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Predictors of compliance with higher dose omega-3 fatty acid supplementation during pregnancy and implications for the risk of prematurity: exploratory analysis of the ORIP randomised trial

Thomas R Sullivan ,[1,2] Lisa N Yelland ,[1,2] Robert A Gibson,[1,3] Sagar K Thakkar,[4] Fang Huang,[5] Karen P Best,[1,6] Surabhi Devaraj,[7] Irma Silva Zolezzi,[4] Maria Makrides[1,6]

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For numbered affiliations see end of article.

Correspondence to
Dr Thomas R Sullivan;
thomas.sullivan@sahmri.com

ABSTRACT

Background Intention-to-treat analyses of the Omega-3 to Reduce the Incidence of Prematurity (ORIP) trial found that omega-3 (n-3) fatty acid supplementation reduces the risk of prematurity in the subgroup of women with a singleton pregnancy and low n-3 status early in pregnancy, but not overall. However, results may have been influenced by less-than-optimal compliance.

Objectives To identify predictors of compliance with n-3 supplementation and determine treatment effects among compliers.

Design Exploratory analyses of a multicentre-blinded randomised trial.

Setting 6 tertiary care centres in Australia.

Participants 5328 singleton pregnancies.

Interventions Daily capsules containing 900 mg n-3 long-chain polyunsaturated fatty acids or vegetable oil, consumed from before 20 weeks gestation until 34 weeks gestation.

Outcome measures Early preterm (<34 weeks gestation) and preterm birth (<37 weeks gestation). Women were considered compliant if they reported missing less than a third of their allocated capsules in the previous week during a mid-pregnancy appointment.

Results Among 2654 singleton pregnancies in the n-3 intervention group, 1727 (65%) were deemed compliant with supplementation. Maternal characteristics associated with compliance included age, years of full-time education, consuming alcohol but not smoking in the 3 months leading up to pregnancy, fewer previous births and taking dietary supplements at enrolment. Based on complier average causal effects, n-3 supplementation reduced the risk of preterm birth in compliers (relative risk=0.76; 95% CI 0.60 to 0.97), but not early preterm birth (relative risk=0.80; 95% CI 0.44 to 1.46). Consistent with intention-to-treat analyses, the lack of an overall effect on early preterm birth in compliers appeared to be due to beneficial effects in women with low n-3 status at enrolment but not women with replete status.

Conclusions Results in compliers were similar to those from intention-to-treat analyses, suggesting that non-compliance was not a major factor in explaining outcomes from the ORIP trial.

Trial registration number ACTRN12613001142729.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This investigation uses data from the largest randomised trial of omega-3 supplementation in pregnancy.
⇒ The study considers complier average causal effects, which attempt to address the shortcomings of classic per-protocol analyses.
⇒ The analyses were exploratory so findings should be interpreted with caution.
⇒ The trial was designed to detect the effect of omega-3 supplementation overall, not in the subgroup of women who complied, hence some comparisons had low statistical precision.

BACKGROUND

Preterm birth (PTB) before 37 weeks of gestation occurs in approximately 15 million pregnancies worldwide annually and is the leading cause of death among children.[1] Despite accounting for just 20% of PTBs, early preterm birth (EPTB) before 34 weeks of gestation is responsible for the largest burden of neonatal deaths and childhood disability.[2] Interventions that can safely lower the risk of PTB, especially EPTB, are a priority in maternal and child health research.

Our recently published Omega-3 to Reduce the Incidence of Prematurity (ORIP) randomised controlled trial evaluated whether n-3 long chain polyunsaturated

fatty acid (LCPUFA) supplementation (≈900 mg daily) during pregnancy reduces the risk of EPTB.[3] While primary results did not support n-3 supplementation for all pregnant women, further analyses indicated that n-3 supplementation was effective at reducing the risk of EPTB in women with a singleton pregnancy and low n-3 status at enrolment (whole blood total n-3 <4.2% of total fatty acids), but not in women with replete n-3 status (>4.9%).[3 4] Together with the results of a recent Cochrane review on n-3 supplementation,[5] ORIP findings are starting to influence clinical practice, with the Australia Pregnancy Care Guidelines now recommending n-3 supplementation for women with low status to reduce the risk of prematurity.[6]

To date, key findings from ORIP have been based on analyses conducted according to the intention-to-treat (ITT) principle, where participants are analysed as randomised, irrespective of protocol compliance.[7] When interpreting results from ITT analyses, consideration should be given to the degree of protocol compliance in the trial. In ORIP, 39% of women with a singleton pregnancy consumed at least 75% of their allocated capsules across the intervention period according to capsule count, and 62% reported consuming more than two-thirds of their allocated capsules during the past week at the 28-week phone appointment. Although compliance with supplementation was likely underestimated by equating missing data with non-compliance (eg, capsules were not returned for counting in 38% of pregnancies, with these pregnancies treated as non-compliant), evidently it was not absolute. A consequence of this suboptimal compliance is that ITT results, which describe the effects of recommending n-3 supplementation at a population level, may underestimate the causal effects of taking n-3 supplements as directed. An awareness of effects in those who comply, along with characteristics associated with compliance, is important to fully understanding and realising the benefits of n-3 supplementation in pregnancy.

Here we use data collected in the ORIP trial to identify predictors of compliance with n-3 supplementation and estimate effects of supplementation in compliers. We focus on women with singleton pregnancies and restrict attention to analyses involving the key clinical outcomes of ORIP, namely EPTB and PTB. In quantifying treatment effects in compliers, trialists often conduct per-protocol analyses where only those participants deemed compliant are retained for analysis. However, the approach breaks randomisation and can introduce bias when the groups of participants being compared no longer have similar characteristics. Given this limitation, we instead consider complier average causal effects (CACEs) in the ORIP data. Because this approach to compliance adjustment is not commonly used, we also take time to outline the rationale for CACEs and the assumptions involved in their calculation.

METHODS

ORIP trial

This investigation used data collected in the ORIP trial, a multicentre, double-blind, randomised controlled trial designed to evaluate whether n-3 LCPUFA supplementation during pregnancy reduces the risk of EPTB. The methods of the trial have been described in detail elsewhere.[3 8] Briefly, pregnant women less than 20 weeks of gestation were randomly allocated to receive three capsules daily, providing a total of ≈900 mg n-3 LCPUFA (~800 mg docosahexaenoic acid (DHA) and ~100 mg eicosapentaenoic acid, intervention group) or vegetable oil with trace amounts of n-3 LCPUFA (control group), with supplementation continuing until 34 weeks of gestation or birth, whichever occurred first. Gestational age at birth was determined according to the date of the last menstrual period and ultrasonographic data collected prior to 20 weeks of gestation, and was defined irrespective of whether onset of labour was spontaneous or induced. In total, 5544 pregnancies (5517 women) from six tertiary care centres in Australia were randomised into ORIP between November 2013 and April 2017. In the current study, we restrict attention to 5328 singleton pregnancies with known gestational age at birth.

Patient and public involvement

Pregnant women consented to, contributed data to and were informed of the main findings of the ORIP trial according to processes described previously.[3 8] There was no direct patient or public involvement in the present study.

Assessment of compliance

Two alternative definitions of n-3 supplementation compliance were pre-specified in the ORIP statistical analysis plan for undertaking ancillary per-protocol analyses, where non-compliant women were simply excluded from the analysis.[3] First, women were considered compliant if they returned their remaining capsules after the intervention period and had consumed at least 75% of the number of capsules expected. Second, women were deemed compliant if they completed the 28-week telephone contact and reported missing less than 7 out of the recommended 21 capsules in the previous week. Using the second definition of compliance resulted in considerably less missing data, particularly in women with an EPTB (missing for 40% vs 83% for compliance based on capsule return); as they had already given birth, most women with an EPTB missed their post-intervention period appointment at 34 weeks when capsule usage was evaluated. Compliance was also indirectly assessed via measures of n-3 LCPUFA status at 34 weeks of gestation in maternal capillary whole blood, collected on chemically treated filter paper as dried blood spots.[9] Importantly, a strong inverse dose–response relationship between the number of capsules missed in the past week at the 28-week telephone contact and DHA in blood at 34 weeks was observed (see online supplemental file 1). Given this

relationship, the relatively low amount of missing data and its pre-specification in the ORIP statistical analysis plan, we selected self-reported compliance at the 28-week telephone contact (missed less than 7 out of 21 capsules) as the primary measure of compliance for the current investigation.

Adjustment for non-compliance and the CACE

Per-protocol and as-treated analyses are often applied to account for non-compliance in randomised trials. Per-protocol analyses include only those participants deemed to be compliant with their randomised assignment, while as-treated analyses ignore randomisation and compare participants according to the treatment they received. Both approaches allow participants to self-select their group for analysis through non-compliance and so are subject to selection bias.[10 11] Given mounting arguments against the usefulness of these approaches, even as a supplement to a primary ITT analysis,[12] we instead sought to account for non-compliance in this investigation by estimating the CACE[13 14]; defined in this context as the effect of n-3 supplementation among the sub-population of women who would comply irrespective of the treatment they were assigned to receive. As this sub-population can be conceptualised as existing before randomisation, the CACE is not prone to selection bias like per-protocol and as-treated analyses, and so has a valid causal interpretation.

A key challenge in estimating the CACE is identifying those participants who would comply irrespective of treatment assignment. While we can determine whether a participant complied with their assigned treatment, often we cannot know whether they would have complied if hypothetically they were assigned to the alternative treatment. Fortunately, this problem of identification is simplified in trials where control group participants are unable to access the intervention, and hence where participants can be considered compliant if assigned to the control treatment. In this case, compliers in the intervention group can be assumed to be representative of the sub-population that would comply irrespective of treatment assignment and the CACE can be estimated using propensity scores.[15 16] The assumption that control group participants were unable to access the intervention was deemed reasonable in ORIP given the limited exposure control group women had to supplements containing n-3 LCPUFA. Of the 2674 women in the control group with a singleton pregnancy and known gestational age at birth, just 11 (0.4%) deviated from the protocol and reported taking supplements containing n-3 LCPUFA at the 28-week telephone contact, and 75 (2.8%) multivitamins containing n-3 LCPUFA. Additionally, the average dose of n-3 LCPUFA taken by these women was likely much lower than that provided to women in the intervention group, an assumption supported by levels of DHA in blood at 34 weeks (mean 2.7% in control group women taking supplements or multivitamins containing n-3 LCPUFA vs 3.2% in the intervention group).

The propensity score approach to estimating the CACE involves first fitting a statistical model (eg, logistic regression model) to identify predictors of compliance in the intervention group. As the sub-population of compliers under either treatment is taken to exist before randomisation, only baseline predictors of compliance require consideration. The fitted model is then used to calculate the probability of compliance with the intervention among participants assigned to the control group, which varies according to their values of the baseline predictors. Lastly, the CACE can be estimated using compliers in the intervention group and all control group participants, with the latter group reweighted according to their probability of compliance with the intervention. The rationale for reweighting is to ensure that control group participants resemble compliers in the intervention group, or equivalently, that the distribution of reweighted outcomes in the control group approximates what would have been observed had it only included women who would comply under either treatment. How well this works in practice, and subsequently the validity of the CACE approach, relies on the inclusion of all important predictors into the statistical model for compliance.[15]

Statistical methods

Statistical analyses were performed in Stata V.16 (College Station, Texas, USA: StataCorp LLC). Predictors of compliance in the intervention group were identified using logistic regression; all baseline characteristics collected in the ORIP trial were considered irrespective of expected associations with compliance. Several baseline characteristics were excluded from the modelling process based on feasibility considerations (eg, infrequent categories, collinearity with other measures) or lack of evidence for an association with compliance in either univariate logistic models or an initial multivariable logistic model using available data (p value for association >0.30). The full list of predictors excluded and justifications for exclusions are provided in online supplemental table 1. The final logistic model for compliance was then used to re-weight observations in the control group, for comparison with compliers in the intervention group. Lastly, the CACE for EPTB and PTB was expressed as a relative risk and estimated using log binomial regression, with adjustment made for variables used to stratify the randomisation (centre and prior n-3 LCPUFA supplement use) and robust variance estimation used to account for uncertainty in the estimated weights. Given previous findings from ITT analyses that the effect of n-3 supplementation on the risk of EPTB depends on baseline n-3 LCPUFA status,[4] we also explored how the CACE was modified by baseline status. Following recommendations in Royston and Sauerbrei,[17] baseline n-3 LCPUFA status was treated as a continuous variable in this analysis and the interaction with randomised group was modelled using two-term fractional polynomials.

A complicating factor for analysis was the extent of missing data on compliance and baseline characteristics

associated with compliance. As the probability of missing data on compliance depended on observed characteristics in the dataset (eg, more likely to be missing compliance if reporting an EPTB), multiple imputation implemented under a missing at random assumption was used to address missing data. Imputation was performed separately by randomised group using chained equations,[18] with linear and logistic models used to impute continuous and binary variables, respectively. To ensure compatibility with the intended analysis, compliance in the intervention group was imputed from a logistic model involving the same baseline characteristics used for reweighting control group observations. EPTB was also added to this model given its association with both compliance and missing data on compliance, while baseline n-3 LCPUFA status was included to facilitate the investigation of effect modification. Following the generation of 100 imputed datasets, the prediction model for compliance in the intervention group and the CACE was estimated within each imputed dataset and the results were combined across datasets using Rubin's rules.[19]

Given the potential dependence of findings on the measure of compliance chosen (missed <7 out of 21 capsules in the past week at the 28-week telephone contact), additional sensitivity analyses were performed adopting stricter (missed <4 capsules) and more relaxed (missed <11 capsules) definitions of compliance.

RESULTS

Of the 5544 pregnancies randomised in the ORIP trial, 5328 singleton pregnancies with known gestational age at birth were included in this analysis; 2654 in the intervention group and 2674 in the control group (figure 1). Capsule consumption at the 28-week telephone contact was available for 2454 out of 2654 pregnancies in the intervention group (92.5%). Following imputation of missing data, 1727 out of 2654 pregnancies in the intervention group (65.1%) were deemed to have met the pre-specified compliance definition of less than 7 of the recommended 21 capsules missed in the previous week. Among compliers, 21 EPTB (1.2%) and 99 PTB (5.7%) were reported. Conversely, among the 927 pregnancies in the intervention group deemed non-compliant with n-3 supplementation, 27 (2.9%) recorded an EPTB and 70 (7.5%) a PTB.

Following the steps outlined in the statistical methods, a predictive model for compliance in the intervention

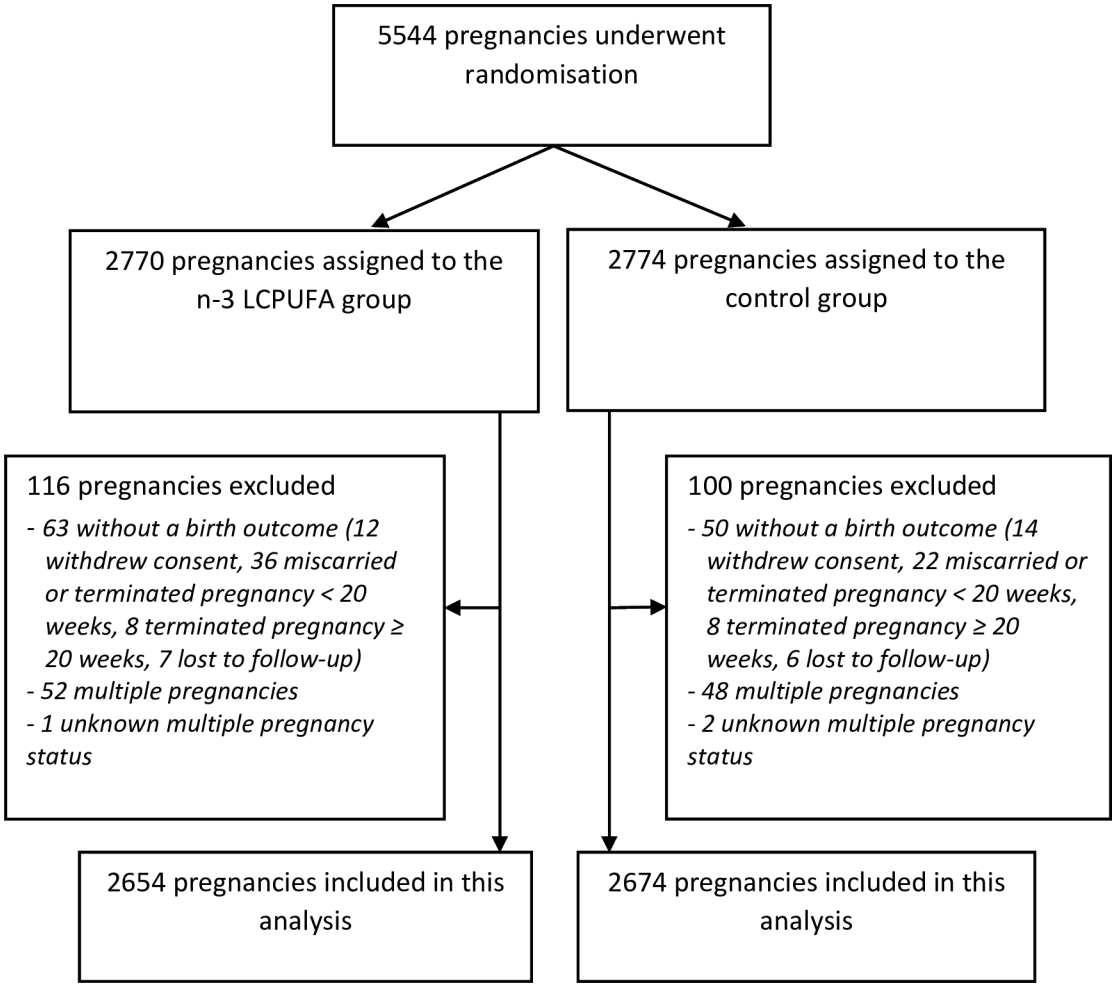

Figure 1 Flowchart of participants in the ORIP trial and the data available for this exploratory analysis. LCPUFA, long chain polyunsaturated fatty acid; ORIP, Omega-3 to Reduce the Incidence of Prematurity.

Table 1 Predictors of compliance with n-3 LCPUFA supplementation in the intervention group*

Baseline characteristic	Adjusted OR for compliance (95% CI)	P value
Gestational age at randomisation (weeks)	1.06 (1.02 to 1.10)	0.003
Mother's age (years)	1.04 (1.02 to 1.06)	<0.001
Years spent in full time education	1.07 (1.03 to 1.11)	0.001
Current height (cm)	1.01 (1.00 to 1.03)	0.09
Smoked cigarettes in 3 months leading up to pregnancy	0.71 (0.56 to 0.91)	0.008
Drank alcohol in 3 months leading up to pregnancy	1.30 (1.07 to 1.58)	0.009
Currently taking dietary supplements	1.64 (1.27 to 2.13)	<0.001
Number of previous births ≥20 weeks	0.84 (0.76 to 0.93)	0.001
Infant sex male	0.83 (0.70 to 0.99)	0.04
Alpha linoleic acid (%)†	1.29 (0.89 to 1.87)	0.19
Eicosapentaenoic acid (%)†	1.58 (1.01 to 2.49)	0.05
Centre (reference=Mater Mothers' Hospital)		0.004‡
Flinders Medical Centre	0.94 (0.69 to 1.28)	0.69
Joondalup Hospital	1.27 (0.77 to 2.10)	0.35
Lyell McEwin Hospital	0.65 (0.42 to 1.02)	0.06
Werribee Mercy Hospital	0.64 (0.46 to 0.88)	0.005
Women's and Children's Hospital	0.81 (0.58 to 1.15)	0.24
SEIFA index of socioeconomic disadvantage quintile (reference=quintile 1, most disadvantaged)		0.02‡
2	0.85 (0.59 to 1.22)	0.38
3	0.65 (0.46 to 0.92)	0.01
4	0.64 (0.45 to 0.91)	0.01
5 (least disadvantaged)	0.57 (0.40 to 0.83)	0.003
Race (reference=Caucasian)		0.04‡
North East Asian	1.94 (1.02 to 3.68)	0.04
Other	0.83 (0.57 to 1.20)	0.32
South East Asian	1.03 (0.66 to 1.61)	0.90
Southern and Central Asian	0.75 (0.55 to 1.02)	0.07

*Results obtained from a multivariable logistic regression model for compliance.
†Baseline fatty acid status measured from maternal capillary whole blood, collected on chemically treated filter paper as dried blood spots.
‡Global p value for categorical characteristics.
LCPUFA, long chain polyunsaturated fatty acid; SEIFA, Socio-Economic Indexes for Areas.

group was developed, with predictors of compliance identified in this process shown in table 1. Adjusting for other characteristics included in the model, the odds of compliance increased with weeks of gestational age at randomisation (OR=1.06; 95% CI 1.02 to 1.10), age in years (OR=1.04; 95% CI 1.02 to 1.06), years of full time education (OR=1.07; 95% CI 1.03 to 1.11), consuming alcohol in the 3 months leading up to pregnancy (OR=1.30; 95% CI 1.07 to 1.58) and taking dietary supplements at randomisation (OR=1.64; 95% CI 1.27 to 2.13). In contrast, characteristics associated with a reduction in compliance included smoking cigarettes in the 3 months leading up to pregnancy (OR=0.71; 95% CI 0.56 to 0.91) and number of previous births (OR=0.84; 95% CI 0.76 to 0.93). Substantial differences in compliance were also seen across the six study centres participating in ORIP (p value=0.004), and there was some evidence to suggest compliance varied by socioeconomic status (p value=0.02) and race (p value=0.04).

As an intermediate step in calculating the CACE, the predictive model for compliance in the intervention group was used to estimate the probability of compliance with n-3 supplementation for pregnancies randomised to the control group. After re-weighting control group pregnancies according to these probabilities, satisfactory balance was observed between compliers in the intervention group and the re-weighted control group on baseline characteristics predictive of compliance (see online supplemental table 2). This suggests that following re-weighting, the control group was representative of compliers in the intervention group, thus supporting the validity of the CACE approach.

After excluding non-compliers in the intervention group and re-weighting the control group, the CACE was calculated for the key clinical outcomes of EPTB and PTB (see table 2). There was little evidence for an overall effect of n-3 LCPUFA supplementation on the risk of EPTB among the sub-population of compliers (relative risk (RR)=0.80; 95% CI 0.44 to 1.46). In contrast, supplementation was associated with a 24% reduction in the risk of PTB in compliers (RR=0.76; 95% CI 0.60 to 0.97). Interestingly, for both outcomes RR estimates for the CACE were smaller in magnitude than corresponding effects from ITT analyses (RR 0.80 vs 1.12 for EPTB and 0.76 vs 0.81 for PTB), although qualitative conclusions based on statistical significance were unchanged.

Our previous ITT analyses of ORIP demonstrated that the effect of n-3 LCPUFA supplementation on the risk of EPTB varies according to baseline n-3 LCPUFA status (interaction p value=0.0009), with supplementation most beneficial in 885 women (17.5% of sample) with low status (whole blood total n-3 <4.2% of total fatty acids; RR=0.23, 95% CI 0.07 to 0.79).[4] When restricted to the sub-population of compliers, evidence for effect modification by baseline n-3 status was less compelling (interaction p value=0.14). As the pattern of effect modification in CACE analyses was very similar to that observed in earlier ITT analyses (figure 2), the lack of a statistically significant interaction test may be a product of the considerably smaller number of EPTBs occurring in the sub-population of compliers.

In sensitivity analyses, we explored the robustness of findings to the definition of compliance adopted. Whereas 1727 pregnancies in the intervention group were deemed compliant according to the primary measure of compliance (missed <7 capsules in the past week), 1505 and 1828 pregnancies satisfied the stricter (missed <4 capsules) and more relaxed (missed <11 capsules) compliance definitions, respectively. In sensitivity analyses, there was little difference in the CACE for PTB across the compliance definitions (RR range 0.75 to

Table 2 Treatment effects for early preterm and preterm birth

Treatment effect	Outcome	n-3 LCPUFA, n (%)	Control, n (%)	Adjusted relative risk (95% CI)	P value
Intention to treat*	Early preterm birth	48/2654 (1.81)	43/2674 (1.61)	1.12 (0.75 to 1.69)	0.58
	Preterm birth	169/2654 (6.37)	209/2674 (7.82)	0.81 (0.67 to 0.99)	0.04
Complier average causal effect†	Early preterm birth	21/1727 (1.22)	26/1730 (1.51)	0.80 (0.44 to 1.46)	0.47
	Preterm birth	99/1727 (5.75)	131/1730 (7.57)	0.76 (0.60 to 0.97)	0.03

*Intention-to-treat estimates are for singleton pregnancies with known gestational age at birth and so do not correspond exactly with previously reported estimates for all randomised pregnancies.
†Complier average causal effect quantifies the effect of n-3 supplementation in the sub-group of compliers; estimated using multiple imputation, with numerators for the n-3 LCPUFA and control groups rounded to the nearest whole number.
LCPUFA, long chain polyunsaturated fatty acid; SEIFA, Socio-Economic Indexes for Areas.

0.77; p value=0.03 for all definitions), while the CACE for EPTB was more pronounced (but not statistically significant) when adopting a stricter compliance definition (RR=0.62; 95% CI 0.31 to 1.23) (see online supplemental table 1). The CACE for EPTB was not significantly modified by baseline n-3 status in the additional sensitivity analyses (interaction p values >0.05), although the pattern of effect modification remained similar to that seen in the ITT analyses (see figure 2).

DISCUSSION

This analysis resolves uncertainties concerning the effects of compliance on ITT estimates in the largest trial of n-3 supplementation in pregnancy. Consistent with ITT results, taking n-3 supplements as directed did not reduce the risk of EPTB overall, but appeared beneficial in women with low n-3 status early in pregnancy. This suggests that the overall null effect of n-3 LCPUFA supplementation on EPTB observed in earlier ITT analyses was not due to a lack of compliance, but instead predominantly a result of differential effects in women according to their n-3 status. In contrast, complying with n-3 supplementation was found to reduce the risk of PTB by 24%, a similar but slightly more pronounced benefit than the 19% reduction observed in ITT analyses. Overall, these results support current evidence for women with a singleton pregnancy and low total n-3 status to take n-3 supplements during pregnancy to reduce their risk of EPTB.[6]

In the analyses presented here, we did not observe large differences between CACE and ITT estimates, particularly for PTB and effect modification by baseline n-3 status on EPTB. This refutes the suggestion[19] that non-compliance

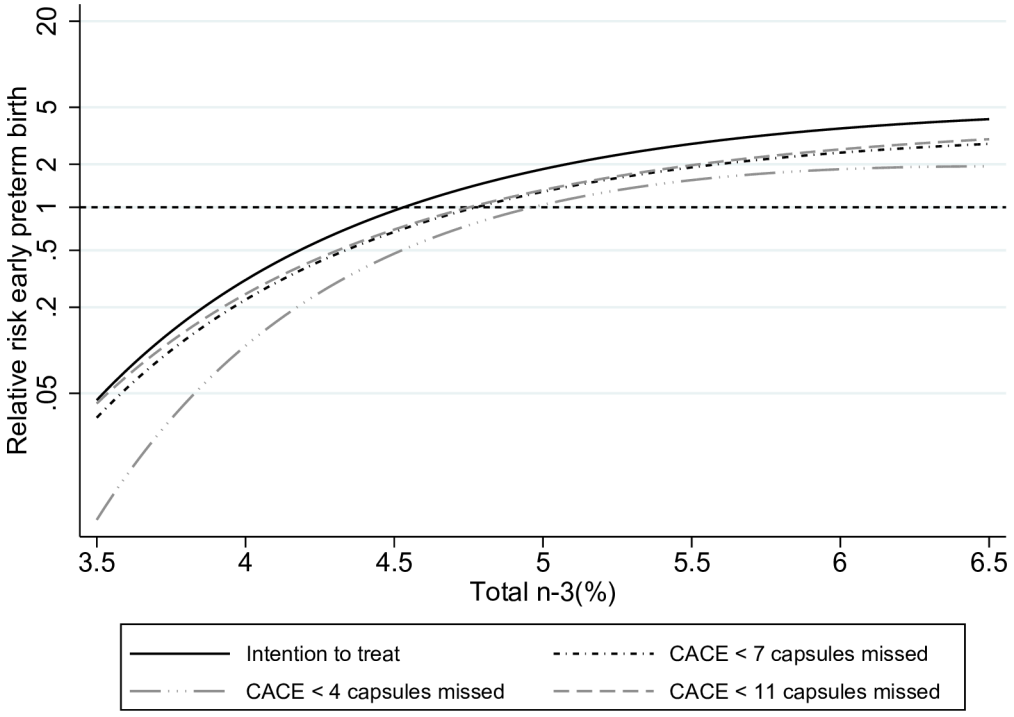

Figure 2 Effect modification by baseline n-3 LCPUFA status, comparison of intention-to-treat and CACE estimates. CACE, complier average causal effect; LCPUFA, long chain polyunsaturated fatty acid.

in ORIP was a major contributor to the null effect observed in the ITT analysis for EPTB. Following imputation, 65% of intervention group women were estimated to have adequately complied with n-3 supplementation, a sufficiently high percentage to expect a reasonable degree of concordance between ITT and CACE estimates. Similarities between these estimates could also be a result of women classified as non-compliant deriving benefits from supplementation. Such a situation could arise if the effective dose of n-3 supplementation for reducing the risk of PTB and EPTB is lower than the ≈900 mg daily dose provided in ORIP.

In a recent secondary analysis of the ADORE randomised trial, Carlson and colleagues explored the effects of complying with DHA supplementation during pregnancy on EPTB risk.[20] Although ADORE involved different treatment regimens (1000 mg/day DHA (high DHA) vs 200 mg/day (low DHA)) and measures of compliance (postpartum red blood cell phospholipid level ≥5.5% or ≥ 8.0%) than ORIP, and their secondary analysis considered per-protocol effects rather than the CACE, similar findings were reported. Notably, they observed a trend towards a lower risk of EPTB among compliers (12/492 (2.4%) in the low DHA arm vs 4/319 (1.3%) in compliers in the high DHA arm (red blood cell phospholipid level ≥8.0%)), with group differences more pronounced among women in the lowest two quartiles of DHA status at enrolment (11/244 (4.5%) in the low DHA arm vs 1/121 (0.8%) in compliers in the high DHA arm). This suggests our findings may generalise to other similar populations and interventions.

Consistent with the findings of Carlson and colleagues,[21] we observed higher rates of compliance with n-3 supplementation among older and more educated women. Owing to the large sample size of ORIP, we also identified several additional predictors of compliance, including consumption of dietary supplements early in pregnancy, drinking alcohol but not smoking in the 3 months leading up to pregnancy, higher socioeconomic status and reporting fewer previous births. Moving from the context of equipoise in a clinical trial to the translation of positive findings into practice, it is difficult to know whether overall rates and predictors of compliance would remain the same. Perhaps the promotion of known benefits or the ability to modify the dosing regimen (eg, to one instead of three capsules daily) could improve compliance with supplementation in practice. Although CACE and ITT results were similar, treatment effects were slightly more pronounced when the analysis was restricted to compliers, and clearly n-3 supplementation can only convey benefit to women who at least commence taking supplements (ie, partially comply). Thus, it may be prudent to monitor and consider strategies to enhance compliance as results are translated into practice, particularly since several of the identified predictors of non-compliance (eg, age, education, socioeconomic status and smoking) have also been associated with elevated risks of PTB.

The major strength of this investigation lies in the design and conduct of the ORIP trial. ORIP assessed n-3 LCPUFA supplementation in a broad population of women, with large numbers, detailed outcome data collection, full allocation concealment and high rates of follow-up. Another strength is the use of rigorous statistical methods in this study, including multiple imputation to address missing data and consideration of the CACE (in preference to per-protocol analyses, which have been extensively criticised). Conversely, limitations include the post-hoc nature of analyses and reduced statistical precision due to the exclusion of non-compliant women during the calculation of the CACE. Of the 48 EPTBs that occurred in the intervention arm and were included in ITT analyses, just 21 were observed in women defined compliant with the intervention. The investigation was also limited by the measures of compliance available in ORIP. Self-reported capsule consumption at the 28 week telephone contact was chosen as the primary indicator of compliance based on its pre-specification in the ORIP statistical analysis plan and the relatively low amount of missing data. Yet the measure only pertained to a single week of the intervention period and may have been subject to reporting and recall bias. However, the strong relationship between capsule consumption at the 28-week telephone contact and DHA in blood at 34 weeks of gestation suggests such biases may have been limited. Finally, we did not explore effect modification by determinants of EPTB other than baseline n-3 status, although we found limited evidence for other effect modifiers in earlier ITT analyses of the ORIP trial.[22]

In conclusion, our results indicate that lack of compliance with n-3 supplementation was not a major factor in explaining the outcomes of the ORIP trial. Rather, they support current recommendations for women with low total n-3 status to take n-3 supplements during pregnancy to reduce their risk of EPTB. The modest increases in suggested benefits with improved compliance indicate that measures to enhance compliance with n-3 supplementation should be considered as n-3 strategies are now translated into practice.

Author affiliations
[1]SAHMRI Women & Kids, South Australian Health and Medical Research Institute Limited, Adelaide, South Australia, Australia
[2]School of Public Health, The University of Adelaide, Adelaide, South Australia, Australia
[3]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, South Australia, Australia
[4]Nestlé Product Technology Center—Nutrition, Societe des Produits Nestle SA, Vevey, Vaud, Switzerland
[5]Nestlé Research, Societe des Produits Nestle SA, Beijing, China
[6]School of Medicine, The University of Adelaide, Adelaide, South Australia, Australia
[7]Nestlé Research, Societe des Produits Nestle SA, Singapore

Twitter Karen P Best @kpb2901

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Contributors TRS, LNY and MM designed the study. TRS performed the statistical analyses and led the first draft of the manuscript. TRS, LNY, SKT, FH, SD, ISZ and MM attended regular meetings to discuss the direction of the study and interpret the results. LNY, RAG, KPB and MM provided input into the selection and validation

of compliance measures. All authors critically revised the manuscript and approved the final version for submission. TRS is responsible for the overall content as guarantor.

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Competing interests RAG has received 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. SKT, FH, SD and ISZ are employees of Société des Produits Nestlé (SPN). MM has received supplies from Croda UK, and prepared supplies for a trial for Efamol/Wassen UK. The other authors do not have any competing interests.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Women's and Children's Health Network Human Research Ethics Committee (HREC/13/WCHN/10). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. De-identified data are available to researchers who provide a methodologically sound research proposal following review and approval by the ORIP trial steering committee and completion of a signed data access agreement. Requests can be made to maria.makrides@sahmri.com or karen.best@sahmri.com.

ORCID iDs
Thomas R Sullivan http://orcid.org/0000-0002-6930-5406
Lisa N Yelland http://orcid.org/0000-0003-3803-8728

REFERENCES

1 March of Dimes. The partnership for maternal, newborn & child health, save the children, world health organization. born too soon: the global action report on Preterm births Geneva World Health Organization; 2012.
2 Martin JA, Osterman MJK. Describing the increase in Preterm births in the United States, 2014-2016. *NCHS Data Brief* 2018;2018:1–8.
3 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;381:1035–45.
4 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;127:975–81.
5 Middleton P, Gomersall JC, Gould JF, *et al*. Omega-3 fatty acid addition during pregnancy. *Cochrane Database Syst Rev* 2018;11:CD003402.
6 Australian Government Department of Health. Clinical practice guidelines, pregnancy care [Canberra]. 2020.
7 Hollis S, Campbell F. What is meant by intention to treat analysis? survey of published randomised controlled trials. *BMJ* 1999;319:670–4.
8 Zhou SJ, Best K, Gibson R, *et al*. Study protocol for a randomised controlled trial evaluating the effect of Prenatal Omega-3 LCPUFA supplementation to reduce the incidence of Preterm birth: the ORIP trial. *BMJ Open* 2017;7:e018360.
9 Liu G, Mühlhäusler 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:S0952-3278(14)00153-7:251–60.:.
10 Mansournia MA, Higgins JPT, Sterne JAC, *et al*. Biases in randomized trials: A conversation between trialists and Epidemiologists. *Epidemiology* 2017;28:54–9.
11 ICH E9 Expert Working Group. Statistical principles for clinical trials international conference on Harmonisation E9 expert working group. *Stat Med* 1999;18:1905–42.
12 White IR. Uses and limitations of randomization-based efficacy Estimators. *Stat Methods Med Res* 2005;14:327–47.
13 Imbens GW, Rubin DB. Bayesian inference for causal effects in randomized experiments with noncompliance. *Ann Statist* 1997;25:23.
14 Little RJ, Rubin DB. Causal effects in clinical and Epidemiological studies via potential outcomes: concepts and Analytical approaches. *Annu Rev Public Health* 2000;21:121–45.
15 Jo B, Stuart EA. On the use of propensity scores in principal causal effect estimation. *Stat Med* 2009;28:2857–75.
16 Herbert RD, Kasza J, Bø K. Analysis of randomised trials with long-term follow-up. *BMC Med Res Methodol* 2018;18:48:48.:.
17 Royston P, Sauerbrei W. A new approach to Modelling interactions between treatment and continuous covariates in clinical trials by using fractional polynomials. *Stat Med* 2004;23:2509–25.
18 White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med* 2011;30:377–99.
19 Rubin DB. Multiple imputation for Nonresponse in surveys. In: *Multiple imputation for nonresponse in surveys*. New York: Wiley & Sons, 9 June 1987.
20 Carlson SE, Gajewski BJ, Valentine CJ, *et al*. Early and late Preterm birth rates in participants adherent to randomly assigned high dose Docosahexaenoic acid (DHA) supplementation in pregnancy. *Clin Nutr* 2023;42:S0261-5614(23)00011-0:235–43.:.
21 Carlson SE, Gajewski BJ, Alhayek S, *et al*. Dose-response relationship between Docosahexaenoic acid (DHA) intake and lower rates of early Preterm birth, low birth weight and very low birth weight. *Prostaglandins Leukot Essent Fatty Acids* 2018;138:S0952-3278(18)30172-8:1–5.:.
22 Yelland LN, Sullivan TR, Gibson RA, *et al*. Identifying women who may benefit from higher dose Omega-3 supplementation during pregnancy to reduce their risk of Prematurity: exploratory analyses from the ORIP trial. *BMJ Open* 2023;13:e070220.