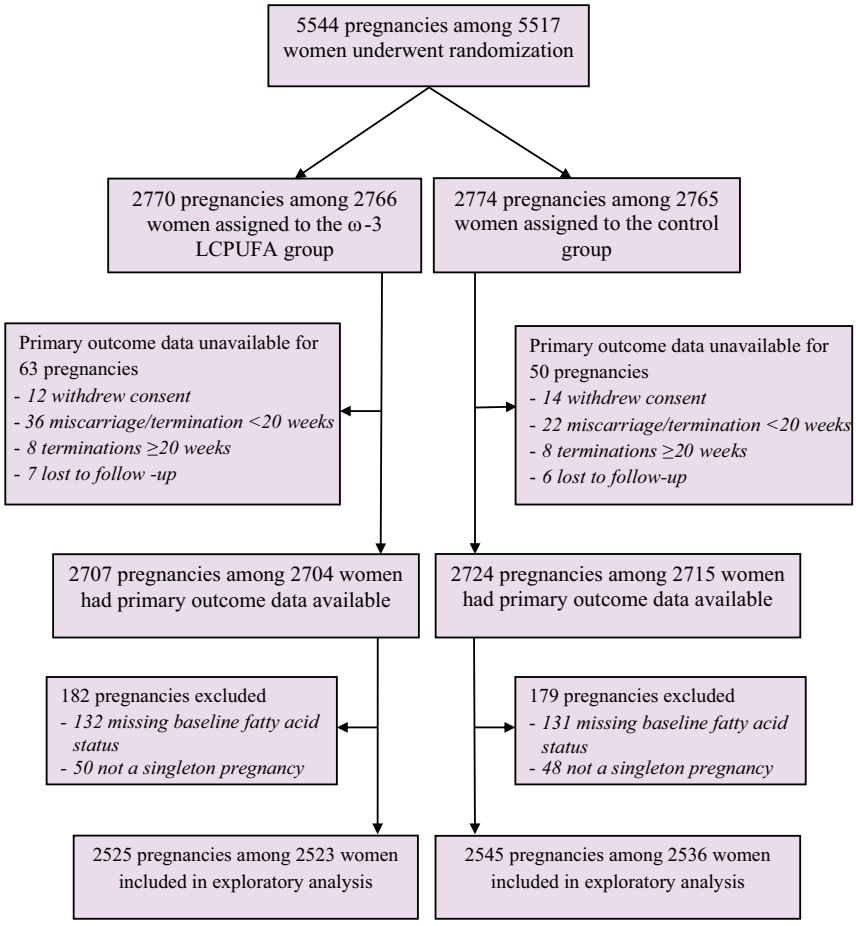

Figure 1. Flowchart of participants through the ORIP trial and the data available for the exploratory analysis.

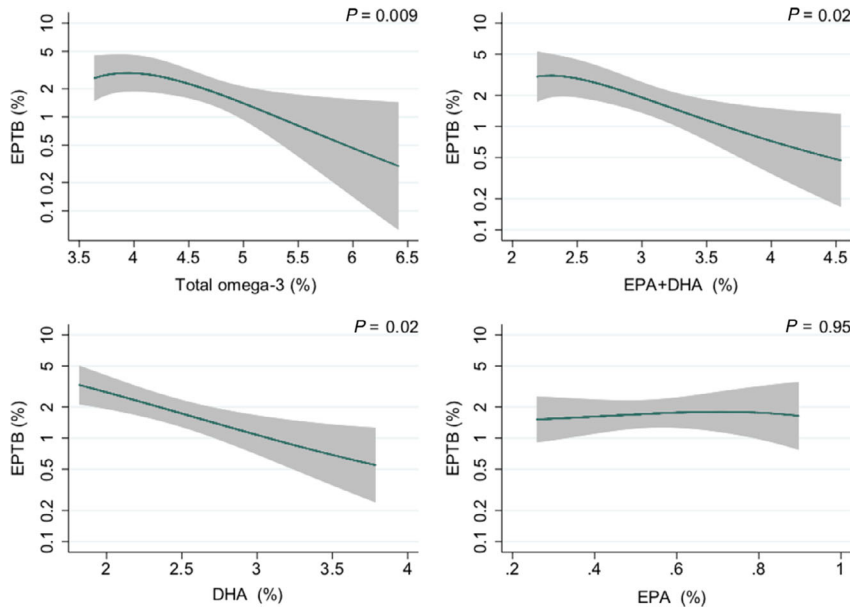

Figure 2. Risk of early preterm birth in the control group by baseline PUFA status.[1-5] (1) Due to small numbers of early preterm births at the extremes, to aid interpretation the plots have been truncated at the 5th and 95th percentiles of each PUFA. (2) The y-axis is on the logarithmic scale. (3) The grey-shaded area on each plot corresponds to a 95% confidence interval for the risk. (4) *P* values are for the association between PUFA status and the risk of early preterm birth. (5) The area under the receiver operating characteristic curve for total omega-3 = 0.67; EPA + DHA = 0.65; DHA = 0.66; EPA = 0.51.

and the risk of preterm birth <37 weeks (see Supporting Information Figure S2).

Effect modification of supplementation by baseline status

The effect of omega-3 supplementation on early preterm birth differed according to baseline total omega-3 PUFA (interaction $P = 0.0009$), EPA + DHA (interaction $P = 0.004$) and DHA (interaction $P = 0.02$) status. As shown in Figure 3, omega-3 supplementation reduced the risk of early preterm birth when baseline status of these omega-3 PUFA biomarkers was low, but increased the risk when their baseline status was high. Of the three biomarkers demonstrating this consistent pattern, total omega-3 PUFA showed the strongest relation (Figure 3). Supplementation reduced early preterm birth risk when total omega-3 status at baseline was <4.5% of total fatty acids in whole blood, with the evidence for a beneficial effect based on 95% confidence limits for the fitted curve becoming stronger for values of 4.1% or lower. Among the 885 (17.5%) women with a total baseline omega-3 PUFA status ≤4.1%, omega-3 supplementation substantially reduced the risk of early preterm birth by 77% compared with control (3/411 [0.73%] vs. 15/474 [3.16%]; RR = 0.23, 95% CI 0.07–0.79). Conversely, there was an increased early preterm birth risk associated with supplementation (25/1138 [2.20%] vs. 11/1139 [0.97%]) among the 2277 women (44.9%) with a baseline status above 4.9% (RR = 2.27, 95% CI 1.13–4.58). The status of EPA, AA and LA at baseline did not influence the effect of omega-3 supplementation on early preterm birth (AA and LA not shown in Figure 3). A similar pattern of results for effect modification was also observed when analyses were performed separately for spontaneous and iatrogenic births or when the seven baseline PUFA biomarkers were grouped into quartiles rather than treated as continuous variables in the analysis (results not shown).

For preterm birth <37 weeks, there was no evidence of the effect of supplementation varying according to baseline status for any of the omega-3 or omega-6 PUFA biomarkers tested (Supporting Information Figure S3).

Discussion

Main findings

For singleton pregnancies, we found that women with a low total omega-3 blood PUFA status were at higher risk of early preterm birth (<34 weeks) and that this risk can be reduced by omega-3 supplementation. However, the data also suggest that supplementing mothers who are already replete in omega-3 PUFA with additional omega-3 increases the risk of early preterm birth.

Strengths and limitations

The low incidence of early preterm birth observed in the ORIP trial and the potential for type I errors due to

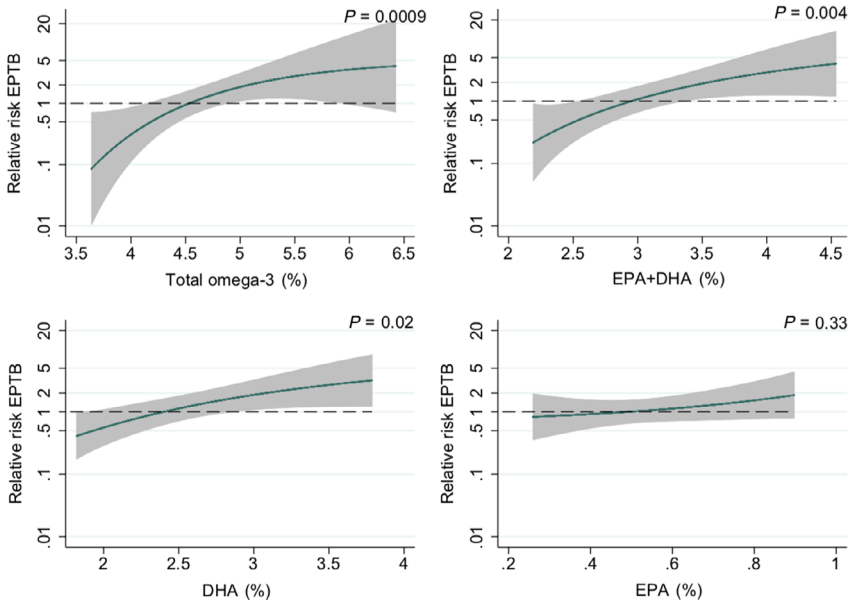

Figure 3. Relative risk of early preterm birth (supplementation versus control) by baseline PUFA status.[1-5] (1) Due to small numbers of early preterm births at the extremes, to aid interpretation the plots have been truncated at the 5th and 95th percentiles of each PUFA. (2) The y-axis is on the logarithmic scale. (3) The grey-shaded area on each plot corresponds to a 95% confidence interval for the treatment effect. (4) Relative risks adjusted for enrolment centre and recent omega-3 supplementation. (5) *P* values are for the interaction between treatment group and PUFA status. For total omega-3, null treatment effect = 4.5%; threshold for benefit ≤4.1%; threshold for harm >4.9%. For EPA + DHA, null treatment effect = 3.0%; threshold for benefit ≤2.5%; threshold for harm >3.4%. For DHA, null treatment effect = 2.4%; threshold for benefit ≤1.9%; threshold for harm >2.9%.

multiple post-hoc comparisons pose some limitations for the interpretation of these findings. Nevertheless, the large numbers of pregnant women, the robustness of the randomisation, the highly significant interaction tests and clinically plausible results for early preterm birth – the primary outcome of the ORIP trial – allow us to begin to understand the pattern of response to omega-3 supplementation in pregnancy for preventing early preterm birth.

Interpretation

Our observation that the effect of omega-3 PUFA supplementation on early preterm birth is altered by baseline omega-3 status is consistent with epidemiological data showing that low fish intake or low omega-3 status is associated with prematurity.[4,7] Our data, within the context of an RCT, add to these observations and demonstrate that correcting low omega-3 status through supplementation can reduce the risk of early preterm birth. Our conservative view is that supplementation of women with a total omega-3 status of ≤4.1% of total fatty acids in whole blood at the end of the first trimester of pregnancy may be a useful strategy to reduce their risk of early preterm birth.

What was less expected was the finding that omega-3 supplementation of women with replete omega-3 status appears to increase risk of early preterm birth. Similar patterns have been seen for several micronutrients and higher risks of adverse health outcomes for both low and high nutrient intakes—a U-shaped relation.[8–12] Although of different design and size compared with ORIP, a secondary analysis of a prenatal omega-3 supplementation RCT suggests that for women at risk of recurrent preterm birth, the probability of preterm birth was highest at low and high intakes, and lowest with moderate fish consumption.[13]

Nearly half (45%) of the women in the ORIP trial had a baseline omega-3 status above 4.9% of total fatty acids in whole blood. Our data suggest that for many women in high-income countries where prenatal supplementation has become common, additional supplementation of 900 mg/day of DHA and EPA may be harmful. Evidence of changing DHA status in pregnant women over time can be seen when comparing the contemporary setting of ORIP with the earlier KUDOS trial,[14] where the baseline mean DHA status of women was approximately 20% lower. It will be important to consider appropriate omega-3 intakes and doses for supplementation, as a 'high' omega-3 status above 4.9% may be achieved with a high-fish intake, or a varied omnivorous diet and an additional 200–400 mg of DHA.[15]

Determining an individual woman's likelihood of benefiting from omega-3 supplementation to reduce her risk of early preterm birth based on her PUFA status would be the most precise way to inform recommendations about whether to supplement. Based on our examination of a

panel of PUFAs in early pregnancy, total omega-3 PUFA status appears to be the strongest candidate clinical biomarker on which to base decision-making around supplementation with omega-3 PUFA in pregnancy to reduce the risk of early preterm birth. To be able to provide tailored advice for women, a suitable omega-3 PUFA testing method is needed. One such method that is less invasive than venous blood puncture, and has advantages in terms of transport and storage of samples, is dried blood spot technology.[5] Although others have suggested that a red blood cell DHA value of <5% of total red blood cell fatty acids requires supplementation,[16] that estimation was based on observational data. Our conservative value of 4.1% of total omega-3 PUFA in whole blood has the benefit of being derived from a systematic study of seven biomarkers in a large RCT.

The consistency of total omega-3 fatty acid status being the best biomarker to influence early preterm birth for both spontaneous as well as iatrogenic deliveries raises two important issues. First, the postulated mechanism by which omega-3 PUFA induces the prolongation of pregnancy has in the past related to the balance of 2- and 3-series prostaglandins derived from AA and EPA, respectively, and almost exclusively relates to spontaneous labour. Our observations indicate that the likely biological mechanisms are broader and may involve the bioactive derivatives, oxylipins, of DHA that are known to alter the function of the placenta, and vascular and immune responses.[17–19] Second, the strength of the biomarker of total omega-3 PUFA also suggests that more complex mechanisms may be at play, since EPA and DHA can be synthesised from ALA and DPA and thus additionally increase omega-3 fatty acid status overall. Further work is needed to understand these underlying mechanisms.

Unlike the data for early preterm birth, the effect of omega-3 supplementation on preterm birth in ORIP was similar regardless of a woman's baseline PUFA status. This lack of effect modification may be due to supplementation ceasing at 34 weeks' gestation. In women with a higher baseline total omega-3 PUFA status (greater than the cross-over point of 4.5%), the harmful effects of the intervention were not apparent after 34 weeks' gestation. Previous research indicates that omega-3 status decreases rapidly after supplementation ceases,[20] and there is some suggestion that any effect on prolongation of gestation also falls away quickly once supplementation stops.[21]

A useful future direction would be to conduct an implementation study of a targeted approach, with women's omega-3 PUFA status evaluated in early pregnancy and only women who are depleted, receiving additional DHA. In such a study, it would be helpful to collect repeated measures of PUFA status after supplementation to explore the optimal target range of omega-3 status. Furthermore, it would be worthwhile to investigate whether effect modification by baseline PUFA status is explained to some degree by associated maternal comorbidities, genetic polymorphisms, or social or clinical characteristics.

Conclusions

The proportion of total omega-3 PUFA in the total fatty acids in maternal capillary blood was identified as the best biomarker to distinguish which women with singleton pregnancies are at increased risk of early preterm birth and are most likely to benefit from omega-3 supplementation to reduce this risk. Women with a low omega-3 status in early pregnancy are at higher risk of early preterm birth and are most likely to benefit from omega-3 supplementation to reduce this risk. Conversely, women with higher total omega-3 status in early pregnancy are at lower risk of early preterm birth and additional omega-3 supplementation may increase their risk.

Disclosure of interests

Dr Liu holds a patent (WO2013/10 40 25 A1) on stabilising and analysing fatty acids in a biologic sample stored on solid media, owned by Adelaide Research and Innovation, the University of Adelaide, and licensed to Xerion. Dr Gibson has received advisory board fees from Fonterra Co-operative Group, supplies from Croda UK, prepared supplies for a trial for Efamol/Wassen UK and holds a patent (WO2013/10 40 25 A1) on stabilising and analysing fatty acids in a biologic sample stored on solid media, owned by Adelaide Research and Innovation, the University of Adelaide, and licensed to Xerion. Dr Makrides has received advisory board fees paid to her institution from Fonterra Co-operative Group and Nestlé Nutrition Institute, supplies from Croda UK, and prepared supplies for a trial for Efamol/Wassen UK. Drs Simmonds, Sullivan, Skubisz, Middleton, Best, Yelland, Quinlivan, Zhou and McPhee have no conflicts of interest to disclose. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

KPB, LNY, JQ, SJZ, AJM and RAG conceived and designed the study. LAS, TRS, MS, PFM, GL, RAG and MM were involved in the analysis and interpretation of data. TRS carried out the statistical analyses. LAS and MS drafted the manuscript. All authors critically revised and edited the manuscript, and approved the final submitted version. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Details of ethics approval

Written informed consent was obtained from all participants in the original study and all procedures were conducted in accordance with the approval of the relevant Human Research Ethics Committees (HREC) at the six sites

involved in the multi-centre trial. The HREC at the lead study site was the Women's and Children's Health Network Human Research Ethics Committee, which provided approval (HREC/13/WCHN/10) on 1 May 2013 for the original study and the analysis reported in the present paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Histograms for the key fatty acids: total omega-3 PUFA1, EPA + DHA, DHA and EPA.

Figure S2. Risk of preterm birth in the control group by baseline PUFA status[1-5].

Figure S3. Relative risk of preterm birth (supplementation versus control) by baseline PUFA status[1-5].

Table S1. Polyunsaturated fatty acid (PUFA) status at trial entry[1]. ■

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