American Journal of
Preventive Medicine

RESEARCH ARTICLE

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

Lucía Iglesias-Vázquez, MSc,[1,2] Núria Voltas, PhD,[1,3,4] Carmen Hernández-Martínez, PhD,[1,3] Josefa Canals, PhD,[1,3] Pilar Coronel, MSc,[5] Mercedes Gimeno, MSc,[5] Josep Basora, PhD,[2,6,7] Victoria Arija, PhD[1,2,8]

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 μg/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 μg/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 μg/L.

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

From the [1]Nutrition and Mental Health (NUTRISAM) research group, Rovira i Virgili University, Reus, Spain; [2]Pere Virgili Institute of Health Research (IISPV), Reus, Spain; [3]Department of Psychology, Faculty of Education Sciences and Psychology, Research Centre for Behavioral Assessment (CRAMC), Rovira i Virgili University, Tarragona, Spain; [4]Serra Húnter Fellow, Department of Psychology, Faculty of Education Sciences and Psychology, Rovira i Virgili University, Tarragona, Spain; [5]Meiji Pharma Spain ES (formerly Tedec-Meiji Farma S.A), Alcalá de Henares, Madrid, Spain; [6]University Institute for Primary Health Care Research Foundation Jordi Gol i Gurina (IDIAPJGol), Barcelona, Spain; [7]CIBER Consortium, Physiopathology of Obesity and Nutrition (CIBERObn), Health Institute Carlos III (ISCIII), Madrid, Spain; and [8]Collaborative Research Group on Lifestyles, Nutrition, and Smoking (CENIT), Tarragona-Reus Research Support Unit, Primary Care Research Institute (IDIAP) Jordi Gol, Tarragona, Spain

Address correspondence to: Victoria Arija, PhD, Nutrition and Mental Health (NUTRISAM) research group, Rovira i Virgili University, C/ Sant Llorenç 21, 43201 Reus, Spain. E-mail: victoria.arija@urv.cat.

0749-3797/$36.00
https://doi.org/10.1016/j.amepre.2023.02.006

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 metabolism[8] 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)-IV[23] 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),

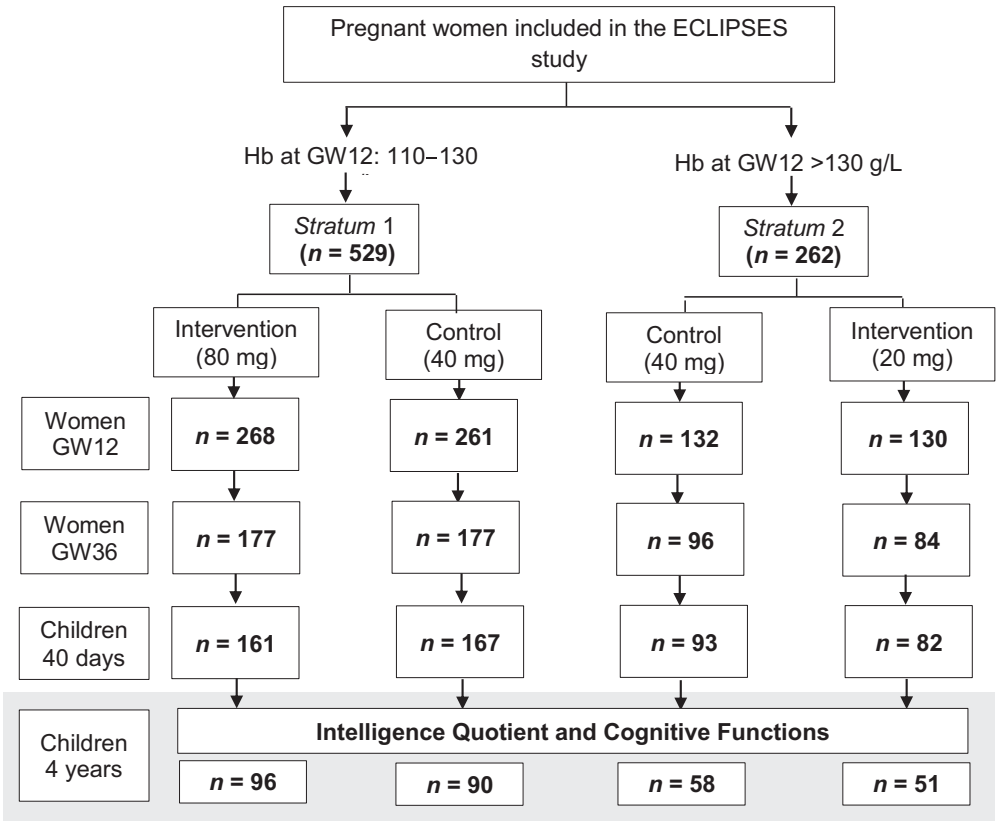

Figure 1. Flowchart of the study.

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 B_{12} 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 μg/L, ID; SF 15−65 μg/L, adequate iron stores; SF>65 μg/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 B_{12}, 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 B_{12} 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 B_{12}, 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 μg/L, whereas they scored lower on VCI, WMI, PSI, and VAI scales when their mothers' baseline SF levels were above 65 μg/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 μg/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 μg/L. By contrast, when mothers showed SF>65 μg/L at the beginning of gestation, a negative association was found with VCI (β= −9.02, 95% CI= −9.13, −8.91), WMI (β= −17.55, 95% CI= −19.53, −15.56), PSI (β= −3.48, 95% CI= −3.99, −2.97), and VAI (β= −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 (β=11.63, 95% CI=4.24, 22.83) and the full IQ (β=3.64, 95% CI=2.98, 4.31) when women started pregnancy with SF>65 μg/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.
[a]Measured by STAI, State-Trait Anxiety Inventory.
[b]Measured 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

	SF<15 μg/L			SF 15–65 μg/L			SF>65 μg/L		
Stratum 1 (Hb 110–130 g/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 (170)	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.
[a]Medium effect size (>0.3–0.8).
[b]Large effect size (>0.8).
[c]Low 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

	SF<15 μg/L (*n*=29)			SF 15–65 μg/L (*n*=124)			SF>65 μg/L (*n*=29)		
Stratum 1 (0: 40 mg/d, 1: 80 mg/d)	***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 B_{12}, folate), serum concentrations of Hb, vitamin D, PUFAs, and CRP at the first and third trimester of pregnancy; RBC, folate, and vitamin B_{12} 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 B_{12}, 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≥65 μg/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≥65 μg/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 μg/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.

ACKNOWLEDGMENTS

The authors thank the Jordi Gol Research Institute in Primary Care (Institut d'Investigació en Atenció Primària [IDIAP]) for their guidance regarding ethical matters. The authors thank to the entities and participants in the ECLIPSES study: Nutrition and Mental Health Research Group (NUTRISAM), Universitat Rovira i Virgili (Reus, Spain) (Victoria Arija, Josefa Canals, Lucía Iglesias-Vázquez, Cristina Bedmar, Cristina Jardí, Carmen Hernández-Martínez, Núria Voltas); Sexual and Reproductive Health Care Services of Tarragona, Spain (Francesc Fargas, Francisca Ruiz, Gemma March, Susana Abajo); the team of midwives recruited for the study (Irene Aguilar, Sònia Aguiles, Rosa Alzúria, Judit Bertrán, Carmen Burgos, Elisabet Bru, Montserrat Carreras, Beatriz Fernández, Carme Fonollosa, María Leiva, Demetria Patricio, Teresa Pinto, María Ramírez, Eusebia Romano, Inés Sombrero); the Research support Unit-Tarragona (Josep Basora, Meritxell Pallejà); the Central Unit-Barcelona (Rosa Morros) of the Institut d'Atenció Primària IDIAP Jordi Gol, Institut Català de la Salut; and the Laboratory of Institut Català de la Salut, University Hospital of Tarragona Joan XXIII (Tarragona, Spain) (Núria Serrat). The authors thank Pilar Coronel and Mercedes Gimeno of Meiji Pharma Spain S.A. for logistic assistance and for providing iron supplements free of charge. The authors also thank the Universitat Rovira i Virgili and the Diputación de Tarragona for their financial support through the predoctoral fellowship Martí Franquès 2020-PMF-PIPF-10. The ECLIPSES study was registered at www.clinicaltrialsregister.eu as EudraCT number 2012-005480-28 on May 21, 2013. The study was designed in agreement with the Declaration of Helsinki/Tokyo. All procedures involving human subjects were approved by the Clinical Research Ethics Committee of the Jordi Gol University Institute for Primary Care Research (IDIAP), the Pere Virgili Health Research Institute (Institut d'Investigació Sanitària Pere Virgili), and 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.

The work was supported by the Instituto de Salud Carlos III, Fondo de Investigación Sanitaria, Ministerio de Sanidad y Consumo (Grant Numbers PI12/02777 and PI17/01754) and cofunded by the European Union (ERDF/ESF, "A way to make Europe"/ "Investing in your future").

No financial disclosures were reported by the authors of this paper.

CREDIT AUTHOR STATEMENT

Lucía Iglesias-Vázquez: Formal analysis, Investigation, Visualization, Writing — original draft, Writing — review & editing. Núria Voltas: Investigation. Carmen Hernández-Martínez: Investigation. Josefa Canals: Conceptualization, Investigation, Methodology, Writing — review & editing. Pilar Coronel: Investigation, Resources. Mercedes Gimeno: Investigation, Resources. Victoria Arija: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing — review & editing.

SUPPLEMENTAL MATERIAL

Supplemental materials associated with this article can be found in the online version at https://doi.org/10.1016/j.amepre.2023.02.006.

REFERENCES

1. Cusick SE, Georgieff MK. The role of nutrition in brain development: the golden opportunity of the "first 1000 days". *J Pediatr.* 2016;175:16–21. https://doi.org/10.1016/J.JPEDS.2016.05.013.
2. Fitzgerald E, Hor K, Drake AJ. Maternal influences on fetal brain development: the role of nutrition, infection and stress, and the potential for intergenerational consequences. *Early Hum Dev.* 2020;150:105190. https://doi.org/10.1016/j.earlhumdev.2020.105190.
3. McCann S, Perapoch Amadó MP, Moore SE. The role of iron in brain development: a systematic review. *Nutrients.* 2020;12(7):2001. https://doi.org/10.3390/NU12072001.
4. Hernández-Martínez C, Canals J, Aranda N, Ribot B, Escribano J, Arija V. Effects of iron deficiency on neonatal behavior at different stages of pregnancy. *Early Hum Dev.* 2011;87(3):165–169. https://doi.org/10.1016/j.earlhumdev.2010.12.006.
5. Radlowski EC, Johnson RW. Perinatal iron deficiency and neurocognitive development. *Front Hum Neurosci.* 2013;7:585. https://doi.org/10.3389/fnhum.2013.00585.
6. Gaillard R, Eilers PHC, Yassine S, Hofman A, Steegers EAP, Jaddoe VW. Risk Factors and consequences of maternal anaemia and elevated haemoglobin levels during pregnancy: a population-based prospective cohort study. *Paediatr Perinat Epidemiol.* 2014;28(3):213–226. https://doi.org/10.1111/ppe.12112.
7. Markova V, Holm C, Pinborg AB, Thomsen LL, Moos T. Impairment of the developing human brain in iron deficiency: correlations to findings in experimental animals and prospects for early intervention therapy. *Pharmaceuticals (Basel).* 2019;12(3):120. https://doi.org/10.3390/ph12030120.
8. Beard J. Iron deficiency alters brain development and functioning. *J Nutr.* 2003;133(5 suppl 1):1468S–1472S. https://doi.org/10.1093/jn/133.5.1468S.
9. Greminger AR, Lee DL, Shrager P, Mayer-Pröschel M. Gestational iron deficiency differentially alters the structure and function of white and gray matter brain regions of developing rats. *J Nutr.* 2014;144(7):1058–1066. https://doi.org/10.3945/jn.113.187732.
10. Wu LL, Zhang L, Shao J, Qin YF, Yang RW, Zhao ZY. Effect of perinatal iron deficiency on myelination and associated behaviors in rat pups. *Behav Brain Res.* 2008;188(2):263–270. https://doi.org/10.1016/j.bbr.2007.11.003.
11. Wessling-Resnick M. Excess iron: considerations related to development and early growth. *Am J Clin Nutr.* 2017;106(suppl 6):1600S–1605S. https://doi.org/10.3945/ajcn.117.155879.
12. Jayasinghe C, Polson R, van Woerden HC, Wilson P. The effect of universal maternal antenatal iron supplementation on neurodevelopment in offspring: a systematic review and meta-analysis. *BMC Pediatr.* 2018;18(1):150. https://doi.org/10.1186/s12887-018-1118-7.
13. Parsons AG, Zhou SJ, Spurrier NJ, Makrides M. Effect of iron supplementation during pregnancy on the behaviour of children at early school age: long-term follow-up of a randomised controlled trial. *Br J Nutr.* 2008;99(5):1133–1139. https://doi.org/10.1017/S0007114507853359.
14. Zhou SJ, Gibson RA, Crowther CA, Baghurst P, Makrides M. Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial. *Am J Clin Nutr.* 2006;83(5):1112–1117. https://doi.org/10.1093/ajcn/83.5.1112.
15. Prado EL, Alcock KJ, Muadz H, Ullman MT, Shankar AH, SUMMIT Study Group. Maternal multiple micronutrient supplements and child cognition: a randomized trial in Indonesia. *Pediatrics.* 2012;130(3):e536–e546. https://doi.org/10.1542/peds.2012-0412.
16. Hanieh S, Ha TT, Simpson JA, et al. The effect of intermittent antenatal iron supplementation on maternal and infant outcomes in rural Viet Nam: a cluster randomised trial. *PLoS Med.* 2013;10(6):e1001470. https://doi.org/10.1371/journal.pmed.1001470.
17. Li Q, Yan H, Zeng L, et al. Effects of maternal multimicronutrient supplementation on the mental development of infants in rural western China: follow-up evaluation of a double-blind, randomized, controlled trial. *Pediatrics.* 2009;123(4):e685–e692. https://doi.org/10.1542/peds.2008-3007.
18. Quezada-Pinedo HG, Cassel F, Muckenthaler MU, et al. Ethnic differences in adverse iron status in early pregnancy: a cross-sectional population-based study. *J Nutr Sci.* 2022;11:e39. https://doi.org/10.1017/jns.2022.35.
19. Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. *Am J Epidemiol.* 2001;154(3):193–206. https://doi.org/10.1093/aje/154.3.193.
20. Rasmussen S, Bergsjø P, Jacobsen G, Haram K, Bakketeig LS. Haemoglobin and serum ferritin in pregnancy - Correlation with smoking and body mass index. *Eur J Obstet Gynecol Reprod Biol.* 2005;123(1):27–34. https://doi.org/10.1016/j.ejogrb.2005.02.012.
21. Beard JL. Effectiveness and strategies of iron supplementation during pregnancy. *Am J Clin Nutr.* 2000;71(5):1288S–1294S suppl. https://doi.org/10.1093/ajcn/71.5.1288s.
22. Iglesias Vázquez L, Arija V, Aranda N, et al. The effectiveness of different doses of iron supplementation and the prenatal determinants of maternal iron status in pregnant Spanish women: ECLIPSES study. *Nutrients.* 2019;11(10):2418. https://doi.org/10.3390/nu11102418.
23. Wechsler D. WPPSI-IV. Escala de Inteligencia de Wechsler Para Preescolar y Primaria (4a Edición). London, United Kingdom: Pearson. https://www.pearsonclinical.es/wppsi-iv-escala-de-inteligencia-de-wechsler-para-preescolar-y-primaria. Published 2014. Accessed March 1, 2023.
24. Korkman M, Kirk U, Kemp S. NEPSY-second edition (NEPSY-II). *J Psychoeduc Assess.* 2007;28(2):175–182. https://doi.org/10.1177/0734282909346716.
25. Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. Validation of a short questionnaire on frequency of dietary intake: reproducibility and validity. *Nutr Hosp.* 2008;23(3):242–252. http://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0212-16112008000300011.
26. Aparicio E, Jardí C, Bedmar C, et al. Nutrient intake during pregnancy and post-partum: ECLIPSES study. *Nutrients.* 2020;12(5):1325. https://doi.org/10.3390/NU12051325.
27. Wechsler D. *Wechsler Adult Intelligence Scale—fourth edition (WAIS-IV).* Apa PsycTests; 2008. https://www.pearsonassessments.com/store/usassessments/en/Store/Professional-Assessments/Cognition-%26-Neuro/Wechsler-Adult-Intelligence-Scale-%7C-Fourth-Edition/p/100000392.html. Accessed March 1, 2023.
28. Voltas N, Canals J, Hernández-Martínez C, Serrat N, Basora J, Arija V. Effect of vitamin D status during pregnancy on infant neurodevelopment: the eclipses study. *Nutrients.* 2020;12(10):3196. https://doi.org/10.3390/nu12103196.
29. Zou R, el Marroun H, Voortman T, Hillegers M, White T, Tiemeier H. Maternal polyunsaturated fatty acids during pregnancy and offspring brain development in childhood. *Am J Clin Nutr.* 2021;114(1):124–133. https://doi.org/10.1093/ajcn/nqab049.
30. Aparicio E, Martín-Grau C, Hernández-Martinez C, Voltas N, Canals J, Arija V. Changes in fatty acid levels (saturated, monounsaturated and polyunsaturated) during pregnancy. *BMC Pregnancy Childbirth.* 2021;21(1):778. https://doi.org/10.1186/s12884-021-04251-0.
31. Díaz-López A, Jardí C, Villalobos M, Serrat N, Basora J, Arija V. Prevalence and risk factors of hypovitaminosis D in pregnant Spanish women. *Sci Rep.* 2020;10(1):15757. https://doi.org/10.1038/s41598-020-71980-1.
32. Brannon PM, Taylor CL. Iron supplementation during pregnancy and infancy: uncertainties and implications for research and policy. *Nutrients.* 2017;9(12):1327. https://doi.org/10.3390/nu9121327.
33. Angulo-Barroso RM, Li M, Santos DCC, et al. Iron supplementation in pregnancy or infancy and motor development: a randomized controlled trial. *Pediatrics.* 2016;137(4):e20153547. https://doi.org/10.1542/peds.2015-3547.

34. Al-Naseem A, Sallam A, Choudhury S, Thachil J. Iron deficiency without anaemia: a diagnosis that matters. *Clin Med (Lond).* 2021;21(2):107–113. https://doi.org/10.7861/CLINMED.2020-0582.
35. Yeung MK. A systematic review and meta-analysis of facial emotion recognition in autism spectrum disorder: the specificity of deficits and the role of task characteristics. *Neurosci Biobehav Rev.* 2022;133:104518. https://doi.org/10.1016/j.neubiorev.2021.104518.
36. Zerem A, Ben-Sira L, Vigdorovich N, et al. White matter abnormalities and iron deposition in prenatal mucolipidosis IV- fetal imaging and pathology. *Metab Brain Dis.* 2021;36(7):2155–2167. https://doi.org/10.1007/s11011-021-00742-3.
37. Lavezzi AM, Mohorovic L, Alfonsi G, Corna MF, Matturri L. Brain iron accumulation in unexplained fetal and infant death victims with smoker mothers-The possible involvement of maternal methemoglobinemia. *BMC Pediatr.* 2011;11(1):62. https://doi.org/10.1186/1471-2431-11-62.