Epidemiology and Psychiatric Sciences (2017), **26**, 157–168. © Cambridge University Press 2016
doi:10.1017/S204579601500116X

ORIGINAL ARTICLE

Serum *n*-3 polyunsaturated fatty acids are inversely associated with longitudinal changes in depressive symptoms during pregnancy

T. J. P. Pinto[1,2], A. A. F. Vilela[2], D. R. Farias[2], J. Lepsch[2], G. M. Cunha[1], J. S. Vaz[2,3], P. Factor-Litvak[4] and G. Kac[2]*

[1] *National School of Public Health, Oswaldo Cruz Foundation, Rua Leopoldo Bulhões, 1480 – Manguinhos, Rio de Janeiro, RJ, Brazil*
[2] *Nutritional Epidemiology Observatory, Department of Social and Applied Nutrition, Institute of Nutrition Josué de Castro, Avenida Carlos Chagas Filho, 367/CCS – Bloco J – 2° andar, sala 29. Cidade Universitária – Ilha do Fundão, Rio de Janeiro, RJ, Brazil*
[3] *Faculty of Nutrition, Federal University of Pelotas, Rua Gomes Carneiro, 1 – Campus Porto, Pelotas, RS, Brazil*
[4] *Department of Epidemiology, Mailman School of Public Health, Columbia University, New York NY10032, USA*

Backgrounds. *N*-3 polyunsaturated fatty acids (PUFAs) have been hypothesised to be protective for depression during pregnancy. However, there are few data and no consensus regarding this association. In this line, we aim to evaluate if the concentration of *n*-3 and *n*-6 PUFAs, and their ratio, are associated with depressive symptoms throughout pregnancy.

Method. A prospective cohort of 172 Brazilian women was followed at 5–13th, 20–26th and 30–36th weeks of gestation. The presence of depressive symptoms was evaluated using the Edinburgh Postnatal Depression Scale (EPDS) at each pregnancy trimester. Depression was defined as an EPDS score ≥11. The concentrations of *n*-3 [α-linolenic acid; eicosapentaenoic acid (EPA); docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)] and *n*-6 PUFAs [linoleic acid; γ linolenic acid; eicosadienoic acid; eicosatrienoic acid; arachidonic acid; docosatetraenoic acid and docosapentaenoic acid] were expressed as absolute (μg/ml) values. The total *n*-6/*n*-3 ratio was calculated. Statistical analyses were performed using univariate and adjusted random intercept logistic model for each fatty acid (FA) considering the longitudinal nature of data. Covariates were selected as potential confounders based on their biological plausibility of having an association with the concentration of FA and depressive symptoms during pregnancy.

Results. The prevalence of depressive symptoms was high in all pregnancy trimesters (1st = 33.7%; 2nd = 18.9%; 3rd = 17.4%). We did not find differences in means FA concentrations by depressive symptom classification, for each follow-up visit. The women presented a 5% decrease in the odds of having depressive symptoms for each one-week increase in the gestational age. As individual women progressed through pregnancy, higher concentrations of EPA (odds ratio (OR) = 0.92; 95% CI: 0.86–0.99), DHA (OR = 0.96; 95% CI: 0.93–0.99), DPA (OR = 0.87; 95% CI: 0.77–0.99) and total *n*-3 (OR = 0.98; 95% CI: 0.96–0.99) were associated with a lower odds of depressive symptoms, while higher total *n*-6/*n*-3 ratio were associated with greater odds of depressive symptoms (OR = 1.40; 95% CI: 1.09–1.79). We detected a decrease in the probability of depressive symptoms as concentrations of total *n*-3 FA, α-linolenic acid, DPA, and DHA increased. We also observed a sharper decline for women with initial greater chance of depressive symptoms compared with those with lower chance of having these symptoms.

Conclusions. We found a high prevalence of depressive symptoms in low-income Brazilian pregnant women and no significant associations between *n*-6 FA and depressive symptoms. Lower serum concentrations of DHA, EPA and DPA and a higher *n*-6/*n*-3 ratio at each pregnancy trimester were associated with higher odds of depressive symptoms throughout pregnancy.

Received 28 July 2015; Accepted 13 December 2015; First published online 30 March 2016

Key words: Depression, mental health, nutrition, pregnancy, prospective study.

Introduction

Gestational depression is a common mental disorder and represents an important clinical and public health issue, increasing the risk of adverse outcomes for the mother and the newborn, including postpartum depression (PPD) and low birth weight (Pereira *et al.* 2011; Bauer *et al.* 2015). The prevalence of depression varies during pregnancy trimesters, according to the

* Address for correspondence: G. Kac, Department of Social and Applied Nutrition, Rio de Janeiro Federal University, Avenida Carlos Chagas Filho, 373, CCS – Bloco J2, Cidade Universitária – Ilha do Fundão, Rio de Janeiro, RJ 21941-902, Brazil.
(Email: gilberto.kac@gmail.com)

population, and according to the diagnostic tool used (Bennett *et al.* 2004; Gavin *et al.* 2005; Pereira *et al.* 2011). Banti *et al.* (2011) evaluated 1066 Italian women and found a prevalence of 12.4% in three points during pregnancy (third, sixth and eighth month of pregnancy). A systematic review reported that around 20% of the pregnant women living in developing countries (including Brazil) are affected by depression during pregnancy (Pereira *et al.* 2011).

The literature concerning how serum polyunsaturated fatty acids (PUFAs) change throughout pregnancy has been poorly characterised. A recent study reveals a pattern of change characterised by an increase in the first period (1st to 2nd trimester) followed by a slight increase in the second period (2nd to 3rd trimester). The authors observed a tendency for concentrations to stabilise or for the rate of increase to slow between the second and third trimesters. The *n*-6/*n*-3 ratio showed a lower rate of increase from the 1st to the 2nd trimester compared with the rate in the second period (Pinto *et al.* 2015).

Depressive disorders during pregnancy have a multifactorial aetiology and epidemiological studies find associations between demographic and psychosocial factors such as women's age, previous history of depression, marital status and unplanned pregnancy on gestational depression (Rich-Edwards, 2006; Pereira *et al.* 2009; Räisänen *et al.* 2014). PUFAs intake has been associated with depressive symptoms in major depressive patients, but results on PPD are controversial (Grosso *et al.* 2014*a*). In fact, only recently the association between biomarkers of PUFAs consumption and occurrence of depression during pregnancy has been investigated (Das, 2008). The role of PUFAs is an active area of research in that they are hypothesised to modulate important monoamine neurotransmitters, such as serotonin and dopamine, which are involved in the neurophysiology of depression and others mental illness (Levant, 2013; Su *et al.* 2013, 2015). Moreover, *n*-3 and *n*-6 PUFAs have been hypothesised to exert anti- and pro-inflammatory actions, respectively, which may influence the inflammatory status characterising depressive disorders (Grosso *et al.* 2014*b*). In the current study, we hypothesised that the concentration of *n*-3 PUFAs are negatively associated with depressive symptoms while *n*-6 PUFAs are positively associated with depressive symptoms throughout pregnancy.

Recent studies have evaluated the association between the serum levels of PUFAs or the phospholipid fatty acids (FA) composition and depressive disorders during pregnancy; however, the results are contradictory and inconclusive (Rees *et al.* 2009; Bodnar *et al.* 2012; Sallis *et al.* 2014; Shiraishi *et al.* 2015). Parker *et al.* (2014) evaluated 895 Australian women and found that, at the 36th week of pregnancy, depressed women presented significant lower concentrations of total *n*-3, docosahexaenoic acid (DHA), DHA plus eicosapentaenoic acid (EPA) and higher *n*-6/*n*-3 ratio, compared with those without depression. In contrast, Bodnar *et al.* (2012) in a sample of 135 American women did not find any significant association between arachidonic acid (AA), DHA or EPA concentrations and major depressive disorder during pregnancy.

Considering the importance of maternal mental health and the high prevalence of depressive disorders during pregnancy, and the lack of consensus concerning their association with PUFAs, the aim of this study was to evaluate the association between maternal serum concentrations of *n*-3 and *n*-6 PUFAs and depressive symptoms in a group of Brazilian women followed throughout pregnancy.

Methods

Study protocol and design

This study is based on a prospective observational cohort of pregnant women, who were followed at a public health care centre, located in Rio de Janeiro, Brazil. Women were invited to participate in this study if they met the following eligibility criteria: (i) between 5 and 13 weeks of gestation at the time of enrolment; (ii) between 20 and 40 years of age; (iii) free from any known chronic diseases (other than obesity); (iv) residing in the study catchment area; and (v) intending to continue prenatal care in the public health centre. The study consisted of three follow-up waves: at 5–13 (baseline), 20–26 and 30–36 gestational weeks. A total of 299 pregnant women underwent screening between November/2009 and October/2011.

Women were excluded if they presented more than 13 gestational weeks ($n = 15$) at enrolment, were diagnosed with an infectious ($n = 9$) or non-communicable disease ($n = 12$), presented twin pregnancies ($n = 4$), reported a miscarriage in current pregnancy ($n = 25$), currently used antidepressants ($n = 3$), did not have depressive symptoms assessments in the first trimester or presented missing data for confounders variables at baseline ($n = 12$), and did not have information for FA at some point during pregnancy ($n = 42$). We also excluded outlier subjects based on the visual graphical analyses of the FA evaluated ($n = 5$). The sample at the baseline, after exclusions, was comprised of 172 healthy pregnant women.

In the second trimester, a subsample of 41 women identified as being at risk for PPD [as evidenced by a possible history of depression or by a score 9 on the Edinburgh Postnatal Depression Scale (EPDS) at the

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

baseline] were invited to participate in a clinical trial nested within the main cohort. This sub-study aimed to test the efficacy of omega-3 supplementation during pregnancy to prevent depressive symptoms during the postpartum period. These women were randomly assigned after the second follow-up visit to receive gelatin capsules containing either omega-3 (fish oil) or placebo, composed by soybean oil, the most commonly used cooking oil in the Brazilian population (Levy *et al.* 2012). The capsules for the treatment group contained a total dosage of 1.8 g of omega-3 per day. Considering the main objective of the present study, we excluded from the third trimester analyses all women who received supplements, regardless of their treatment group. Detailed information on the analytical approach to cope with the clinical trial was extensively described in Teofilo *et al.* (2014).

Depressive symptoms

The EPDS was administered by trained interviewers and was used to measure depressive symptoms during each pregnancy trimester. Cox *et al.* (1987) developed the EPDS to investigate the depressive symptoms in the postpartum period. The instrument contains ten-items that inquire about the mother's mood in the past 7 days. Each item of EPDS has four answer options that are assigned a score from 0 to 3 (total scores range from 0 to 30). Murray & Cox (1990) validated this scale for use in pregnancy.

In Brazil, the EPDS was translated into Portuguese and validated in a sample of mothers from Pelotas, southern Brazil (Santos *et al.* 2007). These authors observed that the cut-off point ≥11 (83.8% of sensitivity and 74.7% of specificity) performed better for screening moderate and severe cases of PPD. Therefore, the depressive symptoms in the current study were dichotomised as EPDS scores <11 *v.* ≥11.

Fatty acids analysis

Fasting blood samples (5 ml) were collected by a technician during all three gestational trimesters using vacutainer tubes containing separator gel. Women were advised to fast for 12 h. After blood was drawn, samples were centrifuged for 5 min (5000 rpm) and serum was separated and stored at −80°C for approximately 2 years prior to analysis.

The serum samples were shipped in dry ice to the Section of Nutritional Neurosciences, Laboratory of Membrane Biochemistry and Biophysics of the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health (NIH), where the FA composition of serum was determined. The FA composition was analysed by a trained technician using a high-throughput robotic direct methylation method coupled with fast gas-liquid chromatography developed and validated by the NIH. This method showed an inter-assay variance of <5%, as previously described (Masood & Salem, 2007; Lin *et al.* 2012).

The concentrations of *n*-3 [α-linolenic acid (18 : 3 *n*-3); EPA (20 : 5 *n*-3); docosapentaenoic acid (22 : 5 *n*-3; DPA) and DHA (22 : 6 *n*-3)] and *n*-6 PUFAs [linoleic acid (18 : 2 *n*-6); γ linolenic acid (18 : 3 *n*-6); eicosadienoic acid (20 : 2 *n*-6); eicosatrienoic acid (20 : 3 *n*-6); AA (20 : 4 *n*-6); docosatetraenoic acid (22 : 4 *n*-6); docosapentaenoic acid (22 : 5 *n*-6)] were expressed as absolute (μg/ml) values. The total *n*-6/total *n*-3 ratio was calculated.

Covariates assessment

A structured questionnaire was administered at baseline to collect the following information: age (years), schooling (years), previous history of depression (no/yes), marital status (married or stable partnership/single), smoking habit (no/yes), alcohol consumption (no/yes), monthly per-capita family income (American dollars), inter-partum interval (nulliparous and ≥48 months/<48 months) and planned pregnancy (no/yes). Possible previous history of depression was ascertained based on self-reported feelings of loss of interest/pleasure in almost all activities nearly every day for a period of two consecutive weeks.

The pre-pregnancy body mass index (BMI) was determined from self-reported pre-pregnancy weight and measured height at baseline. The height was collected in duplicate using a portable stadiometer (Seca Ltd., Hamburg, Germany). This measure was performed according to standardised procedures and was recorded by the trained interviewers (Lohman *et al.* 1988).

Statistical analysis

Means and standard deviations (S.D.) (for continuous variables) and proportions (for categorical variables) were used to describe socioeconomic, demographic, and anthropometric characteristics in the baseline, and FA concentration, at each follow-up visit. Both analyses were stratified by depressive symptoms (<11 *v.* ≥11). Comparisons between groups were performed using Student's *t* test or χ^2 test for proportions.

We detected a potential correlation between the FA (time-dependent variables) and the binary response (depressive symptoms) in the exploratory analysis. We observed that the probability of depressive symptoms tended to decrease with increasing gestational age, while the FA concentration increased as pregnancy progresses. To evaluate the association between

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

longitudinal serum FA concentration and depressive symptoms during pregnancy, we employed a random intercept logistic regression model, which considers the correlation structure between the measures in a same woman during time. The time-dependent variables (*n*-3 and *n*-6 FA) included in the model were decomposed into two terms to facilitate their interpretation. The parameter associated to the first term ($\bar{x}_{1i}$) represents the variation 'between' individuals (with respect to the variation of the independent variable in a subject compared with another subject) and the parameter associated with the second term ($x_{1i}-\bar{x}_{1i}$) the variation 'within' individuals (representing the effect of the independent variable variation within a subject on changes in depressive symptoms classification during pregnancy) (Lalonde *et al.* 2013). In the first term, we calculated the mean FA values for each woman and in the second term we subtracted the FA concentration from their mean values. The parameters associated to the first and to the second terms were included in a same model. Univariate and adjusted random intercept logistic model were estimated for each FA.

To select potential confounders, we have considered the covariates mentioned above (covariates assessment section) based on their biological plausibility of having an association with the concentration of FA and depressive symptoms during pregnancy. These covariates were assessed using longitudinal univariate logistic regression models that included individual confounders and the outcome. The individual confounders with *p*-value <0.2 were included in the full model. The variables with *p*-value >0.05 were removed from the model in descending order of *p*-value.

Gestational age was considered as a time-dependent variable (variables that varied throughout pregnancy), while previous history of depression, planned pregnancy, pre-pregnancy BMI, marital status and inter-partum interval were considered as time-independent variables. These variables were statistically significant and kept in the final model. In additional analyses, we categorised EPDS as ≥12 and ≥13.

We compared key variables between women who participated in the entire study and those who were lost to follow-up. The Student's *t* test or the χ^2 test for proportion was used to assess patterns of loss to follow-up.

Statistical analyses were performed with Stata version 12.0 and R version 3.1. A *p*-value <0.05 was considered significant.

Results

Baseline results revealed that women with depressive symptoms presented higher pre-pregnancy BMI [26.0 (S.D. = 4.9) *v.* 23.8 (S.D. = 3.7) kg/m^2] compared with those without symptoms at baseline. We also observed a higher frequency of possible previous history of depression (67.2 *v.* 34.2%), single marital status (32.8 *v.* 14.9%), inter-partum interval <48 months (27.6 *v.* 12.3%) and unplanned pregnancy (41.4 *v.* 14.0%) in women with depressive symptoms compared with those without (Table 1). The prevalence of depressive symptoms at the first, second and third pregnancy trimesters were: 33.7% (*n* = 58/172), 18.9% (*n* = 32/169) and 17.4% (*n* = 24/138), respectively (data not shown).

We did not find differences in means FA concentrations by depressive symptom classification, for each follow-up visit (Table 2).

We observed a 5% decrease (95% CI: 0.92–0.98) in the odds of having depressive symptoms for each one-week increase in gestational age. The association between FA and depressive symptoms was attenuated after controlling for potential confounders. However, it remained statistically significant. As individual women progressed through pregnancy, higher concentrations of EPA (OR = 0.92; 95% CI: 0.86–0.99), DHA (OR = 0.96; 95% CI: 0.93–0.99), *n*-3 DPA (OR = 0.87; 95% CI: 0.77–0.99) and total *n*-3 (OR = 0.98; 95% CI: 0.96–0.99) were associated with a lower odds of depressive symptoms, while higher *n*-6/*n*-3 ratio was associated with a greater odds of depressive symptoms during pregnancy (OR = 1.40; 95% CI: 1.09–1.79). No associations were found between *n*-6 FA and the occurrence of depressive symptoms during pregnancy (Table 3).

We detected a decrease in the probability of depressive symptoms as concentrations of *n*-3 FA (α-linolenic acid, DPA, DHA, total *n*-3) increased. We also observed a sharper decline for women with initial greater chance of depressive symptoms compared with those with lower chance of having these symptoms (Figs 1, 2).

In the analyses using the EPDS cut-offs ≥12 or ≥13 to classify the presence of depressive symptoms, we only found significant association for the term accounting for 'within' women variation. The DHA (OR = 0.96; 95% CI: 0.93–0.99), total *n*-3 (OR = 0.97; 95% CI: 0.95–0.99) and the *n*-6/*n*-3 ratio (OR = 1.37; 95% CI: 1.05–1.80) were significantly associated with depressive symptoms in the analysis using the EPDS cut-off ≥12. As for the cut-off ≥13, the *n*-6/*n*-3 ratio (OR = 1.40; 95% CI: 1.06–1.86) and the total *n*-3 (OR = 0.97; 95% CI: 0.95–0.99) were associated with depressive symptoms.

The final rate of loss to follow-up was 21.5% (37/172). The analysis of data from the study participants who were lost to follow-up showed no departure from randomness for any variables except for EPDS. Women who were lost to follow-up presented higher EPDS scores than those who remained in the study

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

Table 1. *Baseline characteristics of pregnant women with and without depressive symptoms followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012*

	EPDS <11 ($n = 114$)		EPDS ≥11 ($n = 58$)		
Continuous variables	Mean	S.D.	Mean	S.D.	*p*-value*
Pre-pregnancy BMI (kg/m^2)†	23.79	3.72	26.02	4.90	**<0.001**
Monthly per-capita income (USD$)	326.26	189.37	289.12	194.52	0.116
Categorical variables	*n* (%)		*n* (%)		***p*-value‡**
Previous history of depression§					**<0.001**
No	75 (65.79)		19 (32.76)		
Yes	39 (34.21)		39 (67.24)		
Marital status					**0.007**
Married or stable partnership	97 (85.09)		39 (67.24)		
Single	17 (14.91)		19 (32.76)		
Inter-partum interval (months)‖					**0.012**
Nulliparas and ≥48	100 (87.72)		42 (72.41)		
<48	14 (12.28)		16 (27.59)		
Planned pregnancy					
No	16 (14.04)		24 (41.38)		**<0.001**
Yes	98 (85.96)		34 (58.62)		

EPDS, Edinburgh Postnatal Depression Scale; S.D., standard deviation; BMI, body mass index; PUFAs , polyunsaturated fatty acids.
**p*-value statistical significance based on Student's *t* test.
†Pre-pregnancy BMI was calculated based on the pre-pregnancy weight reported by the mother, at the study baseline.
‡*p*-value statistical significance based on the χ^2 test.
§Nine women did not have information about pre-pregnancy BMI – five in EPDS group <11 and four in the EPDS group ≥11.
‖Three women did not respond to questions on the monthly per-capita income – three in EPDS group <11.
The significance of bold values is $p < 0.05$.

(online supplemental file Table S1), likely due to their participation in the clinical trial.

Discussion

The present study has three main findings. First, we observed that the pregnant women presented a high prevalence of depressive symptoms during pregnancy. Secondly, we observed that social, economic, nutritional and obstetric factors were strongly associated with depressive symptoms in our sample. Finally and more importantly, we observed that higher serum concentration of EPA, DHA, DPA and total *n*-3 were associated with lower odds of depressive symptoms when comparing the longitudinal change in the same individual during pregnancy, while the total *n*-6/*n*-3 ratio represented greater odds of depressive symptoms. Moreover, we observed that in women with depressive symptoms the negative association between DHA concentration and depressive symptoms was more pronounced. As far as we know, this is the first study that evaluated the association between repeated measures of FA and depressive symptoms in the same women during all pregnancy trimesters.

The present study has some limitations that should be addressed. The first is related to the loss to follow up, which is inherent to cohort studies. We observed a non-random loss to follow-up for women with EPDS score ≥11. The pregnant women were selected to participate in the clinical trial based on a possible past history of depression or EPDS score ≥9 in the baseline, which may explain the difference in EPDS scores between the women who completed the study and the loss to follow-up. Despite this potential bias, it is important to highlight the association found between *n*-3 PUFAs and depressive symptoms, which were observed even in healthy women with lower risk for depression. It is reasonable to think that our results are even stronger due to the loss of higher risk women. The second limitation is the potential for misclassification of the outcome. The EPDS is widely used by the majority of the studies around the world. Its wide use is a major advantage once enables the comparison of the current study with the previous ones, although this instrument has been considered prone to misclassification (Stuart *et al.* 1998; Gavin *et al.* 2005; Husain *et al.* 2014). However, it is important to note that very few studies have

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

Table 2. *Characteristics of pregnant women with and without depressive symptoms per waves of follow-up assessed at a public health centre in Rio de Janeiro, Brazil, 2009–2012*

	Follow-up period (gestational weeks)								
	5–13			20–26			30–36		
PUFAs (μg/ml)	EPDS <11 (n = 114) Mean (S.D.)	EPDS ≥11 (n = 58) Mean (S.D.)	p-value*	EPDS <11 (n = 137) Mean (S.D.)	EPDS ≥11 (n = 32) Mean (S.D.)	p-value*	EPDS <11 (n = 114) Mean (S.D.)	EPDS ≥11 (n = 24) Mean (S.D.)	p-value*
n-6 PUFA									
18 : 2 *n*-6	709.76 (124.25)	696.61 (135.60)	0.263	973.14 (162.68)	991.70 (174.65)	0.284	1110.66 (206.45)	1171.85 (189.52)	0.090
18 : 3 *n*-6	8.07 (4.83)	8.40 (4.36)	0.332	8.83 (4.14)	8.24 (3.81)	0.233	8.02 (3.66)	8.60 (4.06)	0.247
20 : 2 *n*-6	7.54 (2.44)	7.24 (2.36)	0.222	13.50 (3.50)	13.29 (3.52)	0.379	14.78 (3.88)	16.01 (4.31)	0.084
20 : 3 *n*-6	44.42 (16.82)	42.99 (15.57)	0.295	65.31 (20.91)	59.30 (18.76)	0.070	65.40 (20.45)	68.16 (21.93)	0.277
20 : 4 *n*-6	209.10 (53.94)	214.86 (44.71)	0.242	233.86 (48.55)	249.06 (42.37)	0.052	240.11 (50.94)	245.01 (47.89)	0.333
22 : 4 *n*-6	9.20 (3.72)	8.92 (3.00)	0.307	11.81 (3.41)	11.29 (3.11)	0.217	11.67 (2.95)	12.54 (3.48)	0.103
22 : 5 *n*-6	8.48 (3.94)	7.82 (3.34)	0.138	14.06 (4.73)	13.31 (3.82)	0.202	15.08 (5.15)	15.68 (5.58)	0.304
Total	996.57 (175.31)	986.83 (179.41)	0.366	1320.52 (199.97)	1346.20 (211.29)	0.259	1465.73 (245.46)	1537.86 (220.82)	0.093
n-3 PUFA									
18 : 3 *n*-3	14.94 (5.21)	14.87 (5.67)	0.467	24.31 (7.47)	23.54 (7.08)	0.298	27.92 (8.58)	30.36 (8.10)	0.102
20 : 5 *n*-3	9.92 (6.08)	10.08 (4.04)	0.430	10.32 (6.11)	9.16 (4.43)	0.156	8.95 (5.18)	8.99 (5.35)	0.486
22 : 5 *n*-3	12.28 (3.99)	12.25 (3.36)	0.485	12.92 (4.00)	12.14 (4.16)	0.164	12.17 (3.71)	12.43 (3.96)	0.381
22 : 6 *n*-3	56.62 (16.20)	56.16 (15.58)	0.430	73.65 (19.30)	73.85 (13.52)	0.478	76.21 (20.68)	75.61 (15.18)	0.447
Total	93.76 (27.09)	93.37 (22.10)	0.462	121.21 (30.18)	118.70 (23.97)	0.331	125.25 (31.79)	127.38 (23.30)	0.378

EPDS, Edinburgh Postnatal Depression Scale; S.D., standard deviation; PUFAs, polyunsaturated fatty acids.
*p-value statistical significance based on Student's t test.

Table 3. *Random intercept logistic model of n-6 and n-3 polyunsaturated FA and their associations with depressive symptoms measured prospectively in women followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012*

	EPDS score (< 11/≥11)			
	Random intercept logistic model			
	Univariate		Adjusted for covariates *	
Variables	Between effect OR (95% CI)	Within effect OR (95% CI)	Between effect OR (95% CI)	Within effect OR (95% CI)
Gestational age (weeks)	**0.95 (0.92–0.98)**	**0.95 (0.92–0.98)**	**0.95 (0.92–0.98)**	**0.95 (0.92–0.98)**
Main independent variables				
Omega-6 PUFAs (μg/ml)				
18 : 2 *n*-6	1.00 (0.99–1.00)	0.99 (0.99–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.01)
18 : 3 *n*-6	1.03 (0.92–1.15)	0.95 (0.86–1.06)	1.04 (0.95–1.14)	0.93 (0.83–1.04)
20 : 2 *n*-6	0.96 (0.82–1.12)	0.89 (0.83–0.96)	0.91 (0.80–1.03)	0.98 (0.86–1.12)
20 : 3 *n*-6	0.99 (0.96–1.01)	0.97 (0.95–0.99)	0.99 (0.97–1.01)	0.99 (0.96–1.02)
20 : 4 *n*-6	1.00 (0.99–1.01)	0.99 (0.98–1.00)	1.00 (1.00–1.01)	1.00 (0.99–1.02)
22 : 4 *n*-6	0.96 (0.83–1.12)	0.86 (0.76–0.96)	0.99 (0.88–1.12)	0.94 (0.81–1.08)
22 : 5 *n*-6	0.94 (0.84–1.04)	0.88 (0.81–0.95)	0.97 (0.89–1.06)	0.95 (0.85–1.07)
Total *n*-6	1.00 (0.99–1.00)	0.99 (0.99–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
Omega-3 PUFAs (μg/ml)				
18 : 3 *n*-3	1.02 (0.95–1.09)	0.93 (0.89–0.97)	0.99 (0.93–1.04)	0.97 (0.90–1.04)
20 : 5 *n*-3	1.06 (0.97–1.15)	0.94 (0.88–1.01)	1.04 (0.96–1.11)	**0.92 (0.86–0.99)**
22 : 5 *n*-3	1.04 (0.93–1.17)	0.88 (0.77–0.99)	1.06 (0.97–1.17)	**0.87 (0.77–0.99)**
22 : 6 *n*-3	1.01 (0.98–1.03)	0.95 (0.93–0.98)	1.02 (0.99–1.04)	**0.96 (0.93–0.99)**
Total *n*-3	1.01 (0.99–1.02)	0.97 (0.96–0.98)	1.01 (0.99–1.02)	**0.98 (0.96– 0.99)**
Ratio				
Total *n*-6/total *n*-3	0.87 (0.71–1.07)	1.13 (0.91–1.42)	0.84 (0.70–1.01)	**1.40 (1.09–1.79)**

EPDS, Edinburgh Postnatal Depression Scale; PUFAs, polyunsaturated fatty acids.

*Adjusted for gestational age, previous history of depression, marital status, inter-partum interval, pre-pregnancy BMI and planned pregnancy, using logistic models with random intercept.

The significance of bold values is $p < 0.05$.

Note: In the first term, it was calculated the mean FA values for each woman and in the second term, it was subtracted the FA concentrations from their mean values.

investigated the EPDS sensitivity and specificity (Santos *et al*. 2007; Su *et al*. 2007; Husain *et al*. 2014). A third limitation refers to the potential presence of residual confounding. We could not evaluate either FA concentrations or depressive symptoms prior to pregnancy, which did not allow us to measure the onset of depression nor the baseline FA profile. In contrast, there are important strengths in the present study especially with regard to the longitudinal assessment of FA status and depressive symptoms during pregnancy. Studies on depression during pregnancy with repeated measures and appropriate statistical analyses are scarce. The random intercept logistic regression model employed in the current study is appropriate as it accounts for the dependence between repeated observations and takes into consideration the longitudinal measure over pregnancy of both FA and depressive symptoms.

Pinto *et al.* (2015) have analysed PUFAs trends throughout pregnancy in a previous paper based on data from the same cohort. These authors observed that total *n*-6 and *n*-3 PUFAs and DHA concentrations increased significantly throughout pregnancy. The *n*-6/*n*-3 ratio trend revealed a lower rate of increase from the 1st to the 2nd trimester compared with the rate in the second period. The mean EPA concentrations were constant from the 1st to the 2nd trimester and decreased significantly from the 2nd to the 3rd trimester. A previous study of Stewart *et al.* (2007) found an increase in the FA composition during pregnancy (mean gestational weeks of 12.5, 26.1 and 35.5) in a sample of 47 healthy women. They observed a less evident rise at the end of gestation, which is in line with the pattern of change described in the study of Pinto *et al.* (2015). We did not detect differences in the mean PUFAs concentrations at each follow-up visit,

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

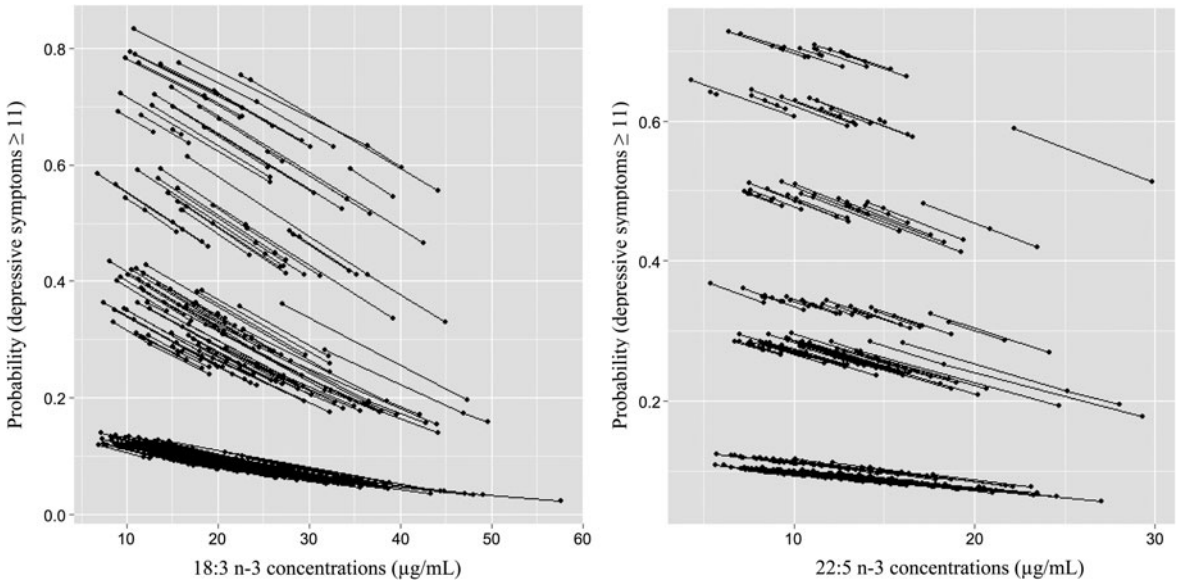

Fig. 1. Predicted probabilities of depressive symptoms according to 18:3 (α-linolenic) and 22:5 (docosapentaenoic) n-3 fatty acids concentrations in women followed at a public health center in Rio de Janeiro, Brazil, 2009–2012.
Note: Each line represents the estimated probability of depressive symptoms throughout pregnancy for each woman. The number of dots at each line corresponds to the number of observations throughout pregnancy.

stratifying by depressive symptoms classification. These findings are in accordance with the results from the random intercept logistic model, which identified a longitudinal association between PUFAs and depressive symptoms 'within' individuals.

We observed that each one-unit increase in EPA, DHA, DPA or total *n*-3 was associated with a 8.0, 4.0, 13.0 or 2.0% reduction in the odds of depressive symptoms when comparing the longitudinal change in the same individual during pregnancy, respectively, while one-unit increase in the total *n*-6/*n*-3 ratio represented a 40.0% greater odds of depressive symptoms. Other studies have evaluated the association of *n*-6/*n*-3 ratio and *n*-3 FA with depressive disorders, with

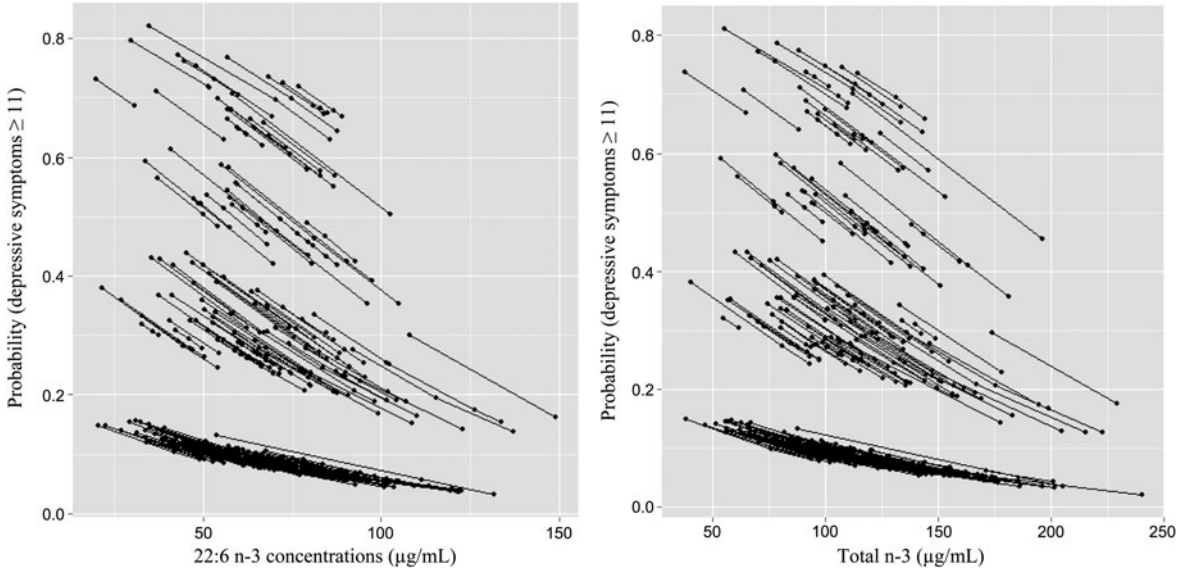

Fig. 2. Predicted probabilities of depressive symptoms according to 22:6 (docosahexaenoic) and total n-3 fatty acids concentrations in women followed at a public health center in Rio de Janeiro, Brazil, 2009–2012.
Note: Each line represents the estimated probability of depressive symptoms throughout pregnancy for each woman. The number of dots at each line corresponds to the number of observations throughout pregnancy.

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

mixed results and diversified methodological approaches (Su *et al.* 2008; Rees *et al.* 2009; Bodnar *et al.* 2012; Sallis *et al.* 2014; Shiraishi *et al.* 2015). Rees *et al.* (2009) assessed *n*-3 and *n*-6 PUFAs in plasma phospholipids and found lower and statistically significant per cent of total *n*-3 and DHA and higher *n*-6/*n*-3 ratio in depressed women ($n = 16$) compared with non-depressed controls ($n = 22$), all in the third trimester of pregnancy. A cross-sectional study of Shiraishi *et al.* (2015) evaluated 329 women between 19 and 23 weeks' gestation and identified a negative association between plasma concentrations of DHA and depressive symptoms. The results of both studies corroborate our findings. However, a longitudinal study with 135 pregnant women failed to find association between essential FA assessed in red blood cells (at approximately 20 weeks of gestation) and occurrence of major depressive disorder (at 20th, 30th and 36th week) (Bodnar *et al.* 2012). In that study, the FA were assessed only in the second trimester and a principal component analysis was used to obtain a factor score combining AA, EPA and DHA. This methodological approach and the presence of a unique measure of FA may explain the differences between this particular longitudinal study and our results.

The *n*-3 and *n*-6 FA participates in the same metabolic chain. Therefore, their anti- and pro-inflammatory actions, respectively, depend on the balanced amount of each FA precursor and/or product (Arterburn *et al*. 2006). The estimated dietetic *n*-6/*n*-3 ratio for occidental populations varies between 10 : 1 and 20 : 1, which is incredible higher than the 4 : 1 ratio considered ideal for the major conversion of the α-linolenic acid (18 : 3 *n*-3) to DHA (Simopoulos, 2000; Da Rocha & Kac, 2012; Grosso *et al*. 2014*a*, *b*). In this context, the interest on the investigation of the *n*-6/*n*-3 ratio is guided by the described association between high *n*-6/*n*-3 ratio and raised concentrations of pro-inflammatory eicosanoids produced from the metabolism of AA, which favours the development of inflammatory diseases such as depression (Simopoulos, 2002; Kiecolt-Glaser *et al*. 2007; Da Rocha & Kac, 2012).

We have not identified studies that find associations between *n*-3 DPA and depressive disorders. However, Hamazaki *et al.* (2013) found decreased *n*-3 DPA content in the post-mortem entorhinal cortex from patients with major depressive disorder ($n = 15$) compared with controls ($n = 15$). In addition, beneficial effects of this *n*-3 FA, e.g., reduced inflammation and increased neuroprotective effects in rats, have been reported (Kaur *et al.* 2011), suggesting the need of experimental and observational studies in humans and pregnant women.

Our results suggest that the associations between increases in DHA and DPA content and reduction in depressive symptoms may represent a biologically plausible relationship because the association persists after control for a variety of potentially confounding variables. Other studies have investigated the neurobiological mechanisms underlying the association between low levels of PUFAs and depressive disorders (Shapiro *et al.* 2012; Levant, 2013). An extensive literature review suggested that alterations in the *n*-3 PUFAs status may be related to decreased activity of important neurotransmitters (serotonin and dopamine) and dysregulation in hypothalamic–pituitary–adrenal axis (Levant, 2013). One possible mechanism may involve the brain-derived neurotrophic factor (BDNF). In an experimental study of 90-days of supplementation with fish-oil containing EPA and DHA, an antidepressant effect in adult rats was observed. Increased expression of BDNF and augmented concentrations of serotonin in the cortex and hippocampus were found (Vines *et al.* 2012). A recent review of Su *et al.* (2015) highlights the effect of omega-3 PUFAs treatment, not only considering the antidepressant therapy. The authors have mentioned the neuroprotective effect and the anti-inflammatory action achieved by the high fish intake. Levant *et al.* (2008) found a decreased brain DHA content associated with reduced BDNF gene expression in rats after administration of α-linolenic acid-deficient diet. It has been proposed that the decrease in BDNF expression cause hippocampal atrophy and consequently impairs neurogenesis (Sheline *et al.* 1996).

We found a high prevalence of depressive symptoms, especially at baseline. There are very few studies that evaluated depressive symptoms in early pregnancy (Banti *et al.* 2011; Bödecs *et al.* 2013). Bödecs *et al.* (2013) investigated 503 Hungarian pregnant women with a mean of 8 weeks and reported a lower prevalence of depressive symptoms (19.9%) measured by the Beck Depression Inventory, when compared with our results. These authors used a different instrument to measure depressive symptoms compared with the one used in our study, which may, in part, explain the divergent findings. De Almeida *et al.* (2012) assessed low-income Brazilian pregnant women ($n = 712$) between the 16th and 36th week and revealed a prevalence of depressive symptoms (21.6%) similar to ours at the second trimester. Husain *et al.* (2012) assessed a sample of 714 British Pakistani women in the third gestational trimester who were screened with depressive symptoms by EPDS. These authors observed an EPDS of 12 or more for 36.6% of the women, i.e., higher in comparison with our results for the same gestational trimester.

https://doi.org/10.1017/S204579601500116X Published online by Cambridge University Press

In the present study, potential confounders at baseline were more frequent in women with depressive symptoms compared with those without these symptoms. Our findings are in line with the study of Pereira *et al.* (2009) that evaluated 331 Brazilian women in the third pregnancy trimester and found a positive association between previous history of depression and major depression disorder. In a Finnish cross-sectional study ($n = 511\,938$), single marital status, previous history of depression and low socioeconomic status increased the chance of major depression during pregnancy (Räisänen *et al.* 2014). Our result also corroborates the study conducted in a rural zone of Southwestern Ethiopia (Dibaba *et al.* 2013). These authors observed that women who did not want the current pregnancy were more likely to report depressive symptoms during pregnancy. A longitudinal study also found a higher likelihood of gestational depression in women with high pre-pregnancy BMI, suggesting a risk of adverse outcomes for the mother and foetus (Bodnar *et al.* 2009).

Conclusion

This prospective study found a high prevalence of depressive symptoms in low-income Brazilian pregnant women and highlighted the attention needed to cope with this serious public health problem that is often overlooked by public health practitioners. We observed that lower serum concentrations of DHA, EPA and *n*-3 DPA and a higher *n*-6/*n*-3 ratio were associated with greater odds of depressive symptoms when comparing the longitudinal change in the same individual during pregnancy. These results suggest that the *n*-3 FA may be involved in the pathophysiology of depression.

Observational and experimental investigations, with more heterogeneous populations and with larger samples, are still needed to confirm the observed associations and to evaluate and elucidate the aetiology of the depressive disorder and the biological effect of FA on the development of this mental disorder in pregnant women. In addition, our study and future investigations are essential, in long-term, to provide direction for the development of public policies focusing on primary prevention and treatment strategies that shall include humanisation of prenatal care and gestational planning combined with prenatal nutritional (dietetic) counselling.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S204579601500116X

Acknowledgements

We would like to thank the organisations that funded our research. TJPP received scholarship from the Carlos Chagas Filho Foundation for Research Support of Rio de Janeiro State (FAPERJ in the Portuguese acronym). AAFV, DRF and JL received scholarship from Brazilian Coordination for the Training of University Level Personnel (CAPES in the Portuguese acronym). GK received scholarship from National Council for Scientific and Technological Development (CNPq in the Portuguese acronym).

Author Contribution

Pinto TJP and Kac G formulated the research question, designed the study and wrote the protocol. Author Pinto TJP conducted the statistical analysis, and author Cunha GM provided substantial support for statistical analysis. Pinto TJP, Vilela AAF, Farias DR, Lepsch J, Vaz JS, Factor-Litvak P and Susser E assisted the literature searches and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Financial Support

This study was developed in partnership with the Institute of Psychiatry, Federal University of Rio de Janeiro (IPUB – UFRJ) and the Institute of Nutrition of the University of the State of Rio de Janeiro (UERJ) and received funding from the Research Foundation of the State of Rio de Janeiro (FAPERJ, grant number: E-26/111.400/2010, E_14/2010) and the National Council for Scientific and Technological Development (CNPq, grant number: 471196/2010-0).

Conflict of Interest

None.

Ethical Standards

The study protocol was approved by the Maternity Hospital Ethics Committee (Protocol number: 0023.0.361.000-08), by the Institute of Psychiatry (Protocol number: 0012.0.249.000-09), both from the Federal University of Rio de Janeiro, and the Ethics Committee of the Municipal Secretary of Rio de Janeiro city (Protocol number: 0139.0.314.000-09). All participants signed the informed consent about participation in the study.

References

Arterburn LM, Hall EB, Oken H (2006). Distribution, interconversion, and dose response of *n*-3 fatty acids in humans. *American Journal of Clinical Nutrition* **83**, 1467–1476.

Banti S, Mauri M, Oppo A, Borri C, Rambelli C, Ramacciotti D, Montagnani MS, Camilleri V, Cortopassi S, Rucci P, Cassano GB (2011). From the third month of pregnancy to 1 year postpartum. Prevalence, incidence, recurrence, and new onset of depression. Results from the Perinatal Depression–Research & Screening Unit study. *Comprehensive Psychiatry* **52**, 343–351.

Bauer A, Pawlby S, Plant DT, King D, Pariante CM, Knapp M (2015). Perinatal depression and child development: exploring the economic consequences from a South London cohort. *Psychological Medicine* **45**, 51–61.

Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR (2004). Prevalence of depression during pregnancy: systematic review. *Obstetrics and Gynecology* **103**, 698–709.

Bödecs T, Szilágyi E, Cholnoky P, Sándor J, Gonda X, Rihmer Z, Horváth B (2013). Prevalence and psychosocial background of anxiety and depression emerging during the first trimester of pregnancy: data from a Hungarian population-based sample. *Psychiatria Danubina* **25**, 352–358.

Bodnar LM, Wisner KL, Moses-Kolko E, Sit DKY, Hanusa BH (2009). Prepregnancy body mass index, gestational weight gain, and the likelihood of major depressive disorder during pregnancy. *Journal of Clinical Psychiatry* **70**, 1290–1296.

Bodnar LM, Wisner KL, Luther JF, Powers RW, Evans RW, Gallaher MJ, Newby P (2012). An exploratory factor analysis of nutritional biomarkers associated with major depression in pregnancy. *Public Health Nutrition* **15**, 1078–1086.

Cox JL, Holden JM, Sagovsky R (1987). Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *British Journal of Psychiatry: The Journal of Mental Science* **150**, 782–786.

Da Rocha CMM, Kac G (2012). High dietary ratio of omega-6 to omega-3 polyunsaturated acids during pregnancy and prevalence of post-partum depression. *Maternal and Child Nutrition* **8**, 36–48.

Das UN (2008). Folic acid and polyunsaturated fatty acids improve cognitive function and prevent depression, dementia, and Alzheimer's disease–but how and why? *Prostaglandins, Leukotrienes, and Essential Fatty Acids* **78**, 11–19.

de Almeida MS, Nunes MA, Camey S, Pinheiro AP, Schmidt MI (2012). Mental disorders in a sample of pregnant women receiving primary health care in Southern Brazil. *Cadernos de Saúde Pública* **28**, 385–394.

Dibaba Y, Fantahun M, Hindin MJ (2013). The association of unwanted pregnancy and social support with depressive symptoms in pregnancy: evidence from rural Southwestern Ethiopia. *BMC Pregnancy and Childbirth* **13**, 135.

Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T (2005). Perinatal depression: a systematic review of prevalence and incidence. *Obstetrics and Gynecology* **106**, 1071–1083.

Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, Drago F, Caraci F (2014*a*). Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. *PLoS ONE* **9**, e96905.

Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F, Caraci F (2014*b*). Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxidative Medicine and Cellular Longevity* **2014**, 313570.

Hamazaki K, Hamazaki T, Inadera H (2013). Abnormalities in the fatty acid composition of the postmortem entorhinal cortex of patients with schizophrenia, bipolar disorder, and major depressive disorder. *Psychiatry Research* **210**, 346–350.

Husain N, Cruickshank K, Husain M, Khan S, Tomenson B, Rahman A (2012). Social stress and depression during pregnancy and in the postnatal period in British Pakistani mothers: a cohort study. *Journal of Affective Disorders* **140**, 268–276.

Husain N, Rahman A, Husain M, Khan SM, Vyas A, Tomenson B, Cruickshank KJ (2014). Detecting depression in pregnancy: validation of EPDS in British Pakistani mothers. *Journal of Immigrant and Minority Health* **16**, 1085–1092.

Kaur G, Cameron-Smith D, Garg M, Sinclair AJ (2011). Docosapentaenoic acid (22:5*n*-3): a review of its biological effects. *Progress in Lipid Research* **50**, 28–34.

Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf DQ, Lemeshow S, Glaser R (2007). Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosomatic Medicine* **69**, 217–224.

Lalonde TL, Nguyen AQ, Yin J, Irimata K, Wilson JR (2013). Modeling correlated binary outcomes with time-dependent covariates. *Journal of Data Science* **11**, 715–738.

Levant B (2013). *N*-3 (omega-3) polyunsaturated fatty acids in the pathophysiology and treatment of depression: pre-clinical evidence. *CNS and Neurological Disorders-Drug Targets (Formerly Current Drug Targets – CNS and Neurological Disorders)* **12**, 450–459.

Levant B, Ozias MK, Davis PF, Winter M, Russell KL, Carlson SE, Reed GA, McCarson KE (2008). Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: interactions with reproductive status in female rats. *Psychoneuroendocrinology* **33**, 1279–1292.

Levy RB, Claro RM, Mondini L, Sichieri R, Monteiro CA (2012). Regional and socioeconomic distribution of household food availability in Brazil, in 2008–2009. *Revista de Saúde Pública* **46**, 6–15.

Lin YH, Salem N, Wells EM, Zhou W, Loewke JD, Brown JA, Lands WEM, Goldman LR, Hibbeln JR (2012). Automated high-throughput fatty acid analysis of umbilical cord serum and application to an epidemiological study. *Lipids* **47**, 527–539.

Lohman TG, Roche AF, Martorell R (1988). *Anthropometric Standardization Reference Manual*. Human Kinetics Books: Champaign, IL.

Masood MA, Salem N (2007). High-throughput analysis of plasma fatty acid methyl esters employing robotic transesterification and fast gas chromatography. *Lipids* **43**, 171–180.

Murray D, Cox JL (1990). Screening for depression during pregnancy with the edinburgh depression scale (EDDS). *Journal of Reproductive and Infant Psychology* **8**, 99–107.

Parker G, Hegarty B, Granville-Smith I, Ho J, Paterson A, Gokiert A, Hadzi-Pavlovic D (2014). Is essential fatty acid status in late pregnancy predictive of post-natal depression? *Acta Psychiatrica Scandinavica* **131**, 148–156.

Pereira PK, Lovisi GM, Pilowsky DL, Lima LA, Legay LF (2009). Depression during pregnancy: prevalence and risk factors among women attending a public health clinic in Rio de Janeiro, Brazil. *Cadernos de Saúde Pública* **25**, 2725–2736.

Pereira PK, Lovisi GM, Lima LA, Legay LF, de Cintra Santos JF, Santos SA, Thiengo DL, Valencia E (2011). Depression during pregnancy: review of epidemiological and clinical aspects in developed and developing countries. *Psychiatric Disorders – Trends and Developments*, 267.

Pinto TJP, Farias DR, Rebelo F, Lepsch J, Vaz JS, Moreira JD, Cunha GM, Kac G (2015). Lower inter-partum interval and unhealthy life-style factors are inversely associated with *n*-3 essential fatty acids changes during pregnancy: a prospective cohort with Brazilian women. *PLoS ONE* **10**, e0121151.

Räisänen S, Lehto SM, Nielsen HS, Gissler M, Kramer MR, Heinonen S (2014). Risk factors for and perinatal outcomes of major depression during pregnancy: a population-based analysis during 2002–2010 in Finland. *BMJ Open* **4**, e004883.

Rees A-M, Austin M-P, Owen C, Parker G (2009). Omega-3 deficiency associated with perinatal depression: case control study. *Psychiatry Research* **166**, 254–259.

Rich-Edwards JW (2006). Sociodemographic predictors of antenatal and postpartum depressive symptoms among women in a medical group practice. *Journal of Epidemiology and Community Health* **60**, 221–227.

Sallis H, Steer C, Paternoster L, Davey Smith G, Evans J (2014). Perinatal depression and omega-3 fatty acids: a Mendelian randomisation study. *Journal of Affective Disorders* **166**, 124–131.

Santos IS, Matijasevich A, Tavares BF, Barros AJD, Botelho IP, Lapolli C, Magalhães PV da S, Barbosa APPN, Barros FC (2007). Validation of the Edinburgh postnatal depression scale (EPDS) in a sample of mothers from the 2004 Pelotas Birth Cohort Study. *Cadernos De Saúde Pública* **23**, 2577–2588.

Shapiro GD, Fraser WD, Séguin JR (2012). Emerging risk factors for postpartum depression: serotonin transporter genotype and omega-3 fatty acid status. *Canadian Journal of Psychiatry. Revue Canadienne De Psychiatrie* **57**, 704–712.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996). Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 3908–3913.

Shiraishi M, Matsuzaki M, Yatsuki Y, Murayama R, Severinsson E, Haruna M (2015). Associations of dietary intake and plasma concentrations of eicosapentaenoic and docosahexaenoic acid with prenatal depressive symptoms in Japan: depressive symptoms and fatty acids. *Nursing and Health Sciences* **17**, 257–262.

Simopoulos AP (2000). Human requirement for N-3 polyunsaturated fatty acids. *Poultry Science* **79**, 961–970.

Simopoulos AP (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy* **56**, 365–379.

Stewart F, Rodie VA, Ramsay JE, Greer IA, Freeman DJ, Meyer BJ (2007). Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum. *Lipids* **42**, 335–344.

Stuart S, Couser G, Schilder K, O'Hara MW, Gorman L (1998). Postpartum anxiety and depression: onset and comorbidity in a community sample. *Journal of Nervous and Mental Disease* **186**, 420–424.

Su KP, Chiu TH, Huang CL, Ho M, Lee CC, Wu PL, Lin CY, Liau CH, Liao CC, Chiu WC, Pariante CM (2007). Different cutoff points for different trimesters? The use of Edinburgh Postnatal Depression Scale and Beck Depression Inventory to screen for depression in pregnant Taiwanese women. *General Hospital Psychiatry* **29**, 436–441.

Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, Chang HC, Pariante CM (2008). Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Psychiatry* **69**, 644–651.

Su KP, Wang SM, Pae CU (2013). Omega-3 polyunsaturated fatty acids for major depressive disorder. *Expert Opinion on Investigational Drugs* **22**, 1519–1534.

Su KP, Matsuoka Y, Pae CU (2015). Omega-3 polyunsaturated fatty acids in prevention of mood and anxiety disorders. *Clinical Psychopharmacology and Neuroscience* **13**, 129–137.

Teofilo MM, Farias DR, Pinto Tde J, Vilela AA, Vaz J dos S, Nardi AE, Kac G (2014). HDL-cholesterol concentrations are inversely associated with Edinburgh Postnatal Depression Scale scores during pregnancy: results from a Brazilian cohort study. *Journal of Psychiatric Research* **58**, 181–188.

Vines A, Delattre AM, Lima MMS, Rodrigues LS, Suchecki D, Machado RB, Tufik S, Pereira SIR, Zanata SM, Ferraz AC (2012). The role of 5-HT1A receptors in fish oil-mediated increased BDNF expression in the rat hippocampus and cortex: a possible antidepressant mechanism. *Neuropharmacology* **62**, 184–191.