Association between maternal plasma ferritin concentration, iron supplement use, and the risk of gestational diabetes: a prospective cohort study

Xu Zhang,[1,2] Meng Wu,[1,2] Chunrong Zhong,[1,2] Li Huang,[1,2] Yu Zhang,[1,2] Renjuan Chen,[1,2] Xuezhen Zhou,[1,2] Shangzhi Xu,[1,2] Qian Li,[1,2] Wenli Cui,[1,2] Xiaoyi Wang,[1,2] Xi Chen,[1,2] Lixia Lin,[1,2] Guofu Zhang,[1,2] Guoping Xiong,[3] Guoqiang Sun,[4] Xuefeng Yang,[1,2] Liping Hao,[1,2] Zhichun Jin,[5] and Nianhong Yang[1,2]

[1]Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; [2]Ministry of Education Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; [3]Department of Obstetrics and Gynecology, The Central Hospital of Wuhan, Wuhan, Hubei, China; [4]Obstetrics Department, Maternal and Child Health Care Hospital of Hubei Province, Wuhan, Hubei, China; and [5]Department of Integrated Traditional and Western Medicine, Hubei Maternal and Child Health Hospital, Wuhan, Hubei, China

ABSTRACT

Background: The association between iron supplementation and gestational diabetes mellitus (GDM) is still inconclusive, and this association has not been extensively studied in relation to plasma ferritin in the early second trimester.

Objectives: We aimed to prospectively examine the independent and combined associations of plasma ferritin concentrations and iron supplement use with GDM.

Methods: We studied 2117 women from the Tongji Maternal and Child Health Cohort in Wuhan, China. Plasma ferritin around 16 weeks' gestation was measured by ELISA kits and information on iron supplement use was collected by questionnaires. GDM was diagnosed by a 75-g oral-glucose-tolerance test (OGTT) at 24–28 weeks' gestation. A log-Poisson regression model was used to estimate the RR of GDM associated with plasma ferritin and iron supplementation.

Results: The median and IQR of plasma ferritin was 52.1 (29.6–89.9) ng/mL, and 863 (40.8%) participants reported use of iron supplements during the second trimester. A total of 219 (10.3%) participants developed GDM. Adjusted RRs (95% CIs) for GDM across increasing quartiles of plasma ferritin were 1.00 (reference), 2.14 (1.37, 3.34), 2.03 (1.30, 3.19), and 2.72 (1.76, 4.21), respectively. After adjustment, supplemental iron ≥60 mg/d during the second trimester was associated with an increased risk of GDM compared with nonusers (RR: 1.37; 95% CI: 1.02, 1.84).

Conclusions: Both elevated plasma ferritin concentrations in the early second trimester and use of ≥60 mg/d of supplemental iron during pregnancy are independently associated with increased risk of GDM. Further clinical trials with precision nutrition approaches considering both baseline iron status and supplement use are needed to evaluate the benefits and risks of iron supplementation during pregnancy. *Am J Clin Nutr* 2021;114:1100–1106.

Keywords: iron stores, iron status, ferritin, iron supplementation, gestational diabetes, Chinese

Introduction

Gestational diabetes mellitus (GDM), a common pregnancy complication, is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (1). Global prevalence of GDM ranges from 1.8% to 25.1% (2) and the estimated incidence in mainland China during 2010–2017 was 14.8% (3). GDM is associated with adverse maternal and neonatal outcomes, such as primary cesarean section, pre-eclampsia, and macrosomia (4). It also increases the risks of obesity, type 2 diabetes, and cardiovascular disease for both mothers and their offspring in later life (5).

Iron deficiency is the most prevalent and widespread micronutrient problem worldwide and iron deficiency during pregnancy is associated with adverse maternal and offspring health outcomes (6, 7). Increased iron intake is recommended to satisfy the biological requirements of pregnant women and their fetuses. However, iron is a potent pro-oxidant and catalyzes production of reactive oxidative species. Excess iron leads to impaired glucose metabolism by causing oxidative damage

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Address correspondence to NY (e-mail: zynh@mails.tjmu.edu.cn).

Abbreviations used: CNY, Chinese yuan; GDM, gestational diabetes mellitus; IADPSG, International Association of Diabetes and Pregnancy Study Groups; OGTT, oral-glucose-tolerance test.

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to the islets of the pancreas, liver, and muscle tissues (8, 9). Existing epidemiological evidence demonstrates that higher serum ferritin, a commonly used biomarker for body iron stores, in early pregnancy is associated with increased risk of GDM (10–14), but results of previous studies on iron supplementation and risk of GDM are partly contradictory. Some studies have suggested that the use of iron supplements was associated with an increased risk of GDM (15–18) while others did not (19–21). The discrepancies are possibly due to the variations in dosage and duration of iron supplementation. In addition, previous studies have focused mainly on associations of either body iron stores or iron intake with respect to GDM. It is unclear whether the effects of iron supplementation on GDM are modified by baseline iron status, because the absorption of iron depends on body iron stores (22).

Therefore, the present study aimed to explore the effect of plasma ferritin and iron intake on the development of GDM by examining the independent and combined associations of plasma ferritin concentrations and iron supplement use with GDM.

Methods

Study population

The present study was embedded in the ongoing Tongji Maternal and Child Health Cohort (TMCHC), a population-based prospective cohort in Wuhan, China, which aims to investigate the effects of maternal dietary and environmental exposures and lifestyle factors on health outcomes of mother–infant pairs. Women aged ≥18 y who initiated prenatal care before 16 weeks' gestation were recruited at 3 designated hospitals from January 2013 to May 2016 and followed up regularly. All participants provided written informed consents at enrollment. The study was approved by the Ethics Review Committee of Tongji Medical College, Huazhong University of Science and Technology.

Women with a singleton fetus who provided blood samples for ferritin measurement during gestational weeks 14–18 were eligible. Women without information on GDM diagnosis ($n = 37$) or iron supplement use in detail ($n = 179$) were excluded. Those who reported pre-existing diabetes ($n = 3$), previous GDM ($n = 3$), infectious disease (e.g., virus hepatitis, tuberculosis) ($n = 43$), polycystic ovary syndrome ($n = 9$), thyroid disorders ($n = 24$), or other systemic diseases ($n = 18$) were also excluded. A total of 2117 participants were involved.

Assessment of plasma ferritin and iron supplement use

Blood samples were collected with EDTA-coated tubes after an overnight fast. After centrifuging at 1620 × g at 4°C for 5 min, plasma samples were placed into aliquots and stored at −80°C for further measurement. Plasma ferritin concentrations were measured using a commercial ELISA kit (Assaypro, Inc.) and the absorbance was read on a microplate reader (BioTek, SYNGENE). The interassay CV was 11.0%. Any other information related to participants was withheld during this assessment.

Information on iron supplement use including brand, dose, starting time (gestational weeks), frequency (days/week), and week of discontinuation was collected by questionnaires at enrollment and the follow-up visits. Detailed information on iron-containing supplements frequently used by pregnant women in the present study is presented in **Supplemental Table 1**. All participants were categorized into 3 groups according to iron supplement use from the time of blood sampling for ferritin measurement until the time of GDM screening. High iron supplementation (supplemental iron ≥60 mg/d) was defined as elemental iron ≥60 mg/d on ≥5 d/wk for at least 4 wk. Nonusers were those who did not report any intake of iron-containing supplements. Those who reported iron supplement use but not meeting the criteria of ≥60 mg/d on at least 5 d/wk for 4 wk were classified as moderate iron supplementation (supplemental iron <60 mg/d).

Ascertainment of GDM

The primary outcome in this study was GDM and has been previously reported (23). During gestational weeks 24–28, participants received a 75-g, 2-h oral-glucose-tolerance test (OGTT) after an overnight fast. The OGTT is a 1-step approach recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (24). According to IADPSG criteria, GDM was diagnosed if fasting plasma glucose is ≥5.1 mmol/L or 1-h plasma glucose is ≥10.0 mmol/L or 2-h plasma glucose is ≥8.5 mmol/L.

Covariates

Data on maternal demographics, socioeconomic status, medical and obstetrical history, and lifestyle characteristics were collected using a structured questionnaire at enrollment. Maternal age, educational level, average income, family history of disease, parity, smoking status, alcohol consumption, and physical activity were included. Hemoglobin concentrations were obtained from medical records. Prepregnancy weight was self-reported, and current weight and height were measured at enrollment. Prepregnancy BMI was calculated using prepregnancy weight (kilograms) divided by height squared (meters squared). Last menstrual period was self-reported and further confirmed by ultrasound in the first trimester. Physical activity was assessed using the International Physical Activity Questionnaire, which identifies the frequency and time spent in walking and engaging in other moderate-to-vigorous-intensity physical activities during the previous 7 d. Regular physical activity during pregnancy was defined as moderate or vigorous intensity activity ≥30 min once with a frequency ≥3 times/wk. Information on dietary intake was obtained by a validated food-frequency questionnaire during gestational weeks 20–28 (25) and intakes of energy and nutrients were calculated based on the China Food Composition Database (26). The average daily intake of each dietary component was analyzed using a specifically designed computer program, which calculated the sum of the nutrient content of each food item. Dietary heme iron was estimated as 40% of the sum of iron from all animal-source products (27, 28), and nonheme iron was calculated by dietary total iron minus heme iron.

Statistical analysis

Baseline characteristics are presented as means ± SDs for normally distributed variables, medians (ranges) for nonparametrically distributed variables, and numbers (percentages) for

categorical variables according to quartiles of plasma ferritin concentrations. We used the log-Poisson regression model to estimate RRs and 95% CIs of GDM across increasing quartiles of plasma ferritin concentrations and categories of iron supplement use (nonusers, moderate iron supplementation, and high iron supplementation). Covariates adjusted in models were selected a priori, including maternal age (years), prepregnancy BMI (kg/m^2), years of education (<16, ≥16), monthly average income per capita [<5000, ≥5000 Chinese yuan (CNY)], nulliparous (yes, no), family history of diabetes (yes, no), and regular physical activity during pregnancy (yes, no). Gestational weeks at blood sampling (weeks) were adjusted in analyses involving plasma ferritin. Plasma ferritin and subsequent iron supplement use were further mutually adjusted. Linear trends across quartiles of plasma ferritin were tested by fitting medians of each quartile in the log-Poisson model as a continuous variable. Similarly, tests for trend across categories of iron supplement use were performed by treating 0, 1, and 2 in models as a continuous variable. To model the association between continuous plasma ferritin concentrations and GDM risk, restricted cubic spline regression was performed using the SAS GLMCURV9 macro (SAS Institute). We further conducted stratified analyses by maternal age (<28, ≥28 y) and prepregnancy BMI (kg/m^2; <21, ≥21), with cutoff values chosen based on the median, to examine whether the associations of plasma ferritin and iron supplement use with GDM were consistent in subgroups.

To examine whether an association between iron supplement use and GDM was influenced by baseline ferritin concentrations (~16 weeks' gestation), participants were stratified into 3 groups based on plasma ferritin concentrations classified as low (quartile 1, ≤29.5 ng/mL), medium (quartile 2 and quartile 3, 29.6–89.9 ng/mL), and high (quartile 4, ≥90.0 ng/mL). The log-Poisson model was performed to estimate RRs and 95% CIs of GDM, with supplement nonusers as a reference in each ferritin group, respectively. Effect modification was tested by performing a likelihood ratio test comparing models with and without categorical cross-product terms of plasma ferritin and iron supplementation. To further examine the combined association of baseline ferritin concentrations and iron supplement use with GDM, we conducted a joint analysis using the log-Poisson model to estimate RRs and 95% CIs of GDM with nonusers in the medium ferritin group as a reference. In sensitivity analyses, we examined the associations in subgroups by excluding participants with age ≥35 y, prepregnancy BMI ≥24, parity ≥1, and family history of diabetes.

Analyses were performed using SAS version 9.4 software (SAS Institute). A P value <0.05 for a 2-tailed test was considered to be statistically significant.

Results

The present study involved 2117 participants in the final analyses (**Supplemental Figure 1**). The median (IQR) of plasma ferritin concentrations in this study population was 52.1 (29.6–89.9) ng/mL, 863 (40.8%) of all participants reported iron supplement use, and 219 (10.3%) developed GDM. Baseline characteristics according to quartiles of plasma ferritin concentrations are shown in **Table 1**.

Either higher plasma ferritin concentrations or higher intake of iron supplements was correlated with increased risk of GDM (**Table 2**) after adjustment for maternal age, prepregnancy BMI, years of education, monthly average income per capita, parity, family history of diabetes, regular physical activity during pregnancy, and gestational weeks at blood sampling (for plasma ferritin only). After mutually adjusting for quartiles of plasma ferritin and categories of iron supplement use, the associations remained significant (Table 2). The adjusted RRs (95% CIs) of GDM across the increasing quartiles of plasma ferritin concentrations were 1.00 (reference), 2.14 (1.37, 3.34), 2.03 (1.30, 3.19), and 2.72 (1.76, 4.21), respectively (P-trend < 0.01). The regression splines demonstrated the continuous association between plasma ferritin and the risk of GDM (**Figure 1**). With respect to iron supplement use, the adjusted RR (95% CI) of GDM in women using supplemental iron ≥60 mg/d was 1.37 (1.02, 1.84) compared with nonusers. When stratified by maternal age (<28, ≥28 y) and prepregnancy BMI (<21, ≥21), the associations of plasma ferritin concentrations and iron supplementation with GDM remained consistent (**Supplemental Table 2**).

Stratified analyses of GDM risk related to iron supplement use by plasma ferritin concentrations showed no effect modification. Among participants with medium plasma ferritin concentrations (quartile 2 and quartile 3, 29.6–89.9 ng/mL), the adjusted RR (95% CI) was 1.64 (1.08, 2.49) for those using supplemental iron ≥60 mg/d compared with nonusers, whereas among women with low (≤29.5 ng/mL) or high (≥90.0 ng/mL) plasma ferritin concentrations, the adjusted RRs (95% CIs) were 1.43 (0.52, 3.97) and 1.09 (0.68, 1.76), respectively. The interaction of plasma ferritin and iron supplementation was tested by likelihood ratio test and no statistical significance was found ($P = 0.26$).

The combined effect of plasma ferritin concentrations and iron supplement use on GDM is shown in **Figure 2**. The combination of high plasma ferritin concentrations (≥90.0 ng/mL) and use of supplemental iron ≥60 mg/d was related to the highest risk of GDM. The adjusted RR (95% CI) was 1.76 (1.09, 2.81) compared with nonusers with medium plasma ferritin concentrations (29.6–89.9 ng/mL). Of note, participants with medium plasma ferritin concentrations and high supplemental iron intake (supplemental iron ≥60 mg/d) were associated with an increased risk of GDM, similar to the participants within the highest quartile of plasma ferritin concentrations (≥90.0 ng/mL) in early pregnancy. In sensitivity analysis, results remained consistent when further restricted to participants aged <35 y, prepregnancy BMI <24, nulliparous, or without family history of diabetes (**Supplemental Figure 2**).

Discussion

To our knowledge, this is the first prospective investigation focused on the combined association of plasma ferritin concentrations and iron supplement intake with the risk of GDM. The present study confirmed that elevated circulating ferritin concentrations in early pregnancy were associated with greater risk of GDM in a dose-response manner, regardless of iron supplements intake thereafter. Use of supplemental iron ≥60 mg/d in midpregnancy was also associated with increased risk of GDM, especially in those with plasma ferritin concentrations between an IQR of 29.6 and 89.9 ng/mL, although the effect modification was not statistically significant. Taken together, the

TABLE 1 Basic characteristics among 2117 participants according to quartiles of plasma ferritin concentrations[1]

Characteristics	Quartiles of plasma ferritin			
	Quartile 1 (≤29.5 ng/mL)	Quartile 2 (29.6–52.0 ng/mL)	Quartile 3 (52.1–89.9 ng/mL)	Quartile 4 (≥90.0 ng/mL)
n	530	528	530	529
Median of plasma ferritin, ng/mL	19.6	40.7	66.7	133.0
Age, y	27.6 ± 3.2	27.8 ± 3.1	28.2 ± 3.1	28.1 ± 3.1
Pregnant adolescents (≤19 y), *n*	1	2	0	0
Height, cm	160.2 ± 4.7	160.0 ± 5.1	160.2 ± 4.9	160.1 ± 5.4
Prepregnancy weight, kg	52.3 ± 6.6	52.9 ± 7.9	53.3 ± 7.2	54.5 ± 8.2
Prepregnancy BMI, kg/m^2	20.4 ± 2.4	20.7 ± 2.8	20.8 ± 2.6	21.3 ± 2.9
Overweight (24 ≤ BMI < 28 kg/m^2), *n* (%)	41 (7.7)	43 (8.1)	55 (10.4)	76 (14.4)
Obesity (BMI ≥28 kg/m^2), *n* (%)	4 (0.8)	10 (1.9)	8 (1.5)	14 (2.6)
Years of education, *n* (%)				
<16 y	239 (45.1)	227 (43.0)	211 (39.8)	221 (41.8)
≥16 y	291 (54.9)	301 (57.0)	319 (60.2)	308 (58.2)
Family monthly income per capita, *n* (%)				
<5000 CNY	206 (38.9)	209 (39.6)	176 (33.2)	186 (35.2)
≥5000 CNY	324 (61.1)	319 (60.4)	354 (66.8)	343 (64.8)
Nulliparous, *n* (%)	434 (81.9)	446 (84.5)	452 (85.3)	469 (88.7)
Family history of diabetes, *n* (%)	45 (8.5)	42 (8.0)	39 (7.4)	41 (7.8)
Regular physical activity during pregnancy,[2] *n* (%)	105 (19.8)	102 (19.3)	103 (19.4)	102 (19.3)
Drinking before pregnancy, *n* (%)	8 (1.5)	9 (1.7)	5 (0.9)	10 (1.9)
Smoking before pregnancy, *n* (%)	20 (3.8)	14 (2.7)	19 (3.6)	10 (1.9)
Iron supplement use,[3] *n* (%)				
Nonusers	339 (64.0)	318 (60.2)	289 (54.5)	308 (58.2)
Iron <60 mg/d	112 (21.1)	110 (20.8)	124 (23.4)	102 (19.3)
Iron ≥60 mg/d	79 (14.9)	100 (18.9)	117 (22.1)	119 (22.5)
Dietary iron intake,[4] mg/d				
n	198	205	247	277
Heme iron	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.9
Nonheme iron	16.9 ± 5.6	16.7 ± 5.6	17.0 ± 5.3	17.1 ± 5.7
Dietary total iron	18.3 ± 5.9	18.1 ± 6.0	18.4 ± 5.7	18.5 ± 6.2
Hemoglobin before 20 gestational wk				
n	510	513	508	508
Gestational age, wk	16.5 ± 1.2	16.5 ± 1.5	16.4 ± 1.5	16.4 ± 1.4
Hemoglobin concentrations, g/L	118.4 ± 8.7	118.8 ± 8.2	119.4 ± 8.4	118.6 ± 8.5
Anemia (Hb <110 g/L), *n* (%)	78 (15.3)	64 (12.5)	53 (10.4)	69 (13.6)
Gestational age at enrollment, wk	12.2 ± 1.7	12.3 ± 1.7	12.2 ± 1.9	12.4 ± 1.6
Gestational age at blood sampling, wk	16.7 ± 0.8	16.6 ± 0.8	16.6 ± 0.8	16.5 ± 0.8

[1] Values are means ± SDs unless otherwise indicated. CNY, Chinese yuan; Hb, hemoglobin.

[2] Regular physical activity during pregnancy was defined as exercise at moderate or vigorous intensity ≥30 min once and frequency ≥3 times/wk.

[3] Iron supplement use was assessed from blood sampling until oral-glucose-tolerance test screening. High iron supplementation (supplemental iron ≥60 mg/d) was defined as elemental iron ≥60 mg/d on ≥5 d/wk for at least 4 wk. Nonusers were those who did not report any intake of iron-containing supplements. Those who reported iron supplement use but not meeting the criteria of ≥60 mg/d on at least 5 d/wk for 4 wk were classified as moderate iron supplementation (supplemental iron <60 mg/d).

[4] Dietary iron intake was obtained by a food-frequency questionnaire during gestational weeks 20–28.

risk of GDM was highest in women with both high plasma ferritin (≥90.0 ng/mL) and use of ≥60 mg/d of supplemental iron.

The positive association between plasma ferritin concentrations and GDM in our study is in accord with most previous studies (10–14, 29). Given the fact that some of the previous studies had relatively small sample sizes with <700 participants (13, 14, 29) or reported only a few cases of GDM, with an incidence ~3.3% (10–12), our data from a large prospective cohort study provide strong scientific evidence in a Chinese population. In our study, median plasma ferritin concentrations were comparable to middle levels in most previous studies (30), in which the means/medians ranged from 36 to 66 ng/mL in early pregnancy (6–20 weeks' gestation) with participant numbers >300. The consistency between studies indicates a positive dose-response association between circulating ferritin concentrations and GDM persisting in various populations.

To date, the relation between iron supplement use and GDM remains inconclusive. A case-control study consisting of 500 pairs of GDM cases and controls found that women who used iron supplements in midpregnancy for ≥2 wk had a higher risk of GDM (OR: 3.36; 95% CI: 1.50, 7.53) compared with nonusers (15). Two additional observational studies also reported associations between higher total iron intake and/or iron supplementation during pregnancy and elevated risk of GDM,

TABLE 2 Associations of plasma ferritin concentrations and iron supplement use with risk of GDM[1]

Variables	GDM, *n* (%)	Crude RR (95% CI)	Adjusted RR (95% CI)[2]	Mutually adjusted RR (95% CI)[3]
Quartiles of plasma ferritin, median (ng/mL)				
Quartile 1 (19.6)	25 (4.7)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Quartile 2 (40.7)	57 (10.8)	2.29 (1.45, 3.61)	2.17 (1.39, 3.39)	2.14 (1.37, 3.34)
Quartile 3 (66.7)	57 (10.8)	2.28 (1.45, 3.59)	2.09 (1.33, 3.27)	2.03 (1.30, 3.19)
Quartile 4 (133.0)	80 (15.1)	3.21 (2.08, 4.94)	2.78 (1.80, 4.28)	2.72 (1.76, 4.21)
P-trend		<0.001	<0.001	<0.001
Iron supplement use[4]				
Nonusers	112 (8.9)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Iron <60 mg/d	49 (10.9)	1.22 (0.89, 1.68)	1.10 (0.80, 1.52)	1.11 (0.81, 1.53)
Iron ≥60 mg/d	58 (14.0)	1.56 (1.16, 2.11)	1.43 (1.06, 1.92)	1.37 (1.02, 1.84)
P-trend		0.003	0.024	0.043

[1]Tests for trend across quartiles of plasma ferritin were done by fitting median values of each quartile in models as continuous variables. For iron supplement use, categories were coded as 0, 1, and 2, respectively, and fitted in models as continuous variables. CNY, Chinese yuan; GDM, gestational diabetes mellitus; Ref., reference.

[2]Adjusted for maternal age (years), prepregnancy BMI (kg/m^2), years of education (<16, ≥16), monthly average income per capita (<5000, ≥5000 CNY), nulliparous (yes, no), family history of diabetes (yes, no), and regular physical activity during pregnancy (yes, no). Gestational week of blood sampling (weeks) was adjusted in the plasma ferritin model.

[3]For plasma ferritin, the model was further adjusted for categories of iron supplement use. For iron supplement use, the model was further adjusted for quartiles of plasma ferritin concentrations and gestational week at blood sampling.

[4]Iron supplement use was assessed from blood sampling until oral-glucose-tolerance test screening. High iron supplementation (supplemental iron ≥60 mg/d) was defined as elemental iron ≥60 mg/d on ≥5 days/wk for at least 4 wk. Nonusers were those who did not report any intake of iron-containing supplements. Those who reported iron supplement use but not meeting the criteria of ≥60 mg/d on at least 5 d/wk for 4 wk were classified as moderate iron supplementation (supplemental iron <60 mg/d).

especially for nonanemic women (16, 18). However, a prospective cohort study found no association between prepregnancy iron supplementation and GDM, although dietary heme iron intake was positively associated with GDM risk (19). Results from 1 large randomized controlled trial with 1164 participants conducted in Hong Kong also showed no relations between iron supplement use and risk of GDM (21). The discrepancy was possibly due to different body iron stores, dosage, and duration of iron supplementation in the studied populations. In the present study, use of iron supplements ≥60 mg/d during midpregnancy was associated with an increased risk of GDM, and the association was more pronounced among women with medium plasma ferritin concentrations. This could be due to different plasma ferritin concentrations among groups. Iron absorption in women with higher plasma ferritin concentrations was reduced (22), so the limited amount of absorbed iron may not further increase the risk of GDM in women who were already at higher risk (plasma ferritin ≥90 ng/mL). Women with lower plasma ferritin concentrations will benefit from iron supplementation and improve their iron status, but it is less likely to contribute to excessive body iron because of their low iron stores. Thus, iron supplementation among women with low plasma ferritin (≤29.5 ng/mL) was not associated with significantly increased risk of GDM.

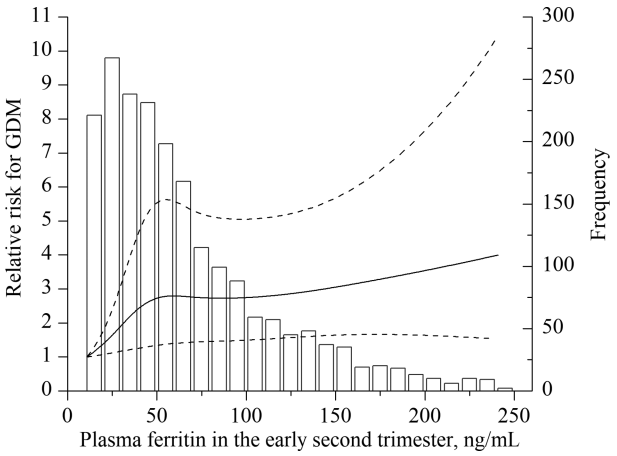

FIGURE 1 Relation between continuous plasma ferritin and GDM risk. Splines represent adjusted RRs and 95% CIs of GDM in relation to plasma ferritin concentrations (percentile 2.5–97.5; $n = 2013$). The solid line represents point estimates of RRs, and the dotted lines represent the 95% CI. The histogram represents frequency of participants according to plasma ferritin concentrations. RRs were calculated by the restricted cubic spline log-Poisson regression model and were adjusted for maternal age (years), prepregnancy BMI (kg/m^2), years of education (<16, ≥16), monthly average income per capita (<5000, ≥5000 CNY), nulliparous (yes, no), family history of diabetes (yes, no), and regular physical activity during pregnancy (yes, no). CNY, Chinese yuan; GDM, gestational diabetes mellitus.

The present study suggests that routine iron supplementation of ≥60 mg/d for pregnant women without iron deficiency may not be suitable with respect to GDM risk. Of note, for participants with medium plasma ferritin concentrations (29.6–89.9 ng/mL), the use of supplemental iron ≥60 mg/d was associated with an elevated risk of GDM, which is close to the risk among participants with high plasma ferritin (≥90.0 ng/mL) in early pregnancy. Thus, assessment of iron status in early pregnancy is needed to make appropriate individualized iron supplementation recommendations (31) to prevent negative outcomes because of maternal iron deficiency and minimize the risk of GDM. For instance, the British Society for Haematology recommends that women with serum ferritin concentrations <30 ng/mL take iron supplements with elemental iron ≥65 mg once a day, rather than recommending routine iron supplementation for all pregnant

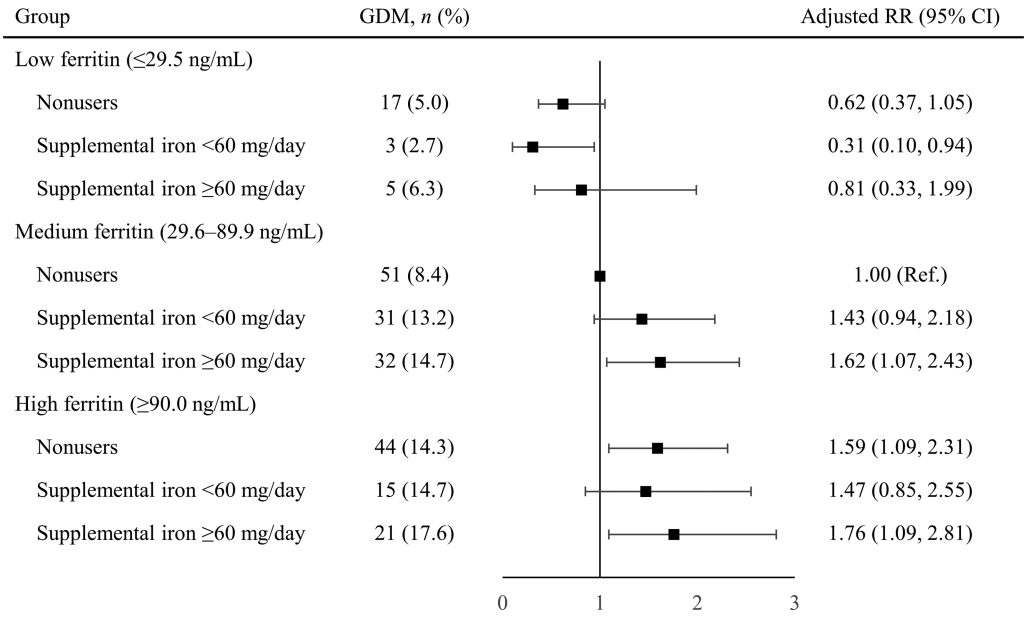

FIGURE 2 Joint association of plasma ferritin concentrations and iron supplement use with GDM. Iron supplement use was assessed from blood sampling until oral-glucose-tolerance test screening. High iron supplementation (supplemental iron ≥60 mg/d) was defined as elemental iron ≥60 mg/d on ≥5 d/wk for at least 4 wk. Nonusers were those who did not report any intake of iron-containing supplements. Those who reported iron supplement use but not meeting the criteria of ≥60 mg/d on at least 5 d/wk for 4 wk were classified as moderate iron supplementation (supplemental iron <60 mg/d). RRs were calculated by the log-Poisson regression model and were adjusted for maternal age (years), prepregnancy BMI (kg/m^2), years of education (<16, ≥16), monthly average income per capita (<5000, ≥5000 CNY), nulliparous (yes, no), family history of diabetes (yes, no), and regular physical activity during pregnancy (yes, no). Nonusers with medium ferritin concentrations were the reference group. CNY, Chinese yuan; GDM, gestational diabetes mellitus; Ref., reference.

women (32). Similarly, recommendations in China also suggest that women with serum ferritin concentrations <30 ng/mL take supplemental iron. In addition, the recommended nutrition intake of iron for pregnant women in the second trimester in China is 24 mg/d. The intake of dietary total iron in our study population was ∼18 mg. Thus, to avoid excessive iron intake, a lower-dose supplementation and/or intermittent iron supplementation rather than daily use of iron supplements might be more appropriate for women without iron deficiency (33, 34).

The current study has several strengths. First, this study was embedded in a prospective cohort with a relatively large sample size, and the time of ferritin measurement was, on average, 2 mo prior to the diagnosis of GDM, which means that concentrations of circulating ferritin would less be influenced by progress of GDM. Second, the information on iron supplement use was detailed, including brand, dose, starting time, frequency, and time of discontinuation. Third, we examined the association of iron supplement use with GDM with the baseline plasma ferritin taken into account.

The study also has limitations meriting discussion. First, we did not measure biomarkers of inflammation. Because serum ferritin concentrations can be influenced by both acute and chronic inflammation (35), the single use of circulating ferritin as an index of iron stores could confound the results. However, it has been reported that the distribution of serum ferritin will be little influenced by a low prevalence of inflammation (36), and we have excluded women with known inflammatory diseases to minimize this influence. Second, only 43.8% of the participants in this study provided information on dietary iron intake. However, as shown in Table 1, dietary heme iron intake, nonheme iron intake, and dietary total iron intake in this subgroup of participants were not significantly different across quartiles of plasma ferritin, and basic characteristics between women who provided dietary information and those who did not were not significantly different (**Supplemental Table 3**), indicating that the relation between plasma ferritin and GDM might not be confounded by dietary iron intake. In addition, it is likely that women using iron supplements had a lower intake of dietary iron, and this could bias our results toward the null. Third, there could be residual confounders due to the nature of observational study. In the present study, major risk factors have been selected a priori from generally considered confounders in previous studies and adjusted in the model, including maternal age, prepregnancy BMI, educational level, average income, parity, family history of diabetes, physical activity, and gestational weeks at blood sampling. Finally, we focused on GDM risk in the study, and further investigations on the relation of iron supplement and maternal body iron stores with fetal iron status are warranted.

In conclusion, elevated plasma ferritin concentrations in early pregnancy and intake of ≥60 mg/d of supplemental iron during the second trimester were each independently associated with increased risk of GDM. Further clinical trials with precision nutrition approaches considering both baseline iron status and iron intakes are warranted to evaluate the benefits and risks of routine iron supplementation during pregnancy.

The authors' responsibilities were as follows—NY and X Zhang: designed the study; X Zhang, CZ, RC, X Zhou, SX, L Huang, QL, XC, WC, XW, and YZ: conducted the study; X Zhang and MW: researched the data and prepared the tables and figures; MW, L Huang, X Zhou, YZ, CZ, RC, MW, GZ, LL, GX, ZJ, GS, XY, and L Hao: explained the data and contributed to

the discussion; X Zhang: drafted the manuscript; NY: edited the manuscript and further contributed to discussion and had primary responsibility for the final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

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

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