ORIGINAL ARTICLE

Maternal early-pregnancy ferritin and offspring neurodevelopment: A prospective cohort study from gestation to school age

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Abstract

Background: Iron plays a role in many key processes in the developing brain. During pregnancy, iron supplementation is widely recommended to prevent and treat iron deficiency; however, the prevalence of iron deficiency and the risk of iron overload vary greatly between populations. Evidence on the role of high levels of maternal ferritin, a storage iron marker during pregnancy in relation to offspring neurodevelopment is lacking.

Objective: Our main objective was to examine if maternal ferritin levels during pregnancy are associated with child cognitive and motor abilities.

Methods: We included Dutch mother-child dyads from the prospective population-based Generation R Study, born in 2002–2006. We compared children whose mothers had high (standard deviation score >+1) or low (standard deviation score <−1) early-pregnancy ferritin to children whose mothers had intermediate ferritin (reference group) using linear regression. Children underwent non-verbal intelligence and language tests at 4–9 years (cognitive abilities), finger-tapping and balancing tests at 8–12 years (motor abilities), and structural magnetic resonance imaging at 8–12 years (brain morphology). Covariates were child age, sex, maternal intelligence quotient estimate, age, body-mass-index, education, parity, smoking and alcohol use.

Results: Of the 2479 mother-child dyads with data on maternal ferritin and at least one child neurodevelopmental outcome, 387 mothers had low (mean = 20.6 µg/L), 1700 intermediate (mean = 64.6 µg/L) and 392 high (mean = 170.3 µg/L) early-pregnancy

ferritin. High maternal ferritin was associated with 2.54 points (95% confidence interval -4.16, -0.92) lower child intelligence quotient and 16.02 cm^3 (95% confidence interval -30.57, -1.48) smaller brain volume. Results remained similar after excluding mothers with high C-reactive protein. Low maternal ferritin was not associated with child cognitive abilities. Maternal ferritin was unrelated to child motor outcomes.

Conclusion: High maternal ferritin during pregnancy was associated with poorer child cognitive abilities and smaller brain volume. Maternal iron status during pregnancy may be associated with offspring neurodevelopment.

KEYWORDS

brain, ferritin, intelligence, iron, motor skills, pregnancy

1 | BACKGROUND

Epidemiological, clinical and experimental studies suggest that suboptimal conditions during intrauterine life impact foetal development and can influence a range of health outcomes.[1] To diagnose iron-deficiency, ferritin is often measured during pregnancy, but is this iron storage marker associated with child outcomes?

Iron, while crucial for erythropoiesis, energy metabolism and cell signalling, is highly toxic in its free form and thus stored within ferritin, a protein whose serum concentration decreases as iron stores are depleted.[2-4] Previous studies have linked low maternal ferritin during pregnancy with poorer child cognitive abilities.[5,6] On the contrary, evidence from animal models and studies on mostly preterm neonates suggest that *high* ferritin and iron overload in infancy may also be associated with disturbances in key neurocellular processes and adverse neurodevelopmental sequelae.[7] Like the prevalence of iron deficiency and low ferritin, also the prevalence of elevated ferritin varies between populations: in the United States, for example approximately 6% of 20–49-year-old women are estimated to have serum ferritin above 150 μg/L, indicating potential risk of iron overload.[4,8] Yet, there is little evidence on what the upper cut-offs of ferritin should be during pregnancy, and whether high maternal ferritin is associated with adverse sequelae for the offspring.

Some indirect evidence to support maternal iron status may be associated with child neurodevelopmental outcomes comes from studies on maternal haemoglobin, which is widely used to screen for (severe) iron-deficiency.[4,9] Previous studies have linked both low and high maternal haemoglobin during pregnancy with perinatal complications, and some evidence suggests both extremes could be associated with poorer motor outcomes in the offspring.[10,11] However, evidence regarding maternal haemoglobin and child developmental outcomes is limited, and should be interpreted with caution, as iron status is only one of many factors that can influence haemoglobin.[4,9,10]

The purpose of the current study was to investigate if maternal ferritin and haemoglobin during early pregnancy are associated with key developmental outcomes in the child: intelligence quotient (IQ) and language and motor abilities in school age. The study was embedded in a population-based, prospective cohort and included 2549 Dutch mothers and their children. To investigate underlying differences in brain development, we also examined global and regional

Synopsis

Study question

Is maternal ferritin associated with child cognitive and motor abilities and brain morphology?

What's already known

Iron plays a role in many key processes in the developing brain. Ferritin is a commonly used marker of iron stores during pregnancy. Low ferritin indicates iron-deficiency, whereas high ferritin can indicate high iron intake and reserves but also inflammation.

What this study adds

In this population where maternal iron deficiency was rare, high rather than low maternal ferritin was associated with poorer offspring cognitive functioning and smaller brain volume in school age. These associations remained after addressing potential confounding by factors such as inflammation, maternal cognitive abilities and smoking.

brain morphology in a subset of children who underwent structural Magnetic Resonance Imaging (MRI). Of the two biomarkers measured in early pregnancy, ferritin is a more reliable indicator of iron status.[4,9] We focussed on ferritin as our main exposure of interest, and analysed haemoglobin as a secondary exposure, with the aim of establishing if either low or high maternal ferritin, or low or high maternal haemoglobin during pregnancy are associated with child neurodevelopment.

2 | METHODS

2.1 | Cohort

This study was conducted within the *Generation R Study*, a previously described population-based prospective cohort.[12] The study was approved by the Medical Ethical Committee of Erasmus MC,

Rotterdam and conducted according to the Declaration of Helsinki. All participating mothers and parents/legal guardians of participating children gave written informed consent.

Briefly, all pregnant women living within Rotterdam, the Netherlands, with expected delivery date between 4/2002 and 1/2006 were invited to participate: 8879 pregnant women were recruited. The Generation R Study is a multi-ethnic cohort: approximately 50% of mothers are of Dutch national origin, that is, both of her parents were born in the Netherlands, while several ethnic minorities make up approximately 50%.[12] To limit confounding and due to the small sample size per ethnic minority group, we only included mothers of Dutch national origin (n = 4096). We excluded women recruited after early pregnancy, when blood samples used to analyse ferritin concentrations were drawn (>18 weeks; n = 602), non-singleton pregnancies (n = 48) and non-live births/neonatal deaths (n = 44).[13] After attrition of 248 mother-child dyads without early-pregnancy ferritin/haemoglobin data, and 605 without cognitive/motor outcome data at 4–12 years, the final analytical sample included 2549 dyads. Of them, 1550 additionally underwent MRI at 8–12 years: after excluding six participants with incidental findings (e.g., large cysts, confirmed by a neuroradiologist) and low-quality data (n = 269), neuroimaging data were available for 1275 children (Figure S1).[14,15]

2.2 | Exposure: Maternal ferritin and haemoglobin

Maternal non-fasting blood samples were drawn at 6–18 weeks of gestation using antecubital venepuncture (mean = 13.2 weeks, SD = 1.8).[13] Maternal ferritin was measured from serum samples stored at −80°C using electrochemiluminescence immunoassay; haemoglobin was measured from fresh plasma.

During early pregnancy, physiological haemodilution occurs, and ferritin and haemoglobin decline (Figure S2).[16] To account for differences in the timing of venepuncture and standardise exposures, we regressed ferritin and haemoglobin on gestational age at venepuncture using linear regression to create standard deviation scores (SDS; mean = 0, SD = 1). Ferritin was square-transformed to reduce skewness before standardisation. Ferritin and haemoglobin were categorised into three groups each: low (SDS <−1), intermediate (SDS −1 to +1), high (SDS >+1).

2.3 | Outcomes

Cognitive abilities were assessed at the 6-year follow-up (mean age = 6.1 years, SD = 0.4, range 4.9–9.0). Firstly, non-verbal IQ was estimated using two subtests (mosaics, categories) of the Dutch non-verbal intelligence test, *Snijders-Oomen Niet-Verbale Intelligentietest*,[17] as described previously.[18] Raw scores were converted into non-verbal IQ estimates using normative data tailored to age.[18] Estimates ≤50 and ≥150 were assigned to 50 and 150, respectively, as this test is not designed to differentiate between individuals beyond these limits. Secondly, language ability was measured

using the comprehension subtest of the Dutch language development test, *Taaltest voor Kinderen*.[19] Children were shown two pictures and asked to choose the picture that matched a given word. Number of correct answers was divided by number of items (37) to yield a total score. To attain normality and for ease of interpretation, we square-transformed and standardised language scores within the cohort.

Motor abilities were assessed at the 9-year follow-up (mean = 9.8 years, SD = 0.3, range 8.5–12.0). Firstly, motor control and fine-motor speed were assessed using a computerised finger-tapping task. Finger-tapping is one of the most frequently used neuropsychological instruments,[20] and has been associated with brain lesions and a range of motor dysfunctions.[21] The test included five trials: Participants were asked to tap their index finger as fast as possible for 10 seconds, starting with (1) right, (2) left, (3) both (alternating), (4) right and (5) left hand: average number of taps across tasks was used as the outcome.[14] Secondly, gross motor skills and balance were assessed using a balancing task, the Walking Backwards task from the Body Coordination Test for Children (*Körperkoordinationstest für Kinder*), validated among school-aged Dutch children.[22,23] Children walked backwards on three 3-metre-long, 5-centimetre-high beams of different widths (6, 4.5, 3 cm). After a practice trial, each child walked twice backwards along each beam, starting with the widest, finishing with the narrowest.[14] The outcome was the total number of correct steps (until the child stepped off/reached a maximum of 8 steps per trial) across all 6 trials.

On all cognitive and motor outcomes, higher scores reflect better performance.

Children underwent MRI at the mean age of 10.1 years (SD = 0.6, range 8.9–12.0), as described previously.[14,15] Images were acquired using the same sequence and scanner (3 Tesla GE 750w Discovery). Following three-plane localiser scans, a high-resolution T1-weighted inversion recovery fast spoiled gradient recalled sequence was acquired (T_R = 8.77 ms, T_E = 3.4 ms, T_I = 600 ms, flip angle = 10°, field of view = 220 × 220 mm, Acquisition Matrix = 220 × 220, slice thickness = 1 mm, number of slices = 230). FreeSurfer v.6.0.0 (http://surfer.nmr.mgh.harvard.edu/) was used to obtain total brain volume, global cortical and subcortical grey matter, global cerebral white matter, total cerebellar volume and total intracranial volume.[14,15]

2.4 | Statistical analysis

In the primary models, we used multivariable linear regression to examine associations between maternal ferritin (high vs. intermediate [reference category] vs. low) and child IQ, language ability, finger-tapping and balancing (continuous). We corrected for multiple testing using the False-Discovery-Rate method (FDR)[24] at α = 0.05 across adjusted primary models, calculating the number of tests (8) based on two exposures (low-versus-reference ferritin, high-versus-reference ferritin) and four outcomes (IQ, language, finger-tapping, balancing).

If analyses suggested associations between ferritin and cognitive or motor abilities (adjusted model, surviving multiple-testing

correction), we followed up with analyses of neuroimaging measures to investigate underlying biological differences. We first examined differences in total brain volume. We then examined cortical grey, subcortical grey, cerebellar and total cerebral white matter volumes: in a further model, these were regressed on total intracranial volumes to examine structural specificity.

We used R-3.3.2 for all analyses.

Covariates were chosen based on prior literature with the help of directed acyclic graphs (see Figure S3).[4,25–30] In the age-and-sex-adjusted model, we included child age at follow-up and sex (male/female) as covariates. In the adjusted model, we added potential maternal confounders. These included age at enrolment, pre-pregnancy body-mass-index (kg/m^2), prospectively self-reported smoking (none/quit when pregnancy was known/continued) and alcohol consumption (none/quit when pregnancy was known/continued occasionally/continued weekly), education at enrolment (tertiary/lower), parity (primipara/multipara) and maternal IQ assessed 5 years after delivery (using Raven's Standard Progressive Matrices[31]).

2.4.1 | Missing data

We imputed forty datasets using multiple imputation by chained equations (MICE) to handle missing covariate data within the analytical sample ($n = 2549$), using predictive mean matching, logistic regression and polytomous logistic regression methods to impute continuous, binary and non-binary nominal covariates, respectively.[32] All available exposure, outcome and covariate data were used for imputing covariate data (Table S1). In all models, estimates were pooled across multiple imputation datasets using Rubin's rules.

2.4.2 | Sensitivity analyses

To address unmeasured confounding, we calculated E-values.[33,34] E-values reflect the minimum strength of association to exposure and outcome that an unmeasured confounder would need to fully explain exposure-outcome associations.[34]

Inflammatory status may increase ferritin independently of iron status: in sensitivity analyses, we only included participants with early-pregnancy C-reactive protein (CRP) <5 mg/L.[4,35]

Folic acid and vitamin supplements can contain iron: in sensitivity analyses, we added self-reported prenatal maternal folic acid (no/yes) or multivitamin (no/yes) supplement use as covariates, to investigate if these factors explained any observed associations.

To further facilitate comparisons between our results and findings from other populations, we re-ran the primary models using maternal ferritin re-categorised into low (<20 μg/L), intermediate (20–150 μg/L) or high (>150 μg/L), to predict child cognitive and motor abilities, based on previously used cut-offs.[3,4]

To test for linear associations, we used ferritin SDS as a continuous predictor. To further assess if associations between iron status and neurodevelopment are U-shaped,[11] we used quadratic terms (ferritin SDS^2).

Furthermore, we re-ran the primary models after replacing ferritin with haemoglobin, a more widely available yet less specific screener for iron status, categorised into low (<−1 SD), intermediate or high (>1 SD). Iron deficiency ultimately leads to decreased haemoglobin, however, in our sample anaemia (haemoglobin <11g/dl, $n = 54$) and iron-deficiency anaemia (haemoglobin <11 g/dl and ferritin <20 μg/L, $n = 8$) were rare, and we refrained from analysing these as predictors.[9]

We describe the characteristics of the Generation R Study cohort members who were otherwise eligible for inclusion in the current study but could not be included in the analytical sample because of missing exposure or outcome data ($n = 853$) (see Figure S1). In sensitivity analyses, we addressed the potential for selection bias owing to non-response by using baseline data (predictors: maternal characteristics during pregnancy, child sex; see Table 1) to calculate inverse probability of attrition weights (outcome: inclusion or exclusion from the analytical sample), which were then used to re-estimate the primary linear regression models.[36]

2.5 | Ethics approval

The study was approved by the Medical Ethical Committee of Erasmus MC, Rotterdam.

3 | RESULTS

Table 1 shows participant characteristics stratified by low, intermediate and high ferritin. Compared to mothers with intermediate ferritin ($n = 1700$, ferritin mean = 64.6 μg/L), mothers with low ferritin ($n = 387$, mean = 20.6 μg/L) were more often multiparous and older, and less often smoked or used alcohol, multivitamins and folic acid, whereas mothers with high ferritin ($n = 392$, mean = 170.3 μg/L) were more often primiparous, had higher BMI and higher education, used alcohol and folic acid supplements more often, and more often had elevated CRP. Table S1 shows characteristics in the entire analytical sample. Table S2 shows characteristics stratified by low, intermediate and high haemoglobin. Spearman's correlation between maternal haemoglobin and ferritin was 0.03.

We report results from adjusted models (see: 'Statistical analysis'), unless otherwise stated.

3.1 | Maternal ferritin and child cognitive abilities

Figure 1 depicts the associations of maternal early-pregnancy ferritin with child IQ and language abilities. Children exposed to *high* maternal ferritin had 2.54 points lower IQ (95% CI −4.16, −0.92), compared to children whose mothers had intermediate ferritin (Figure 1). This association survived correction for multiple testing.

TABLE 1 Characteristics of the analytical sample, stratified by low, intermediate and high maternal ferritin during pregnancy

	Low ferritin (SDS <−1, n = 387)	Intermediate ferritin (n = 1700)	High ferritin (SDS >1, n = 392)
Maternal characteristics			
Ferritin, µg/L, mean (SD)	20.6 (6.6)	64.6 (24.3)	170.3 (53.6)
Haemoglobin, g/dl, mean (SD)	12.5 (0.8)	12.6 (0.8)	12.6 (0.8)
Age at enrolment, years, mean (SD)	31.7 (4.6)	31.4 (4.1)	31.4 (3.6)
Pre-pregnancy BMI, kg/m^2, mean (SD)	23.0 (3.6)	23.2 (4.0)	23.6 (4.0)
Estimated IQ, mean (SD)	101.7 (12.5)	100.9 (12.7)	100.2 (12.5)
Education, tertiary, n (%)	237 (61.6)	1032 (61.6)	248 (64.1)
Parity, primiparous, n (%)	138 (35.8)	1076 (63.4)	325 (82.9)
Smoking during pregnancy			
None, n (%)	290 (80.8)	1144 (73.6)	261 (73.5)
Quit in early pregnancy, n (%)	18 (5.0)	160 (10.3)	49 (13.8)
Continued, n (%)	51 (14.2)	250 (16.1)	45 (12.7)
Alcohol use during pregnancy			
None, n (%)	125 (33.3)	473 (29.2)	84 (22.6)
Quit in early pregnancy	60 (16.0)	272 (16.8)	71 (19.1)
Occasional, n (%)	150 (40.0)	678 (41.9)	156 (41.9)
Weekly, n (%)	40 (10.7)	196 (12.1)	61 (16.4)
Prenatal folic acid supplementation, n (%)	288 (88.9)	1270 (91.4)	298 (94.3)
Prenatal multivitamin supplementation, n (%)	101 (29.8)	577 (38.8)	122 (35.9)
Early-pregnancy CRP <5 mg/L, n (%)	232 (61.9)	973 (58.6)	214 (55.6)
Child characteristics			
Sex, female, n (%)	183 (47.3)	863 (50.8)	196 (50.0)
Characteristics at the 6-year follow-up			
Age at cognitive assessment, years, mean (SD)	6.1 (0.4)	6.1 (0.4)	6.1 (0.4)
Non-verbal IQ, points, mean (SD)	106.4 (13.2)	105.1 (14.4)	103.0 (15.0)
Language ability score, points, mean (SD)	0.24 (0.93)	0.26 (0.86)	0.21 (0.94)
Characteristics at the 9-year follow-up			
Age at motor assessment, years, mean (SD)	9.8 (0.3)	9.8 (0.3)	9.8 (0.3)
Finger-tapping score, taps, mean (SD)	38.2 (6.5)	38.3 (6.4)	37.9 (6.5)
Balance performance score, steps, mean (SD)	27.1 (8.1)	26.8 (8.6)	26.0 (8.9)
Characteristics at the MRI visit			
Age at MRI, years, mean (SD)	10.1 (0.6)	10.1 (0.6)	10.2 (0.6)
Total brain volume, cm^3, mean (SD)	1232.2 (102.6)	1235.2 (105.8)	1223.1 (117.4)
Cortical grey matter volume, cm^3, mean (SD)	592.4 (49.3)	592.7 (51.5)	586.6 (55.6)
Subcortical grey matter volume, cm^3, mean (SD)	60.8 (4.3)	61.2 (4.4)	60.9 (4.7)
Cerebral white matter volume, cm^3, mean (SD)	430.5 (47.3)	432.2 (48.2)	427.6 (52.6)
Cerebellar volume, cm^3, mean (SD)	146.3 (13.3)	147.0 (12.6)	145.7 (14.0)

Abbreviations: %, proportion of cases in relation to all participants with data available; BMI, Body-mass-index; CRP, C-reactive protein; IQ, Intelligence Quotient; MRI, Magnetic resonance imaging; n, number of cases; SD, Standard deviation; SDS, standard deviation score (ferritin and haemoglobin were standardised for gestational age at the time of venepuncture).

High maternal ferritin was not associated with child language abilities (−0.08 SD units, 95% CI −0.19, 0.02) (Figure 1).

Children whose mothers had *low* ferritin had 1.40 points higher IQ (95% CI −0.25, 3.05) and 0.04 SD units higher language scores (95% CI −0.07, 0.14) (Figure 1). This suggests that low maternal ferritin was not associated with adverse child cognitive outcomes.

E-value point estimates were 1.63 and 1.41 for the associations of child IQ with high and low maternal ferritin, respectively, and 1.41 and 1.24 for the associations of language ability with high and low maternal ferritin, respectively. These describe the effect magnitude of an unmeasured confounder needed to explain away the associations. Of note, the changes in effect estimates in the
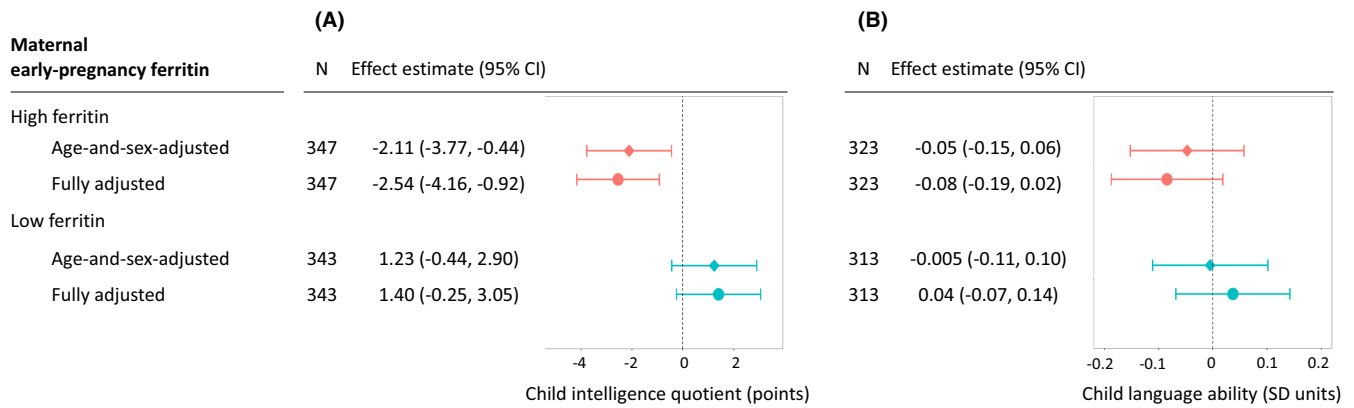
FIGURE 1 Maternal early-pregnancy ferritin level and child intelligence quotient (panel A) and language ability (panel B) at the 6-year-follow-up. Panel A. Child intelligence quotient (points). Panel B. Child language ability (SD units). In the age-and-sex-adjusted model (squares), associations were adjusted for child sex and age at the time of assessment. In the adjusted model (circles), we added as covariates the following maternal factors: age at enrolment (years), pre-pregnancy body-mass-index (kg/m^2), prospectively self-reported smoking (none/quit when pregnancy was known/continued) and alcohol consumption (none/quit when pregnancy was known/continued occasionally/continued weekly), education at enrolment (tertiary/lower), parity (primipara/multipara) and maternal intelligence quotient estimate (continuous). Maternal ferritin was standardised for gestational age at the time of venepuncture. CI, Confidence Interval; Effect estimate: difference in intelligence quotient points (panel A) or language ability SD units (panel B), among children exposed to high ferritin (ferritin standard deviation score >1), or low ferritin (ferritin standard deviation score <−1), compared to reference group with intermediate ferritin (ferritin standard deviation score between −1 and +1; $n = 1517$ and $n = 1415$ for intelligence quotient and language ability analyses, respectively); N, group size; SD, standard deviation [Colour figure can be viewed at wileyonlinelibrary.com]
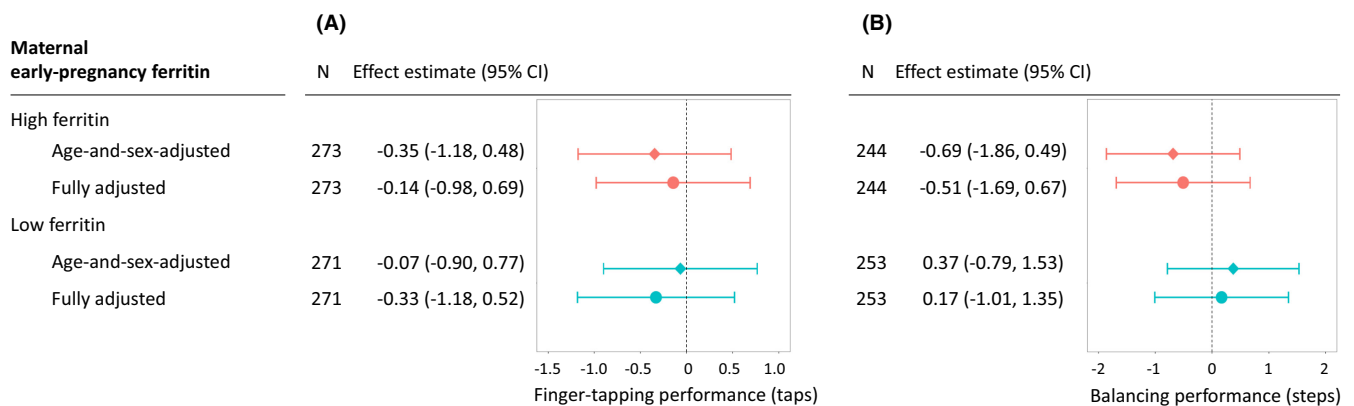

FIGURE 2 Maternal early-pregnancy ferritin level and child motor abilities: finger-tapping (panel A) and balancing task (panel B) performance at the 9-year-follow-up. Panel A. Child finger-tapping performance (taps). Panel B. Child balancing performance (steps). In the age-and-sex-adjusted model (squares), associations were adjusted for child sex and age at the time of assessment. In the adjusted model (circles), we added as covariates the following maternal factors: age at enrolment (years), pre-pregnancy body-mass-index (kg/m^2), prospectively self-reported smoking (none/quit when pregnancy was known/continued) and alcohol consumption (none/quit when pregnancy was known/continued occasionally/continued weekly), education at enrolment (tertiary/lower), parity (primipara/multipara) and maternal intelligence quotient estimate (continuous). Maternal ferritin was standardised for gestational age at the time of venepuncture. CI, Confidence Interval; Effect estimate: difference in taps (panel A) or steps (panel B), among children exposed to high ferritin (ferritin standard deviation score >1), or low ferritin (ferritin standard deviation score <−1), compared to reference group with intermediate ferritin (ferritin standard deviation score between −1 and +1; $n = 1200$ and $n = 1109$ for finger-tapping and balancing analyses, respectively); N, group size; SD, standard deviation [Colour figure can be viewed at wileyonlinelibrary.com]

age-and-sex-adjusted versus fully adjusted models were small (Figure 1).

In sensitivity analyses, we excluded mothers with CRP ≥5 mg/L (Table S3), added maternal antenatal folic acid or multivitamin supplements as covariates (Table S4), and used alternative cut-offs that are not tailored to gestational age, to categorise maternal ferritin into low (<20 μg/L, $n = 176$), intermediate (20–150 μg/L, $n = 2085$) or high (>150 μg/L, $n = 218$) (Table S5). The results remained similar: children whose mothers had high ferritin had lower IQ.

Secondary analyses of maternal ferritin SDS as a continuous variable supported the primary findings. Per 1 SD-unit increase in

maternal ferritin, child IQ decreased by 0.61 points (95% CI −1.21, −0.002) and language score by 0.04 SD units (95% CI −0.07, 0.002). Adding quadratic terms did not suggest U-shaped associations between ferritin and IQ or language abilities.

3.2 | Maternal ferritin and child motor abilities

We found no associations between maternal ferritin and child finger-tapping or balancing scores (Figure 2). E-value point estimates were 1.17 and 1.27 for associations of finger-tapping with high and low maternal ferritin, respectively, and 1.30 and 1.15 for associations of balancing with high and low maternal ferritin, respectively.

The results remained similar when excluding mothers with CRP ≥5 mg/L (Table S3), adding maternal antenatal folic acid or multivitamin supplements as covariates (Table S4), or using alternative cut-offs to categorise maternal ferritin into low (<20 μg/L), intermediate or high (>150 μg/L) (Table S5). There was no indication of linear associations between ferritin SDS and finger-tapping (per 1 SD increase in ferritin, score increased by 0.09 taps, 95% CI −0.23, 0.40) or balancing (0.13 steps, 95% CI −0.31, 0.57). Adding quadratic terms did not suggest U-shaped associations between ferritin and finger-tapping or balancing.

3.3 | Maternal ferritin and child brain morphology

After observing an association between maternal high ferritin and child IQ, we tested for associations between high maternal ferritin and child brain morphology (Table 2). Children exposed to high maternal ferritin had 16.02 cm^3 smaller total brain volume (95% CI −30.57, −1.48 cm^3) compared to children in the intermediate ferritin (reference) group. E-value point estimate for this model was 1.56. When examining brain structures separately, the association (of high ferritin) with cortical grey matter volume seemed most pronounced; however, after residualising the outcomes on total intracranial volume, no evidence of structural specificity remained (Table 2).

3.4 | Maternal haemoglobin and child outcomes

Maternal haemoglobin was mostly unrelated to child cognitive and motor abilities (Table S6). Children of mothers with low haemoglobin (SDS <−1, n = 257) had lower language abilities scores (−0.13 SD, 95% CI −0.24, −0.01) than children whose mothers had intermediate haemoglobin (n = 1123).

3.5 | Non-response analyses

Figure S1 is a flowchart describing the exclusions and loss to follow-up. Table S7 shows that compared to eligible mother-child dyads who were lost to follow-up (n = 853), mothers in the analytical sample (n = 2549) more often had tertiary education, were primiparous, and used folic acid supplements, were older, and less often continued smoking; children were more often girls. When we used inverse probability of attrition weights, calculated based on maternal baseline characteristics and child sex, to re-estimate the associations between maternal ferritin and child neurodevelopment, the results remained unchanged (Table S8).

TABLE 2 Brain morphology at the mean age of 10.1 years among children exposed to high maternal ferritin (n = 200), compared to intermediate maternal ferritin (n = 848) during early pregnancy

Brain outcome	Outcome treated as natural units (cm^3) Effect estimate (95% CI)	Outcome regressed on intracranial volume (SD units) Effect estimate (95% CI)
Total brain volume	−16.02 (−30.57, −1.48)	n/a
Cortical grey matter	−7.43 (−14.64, −0.22)	−0.06 (−0.22, 0.10)
Subcortical grey matter	−0.41 (−1.03, 0.22)	0.02 (−0.13, 0.18)
Cerebral white matter	−6.51 (−1327, 0.25)	−0.05 (−0.21, 0.11)
Cerebellar volume	−1.75 (−3.59, 0.10)	−0.07 (−0.23, 0.09)

Note: All associations were adjusted for child sex and age at the time of assessment, and the following maternal factors: age at enrolment (years), pre-pregnancy body-mass-index (kg/m^2), prospectively self-reported smoking (none/quit when pregnancy was known/continued) and alcohol consumption (none/quit when pregnancy was known/continued occasionally/continued weekly), education at enrolment (tertiary/lower), parity (primipara/multipara) and intelligence quotient (continuous, assessed among mothers approximately 5 years after delivery).

Abbreviation: CI, Confidence Interval;

Effect estimate: difference in brain volumetric outcomes from magnetic resonance imaging data in natural units (cm^3), or in SD units (after the outcome in question has been regressed on total intracranial volume to reveal tissue- and region-specific differences), among children exposed to high maternal ferritin (ferritin SD score >1), compared intermediate ferritin (ferritin SD score between −1 and +1). Maternal ferritin was standardised for gestational age at the time of venepuncture.

4 | COMMENT

4.1 | Principal findings

The current study showed that high maternal ferritin in early pregnancy is associated with lower child IQ in school age. This association remained when adjusting for maternal IQ, age, BMI, smoking, alcohol use, education and parity, and survived correction for multiple testing. The finding was supported by neuroimaging measures, which suggested that children whose mothers had high ferritin during pregnancy had smaller brain volume in preadolescence. High maternal ferritin was not associated with child language or motor abilities. In contrast to high maternal ferritin, low maternal ferritin was not associated with child neurodevelopmental outcomes.

4.2 | Strengths of the study

The large, prospective cohort study design and population-based neuroimaging approach are strengths of the current study. Further, we had detailed, high-quality follow-up data including cognitive, motor and neuroimaging outcomes in school age and were able to control for many maternal factors that have been proposed to affect both ferritin levels and offspring development.

4.3 | Limitations of the data

Ethnic homogeneity, while a strength in controlling for confounding, limits generalisability to other ethnicities. Follow-up rates were higher among more highly educated mothers, but the potential for selective attrition that could be addressed based on available data had little impact on the findings. While using pooled estimates from multiple imputation models partly addresses selective attrition and estimation uncertainty, it assumes data are missing at random given all observed values. This assumption cannot be tested, and its violation could lead to biased estimates. Maternal inflammation, metabolic diseases and substance use could elevate ferritin and influence child neurodevelopment, and we may not fully capture these phenomena by adjusting for self-reported smoking and alcohol use and BMI or excluding women with elevated CRP. While unmeasured confounders would need to have a considerable effect on both the exposure and the outcome to explain away the association between high ferritin and child IQ or brain volume—which changed only little when observed potential confounders were added as covariates—the risk of residual confounding inevitably remains, and non-observational data would be needed to overcome this limitation. Further, we had no data on iron supplements, and iron overload or its aetiology could not be confirmed with liver biopsies, genetic analyses or complimentary parameters such as fasting transferrin saturation or soluble transferrin receptor. Finally, extreme iron overload, for example due to hereditary haemochromatosis, where average serum ferritin can be >1000 μg/L, were beyond the scope of this study: studies with access to clinical rather than population-based samples may be better suited to investigate such rare disorders.[3,4,37]

4.4 | Interpretation

High ferritin can indicate high storage iron levels, for example due to an iron-rich diet, and ferritin is generally considered a reliable marker of iron status during pregnancy.[3,4,37] However, as an active phase protein, ferritin can also increase through inflammation, thus measurements of inflammatory markers including CRP can be used in combination with ferritin when assessing iron status.[4] In the current study, findings remained similar after excluding mothers with elevated CRP, which makes it less likely that inflammation-related increase in ferritin explained the findings. Further, factors such as overweight and alcohol are associated with higher ferritin.[4,30] In our study, associations between maternal ferritin and child neurodevelopment remained similar after adding maternal BMI, IQ, age, smoking, alcohol use, education and parity as covariates. Taken together, these results offer some support that maternal iron status is associated with child neurodevelopment; however, we advise caution in drawing causal conclusions based on observational data.

Excess iron exposure could lead to oxidative stress, interact with other nutrients and decrease key neurotransmitter levels in the developing brain.[7] However, prior evidence mostly comes from animal models and postnatal iron overload among preterm infants.[7] Some studies suggest that *low* maternal iron intake/anaemia during pregnancy is associated with neonatal alterations in hippocampal morphogenesis,[38] serum brain-derived neurotrophic factor levels,[38] and white matter maturation.[39] In our sample, high maternal ferritin during pregnancy was associated with smaller child brain volume, which is in line with our finding that high maternal ferritin during pregnancy is associated with lower IQ. This is in accordance with the consistent finding in the neuroscience of intelligence that larger brain volume is associated with better cognitive abilities; however, the basis of this correlation is not fully understood.[27] We found no evidence of structural specificity (differences specific to cortical or subcortical grey matter, white matter or cerebellar volume), after taking into account an overall reduction in brain volume, which is plausible considering the early timing of the exposure. Taken together, our neuroimaging and IQ findings are consistent and support the hypothesis that maternal iron status is associated with offspring brain development.

High maternal ferritin during pregnancy was associated with poorer child cognitive abilities in the current study, while some previous studies have suggested that *low* maternal ferritin during pregnancy predicts poorer child cognitive abilities.[5,6] This may seem like a discrepancy; however, some differences in study populations may help elucidate how our findings add to the existing literature, rather than contradict it. The current study was carried out in a population that was well-suited to detect associations with high ferritin: a relatively high proportion of mothers (~9%) had ferritin concentrations >150 μg/L.[4] Conversely, severe iron deficiency was rare, thus we may be unable to detect adverse effects driven by severe iron-deficiency,

which may be a true concern in more iron-deficient populations.[40] Further, our population had access to routine haemoglobin controls during pregnancy and, if anaemic, to iron supplementation through the Dutch antenatal care system.[41] This may have mitigated potentially adverse effects of iron deficiency on offspring neurodevelopment.

This study does not offer proof of underlying causal mechanisms, nor should its results be directly translated into clinical recommendations. Importantly, our results are not evidence that iron supplementation during pregnancy is unnecessary or harmful. It remains important to identify and treat iron-deficiency anaemia.[42] For non-targeted antenatal iron supplementation, the long-term effects remain unclear and most likely vary by population: in low-income settings with high rates of iron-deficiency, routine antenatal iron supplementation may be associated with some benefits.[40,43] We encourage future studies to analyse data on maternal iron intake, particularly from randomised controlled trials, to see if there is an optimal level of intake—not too low and not too high—that we should target, to optimise maternal and child health.

We are not aware of prior studies showing an association between high ferritin during pregnancy and child cognitive abilities or brain morphology and encourage replication of our findings. Prenatal MRI studies could elucidate the underlying mechanisms further: we refrained from testing mediation of ferritin on IQ through morphology, as children underwent MRI after cognitive tests. Repeated maternal measures and measuring maternal *and* child ferritin could elucidate timing-specificity and mediation through child iron stores.

In our study, higher ferritin was associated with lower IQ and smaller brain volume, while associations with language abilities were less clear, and motor skills seemed unrelated to maternal ferritin. This could suggest outcome-specificity; however, IQ and language abilities were assessed earlier in life, and the sensitivity of tests aimed at measuring different phenotypes may vary. Nonetheless, we encourage research into possible outcome-specificity, and note that offspring mental health outcomes could also be of interest.[44,45]

Finally, in the current study, we focussed on maternal ferritin; however, we also tested if haemoglobin was associated with child neurodevelopmental outcomes. While iron deficiency ultimately leads to low haemoglobin, haemoglobin is a less specific marker of iron status, compared to ferritin. In the current study, we observed an association between low maternal haemoglobin and child language abilities. This association, while in line with some studies linking maternal anaemia with offspring neurodevelopment,[46,47] seemed not to be driven by iron status. Thus, to understand the associations between maternal haemoglobin or anaemia and child neurodevelopment, we encourage research into the many genetic and environmental factors that can affect haemoglobin, beyond just iron-deficiency.[42]

5 | CONCLUSIONS

In the current study, we showed that high maternal ferritin during pregnancy was associated with lower IQ and smaller brain volume among school-aged children. The results support the hypothesis that maternal iron status is associated with offspring brain development, but these associations may be more complex than previous research on iron deficiency has shown.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest to report.

DATA AVAILABILITY STATEMENT

The individual-level data are not publicly available due to privacy and ethical restrictions. Requests for data access can be send to datamanagementgenr@erasmusmc.nl and will be discussed in the Generation R Study Management Team.

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