

# Arachidonic acid and DHA status in pregnant women is not associated with cognitive performance of their children at 4 or 6–7 years



**Arachidonic and docosahexaenoic acid status in pregnant women is not associated with cognitive performance of their children at 4 or 6 - 7 years**

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*Abbreviations:* ARA, arachidonic acid; BMI, body-mass-index; CANTAB®, Cambridge Neuropsychological Test Automated Battery; DAG, direct acyclic graph; DHA, docosahexaenoic acid; DMS, delayed matching to sample; FAME, fatty acid methyl esters; FFQ, food frequency questionnaire; IED, intra/extra-dimensional shift; IST, Information Sampling Task; PC, phosphatidylcholine; SSP, Spatial Span; WASI, Wechsler Abbreviated Scale of Intelligence; WPPSI, Wechsler Preschool and Primary Scale of Intelligence.

**Abstract**

Arachidonic (ARA) and docosahexaenoic (DHA) acids, supplied primarily from the mother, are required for early development of the central nervous system. Thus, variations in maternal ARA or DHA status may modify neurocognitive development. We investigated the relationship between maternal ARA and DHA status in early (11.7 wk) or late (34.5 wk) pregnancy on neurocognitive function at age 4 y or 6-7 y in 724 mother-child pairs from the Southampton Women's Survey cohort. Plasma phosphatidylcholine fatty acid composition was measured in early and late pregnancy. ARA concentration in early pregnancy predicted 13% of the variation in ARA concentration in late pregnancy ($\beta = 0.36$, $P < 0.001$). DHA concentration in early pregnancy predicted 21% of the variation in DHA concentration in late pregnancy ($\beta = 0.46$, $P < 0.001$). Children's cognitive function at age 4 y was assessed by the Wechsler Preschool and Primary Scale of Intelligence and at age 6-7 y by the Wechsler Abbreviated Scale of Intelligence. Executive function at age 6-7 y was assessed using elements of the Cambridge Neuropsychological Test Automated Battery. Neither DHA nor ARA concentrations in early or late pregnancy were associated significantly with neurocognitive function in children at age 4 y or age 6-7 y. These findings suggest that ARA and DHA status during pregnancy in the range found in this cohort are unlikely to have major influences on neurocognitive function in healthy children.

## INTRODUCTION

The polyunsaturated fatty acids (PUFA) arachidonic acid (ARA) and docosahexaenoic acid (DHA) are major components of neural cell membrane phospholipids [1, 2]. In humans, there is substantial accumulation of ARA and DHA into the fetal brain during the third trimester of pregnancy [1, 2]. The human fetus is dependent largely on transfer of pre-formed ARA and DHA from the mother across the placenta. Human term infants fed milk formula without preformed DHA exhibit low DHA concentrations in brain [3] and plasma phospholipids [4]. Studies in non-human primates have shown that maternal diets deficient in omega-3 PUFA are associated with impaired cognition and abnormal behaviour in their offspring [5; 6]. It is therefore considered important to ensure adequate provision of DHA and ARA during brain development [7].

There have been relatively few studies of the effect of maternal or neonatal ARA and DHA status on neurocognitive function in children. ARA and DHA status at birth has been shown not to be associated with cognitive development at age 4 y [8], or with problem behaviour [9] and cognitive development [10] at age 7 y, although there was a positive association with motor function [10]. In contrast, maternal fish intake, a proxy measure of DHA intake, was associated positively with developmental milestones at 6 and 18 months [11] and with cognition at age 3 y [12]. Maternal sea food intake has also been associated positively with verbal intelligence quotient in children [13], although others have concluded that maternal fish intake during pregnancy had little long-term effect on the neurodevelopment of the child [14]. However, these studies did not report maternal ARA or DHA status.

The primary purpose of the present study was to determine the relationship between maternal ARA and DHA concentrations in early and late pregnancy, and neurocognitive outcomes in their children at age 4 y or at age 6 - 7 y. PUFA concentrations were measured at two time points in gestation because DHA concentration increases physiologically from mid pregnancy [29,30, 31] due to adaptions to maternal hepatic phospholipid [32] and PUFA metabolism [33]. We also tested the relationship between the change in ARA and DHA status during pregnancy, as a surrogate measure of the mother's capacity to adapt her PUFA metabolism, and neurocognitive function in children.

## METHODS

*Ethical statement*

The SWS was approved by the Southampton and South West Hampshire Local Research Ethics Committee (307/97, 153/99w, 005/03/t and 06/Q1702/104), and all participants gave written informed consent.

*Study sample*

The Southampton Women's Survey (SWS) is a prospective cohort study of the impact of the early life environment on patterns of health throughout the life course in which the diet, body composition, physical activity, and social circumstances of non-pregnant women aged 20 to 34 years living in the city of Southampton, UK, were characterised [34]. Women were recruited through primary healthcare practices across the city between April 1998 and December 2002. Women who subsequently became pregnant with singleton fetuses were followed throughout pregnancy; detailed interviews were conducted at 11 and 34 wks gestation, when blood samples were collected for fatty acid analysis after an overnight fast. The growth and development of the SWS children were assessed during infancy and childhood.

A total of 3158 women became pregnant and delivered a live-born singleton infant within the study period (Supplementary Fig. 1). Eight infants died in the neonatal period. Subsets of children were followed up at age 4 and at age 6-7y. 1207 offspring had data collected about cognitive development at age 4 y or at age 6 - 7 y. 724 mothers did not have exposure data on plasma PC fatty acid composition, leaving an analysis sample of 724 mother-child pairs. Of these, 584 gave blood samples in early pregnancy and 331 gave blood samples in late pregnancy. 191 women provided blood samples in both early and late pregnancy in early (median 11.7 (IQR 11.4, 12.2) wk), before the start of the physiological increase in plasma PC DHA concentration [29] and in late (34.5 (34.2, 34.8) wk) pregnancy, corresponding to maximum plasma PC DHA concentration [29]. Details of mothers' educational attainment (defined in 6 groups according to highest academic qualification) were obtained at the pre-pregnancy interview. Height was measured with a portable stadiometer (Harpenden; CMS Weighing Equipment Ltd, London, UK) to the nearest 0.1 cm with the head in the Frankfort plane. Weight was measured using calibrated electronic scales (Seca, Hamburg, Germany) to the nearest 0.1 kg (after removal of shoes and heavy clothing or jewellery). These measurements were used to calculate body mass index (BMI). Among women who became pregnant, smoking status was ascertained. Maternal IQ was assessed when her children were aged age 4 y and age 6 - 7 y using the Wechsler Abbreviated Scale of Intelligence (WASI) scale.

*Maternal sample collection and plasma fatty acid composition*

Venous blood samples were collected into tubes containing lithium heparin in early and late pregnancy. Plasma was separated from cells by centrifugation and stored at -80ºC. Plasma PC fatty acid composition was measured essentially as described [35]. Briefly, frozen plasma (0.8ml) was thawed, dipentadecanoyl PC (100 µg) internal standard was added and total lipids were then extracted with chloroform and methanol. Lipid extracts were dried under $N_2$, dissolved in chloroform (1.0 ml) and applied to a BondElut aminopropylsilica cartridge (100 mg) (Agilent Technologies). Unbound lipids were removed by washing with chloroform and PC was then eluted with chloroform/methanol (60:40, v/v). Purified PC was dissolved in toluene and fatty acid methyl esters (FAME) were synthesised by heating at 50°C in the presence of methanol containing 2 % (v/v) sulphuric acid. FAME were recovered with hexane and resolved on a BPX-70 fused silica capillary column (32 m×0·25 mm×25 µm; SGE Analytical Science) using an Agilent 6890 gas chromatograph equipped with flame ionisation detection (Agilent Technologies Ltd). The concentrations of ARA and DHA were calculated from the ratio of their peak areas to the peak area of the internal standard, multiplied by the amount of standard and corrected for the volume of plasma extracted.

*Assessment of cognitive function in children*

IQ was assessed at age 4 y using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) [36] and at age 6 - 7 y using the Wechsler Abbreviated Scale of Intelligence (WASI) [37]. Executive functioning was tested at age 6 - 7 y using the Cambridge Neuropsychological Test Automated Battery (CANTAB®), with 4 specific tests and outcomes chosen based on the published literature: these were 1) delayed matching to sample (DMS, i) total correct, to test visual working memory, 2) intra/extra-dimensional shift (IED, ii) total errors, iii) adjusted errors, and iv) stages completed, to test rule learning and cognitive flexibility through efficiency of completing the test, 3) Spatial Span (SSP) length, to test working memory), and 4) Information Sampling Task (IST), vi) pre-extradimensional shift errors, vii) extradimensional shift errors and viii) adjusted IED total errors, to test impulsivity and decision making [38].

*Statistical analysis*

Children's IQ was the primary study outcome for which we calculated the statistical power of the analysis. Two hundred and sixty participants had IQ measured at 4 years; of these, 146

participants had measures of early pregnancy fatty acid status and 253 had measures of late pregnancy fatty acid status. Since these were all the participants in the SWS cohort with these measurements, further data collection is not feasible. Consequently, we have determined minimally detectable effect sizes. Our calculations show that these numbers have 80% power to detect regression coefficients of 2.9 and 2.2 at a 5% significance level, in early and late pregnancy respectively. Thus, we had sufficient numbers to detect a change in IQ of 2.9 (or 2.2) points for each standard deviation change in maternal fatty acid status. An increase in IQ of 2.9 or 2.2 points equates to a change in the distribution of IQ in a favourable direction of approximately 0.2 of a standard deviation (based on the standard deviation at age 4). This difference in IQ would have only a modest impact at an individual level. However, according to Rose's theory of prevention [39], a shift in the population mean IQ of that magnitude would potentially have a marked effect on cognitive ability in that population as it would prevent many individuals having cognitive problems

Summary statistics are presented as mean (SD) or median (IQR) for continuous variables and percentages for categorical variables. T-tests (for normally distributed continuous variables), Mann-Whitney U-Tests (for non-normally distributed continuous variables) and Chi-squared tests (for categorical variables) were used to compare the distributions of characteristics between omnivores and vegetarians. Maternal ARA and DHA levels, and changes in DHA and ARA concentrations in both early and late pregnancy were log transformed to normality before analysis. To assist with their interpretation, these logged variables were standardised so that the variables have an SD of 1. Maternal BMI was also log transformed before analysis. Additional analyses used maternal ARA and DHA without transformation.

IED pre-EDS errors, IED EDS errors and IED total errors (adjusted) were all transformed using Fisher-Yates transformations [40], so the resulting variable has SD units. It was not possible to transform IED total errors (stage 1), IED total errors (stage 8) and IST mean probability correct so these were grouped into five groups. Similarly, IED stages completed was grouped into four groups (five groups were inappropriate here due to the distribution of responses). It was not necessary to transform DMS total correct, or SSP span length so these are in original units.

Linear regression models were fitted to assess the association between dietary exposures and cognitive development outcomes. Models were fitted unadjusted and adjusted for confounders. We used the directed acyclic graph (DAG) approach [41] to select suitable confounders (Supplementary Fig. 2). This approach provides a robust and objective means of

identifying confounders in observational studies. DAGs are specified before data analysis based on prior knowledge. A graphical representation of causal effects between variables is generated in order to identify a set of variables that should be adjusted for in a multivariate analysis to minimise confounding bias [41]. The confounders identified by the DAG for the association between maternal fatty acid status and childhood cognitive development were maternal body-mass-index (BMI), maternal IQ, maternal education and maternal smoking. In addition, all models were adjusted for maternal, BMI, IQ and smoking and for child's sex and in the case of the CANTAB outcomes, and age (the WASI and WPSSI outcomes are already adjusted for age) in order to improve the precision of the models.

## RESULTS

*PC ARA and DHA concentrations in pregnant women*

Maternal ARA concentration was 34% lower in late pregnancy ($P = 0.004$) than in early pregnancy (Table 1). DHA concentration was 32% lower in late pregnancy than in early pregnancy, although this did not reach statistical significance (Table 1). Maternal ARA and DHA concentrations in early pregnancy were significantly correlated with their concentrations in late pregnancy (both $P < 0.001$) such that ARA concentration in early pregnancy predicted 13% of the variation in ARA concentration in late pregnancy ($\beta = 0.36$), and DHA concentration in early pregnancy predicted 21% of the variation in DHA concentration in late pregnancy ($\beta = 0.46$).

*The relationship between ARA and DHA concentration in maternal plasma PC and cognitive function in their children*

Unadjusted and adjusted data are summarised in Tables 2-3. There were no significant associations between maternal ARA concentrations in early or late pregnancy and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI IQ) composite IQ score adjusted or unadjusted at age 4 y (Table 2).

There were no significant associations between maternal ARA concentration in early or late pregnancy and any of the measures of cognitive function in the children at 6 - 7 y after adjustment for confounders (Table 2).

There were no significant associations between maternal plasma PC DHA concentration in early or late pregnancy, and the change in DHA concentration between early and late pregnancy, and cognitive function in the children at either age 4 y or age 6 - 7 y of age after adjustment for confounders (Table 3).

In additional analyses, untransformed maternal ARA and DHA were considered as predictors of offspring IQ at both 4 and 6 years of age (Supplementary Table 1); none of the associations were statistically significant. These findings are exemplified as follows; a 10 μg/ml increase in early pregnancy ARA was associated with a -0.37 IQ point decrease (95% CI -0.80, 0.07) at age 4 years (P = 0.10), whereas a 10 μg/ml increase in early pregnancy DHA was associated with a -0.03 (-0.80, 0.74) IQ point decrease at age 4 years (P = 0.94).

**Discussion**

The findings of this study quantify for the first time a modest association between maternal ARA and DHA concentrations in early and late pregnancy. However, there were no statically significant associations between maternal ARA or DHA concentrations during pregnancy, and their children's IQ or executive function.

The human fetus accumulates LC PUFA throughout gestation, although this occurs most rapidly during the last 5 weeks [(1)] and is dependent primarily on supply of preformed ARA and DHA from the mother. Deprivation of n-3 PUFA during pregnancy in non-human primates has been shown to induce impaired neurological development in their offspring [(5)]. Thus, it may be anticipated that variation in maternal ARA and DHA status, particularly during the third trimester, would be associated with differences in neurocognitive development. Previous studies that have shown longitudinal changes in DHA and ARA concentrations during pregnancy [(29,30)]. However, they did not report the relationship between maternal DHA or ARA status in early and late gestation. Both studies showed an increase in DHA concentration between early and late gestation, with the exception of Hungarian and Ecuadorian cohorts [(30)]. In contrast to cohorts studied previously in the UK [(29,30)], we found that maternal plasma ARA and DHA concentrations decreased during pregnancy by 34% and 32%, respectively, although this change in DHA was not significant. The reason for this decrease could not be deduced from the present data. However, these findings suggest a reduction in capacity to supply these PUFA to the developing fetus during a period in which the developing brain acquires substantial amounts of ARA and DHA [(1)].

The present study reports for the first time that there were no significant associations between maternal ARA and DHA status in early or late pregnancy, and measures of executive function and IQ in children. These findings suggest that, within the range of this cohort, variation in concentrations of these fatty acids in maternal blood during pregnancy exerts at most a minor influence on neurocognitive development in children. This suggestion is supported by the findings of studies in which pregnant women took a DHA supplement

during pregnancy which showed no significant effect on psychomotor, mental development or behavioural scores at 18 months [42; 43], or on executive function at age 2 y [43]. However, others have reported improved attention at age 5 y [46]. Moreover, a systematic review of 8 randomised controlled trials failed to detect a significant effect of maternal supplementation with DHA during breastfeeding on neurocognitive outcomes [46]. However, because this study did not investigate the nutrition of the children in the period between birth and the ages at which they were studied, postnatal dietary intakes of pre-formed ARA and DHA may have ameliorated any deficit in accumulation of these fatty acids in the central nervous system. For example, diet quality has been shown to be associated positively with neurodevelopment at age 4 y in the present cohort [37] and this may compensate for variations in DHA and ARA status in pregnancy

One possible explanation for the absence of significant associations between maternal ARA and DHA status and neurocognitive outcomes in the children is that the range of concentrations of these fatty acids reported here were sufficient to support normal brain development. Alternatively, it is possible that physiological processes may compensate for low PUFA concentrations in the mothers, thus protecting the development of the fetal brain from any negative effects of sub-optimal accumulation of DHA or ARA. For example, women have greater capacity for DHA synthesis [47], and maintain higher ARA and DHA status than men [48] and so conversion of essential fatty acids to longer chain PUFA may compensate for low dietary intakes of pre-formed DHA and ARA. Furthermore, pregnancy has been associated with specific increase in DHA in plasma PC [29, 30, 53], which has been shown in animal models to involve changes in the specificity of phospholipid biosynthesis [51] and increased expression of genes involved in conversion of essential fatty acids to longer chain PUFA [50; 51]. There is also evidence of biomagnification of DHA by the placenta leading to a higher concentration in the fetus compared to the mother [52].

Strengths of the study include assessment of a range of cognitive outcomes and the availability of measurements of maternal PUFA status in both early and late pregnancy. There was detailed information about potential maternal confounding factors known to influence the cognition of children including maternal education and IQ, smoking and BMI. Limitations of the study include that there was no information about the home environment. Consequently, we were not able to take into account factors that can influence IQ such as parenting style and the cognitive stimulation of the children. The children follow up were a sub-sample of the original cohort and some did not participate in all the tests. Since the present findings are from data collected in a cohort study and all the participants with data on

fatty acid composition and cognitive function were included in our analysis, we were not able to collect further data to increase sample size; our modest sample size could have contributed to the null findings.

Overall, the findings of this study suggest that maternal ARA and DHA status in early or late pregnancy in the range found in this cohort are unlikely to have major influences on neurocognitive function in the children. Consequently, in this group of healthy children of mothers consuming an omnivorous diet, maternal DHA and ARA status during pregnancy appeared to be adequate for development of cognitive function.

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*Author's responsibilities:* The author's responsibilities were as follows – GCB: had primary responsibility for the final content of the manuscript; GCB, KMG and SRC wrote the manuscript and participated in the design of the study; SRC analysed the data; CMB, HLF, CG, SMR, HMI, JB, NCH and the SWS study group conducted the research; PCC, GCB, KMG and CC oversaw the study. All authors contributed to the interpretation and discussion of the results, and read and approved the final version of the manuscript.

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**Table 1.** Characteristics of 724 mothers and children studied

| | Mother-child pairs studied | | |
|---|---|---|---|
| | *n* | Mean, median or number | IQR, SD or % |
| Mother | | | |
| Age at child's birth, (years), mean (SD) | 724 | 31.1 | 3.6 |
| Educational attainment; qualifications ≥ A-level, n (%) | 723 | 456 | 63.1% |
| IQ, child age 4 y, mean (SD) | 260 | 107.8 | 12.6 |
| IQ, child age 6 y, mean (SD) | 458 | 104.2 | 15.8 |
| Smoked in pregnancy, n (%) | 712 | 110 | 15.5% |
| BMI ($kg/m^2$), median (IQR) | 722 | 24.4 | 21.9, 27.3 |
| Multiparous, n(%) | 724 | 312 | 43.1% |
| Duration breastfeeding (weeks), median (IQR) | 688 | 13.0 | 1.4, 30.4 |
| Early pregnancy plasma ARA concentration (μg/ml), median (IQR) | 584 | 172 | 142, 212 |
| Late pregnancy plasma ARA concentration (μg/ml), median (IQR) | 331 | 113 | 86, 147 |
| Early pregnancy plasma DHA concentration (μg/ml), median (IQR) | 584 | 86 | 67, 107 |
| Late pregnancy plasma DHA concentration (μg/ml), median (IQR) | 331 | 58 | 44, 77 |
| Child | | | |
| Female, n (%) | 724 | 346 | 47.8% |
| Gestation at birth (weeks), median (IQR) | 724 | 40.0 | 39.0-41.0 |
| BMI at 4 years (kg/m2), median (IQR) | 260 | 15.9 | 15.1-16.7 |
| BMI at 6-7 years (kg/m2), median (IQR) | 411 | 15.7 | 14.9, 16.9 |

| | | | |
|---|---|---|---|
| Age at 4 years (years), mean (SD) | 260 | 4.4 | 0.1 |
| Age at 6-7 years (years), mean (SD) | 419 | 7.0 | 0.2 |

Sample sizes vary due to outcome-specific missing values. Values are n (%), mean (standard deviation) or median (IQR, interquartile range).

**Table 2.** Maternal plasma PC ARA concentration as predictor of cognitive outcomes

| | | Unadjusted | | | | Adjusted | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | β | 95% CI | *P* | n | β | 95% CI | *P* | n |
| 4 y WPPSI IQ | | | | | | | | | |
| | Early pregnancy, SD | -2.24 | -4.56;0.07 | 0.06 | 146 | -2.00 | -4.33;0.34 | 0.09 | 146 |
| | Late pregnancy, SD | 0.40 | -1.22;2.02 | 0.63 | 253 | 0.39 | -1.16;1.94 | 0.62 | 253 |
| | Late-early pregnancy change, SD | 3.10 | 0.36;5.85 | 0.03 | 139 | 2.74 | -0.05; 5.52 | 0.05 | 139 |
| 6-7 y WASI | | | | | | | | | |
| | Early pregnancy, SD | -0.30 | -1.74;1.14 | 0.68 | 432 | 0.14 | -1.18; 1.45 | 0.84 | 414 |
| | Late pregnancy, SD | -2.15 | -6.53;2.22 | 0.33 | 77 | -1.27 | -5.78; 3.25 | 0.58 | 76 |
| | Late-early pregnancy change, SD | -0.23 | -4.59;4.13 | 0.92 | 51 | 0.66 | -3.57; 4.89 | 0.75 | 50 |
| 6-7 y CANTAB DMS total correct (12 sec delay) | | | | | | | | | |
| | Early pregnancy, SD | 0.04 | -0.08;0.16 | 0.47 | 393 | 0.06 | -0.06;0.19 | 0.32 | 375 |
| | Late pregnancy, SD | -0.06 | -0.39;0.27 | 0.73 | 73 | -0.00 | -0.38;0.37 | 0.98 | 72 |
| | Late-early pregnancy change, SD | 0.07 | -0.44;0.58 | 0.79 | 47 | 0.24 | -0.42;0.90 | 0.47 | 46 |
| 6-7 y CANTAB IED pre-EDS errors (z-score) | | | | | | | | | |
| | Early pregnancy, SD | 0.02 | -0.07;0.12 | 0.65 | 392 | 0.02 | -0.08;0.12 | 0.69 | 374 |
| | Late pregnancy, SD | 0.20 | -0.08;0.47 | 0.16 | 73 | 0.14 | -0.18;0.46 | 0.37 | 72 |
| | Late-early pregnancy change, SD | 0.23 | -0.19;0.65 | 0.27 | 47 | 0.01 | -0.55;0.57 | 0.96 | 46 |
| 6-7 y CANTAB IED EDS errors | | | | | | | | | |
| | Early pregnancy, SD | 0.08 | -0.02;0.17 | 0.12 | 392 | 0.06 | -0.04;0.16 | 0.22 | 374 |
| | Late pregnancy, SD | 0.11 | -0.14;0.36 | 0.38 | 73 | 0.14 | -0.14;0.43 | 0.32 | 72 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Late-early pregnancy change, SD | 0.11 | -0.31;0.52 | 0.61 | 47 | 0.21 | -0.34;0.76 | 0.44 | 46 |
| 6-7 y CANTAB IED total errors (stage 1) in 5 groups | | | | | | | | |
| Early pregnancy, SD | -0.04 | -0.15;0.07 | 0.43 | 390 | -0.03 | -0.15;0.08 | 0.59 | 372 |
| Late pregnancy, SD | 0.20 | -0.13;0.53 | 0.24 | 73 | 0.23 | -0.15;0.61 | 0.23 | 72 |
| Late-early pregnancy change, SD | 0.57 | 0.08;1.06 | 0.02 | 47 | 0.64 | -0.01;1.29 | 0.05 | 46 |
| 6-7 y CANTAB IED total errors (stage 8) in 5 groups | | | | | | | | |
| Early pregnancy, SD | 0.10 | -0.03;0.24 | 0.13 | 390 | 0.07 | -0.07;0.21 | 0.33 | 372 |
| Late pregnancy, SD | 0.17 | -0.22;0.56 | 0.40 | 73 | 0.25 | -0.19;0.69 | 0.25 | 72 |
| Late-early pregnancy change, SD | 0.19 | -0.44;0.82 | 0.55 | 47 | 0.37 | -0.49;1.23 | 0.39 | 46 |
| 6-7 y CANTAB IED total errors (adjusted) | | | | | | | | |
| Early pregnancy, SD | 0.06 | -0.03;0.16 | 0.17 | 392 | 0.04 | -0.06;0.13 | 0.46 | 374 |
| Late pregnancy, SD | 0.27 | 0.00;0.53 | 0.05 | 73 | 0.27 | -0.04;0.57 | 0.08 | 72 |
| Late-early pregnancy change, SD | 0.24 | -0.15;0.63 | 0.22 | 47 | 0.23 | -0.28;0.75 | 0.37 | 46 |
| 6-7 y CANTAB IED stages completed in 4 groups | | | | | | | | |
| Early pregnancy, SD | -0.09 | -0.18;-0.00 | 0.04 | 392 | -0.06 | -0.15;0.03 | 0.16 | 374 |
| Late pregnancy, SD | -0.22 | -0.48;0.03 | 0.08 | 73 | -0.25 | -0.53;0.04 | 0.09 | 72 |
| Late-early pregnancy change, SD | -0.19 | -0.58;0.21 | 0.35 | 47 | -0.32 | -0.83;0.20 | 0.22 | 46 |
| 6-7 y CANTAB SSP span length | | | | | | | | |
| Early pregnancy, SD | -0.04 | -0.13;0.05 | 0.35 | 374 | -0.01 | -0.10;0.08 | 0.83 | 356 |
| Late pregnancy, SD | -0.11 | -0.37;0.15 | 0.41 | 70 | -0.02 | -0.29;0.25 | 0.89 | 69 |
| Late-early pregnancy change, SD | -0.46 | -0.88;-0.04 | 0.03 | 45 | -0.25 | -0.74;0.24 | 0.31 | 44 |
| 6-7 y CANTAB IST mean prob. correct (win condition fixed) in 5 groups | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Early pregnancy, SD | -0.05 | -0.19;0.10 | 0.52 | 357 | -0.03 | -0.18;0.12 | 0.69 | 340 |
| Late pregnancy, SD | -0.13 | -0.80;0.53 | 0.69 | 27 | 0.33 | -0.47;1.14 | 0.40 | 27 |
| Late-early pregnancy change, SD | -0.19 | -0.93;0.56 | 0.60 | 20 | 0.48 | -0.61;1.56 | 0.36 | 20 |

Sample sizes varied vary for specific variables because of due to outcome-specific missing values. Data were adjusted for maternal BMI, maternal IQ, maternal education, maternal smoking, child's sex and (for CANTAB outcomes) child's age. Values are linear regression coefficient, β, (95% confidence interval).

**Table 3.** Maternal plasma PC DHA concentration as predictor of cognitive outcomes

| | | Unadjusted | | | | Adjusted | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | β | (95% CI) | *P* | *n* | β | (95% CI) | *P* | *n* |
| 4 y WPPSI IQ | | | | | | | | | |
| | Early pregnancy, SD | 0.25 | -2.01;2.51 | 0.83 | 146 | -0.03 | -2.29;2.23 | 0.98 | 146 |
| | Late pregnancy, SD | 1.97 | 0.35;3.60 | 0.02 | 253 | 1.13 | -0.43;2.69 | 0.15 | 253 |
| | Late-early pregnancy change, SD | 2.10 | -0.58;4.78 | 0.12 | 139 | 1.66 | -1.04;4.37 | 0.23 | 139 |
| 6 - 7 y WASI | | | | | | | | | |
| | Early pregnancy, SD | 1.79 | 0.34;3.23 | 0.02 | 432 | 0.87 | -0.46;2.20 | 0.20 | 414 |
| | Late pregnancy, SD | 1.09 | -3.19;5.38 | 0.61 | 77 | -0.86 | -5.03;3.31 | 0.68 | 76 |
| | Late-early pregnancy change, SD | 1.76 | -2.58;6.10 | 0.42 | 51 | 1.02 | -3.10;5.14 | 0.62 | 50 |
| 6 - 7 y CANTAB DMS total correct (12 sec delay) | | | | | | | | | |
| | Early pregnancy, SD | 0.06 | -0.06;0.18 | 0.32 | 393 | 0.07 | -0.06;0.20 | 0.28 | 375 |
| | Late pregnancy, SD | -0.18 | -0.51;0.14 | 0.26 | 73 | -0.15 | -0.50;0.19 | 0.38 | 72 |
| | Late-early pregnancy change, SD | -0.04 | -0.57;0.50 | 0.89 | 47 | -0.09 | -0.70;0.52 | 0.77 | 46 |
| 6 - 7 y CANTAB IED pre-EDS errors (z-score) | | | | | | | | | |
| | Early pregnancy, SD | 0.05 | -0.05;0.14 | 0.31 | 392 | 0.07 | -0.03;0.17 | 0.18 | 374 |
| | Late pregnancy, SD | 0.23 | -0.04;0.50 | 0.09 | 73 | 0.24 | -0.05;0.53 | 0.11 | 72 |
| | Late-early pregnancy change, SD | 0.12 | -0.33;0.56 | 0.60 | 47 | 0.01 | -0.50;0.52 | 0.97 | 46 |
| 6 - 7 y CANTAB IED EDS errors | | | | | | | | | |
| | Early pregnancy, SD | 0.01 | -0.09;0.10 | 0.90 | 392 | 0.01 | -0.09;0.11 | 0.87 | 374 |
| | Late pregnancy, SD | -0.08 | -0.33;0.16 | 0.50 | 73 | -0.07 | -0.33;0.20 | 0.61 | 72 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Late-early pregnancy change, SD | -0.12 | -0.55;0.31 | 0.57 | 47 | -0.14 | -0.65;0.37 | 0.59 | 46 |
| 6 - 7 y CANTAB IED total errors (stage 1) in 5 groups | | | | | | | | |
| Early pregnancy, SD | -0.06 | -0.17;0.05 | 0.28 | 390 | -0.06 | -0.17;0.06 | 0.33 | 372 |
| Late pregnancy, SD | 0.21 | -0.12;0.53 | 0.21 | 73 | 0.19 | -0.16;0.54 | 0.29 | 72 |
| Late-early pregnancy change, SD | 0.40 | -0.13;0.93 | 0.14 | 47 | 0.35 | -0.26;0.97 | 0.25 | 46 |
| 6 - 7 y CANTAB IED total errors (stage 8) in 5 groups | | | | | | | | |
| Early pregnancy, SD | 0.00 | -0.14;0.14 | 0.99 | 390 | -0.01 | -0.15;0.13 | 0.90 | 372 |
| Late pregnancy, SD | -0.23 | -0.61;0.15 | 0.23 | 73 | -0.19 | -0.59;0.22 | 0.37 | 72 |
| Late-early pregnancy change, SD | -0.36 | -1.02;0.29 | 0.27 | 47 | -0.41 | -1.19;0.38 | 0.30 | 46 |
| 6 - 7 y CANTAB IED total errors (adjusted) | | | | | | | | |
| Early pregnancy, SD | 0.03 | -0.06;0.13 | 0.49 | 392 | 0.04 | -0.06;0.13 | 0.43 | 374 |
| Late pregnancy, SD | 0.02 | -0.24;0.29 | 0.85 | 73 | 0.04 | -0.24;0.33 | 0.76 | 72 |
| Late-early pregnancy change, SD | -0.10 | -0.52;0.31 | 0.62 | 47 | -0.17 | -0.64;0.30 | 0.47 | 46 |
| 6 - 7 y CANTAB IED stages completed in 4 groups | | | | | | | | |
| Early pregnancy, SD | -0.03 | -0.12;0.05 | 0.44 | 392 | -0.02 | -0.11;0.07 | 0.62 | 374 |
| Late pregnancy, SD | 0.15 | -0.10;0.40 | 0.24 | 73 | 0.13 | -0.14;0.40 | 0.34 | 72 |
| Late-early pregnancy change, SD | 0.19 | -0.22;0.61 | 0.35 | 47 | 0.16 | -0.31;0.64 | 0.49 | 46 |
| 6 - 7 y CANTAB SSP span length | | | | | | | | |
| Early pregnancy, SD | 0.05 | -0.04;0.14 | 0.29 | 374 | 0.05 | -0.04;0.14 | 0.28 | 356 |
| Late pregnancy, SD | 0.23 | -0.02;0.49 | 0.07 | 70 | 0.19 | -0.06;0.44 | 0.13 | 69 |
| Late-early pregnancy change, SD | -0.27 | -0.73;0.18 | 0.24 | 45 | -0.29 | -0.74;0.15 | 0.19 | 44 |
| 6 - 7 y CANTAB IST mean prob. correct (win condition fixed) in 5 groups | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Early pregnancy, SD | 0.00 | -0.15;0.15 | 0.99 | 357 | -0.02 | -0.18;0.14 | 0.79 | 340 |
| Late pregnancy, SD | 0.12 | -0.44;0.67 | 0.67 | 27 | 0.18 | -0.48;0.84 | 0.57 | 27 |
| Late-early pregnancy change, SD | -0.21 | -1.31;0.88 | 0.68 | 20 | 0.06 | -1.29;1.42 | 0.92 | 20 |

Sample sizes varied vary for specific variables because of due to outcome-specific missing values. Data were adjusted for maternal BMI, maternal IQ, maternal education, maternal smoking, child's sex and (for CANTAB outcomes) child's age. Values are linear regression coefficient, β, (95% confidence interval).