

Maternal Iron Status in Early Pregnancy and Blood Pressure Throughout Pregnancy, Placental Hemodynamics, and the Risk of Gestational Hypertensive Disorders

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ABSTRACT

Background: In nonpregnant populations, higher serum ferritin, which reflects high iron stores, is associated with an increased risk of hypertension. We hypothesized that a dysregulated maternal iron status in early pregnancy may lead to impaired gestational hemodynamic adaptations, leading to an increased risk of gestational hypertensive disorders.

Objectives: We examined the associations of maternal iron status with maternal blood pressure, placental hemodynamic parameters, and the risks of gestational hypertensive disorders.

Methods: In a population-based prospective cohort study among 5983 pregnant women, we measured maternal serum ferritin, transferrin saturation, serum iron, and transferrin concentrations at a median of 13.2 weeks gestation (95% range, 9.6–17.6). Maternal blood pressure was measured in early pregnancy, mid pregnancy, and late pregnancy, and placental hemodynamic parameters in mid pregnancy and late pregnancy were measured by ultrasound. Information on gestational hypertensive disorders was collected from medical records. We examined the associations of maternal early pregnancy iron status with maternal systolic and diastolic blood pressure, placental hemodynamic parameters, and the risks of gestational hypertensive disorders using linear and logistic regression models.

Results: Higher maternal early pregnancy serum ferritin concentrations were associated with higher systolic and diastolic blood pressure throughout pregnancy in the basic models (P values < 0.05). After adjustment for maternal inflammation, sociodemographic and lifestyle factors, higher maternal early pregnancy serum ferritin concentrations were only associated with a higher early pregnancy diastolic blood pressure [0.27 (95% CI, 0.03–0.51) mmHg per SD score increase in serum ferritin] and with a higher mid pregnancy umbilical artery pulsatility index ($P < 0.05$). No associations with the risk of gestational hypertensive disorders were present.

Conclusions: No consistent associations were present of maternal iron status in early pregnancy with gestational hemodynamic adaptations or the risks of gestational hypertensive disorders. Further studies are needed to examine the potential role of iron metabolism in the development of gestational hypertensive disorders within higher-risk populations.

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Keywords: pregnancy, iron, serum ferritin, placental hemodynamics, gestational hypertensive disorders

Introduction

Gestational hypertensive disorders, which include gestational hypertension and preeclampsia, are a leading cause of maternal and neonatal morbidity and mortality (1). Gestational hypertension is characterized by the late onset of hypertension in pregnancy in previously normotensive women, while preeclampsia is defined as gestational hypertension with the presence of high protein levels in the urine (2). Both high and low maternal hemoglobin concentrations in early pregnancy

have been associated with elevated blood pressure levels during pregnancy, impaired placental function, and a higher risk of gestational hypertensive disorders (3–6). The underlying pathophysiological mechanisms for these associations are unclear, but it has been hypothesized that a dysregulated iron status may play a role.

A dysregulated iron status can cause oxidative stress. Iron overload leads to more production of reactive oxygen species (ROS), whereas iron deficiency can cause leakage of ROS

through mitochondrial damage (7, 8). Oxidative stress leads to endothelial damage and impaired vasoreactivity, which may negatively affect placental development and gestational hemodynamic adaptations, predisposing women to the development of gestational hypertensive disorders (9–13). Already in nonpregnant populations, increased serum ferritin concentrations, which reflect high iron stores, have been associated with the risk of hypertension, increased arterial stiffness, and a higher risk of cardiovascular disease (14–24). In pregnant populations, far less is known about the influence of maternal iron status in early pregnancy on gestational hemodynamic adaptations and the risk of gestational hypertensive disorders (25–29). Two observational studies among 484 healthy Polish pregnant women and 57 healthy nulliparous American women reported that lower serum iron concentrations at 12 weeks gestation were associated with a higher risk of gestational hypertensive disorders (25, 26). In contrast, an observational study among 47 pregnant women with diabetes mellitus reported no association of serum iron concentrations at 12 weeks gestation with the risk of preeclampsia (27). On the contrary, iron supplementation in early pregnancy has been associated with a higher risk of gestational hypertensive disorders (28, 29).

We hypothesized that both decreased and increased maternal iron store concentrations are associated with higher maternal blood pressure and impaired placental vascular function throughout pregnancy, leading to a higher risk of gestational hypertensive disorders. Therefore, we examined the associations of maternal early pregnancy iron status with maternal systolic and diastolic blood pressure during pregnancy, placental hemodynamic parameters, and the risks of gestational hypertensive disorders within a population-based cohort of 5983 multi-ethnic pregnant women.

Methods

Study design and study sample

This study was embedded in the Generation R Study, a prospective, population-based cohort study from early pregnancy onwards in Rotterdam, the Netherlands (30). Written informed consent was obtained for all participants. The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center

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None of the supporting sources had any involvement in the design, implementation, analysis or interpretation of the study.

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Supplemental Tables 1–20 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ijn/>.

Abbreviations used: CRP, C-reactive protein; ROS, reactive oxygen species; SDS, standard deviation scores; TSAT, transferrin saturation; UmPI, umbilical artery pulsatility index; UtRI, uterine artery resistance index.

Rotterdam (MEC 198.782/2001/31). In total, 7069 women enrolled in early pregnancy. Data on maternal iron markers in early pregnancy was available for 6159 women. We excluded women with preexistent hypertension ($n = 107$), multiple pregnancies ($n = 68$), and absent outcome data ($n = 1$). The total population for analysis consisted of 5983 women (Figure 1).

Maternal iron status

Serum ferritin, serum iron, and transferrin concentrations were measured from maternal nonfasting venous blood samples that were collected during early pregnancy (median = 13.2 weeks gestation; 95% range, 9.6–17.6) (31). Ferritin was measured by electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche). Iron was measured by colorimetric assay and transferrin by immunoturbidimetric assay, both by the C502 module on the Cobas 8000 (Roche) (32). As serum ferritin is considered the gold standard to assess iron stores, we used serum ferritin concentrations as our primary exposure (33). To obtain further insight into maternal iron status, we additionally used transferrin saturation (TSAT), serum iron, and transferrin as secondary exposures. These measures provide additional information on the bioavailability of iron in the body. TSAT was calculated using serum iron and transferrin concentrations [TSAT = (serum iron \times 100)/(transferrin \times 25.1)] to reflect the iron-bound part of the total iron binding capacity.

We constructed quintiles of all iron markers to assess whether associations were restricted to lower or higher iron stores and to explore potential nonlinear effects. We also constructed standard deviation scores (SDS) of all iron markers to assess the continuous associations across the full range. Serum ferritin was log-transformed prior to the construction of the SDS due to its skewed distribution.

Gestational hemodynamic adaptations

Systolic and diastolic blood pressure were measured in early pregnancy (median = 13.2 weeks gestation; 95% range, 9.6–17.6), mid pregnancy (median = 20.5 weeks gestation; 95% range, 18.7–23.1), and late pregnancy (median = 30.4 weeks gestation; 95% range, 28.6–32.8). An OMRON 907 automated digital oscillometric sphygmomanometer (OMRON Healthcare Europe BV) was used to perform the blood pressure measurements, as previously described (34, 35). Two blood pressure measurements were performed at a 60-second interval, from which the mean blood pressure was calculated (34, 35).

Ultrasounds were performed in mid pregnancy (median = 20.5 weeks gestation; 95% range, 18.7–23.1 weeks) and late pregnancy (median = 30.4 weeks gestation; 95% range, 28.6–32.8 weeks). Mid pregnancy and late pregnancy placental vascular resistance were evaluated with recorded flow velocity waveforms from the uterine and umbilical arteries (36). The umbilical artery pulsatility index (UmPI) was measured in a free-floating loop of the umbilical cord. The uterine artery resistance index (UtRI) was measured in the uterine arteries near the crossover with the external iliac artery. Higher UmPI and UtRI values indicate increased placental vascular resistance. The presence of uterine artery notching was assessed. The presence of uterine artery notching reflects an increase in resistance to blood flowing into the placenta and is used for identification of high-risk pregnancies.

Gestational hypertensive disorders

The gestational hypertensive disorder status was obtained from medical records, which were cross-checked with the original hospital charts (37). Gestational hypertension was defined as a diastolic blood pressure of at least 90 mmHg and/or systolic blood pressure of at least 140 mmHg after 20 weeks of gestation in women without previous hypertension. Preeclampsia was defined as gestational hypertension, including the presence of proteinuria (defined as a 24-hour urine sample containing at least 300 mg of protein, 1 catheter sample reading of at least 1+, or 2 or more dipstick readings of at least 2+) (2).

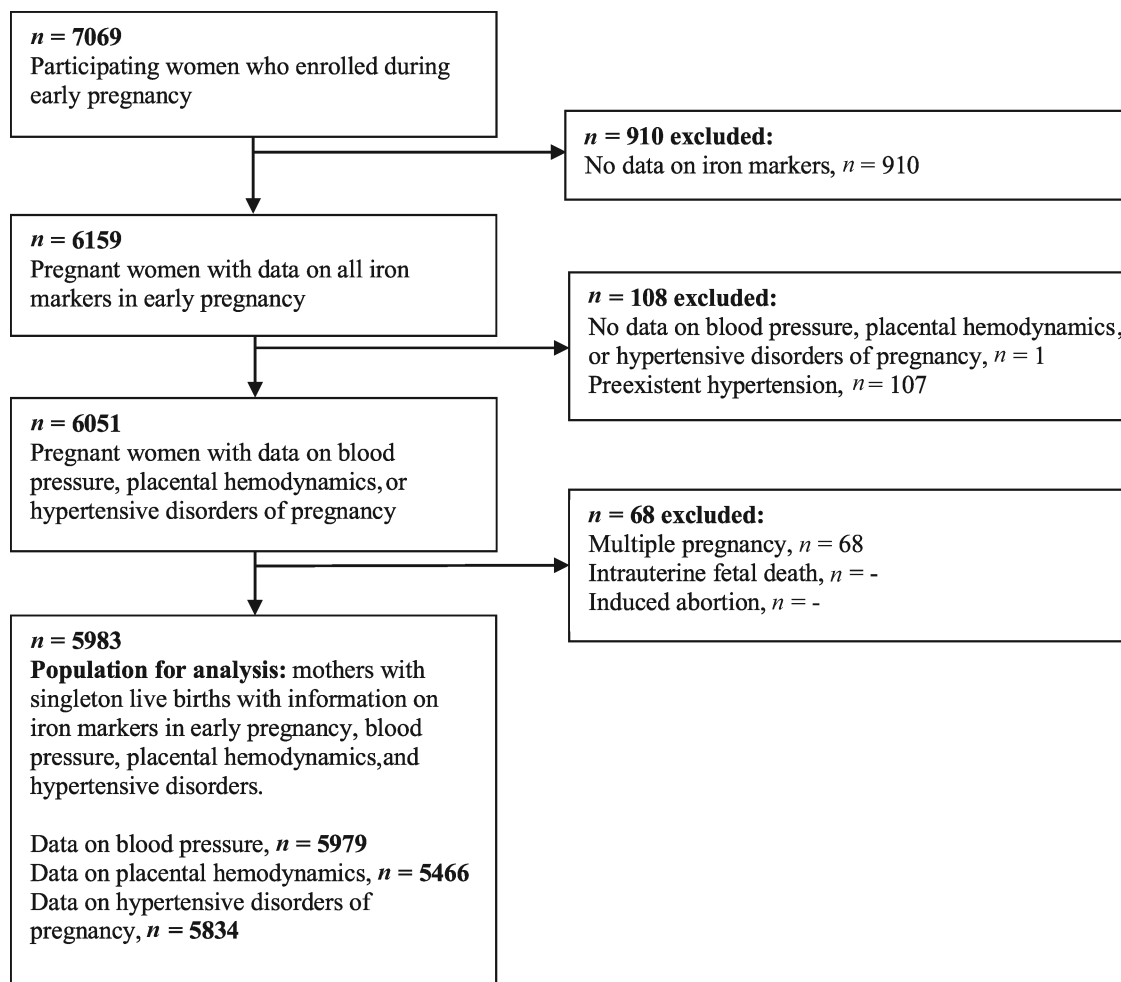


FIGURE 1 Flowchart of the study population.

Covariates

Information on maternal age, ethnicity, prepregnancy weight, educational level, parity, folic acid supplementation, and maternal smoking was obtained from questionnaires. Ethnicity was categorized as European and non-European. European ethnicity included Dutch and other European ethnicities. Non-European ethnicity included Indonesian, Cape Verdian, Moroccan, Antillean, Surinamese, Turkish, African, American (Western and non-Western), Asian (Western and non-Western), and Oceanian ethnicities. Height was measured at the intake visit and was used to calculate the prepregnancy BMI. Maternal hemoglobin and C-reactive protein (CRP) concentrations were measured in the same nonfasting venous blood samples that were used for the measurement of the iron markers, as described previously (38).

Statistical analyses

First, we performed a nonresponse analysis comparing women with information on early pregnancy maternal iron markers to those without. Second, we used 1-way ANOVAs or chi-square tests to compare the participant characteristics across serum ferritin quartiles. Third, we examined the associations of maternal serum ferritin in categories (quintiles) and serum ferritin continuously (SDS) with systolic and diastolic blood pressure in early pregnancy, mid pregnancy, and late pregnancy; and UmPI and UtrI in mid pregnancy and late pregnancy using linear regression models. We examined the associations of maternal serum ferritin quintiles and SDS with the risk of bilateral uterine artery notching using logistic regression models. Fourth, we examined the associations of serum ferritin quintiles and SDS with the risk of gestational hypertensive disorders using logistic regression

analyses. We constructed 2 different models: 1) a basic model that was adjusted for gestational age at the time of blood sampling and gestational age at the time of blood pressure measurement or ultrasound; and 2) a confounder model that was additionally adjusted for maternal sociodemographic factors, lifestyle factors, and inflammation, including maternal age, ethnicity, educational level, parity, prepregnancy BMI, folic acid supplementation, smoking, and CRP concentrations. Confounders were based on the literature and selected if they were associated with serum ferritin and the outcomes of interest or if they led to a change in the effect estimate of >10% when the covariate was included in the univariate model. As the effects of an impaired maternal iron status may be more pronounced among pregnant women who also have abnormal hemoglobin levels, we calculated the interaction terms of maternal serum ferritin with maternal hemoglobin for each outcome. Only the interaction terms for maternal systolic and diastolic blood pressure were significant, and we repeated these analyses with stratification by maternal hemoglobin levels, defined as low (≤ 11 g/dl), normal (> 11 g/dl and < 13.2 g/dl), and high (≥ 13.2 g/dl) (3).

Next, we performed several sensitivity analyses to examine the robustness of our findings. First, we repeated the analyses using maternal early pregnancy TSAT, serum iron, and transferrin concentrations as secondary exposures that further reflect the bioavailability of maternal iron. Second, as higher serum ferritin concentrations may be explained by acute inflammation, we performed a sensitivity analysis excluding all women with CRP concentrations > 10 mg/ml, who may suffer from acute inflammation, while still additionally adjusting for CRP concentrations across the full range to consider the impact of chronic low-grade inflammation. Third, we repeated the analyses with maternal serum ferritin concentrations, additionally adjusted for transferrin

concentrations, as physiological changes in transferrin concentrations occur to regulate the bioavailability of the iron stores (39).

We imputed missing data of the covariates using multiple imputation. The percentage of missing values was <10%, with the exception for smoking (11%), prepregnancy BMI (18%), and folic acid supplementation (24%). *P* values < 0.05 were considered significant. Analyses were performed using Statistical Package of Social Science version 25.0 for Windows (SPSS Inc).

Results

Participant characteristics

Table 1 shows the participant characteristics. Participating women were 30 years old on average, with a mean prepregnancy BMI of 23.5 kg/m²; 40% had higher education, and 57% were nulliparous. The median serum ferritin concentration was 52.2 µg/L (95% range, 9.9–203.7). Iron deficiency was observed in 7.2% (serum ferritin < 15 µg/L) and iron overload was observed in 6.7% (serum ferritin > 150 µg/L) of women (40). No differences in maternal blood pressure, placental hemodynamic parameters, or gestational hypertensive disorders were present for women with data on early pregnancy iron markers compared to those without (**Supplementary Table S1**).

Early pregnancy serum ferritin concentrations and blood pressure throughout pregnancy

In the basic models, higher serum ferritin concentrations across the full range were associated with higher systolic and diastolic blood pressure throughout pregnancy (all *P* values < 0.05; **Supplementary Table S2**). After adjustment for maternal sociodemographic factors, lifestyle factors, and inflammation, no associations of serum ferritin concentrations in quintiles or across the full range with systolic blood pressure throughout pregnancy were present (**Table 2**). Higher serum ferritin concentrations across the full range were only associated with higher early pregnancy diastolic blood pressure [0.27 (95% CI, 0.03–0.51) mmHg per SDS increase in serum ferritin], but not with mid pregnancy or late pregnancy diastolic blood pressure. No associations were present for the serum ferritin quintiles with diastolic blood pressure. Analyses stratified for maternal hemoglobin concentrations showed that the strongest effect for serum ferritin concentrations across the full range with early pregnancy diastolic blood pressure was present for women with high hemoglobin concentrations (*P* value < 0.05; **Supplementary Table S3**).

Early pregnancy serum ferritin concentrations and placental hemodynamic parameters

In the confounder model, as compared to the third serum ferritin quintile, the lowest serum ferritin quintile was associated with a lower midpregnancy UmPI, whereas the highest serum ferritin quintile was associated with a higher mid pregnancy UmPI (all *P* values < 0.05; **Table 3**). Higher serum ferritin concentrations across the full range were also associated with a higher mid pregnancy UmPI [0.010 (95% CI, 0.005–0.016) per SDS increase in serum ferritin]. No associations were present with the UtRI or the risk of bilateral uterine artery notching. In the basic models, similar findings were present (**Supplementary Table S4**).

Early pregnancy serum ferritin concentrations and risks of gestational hypertensive disorders

Table 4 shows that serum ferritin quintiles and serum ferritin concentrations across the full range were not associated with

the risks of any gestational hypertensive disorder in the confounder model. In the basic model, the higher serum ferritin quintiles, as compared to the third quintile, and serum ferritin concentrations across the full range were associated with a higher risk of gestational hypertensive disorders and gestational hypertension, but not preeclampsia (all *P* values < 0.05; **Supplementary Table S5**).

Sensitivity analyses

A higher TSAT was associated with a lower systolic and diastolic blood pressure in early pregnancy (*P* values < 0.05), but not in mid pregnancy or late pregnancy, in the confounder model (**Supplementary Table S6**). Higher serum iron concentrations were associated with a lower systolic blood pressure in early pregnancy (*P* value < 0.05), but not in mid pregnancy or late pregnancy, nor with diastolic blood pressure throughout pregnancy, in the confounder model (**Supplementary Table S7**). Higher transferrin concentrations were associated with higher systolic and diastolic blood pressure throughout pregnancy (*P* values < 0.05) in the confounder model (**Supplementary Table S8**). No consistent associations were found for TSAT, serum iron, and transferrin concentrations with placental hemodynamic parameters or the risks of any gestational hypertensive disorder (**Supplementary Tables S9–S14**). When we excluded women with acute inflammation (CRP > 10mg/L), we found stronger associations for serum ferritin concentrations across the full range with diastolic blood pressure throughout pregnancy as compared to the main analysis for serum ferritin (all *P* values < 0.05; **Supplementary Tables S15–S17**). We observed that higher serum ferritin concentrations across the full range were associated with higher systolic and diastolic blood pressure throughout pregnancy (all *P* values < 0.05) when we additionally adjusted the confounder model for transferrin concentrations, but no associations were present for placental hemodynamic parameters or the risks of gestational hypertensive disorders (**Supplementary Tables S18–S20**).

Discussion

In this population-based, prospective cohort study, we found no consistent associations of maternal early pregnancy iron status with maternal blood pressure and placental vascular function throughout pregnancy or the risks of gestational hypertensive disorders after considering maternal inflammatory, sociodemographic, and lifestyle factors.

Gestational hypertensive disorders are a leading cause of maternal and neonatal morbidity and mortality (1). Increased oxidative stress has been suggested to play a role in impaired placental development and the pathophysiology of gestational hypertensive disorders (41). Both iron overload and iron deficiency can induce increased levels of oxidative stress (7, 8). High levels of oxidative stress can lead to endothelial damage and impaired vasoreactivity, which may negatively affect placental development and gestational hemodynamic adaptations, predisposing women to the development of gestational hypertensive disorders (9–13). We hypothesized that both higher and lower serum ferritin concentrations in early pregnancy might lead to higher maternal blood pressure, impaired placental hemodynamics, and an increased risk of gestational hypertensive disorders.

Already in nonpregnant populations, it has been suggested that higher serum ferritin concentrations are associated with

TABLE 1 Characteristics of the study population by early pregnancy serum ferritin quintiles ($n = 5983$)¹

	Total group $n = 5983$	Serum ferritin quintile 1 $n = 1196$	Serum ferritin quintile 2 $n = 1197$	Serum ferritin quintile 3 $n = 1196$	Serum ferritin quintile 4 $n = 1198$	Serum ferritin quintile 5 $n = 1196$	<i>P</i> value ²
Serum ferritin, range, $\mu\text{g/L}$	2–390	2–26	26–42	42–63	63–96	96–390	
Serum ferritin, $\mu\text{g/L}$	52.2 [9.9, 203.7]	17.3 [6.7, 25.9]	34.2 [26.8, 42.1]	52.2 [42.8, 62.4]	76.2 [63.3, 94.2]	128.4 [96.5, 295.7]	<0.001
TSAT, %	24.5 \pm 10.5	18.4 \pm 8.9	24.0 \pm 8.9	26.1 \pm 9.9	26.3 \pm 10.4	28.0 \pm 11.7	<0.001
Iron, $\mu\text{mol/L}$	17.1 \pm 6.6	14.7 \pm 6.5	17.5 \pm 6.0	17.9 \pm 6.3	17.6 \pm 6.5	18.0 \pm 7.0	<0.001
Transferrin, g/L	2.9 \pm 0.5	3.3 \pm 0.5	3.0 \pm 0.4	2.8 \pm 0.4	2.7 \pm 0.4	2.6 \pm 0.3	<0.001
Hemoglobin, g/dL	12.3 \pm 0.9	12.0 \pm 1.0	12.3 \pm 0.9	12.4 \pm 0.9	12.4 \pm 0.9	12.4 \pm 0.9	<0.001
CRP, mg/L	4.5 [0.6, 25.7]	4.8 [0.7, 23.6]	4.4 [0.6, 22.1]	4.4 [0.7, 24.4]	4.3 [0.6, 29.1]	4.4 [0.5, 32.8]	<0.001
Maternal age at enrollment, years	29.7 \pm 5.1	29.0 \pm 5.6	29.4 \pm 5.3	29.8 \pm 5.0	30.1 \pm 4.8	30.5 \pm 4.4	<0.001
Parity, nulliparous, n (%)	3399 (56.8)	481 (40.2)	602 (50.3)	682 (57.0)	764 (63.8)	870 (72.7)	<0.001
Prepregnancy BMI, kg/m ²	23.5 \pm 4.2	23.5 \pm 4.1	23.3 \pm 4.2	23.3 \pm 4.0	23.6 \pm 4.2	23.9 \pm 4.4	<0.001
Gestational age at blood sampling, weeks	13.2 [9.6, 17.6]	13.9 [7.6, 17.9]	13.5 [9.5, 17.7]	13.2 [9.6, 17.4]	12.9 [9.5, 17.4]	12.8 [9.5, 17.1]	<0.001
Higher education, n (%)	2501 (41.8)	369 (30.9)	467 (39.0)	533 (44.6)	546 (45.6)	586 (49.0)	<0.001
European ethnicity, ³ n (%)	3500 (61.0)	470 (39.3)	668 (55.8)	740 (61.9)	797 (66.5)	825 (69.0)	<0.001
Continued smoking during pregnancy, n (%)	990 (16.5)	188 (15.7)	228 (19.0)	212 (17.7)	195 (16.3)	167 (14.0)	<0.05
Folic acid supplement use, n (%)	3413 (57.0)	532 (44.5)	634 (53.0)	703 (58.8)	755 (63.0)	789 (66.0)	<0.001
Systolic blood pressure, mmHg							
Early pregnancy	115.3 \pm 12.0	113.5 \pm 11.9	114.8 \pm 11.8	115.1 \pm 11.8	115.9 \pm 12.0	116.9 \pm 12.3	<0.001
Mid pregnancy	116.9 \pm 11.8	115.5 \pm 11.6	116.1 \pm 11.6	116.9 \pm 11.5	117.2 \pm 12.1	118.9 \pm 11.9	<0.001
Late pregnancy	118.3 \pm 11.8	116.5 \pm 11.6	117.3 \pm 11.4	118.6 \pm 12.2	119.0 \pm 12.0	119.7 \pm 11.8	<0.001
Diastolic blood pressure, mmHg							
Early pregnancy	67.9 \pm 9.3	66.8 \pm 9.2	67.2 \pm 9.0	67.7 \pm 9.2	68.6 \pm 9.4	69.4 \pm 9.4	<0.001
Mid pregnancy	67.0 \pm 9.2	66.2 \pm 9.3	66.4 \pm 8.6	66.6 \pm 9.1	67.4 \pm 9.5	68.5 \pm 9.3	<0.001
Late pregnancy	68.9 \pm 9.2	67.7 \pm 9.5	68.2 \pm 8.7	68.6 \pm 9.2	69.6 \pm 9.2	70.3 \pm 9.0	<0.001
Uterine artery resistance index							
Mid pregnancy	0.54 \pm 0.09	0.54 \pm 0.08	0.54 \pm 0.09	0.54 \pm 0.09	0.55 \pm 0.09	0.53 \pm 0.09	0.08
Late pregnancy	0.48 \pm 0.08	0.49 \pm 0.08	0.49 \pm 0.08	0.48 \pm 0.08	0.48 \pm 0.08	0.48 \pm 0.08	0.26
Umbilical artery pulsatility index							
Mid pregnancy	1.20 \pm 0.18	1.18 \pm 0.18	1.20 \pm 0.18	1.20 \pm 0.18	1.21 \pm 0.18	1.22 \pm 0.18	<0.05
Late pregnancy	0.98 \pm 0.17	0.98 \pm 0.17	0.98 \pm 0.17	0.98 \pm 0.17	0.98 \pm 0.17	0.98 \pm 0.17	0.94
Bilateral uterine artery notching	108 \pm 1.8	19 \pm 1.6	25 \pm 2.1	22 \pm 1.8	20 \pm 1.7	22 \pm 1.8	0.90
Gestational hypertensive disorders							
Preeclampsia, n (%)	123 (2.1)	20 (1.7)	32 (2.7)	25 (2.1)	20 (1.7)	26 (2.2)	0.38
Gestational hypertension, n (%)	228 (3.8)	26 (2.2)	35 (2.9)	44 (3.7)	47 (3.9)	76 (6.4)	<0.05

¹Values are shown as the mean \pm SD, median [95% range], or count (%). CRP, C-reactive protein; TSAT, transferrin saturation.

²Differences between characteristics in serum ferritin quintiles were tested with a 1-way ANOVA for continuous variables and χ^2 test for categorical variables.

³In the full study population, European ethnicity consisted of 52.5% Dutch and 8.5% other European ethnicities. Non-European ethnicity (39.0%) consisted of 8.5% Surinamese, 8.1% Turkish, 6.0% Moroccan, 4.2% Cape Verdian, 3.0% Indonesian, 2.9% Antillean, 2.7% Asian, 1.9% African, and 0.1% Oceanian.

TABLE 2 Associations of early pregnancy serum ferritin with systolic blood pressure and diastolic blood pressure during pregnancy (*n* = 5979)¹

Early pregnancy serum ferritin	Early pregnancy		Mid pregnancy		Late pregnancy	
	β (95% CI)	<i>n</i>	β (95% CI)	<i>n</i>	β (95% CI)	<i>n</i>
Differences in systolic blood pressure (mmHg)						
Quintile 1 ² , 2–26 $\mu\text{g/L}$	-0.07 (-0.99 to 0.85)	1189	-0.09 (-1.02 to 0.83)	1121	-0.64 (-1.59 to 0.31)	1110
Quintile 2 ² , 26–42 $\mu\text{g/L}$	0.33 (-0.57 to 1.24)	1190	-0.33 (-1.24 to 0.57)	1141	-0.75 (-1.67 to 0.17)	1146
Quintile 3, 42–63 $\mu\text{g/L}$	reference	1187	reference	1132	reference	1127
Quintile 4 ² , 63–96 $\mu\text{g/L}$	0.14 (-0.76 to 1.04)	1190	-0.36 (-1.26 to 0.54)	1156	-0.12 (-1.03 to 0.80)	1144
Quintile 5 ² , 96–390 $\mu\text{g/L}$	0.35 (-0.56 to 1.26)	1184	0.64 (-0.26 to 1.55)	1159	-0.08 (-1.01 to 0.84)	1150
SDS ³	-0.01 (-0.31 to 0.30)	5940	0.20 (-0.10 to 0.51)	5709	0.18 (-0.13 to 0.49)	5709
Differences in diastolic blood pressure, mmHg						
Quintile 1 ² , 2–26 $\mu\text{g/L}$	-0.23 (-0.94 to 0.48)	1189	0.16 (-0.56 to 0.89)	1120	-0.06 (-0.79 to 0.67)	1110
Quintile 2 ² , 26–42 $\mu\text{g/L}$	-0.02 (-0.72 to 0.68)	1190	0.07 (-0.64 to 0.78)	1141	0.0 (-0.72 to 0.72)	1146
Quintile 3, 42–63 $\mu\text{g/L}$	reference	1187	reference	1132	reference	1126
Quintile 4 ² , 63–96 $\mu\text{g/L}$	0.53 (-0.16 to 1.23)	1190	0.38 (-0.33 to 1.08)	1155	0.56 (-0.15 to 1.28)	1144
Quintile 5 ² , 96–390 $\mu\text{g/L}$	0.68 (-0.03 to 1.38)	1184	0.90 (0.18–1.61) ⁴	1159	0.62 (-0.10 to 1.34)	1150
SDS ³	0.27 (0.03–0.51) ⁴	5940	0.23 (-0.01 to 0.47)	5707	0.23 (-0.02 to 0.47)	5676

¹Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, gestational age at time of blood pressure measurements, and CRP levels. CRP, C-reactive protein; SDS, standard deviation score.

²Values are regression coefficients (95% CIs) and reflect the difference in mmHg blood pressure per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ –63 $\mu\text{g/L}$) as a reference. Estimates are from multiple imputed data.

³Values are regression coefficients (95% CI) and reflect the difference in mmHg blood pressure per log serum ferritin SDS.

⁴*P* value < 0.05.

the risk of hypertension, increased arterial stiffness, and a higher risk of cardiovascular disease, but findings are inconsistent (14–24). Two large prospective observational studies showed that higher serum ferritin concentrations were associated with an increased risk of hypertension (14, 16). However, 2 large prospective observational studies among 4509 Chinese and 2895 French adult men and women showed no associations of serum ferritin concentrations with the risk of hypertension after a more thorough adjustment for potential confounding factors (42, 43). In line with these studies in nonpregnant populations, we did not find consistent associations of maternal iron status in early pregnancy with maternal blood pressure development or placental vascular function throughout pregnancy, after adjustment for maternal inflammation, sociodemographic, and lifestyle-related factors. In the basic models, we observed consistent associations of higher early pregnancy serum ferritin concentrations with higher maternal systolic and diastolic pressure throughout pregnancy, a higher UmPI in mid pregnancy, and a higher risk of gestational hypertensive disorders. After adjustment for maternal inflammation, sociodemographic, and lifestyle factors, only the associations of higher maternal early pregnancy serum ferritin concentrations with a higher diastolic blood pressure in early pregnancy and a higher UmPI in mid pregnancy remained. This indicates that the associations of serum ferritin with our outcomes are, for a large part, explained by sociodemographic and lifestyle-related factors, such as maternal age, BMI, and parity. These observed tendencies may be more pronounced in early pregnancy, since the iron measurements were conducted close to these time points. A high iron status may lead to higher blood pressure during pregnancy and an impaired uteroplacental vascular function due to increased levels of oxidative stress (10, 11, 13). A high iron status may also lead to higher hemoglobin levels, which have been previously associated with higher blood pressure throughout pregnancy (3). In the current study, we indeed observed the strongest effects of high serum ferritin concentrations on early pregnancy

diastolic blood pressure among pregnant women who also had a high hemoglobin level. Iron availability is strongly regulated through physiological feedback, which may be even more pronounced in pregnancy, since iron stores are increasingly being mobilized during the course of pregnancy to facilitate placental and fetal development (44). Iron availability in the body is largely influenced by levels of transferrin, which is the main iron carrier in the blood. Transferrin concentrations decrease in the presence of iron overload, but they increase in the presence of iron deficiency to make it readily available for cells to use. We observed that the associations of serum ferritin concentrations with systolic and diastolic blood pressure during pregnancy were stronger when additionally adjusting for transferrin, which may further suggest that relatively higher serum ferritin concentrations negatively affect maternal blood pressure development in pregnancy. However, the observed effect is only small from a clinical perspective, but may be considered important on a population level and from an etiological perspective. We observed no consistent associations for other secondary measures of maternal iron status with blood pressure development in pregnancy. A higher early pregnancy TSAT was associated with lower systolic and diastolic blood pressure in early pregnancy, and higher early pregnancy serum iron was associated with lower systolic blood pressure in early pregnancy only. We cannot explain these findings. They may reflect a chance finding or be related to the measurement of these iron markers. Both TSAT and serum iron are influenced by recent dietary intake and diurnal variation (45). Thus, special care is needed in the interpretation of these findings, since these iron markers were determined from nonfasting blood samples and might not reflect the overall iron status accurately. To summarize, suboptimal maternal iron status in early pregnancy within the normal range was not consistently associated with maternal gestational hemodynamic adaptations after adjustment for inflammatory, sociodemographic, and lifestyle factors. Further studies are needed to explore whether more pronounced iron

TABLE 3 Associations of early pregnancy serum ferritin with umbilical artery pulsatility index, uterine artery resistance index, and third trimester bilateral uterine artery notching (n = 5466)¹

Early pregnancy serum ferritin	Umbilical artery pulsatility index			Uterine artery resistance index			Bilateral uterine artery notching		
	Mid pregnancy	Late pregnancy	n	Mid pregnancy	Late pregnancy	n	Late pregnancy	Late pregnancy	n _{cases}
	β (95% CI)	β (95% CI)		β (95% CI)	β (95% CI)		β (95% CI) ²	OR (95% CI) ²	
Quintile 1 ³ , 2–26 μg/L	-0.019 (-0.036 to -0.002) ⁴	-0.005 (-0.020 to 0.011)	914	0.003 (-0.007 to 0.012)	0.002 (-0.007 to 0.010)	673	0.87 (0.46–1.65)	19	
Quintile 2 ³ , 26–42 μg/L	-0.002 (-0.018 to 0.015)	-0.006 (-0.021 to 0.010)	922	0.005 (-0.004 to 0.015)	0.003 (-0.005 to 0.012)	677	1.13 (0.63–2.05)	25	
Quintile 3, 42–63 μg/L	reference	reference	922	reference	reference	689	reference	22	
Quintile 4 ³ , 63–96 μg/L	0.009 (-0.007 to 0.026)	-0.006 (-0.021 to 0.009)	912	0.009 (0.000–0.018)	0.000 (-0.008 to 0.008)	682	0.88 (0.48–1.63)	20	
Quintile 5 ³ , 96–390 μg/L	0.017 (0.000–0.033) ⁴	-0.006 (-0.022 to 0.009)	925	-0.001 (-0.010 to 0.008)	-0.005 (-0.013 to 0.004)	695	0.94 (0.51–1.73)	22	
SDS ⁴	0.010 (0.005–0.016) ⁴	-0.001 (-0.006 to 0.005)	4595	-0.000 (-0.004 to 0.003)	-0.002 (-0.005 to 0.001)	3430	1.02 (0.83–1.25)	108	

¹Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, foie acid supplementation, smoking habits, gestational age at time of blood sampling, gestational age at time of ultrasound measurements, and CRP levels. CRP, C-reactive protein; SDS, standard deviation score.

²Values are ORs (95% CIs) that reflect differences in risks of third trimester bilateral uterine artery notching per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 μg/L–63 μg/L) as a reference. Estimates are from multiple imputed data.

³Values are regression coefficients (95% CIs) and reflect differences in the umbilical artery pulsatility index and uterine artery resistance index per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 μg/L–63 μg/L) as a reference. Estimates are from multiple imputed data.

⁴P value > 0.05.

⁵Values are regression coefficients (95% CIs) that reflect the difference in the umbilical artery pulsatility index and uterine artery resistance index per log serum ferritin SDS or ORs (95% CI) that reflect differences in risks of third trimester bilateral uterine artery notching per log serum ferritin SDS.

overload or iron deficiency affects gestational hemodynamic adaptations.

In women that manifest preeclampsia during later stages of pregnancy, apparent differences in iron biomarkers are present when compared to healthy pregnant women (46). A few previous studies investigated the association of serum iron in early pregnancy with the risk of gestational hypertensive disorders (25–27). A case-control study from Poland among 484 healthy pregnant women and an observational study from the United States among 57 healthy nulliparous women found that lower serum iron concentrations from fasting samples at 12 weeks gestation were associated with higher risks of gestational hypertensive disorders (25, 26). However, adjustments for confounders were limited in these studies. In contrast, an observational study among 47 pregnant women with pregestational type 1 diabetes mellitus found no association of serum iron concentrations at 12 weeks gestation with preeclampsia (27). As the gestational age at the time of blood sampling was quite similar in our and previous studies, it seems unlikely that differences in gestational age at measurement of iron status explained any discrepancies between our and previous studies. With regards to iron overload, a retrospective study from Thailand among 400 pregnant women showed that iron supplementation in early pregnancy was associated with an increased risk of gestational hypertensive disorders (28). Similarly, a randomized, placebo-controlled trial among 727 Iranian nonanemic pregnant women reported that iron supplementation in early pregnancy was associated with an increased risk of gestational hypertensive disorders, but the baseline iron status was not determined in either of these studies (29). We did not find consistent associations of maternal early pregnancy iron status with the risk of gestational hypertensive disorders. Different findings in these previous studies and our study may be explained by our nonfasting blood samples, which influence the measurement of serum iron, and our relatively healthy population, since in comparison most of these previous studies had a higher percentage of gestational hypertensive disorders cases. Thus, our findings suggest that within our population, iron status in early pregnancy is not associated with the risk of gestational hypertensive disorders.

The response rate for participating in the Generation R Study was 61% for all the eligible women in the study area at the time of enrollment. Biased estimates are unlikely since they are more commonly caused by loss to follow-up rather than from nonresponse at baseline (47). Moreover, selection on the availability of iron status is unlikely to have affected the generalizability of the results, since we observed no substantial differences in the characteristics of women with data on early pregnancy iron markers compared to those without. Due to the design of our study, the gestational age at the measurement of iron status in early pregnancy was relatively broad. During early placental development, the syncytiotrophoblast can adapt to small increases in ROS by producing antioxidants (48). A dysregulated iron status in early pregnancy may increase the risk of gestational hypertensive disorders through small increases in oxidative stress that negatively influence placental development. In our study, iron markers were measured at a median of 13.2 weeks, partly after early placentation. However, we still consider our markers as adequate proxies of iron status from early gestation onwards, since the relative trend of iron status among the participants is likely to remain similar from conception to early pregnancy. For example, women with relatively low iron levels at conception are likely to continue having lower iron levels in early pregnancy compared to the rest of the

TABLE 4 Associations of early pregnancy serum ferritin with hypertensive disorder of pregnancy, gestational hypertension and preeclampsia ($n = 5834$)¹

Early pregnancy serum ferritin	Gestational Hypertensive Disorder		Gestational hypertension		Preeclampsia	
	OR (95% CI)	n_{cases}	OR (95% CI)	n_{cases}	OR (95% CI)	n_{cases}
Quintile 1 ² , 2–26 $\mu\text{g/L}$	0.85 (0.57–1.27)	46	0.86 (0.52–1.44)	26	0.84 (0.45–1.55)	20
Quintile 2 ² , 26–42 $\mu\text{g/L}$	1.10 (0.77–1.57)	67	0.95 (0.60–1.52)	35	1.36 (0.80–2.34)	32
Quintile 3, 42–63 $\mu\text{g/L}$	reference	69	reference	44	reference	25
Quintile 4 ² , 63–96 $\mu\text{g/L}$	0.85 (0.60–1.21)	67	0.90 (0.59–1.39)	47	0.75 (0.41–1.37)	20
Quintile 5 ² , 96–390 $\mu\text{g/L}$	1.20 (0.86–1.66)	102	1.35 (0.91–2.01)	76	0.94 (0.54–1.65)	26
SDS ³	1.08 (0.96–1.22)	351	1.15 (0.99–1.34)	228	0.98 (0.81–1.18)	123

¹Models are adjusted for maternal age, ethnicity, educational level, parity, prepregnancy BMI, folic acid supplementation, smoking, gestational age at time of blood sampling, and CRP levels. CRP, C-reactive protein; SDS, standard deviation score.

²Values are ORs (95% CIs) that reflect differences in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per serum ferritin quintile. Groups are compared to women in quintile 3 (serum ferritin: 42 $\mu\text{g/L}$ –63 $\mu\text{g/L}$) as the reference. Estimates are from multiple imputed data.

³Values are ORs (95% CIs) that reflect differences in risks of gestational hypertensive disorder, gestational hypertension, and preeclampsia per log serum ferritin as SDS.

study population. As individual absolute iron levels might differ depending on gestational age at blood sampling, we adjusted all analyses for gestational age at the time of blood sampling. We were able to adjust our analyses for maternal sociodemographic and lifestyle factors, but due to the observational nature of this study, residual confounding might still be present due to unmeasured factors such as iron supplementation.

We did not observe consistent associations of maternal iron status in early pregnancy with gestational hemodynamic adaptations or the risk of gestational hypertensive disorders. Our study population was mainly composed of relatively young women with higher education and a prepregnancy BMI within the normal range. We excluded women with preexistent hypertension. Pregnant populations with older women with a higher BMI would be at a higher risk for gestational hypertensive disorders. Furthermore, all markers of iron metabolism were mostly within the normal range. The prevalence of iron deficiency (serum ferritin < 15 $\mu\text{g/L}$) in our study population was 7%, and was slightly lower than that in the general Dutch population (49). Together, these factors may reflect a selection towards a relatively healthy and lower-risk pregnant population. Effects on gestational hemodynamic adaptations might be more pronounced in women with evidently low or high iron stores or those at higher risk for the development of gestational hypertensive disorders. In addition, the relatively low numbers of cases of iron deficiency, iron overload, and gestational hypertensive disorders may also have led to reduced statistical power. Studies in higher-risk populations could help to consolidate these initial findings and assess the potential associations of more extreme dysregulations in iron metabolism with hemodynamic adaptations in pregnancy. Moreover, the interpretation of iron status is particularly difficult in pregnancy due to iron stores being increasingly mobilized with gestational age progression in a reaction to higher iron requirements to facilitate placental and fetal development (44). Repeated measurement of fasting blood samples within the same participant are needed to assess longitudinal changes of iron parameters in pregnancy and their effects on maternal gestational hemodynamic adaptations. Since serum ferritin concentrations can be influenced by an inflammatory state, other important factors involved in the regulation of iron status, such as hepcidin, IL-6, and erythropoietin, in combination with other markers of iron metabolism that are less affected by inflammation, like the soluble transferrin receptor, could have aided in the interpretation of our results (50, 51). This is especially important in the context of this study, since inflammatory processes are suggested to be involved in

the pathophysiology of gestational hypertensive disorders (52). Unfortunately, these markers were not available in our cohort due to the considerable costs of these measurements. Further studies including these markers could provide a broader picture of the role of maternal iron metabolism in the development of gestational hypertensive disorders.

In conclusion, we found no consistent associations of early pregnancy serum ferritin concentrations with maternal blood pressure, placental hemodynamic parameters, or the risks of gestational hypertensive disorders after considering maternal inflammation, sociodemographic, and lifestyle-related factors. Further studies are needed to investigate the potential role of iron metabolism on gestational hemodynamic adaptations and the development of gestational hypertensive disorders within higher-risk populations.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon reasonable request.

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