

Importance of Maternal Iron Status on the Improvement of Cognitive Function in Children After Prenatal Iron Supplementation

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Introduction: The effectiveness of prenatal iron supplementation improves maternal hematological outcomes, but little research has focused on child outcomes. The objective of this study was to assess whether prenatal iron supplementation adjusted to maternal needs improves children's cognitive functioning.

Methods: The analyses included a subsample of nonanemic pregnant women recruited in early pregnancy and their children aged 4 years ($n=295$). Data were collected between 2013 and 2017 in Tarragona (Spain). On the basis of hemoglobin levels before the 12th gestational week, women receive different iron doses: 80 vs 40 mg/d if hemoglobin is 110–130 g/L and 20 vs 40 mg/d if hemoglobin >130 g/L. Children's cognitive functioning was assessed using the Wechsler Preschool and Primary Scale of Intelligence-IV and Developmental Neuropsychological Assessment-II tests. The analyses were carried out in 2022 after the completion of the study. Multivariate regression models were performed for assessing the association between different doses of prenatal iron supplementation and children's cognitive functioning.

Results: Taking 80 mg/d of iron was positively associated with all the scales of the Wechsler Preschool and Primary Scale of Intelligence-IV and Neuropsychological Assessment-II when mothers had initial serum ferritin <15 $\mu\text{g/L}$, but it was negatively associated with Verbal Comprehension Index, Working Memory Index, Processing Speed Index, and Vocabulary Acquisition Index from Wechsler Preschool and Primary Scale of Intelligence-IV and verbal fluency index from Neuropsychological Assessment-II when mothers showed initial serum ferritin >65 $\mu\text{g/L}$. In the other group, taking 20 mg/d of iron was positively associated with Working Memory Index, Intelligence Quotient, verbal fluency, and emotion recognition indices when women had initial serum ferritin >65 $\mu\text{g/L}$.

Conclusions: Prenatal iron supplementation adjusted to the maternal hemoglobin levels and baseline iron stores improves cognitive functioning in children aged 4 years.

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INTRODUCTION

Cognitive development is based on brain formation and maturation, highly sensitive processes that begin at the fetal stage.¹ The prenatal environment, particularly maternal nutritional status, plays a key role in fetal brain development.² Maternal iron status during pregnancy is a major public health concern because it can have an impact on maternal and child health in many ways, including child neurodevelopment.³ Several animal and human studies have shown that both iron deficiency (ID) and excess can impair cognitive abilities in the short and long term.^{4–7} Whereas perinatal ID has been associated with alterations in brain energy and dopamine metabolism⁸ and myelination in various brain regions,^{9,10} iron excess can be toxic by forming deposits that lead to cellular damage, although there are still significant knowledge gaps.¹¹

Prenatal iron supplementation has been successful in improving maternal iron status in late pregnancy, but it has not yet shown clear benefits for children's cognitive development.¹² Studies conducted to date have examined a single dose of iron compared with a placebo^{13,14} or in combination with other micronutrients.^{15–17} However, because several factors are related to maternal iron status and because women's iron requirements may vary accordingly,^{18–20} some experts have long advocated adjusting prenatal iron supplementation to the needs of individual women, considering both their iron stores and other conditions.²¹ Indeed, in the previously published results of the ECLIPSES study, it was found that adjusting prenatal iron supplementation in nonanemic women to their initial iron status was a good strategy to prevent ID and iron excess in women who are at risk.²² However, in clinical practice, the same dose is still usually prescribed to all women, leaving many women at risk of iron imbalance. This can not only harm the mother's health but can also have lasting consequences on the baby's health. This study aimed to investigate whether the benefits of adjusting prenatal iron supplementation to mothers' needs previously observed in their iron status at the end of pregnancy extend to children's cognitive functions.

METHODS

Study Sample

The ECLIPSES study is a population-based RCT conducted in 2013–2017 in Tarragona, Spain, that aimed to evaluate the effectiveness of prescribing different doses of prenatal iron supplementation to nonanemic women in early pregnancy on their iron status at the end of gestation. Participating women were recruited before the 12th gestational week and allocated into 2 groups

according to their hemoglobin (Hb) levels. Women in Stratum 1 (initial Hb=110–130 g/L) were randomly assigned to receive a daily dose of 40 mg or 80 mg of iron aiming to prevent an ID, whereas those in Stratum 2 (initial Hb>130 g/L) received 20 mg or 40 mg of iron daily aiming to prevent the risk of developing iron excess (Figure 1). Because the usually prescribed dose of iron is 40 mg daily, women in each stratum who received this dose were considered the control group on which the higher and lower iron doses were tested. The intervention was triple blinded, meaning that neither the researchers, the supplement providers, nor healthcare workers knew the dose of each woman's iron supplement until the end of the study. The adherence to the intervention was determined by comparing the number of leftover pills participants brought back at each visit with self-reported compliance and was rated good if women forgot to take the pill less than twice a week and low if they forgot to take it 2 or more times a week at any of the study visits. Later, the ECLIPSES-NEN study consisted of a follow-up of the women's children at age 4 years intending to assess the children's cognitive functioning. This analyses, carried out in 2022, were based on a subsample from the main study including children at age of 4 years and their mothers.

The study was designed in agreement with the Declaration of Helsinki/Tokyo. All procedures were approved by the Clinical Research Ethics Committee of the Jordi Gol University Institute for Primary Care Research (Institut d'Investigació en Atenció Primària), of the Pere Virgili Health Research Institute (Institut d'Investigació Sanitària Pere Virgili), and of the Spanish Agency for Medicines and Medical Devices (Agencia Española del Medicamento y Productos Sanitarios). Signed informed consent was obtained from all women participating in the study.

Measures

Individualized cognitive assessment of children aged 4 years was performed in a Primary Care Centre facility, by 2 trained psychologists, with parents present, using the Spanish version of The Wechsler Preschool and Primary Scale of Intelligence (WPPSI)-IV²³ and some parts of the NEPSY-II: A Developmental Neuropsychological Assessment.²⁴

The WPPSI-IV assesses cognitive abilities using 15 subtests, from which 5 primary indices, 4 secondary indices, and the full-scale intelligence quotient (IQ) can be obtained. The following primary indices were obtained: Verbal Comprehension Index (VCI) (the ability to verbally reason, influenced by semantic knowledge), Fluid Reasoning Index (FRI) (the ability to think logically, identifying abstract relationships between pairs of words or images), Working Memory Index (WMI) (the ability to hold information temporarily and then process it), and Processing Speed Index (PSI) (the speed to understand information and begin to respond). As for visuospatial ability, the Block Design subtest was used, which measures an individual's ability to analyze, synthesize, and reproduce an abstract design. As secondary indices, the Vocabulary Acquisition Index (VAI) (the ability to acquire new vocabulary skills) was considered. Finally, the full-scale IQ provides a general measure of cognitive and intellectual performance. All indices have a mean of 100 and an SD of 15, whereas the subtests have a mean of 10 and an SD of 2.

The NEPSY-II is a comprehensive neuropsychological assessment tool. The verbal fluency subtest (language domain),

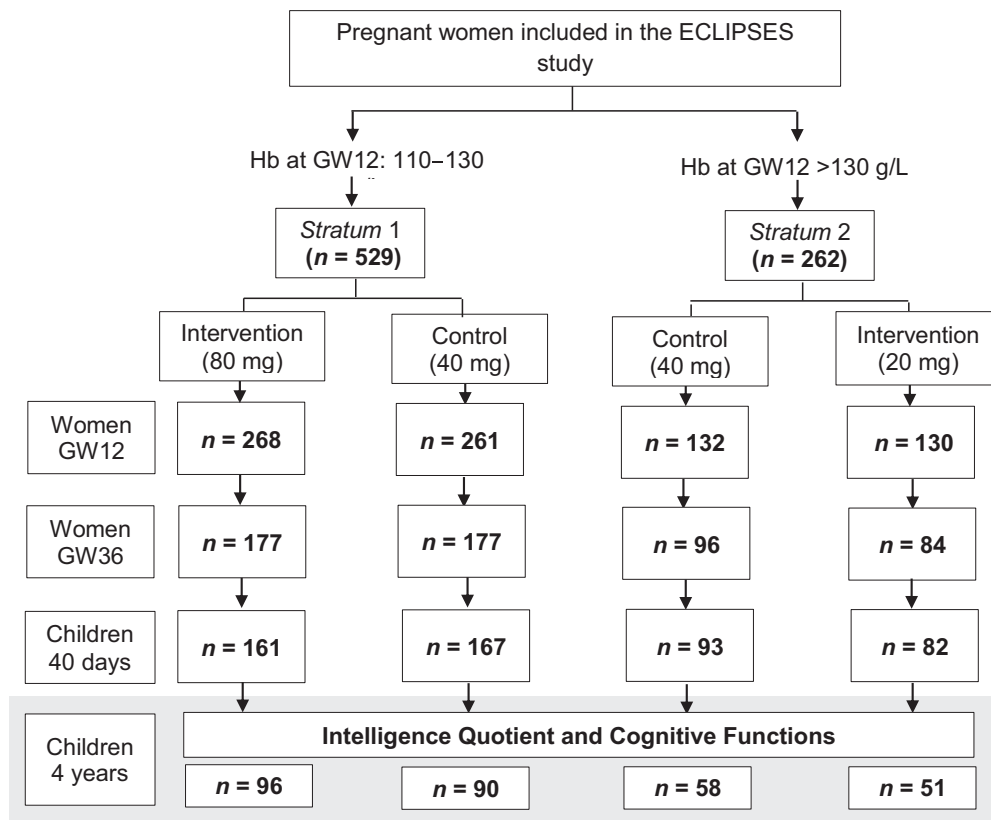


Figure 1. Flowchart of the study.

Hb, hemoglobin; GW, gestational week.

the visual-motor precision subtest (sensorimotor domain), and the emotion recognition subtest (social perception domain) were used to assess some cognitive functions that complement the WPPSI-IV results. The NEPSY-II subtests have a mean of 10 and an SD of 3.

Women were visited once in each trimester of pregnancy, and a wide range of information was recorded. Clinical and obstetric history was requested, including maternal age, parity, and pregnancy planning. Anthropometric measurements (weight and height) were taken, from which BMI was calculated. Dietary habits were assessed using a self-administered food frequency questionnaire (FFQ) previously validated in the study population.²⁵ In this process, participants retrospectively provided information on their usual food consumption at Weeks 12, 24, and 36 of pregnancy; 40 days after delivery; and at follow-up when the children were aged 4 years. The FFQ was reviewed and analyzed by trained dietitians who calculated the daily food intake, energy content, and various nutrients.²⁶ Maternal lifestyle information included smoking and physical activity. Family SES was calculated using participants' and partners' educational levels and occupational status.²² The parental IQ approach was assessed using the Matrix Reasoning subscale of the Wechsler Adult Intelligence Scale, 4th edition.²⁷ Maternal anxiety status, measured by the State–Trait Anxiety Inventory, and postpartum depression, assessed by the Edinburgh Postnatal Depression Scale, provided information about women's emotional status during pregnancy and after delivery. The State–Trait Anxiety Inventory test assessed 2 separate

concepts of anxiety: state and trait. State anxiety was included, which assesses a transient emotional state characterized by subjective, consciously perceived feelings of alertness and apprehension and autonomic-nervous-system hyperactivity. Detailed information can be found elsewhere.²²

Blood samples were collected in each trimester of pregnancy. Biochemical determinations of Hb and serum ferritin (SF) were done by immunochemiluminescence while the serum concentration of C-reactive protein was measured by immunoturbidimetry. Plasma polyunsaturated fatty acids (PUFAs) and serum vitamin D concentrations were quantified because they are involved in brain development, so maternal levels during pregnancy may affect fetal neurodevelopment.^{28,29} Then, PUFA concentrations were measured by gas chromatography-mass spectrometry,³⁰ and serum concentrations of vitamin D were quantified by an automated chemiluminescent immunoassay method.³¹ Folate and vitamin B₁₂ measurements were also performed in the first trimester of pregnancy. Red blood cell folate concentrations were then calculated using the following formula: (serum folate in hemolyzed whole blood × dilution factor in hemolysis × 100)/hematocrit. Genetic determinations of *HFE* gene mutations were performed.

As for information about the children, the following data were recorded. At birth, sex, gestational age (calculated from the time since the first day of the last menstrual period), and Apgar test score were obtained. Anthropometric measurements were recorded at birth and at age 40 days (length, head circumference,

and weight) and repeated at age 4 years (height and weight). Similarly, the feeding mode was recorded at birth and age 40 days, and children's diet at age 4 years was reported by parents using the same FFQ as for participating women.²⁶ All children were schooled by the time of the assessment.

Statistical Analyses

Analyses were performed per protocol and stratified first according to baseline Hb concentrations following the study design and then within each stratum according to women's baseline iron stores, defined by their SF levels (SF<15 $\mu\text{g/L}$, ID; SF 15–65 $\mu\text{g/L}$, adequate iron stores; SF>65 $\mu\text{g/L}$, normal-high iron stores). The SF threshold for normal-high iron stores corresponded to the 85th percentile.

Descriptive analyses of the variables studied were performed using the Student's *t* test and the ANOVA test for continuous variables and the chi-square test for categorical variables. The natural logarithm transformation was applied to the SF concentration to normalize its distribution. For statistically significant differences, the effect size was assessed using Cohen's *d*. Multivariate regression models provided estimates of the effect of different doses of prenatal iron supplementation (Stratum 1: 80 mg vs control, Stratum 2: 20 mg vs control) on the child's cognitive functioning. The models were adjusted a priori for those covariates that might influence this association. Maternal covariates include age, BMI in early pregnancy, parity (yes, meaning having previous children, or no, meaning having no previous children), pregnancy planning (yes or no), type of delivery (eutocic or dystocic), family SES (low, middle, high), smoking (yes or no), emotional status, postpartum depression, parental IQ approach, *HFE* gene mutations (yes or no), energy intake, dietary intake of iron and nutrients related to its metabolism (fiber, calcium, vitamin C), dietary intake of nutrients related to brain development (PUFAs, vitamin B₁₂, folate), serum concentrations of Hb, vitamin D, PUFA, and C-reactive protein at the first and third trimester of pregnancy and serum concentrations of red blood cell, folate, and vitamin B₁₂ at the first trimester of pregnancy. As for the child's covariates, the following were included: gestational age, sex, head circumference at birth, and dietary intake at age 4 years (energy, iron, PUFAs, vitamin B₁₂, and folate). The statistical analyses were done using the SPSS software (Version 27.0 for Windows; SPSS Inc., Chicago, IL).

RESULTS

This analyses were based on a sample of 295 mother–child pairs (Figure 1). Table 1 shows the main characteristics of the participants included and not included in the present sample after losses of follow-up, with no differences between them. Maternal characteristics of the participants included in this analyses are described according to their baseline iron status and dose of iron supplementation in Appendix Table 1 (available online). The change in maternal iron status after prenatal iron supplementation is also shown in Appendix Table 2 (available online).

Scores obtained by children at age 4 years on the WPPSI-IV and NEPSY-II tests for the intervention (80

or 20 mg/d iron) and control (40 mg/d iron) group according to the mother's baseline iron stores in each stratum are shown in Table 2. In the unadjusted analyses, some differences were found between the control and intervention groups and in the effect of iron doses according to maternal iron stores at baseline. For the WPPSI-IV, children of women in Stratum 1 (baseline Hb of 110–130 g/L) intervention group (80 mg/d iron) scored higher on FRI than those in the control group when their mothers started pregnancy with SF levels below 15 $\mu\text{g/L}$, whereas they scored lower on VCI, WMI, PSI, and VAI scales when their mothers' baseline SF levels were above 65 $\mu\text{g/L}$. Regarding the NEPSY-II, children of women in Stratum 1 intervention group obtained higher scores than their control peers on verbal fluency, visual-motor precision, and emotion recognition when their mothers were iron deficient in early pregnancy. For the visual-motor precision scale, the intervention resulted in higher scores than in the control group when women started pregnancy with SF>65 $\mu\text{g/L}$. As for Stratum 2, the tested dose of 20 mg/d of iron daily during pregnancy led children to obtain better scores in emotion recognition than those whose mothers received 40 mg/d of iron prenatally daily.

Multivariate adjusted analyses provided estimates of the effect of different doses of prenatal iron supplementation on children's cognitive functioning. First, no difference was found in the WPPSI-IV and NEPSY-II scores when comparing the intervention and control group in each stratum by the mother's baseline Hb levels without considering their baseline iron stores (data not shown). However, the analyses stratified by the women's baseline iron stores, classified according to their SF concentrations, unveiled various associations between different doses of prenatal iron supplementation on the child's cognitive functions and IQ (Table 3). Regarding the WPPSI-IV scores, the tested iron dose (80 mg/d) in Stratum 1 compared with 40 mg/d was positively associated with all the scales and full IQ when mothers started pregnancy with SF<15 $\mu\text{g/L}$. By contrast, when mothers showed SF>65 $\mu\text{g/L}$ at the beginning of gestation, a negative association was found with VCI ($\beta = -9.02$, 95% CI = -9.13, -8.91), WMI ($\beta = -17.55$, 95% CI = -19.53, -15.56), PSI ($\beta = -3.48$, 95% CI = -3.99, -2.97), and VAI ($\beta = -7.69$, 95% CI = -8.35, -7.02). In Stratum 2, the tested iron dose of 20 mg/d compared with 40 mg/d was positively associated with WMI ($\beta = 11.63$, 95% CI = 4.24, 22.83) and the full IQ ($\beta = 3.64$, 95% CI = 2.98, 4.31) when women started pregnancy with SF>65 $\mu\text{g/L}$. Similar results were found for the NEPSY-II scores. In Stratum 1, the tested iron dose was positively associated with all the indices compared with the control dose when women started pregnancy with ID. Conversely,

Table 1. Maternal Characteristics of Participants Included and Not Included in the Present Analyses

Maternal characteristics	Included (n=295)	Not included (n=496)	p-value
Baseline			
Age, years	32±6	31±7	0.098
Parity, yes	55.8 (164)	57.1 (283)	0.410
Pregnancy planning, yes	84.0 (248)	82.3 (408)	0.201
Body mass index			0.881
Underweight	1.9 (6)	1.7 (8)	
Normal weight	56.4 (166)	58.5 (290)	
Overweight	27.9 (82)	25.4 (126)	
Obesity	13.8 (41)	14.4 (72)	
Smoking, yes	16.9 (50)	18.4 (91)	0.770
Family socioeconomic status			0.235
High	22.9 (68)	18.7 (93)	
Middle	66.5 (196)	67.4 (334)	
Low	10.7 (31)	13.9 (69)	
HFE gene mutation, yes	32.3 (95)	33.6 (167)	0.732
Whole pregnancy			
Physical activity			0.419
Low	26.7 (79)	22.6 (112)	
Moderate	68.1 (200)	70.1 (348)	
High	5.2 (16)	7.3 (36)	
Anxiety assessment ^a			
Trait	14.49 (8.52)	13.55 (8.06)	0.065
State	15.15 (7.31)	14.94 (7.65)	0.158
Type of delivery			0.354
Eutocic	66.8 (198)	60.4 (300)	
Dystocic	32.9 (97)	39.6 (196)	
After delivery			
Postpartum depression ^b	6.82 (4.93)	6.87 (4.98)	0.925

Note: Data are expressed in mean (SD) for continuous normally distributed variables, median±IQR for continuous non-normally distributed variables, and % (n) for categorical variables.

^aMeasured by STAI, State-Trait Anxiety Inventory.

^bMeasured by EPDS Scale, Edinburgh Postnatal Depression Scale.

when initial SF levels were above 65 µg/L, taking 80 mg/d of iron instead of 40 mg/d was associated with lower verbal fluency scores and visual-motor precision scores. Regarding Stratum 2, the dose of 20 mg/d of iron compared with the control dose was positively associated with verbal fluency when women's SF levels were 15–65 µg/L and SF>65 µg/L in early pregnancy, and with emotion recognition only for the latest ones. On the contrary, the intervention resulted in a worse performance of visual-motor precision in children from women with baseline SF levels of 15–65 µg/L.

Although the scores obtained by most of the children on the WPPSI-IV indices were in the normal range, a percentage of them scored below the threshold for optimal cognitive functioning (<85 points), as follows: 5.1% for the VCI, 7.2% for the FRI, 16% for the WMI, 20.7% for the PSI, 5.8% for the full-scale IQ, and 13.1% for the

VAI. Logistic regressions showed no statistically significant difference between the control and tested doses of iron in each stratum on the WPPSI-IV scores (data not shown).

DISCUSSION

The main finding of this study was that adapting prenatal iron supplementation in nonanemic women not only to their Hb concentrations but also to their iron reserves in early pregnancy improved neuropsychological functioning in children at age 4 years. As far as is known, this is the first study to assess the effects of different doses of prenatal iron, depending on maternal iron stores in early pregnancy on child cognitive development, which makes it difficult to compare the current results. In evaluating the effects of routine prenatal iron

Table 2. Scores From WPPSI-IV and NEPSY-II Tests by Prenatal Iron Supplementation According to Maternal Iron Stores

Stratum 1 (Hb 110–130 g/L)	SF <15 µg/L			SF 15–65 µg/L			SF >65 µg/L		
	40 mg/d (n=16)	80 mg/d (n=13)	p-value	40 mg/d (n=63)	80 mg/d (n=61)	p-value	40 mg/d (n=11)	80 mg/d (n=18)	p-value
WPPSI-IV									
Verbal Comprehension Index	101.13 (12.55)	104.64 (15.03)	0.491	105.33 (12.04)	102.86 (15.18)	0.312	106.73 (11.62)	102.94 (11.84)	0.015^a
Fluid Reasoning Index	95.50 (13.42)	106.77 (9.64)	0.014^b	103.81 (13.45)	100.16 (14.18)	0.044^c	106.64 (9.80)	106.94 (13.82)	0.949
Working Memory Index	98.63 (13.13)	102.08 (13.10)	0.674	98.35 (11.92)	96.05 (12.49)	0.765	98.55 (10.74)	95.28 (7.76)	0.029^a
Processing Speed Index	89.19 (13.39)	98.85 (13.50)	0.065	94.25 (13.07)	91.84 (12.52)	0.043^c	97.15 (12.89)	95.17 (11.00)	0.046^c
Full intelligence quotient	97.44 (13.73)	105.77 (11.33)	0.091	102.49 (11.98)	100.54 (12.83)	0.384	102.45 (10.11)	102.17 (10.03)	0.658
Vocabulary Acquisition Index	90.50 (12.73)	97.86 (16.11)	0.174	99.42 (13.39)	94.88 (14.44)	0.061	99.27 (12.19)	95.84 (15.66)	0.038^c
Block Design subtest	10.69 (10.69)	11.79 (1.93)	0.061	10.79 (2.18)	11.40 (2.22)	0.054	12.08 (2.39)	11.00 (1.76)	0.018^a
NEPSY-II									
Verbal fluency	8.33 (3.33)	9.69 (2.84)	0.034^a	9.16 (2.69)	8.34 (2.89)	0.116	10.73 (2.32)	8.44 (3.07)	0.060
Visual-motor precision	9.44 (3.44)	11.85 (4.12)	0.028^a	10.11 (2.50)	9.64 (3.64)	0.407	9.04 (2.42)	10.94 (2.86)	0.017^a
Emotion recognition	8.44 (2.13)	9.69 (2.29)	0.038^a	9.37 (2.68)	8.42 (2.71)	0.052^a	10.36 (1.21)	8.94 (2.07)	0.952
Stratum 2 (Hb >130 g/L)									
	40 mg/d (n=8)	20 mg/d (n=7)		40 mg/d (n=39)	20 mg/d (n=38)		40 mg/d (n=9)	20 mg/d (n=12)	
WPPSI-IV									
Verbal Comprehension Index	106.88 (10.97)	97.00 (5.34)	0.090	105.14 (12.21)	107.10 (14.25)	0.521	110.67 (9.63)	105.27 (13.86)	0.337
Fluid Reasoning Index	103.13 (13.89)	105.60 (14.03)	0.761	103.05 (12.97)	104.68 (10.58)	0.550	104.55 (12.87)	96.11 (16.21)	0.210
Working Memory Index	92.25 (12.15)	98.60 (6.99)	0.315	97.16 (12.15)	98.22 (13.07)	0.720	97.11 (11.90)	100.70 (11.55)	0.054
Processing Speed Index	95.13 (12.41)	94.20 (13.92)	0.903	97.78 (10.72)	97.08 (12.90)	0.800	99.70 (11.00)	99.22 (12.47)	0.930
Full intelligence quotient	101.88 (9.88)	96.60 (8.26)	0.342	102.35 (10.95)	103.08 (10.28)	0.767	103.10 (12.89)	104.33 (10.87)	0.300
Vocabulary Acquisition Index	101.00 (14.68)	91.00 (10.56)	0.184	99.37 (14.20)	99.31 (12.87)	0.984	99.70 (12.37)	97.60 (15.48)	0.741
Block Design subtest	10.75 (1.83)	9.43 (1.72)	0.175	11.31 (1.88)	11.51 (2.42)	0.675	11.18 (2.12)	11.82 (2.36)	0.353
NEPSY-II									
Verbal fluency	10.50 (1.69)	8.50 (2.43)	0.093	8.69 (2.60)	9.50 (3.09)	0.041^c	9.50 (2.32)	10.27 (1.70)	0.065
Visual-motor precision	10.50 (3.82)	10.80 (3.90)	0.894	10.81 (3.67)	9.69 (2.46)	0.069	11.20 (2.47)	10.30 (3.27)	0.554
Emotion recognition	8.88 (2.95)	11.60 (1.52)	0.085	9.65 (2.35)	8.95 (2.58)	0.222	9.00 (2.40)	10.00 (2.87)	0.020^a

Note: Boldface indicates statistical significance ($p < 0.05$).

Data are expressed in mean (SD). Cohen's d for assessing effect size was indicated as described by the superscripted letters.

^aMedium effect size (>0.3 – 0.8).

^bLarge effect size (>0.8).

^cLow effect size (0.2 – 0.3).

NEPSY, Developmental Neuropsychological Assessment; SF, serum ferritin; WPPSI, Wechsler Preschool and Primary Scale of Intelligence.

Table 3. Adjusted Estimates of the Effect of Different Doses of Prenatal Iron Supplementation on Cognitive Functions at age 4 Years

Stratum 1 (0: 40 mg/d, 1: 80 mg/d)	SF <15 µg/L (n=29)			SF 15–65 µg/L (n=124)			SF >65 µg/L (n=29)		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WPPSI-IV									
Verbal Comprehension Index	12.06	10.37, 21.75	0.007	-2.98	-10.62, 4.66	0.426	-9.02	-9.13, -8.91	0.001
Fluid Reasoning Index	35.90	35.66, 36.13	<0.001	-10.89	-18.28, -3.51	0.006	-6.66	-15.96, 2.64	0.107
Working Memory Index	24.39	18.35, 30.62	0.003	-6.34	-17.27, 4.59	0.240	-17.55	-19.53, -15.56	0.001
Processing Speed Index	26.50	19.76, 28.41	0.013	-10.31	-18.94, -1.68	0.022	-3.48	-3.99, -2.97	0.007
Full intelligence quotient	27.38	25.96, 28.80	0.003	-7.79	-19.15, 3.56	0.168	-1.86	-2.72, 1.51	0.123
Vocabulary Acquisition Index	24.73	23.37, 26.09	0.003	-8.79	-19.15, 1.57	0.092	-7.69	-8.35, -7.02	0.004
Block Design subtest	2.86	0.60, 5.13	0.023	2.14	0.37, 3.90	0.021	-4.26	-4.92, -3.60	0.008
NEPSY-II									
Verbal fluency	1.81	1.31, 2.31	0.001	0.46	-1.89, 2.81	0.689	-0.93	-1.16, -0.69	0.013
Visual-motor precision	3.17	3.08, 3.26	<0.001	-1.04	-3.42, 1.34	0.370	2.67	1.93, 4.41	0.012
Emotion recognition	2.56	2.36, 2.77	<0.001	1.44	-3.68, 0.81	0.197	-0.75	-4.41, 2.91	0.599
Stratum 2 (0: 40 mg/d, 1: 20 mg/d)				(n=77)			(n=21)		
WPPSI-IV									
Verbal Comprehension Index	—	—	—	2.37	-5.90, 10.63	0.557	-3.83	-17.37, 9.71	0.434
Fluid Reasoning Index	—	—	—	5.36	-3.66, 14.38	0.230	-9.23	-24.64, 6.18	0.123
Working Memory Index	—	—	—	6.39	-4.20, 16.98	0.224	11.63	4.24, 22.83	0.047
Processing Speed Index	—	—	—	-3.65	-10.63, 3.33	0.289	9.51	-0.25, 16.59	0.095
Full IQ	—	—	—	4.72	-2.03, 11.47	0.160	3.64	2.98, 4.31	0.009
VAI	—	—	—	-4.94	-17.12, 7.23	0.405	-1.50	-12.68, 9.68	0.622
Block Design subtest	—	—	—	0.12	-0.77, 1.01	0.778	0.68	-5.67, 3.96	0.954
NEPSY-II									
Verbal fluency	—	—	—	1.99	0.06, 3.92	0.044	1.82	1.16, 2.49	0.018
Visual-motor precision	—	—	—	-2.51	-4.57, -0.47	0.019	-0.33	-1.21, 0.55	0.131
Emotion recognition	—	—	—	-1.89	-3.37, 0.42	0.104	4.19	3.76, 4.62	0.005

Note: Boldface indicates statistical significance ($p < 0.05$).

Models adjusted by maternal age, BMI in early pregnancy, parity (yes/no), pregnancy planning (yes/no), type of delivery (eutocic or dystocic), family SES (low, middle, high), smoking at recruitment (yes/no), maternal emotional status during pregnancy, postpartum depression, parental IQ approximation, *HFE* gene mutations (yes/no), maternal diet (energy, fiber, iron, PUFAs, calcium, vitamin C, vitamin B₁₂, folate), serum concentrations of Hb, vitamin D, PUFAs, and CRP at the first and third trimester of pregnancy; RBC, folate, and vitamin B₁₂ concentrations at the first trimester of pregnancy; SF concentrations at the third trimester of pregnancy; gestational age, child sex, head circumference at birth, and child diet at age 4 years (energy, iron, polyunsaturated fatty acids, vitamin B₁₂, folate). CRP, C-reactive protein; Hb, hemoglobin; IQ, intelligence quotient; NEPSY, Developmental Neuropsychological Assessment; PUFA, polyunsaturated fatty acid; RBC, red blood cell; SF, serum ferritin; VPI, Vocabulary Acquisition Index; WPPSI, Wechsler Preschool and Primary Scale of Intelligence.

supplementation, in which all women receive a single dose of iron, no clear evidence of benefit to offspring cognitive development has been found in previous studies.¹² At this point, it is worth noting the “U”-shaped risk for iron status, which suggests that both ID and iron excess have adverse health effects and would justify prescribing appropriate prenatal iron supplementation to avoid both situations.³² This may be one of the reasons that studies examining the effect of a single iron dose compared with that of no supplementation found no significant differences^{16,33} or even an adverse effect.¹³ The dose tested may be inadequate or too high, depending on the case.

All women in this study started pregnancy without anemia because this was an exclusion criterion for eligibility, but some of them had ID. This has been referred to as ID without anemia, which has recently emerged and suggests that even intermediate ID states are potentially harmful to health and should receive more attention in clinical practice.³⁴ Indeed, the differences found in the effect of prenatal iron doses (the usual dose of 40 mg/d in Spain versus a higher and a lower dose of 80 and 20 mg/d, respectively) on the neuropsychological functions of the children depended not only on the initial maternal Hb levels (normal: 110–130 g/L or normal-high: >130 g/L) but also on their iron stores at the beginning of pregnancy. Specifically, prenatal administration of high-dose iron (80 mg/d) in Stratum 1 women with ID in early pregnancy improved all neuropsychological functions and IQ in their children at age 4 years. This finding highlights that optimal iron status from early pregnancy, which in this case compensates for low iron stores through adequate supplementation, is essential for the child's cognitive and neuropsychological functions assessed on the WPPSI-IV. By contrast, prenatal administration of the commonly prescribed iron dose (40 mg/d) in women from Stratum 1 who began pregnancy with adequate iron stores resulted in improved indices of executive function in their children, including WMI and PSI from the WPPSI-IV. This indicates that not only is the prevention of ID important, but also the prevention of possible iron excess leads to better neurocognitive development of the child.

In support of this point, the results in Stratum 2 women with SF \geq 65 μ g/L showed better performance in WMI and IQ in children whose mothers had received low-dose iron supplementation (20 mg/d) prenatally, again indicating the need for prevention of iron excess. Another notable finding is that the children of women from Stratum 2 with SF \geq 65 μ g/L at baseline who

received low-dose iron prenatally showed significant improvement in the NEPSY-II emotion recognition index, an item related to some aspects of psychological problems such as autism spectrum disorders.³⁵ On the basis of this observation, it could be suggested that preventing iron excess by considering both women's initial Hb concentration and iron stores when adjusting the dose of iron supplements has positive effects on children.

It was expected that a high prenatal iron dose would have a positive effect on the development of the child if women started the pregnancy with ID. In contrast, the deleterious effect that high prenatal iron intake may have on the children of iron-repleted women has not been as clear. It can be hypothesized that this may be because women who had higher iron stores at the beginning of pregnancy are more likely to have excess iron, which in turn may impair fetal brain development by causing iron deposition in specific areas involved in some cognitive functions. Although this is a process that occurs naturally with aging and is usually associated with neurodegeneration, the results of some recent studies suggest that maternal iron overload during pregnancy may have a similar effect on the fetal brain, affecting its formation and development as well as the child's later cognitive functions.^{36,37} Given the physiology of iron during pregnancy and its high requirement, this does not mean that women who have good iron stores at the beginning of pregnancy do not need supplemental iron but that low iron doses are sufficient to provide benefits for infant cognitive development, as shown by the present results. These findings are of great importance for clinical practice because if iron stores in early pregnancy are not assessed by determining concentrations at SF, crucial data are lacking to adjust the dose of prenatal iron supplementation to the actual needs of individual women. Overall, they underscore the importance of considering not only the presence of anemia, as is the common practice, but also maternal iron stores when prescribing prenatal iron supplementation. To this end, physicians and midwives should consider beginning routine measurement of SF concentration beyond Hb early in pregnancy and even earlier as part of pregnancy planning programs.

Limitations

This study has several strengths worth mentioning, including (1) the original study design, which was a triple-blinded, population-based, RCT; (2) the comprehensive data collection from mothers, including sociodemographic, clinical, emotional, and lifestyle information; and (3) the comprehensive and detailed

assessment of children's neuropsychological functioning using internationally accepted and reliable tests. However, some limitations must also be considered when interpreting the results of this study. First, the high number of dropouts may have affected the statistical accuracy and impact of the intervention. Nonetheless, selection bias was accounted for by comparing the characteristics of participants included and not included in this analyses. Second, the sample size in the conducted analyses was small, which might weaken statistical power. Finally, there may be some residual confounding attributable to unmeasured or unknown risk factors after adjustment for known potential confounders.

CONCLUSIONS

Adjusting prenatal iron supplementation by considering maternal baseline Hb concentration and iron stores together, even in nonanemic women, can improve cognitive functioning in children aged 4 years. The best results in neuropsychological development of children aged 4 years were found in children of mothers who received a higher (80 mg/d) than usually prescribed iron dose when they had normal Hb concentrations at the beginning of pregnancy but ID, in infants of mothers who received the standard iron dose (40 mg/d) when they had normal Hb levels and no ID in early pregnancy, and in infants of mothers who received the lower than the usual iron dose (20 mg/d) when they had normal-high Hb concentrations and SF > 65 $\mu\text{g/L}$ at the beginning of pregnancy. The present results experimentally support that high-dose prenatal iron supplementation is beneficial for nonanemic women with low initial iron stores, whereas on the contrary, it seems to have a negative effect on women with full iron stores who would benefit from low doses. Given the great importance of ID even in intermediate phases, routine determination of SF concentration in addition to Hb level in early pregnancy would allow physicians to prescribe appropriate prenatal iron supplementation. Further studies with larger populations and greater statistical power would be useful to verify these findings.

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SUPPLEMENTAL MATERIAL

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