Long-Chain Polyunsaturated Fatty Acid Status During Pregnancy and Maternal Mental Health in Pregnancy and the Postpartum Period:
Results From the GUSTO Study

Mary F. F. Chong, PhD[a,b,e,*]; Yi-Lin Ong, BSc[b]; Philip C. Calder, PhD[i,k]; Marjorelee Colega, BSc[b]; Jocelyn X. Y. Wong, BSc[b]; Chuen Seng Tan, PhD[c]; Ai Lin Lim, PhD[b]; Helena L. Fisk, BSc[i]; Shirong Cai, PhD[f]; Wei Wei Pang, PhD[f]; Birit F. P. Broekman, PhD[b,d]; Seang Mei Saw, PhD[c]; Kenneth Kwek, MBBS[g,h]; Keith M. Godfrey, PhD[j]; Yap-Seng Chong, MD[b,f]; Peter Gluckman, PhD[a,b,l]; Michael J. Meaney, PhD[b,m]; and Helen Chen, MMedPsych, DipPsychotherapy[g,h]; on behalf of the GUSTO Study Group

ABSTRACT

Objective: Studies have demonstrated a relationship between lower omega-3 long-chain polyunsaturated fatty acid (LC-PUFA) status and anxiety and depression. It is uncertain whether similar associations occur in pregnant women, when anxiety and depression could have long-term effects on the offspring. We examined the associations between plasma LC-PUFA status during pregnancy and perinatal mental health.

Method: At 26–28 weeks' gestation, plasma LC-PUFAs were measured in mothers of the Growing Up in Singapore Toward healthy Outcomes (GUSTO) mother-offspring cohort study, who were recruited between June 2009 and September 2010. Maternal symptoms of anxiety and depression were assessed with the State-Trait Anxiety Inventory (STAI) and Edinburgh Postnatal Depression Scale (EPDS) during the same period and at 3 months' postpartum. The STAI-state subscale was used as a continuous measure of current anxiety, while EPDS scores ≥ 15 during pregnancy or ≥ 13 postpartum were indicative of symptoms of probable depression.

Results: In adjusted regression analyses (n = 698), lower plasma total omega-3 PUFA concentrations (β = −6.49 STAI-state subscale scores/unit increase of omega-3 fatty acid; 95% CI, −11.90 to −1.08) and higher plasma omega-6:omega-3 PUFA ratios (β = 6.58 scores/unit increase of fatty acid ratio; 95% CI, 1.19 to 12.66), specifically higher arachidonic acid (AA):docosahexaenoic acid, AA:eicosapentaenoic acid, and AA:docosapentaenoic acid ratios, were associated with increased antenatal anxiety ($P < .05$ for all), but not postpartum anxiety. There was no association between plasma PUFAs and perinatal probable depression.

Conclusions: No association was found with probable depression in pregnancy or postpartum. Lower plasma omega-3 fatty acids and higher omega-6:omega-3 fatty acid ratios were associated with higher antenatal anxiety, but not postpartum anxiety. Replication in other studies is needed to confirm the findings and determine the direction of causality.

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[a]Clinical Nutrition Research Centre, [b]Singapore Institute for Clinical Sciences, A*STAR
[c]Saw Swee Hock School of Public Health, Departments of [d]Psychological Medicine, [e]Paediatrics, and [f]Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System
[g]KK Women's and Children's Hospital, Singapore
[h]Duke-National University of Singapore
[i]Human Development and Health Academic Unit, and [j]MRC Lifecourse Epidemiology Unit, NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom
[k]Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[l]Liggins Institute, Auckland, New Zealand
[m]Ludmer Centre for Neuroinformatics and Mental Health, McGill University, Montreal, Canada

**Corresponding author:* Mary F. F. Chong, PhD, Singapore Institute for Clinical Sciences, Brenner Centre for Molecular Medicine, 30 Medical Drive, Singapore 117609 (mary_chong@sics.a-star.edu.sg).

Maternal anxiety and depression during and immediately following pregnancy are increasingly recognized as major public health issues, with prevalence rates of 13%–25% in developed countries.[1] The negative effects of poor maternal mental well-being on fetal development are widely known. Antenatal anxiety has been linked to shorter gestational length,[2] lower birth weight offspring,[3] and adverse effects on infant neurodevelopment[4] and stress regulation,[5] and it has been found to be predictive of child behavioral and emotional problems.[6] Similarly, perinatal depression has been associated with developmental problems[7–12] in the offspring, which are further compounded by its negative impact on mother-child interaction.[13,14] It is thus critical to understand the basis for individual differences in mood disorders over the gestational period.

There is growing interest in the effects of long-chain polyunsaturated fatty acids (LC-PUFAs) on affective disorders, such as anxiety and depression and their related symptoms. Long-chain polyunsaturated fatty acids consist of omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and omega-6 fatty acids such as arachidonic acid (AA), which are vital for fetal neural and retinal development.[15] During pregnancy, accretion of maternal, placental, and fetal tissue leads to higher LC-PUFA requirements.[16] Consequently, maternal omega-3 PUFA depletion can occur during pregnancy, which has been postulated to increase the risk of anxiety and depression during pregnancy and the postpartum period. Indeed, affective disorders have been linked to poor omega-3 PUFA status, with supplementation studies[17,18] demonstrating the effectiveness of omega-3 PUFAs as treatment.

There are candidate pathways that suggest biologically plausible influences of PUFAs in

Clinical Points

- Current studies do not offer consistent or conclusive evidence on the associations between maternal long-chain polyunsaturated fatty acid (PUFA) status during pregnancy and symptoms of perinatal anxiety and depression.
- Similar to findings in a growing number of studies, maternal depression during the antenatal and postpartum period was not found to be associated with maternal fatty acid status during pregnancy in this cohort.
- Lower maternal omega-3 PUFA plasma concentrations and higher omega-6:omega-3 fatty acid ratios during late pregnancy were associated with symptoms of antenatal anxiety, but it is still unclear if this altered PUFA status is a cause or consequence of antenatal anxiety.

the development of affective disorders, including anxiety and depression. The omega-3 PUFAs are important players in signal transduction, affecting the release and function of dopamine and serotonin in neural systems,[19] which mediate the processes critical for mental health.[20] Various neuroinflammatory processes have also been suggested, in which derivatives of neuronal omega-3 and omega-6 PUFAs play a role. Both DHA and EPA possess anti-inflammatory and neuroprotective properties, which are important in counteracting the proinflammatory responses to AA and other omega-6 fatty acids.[20] Consequently, this cytoprotective function is heavily dependent on the omega-6:omega-3 PUFA ratios or, specifically, AA:DHA and AA:EPA ratios, which have shown to be consistently associated with various affective disorders and other mental disorders.[20] It has also been suggested that lower omega-3 fatty acids may facilitate excessive stress responses and hypothalamic pituitary adrenal hyperactivity by its association with higher levels of corticotropin-releasing hormone (CRH).[21] Increased concentrations of CRH have been reported in both anxiety and depression.[22]

Results from studies examining the relationships between maternal LC-PUFA levels and perinatal mood are inconsistent. While large observational cohort studies such as the Osaka Maternal and Child Health Study[23] (n = 865) and Danish National Birth Cohort[24] (n = 54,202) have reported no significant associations between total omega-3 or total omega-6 PUFA intakes, or omega-3:omega-6 ratios during pregnancy and risk of postpartum depression, a few smaller observational studies (sample sizes range from 48 to 529) have found low plasma omega-3 PUFA concentrations or intakes during pregnancy to be associated with increased risk of postpartum depression.[25–27] Supplementation studies examining perinatal depression also give mixed results, with a study[28] reporting improved perinatal depressive symptoms after omega-3 PUFA supplementation, while others[29–34] reporting no improvement after fish oil, omega-3 PUFA, DHA, or EPA supplementation. In contrast, fewer studies examine the association of omega-3 PUFAs with perinatal anxiety, with the only one known cohort study documenting an association between increased dietary omega-3 PUFA intake during pregnancy and reduced symptoms of antenatal anxiety.[35] Evidence for the effect of omega-3 PUFAs in ameliorating anxiety states and disorders comes mainly from supplementation studies in patients with cardiac conditions, drug abusers, alcoholics, and nondepressed students.[36–39]

In this study, we examined the associations of maternal plasma LC-PUFA status during pregnancy with antenatal and postpartum symptoms of anxiety and depression in a prospective cohort consisting of Asian mothers. We also examined the associations with specific omega-6 to omega-3 fatty acid ratios. We postulated that lower omega-3 fatty acid status and higher omega-6 to omega-3 fatty acid ratios during pregnancy would be associated with higher risk of probable anxiety and depression at both antenatal and postpartum periods.

METHOD

Study Design

We used data from the Growing Up in Singapore Toward healthy Outcomes (GUSTO) study,[40] the first prospective mother-offspring cohort study in Singapore, which consisted of 1,247 women recruited between June 2009 and September 2010. The GUSTO study aims to evaluate the role of early life influences on offspring epigenetic state and later risk of metabolic disease. Further details of the study can be obtained from Soh et al.[40] In brief, this study was granted ethical approval by the institutional review boards of the respective hospitals involved (ClinicalTrials.gov identifier: NCT01174875). The inclusion criteria included pregnant women who were within the age range of 18–50 years and were recruited from 2 major public maternity units in National University Hospital (NUH) and Kedang Kerbau Women's and Children's Hospital (KKH). Participants recruited were Singaporean citizens or permanent residents of Chinese, Malay, or Indian ethnicity with parents of homogenous ethnic background, with the intention of delivering in KKH or NUH and residing in Singapore for the next 5 years and who were willing to donate birth tissues including cord, placenta, and cord blood at delivery. Exclusion criteria included preexisting health conditions such as type 1 diabetes mellitus, depression, or mental health-related disorders self-reported during recruitment. Written informed consent was collected from all participants upon recruitment.

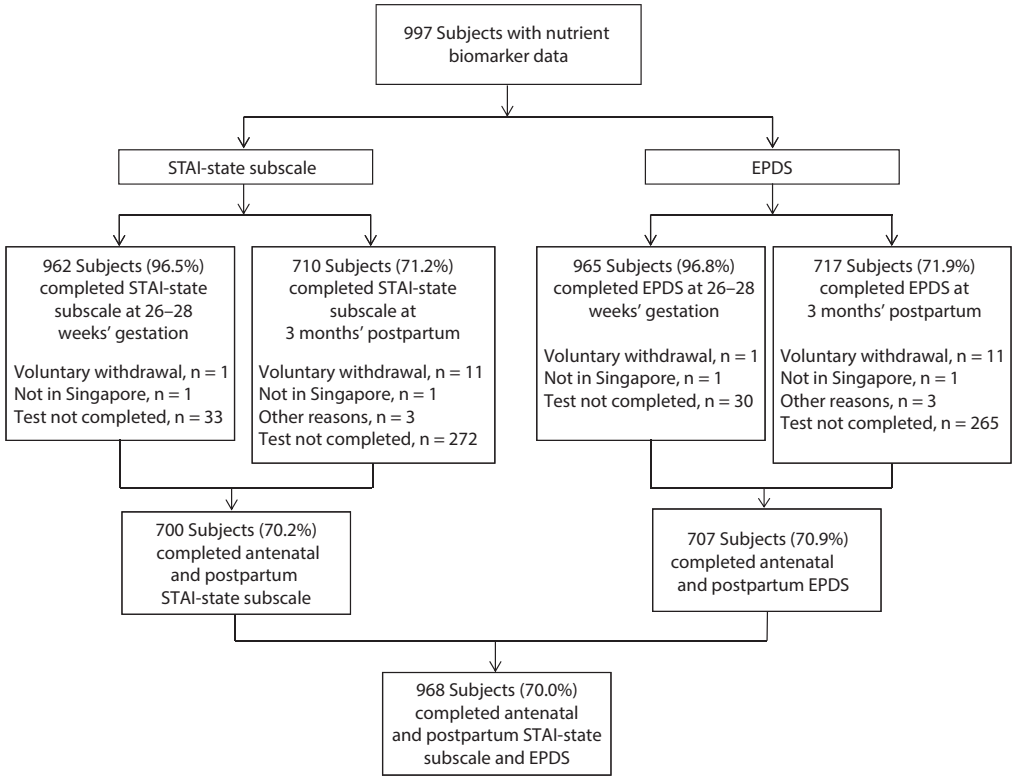
Maternal Characteristics

Demographic data including socioeconomic status, physical activity, smoking and alcohol status, and self-reported obstetric and medical history data were collected from the women at 26–28 weeks' gestation during the follow-up clinic visit. Information including obstetric and neonatal complications, infant sex, and mode of delivery was obtained at delivery.

Assessment of Perinatal Mental Health

Perinatal anxiety was assessed with the State-Trait Anxiety Inventory (STAI). The STAI-state subscale was used as a

Figure 1. Flowchart of Study Participants Who Completed State-Trait Anxiety Inventory (STAI)-State Subscale and Edinburgh Postnatal Depression Scale (EPDS) Tests at 26–28 Weeks' Gestation and at 3 Months' Postpartum



continuous measure of current anxiety, as opposed to the trait subscale, which measures trait-anxiety. Previous studies[41,42] carried out on antenatal and postpartum assessment of maternal anxiety indicated good internal consistency, with Cronbach α values between .94 and .95. Perinatal depressive symptoms were measured with the Edinburgh Postnatal Depression Scale (EPDS). The EPDS is a widely used, self-reported, 10-item screening questionnaire for postpartum depression.[43] Based on Spitzer's Research Diagnostic Criteria[44] and the *Diagnostic and Statistical Manual of Mental Disorders*, scores above a cutoff of 12/13 and 14/15 were used to identify probable postpartum depression and probable antenatal depression, respectively.[45] In this study, subjects with probable postpartum depression and probable antenatal depression were identified as having EPDS scores of ≥ 13 and ≥ 15, respectively. Both the STAI and EPDS were self-administered by the women at 26–28 weeks' gestation and at 3 months' postpartum.

Plasma LC-PUFA Concentrations

Among the 1,162 mothers who conceived naturally, 997 mothers with singleton live births had available blood samples for plasma PUFA measurement. Blood samples taken at 26–28 weeks' gestation were processed and plasma stored at −80°C. Total lipid extraction was carried out with chloroform/methanol (2:1 vol/vol) (Fisher Scientific), and phosphatidylcholine (PC) (Avanti Polar Lipids), which contributes about 75% plasma phospholipids, was isolated by solid-phase extraction[46] on aminopropyl silica cartridges (Agilent) and eluted with chloroform/methanol (3:2 vol/vol). Fatty acid methyl esters were generated by reaction of purified PC with 2% sulfuric acid (vol/vol) (Sigma-Aldrich) at 50°C for 2 hours, extracted into hexane, and separated by gas chromatography. A BPX-70 column (30 m × 220 μm; film thickness, 0.25 μm) (SGE Analytical Science) fitted to a Hewlett-Packard HP6890 gas chromatograph was used for separation with helium as the running gas, and detection of fatty acid methyl esters by flame ionization before quantification was conducted with the ChemStation software (Agilent). Data were expressed as absolute concentration (μg/mL plasma). For all fatty acids within plasma PC, the coefficient of intra-assay variation was < 3% and coefficient of interassay variation was < 6%.

Study Population

Among the 997 participants with available plasma PUFA data, 962 participants (96.5%) completed the STAI-state subscale at 26–28 weeks' gestation and 710 (71.2%) at 3 months' postpartum. Additionally, 965 participants (96.8%) completed the EPDS at 26–28 weeks' gestation and 717 (71.9%) at 3 months' postpartum. In total, 698 eligible participants (70.0%) completed both questionnaires at both time points and had measurements of LC-PUFAs (Figure 1 provides details of excluded participants). A

comparison between the characteristics of the participants who completed both questionnaires at both time points and those who failed to complete any showed no significant difference in sociodemographic profile, obstetric and medical history, as well as lifestyle (see Supplementary eTable 1 at Psychiatrist.com).

From the 698 eligible participants with completed measures, frequencies of missing demographic data were low (< 5%) and, hence, imputed to retain good population representation. Data were assumed to be missing at random, and ordinal variables were imputed as categories with the highest observed frequency in the sample population.

Statistical Analysis

Statistical analyses were carried out on the 698 participants to determine associations between plasma PC PUFA levels at 26–28 weeks' gestation and perinatal mental well-being. STAI-state subscale scores were used as a continuous measure of anxiety in multiple linear regression analysis, while high EPDS scores were used as a categorical measure of symptoms of probable depression in multiple logistic regression analysis. Both analyses were carried out on scores at 26–28 weeks' gestation and 3 months' postpartum. Separate regression models were used for associations with various plasma PUFA concentrations. These include total plasma omega-3 and omega-6 PC PUFA concentrations (model 1), plasma omega-6:omega-3 PC PUFA ratio (model 2), and ratios AA:DHA (model 3), AA:EPA (model 4), and AA:docosapentaenoic acid (DPA) (model 5). The effect of mutual adjustment for plasma ratios of AA:DHA, AA:EPA, and AA:DPA (model 6) was also investigated. Docosapentaenoic acid is a PUFA metabolite synthesized endogenously from EPA. It accounts for nondietary processes and has been shown to correlate strongly with circulating EPA levels instead of dietary intake.[47] The omega-6:omega-3 PUFA ratio was calculated with the sum of all plasma omega-6 PUFA concentrations (μg/mL) as the numerator and the sum of all plasma omega-3 PUFA concentrations as the denominator. Plasma fatty acid concentrations and ratios were normalized by log transformation to ensure normal distributions of the data before inclusion in regression models. To further examine dose response, total omega-3 PC PUFA concentrations were modeled as tertiles and included in linear regression models.

In the regression models, outcomes were adjusted for potential confounders, including ethnicity, parity, education level, marital status, maternal body mass index (BMI) at 26–28 weeks' gestation, maternal age, employment status, and variables associated with maternal and infant health status, including obstetric and neonatal complications; smoking status and smoke exposure before and during pregnancy; alcohol consumption before and during pregnancy; history of abortion, miscarriage, and stillbirth; frequency of exercise; and reported fish oil supplementation. Smoke exposure was defined as living with at least 1 person smoking on a daily basis for 6 months or more. Regular smoking was defined as smoking on a daily basis for a year or more. Outcomes at 3 months' postpartum were further adjusted for infant sex,[48–51] mode of delivery,[52–54] and respective STAI-state or EPDS scores at 26–28 weeks' gestation.

All statistical analyses were performed on standard statistical software (SPSS Version 16.0, SPSS Inc). Analyses are presented with 95% confidence intervals (CIs), and statistical significance was identified by P values < .05.

RESULTS

Characteristics of Participants

Of the 698 mothers analyzed, the median (range) of total omega-3 and omega-6 PUFAs in plasma PC were 142.4 μg/mL (36.2–690.4) and 799.4 μg/mL (271.8–2,186.3), respectively. Mothers with lower educational levels who smoked or were exposed to smoke before and during pregnancy and who reported symptoms of probable antenatal and postpartum depression had higher antenatal and postpartum anxiety scores than their counterparts. Mothers who did not engage in exercise and were of Malay ethnicity also tended to have higher antenatal anxiety scores (P < .05 for all) (Table 1). No significant differences in perinatal anxiety scores were observed for the other maternal characteristics (Supplementary eTable 2).

For probable depression, 7.2% (n = 50) were identified as having EPDS scores ≥ 15 at 26–28 weeks' gestation, and 10.3% (n = 72) had EPDS scores ≥ 13 at postpartum. Mean ± SD plasma PC omega-3 PUFA concentrations were not significantly different between those with probable antenatal depression and those without (161.0 ± 81.3 vs 154.6 ± 81.0 μg/mL; P = .59) and between those with probable postnatal depression and those without (159.9 ± 81.0 vs 165.9 ± 84.2 μg/mL; P = .55). This was similar for the maternal plasma PC omega-6 PUFA concentrations and omega-6:omega-3 PUFA ratios (Supplementary eTables 2 and 3). Mothers with probable antenatal depression had lower educational levels, were more likely to have smoked or been exposed to smoke before and during pregnancy, were younger, and were of Indian ethnicity (P < .05 for all). Mothers with postpartum depression were more likely to have lower educational levels. No significant difference in EPDS scores in pregnancy and postpartum were observed for the other maternal characteristics as for perinatal anxiety (Supplementary eTables 3 and 4).

Associations With Perinatal Symptoms of Anxiety and Depression

After potential confounders were adjusted, lower total plasma PC omega-3 fatty acid concentrations (β = −6.49 for STAI-state subscale scores/unit increase of omega-3 fatty acid; 95% CI, −11.90 to −1.08) and higher plasma PC omega-6:omega-3 fatty acid ratios (β = 6.58 for STAI-state subscale scores/unit increase of fatty acid ratios; 95% CI, 1.19 to 11.98) were independently associated with increased antenatal anxiety (Table 2). Specific plasma fatty acid ratios AA:DHA, AA:EPA, and AA:DPA were also found to be associated with increased antenatal anxiety (P < .05 for all).

Table 1. Comparison of State-Trait Anxiety Inventory (STAI)-State Subscale Scores of Mothers Who Completed Both STAI and Edinburgh Postnatal Depression Scale at 26–28 Weeks' Gestation and at 3 Months' Postpartum

Variable	STAI-State Subscale Scores at 26–28 Weeks' Gestation (n = 698)[a,b] Mean	SD	*P* Value	STAI-State Subscale Scores at 3 Months' Postpartum (n = 698)[a,b] Mean	SD	*P* Value
Ethnicity			**.022**			.472
Chinese	32.9*	9.6		33.8*	10.2	
Malay	35.3†	10.4		33.0†	10.3	
Indian	33.6*	10.0		34.3*	10.8	
Education			**< .001**			**.001**
Primary and secondary	36.5*	10.5		35.9*	10.5	
Postsecondary	33.8†	9.9		33.1†	10.7	
University	31.1‡	8.8		32.5‡	9.6	
Obstetric and neonatal complications			.260			**.029**
No complications	33.9	10.2		34.2	10.2	
Had complications	33.0	9.3		32.4	10.5	
Smoked regularly before pregnancy			**.006**			**.026**
No	33.3	9.8		33.3	10.1	
Yes	36.3	10.7		35.9	11.3	
Smoked regularly during pregnancy			**.025**			**.006**
No	33.5	9.9		33.5	10.2	
Yes	38.8	9.7		40.2	12.6	
Smoke exposure before pregnancy			**< .001**			**.014**
No	32.1	9.2		32.8	10.0	
Yes	35.7	10.4		34.8	10.7	
Smoke exposure during pregnancy			**< .001**			**.016**
No	32.0	9.5		33.0	10.1	
Yes	36.4	10.1		34.9	10.5	
Exercise extent			**.031**			.229
No exercise	36.4*	10.4		34.3*	11.4	
Only gentle exercise	33.9†	9.9		33.9†	10.2	
≤ 150 min moderate exercise/wk	31.8†	10.0		32.1†	9.9	
> 150 min moderate exercise/wk	33.7†	9.2		34.9†	10.8	
With probable antenatal depression			**< .001**			**< .001**
No	32.7	9.3		32.9	9.8	
Yes	46.3	9.7		43.4	11.9	
With probable postpartum depression			**< .001**			**< .001**
No	32.8	9.5		32.0	9.1	
Yes	41.5	10.5		48.2	8.5	

[a]Missing values imputed for confounders include education (9 imputed as "postsecondary"), smoked regularly before pregnancy (1 imputed as "no"), smoke exposure before pregnancy (11 imputed as "no"), and exercise extent (2 imputed as "only gentle exercise").
[b]Mean values within column with unlike symbols were significantly different ($P < .05$) based on Bonferroni test. *P* values indicating significant differences within each variable are in bold.

After mutual adjustment of the 3 fatty acid ratios, only increased AA:DHA ratio showed a persisting trend for an association with increased antenatal anxiety ($P = .061$), while AA:EPA and AA:DPA ratios were no longer significantly associated. No plasma fatty acids or their ratios were associated with postpartum symptoms of anxiety.

In grouped analysis, compared with the highest tertile of plasma PC omega-3 PUFA concentration (median = 230.7 μg/mL; range, 174.3–690.4), the lowest tertile of plasma PC omega-3 PUFA concentration (median = 91.3 μg/mL; range, 36.2–115.5) was associated with higher antenatal anxiety scores (β = 2.02; 95% CI, 0.23–3.80; *P* value of trend = .037) (Figure 2). No significant associations were observed with postpartum anxiety (data not shown).

For symptoms of probable antenatal and postpartum depression, neither univariate (Supplementary eTables 2 and 3) nor adjusted multivariate logistic regression models (Table 3) showed any association with plasma PC PUFAs or their ratios ($P > .1$ for all).

DISCUSSION

Our study found that lower maternal plasma PC omega-3 PUFA concentrations and higher omega-6:omega-3 PUFA ratios measured at 26–28 weeks' gestation were associated with antenatal anxiety symptoms but not with postpartum anxiety symptoms. We found no association between plasma PC PUFAs measured at 26–28 weeks' gestation and symptoms of probable depression during pregnancy and at postpartum.

This study is, to our knowledge, the first to report an association between maternal LC-PUFAs and antenatal anxiety symptoms using plasma LC-PUFAs as a biomarker. One other study[35] on pregnant women reported a similar association but used dietary intakes of omega-3 PUFAs from seafood sources. Other related findings come from cross-sectional studies[55,56] investigating patients with mental disorders. Total omega-3 fatty acid concentrations and ratios such as DHA:omega-6 DPA and DHA:omega-3 DPA were lower, while the total omega-6 to omega-3 PUFA ratio was higher in patients with social anxiety disorder compared to healthy controls.[55] However, in older people with previous history of depression, no association between specific omega-3 fatty acid concentrations and residual anxiety symptoms was seen.[57]

In results consistent with previous observational cohort studies[23,24,58] and the majority of supplementation studies,[29–34] we did not find any associations between plasma PC omega-3 PUFA concentrations at 26–28 weeks' gestation and symptoms of probable perinatal depression. While some smaller observational studies[25–27,59] did report associations, data in these studies were adjusted for a less comprehensive list of confounders. Factors that are strong predictors of depression, such as smoking status and alcohol consumption before pregnancy and smoke exposure, were excluded. Meta-analyses[60,61] on intervention studies report that limitations of existing supplementation studies, such as small sample sizes, disparities in treatment, outcome measures, and population, prevent reliable conclusions on the effect of omega-3 PUFAs on perinatal depression to be drawn.

Mechanisms

Our results provide a unique demonstration of the relationships of the various fatty acid ratios with increased antenatal anxiety, which are consistent with the various postulated mechanisms. Anxiety has been postulated to arise from neuroinflammatory processes in the brain,[62] where the omega-3–derived

Table 2. Multivariate Linear Regression Models of Associations of Maternal Plasma Phosphatidycholine Omega-3 and Omega-6 Fatty Acids and Risk Factors of Interest With Antenatal and Postpartum Anxiety

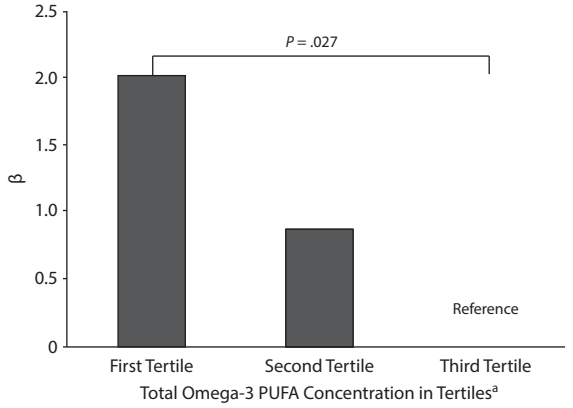
	26- to 28-Week Antenatal Model (n = 698)[a]			3-Month Postpartum Model (n = 698)[b]		
Model	β	95% CI	*P* Value	β	95% CI	*P* Value
Model 1						
Log plasma total omega-3 (μg/mL)	−6.49	−11.90 to −1.08	**.019**	−0.42	−5.60 to 4.80	.874
Log plasma total omega-6 (μg/mL)	5.03	−2.60 to 12.66	.196	0.25	−7.11 to 7.60	.948
Model 2						
Log plasma omega-6:omega-3 ratio	6.58	1.19 to 11.98	**.017**	0.44	−4.78 to 5.65	.870
Model 3						
Log plasma AA:DHA ratio	7.54	2.39 to 12.70	**.004**	0.63	−4.37 to 5.62	.805
Model 4						
Log plasma AA:EPA ratio	3.44	0.72 to 6.16	**.013**	−0.50	−3.12 to 2.12	.708
Model 5						
Log plasma AA:DPA ratio	5.36	0.66 to 10.06	**.025**	−0.72	−5.25 to 3.80	.753
Model 6						
Log plasma AA:DHA ratio	5.62	−0.25 to 11.50	.061	1.38	−4.30 to 7.06	.633
Log plasma AA:EPA ratio	1.96	−1.52 to 5.43	.269	−0.51	−3.86 to 2.83	.763
Log plasma AA:DPA ratio	0.95	−5.27 to 7.16	.765	−0.75	−6.73 to 5.23	.805

[a]Values are based on a linear regression model and reflect change (95% CI) in STAI-state subscale scores, used as a continuous measure of anxiety. Model adjusted for potential confounders: ethnicity; parity; education level; marital status; maternal body mass index at 26–28 weeks' gestation; maternal age; employment status; obstetric and neonatal complications; smoking status and smoke exposure before and during pregnancy; alcohol consumption before and during pregnancy; history of abortion, miscarriage and stillbirth; exercise frequency; and reported fish oil supplementation.

[b]Values are based on a linear regression model and reflect change (95% CI) in STAI-state subscale scores, used as a continuous measure of anxiety. Model adjusted for confounders in antenatal model with addition of infant sex, mode of delivery, and corresponding STAI-S scores at 26–28 weeks' gestation.

Abbreviations: AA = arachidonic acid, DHA = docosahexaenoic acid, DPA = docosapentaenopic acid, EPA = eicosapentaenoic acid, STAI = State-Trait Anxiety Inventory.

Figure 2. Associations of Total Plasma Phosphatidycholine Omega-3 Polyunsaturated Fatty Acids (PUFAs) With Odds of Antenatal Anxiety



[a]Tertile concentration range (minimum–maximum): first tertile (36.2–115.5 μg/mL), second tertile (115.5–174.2 μg/mL), T3 (174.3–690.4 μg/mL); third tertile is the reference tertile. *P* value of trend = .037.

eicosanoids and docosanoids have been suggested to modulate the inflammatory effects of omega-6–derived eicosanoids.[20] Imbalanced omega-6:omega-3 PUFA ratios alter membrane fluidity, affecting specific gene expression and inflammatory signaling processes.[63,64] Thus, plasma omega-6:omega-3 PUFA ratio appears to be a stronger indicator of inflammation compared to individual plasma PUFA concentrations. In our study, the associations of high total omega-3 PUFA concentrations and high plasma omega-6:omega-3 PUFA ratios with higher anxiety scores add support to possible mediation of effects via inflammatory pathways.

On similar lines, AA (omega-6 fatty acid) is the precursor of eicosanoids like prostaglandin E_2 (PGE_2) and may promote synthesis of proinflammatory cytokines, which in turn stimulate corticosterone secretion, triggering neuroinflammation,[65,66] while EPA and its derivatives (DHA and DPA) reduce interleukin-1–induced PGE_2 secretion and corticosterone levels, which can attenuate anxiety-like behavior.[67–69] In the current study, AA:EPA, AA:DHA, and AA:DPA ratios were associated with antenatal anxiety symptoms, further supporting the hypothesis that metabolites along the EPA-DPA-DHA pathway may be involved in neuroinflammatory processes of anxiety.

While the underlying mechanisms of LC-PUFAs in depression appear to be similar to anxiety,[22,70,71] it has been suggested that the release of other peptides or hormones of the hypothalamic pituitary adrenal axis could be regulated differently in the 2 disorders.[22] This area of research is, however, still poorly understood but may explain the lack of significant associations of depressive symptoms with plasma PUFA levels and ratios found in our study.

Limitations of Study

While mechanistic studies, animal studies,[72–74] and human trials of omega-3 PUFA supplementation[36–39] provide evidence of the anxiolytic benefits of omega-3 PUFAs, the cross-sectional nature of the current analysis limits us from concluding any direction of causality. It is also possible that symptoms of greater anxiety could lead to poor dietary intake

Table 3. Multivariate Logistic Regression Models of Associations of Maternal Plasma Phosphatidycholine Omega-3 and Omega-6 Fatty Acids and Risk Factors of Interest With Probable Antenatal and Postpartum Depression

	26- to 28-Week Antenatal Model (n = 698)[a]			3-Month Postpartum Model (n = 698)[b]		
Model	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Model 1.1[c]						
Log plasma total omega-3 (μg/mL)	1.40	0.29 to 6.73	.675	1.48	0.40 to 5.55	.560
Model 1.2[c]						
Log plasma total omega-6 (μg/mL)	4.48	0.51 to 39.68	.178	1.63	0.24 to 10.97	.618
Model 2						
Log plasma omega-6:omega-3 ratio	2.75	0.27 to 27.87	.392	0.70	0.09 to 5.14	.722
Model 3						
Log plasma AA:DHA ratio	1.71	0.18 to 16.02	.639	0.90	0.13 to 6.32	.913
Model 4						
Log plasma AA:EPA ratio	1.66	0.52 to 5.36	.394	0.80	0.30 to 2.13	.653
Model 5						
Log plasma AA:DPA ratio	2.36	0.32 to 17.37	.400	1.73	0.30 to 10.04	.542
Model 6						
Log plasma AA:DHA ratio	1.05	0.08 to 13.44	.969	0.74	0.08 to 6.69	.790
Log plasma AA:EPA ratio	1.38	0.31 to 6.18	.677	0.54	0.16 to 1.85	.323
Log plasma AA:DPA ratio	1.65	0.12 to 23.39	.712	3.81	0.39 to 37.06	.249

[a]Values are based on a logistic regression model and reflect the ORs (95% CI) for antenatal depression, defined as having and EPDS score ≥ 15. Model adjusted for potential confounders: ethnicity; parity; education level; marital status; maternal body mass index at 26–28 weeks' gestation; maternal age; employment status; obstetric and neonatal complications; smoking status and smoke exposure before and during pregnancy; alcohol consumption before and during pregnancy; history of abortion, miscarriage, and stillbirth; exercise frequency; and reported fish oil supplementation.

[b]Values are based on a logistic regression model and reflect the ORs (95% CI) for postpartum depression, defined as having and EPDS score ≥ 13.Model adjusted for confounders in the 26- to 28-week antenatal model with addition of infant sex, mode of delivery, and corresponding EPDS scores at 26–28 weeks' gestation.

[c]Due to the small number of depression cases, modeling log plasma total omega-3 and omega-6 concentrations in separate regression models was found to be more appropriate.

Abbreviations: AA = arachidonic acid, DHA = docosahexaenoic acid, DPA = docosapentaenopic acid, EPA = eicosapentaenoic acid, EPDS = Edinburgh Postnatal Depression Scale.

in the mothers and lowered levels of plasma omega-3 fatty acids. In addition, measurement of plasma fatty acid status during the early postpartum period was not made. Given the intense maternal fatty acid mobilization in the third trimester,[75] an essential period of fetal brain development and growth, the antenatal fatty acid status measured at 26–28 weeks of gestation may not accurately reflect the postpartum status, and this may explain the lack of significant findings with the postpartum measure.

It is acknowledged that while our study has adjusted for many potential confounding factors, there could still be residual confounding arising from factors such as interpersonal conflicts, emotional support, and confinement practices and experiences,[76] which were not collected as part of the main GUSTO study design. Last, that women with self-reported mental health history were excluded from the study population limits the generalizability of our study results, and further studies in clinically depressed or anxious peripartum women are needed.

CONCLUSIONS AND CLINICAL IMPLICATIONS

Consistent with the other larger observational studies and the majority of supplemental studies, maternal PUFA status during pregnancy was not found to be associated with symptoms of probable depression in pregnancy and postpartum in this study. In contrast, lower omega-3 PUFA concentrations and higher omega-6:omega-3 fatty acid ratios in maternal PC plasma measured at 26–28 weeks' gestation were associated with symptoms of antenatal anxiety, but not postpartum anxiety. It remains unclear whether altered PUFA status is a cause or consequence of antenatal anxiety, but our findings suggest possible links via postulated mechanisms of neuroinflammation. Future studies are required to draw more definitive inferences on the direction of causality.

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Author contributions: Dr M. F. F. Chong and Ms Ong are joint first authors. Drs M. Chong and Chen designed the study; Drs M. F. F. Chong, Wong, Colega, and Fisk contributed to the analyses of the data; Drs Lim, Cai, and Pang coordinated blood samples and study data; Dr Tan provided statistical support; and Dr M. F. F. Chong and Ms Ong performed statistical analyses, wrote the paper, and had primary responsibility for the final content. Drs Calder, Broekman, Godfrey, Meaney, Saw, Kwek, Y-S. Chong, Gluckman, and Chen provided intellectual contribution to the study and manuscript. The GUSTO study was designed and led by the principal investigators Drs Saw, Kwek, Y-S. Chong, and Gluckman. All the authors read and approved the final manuscript.

Potential conflicts of interest: **Drs Gluckman**, **Godfrey,** and **Y-S**. **Chong** have received reimbursement for speaking at conferences sponsored by Nestec, Abbott Nutrition, and Danone. **Drs Godfrey** and **Y-S. Chong** are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec, and Danone. **Drs M. F. F. Chong**, **Calder**, **Tan**, **Lim**, **Cai**, **Pang**, **Broekman**, **Saw**, **Kwek**, **Meaney**, and **Chen** and **Mss Ong**, **Colega**, **Wong**, and **Fisk** have no financial or personal conflict of interests.

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Supplementary material follows this article.

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Supplementary Material

Article Title: Long-Chain Polyunsaturated Fatty Acid Status During Pregnancy and Maternal mental health in Pregnancy and the Postpartum Period: Results From the GUSTO Study

Authors: Mary F. F. Chong, PhD; Yi-Lin Ong, BSc; Philip C. Calder, PhD; Marjorelee Colega, BSc; Jocelyn X. Y. Wong, BSc; Chuen Seng Tan, PhD; Ai Lin Lim, PhD; Helena L. Fisk, BSc; Shirong Cai, PhD; Wei Wei Pang, PhD; Birit F. P. Broekman, PhD; Seang Mei Saw, PhD; Kenneth Kwek, MBBS; Keith M. Godfrey, PhD; Yap-Seng Chong, MD; Peter Gluckman, PhD; Michael J. Meaney, PhD; and Helen Chen, MMedPsych, DipPsychotherapy; on behalf of the GUSTO Study Group

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List of Supplementary Material for the article

1. eTable 1 — Comparison between characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum with mothers who did not complete all tests
2. eTable 2 — Comparison of STAI-S scores of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum ($p>0.05$)
3. eTable 3 — Characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum, stratified by risk of probable antenatal depression
4. eTable 4 — Characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum, stratified by risk of probable postpartum depression

Disclaimer

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Supplementary eTable 1. Comparison between characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum with mothers who did not complete all tests

	Completed STAI-S and EPDS at both time points *n=698*		**Did not complete STAI-S or EPDS at either time point** *n=299*		***p-value***
	Mean/N[1]	SD/%[2]	Mean/N[1]	SD/%[2]	
Age[3]	30.8	5.2	30.2	5.1	0.093
Ethnicity					0.206
Chinese	384	70.2	163	29.8	
Malay	192	73.0	71	27.0	
Indian	122	65.2	65	34.8	
Parity					0.380
1	298	71.6	118	28.4	
>1	400	68.8	181	31.2	
Education					0.230
Primary and Secondary	202	67.6	97	32.4	
Post-Secondary	255	68.9	115	31.1	
University	241	73.5	87	26.5	
Current employment					0.935
Unemployed	195	69.6	85	30.4	
Employed	503	70.2	214	29.8	
Abortion history					0.635
No	656	69.8	284	30.2	
Yes	42	73.7	15	26.3	
Miscarriage history					1.000
No	640	70.0	274	30.0	
Yes	58	69.9	25	30.1	
Stillbirth history					0.789
No	693	69.9	298	30.1	
Yes	5	83.3	1	16.7	
Obstetric and Neonatal Complications					0.228
No complications	493	71.2	199	28.8	
Had complications	205	67.2	100	32.8	
Alcohol consumption before pregnancy					0.534
No	471	70.7	195	29.3	
Yes	227	68.6	104	31.4	
Alcohol consumption during pregnancy					0.085
No	680	69.6	297	30.4	
Yes	18	90.0	2	10.0	
Smoked regularly before pregnancy					0.920
No	606	69.9	261	30.1	
Yes	92	70.8	38	29.2	
Smoked regularly during pregnancy					1.000
No	680	70.0	292	30.0	
Yes	18	72.0	7	28.0	
Smoke exposure before pregnancy					0.552
No	395	69.2	176	30.8	
Yes	303	71.1	123	28.9	

Smoke exposure during pregnancy					0.757
No	437	69.6	191	30.4	
Yes	261	70.7	108	29.3	
Marital status					0.061
Married and living with husband	668	69.4	294	30.6	
Single and not living with baby's father	30	85.7	5	14.3	
Maternal BMI					0.380
≤ 23	169	72.5	64	27.5	
>23	529	69.2	235	30.8	
Exercise extent					0.224
No exercise	52	69.3	23	30.7	
Only gentle exercise	441	68.1	207	31.9	
<150 mins moderate exercise/week	134	74.0	47	26.0	
>150mins moderate exercise/week	71	76.3	22	23.7	
Reported Fish Oil supplementation					0.221
No supplements containing fish oil	413	68.5	190	31.5	
Took supplements containing fish oil	285	72.3	109	27.7	
Infant sex[3]					0.492
Female	342	72.0	133	28.0	
Male	356	69.8	154	30.2	
Delivery Mode					0.936
Vaginal	496	70.2	211	29.8	
Caesarean	202	69.7	88	30.3	

[1] reflects mean of continuous variables or frequency for categorical variables
[2] reflects standard deviation of continuous variables or percentages of categorical variables
[3] total numbers do not add up due to missing data in the "Did not complete STAI-S or EPDS at either time point" category

Supplementary eTable 2. Comparison of STAI-S scores of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum (p>0.05)

	STAI-S scores at 26-28 weeks gestation[1] *n=698*		***p-value***	**STAI-S scores at 3 months postpartum[1]** *n=698*		***p-value***
	Mean	SD		Mean	SD	
Age[2]			0.196			0.806
≤ 31	34.1	10.2		33.6	10.3	
>31	33.1	9.6		33.8	10.3	
Parity			0.432			0.305
1	34.0	9.9		34.2	10.0	
>1	33.4	9.9		33.3	10.5	
Current employment			0.314			0.079
Unemployed	34.3	10.2		34.8	10.1	
Employed	33.4	9.8		33.3	10.4	
Abortion history			0.123			0.330
No	33.5	10.0		33.6	10.2	
Yes	36.0	9.2		35.2	11.7	
Miscarriage history			0.295			0.977
No	33.5	10.0		33.7	10.3	
Yes	35.0	9.6		33.7	10.8	
Stillbirth history			0.327			0.882
No	33.6	9.9		33.7	10.3	
Yes	38.0	11.4		33.0	7.0	
Alcohol consumption before pregnancy			0.150			0.976
No	34.0	9.9		33.7	10.3	
Yes	32.9	10.0		33.7	10.4	
Alcohol consumption during pregnancy			0.474			0.638
No	33.7	10.0		33.7	10.4	
Yes	32.0	8.5		32.6	9.2	
Marital status			0.862			0.431
Married and living with husband	33.6	9.9		33.8	10.3	
Single and not living with baby's father	34.0	9.9		32.2	9.9	
Maternal BMI			0.541			0.532
≤ 23	34.1	9.7		34.1	9.7	
>23	33.5	10.0		33.5	10.5	
Fish Oil supplements			0.075			0.261
no supplements containing fish oil	34.2	9.9		34.1	10.4	
took supplements containing fish oil	32.9	10.0		33.2	10.1	
Infant Sex[3]						0.328
Female				34.1	10.5	
Male				33.3	10.1	
Delivery Mode[3]						0.914
Vaginal				33.7	10.4	
Caesarean				33.8	10.1	

[1] Missing values imputed for confounders include: Parity (15 missing: 5 imputed as parity "1", 10 imputed as parity ">1" based on birth order data); Current employment (16 imputed as "Employed"); Abortion history (30 imputed as "No"); Miscarriage history (30 imputed as "No"); Stillbirth history (30 imputed as "No"); Alcohol consumption during pregnancy (23 imputed as "No"); Marital status (15 imputed as "Married and living with husband"); Maternal BMI (12 imputed as "BMI>23").

[2] Subjects were stratified based on the median age (31 years) in subject population (n=698)

[3] Only applicable to postpartum anxiety scores

Supplementary eTable 3. Characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum, stratified by risk of probable antenatal depression

	Normal (EPDS<15) *n=648*		**With probable antenatal depression (EPDS≥15)** *n=50*		***p-value***
	Mean/N[1]	SD/%[2]	Mean/N[1]	SD/%[2]	
Age	30.9	5.0	28.5	6.5	**0.014**
Ethnicity					**0.007**
Chinese	367	95.6	17	4.4	
Malay	173	90.1	19	9.9	
Indian	108	88.5	14	11.5	
Parity					1.000
1	277	93.0	21	7.0	
>1	371	92.8	29	7.3	
Education					**0.012**
Primary and Secondary	179	88.6	23	11.4	
Post-Secondary	238	93.3	17	6.7	
University	231	95.9	10	4.1	
Current employment					0.070
Unemployed	175	89.7	20	10.3	
Employed	473	94.0	30	6.0	
Abortion history					1.000
No	609	92.8	47	7.2	
Yes	39	92.9	3	7.1	
Miscarriage history					0.854
No	595	93.0	45	7.0	
Yes	53	91.4	5	8.6	
Stillbirth history					1.000
No	643	92.8	50	7.2	
Yes	5	100.0	0	0.0	
Obstetric and Neonatal Complications					0.177
No complications	453	91.9	40	8.1	
Had complications	195	95.1	10	4.9	
Alcohol consumption before pregnancy					0.698
No	439	93.2	32	6.8	
Yes	209	92.1	18	7.9	
Alcohol consumption during pregnancy					0.465
No	630	92.6	50	7.4	
Yes	18	100.0	0	0.0	
Smoked regularly before pregnancy					**0.003**
No	570	94.1	36	5.9	
Yes	78	84.8	14	15.2	
Smoked regularly during pregnancy					0.262
No	633	93.1	47	6.9	
Yes	15	83.3	3	16.7	

Smoke exposure before pregnancy					**0.001**
No	378	95.7	17	4.3	
Yes	270	89.1	33	10.9	
Smoke exposure during pregnancy					**0.001**
No	417	95.4	20	4.6	
Yes	231	88.5	30	11.5	
Marital status					1.000
Married and living with husband	620	92.8	48	7.2	
Single and not living with baby's father	28	93.3	2	6.7	
Maternal BMI					1.000
≤ 23	157	92.9	12	7.1	
>23	491	92.8	38	7.2	
Exercise extent					0.136
No exercise	48	92.3	4	7.7	
Only gentle exercise	407	92.3	34	7.7	
<150 mins moderate exercise/week	130	97.0	4	3.0	
>150mins moderate exercise/week	63	88.7	8	11.3	
Reported Fish Oil supplementation					0.077
No supplements containing fish oil	377	91.3	36	8.7	
Took supplements containing fish oil	271	95.1	14	4.9	
Antenatal STAI-S score	32.7	9.3	46.3	9.7	**<0.001**
Postpartum STAI-S score	32.9	9.8	43.4	11.9	**<0.001**
Total n-3 concentration (μg/mL)	161.0	81.3	154.6	81.0	0.591
Total n-6 concentration (μg/mL)	834.8	273.3	878.5	297.6	0.280
Plasma n6:n3 ratio	5.8	2.0	6.3	1.9	0.067

[1] reflects mean of continuous variables or frequency for categorical variables
[2] reflects standard deviation of continuous variables or percentages of categorical variables

Supplementary eTable 4. Characteristics of mothers who completed both STAI and EPDS at 26-28 weeks gestation and 3 months postpartum, stratified by risk of probable postpartum depression

	Normal (EPDS<13) *n=626*		**With probable postpartum depression (EPDS≥13)** *n=72*		***p-value***
	Mean/N[1]	SD/%[2]	Mean/N[1]	SD/%[2]	
Age	30.7	5.1	31.5	5.7	0.208
Ethnicity					0.500
Chinese	349	90.9	35	9.1	
Malay	170	88.5	22	11.5	
Indian	107	87.7	15	12.3	
Parity					0.415
1	271	90.9	27	9.1	
>1	355	88.8	45	11.3	
Education					**0.001**
Primary and Secondary	168	83.2	34	16.8	
Post-Secondary	237	92.9	18	7.1	
University	221	91.7	20	8.3	
Current employment					0.224
Unemployed	170	87.2	25	12.8	
Employed	456	90.7	47	9.3	
Abortion history					0.257
No	591	90.1	65	9.9	
Yes	35	83.3	7	16.7	
Miscarriage history					0.494
No	576	90.0	64	10.0	
Yes	50	86.2	8	13.8	
Stillbirth history					1.000
No	622	89.8	71	10.2	
Yes	4	80.0	1	20.0	
Obstetric and Neonatal Complications					0.204
No complications	437	88.6	56	11.4	
Had complications	189	92.2	16	7.8	
Alcohol consumption before pregnancy					0.298
No	418	88.7	53	11.3	
Yes	208	91.6	19	8.4	
Alcohol consumption during pregnancy					0.779
No	609	89.6	71	10.4	
Yes	17	94.4	1	5.6	
Smoked regularly before pregnancy					0.140
No	548	90.4	58	9.6	
Yes	78	84.8	14	15.2	
Smoked regularly during pregnancy					0.197
No	612	90.0	68	10.0	
Yes	14	77.8	4	22.2	

Smoke exposure before pregnancy					0.573
No	357	90.4	38	9.6	
Yes	269	88.8	34	11.2	
Smoke exposure during pregnancy					0.507
No	395	90.4	42	9.6	
Yes	231	88.5	30	11.5	
Marital status					0.804
Married and living with husband	600	89.8	68	10.2	
Single and not living with baby's father	26	86.7	4	13.3	
Maternal BMI					0.757
≤ 23	150	88.8	19	11.2	
>23	476	90.0	53	10.0	
Exercise extent					0.127
No exercise	50	96.2	2	3.8	
Only gentle exercise	389	88.2	52	11.8	
<150 mins moderate exercise/week	125	93.3	9	6.7	
>150mins moderate exercise/week	62	87.3	9	12.7	
Reported Fish Oil supplementation					0.631
No supplements containing fish oil	368	89.1	45	10.9	
Took supplements containing fish oil	258	90.5	27	9.5	
Infant sex					0.121
Female	300	87.7	42	12.3	
Male	326	91.6	30	8.4	
Delivery Mode					1.000
Vaginal	445	89.7	51	10.3	
Caesarean	181	89.6	21	10.4	
Antenatal EPDS score	6.7	4.2	11.8	4.6	**<0.001**
Antenatal STAI-S score	32.8	9.5	41.5	10.5	**<0.001**
Postpartum STAI-S score	32.0	9.1	48.2	8.5	**<0.001**
Total n-3 concentration (μg/mL)	159.9	81.0	165.9	84.2	0.554
Total n-6 concentration (μg/mL)	833.6	271.6	875.8	303.6	0.218
Plasma n6:n3 ratio	5.8	2.0	5.9	2.2	0.666

[1] reflects mean of continuous variables or frequency for categorical variables
[2] reflects standard deviation of continuous variables or percentages of categorical variables