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Antenatal vitamin D status is not associated with standard neurodevelopmental assessments at five years in a well-characterised prospective maternal-infant cohort

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Running title Antenatal 25(OH)D and childhood neurodevelopment

Clinical Trial Registration BASELINE Study NCT01498965 (www.clinicaltrials.gov); SCOPE Study ACTRN12607000551493 (<http://www.anzctr.org.au>)

Abbreviations used BASELINE: Babies after SCOPE: Evaluating the Longitudinal Impact using Neurological and Nutritional Endpoints; CBCL: Child Behaviour Checklist; IQ: intelligence quotient; KBIT-2: Kaufman Brief Intelligence Test, 2nd Edition; SCOPE: Screening for Pregnancy Endpoints; 25(OH)D: 25-hydroxyvitamin D; 25(OH)D₃: 25-hydroxyvitamin D₃; 25(OH)D₂: 25-hydroxyvitamin D₂; 3-epi-25(OH)D₃: 3-epi-25-hydroxyvitamin D₃.

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Conflict of interest The authors have no conflicts of interest to declare.

1 ABSTRACT

2 **Background** Although animal studies show evidence for a role of vitamin D during brain
3 development, data from human studies show conflicting signals.

4 **Objective** We aimed to explore associations between maternal and neonatal vitamin D status with
5 childhood neurodevelopmental outcomes.

6 **Methods** Comprehensive clinical, demographic and lifestyle data were collected prospectively in 734
7 maternal-infant dyads from the Cork BASELINE Birth Cohort Study. Serum 25-hydroxyvitamin D
8 (25(OH)D) concentrations were quantified at 15 weeks' gestation and in umbilical cord sera at birth
9 using a CDC-accredited LC-MS/MS method. Children were assessed at five years using the Kaufman
10 Brief Intelligence Test (2nd Edition, KBIT-2) and the Child Behaviour Checklist (CBCL). Linear
11 regression was used to explore associations between 25(OH)D and neurodevelopmental outcomes.

12 **Results** 25(OH)D concentrations were <30nmol/L in 15% of maternal and 45% of umbilical cord sera
13 and <50nmol/L in 42% of mothers and 80% of cords. At five years, the mean (SD) KBIT-2 IQ
14 composite score was 104.6 (8.6); scores were 107.2 (10.0) in verbal and 99.8 (8.8) in non-verbal
15 tasks. Developmental delay (scores <85) was seen in <3% of children across all domains. The mean
16 (SD) CBCL total problem score was 21.3 (17.5); scores in the abnormal/clinical range for internal,
17 external and total problem scales were present in 12%, 4% and 6% of participants. KBIT-2 and
18 CBCL subscale scores at five years were not different between children exposed to low antenatal
19 vitamin D status, either at 30 or 50nmol/L 25(OH)D thresholds. Neither maternal nor cord 25(OH)D
20 (per 10nmol/L) were associated with KBIT-2 IQ composite scores (adjusted β (95% CI): maternal -
21 0.01 (-0.03, 0.02); cord 0.01 (-0.03, 0.04)) or CBCL total problem scores (maternal 0.01 (-0.04, 0.05);
22 cord 0.01 (-0.07, 0.09)).

23 **Conclusions** In this well-characterized prospective maternal-infant cohort, we found no evidence that
24 antenatal 25(OH)D concentrations are associated with neurodevelopmental outcomes at five years.

25 **KEYWORDS** vitamin D, serum 25-hydroxyvitamin D, neurodevelopment, intelligence, antenatal.

26 INTRODUCTION

27 Vitamin D deficiency is a public health concern, with pregnant women and their infants at particular
28 risk (1, 2). A recent systematic review summarizing maternal and neonatal vitamin D status globally
29 reported that over half of pregnant women and three-quarters of neonates have serum 25-
30 hydroxyvitamin D (25(OH)D) concentrations <50 nmol/L (3). We have published similar findings in
31 Ireland, indicating that 17% of mothers in their 2nd trimester and 46% of their neonates at birth have
32 25(OH)D concentrations <30 nmol/L (4, 5). This is concerning given that low 25(OH)D
33 concentrations during pregnancy have been associated with an increased risk of pregnancy
34 complications, including gestational diabetes, preeclampsia and small-for-gestational age infants (6).
35 Additionally, as neonatal 25(OH)D concentrations are dependent on maternal concentrations, infants
36 born to vitamin D deficient mothers are at an increased risk of neonatal deficiency and its associated
37 consequences for infant and long-term health (7, 8).

38 One potential consequence of early life vitamin D deficiency for infant health is brain development
39 and function. In vitro studies have provided compelling evidence for a potential role of vitamin D
40 during fetal brain development. Both the vitamin D receptor and CYP27B1 are expressed in the
41 human brain (9). Vitamin D metabolites have also been shown to cross the blood-brain barrier (10).
42 Furthermore, animal models have illustrated that vitamin D influences the developing brain through
43 the regulation of important processes, including the maintenance of calcium balance, enhancement of
44 signal transmission and synaptic plasticity, neuroprotection and modulation of neuronal
45 differentiation, maturation and growth (11, 12). These rodent models also suggest that vitamin D
46 deficiency *in utero* can modify the expression of multiple genes and proteins in the brain resulting in
47 altered brain structure and function (10). However, the translation of this evidence into humans is
48 unclear.

49 To date, 10 observational studies in humans have investigated associations between 25(OH)D
50 concentrations either in early/mid (13-15) or late pregnancy (16-19) and/or in umbilical cord blood at
51 birth (20-22) and measures of childhood neurodevelopment. Findings have been mixed and

52 inconclusive, due mainly to the substantial variability in study design, as summarised in **Table 1**. A
53 number of these studies have also been restricted to historical data, while only one study has
54 investigated the influence of 25(OH)D concentrations in both the fetal and early neonatal period (20).
55 Given the high prevalence of vitamin D deficiency in pregnant women and their infants, its potential
56 impact on childhood neurodevelopment is an important consideration. Therefore, the aim of the
57 current study was to explore associations between maternal and neonatal serum 25(OH)D
58 concentrations and neurodevelopmental outcomes in children aged five years in a prospective
59 maternal-infant birth cohort in Ireland.

60 **METHODS**

61 **Study design and participants**

62 Participants were recruited to the Cork BASELINE (Babies after SCOPE: Evaluating the
63 Longitudinal Impact using Neurological and Nutritional Endpoints) Birth Cohort Study
64 (www.clinicaltrials.gov NCT01498965) between March 2008 and January 2011. The BASELINE
65 Study is a follow-on to the SCOPE (Screening for Pregnancy Endpoints) Ireland pregnancy study
66 (<http://www.anzctr.org.au> ACTRN12607000551493), where low risk, nulliparous women with a
67 singleton pregnancy were recruited before 15 weeks' gestation from Cork University Maternity
68 Hospital, as part of an international multicentre study aimed at investigating early indicators of
69 pregnancy complications (23). At 15 weeks' gestation, research midwives collected information on
70 maternal socioeconomic status, occupation, education, relationship status and a complete medical
71 history. Information on nutritional supplement use, recreational activity, cigarette, drug and alcohol
72 use were recorded for the three-month period prior to conception and during the first trimester.
73 Maternal anthropometric and clinical measurements were also collected prospectively during
74 pregnancy.

75 Women in the SCOPE Ireland study ($n = 1537$) provided written informed consent to the BASELINE
76 Study for their infants at 20 weeks' gestation. Their infants were followed prospectively from birth,
77 with assessments at day 2 and at 2, 6, 12 and 24 months. Assessments at five years of age were

78 completed in December 2016. Detailed information on early life environment, diet, lifestyle, health,
79 growth and development was gathered by interviewer-led questionnaires and clinical assessments
80 performed by trained researchers in accordance with the Declaration of Helsinki, with further
81 information on study design and procedures reported previously (24). Ethical approval for both
82 SCOPE Ireland and the Cork BASELINE Birth Cohort Study was granted by the Clinical Research
83 Ethics Committee of the Cork teaching hospitals (SCOPE: ECM 5(10) 05/02/2008, BASELINE:
84 ECM 5(9) 01/07/2008).

85 **Neurodevelopmental assessments**

86 At the study's five year assessment, participants completed two neurodevelopmental assessments, 1)
87 the Kaufman Brief Intelligence Test, 2nd Edition (KBIT-2) and 2) the Child Behaviour Checklist
88 (CBCL).

89 The KBIT-2 is designed as a brief, individualised test to measure verbal and non-verbal intelligence in
90 children and adults, from age 4-90 years (25). It is used to screen the intellectual abilities of an
91 individual and identify those who may be at risk of academic problems. The assessment consists of
92 three subtests, two of which are verbal (Verbal Knowledge and Riddles) and one non-verbal
93 (Matrices). The subtests involve individually administered verbal and non-verbal tasks that do not
94 require reading or spelling but consist of verbal questions, illustrations and visual stimuli. The verbal
95 subtests assess verbal concept formation, word meaning and reasoning, while the non-verbal subtest
96 assesses fluid reasoning, visual processing and problem solving. The assessment was administered by
97 a research nurse trained in administration and interpretation of the test. After the examination was
98 complete, the verbal and non-verbal scales were tallied, standardized for age and transformed into a
99 composite IQ score. The standard score for each component has a mean of 100 and a standard
100 deviation of 15, with scores less than 85 considered abnormal or represent developmental delay.

101 Emotional and behavioural problems were assessed by the CBCL for ages 1.5-5 years (26). The
102 CBCL is a 99-item validated screener checklist completed by parents/caregivers, indicating the
103 frequency of particular behaviours in their child over the past two months on a three-point scale (not

104 true, sometimes true or very/often true), with increasing scores indicating increasing behavioural
105 issues/problems. The CBCL comprises of two broadband scales, Internal Problem Score and External
106 Problem Score. The Internal Problem Score is made up of scores from four individual syndrome
107 scales: Emotionally Reactive, Anxious/depressed, Somatic Complaints (physical complaints such as
108 nausea, headaches etc.) and Withdrawn. The External Problem Score is made up of scores from two
109 individual syndrome scales: Attention Problems and Aggressive Behaviour. Summing the Internal
110 Problem Score and the External Problem Score with two further individual scale scores: Sleep
111 Problems and Other Problems, provides a Total Problem Score. For all scales, scores $\geq 93^{\text{rd}}$ percentile
112 were designated as borderline abnormal and scores $\geq 98^{\text{th}}$ percentile as clinically abnormal. For this
113 analysis, all scores $\geq 93^{\text{rd}}$ percentile were denoted as abnormal, indicating significant behavioural
114 problems.

115 **Biological samples and analytical methods**

116 Blood samples were collected from mothers at 15 weeks' gestation and from the umbilical cord at
117 birth and were processed to serum within three hours of collection and stored at -80°C until use.
118 Circulating 25-hydroxyvitamin D₃ (25(OH)D₃), 25-hydroxyvitamin D₂ (25(OH)D₂) and 3-epi-25-
119 hydroxyvitamin D₃ (3-epi-25(OH)D₃) concentrations were measured at the Cork Centre for Vitamin D
120 and Nutrition Research laboratory with the use of a liquid chromatography–tandem mass
121 spectrometry (LC-MS/MS) method that has been described in detail previously (4, 27). The
122 instrument used was a Waters Acquity UPLC system coupled to an Acquity Triple Quadrupole TQD
123 mass-spectrometer detector (Waters, Dublin 9, Ireland). Concentrations of 25(OH)D₃ and 25(OH)D₂
124 were quantified individually and their values were summed to generate total 25(OH)D.
125 Chromatographic separation and quantitation of 3-epi-25(OH)D₃ was also achieved. Four amounts of
126 serum-based National Institute of Standards and Technology (NIST)-certified quality-assurance
127 material (standard reference material 972) were used for method validation, while quality-control
128 materials that were assayed in parallel to all samples were purchased from Chromsystems (Germany).
129 NIST calibrators were used throughout the analysis (standard reference material 2972). The intra- and
130 inter-assay coefficients of variation were not greater than 6 and 5%, respectively, for all metabolites.

131 The limit of detection for 25(OH)D₃, 3-epi-25(OH)D₃, and 25(OH)D₂ were 0.31, 0.20, and 0.44
132 nmol/L, respectively and the limit of quantitation was 1.03, 0.66, and 1.43 nmol/L, respectively. The
133 quality and accuracy of the vitamin D metabolite analysis in our laboratory is assessed on an on-going
134 basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charing
135 Cross Hospital, London UK). We also participate in the CDC Vitamin D Standardization
136 Certification program, which reports accuracy and bias for total 25(OH)D, 25(OH)D₃, 3-epi-
137 25(OH)D₃ and 25(OH)D₂, since 2013.

138 **Data analysis**

139 Data were analysed using IBM SPSS® for Windows™ version 23 (IBM Corp., Armonk, NY, USA)
140 and Statistical Analysis System (SAS) version 9.4 (SAS Institute Inc.). Descriptive statistics (mean,
141 standard deviation (SD), median, quartiles (IQR), frequencies and percentages) were generated.
142 Comparisons between categorical variables were made using Chi square (χ^2) tests, while independent
143 t-tests or Mann-Whitney U tests were employed for continuous variables, depending on their
144 distribution. Multiple linear regression was used to explore associations between maternal and
145 neonatal 25(OH)D concentrations and neurodevelopmental outcomes at five years of age. Serum
146 25(OH)D concentrations were analysed firstly as continuous variables and secondly, to investigate a
147 potential threshold effect, both maternal and neonatal 25(OH)D were divided into three categories
148 (<30 nmol/L, 30-<50 nmol/L, \geq 50 nmol/L). The categories were decided upon based on the
149 thresholds for deficiency/sufficiency proposed by the US Institute of Medicine (28) and the vitamin D
150 literature, given the lack of reference intervals for umbilical cord 25(OH)D concentrations in
151 particular. Separate linear regression models (24 in total) were built for each predictor-outcome
152 association with adjustment for covariates based on both statistical significance and clinical and
153 theoretical knowledge. In each model, initial associations between serum 25(OH)D concentrations
154 (and other potential confounders) with the outcomes (KBIT-2 and CBCL scores) were assessed by
155 univariable linear regressions in which the significance level was set at $\alpha=0.25$. Multivariable
156 models that included serum 25(OH)D and other covariates that were significant in the univariable
157 analysis were then built and assessed. At this stage, any non-significant covariates at $\alpha=0.05$ were

158 either kept in the model if clinically relevant or dropped. Linearity and constant error variance were
159 then evaluated visually, through scatter plots, and statistically, through the White test, for both the
160 outcome and each of the predictors in the model. Normality of distribution of residuals was also
161 assessed both visually, through histograms and normal probability plots, and statistically, through the
162 Shapiro-Wilk test. Final model selection between sets of potential covariates was also aided by
163 Mallows' Cp criterion and PRESS statistic. Associations were expressed as adjusted estimates with
164 95% confidence intervals (95% CI) and $P < 0.05$ was considered significant in final models.

165 RESULTS

166 Of the 920 firstborn children that attended the study's five year assessment, 83% ($n = 763$) completed
167 both the KBIT-2 and the CBCL. Children that were born premature (<37 weeks' gestation, $n = 29$)
168 were excluded, providing a final sample size for this study of 734 (**Figure 1**). Principal
169 characteristics of the mothers and their infants are presented in **Table 2**. The median [IQR] age of
170 mothers at delivery was 31.0 [29.0, 33.0] years and most were Caucasian. Vitamin D supplements
171 (dose ranged from 2.5 to 10 $\mu\text{g/day}$) were taken by 42% of women at 15 weeks' gestation.

172 Serum 25(OH)D concentrations were measured in all 734 mothers at 15 weeks' gestation and in 547
173 umbilical cords. Mean (SD) serum 25(OH)D concentrations in mothers and infants were 58.3 (25.8)
174 nmol/L and 35.1 (18.2) nmol/L, respectively. Vitamin D deficiency (<30 nmol/L) was observed in
175 15% of mothers, while 42% had 25(OH)D concentrations <50 nmol/L. Almost half (45%) of infants
176 were born deficient (34% were <25 nmol/L) and 80% had concentrations <50 nmol/L. Both maternal
177 and neonatal mean (SD) 25(OH)D concentrations were higher in summer (maternal: 67.0 (23.7)
178 nmol/L, neonatal: 44.5 (17.7) nmol/L) than in winter (maternal: 52.0 (25.5) nmol/L, neonatal: 28.0
179 (15.1) nmol/L, both $P < 0.0001$), with 63% of infants born deficient in winter compared to 22% in
180 summer ($P < 0.0001$). Only two mothers, and no infants had 25(OH)D >125 nmol/L.

181 At five years, the mean (SD) IQ composite score was 104.6 (8.6), with higher scores reported in
182 verbal tasks (107.2 (10.0)) than non-verbal tasks (99.8 (8.8)). The prevalence of developmental delay,
183 indicated by scores <85 on the KBIT-2 was <3% across all domains. The mean (SD) CBCL total

184 problem score for the study population was 21.3 (17.5), with scores in the clinical/abnormal range in
185 the internal, external and total problem scales observed in 12%, 4% and 6% of participants,
186 respectively.

187 KBIT-2 and CBCL subscales scores at five years did not differ between those with maternal or cord
188 25(OH)D concentrations above or below 30 nmol/L or 50 nmol/L. **Supplemental Figure 1** presents
189 the distribution of maternal and cord serum 25(OH)D concentrations with neurodevelopmental
190 outcomes. There was no evidence of an association between maternal serum 25(OH)D concentrations
191 and intelligence or behavioural outcomes assessed by the KBIT-2 and CBCL, either in unadjusted or
192 adjusted multivariable linear regression models (**Table 3**). When maternal 25(OH)D concentrations
193 were categorised, using the lower threshold of <30 nmol/L as the reference group, no significant
194 differences in KBIT-2 or CBCL subscale scores between 25(OH)D categories were observed (Table
195 3). Cord 25(OH)D at birth was not associated with intelligence or behavioural outcomes at five years
196 and when cord 25(OH)D was divided into categories, there was also no evidence of an association
197 with KBIT-2 or CBCL subscale scores (**Table 4**).

198 **DISCUSSION**

199 In this prospective maternal-infant birth cohort, with a high prevalence of low vitamin D status among
200 pregnant women and new-borns, we found no evidence to suggest that antenatal 25(OH)D
201 concentrations are associated with childhood neurodevelopmental outcomes at five years.

202 Our observation that maternal 25(OH)D concentrations at 15 weeks' gestation were not associated
203 with childhood intelligence scores at five years was consistent with reports from two similar maternal-
204 infant cohorts in the UK (16) and Denmark (18), although in both of those studies, maternal vitamin D
205 status was measured in the 3rd trimester. The 2nd trimester has been suggested as a potentially
206 important period of vulnerability to vitamin D deficiency during fetal brain development. In the
207 Australian Raine cohort, using a quartile analysis, children born to women with 25(OH)D \leq 46 nmol/L
208 during their 2nd trimester had an almost twofold increased risk of language difficulties at five and 10
209 years of age compared to those whose mothers had concentrations >70 nmol/L (14). In a racially and

210 socioeconomically diverse birth cohort in North America, Tylavsky and colleagues also reported a
211 small, positive association with language development in two year olds (15). In contrast, Keim *et al.*
212 observed no association between maternal 25(OH)D in the 2nd trimester and reading or spelling
213 achievement (20), albeit within a different timeframe. With regard to motor development, modest
214 associations with maternal 25(OH)D in preschool-age children have been observed in studies in Spain
215 and the UK (13, 19), however this could be due to an effect of maternal 25(OH)D on fetal
216 musculoskeletal development and/or brain development, resulting in altered motor function. Given
217 these contrasting findings, the literature describing any influence of maternal vitamin D status during
218 pregnancy on fetal brain development is immature and requires careful study.

219 Associations between cord 25(OH)D concentrations and childhood neurodevelopmental outcomes
220 have been described previously in three studies (20-22), although ours is the first report from a
221 European cohort. In contrast to these studies, we observed no significant association between cord
222 25(OH)D and intelligence at five years of age. Zhu and colleagues in China reported an inverted U-
223 shaped relationship between cord 25(OH)D and mental and psychomotor development at 16-18
224 months (21), although these data should be interpreted with caution given the study's relatively small
225 sample size and use of radioimmunoassay to measure cord 25(OH)D concentrations. In the secondary
226 analysis of historical data from the US Collaborative Perinatal Project (1959-73) performed by Keim
227 and colleagues, the modest, positive association observed with intelligence at four and seven years
228 was inconsistent and attenuated following adjustment for confounders (20). In mother-child dyads
229 recruited as part of an antenatal docosahexaenoic acid RCT, Gould *et al.* reported a small, positive
230 association with language development at 18 months and four years, although a 10 nmol/L increase in
231 cord 25(OH)D was only associated with a 0.60-0.67 increase in language scores (22). While these
232 studies have observed relatively small associations between cord 25(OH)D and neurodevelopmental
233 outcomes, the study designs were heterogeneous and importantly, the magnitude of the reported
234 associations was very small.

235 Our finding of no association between either maternal or cord 25(OH)D with behavioural outcomes at
236 five years is in accordance with previous reports. Parent-report assessments of behaviour similar to

237 those used in the current study have been employed in three other studies, with all studies reporting no
238 association with either maternal or cord 25(OH)D (14, 16, 19). The Strengths and Difficulties
239 Questionnaire was used by Gale *et al.* (16) and Darling *et al.* (19) in the UK, while in the Raine
240 cohort, no association between maternal 25(OH)D and behavioural outcomes, as assessed by the
241 CBCL, were observed throughout childhood to the age of 17 years (14). Studies that have used more
242 objective, psychologist administered assessments, such as the Bayley Scales of Infant and Toddler
243 Development, have also reported no association with maternal or cord 25(OH)D concentrations (17,
244 20, 22). Altogether, there seems to be little evidence to suggest that either maternal or neonatal
245 vitamin D status influences behavioural or emotional development.

246 While animal studies have provided a plausible biological basis indicating a role for vitamin D during
247 fetal brain development, the evidence from human studies continues to show conflicting signals.
248 Significant heterogeneity in study design, as summarised in Table 1, has contributed largely to the
249 mixed findings, particularly in the timing and methods employed for both the exposure and outcome
250 assessments and the statistical analysis applied with respect to the use of cut-offs for 25(OH)D
251 concentrations and potential confounders. Therefore, the timing and duration, or indeed the presence
252 of, a critical window of vulnerability and susceptibility to vitamin D deficiency or insufficiency
253 during brain development is yet to be fully determined. Importantly, this critical window could be
254 later in the postnatal period, as early infancy is another crucial period of rapid brain development.
255 The plasticity of the young brain in the postnatal period and its ability for repair should also be
256 acknowledged, as although almost half of our cohort had a 25(OH)D concentration <30 nmol/L at
257 delivery, indicating a high risk of nutritional deficiency, fewer than 5% were <30 nmol/L at two and
258 five years (29). Further consideration of these issues will enable more targeted and specific
259 assessments of the developmental outcomes that are most likely to be affected by vitamin D
260 deficiency. However, reliance on global developmental assessments in early childhood is still a
261 limitation of this research field as such assessments may not be sensitive enough to identify specific
262 developmental processes that are affected by nutritional factors including vitamin D (30).

263 Apart from the study by Keim *et al.* that utilised data from a 1950's US cohort (20), our study is the
264 only other to report the effects of vitamin D status in both the fetal and early neonatal period on
265 childhood neurodevelopmental outcomes. The prospective design of the Cork BASELINE Birth
266 Cohort Study, with its multidisciplinary team and use of validated neurodevelopmental assessments
267 are strengths of this study. The sample size, extensively characterised participants and use of the gold
268 standard CDC-accredited method for measuring serum 25(OH)D concentrations are other advantages.
269 The generalizability of our results may be limited, given the regional recruitment of the cohort and
270 predominantly Caucasian sample; however, the findings are still generalizable to other healthy,
271 Caucasian, low risk maternal-infant populations. Parental intelligence, considered an important
272 determinant of child development was not measured directly in this study; however maternal
273 educational attainment and household income were considered as proxy measures in the analysis. The
274 overall normal developmental profile observed in our cohort is unsurprising and is reflective of the
275 high-resource population studied.

276 To conclude, in this prospective maternal-infant birth cohort in Ireland, we found no evidence of an
277 association between antenatal 25(OH)D concentrations and intelligence or behavioural outcomes in
278 five-year-old children. Further research is required to identify and define the periods in brain
279 development that vitamin D is critical for. Longitudinal studies with vitamin D status measured at
280 multiple time-points throughout gestation and the early neonatal period, along with long-term follow-
281 up of neurodevelopmental outcomes using appropriate validated assessments are required to ascertain
282 this.

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Table 1 Summary of observational studies exploring associations between antenatal 25-hydroxyvitamin D (25(OH)D) concentrations and childhood neurodevelopment outcomes

	Study type	No. of participants¹	Sampling for 25(OH)D	25(OH)D analytical method	Neurodevelopmental assessment (age at assessment)
Morales <i>et al.</i> , 2012 [Spain] (13)	Prospective cohort Recruited: 2003-08	1820	13.5 weeks gestation	HPLC	BSID (14 months)
Whitehouse <i>et al.</i> , 2012 [Australia] (14)	Prospective cohort Recruited: 1989-91	743	18 weeks gestation	Enzyme immunoassay	CBCL (2, 5, 8, 10, 14, 17 years) Peabody Picture Vocabulary Test (5, 10 years)
Tylavsky <i>et al.</i> , 2015 [USA] (15)	Prospective cohort Recruited: 2006-11	1020	2 nd trimester	Enzyme immunoassay	BSID (2 years)
Gale <i>et al.</i> , 2008 [UK] (16)	Prospective cohort Recruited: 1991-92	178	3 rd trimester	Radioimmunoassay	Wechsler Intelligence Scale (9 years) Strengths and Difficulties (9 years)
Hanieh <i>et al.</i> , 2014 [Vietnam] (17)	Antenatal micronutrient RCT Recruited: 2010-12	960	32 weeks gestation	LC-MS/MS	BSID (6 months)
Strom <i>et al.</i> , 2014 [Denmark] (18)	Prospective cohort Recruited: 1988-89	798	30 weeks gestation	LC-MS/MS	Scholastic achievement results (15-16 years) obtained from national registry
Darling <i>et al.</i> , 2017 [UK] (19)	Prospective cohort Recruited: 1991-92	7065	30 weeks gestation	HPLC and LC-MS/MS	Parent-report tests (6, 18, 30, 42 months) Strengths and Difficulties (7 years) Wechsler Intelligence Scale (8 years) Neale Analysis of Reading Ability (9 years)

Keim <i>et al.</i> , 2014 [USA] (20)	Prospective cohort Recruited: 1959-65	3896	≤26 weeks and umbilical cord	LC-MS/MS	BSID (8 months) Stanford-Binet Intelligence Scale (4, 7 years) Wechsler Intelligence Scale (4, 7 years) Wide Range Achievement Test (7 years) Psychologist assessed behaviour (4, 7 years)
Zhu <i>et al.</i> , 2015 [China] (21)	Prospective cohort Recruited: 2008	363	Umbilical cord	Radioimmunoassay	BSID (16-18 months)
Gould <i>et al.</i> , 2017 [Australia] (22)	Antenatal DHA RCT Recruited: 2005-08	337	Umbilical cord	LC-MS/MS	BSID (18 months) Differential Ability Scales (4 years) Clinical Evaluation of Language Fundamentals (4 years)

¹Mother-child dyads with both exposure and outcome of interest measured. BSID, Bayley Scales of Infant and Toddler Development; CBCL, Child Behaviour Checklist; DHA, docosahexaenoic acid; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectroscopy; RCT, randomised controlled trial.

Table 2 Maternal and infant characteristics of the study population¹

Maternal	
Age at delivery (years)	31.0 [29.0, 33.0]
Caucasian	99 (728)
Attended university/third level education	89 (652)
Relationship status, single	5 (36)
Household income <€1,000 per annum	5 (34)
<i>Pregnancy-related factors²</i>	
Obesity (BMI >30 kg/m ²)	12 (91)
Smoking	7 (52)
Vitamin D supplement user	42 (306)
Serum 25(OH)D concentrations (nmol/L)	56.1 [38.1, 76.6]
Infant	
Gender, male	51 (377)
Birth weight (kg)	3.5 [3.2, 3.8]
Gestational age (weeks)	40.4 [39.6, 41.1]
Cord serum 25(OH)D concentrations (nmol/L)	32.1 [20.8, 46.3]
<i>Infant feeding</i>	
Breastfed at hospital discharge	75 (547)
Duration of breastfeeding (weeks)	16.0 [0.1, 99.0]
Age first weaned onto solids (weeks)	20.0 [17.0, 22.0]
Vitamin D supplement user (in first year)	60 (443)

¹Values are medians [interquartile range] or frequencies (percentages), study population $n = 734$ (cord serum 25(OH)D measured in 547 infants only). BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

²Maternal data collected at 15 weeks' gestation unless otherwise stated.

Table 3 Association between maternal serum 25(OH)D concentrations (continuous per 10 nmol/L and categorised) at 15 weeks' gestation and offspring neurodevelopmental outcomes at five years¹

	Continuous measure (per 10 nmol/L increment)		Categorical measure (reference category = 25(OH)D <30 nmol/L)			
	Unadjusted	Adjusted	25(OH)D 30-<50 nmol/L		25(OH)D ≥50 nmol/L	
			Unadjusted	Adjusted	Unadjusted	Adjusted
<i>Kaufman Brief Intelligence Test</i>						
Verbal standard score	0.04 (-0.24, 0.32)	-0.01 (-0.03, 0.03) ²	1.02 (-0.58, 2.61)	0.91 (-1.38, 3.20) ²	-0.13 (-1.57, 1.30)	0.42 (-1.66, 2.49) ²
Non-verbal standard score	0.01 (-0.24, 0.25)	0.01 (-0.02, 0.03) ³	0.78 (-0.69, 2.25)	1.21 (-0.88, 3.30) ³	0.50 (-0.82, 1.81)	1.29 (-0.60, 3.17) ³
IQ composite score	0.02 (-0.23, 0.26)	-0.01 (-0.03, 0.02) ²	1.18 (-0.22, 2.58)	1.39 (-0.58, 3.37) ²	0.19 (-1.07, 1.44)	0.94 (-0.85, 2.72) ²
<i>Child Behaviour Checklist</i>						
Internal problem score	0.04 (-0.13, 0.21)	0.01 (-0.01, 0.02) ⁴	0.44 (-1.84, 0.95)	-0.30 (-1.73, 1.13) ⁴	0.14 (-0.75, 1.03)	-0.01 (-1.29, 1.29) ⁴
External problem score	0.01 (-0.18, 0.19)	-0.01 (-0.02, 0.02) ⁴	-0.91 (-2.41, 0.59)	-0.73 (-2.24, 0.79) ⁴	0.15 (-0.81, 1.10)	-0.40 (-1.77, 0.96) ⁴
Total problem score	0.04 (-0.45, 0.53)	0.01 (-0.04, 0.05) ⁴	-2.26 (-6.26, 1.73)	-1.75 (-5.77, 2.26) ⁴	0.35 (-2.20, 2.90)	-0.71 (-4.34, 2.91) ⁴

¹Values are β coefficients (95% confidence interval), total $n = 734$.

²Model adjusted for infant sex, birth weight, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

³Model adjusted for infant sex, birth weight, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), maternal smoking at 15 weeks' gestation and duration of breastfeeding.

⁴Model adjusted for infant sex, marital status, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

Table 4 Association between cord serum 25(OH)D concentrations (continuous per 10 nmol/L and categorised) at birth and neurodevelopmental outcomes at five years¹

	Continuous measure (per 10 nmol/L increment)		Categorical measure (reference category = <30 nmol/L)			
	Unadjusted	Adjusted	25(OH)D 30-<50 nmol/L		25(OH)D ≥50 nmol/L	
			Unadjusted	Adjusted	Unadjusted	Adjusted
<i>Kaufman Brief Intelligence Test</i>						
Verbal standard score	-0.10 (-0.56, 0.35)	-0.02 (-0.06, 0.03) ²	-0.03 (-0.05, -0.01)	-0.01 (-1.87, 1.85) ²	-0.03 (-0.05, -0.01)	-0.43 (-2.63, 1.78) ²
Non-verbal standard score	0.28 (-0.11, 0.67)	0.02 (-0.02, 0.06) ³	-0.03 (-0.05, -0.01)	0.14 (-1.50, 1.78) ³	-0.03 (-0.05, -0.01)	0.95 (-1.02, 2.92) ³
IQ composite score	0.11 (-0.27, 0.49)	0.01 (-0.03, 0.04) ⁴	-0.04 (-0.06, -0.02)	0.56 (-0.97, 2.08) ⁴	-0.04 (-0.06, -0.02)	0.52 (-1.29, 2.33) ⁴
<i>Child Behaviour Checklist</i>						
Internal problem score	-0.09 (-0.37, 0.20)	-0.01 (-0.03, 0.03) ⁵	-0.36 (-1.06, 0.35)	-0.67 (-1.82, 0.48) ⁵	0.36 (-0.35, 1.07)	0.05 (-1.31, 1.41) ⁵
External problem score	-0.08 (-0.39, 0.22)	0.01 (-0.02, 0.04) ⁶	0.03 (-0.73, 0.79)	0.28 (-0.96, 1.52) ⁶	-0.03 (-0.79, 0.73)	0.32 (-1.14, 1.78) ⁶
Total problem score	-0.28 (-1.09, 0.53)	0.01 (-0.07, 0.09) ⁷	-0.33 (-2.35, 1.69)	-0.41 (-3.70, 2.88) ⁷	0.34 (-1.68, 2.36)	0.45 (-3.42, 4.32) ⁷

¹Values are β coefficients (95% confidence interval), total $n = 547$.

²Model adjusted for infant sex, birth weight, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

³Model adjusted for infant sex, birth weight, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), maternal smoking at 15 weeks' gestation and duration of breastfeeding.

⁴Model adjusted for infant sex, birth weight, marital status, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

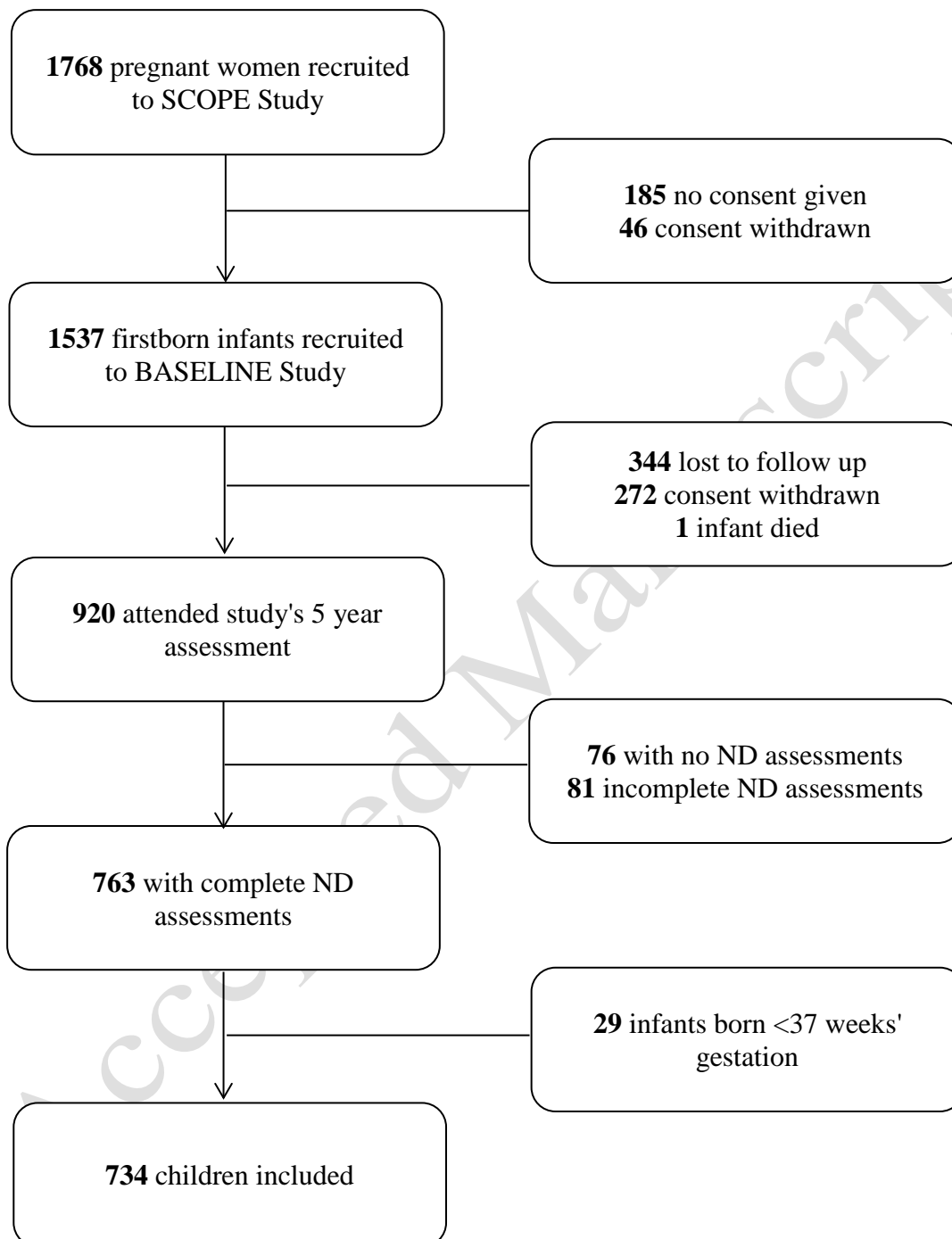
⁵Model adjusted for infant sex, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log) and age to weaning onto solids.

⁶Model adjusted for infant sex, marital status, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

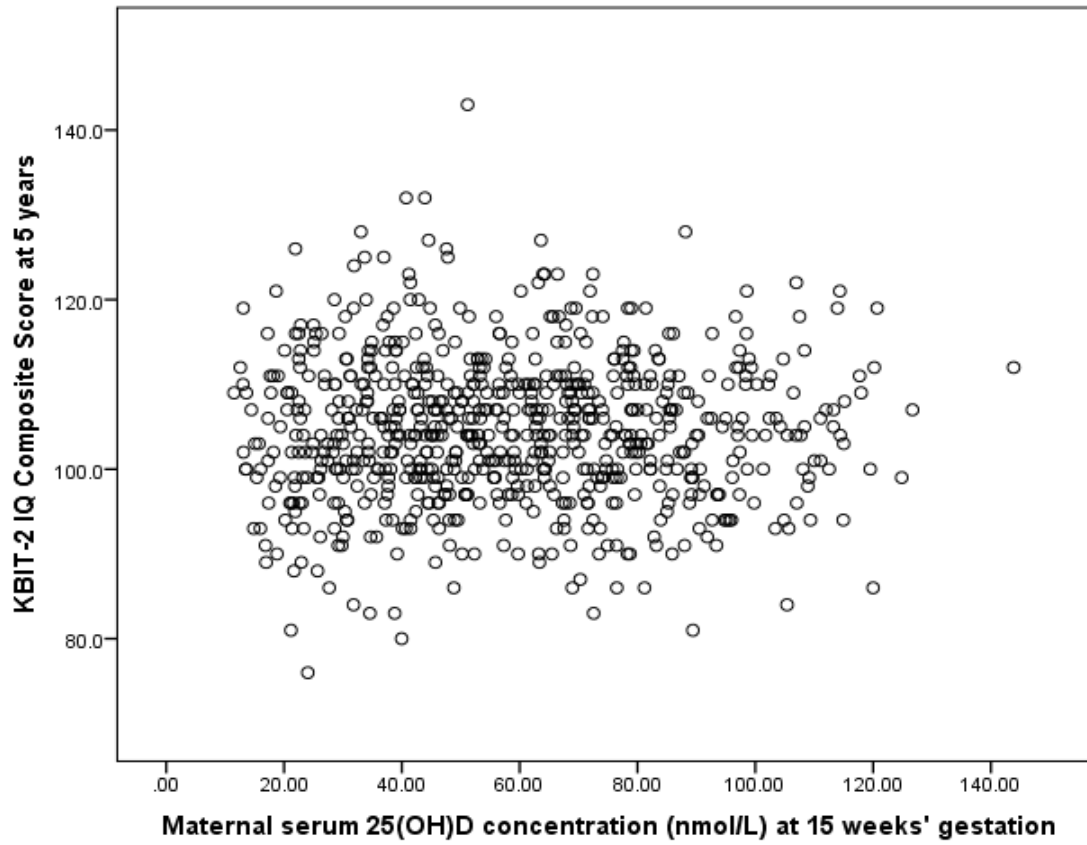
⁷Model adjusted for infant sex, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

Figure 1 Flow chart of study participants

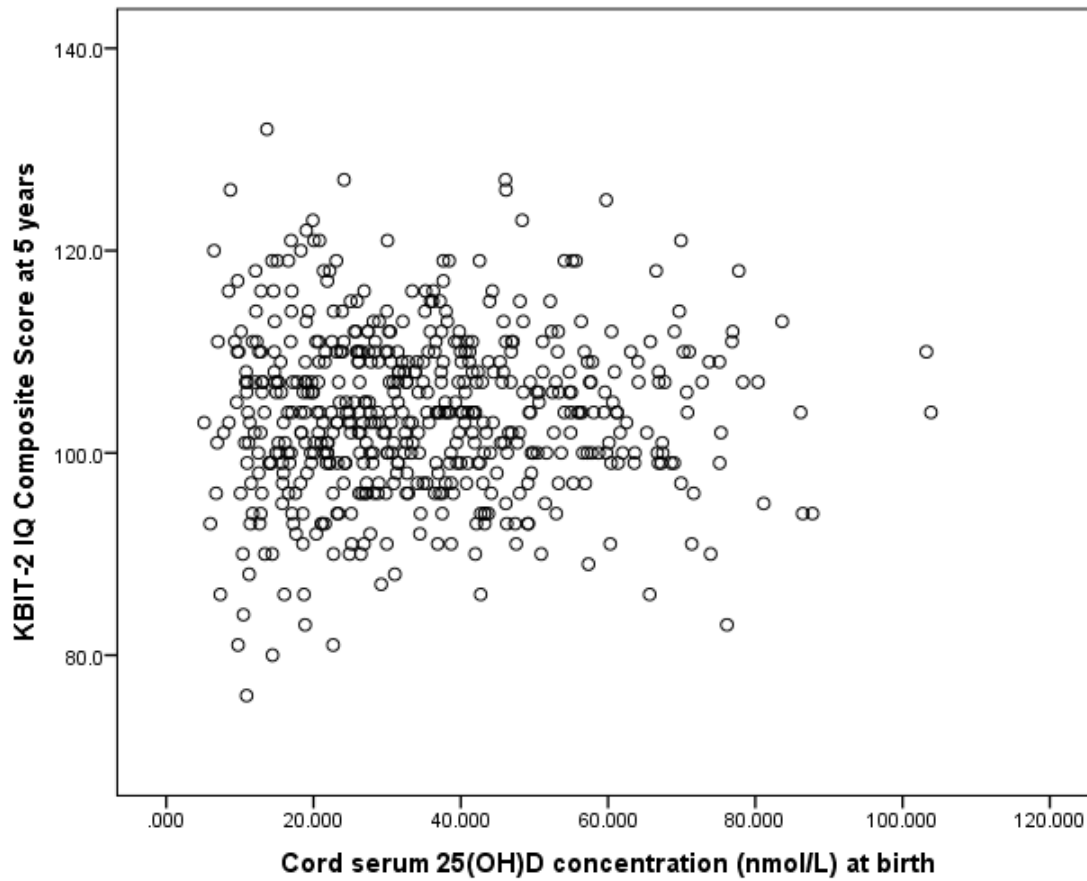
ND: neurodevelopment



A



B



Supplemental Figure 1 Distribution of (A) maternal serum 25-hydroxyvitamin D (25(OH)D) concentrations at 15 weeks' gestation and (B) cord serum 25(OH)D concentrations at birth with Kaufman Brief Intelligence Test, 2nd Edition (KBIT-2) IQ composite scores at five years.

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