Original Article

Dietary intake of heme iron and body iron status are associated with the risk of gestational diabetes mellitus: a systematic review and meta-analysis

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Background and Objectives: Some potential role of iron overload in the development of diabetes mellitus have been suggested. Our study aimed to systematically assess the association between the risk of gestational diabetes mellitus (GDM) and iron intakes/body iron status. **Methods and Study Design:** PubMed and Web of Science were searched for relevant articles. Relative risks (RR) of GDM in relation to dietary iron intakes and body iron stores were pooled with the random-effects model. Weighted mean differences of iron blood markers between GDM and non-GDM individuals were also analyzed. **Results:** Twenty-five studies were included in the qualitative analysis, and 23 studies with 29,378 participants and 3,034 GDM patients were included in the quantitative analysis. Dietary intake of heme iron was significantly associated with GDM risk (RR=1.65, 95% CI: 1.28 to 2.12), and the pooled RR for each 1mg/day increment of heme iron intake was 1.38 (95% CI: 1.19 to 1.61). No association between GDM and the intakes of nonheme iron, total iron, or supplemental iron was detected. Body iron stores, as represented by serum ferritin level, were correlated with GDM risk (RR=1.64, 95% CI: 1.27 to 2.11). Moreover, the concentrations of both serum ferritin and serum iron were increased in GDM patients, compared with non-GDM individuals. **Conclusions:** Increased dietary intake of heme iron and body iron status are positively associated with the risk of GDM development in pregnant women. Future studies are warranted to better understand the role of iron in GDM development.

Key Words: pregnancy, diabetes mellitus, heme iron, nonheme iron, supplemental iron

INTRODUCTION

Gestational diabetes mellitus (GDM) is a pregnancy complication, defined as glucose intolerance with onset or first recognition during pregnancy.[1] During pregnancy, progressive insulin resistance is normally detected, likely to be caused by maternal adiposity and placental hormones.[2] For most women, insulin resistance can be compensated by elevated insulin secretion from pancreatic β-cells. However, chronic β-cell dysfunction may take place in certain women and result in the development of GDM. The mechanisms causing β-cell injury are not clear, which possibly involve chronic insulin resistance, autoimmune damages and genetic factors.[1] Increased perinatal risks have been associated with GDM, including macrosomia, infant mortality and higher rates of cesarean deliveries.[3]

Recent studies suggested that iron overload may impair the regulation of glucose metabolism in the body. Iron is an essential trace element required for pivotal functions of the body, such as oxygen transport and energy production. However, as a redox-active metal, excessive iron leads to elevated levels of reactive oxygen and oxidative stress, which may cause pancreatic β-cell dysfunction and insulin resistance.[4] A recent meta-analysis concluded that higher iron intake and increased body stores were significantly associated with a greater risk of type 2 diabetes.[5] Moreover, the protective role of dietary iron restriction

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against diabetes development has been observed in animal studies.[6,7]

Whether iron is associated with the development of GDM is not clear. The association between body iron status and impaired glucose tolerance during pregnancy was firstly reported by Lao et al in 1997,[8] and was supported by several subsequent studies.[9,10] Nevertheless, insignificant changes of iron status in GDM patients were also suggested by other research groups,[11,12] The association between iron intakes and GDM risk was also examined by several studies,[13-17] yet no consensus has been reached. A recent meta-analysis study suggested a positive relationship between body iron and GDM in 14 cohort and case-control studies.[18] In this study, we aimed to systematically investigate the associations between iron intakes, body iron status, and the risk of GDM development from all available studies in the field, without limiting the type of studies, and then we quantitatively assessed the data by meta-analysis.

METHODS

Data sources and searches

The criteria for conducting and reporting meta-analysis for observational studies were followed as previously reported.19 Two investigators (LZ and JL) independently conducted the literature search in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Web of Science [v.5.17] (http://webofknowledge.com/WOS) up to May 4th 2016, using the search terms (iron or ferritin or heme) and (GDM or gestational diabetes or (pregnant* and glucose)). The references of any relevant original papers and review articles were screened to identify potential publications. Only studies written in English were included.

Study selection

Study inclusion criteria were: 1) studies that evaluated the association between iron intake or body iron status and GDM risk; 2) studies that reported the risk estimates and corresponding 95% CIs of GDM in populations with different iron intake or body iron status; 3) studies that reported mean and standard deviation (SD) (or median and ranges/interquartile ranges) of iron hematological markers in GDM and non-GDM populations. Figure 1 presents the flowchart of publication selection.

Exclusion criteria were: 1) studies that did not report iron intake or body iron stores as exposure; 2) studies that did not include GDM as the outcome; 3) studies that did not report sufficient quantitative data for the risk of GDM or the concentrations of iron hematological markers; 4) studies in animals or cell lines; 5) non-original researches (reviews, editorials, meetings or commentaries); abstracts; unpublished studies, and duplicated studies.

Data extraction and quality assessment

From each included article, the following data were extracted: basic information (title, first author's name, publication year), study characteristics (name of the study, study design, country, duration of follow-up (for prospective studies)), participant characteristics (sample size, number of GDM cases, age, gestational age), assessment of iron intakes and body iron stores, ascertainment of GDM, prevalence or risk estimates and 95% CIs of GDM, any covariates adjusted in the multivariate analysis, mean and SD (or median and ranges/interquartile ranges) of iron hematological markers. The most adjusted risk estimate was selected, when a study had multiple adjustment models for potentially confounding variables.

The quality of each study was assessed by two investigators, independently, according to the Cochrane Collaboration's tool for assessing risk of bias[20] for randomized controlled studies and the Newcastle-Ottawa quality assessment Scale[21] for non-randomized studies. Discrepancies were resolved by discussions.

Data synthesis and analysis

As the incidence of GDM was low for the assumption of rare disease (<10%),[22] OR was assumed to approximate the relative risk (RR) in each case. Thus we combined the ORs with RRs in meta-analysis. To analyze the association between GDM risk and iron intakes or body iron status, we used the RR comparing GDM risk between the high and the low iron intake/status groups, as the major outcome for our meta-analysis. Some studies categorized iron intake or body iron status into multiple levels, in such cases we selected the RR corresponding to the highest versus the lowest iron intake/status category comparison. The risk estimates adjusted for most covariates were selected. Original data were used to calculate a crude risk estimate, when adjusted covariates were unavailable. The overall RRs and corresponding 95% CIs were assessed in a random-effects model (DerSimonian-Laird method),[23] which incorporates between-study heterogeneity of effects. The dose-response analysis was performed using the Grennland-Longnecker method[24] and the publicly available Stata code written by Orsini et al[25] For this method, categorical midpoint was used when neither medians nor means were reported for an exposure category. If the highest or lowest category was open-ended, the midpoint of the category was set by assuming the width of the category as the same as the next adjacent category. In the tests of hematological iron markers, sample size, mean and SD were analyzed. Median and range/interquartile ranges were converted to mean and SD, as in previous work.[26,27] The differences of the concentrations of iron hematological markers were calculated using weighted mean difference (WMD), and then assessed in a random-effects model.

Heterogeneity across different studies was evaluated by the I^2 statistic, which describes the percentage of variation across studies, caused by heterogeneity rather than by chance.[28,29] I^2 values of 25%, 50%, and 75% were considered as low, medium, and high heterogeneity, respectively. Publication bias was assessed by Begg's rank correlation test[30] and funnel plots.[31] Sensitivity analysis was performed to examine the influence of individual studies, in which the meta-analysis estimate was computed, omitting one study at a time.[32] Publication bias and sensitivity analyses were performed for meta-analyses with at least three individual studies. All statistical analyses were conducted by STATA version 12.0 (STATA Corp, College Station, Texas) and R program.[33]

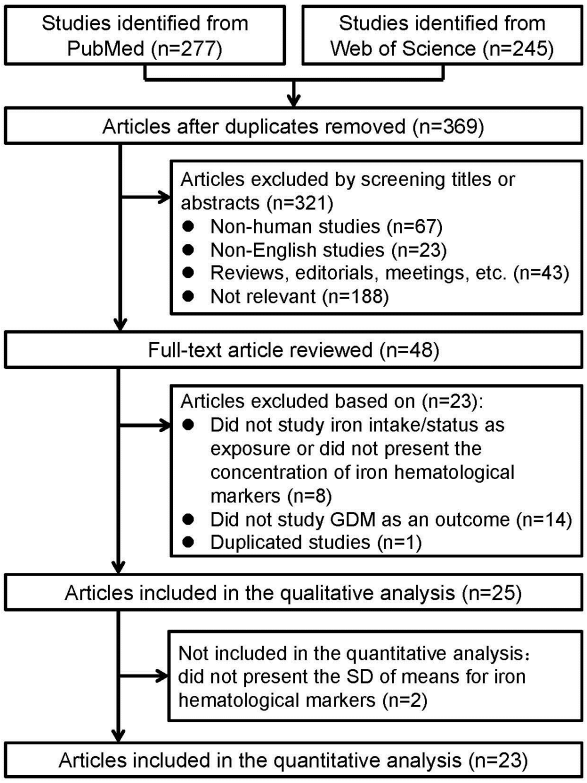

Figure 1. Flowchart of literature search and publication selection in the meta-analysis.

RESULTS

Characteristic of included studies

Our initial search identified 369 potentially relevant records. After screening titles and abstracts, we identified 48 articles for further evaluation (Figure 1). Twenty-three studies were ultimately included in the quantitative meta-analysis, with 12 case-control studies,[10-12,34-42] 9 cohort studies,[9,13-16,43-46] and 2 randomized controlled studies (RCT).[47,48] In total, 3,034 GDM patients and 26,344 non-GDM pregnant women were involved. Six studies examined the association between iron intakes and the risk of GDM,[13-16,47,48] and the other 17 studies assessed the relationship between body iron status and GDM. Among the 17 studies examining body iron status, 4 studies evaluated the risk of GDM of individuals with different levels of iron status,[9,44-46] 11 studies compared the concentration of iron hematological markers between GDM patients and control groups, and the remaining 2 studies performed both analyses.[10,37] Five studies analyzed the concentrations of both serum ferritin and iron between GDM and non-GDM women,[34,37,38,40,42] four studies only reported serum ferritin levels,[10-12,39] and the remaining four studies only examined serum iron.[35,36,41,43] In addition, two articles closely related to our study topic were included in the qualitative analyses.[49,50] Among the 18 articles which provided detailed GDM diagnosis methods, 16 of them adopted the American Diabetes Association standards, suggesting a high consistency of GDM diagnosis in included studies. Detailed information of all included studies is summarized in Table 1 and Table S1.

The two RCT trials were evaluated as low risk of bias using the Cochrane Collaboration's method,[20] and the quality scores of included non-randomized studies ranged from 4 to 8, with an average score of 6.7, using the Newcastle-Ottawa method 21 (Table S2-3).

Associations between iron intakes and the risk of GDM

Dietary iron intakes

Six studies examined the association of GDM risk with different types of iron intakes (Table 2). Dietary iron exists as heme iron or nonheme iron. The Bowers et al study[13] and the Qiu et al study[14] assessed the relative risk of developing GDM in two large cohorts of pregnant women, with different levels of heme and nonheme iron intakes. Both studies reported significant association between heme iron intake and GDM risk. Consistently, our meta-analysis showed that the pooled RR (95% CI) of GDM in pregnant women with high intake of heme iron was 1.65 (95% CI: 1.28 to 2.12) (Figure 2A). However, neither study found significant association between GDM and nonheme iron intake. The pooled RR (95% CI) of GDM in women with high intake of nonheme iron was 0.86 (95% CI: 0.58 to 1.28). No evidence for significant heterogeneity (heme iron: I^2=0.0%; nonheme iron: I^2=40.3%) was found in the included studies.

Dose-response analyses suggested that every 1mg/day increment of heme iron intake was significantly associated with a higher risk of GDM (RR=1.38, 95% CI: 1.19 to 1.61) (Figure 2B). We did not detect any obvious changes of GDM risk for every 5mg/day increment of nonheme iron intake (RR=0.95, 95% CI: 0.84 to 1.09) (Figure 2B). Low to medium levels of study heterogeneity were shown in our analyses (heme iron: I^2=0.0%; nonheme iron: I^2=68.3%).

Supplemental iron and total iron intakes

The association between supplemental iron and GDM was examined in four articles, including two cohort studies[13,16] and two RCTs.[47,48] Only the Bo et al study[16] reported increased risk of GDM in iron supplement users, compared with non-users. The pooled result for cohort studies did not suggest significant correlation between supplemental iron intake and GDM risk (RR=1.75, 95% CI: 0.56 to 5.47) (Figure 2A). A high heterogeneity was detected in the analysis (I^2=86.8%). In the two RCTs, 60 or 100 mg/day elemental iron supplement were given to the experimental group as a routine treatment from early pregnancy to delivery. Placebos or no treatment was given to the control groups. Noticeably, out of ethical concerns, the research assistants of both RCTs were not blinded. Iron supplement was also provided to women in the control groups once they developed anemia. No significant association between iron supplementation and GDM risk was reported in these studies. The pooled RR (95% CI) of GDM in iron supplement group was 0.88 (95% CI: 0.72 to 1.07), compared with control groups. No evidence for significant heterogeneity was found in the analysis (I^2=0.0%).

The association between total iron intake (from both diet and supplements) was also examined by two studies (Table 2). No significant relationship was detected by either study.[13,15] The pooled RR (95% CI) of GDM in women with high intake of total iron was 1.12 (95% CI: 0.63 to 1.98), compared with the low intake group (Figure 2A). Medium levels of heterogeneity was found

Table 1. Characteristics of the 23 studies included in the quantitative analysis

Author	Year	Country	Study design	Sample size*	Age	Assessment of iron intakes or iron status/ gestational age	Ascertainment of GDM/ gestational age	QS†
Iron intakes and GDM (n=6)								
Bo et al[16]	2009	Italy	Cohort‡	500/1000	28-41	Structured interview/24-28w	OGTT (ADA)§/24-28w	8
Bowers et al[13]	2011	USA	Cohort	867/13,475	28-35	Structured interview (FFQs)/na	Self-report / na	8
Chan et al[48]	2009	China	RCT	116/1,164	30-33	Iron supplement and placebo/around 11w to delivery	OGTT (WHO)‖/28w,36w	LR
Helin et al[15]	2012	Finland	Cohort	72/399	24-35	Structured interview (FFQs)/26-28w	OGTT (ADA)§/26-28w	7
Kinnunen et al[47]	2016	Finland	RCT	323/2,694	22-35	Routine and selective iron supplement/ around 11w to delivery	Medical records review/na	LR
Qiu et al[14]	2011	USA	Cohort	158/3,158	18 and up	Structured interview (FFQs)/before conception to 12w	OGTT(ADA)§/24-28w	7
Body iron stores and GDM (n=16)								
Afkhami-Ardekani et al[34]	2009	Iran	Case-control	34/34	NA	Ferritin (IRMA); Serum iron (CMA)/24-28w	OGTT(ADA)§/24-28w	7
Al-Saleh et al[35]	2004	Kuwait	Case-control	15/15	23-34	Serum iron (AAS)/at delivery	Standard criteria¶/na	7
Al-Saleh et al[36]	2007	Kuwait	Case-control	11/10	28-34	Serum iron (AAS)/at delivery	Standard criteria¶/na	7
Amiri et al[37]	2013	Iran	Case-control	100/100	20-31	Ferritin (IRMA); Serum iron(CMA)/24-28w	OGTT(ADA)§/24-28w	7
Behboudi-Gandevani et al[43]	2013	Iran	Cohort	72/1,033	20-35	Serum iron (AAS)/24-28w	OGTT(ADA)§/24-28w	8
Chen et al[9]	2006	USA	Cohort	356/1,456	21-23	Ferritin (IRMA)/14-16w	OGTT(ADA)§/~28w	8
Derbent et al[38]	2013	Turkey	Case-control	30/72	23-37	Ferritin (CLA); Serum iron (CMA)/24-28w	OGTT(ADA)§/24-28w	5
Gungor et al[11]	2007	Turkey	Case-control	56/56	21-34	Ferritin (MEIA)/28-30w	OGTT(ADA)§/24-28w	5
Javadian et al[39]	2014	Iran	Case-control	52/50	22-38	Ferritin (IRMA)/24-28w	OGTT(ADA)§/24-28w	5
Kaygusuz et al[40]	2013	Turkey	Case-control	30/28	28-33	Ferritin (CLA); Serum iron (CMA)/24-28w	OGTT(ADA)§/24-28w	6
Khambalia et al [46]	2015	Australia	Cohort	129/3776	NA	Ferritin (ELISA)/~12w	Standard criteria¶/na	8
Ozyer et al[12]	2014	Turkey	Case-control	35/70	25-36	Ferritin (CLA)/24-28w	OGTT(ADA)§/24-28w	7
Sharifi et al[10]	2010	Iran	Case-control	64/64	25-35	Ferritin (IRMA)/24-28w	OGTT(ADA)§/24-28w	7
Soubasi et al[44]	2010	Greece	Cohort	6/63	24-37	Ferritin (ELISA)/at delivery	OGTT(ADA)§/24-28w	6
Wang et al[41]	2002	China	Case-control	46/90	NA	Serum iron (ICP-AES)/na	OGTT(ADA)§/na	5
Yeniel et al[42]	2012	Turkey	Case-control	29/94	23-33	Ferritin (CLA); Serum iron (CMA)/12w	OGTT(ADA)§/24w	7
Zein et al[45]	2015	Lebanon	Cohort	16/104	20-33	Ferritin (CLA)/6-11w	OGTT(IADPSG)#/24-28w	8

AAS: atomic absorption spectrophotometry; CLA: chemiluminescence assay; CMA: colorimetric assay; ELISA: enzyme-linked immunosorbent assay; FFQs: food frequency questionnaires; ICP-AES: inductively coupled plasma-atomic emission spectrometry; IRMA: immunoradiometric assay; LR: low risk; MEIA: microparticle enzyme immunoassay; NA: not available; OGTT: tolerance test; QS: quality score; RCT: randomized controlled studies; w: weeks.

*Sample size: numbers of cases/controls for case-control studies; numbers of cases/participants for cohort and randomized controlled studies.

†Quality score: the quality assessment of cohort and case-control studies followed the Newcastle-Ottawa method.[21] The quality of randomized controlled studies was assessed according to the Cochrane Collaboration's method.[20]

‡A nested case control study.

§ADA, American diabetes association standard, Gestational diabetes mellitus. Diabetes care, 27 Supplement (1), 2004. ADA recommend a diagnostic 100 g OGTT test (≥95 mg/dL fasting, ≥180 mg/dL at 1 h, ≥155 mg/dL at 2 h, and ≥140 mg/dL at 3 h). Two or more of the plasma glucose values must be met or exceeded for a positive diagnosis.

‖ WHO, World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part1: diagnosis and classification of diabetes mellitus. WHO/NCD/NCS. Geneva, Switzerland, World Health Organization; 1999.[63] According to the WHO standards, impaired glucose tolerance (75 g OGTT test: ≥ 140 mg/dL and < 200 mg/dL at 2 h) and diabetes (75 g OGTT test: ≥ 200 mg/dL) were both considered as GDM.

¶The detailed diagnosing methods for GDM is not described in these studies.

#IADPSG, international association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care, 33(3), 2010.[64] IADPSG. IADPSG recommends two-phase detection (prenatal visit and 24-28 week's gestation). In the Zeinet al.[45] paper, only the later phase was examined (75 g OGTT test: ≥ 126 mg/dL fasting, ≥ 180 mg/dL at 1 h, ≥153 mg/dL at 2 h).

Table 2. Associations between iron intakes or body iron status with the risk of GDM in the included studies

Source	Comparison	RRs (95% CI)	Adjusted covariates
Iron intakes and GDM (n=4)			
Bo et al, 2009[16]	Supplemental iron intake: high (105 mg of elemental iron) vs low (0 mg of elemental iron) intake	3.36 (1.50-7.53)	Age, diabetes in first-degree relatives, pregnancy BMI, education level, smoking, parity, duration of iron supplementation, and current employment
Bowers et al, 2011[13]	Heme iron intake: highest (median 1.60 mg/day) vs lowest (median 0.66 mg/day) quintile	1.58 (1.21-2.08)	Age, parity, BMI, physical activity, glycemic load, polyunsaturated fat intake, cereal fiber, smoking, alcohol, total calories, and family history of diabetes
	Nonheme iron intake: highest (median 45.33 mg/day) vs lowest (median 7.58 mg/day) quintile	0.97 (0.78-1.20)	
	Supplemental iron intake: highest (median 60 mg/day) vs lowest (median 0 mg/day) quintile	1.04 (0.84-1.28)	
	Total iron intake: highest (median 49.80 mg/day) vs lowest (median 10.70mg/day) quintile	0.90 (0.72-1.12)	
Chan et al, 2009[48]	Supplemental iron intake: experimental group (60 mg/day elemental iron) vs placebo group	1.04 (0.70-1.53)	None.
Helin et al, 2012[15]	Total iron intake: high (>110.0 mg/day) vs low (<110.0 mg/day)	1.66 (0.84-3.30)	BMI, age, diabetes in first-degree or second-degree relatives, GDM or macrosomia in a previous pregnancy, total energy intake, dietary fiber, saturated fatty acids and total gestational weight gain.
Kinnunenet al, 2016[47]	Supplemental iron intake: routine iron group (100 mg/day elemental iron) vs selective iron group (100 mg/day elemental iron only when anemic)	0.83 (0.66-1.05)	None.
Qiu et al, 2011[14]	Heme iron intake: highest (≥1.12 mg/day) vs lowest (<0.48 mg/day) quartile	2.15 (1.09-4.27)	Daily energy intake, maternal age, race/ethnicity, parity, physical activity, prepregnancy BMI, and dietary fiber, vitamin C, saturated fat, cholesterol, , and red and processed meat intake.
	Nonheme iron intake: highest (≥12.98 mg/day) vs lowest (<0.10 mg/day) quartile	0.61 (0.31-1.18)	
Body iron stores and GDM (n=5)			
Amiri et al, 2013[37]	High ferritin (>80 ng/mL) vs low ferritin (<20 ng/mL)	2.37 (0.80-7.01)	BMI
Chen et al, 2006[9]	High ferritin (>58.5 ng/mL) vs low ferritin (≤58.5 ng/mL)	1.84 (0.95-3.58)	Age, ethnicity, parity, family history of diabetes in a first-degree relative, gestational age at blood collection, cigarette smoking, BMI.
Khambalia et al, 2015[46]	Ferritin (μg/L) examined as a continuous variable	1.41 (1.11-1.78)	Age, country of birth, parity, maternal weight, smoking during pregnancy, hypertensive disorders in pregnancy and C-reactive protein.
Sharifi et al, 2010[10]	High ferritin (>82.8 ng/mL) vs low ferritin (<82.8 ng/mL)	2.40 (1.20-6.80)	C-reactive protein, BMI, blood pressure, history of GDM and family history of diabetes.
Soubasi et al, 2010[44]	High ferritin (>60 ng/mL) vs low ferritin (<60 ng/mL)	10.00(1.09-91.8)	None.
Zein et al, 2015[45]	High ferritin (≥38.5 ng/mL) vs low ferritin (<38.5 ng/mL)	2.04 (0.66-6.30)	None.

BMI: body mass index; w: weeks.

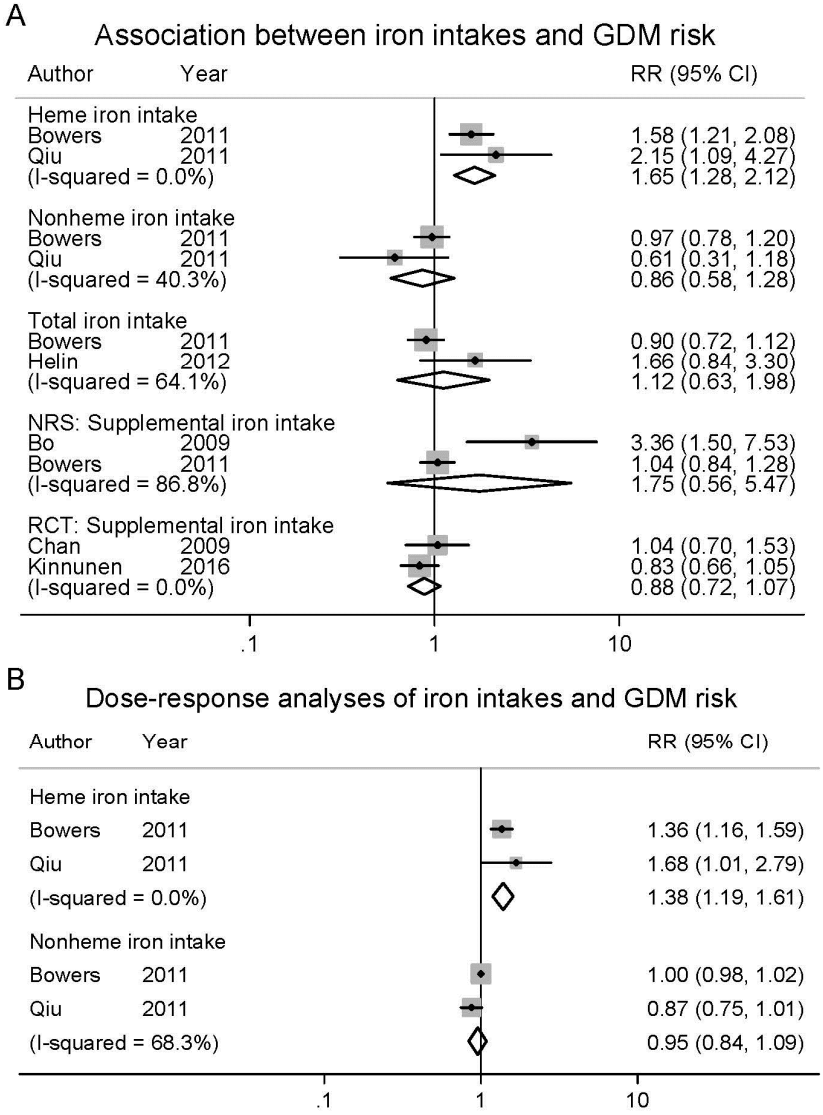

Figure 2. Associations between intakes of heme iron, nonheme iron, total iron, supplemental iron, and the risk of GDM in the included studies (A).Dose-response analyses of heme iron, nonheme iron intakes and the risk of GDM (B). The relative risks of GDM with every 1mg/day increment of heme iron intake, or 5mg/day increment of nonheme iron intake was plotted. The black dot, the horizontal line and the grey box represent the relative risks, 95% CI and the weight percentage of the corresponding study, respectively. The diamond shape represents the pooled relative risks and 95% CI. The random-effects model was adopted.

(I^2=64.1%). Nevertheless, after excluding participants with low hemoglobin levels (≤12 g/dL), the Helin et al study[15] observed a strong correlation between high total iron intake and GDM risk. A dose-response test was not performed for analysis of supplemental or total iron intakes because of insufficient categorical data.

Association between body iron stores and the risk of GDM

Six included studies assessed the risk of GDM between pregnant women with high ferritin (a major iron storage protein) concentration and low ferritin concentration in the blood (Table 2). Our meta-analysis detected evident association between GDM risk and high ferritin concentration. The pooled RR (95% CI) of GDM in women with higher levels of serum ferritin was 1.64 (95% CI: 1.27 to 2.11), compared with those with lower ferritin levels (Figure 3A). No evidence for significant heterogeneity (I^2=7.0%) or publication bias (Begg's test: p=0.260) was found in the included studies (Figure S1). Sensitivity analysis suggested that the RR between high ferritin and GDM incidence was even higher with the removal of the Khambalia et al study[46] (Figure S2). A dose-response test was not performed for analysis of body iron stores, because of insufficient categorical data.

Body iron status in GDM patients

We further compared the status of body iron between GDM and non-GDM pregnant women, with the data extracted from 13 studies. Both the serum concentrations of ferritin and iron were evidently higher in GDM patients (serum ferritin: mean difference=10.4, 95% CI: 4.09 to 16.7, unit: ng/mL; serum iron: mean difference=13.4, 95% CI: 0.92 to 25.9, unit: μg/dL), compared with non-GDM controls (Figure 3B, C). High heterogeneities were detected in the analyses (serum ferritin: I^2=93.6%; serum iron: I^2=88.4%), potentially due to varied sample selection and analytical techniques. Publication bias was detected by neither funnel plots (Figure S1) nor Begg's test (serum ferritin: p=0.466; serum iron: p=0.917). Sensitivi-

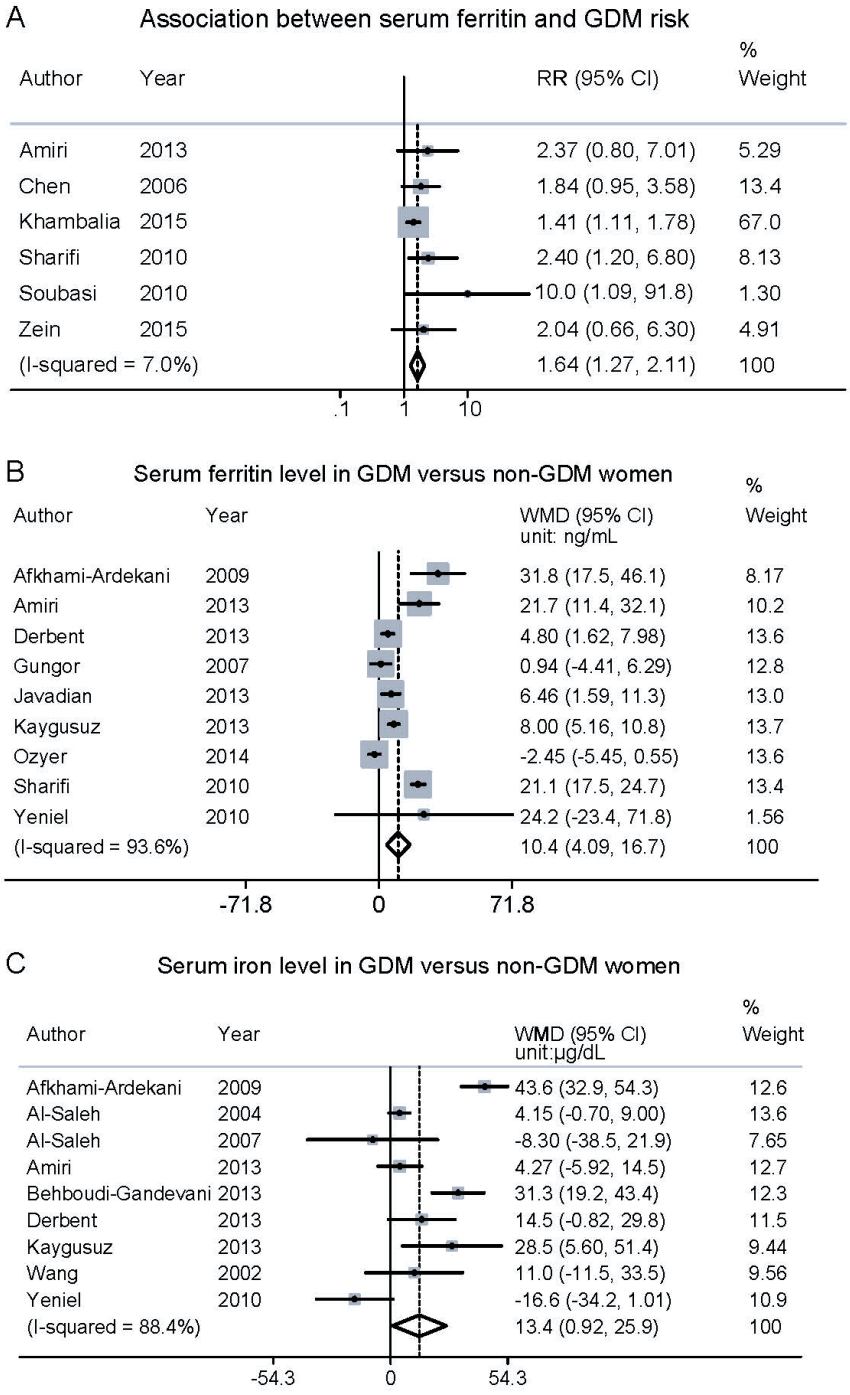

Figure 3. Associations between the concentration of serum ferritin and the risk of GDM in the included studies (A). Meta-analysis of studies comparing the concentrations of serum ferritin (B), and serum iron (C) between GDM and non-GDM women. The black dot, the horizontal line and the grey box represent the relative risks, 95% CI and the weight percentage of the corresponding study, respectively. A vertical dashed line indicates the location of the pooled effect estimate, and the diamond shape represents the pooled relative risk and its 95% CI. The random-effects model was adopted. Weighted mean difference was analyzed for serum ferritin and serum iron. WMD: weighted mean difference.

ty test detected no evident impact of any single study on the pooled results of both analyses (Figure S2).

In addition, two other studies examined the status of serum ferritin and serum iron in GDM patients. The Lao et al study[49] reported significantly higher levels of serum ferritin and iron in GDM patients, whereas the Maitland et al study[50] did not observe any difference of serum ferritin between GDM and non-GDM groups. These studies were not included in our analysis because mean and SD were not available in the results.

DISCUSSION

In this study, we observed that the risk of GDM development in pregnant women was positively associated with increased dietary intake of heme iron, as well as body iron stores. No significant relationship was detected between the risk of GDM and the intakes of nonheme iron, total iron or iron supplement.

Iron may participate in the development of diabetes in multiple ways. First of all, excessive iron stimulates the elevation of reactive oxygen species and oxidative stress,

which leads to cellular injury and apoptosis of β-cells.[51] Besides, animal studies have suggested that iron overload inhibits glucose oxidation in skeletal muscle, and results in increased insulin resistance and hepatic glucose production.[52] Furthermore, excessive iron may competitively inhibit the mobilization of chromium and reduce insulin sensitivity.[4]

Heme iron and nonheme iron come from different food sources. Heme iron is derived from hemoglobin and myoglobin in animal foods, such as red meat, seafood and poultry, whereas nonheme iron is found mainly in plant foods.[53] Our analyses concluded a positive relationship between heme iron intake and GDM risk. We found that every 1 mg/day increment of heme iron intake, which is approximately the content in a one-ounce portion of lean beef,[53] was associated with 38% higher risk of GDM. Consistent with our results, two cohort studies examined the association between GDM risk and red meat intake, and reported that women with more than one serving of red meat per day had increased risk of developing GDM, compared with women with less than a 0.2 serving/day.[54,55] In both studies, groups with the highest level of red meat intake also reported the highest levels of heme iron intake. We did not detect a significant relationship between GDM and the intake of nonheme iron. The cause for the different impact of heme and nonheme iron intakes on GDM development is not clear. One possible reason is that the absorption rates of heme iron and nonheme iron are different. Heme iron absorption ranges from 15% to 35%, with no significant inhibitors except calcium. In comparison, the absorption rate of nonheme iron ranges from 2% to 20%, and is influenced by many other components in the diet.[56]

The potential roles of iron supplements in GDM development is an issue with important clinical concerns. Pregnant women have a significantly increased demand for iron, because of physiological expansion of blood volume, fetal iron requirements, placental growth and delivery-associated iron loss.[57] Iron deficiency anemia was estimated to be presented in 19.2% of pregnant women worldwide, which was associated with increased prevalences of low birth weight, prematurity and perinatal mortality.[58] In 2012, the World Health Organization advised that all pregnant women should be provided with daily oral iron supplementation (30-60 mg of elemental iron) throughout pregnancy.[59] Although we did not detect significant association between supplemental iron intake and GDM risk in the meta-analysis, a clear conclusion cannot be drawn.

There are several problems in the previous studies analyzing the correlation between iron supplementation and GDM risk. First of all, the two major confounders - iron status and dietary iron intakes of participants -were not addressed in most studies.[13,16,47] This may be at least partially responsible for the inconsistent findings reported in previous studies. The Chan et al study[48] is the only trial which controlled for both confounders. Nevertheless, nearly half of their study population had taken additional supplements during the trial, which possibly included iron. Besides, the overall compliance rate in the Chan et al trial was low.[48] Moreover, in previous RCTs, a portion of women in the control group developed iron deficiency anemia and received iron supplement as clinically indicated.[47,48] These individuals were included in the final analysis of both studies, which may mask the impact of supplemental iron intake on GDM risk. Therefore, future RCT with improved experimental designs is warranted to determine the association between supplemental iron intake and GDM risk.

We examined the status of body iron through two iron hematological markers: serum ferritin and serum iron. Serum ferritin is an iron storage protein that is widely used for determining body iron stores. Nonetheless, serum ferritin is also an acute-phase protein, whose plasma concentrations increase in response to inflammation, infection, malignancies and liver disease.60 In comparison, serum iron measures the amount of circulating iron, the level of which can be decreased in circumstances such as chronic inflammation, through the function of hepcidin.[61,62] Our analysis observed increased levels of both serum ferritin and serum iron in GDM patients, suggesting the elevation of body iron status.

Our study had several limitations. Firstly, the number of available studies included in the analyses of iron intakes and GDM risk was small. Future studies are needed to better understand the impact of iron intakes on gestational glucose metabolism. Besides, the functions of dietary iron are difficult to differentiate from other food components. The intakes of heme iron are closely related to the intakes of red meat. Other nutrient factors in meat, such as fatty acids and animal protein, are potentially related to diabetes development and thus can be confounding factors. Nevertheless, our findings of the association between body iron stores and GDM, as well as the biological roles of excessive iron in oxidative stress, support the link between heme iron intake and GDM. Moreover, high heterogeneity exists in the tests comparing the concentrations of iron blood markers between GDM and non-GDM individuals. Factors such as analytical techniques or gestational age may contribute to high heterogeneity. However, the number of studies was not sufficient for a meta-regression analysis. Finally, most of the studies examining body iron status were conducted in Middle East populations, whereas the studies of iron intakes were mostly conducted in Western populations. Although the meta-analysis results from the two regions are consistent with each other, future studies in more diverse geographical regions and ethnic groups are anticipated.

Despite these limitations, our study holds significance in that it systematically reviewed the association between iron and GDM development. We investigated the influence of iron on GDM development through multiple perspectives, including iron intakes, iron stores and iron concentrations. We validated significant correlations between increased heme iron intakes and body iron status with GDM risk. Based on our findings, we have suggested that pregnant women should avoid intakes of excessive heme iron enriched food, especially for those with other known GDM risk factors, such as obesity, family history of diabetes and advanced maternal age. Future interventional studies with sufficient controls in dietary factors and body iron status are warranted to test the relationship between supplemental iron and GDM.

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AUTHOR DISCLOSURES

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Supplementary tables and figures

Table S1. Characteristics of the 2 studies included in the qualitative analysis

Author	Year	Country	Study design	Sample size[†]	Age	Assessment of iron intakes or iron status/ gestational age	Ascertainment of GDM/ gestational age	Reasons for not included in quantitative analysis
Lao et al	2001	China	Cohort	97/401	27-38	Ferritin (MEIA), serum iron (CMA)/ 28-31w	OGTT(WHO 1980)[‡]/28-31w	Did not present the SD of the means
Maitland et al	2014	UK	Case-control	29/77	31-36	Ferritin (CLA)/ first trimester	OGTT(IADPSG)[§]/28w	Did not present the mean and its SDs

CLA: chemiluminescence assay; CMA: colorimetric assay; MEIA: microparticle enzyme immunoassay; OGTT: tolerance test; w: weeks.
[†]Sample size: numbers of cases/controls for case-control studies; numbers of cases/participants for cohort studies.
[‡]World Health Organization expert committee on diabetes mellitus.Technical report series 646, Geneva, Switzerland, World Health Organization; 1980[65].
[§]IADPSG, international association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care, 33(3), 2010[64].

Table S2. Quality assessments of 21 non-randomized studies using the Newcastle-Ottawa scale

Author	Year	Selection				Comparability		Exposure			
Case-control studies											
		Case Definition	Representativeness of cases	Controls selection	Controls definition	Study controls for age	Study controls for other factors	Ascertainment of exposure	Same ascertainment	Non-response rate	Total score
Afkhami-Ardekani et al	2009	1	1	0	1	1	1	1	1	0	7
Al-Saleh et al	2004	1	1	0	1	1	1	1	1	0	7
Al-Saleh et al	2007	1	1	0	1	1	1	1	1	0	7
Amiri et al	2013	1	1	0	1	1	1	1	1	0	7
Derbent et al	2013	1	1	0	1	0	0	1	1	0	5
Gungor et al	2007	1	1	0	1	0	0	1	1	0	5
Javadian et al	2013	1	1	0	1	0	0	1	1	0	5
Kaygusuz et al	2013	1	1	0	1	1	0	1	1	0	6
Ozyer et al	2014	1	1	0	1	1	1	1	1	0	7
Sharifi et al	2010	1	1	0	1	1	1	1	1	0	7
Wang et al	2002	1	1	0	0	1	0	1	1	0	5
Yeniel et al	2012	1	1	0	1	1	1	1	1	0	7
Cohort studies											
		Representative-ness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	No outcome of interest at the start	Study controls for age	Study controls for other factors	Assessment of outcome	Long follow-up	Adequacy of follow up	
Behboudi-Gandevani et al	2013	1	1	1	1	1	1	1	0	1	8
Bo et al	2009	1	1	1	1	1	1	1	0	1	8
Bowers et al	2011	0	1	1	1	1	1	1	1	1	8
Chen et al	2006	1	1	1	1	1	1	1	0	1	8

Table S2. Quality assessments of 21 non-randomized studies using the Newcastle-Ottawa scale (cont.)

Cohort studies											
		Representative-ness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	No outcome of interest at the start	Study controls for age	Study controls for other factors	Assessment of outcome	Long follow-up	Adequacy of follow up	
Helin et al	2012	1	1	1	0	1	1	1	0	1	7
Khambalia et al	2015	1	1	1	1	1	1	1	0	1	8
Qiu et al	2011	1	1	1	1	1	1	1	0	1	8
Soubasi et al	2010	1	1	1	0	0	0	1	1	1	6
Zein et al	2015	1	1	1	1	1	1	1	0	1	8

The quality score of included studies was assessed by the Newcastle-Ottawa scale.[19] A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories and a maximum of two stars for Comparability.
For case-control studies:
Selection: 1) definition of cases: 1, adequate definition with independent validation; 0, definition as record linkage or self-reports, or no description on case definition. 2) representativeness of the cases: 1, consecutive or obviously representative series of cases; 0, potential for selection biases or not stated.3) selection of controls: 1, community controls; 0, hospital controls or no description.4) definition of controls: 1, no history of disease; 0, no description of source.
Comparability: 1) 1, study controls for maternal age; 1, study controls for any additional factor.
Exposure: 1) ascertainment of exposure: 1, secure ascertainment; 0, no description on exposure ascertainment. 2) same method of ascertainment for cases and controls: 1, yes; 0, no. 3) non-response rate: 1, same rate for both groups; 0, no description or different rates and no designation for case and controls.
For cohort studies:
Selection: 1) Representativeness of the exposed cohort: 1, truly representative or somewhat representative of the average pregnant women in the community; 0, selected group of users eg nurses, volunteers, or no description of the derivation of the cohort. 2) Selection of the non-exposed cohort: 1, drawn from the same community as the exposed cohort; 0, drawn from a different source, or no description of the derivation of the non-exposed cohort. 3) Ascertainment of exposure: 1, secure record (eg surgical records) or structured interview; 0, written self-report or no description. 4) Demonstration that outcome of interest was not present at start of study: 1, yes; 0, no.
Comparability: 1) Comparability of cohorts on the basis of the design or analysis: 1, study controls for maternal age; 1, study controls for any additional factor.
Outcome: 1) Assessment of outcome: 1, independent blind assessment or record linkage; 0, self-report or no description. 2) Was follow-up long enough for outcomes to occur: 1, yes: till delivery; 0, no. 3) Adequacy of follow up of cohorts: complete follow up or subjects lost to follow up unlikely to introduce bias; 0, low follow up rate, no description of those lost, or no statement.

Table S3. Quality assessments of 2 randomized controlled studies using the Cochrane Collaboration's tool for assessing risk of bias

Author	Year	Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
		Random sequence generation	Allocation con-cealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete out-come data	Selective reporting	Other sources of bias
Chan et al	2009	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Kinnunen et al	2016	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

The quality score of included studies was assessed by the Cochrane Collaboration's tool for assessing risk of bias. The definition of sources of bias and methods of assessing risk of bias can be found in Cochrane Handbook for Systematic Reviews of Interventions.[18]

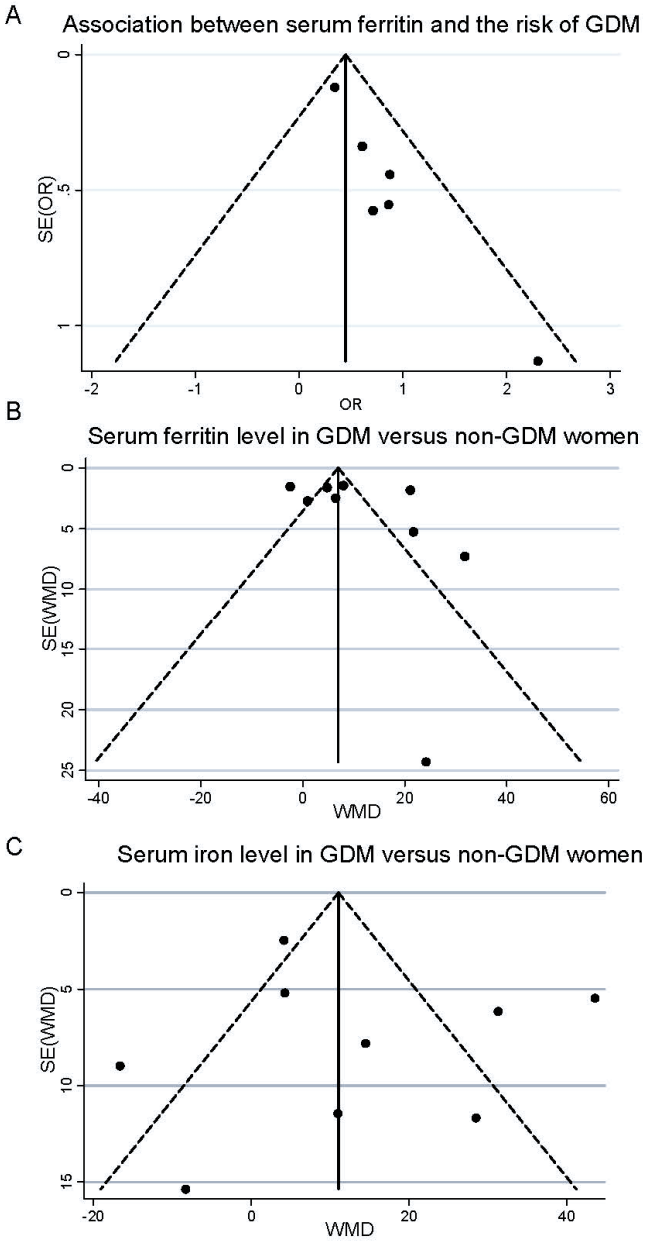

Figure S1. No evidence of publication bias is present in the dataset: Funnel plots with pseudo 95% confience limits for the identification of the publication bias of included studies. The RRs were plotted against their standard errors for studies reporting the associations between serum ferritin and the risk of GDM (A). The mean differences were plotted against their standard errors for studies examining the concentrations of serum ferritin (B) and serum iron (C) in GDM and non-GDM population. One black dot represents one included study. SE: standard error; WMD: weighted mean difference.

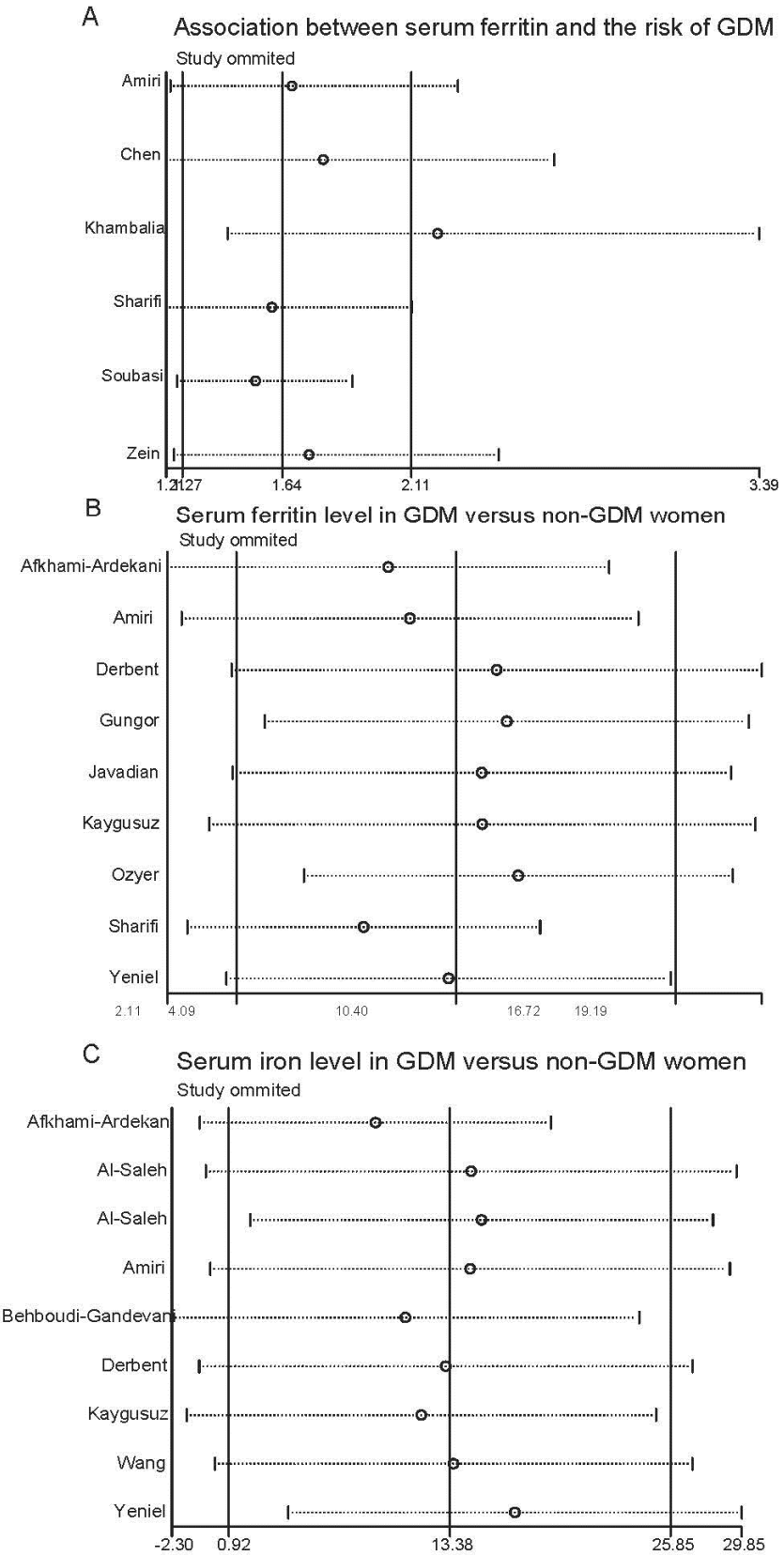

Figure S2. Sensitivity analysis for all included studies in the meta-analyses. The results of sensitivity test was shown for analyses of association between serum ferritin and GDM (A), and the analyses of concentrations of serum ferritin (B) and serum iron (C) in GDM and non-GDM population. In each panel, one individual study was omitted from the pooled analysis in turn, to check its influence on the total results. One circle and its corresponding horizontal dashed line represent the effect size and its 95%CI, after one corresponding study was omitted. The three vertical lines display the positions of the effect size, the upper and lower limits of 95% CI of the pooled results.