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High maternal iron status, dietary iron intake and iron supplement use in pregnancy and risk of gestational diabetes mellitus: In-house study and systematic review.

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ABSTRACT

Background: High iron measured using dietary and serum biomarkers have been associated with type 2 diabetes; however it is uncertain whether a similar association exists for gestational diabetes mellitus (GDM).

Objectives: To conduct a cohort study examining first trimester body iron stores and subsequent risk of GDM and to include these findings in a systematic review of all studies examining the association between maternal iron status, iron intake (dietary and supplemental) and the risk of GDM.

Methods: Serum samples for women with first trimester screening were linked to birth and hospital records for data on maternal characteristics and GDM diagnosis. Blood was analysed for ferritin, soluble transferrin receptor (sTfR) and C-reactive protein (CRP). Associations between iron biomarkers and GDM were assessed using multivariate logistic regression. A systematic review and meta-analysis, registered with PROSPERO (CRD42014013663) included all studies published in English from Jan 1995 to March 2014 that examined the association between iron and GDM and included an appropriate comparison group.

Results: Of 3, 776 women, 3.4% subsequently developed GDM. Adjusted analyses found increased odds of GDM for ferritin (OR 1.41; 95% CI: 1.11, 1.78) but not for sTfR (OR 1.00, 95% CI: 0.97, 1.03) levels. Two trials of iron supplementation in early pregnancy found no association with GDM. Increased risk of GDM was associated with higher levels of maternal ferritin and serum iron and dietary heme iron intakes.

Conclusions: Increased risk of GDM among women with high serum ferritin and iron levels and dietary heme iron intakes warrants further investigation.

INTRODUCTION

Iron is a transitional metal that is essential for several physiological functions in the body (a micronutrient), but excessive levels can be pathological.[1] The role of iron in diabetic pathogenesis was first identified by increased rates of diabetes (25-60%) in individuals with hereditary haemochromatosis, an inherited iron overload syndrome.[2] Even in the absence of significant iron overload, studies in general populations have found that high dietary intakes of red meat and heme iron (animal sources) are associated with risk of type 2 diabetes[3-5] and that moderately elevated ferritin levels (biomarker of iron stores) are associated with increased insulin secretion, decreased insulin sensitivity and type 2 diabetes.[3,4,6]

The association between excess iron and type 2 diabetes mellitus has lead to concern that these may also affect gestational diabetes mellitus (GDM). In pregnant women, there has been concern that high intakes of supplemental iron by iron-replete pregnant women may lead to increased amounts of unabsorbed iron in the intestine and result in local oxidative stress,[7] damage of pancreatic beta cells, increased insulin resistance and subsequently the development of diabetes.[8]

Results from previous studies examining the risk of GDM in relation to elevated iron, measured as iron intakes (dietary and supplemental)[9-11] or serum ferritin levels have been inconsistent.[12-17] Differences in study findings may be due to differences in study populations, timing of data collection for iron exposure, thresholds for elevated iron levels, and adjustment for confounders including inflammation.

While there have been a number of systematic reviews examining the association between iron intake, body iron stores and the risk of type 2 diabetes in general populations,[3,4,6] to our

knowledge, a systematic review has not yet been performed that examines maternal serum iron biomarkers, iron intake and risk of GDM. Therefore, the aims of this study were twofold: i) to conduct a cohort study examining first trimester body iron stores and subsequent risk of GDM and; ii) to systematically review studies of all designs examining the association between maternal iron status, iron intake (dietary and supplemental), and the risk of GDM.

METHODS

In-house study

The study population included pregnant women who attended first trimester Down syndrome screening between January and October 2007 and had their sera analyzed and subsequently archived by Pathology North laboratory, a state-wide public screening service in New South Wales, Australia.

For this study, sera were thawed and analyzed to assess serum ferritin (µg/L), soluble transferrrin receptor (sTfR; nmol/L) and C-reactive protein (CRP; mg/L) using commercial assays. Serum ferritin was measured using a solid phase direct sandwich ELISA method (Calbiotech, Inc, CA, USA) with an interassay CV of 6.2%. sTfR was measured using an enzyme-linked immunosorbent assay (Quantikine IVD, Human sTfR Immunoassay, R & D Systems, Minneapolis, MN, USA) with an interassay CV of 6.4%. CRP was measured using the quantitative sandwich enzyme immunoassay technique (QUANTIKINE™, Minneapolis, USA) with an interassay CV of 13.3%.

Maternal information and first trimester screening results derived from the laboratory database were combined via record linkage with women's corresponding health records from

routinely collected birth and hospital databases to obtain information on their pregnancy and infant outcomes. 'Birth data' were sourced from the NSW Perinatal Data Collection (PDC) and 'hospitalisation data' from the NSW Admitted Patients Data Collection (APDC). The PDC is a statutory population-based collection of all births in NSW of at least 400 grams birth weight or at least 20 weeks of gestation, and includes information on maternal and infant characteristics, pregnancy, labor, delivery and infant characteristics at birth. The APDC is a census of all admissions in NSW public and private hospitals. Up to 50 diagnosis and procedures for each separation are coded according to the 10th revision of the International Classification of Diseases, Australian Modification (ICD-10-AM).[18] Only variables known to be reliably reported in birth and/or hospital data were included in the analysis. For these variables, reporting in both datasets had high specificity (>99%) indicating few false positive reports and validation studies of the PDC and the APDC showed excellent level of agreement with the hospital medical record and low rates of missing data.[19,20] The NSW Centre for Health Record Linkage (CHeReL) performed probabilistic record linkage between the three datasets.[21] The CHeReL assesses the linkage quality for each study and, for this study, reported <5/1000 missed links and <2/1000 false positive links. Only de-identified data were provided to the researchers. The study was approved by the NSW Population and Health Services Research Ethics Committee (HREC/09/CIPHS/52).

The primary outcome was gestational diabetes mellitus (GDM). Information on GDM and pre-existing diabetes were identified from hospital records based on diagnosis by the attending clinician.[20,22,23] Validation studies of the hospital data indicate 69%-96% ascertainment of GDM with few false positives, and 100% ascertainment of pre-existing diabetes with no false positives.[20,22] These sensitivity and specificity values are in keeping

with those reported by other international validation studies of birth and hospital records for identification of GDM and pre-existing diabetes.[23]

The primary exposure of interest was serum ferritin. As there is no established cut-off for elevated ferritin levels, we assessed serum ferritin concentrations as a continuous measure and using three cut-offs based on the highest tertile (>66th percentile), highest quartile (>75th percentile) and highest quintile (>80th percentile). These cut-offs were selected to allow us to compare our results with those used in previous GDM studies.[15,16]

Explanatory variables included in the analyses were: maternal age, country of birth, parity, maternal weight, smoking during pregnancy, hypertensive disorders of pregnancy and CRP concentrations. Countries of birth classified as high-risk for GDM included Oceania, Southern and Eastern Europe, Middle East and North Africa, South-East Asia, Southern and Central Asia.[24] Maternal postcode of residence was used to derive an indicator of socioeconomic status (SES). An Index of Relative Disadvantage, produced by the Australian Bureau of Statistics, was assigned to each postcode and quintiles produced with women in the lowest 20th percentile classified as most disadvantaged. Hypertensive disorders in pregnancy were defined as women with the onset of hypertension from 20 weeks gestation including gestational hypertension, preeclampsia and eclampsia.[25]

Descriptive statistics were used to assess maternal and pregnancy characteristics as well as iron and inflammatory biomarker concentrations by GDM status. The distribution of biomarkers was assessed and those without normal distribution (ferritin and CRP) were log-transformed. Multivariate logistic regression analysis was performed to determine the association between serum ferritin and sTfR concentrations and risk of GDM adjusting for

explanatory variables and CRP levels. Results are presented using odds ratio (OR) and 95% confidence intervals (CI). Statistical analyses were performed using SAS for Windows version 9.3 (SAS Institute Inc, Carey, North Carolina).

Systematic review and meta-analyses

The systematic review and meta-analysis was conducted and reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA),[26] and where applicable, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines.[27] This systematic review has been registered with the international prospective register of systematic reviews (PROSPERO) as number CRD42014013663.

We searched PubMed, MEDLINE and EMBASE and CINAHL from 01 January 1995 to 01 March 2014. Electronic searches combined keyword and MeSH search terms related to diabetes, gestational, hyperglycemia, glucose, insulin, iron, and iron-binding proteins. We also reviewed the reference lists of identified articles. The search was restricted to studies of humans, and those published in English. Full search strategies are provided in **Supplementary Table 1.**

To be included, studies had to restrict their study population to pregnant women, defined GDM as the primary outcome, include an appropriate control or comparison group, and examine iron as the exposure of interest. Excess or additional iron could be defined as an intervention (i.e. iron supplement) or measured using at least one iron biomarker or dietary intake data. Eligible study designs included trials, cohort, case-control and cross-sectional studies. Two investigators (AK, PN) independently evaluated the eligibility of all retrieved studies. Where there was disagreement at this stage, the article remained included until the

full text was reviewed. Each full text article was assessed independently by two investigators using the aforementioned inclusion criteria and any disagreement regarding eligibility of an article was discussed to reach agreement by consensus. Where information pertinent to the inclusion criteria was not reported in the article, efforts were made to contact the listed corresponding author. Where no reply was received, the article was excluded.

Data were extracted by two investigators (AK, AA) on the article (author, publication year, journal name), study characteristics (study design, geographical location, population source, duration of follow-up), participant characteristics (sample size, age, number of GDM events, numbers of controls), assessment of iron (dietary intakes, plasma/serum, dose/frequency of iron supplement), ascertainment of GDM, measurement and adjustment for inflammatory markers such as C-reactive protein (CRP), statistical methods used for the analysis, comparison group, risk estimates and 95% CIs, and any covariates that were matched on, or adjusted for, in the multivariate analyses.

Quality assessment was conducted by two independent reviewers (AK, AA) using standardised measures. Randomised studies were evaluated using the Cochrane Collaboration's tool for assessing risk of bias.[28] This tool provides a model to evaluate the risk of bias across a number of domains: how a study selects participants, measures performance, blinds participants and investigators, explores attrition, and reports findings. Risk of bias for each domain was allocated a ranking of "low" (score=2), "unclear" (score=1), or "high" (score=0). Study quality for cohort and case-control studies was assessed using the nine-star Newcastle–Ottawa Scale for observational studies.[29] Independent scores from each assessor were averaged and expressed as a percentage.

Study findings were summarized descriptively by study design and where possible sub-group comparisons presented in tabular form for studies with comparable exposure measurements and reporting of findings. Descriptive and outcome data from the in-house study were included for comparison with other iron biomarker studies. Iron biomarker concentrations were converted to the same units (mean values) and mean differences were computed between cases and controls for each study so that data could be summarized using forest plots. Forest plots were performed for all studies with available data and in sub-groups based on common factors. The extent of heterogeneity was measured using I^2 statistic, a measure of the proportion of total variability explained by heterogeneity and expressed as a percentage of heterogenity, with 0–40% indicating might not be important, 30-60% indicating may represent moderate heterogenity, 50-90% indicating may represent substantial heterogenity and 75-100% indicating considerable heterogenity.[30] Data were analysed using RevMan, version 5.3.

RESULTS

In-house study

A total of 3, 776 women were included in the analysis after excluding 124 women with pre-existing diabetes, a twin pregnancy, medical abortion, infant with a major congenital anomaly or an undetectable ferritin measurement. There were 129 women (3.4%) diagnosed with GDM and 3, 647 women who were not diagnosed with GDM. Women with GDM were more likely to be older, from a country identified as high risk for GDM, heavier and diagnosed with hypertensive disorders of pregnancy (**Table 1**). They also had significantly higher median serum ferritin concentrations (32.8 vs. 24.8 μg/L, P=0.001) and were less likely to have iron deficiency (<12 μg/L) (9.3% vs. 19.9%, P=0.003). Women with GDM were also more likely to have CRP levels >90th percentile (2.8 mg/L) indicative of inflammation

(14.8% vs. 9.5%, P=0.05). There were no differences between GDM and non-GDM women in median TfR concentrations (15.7 vs. 15.1 nmol/L, P=0.11).

Results for univariate and multivariate logistic regression analyses examining the odds of GDM for maternal serum ferritin and sTfR concentrations are presented in **Table 2**. Multivariate analyses found increased risk of GDM when ferritin was examined as a continuous variable (adjusted odds ratio (AOR): 1.41; 95% CI: 1.11, 1.78) and when elevated ferritin was defined using the highest tertile (≥35 µg/L) (AOR 1.60, 95% CI: 1.03-2.49). Increased odds of GDM but did not reach statistical significance for ferritin levels defined as the highest quartile (≥43 µg/L) (AOR: 1.39; 95% CI: 0.93, 2.06) or the highest quintile (≥48 µg/L) (AOR: 1.43, 95% CI: 0.95, 2.16). There was no association between sTfR concentrations and GDM (AOR 1.00, 95% CI: 0.97, 1.03).

Systematic review and meta-analyses

Of 714 citations identified in the search strategy, 18 articles met the inclusion criteria (**Figure 1**). There were two randomized controlled trials, three cohort studies and 13 case-control studies. Tables 3a-c presents study characteristics and quality assessment scores categorised by different measurements of iron exposure. Both trials assessed iron supplement use in early pregnancy and found no association with risk of GDM (**Table 3a**).[10,31] The Chan et al. trial was well-designed and scored high (92%) in the quality assessment.[10] The other trial scored poorly on the quality assessment (58%) and did not report on method of randomization or blinding, suffered from considerable loss to follow-up and measured the primary outcome of GDM using a questionnaire.[32]

There were three cohort studies which examined dietary iron intake during pregnancy and risk of GDM (**Table 3b**).[9,11,33] All three studies had moderate to high quality assessment scores (72-95%). Each of these studies used a different threshold for elevated dietary iron intake. The Finnish study by Helin et al. found no significance difference in total iron intake using >80 percentile cut-point (AOR: 1.66, 95% CI: 0.84 - 3.30).[11] The other two studies found a significant association for dietary heme iron intake but not for non-heme iron.[9,33] Bowers et al., using data from the Nurses' Health Study in the USA, found the adjusted relative risks (RR) (95% CIs) of GDM across increasing quintiles of heme iron were 1.0 (lowest reference), 1.11 (0.87, 1.43), 1.31 (1.03, 1.68), 1.51 (1.17, 1.93), and 1.58 (1.21, 2.08), respectively (P for linear trend <0.0001). For every 0.5-mg per day of increase in iron intake, the adjusted RR of GDM increased by 1.22 (95% CI: 1.10, 1.36).[9] Qiu, 2011, using data from the Omega Study also conducted in the USA, found adjusted RR across increasing quartiles of heme iron were 1.0 (reference), 1.27 (0.77, 2.09), 1.41 (95% CI: 0.81, 2.44) and 2.15 (95% CI: 1.09, 4.27), respectively (P for linear trends 0.04).[33] The multivariate adjusted RR for GDM associated with 1-mg per day increase in heme iron intake was 1.51 (95% CI: 0.99, 2.36).

There were 13 case-control studies which examined the association between high serum iron levels and GDM (**Table 3c)**. The quality assessment scores varied; with only three studies scoring higher than 75%.[16,34,35] Only one case-control study measured serum ferritin in early pregnancy,[15] and two studies[15,16] assessed an inflammatory biomarker, which in both studies was CRP. Overall, limitations of these studies included non-representative study populations from a single clinic or hospital setting; small sample sizes ranging from 6 to 64 GDM cases, inconsistent thresholds for elevated ferritin levels and lack of data on other iron and inflammatory biomarkers.

Most iron biomarker studies used descriptive statistics to compare mean or median biomarker concentrations in GDM and non-GDM pregnancies; with inconsistent findings across studies (**Table 4**).[12-17] Only 3 of the 14 studies adjusted for confounders.[14-16] Behboudi-Gandevani et al. found serum iron levels examined on a continuous scale were associated with GDM (AOR 1.01, 95% CI 1.00–1.01).[14] Chen et al. found women with the highest tertile of ferritin levels (≥92.1 μgl/L) were not significantly at increased risk of GDM (adjusted odds ratio (AOR 1.88, 95% CI 0.81–4.36),[15] and Sharifi et al. found women in the highest quartile of serum ferritin (>84.7 μgl/L) had a greater than two-fold increased risk of GDM (AOR 2.30, 95% CI 1.06–5.10).[16]

Including serum ferritin data from the in-house study, results from pooled analyses reveal that women with GDM had higher concentrations of serum ferritin (mean $\text{difference}_{\text{pooled}}$ 23.6 pmol/L, 95% CI 21.1, 26.1, I^2: 96%, test for overall effect P<0.00001) (**Figure 2a**). To examine heterogeneity, pooled analyses of serum ferritin concentrations were performed excluding 3 studies that had a quality assessment score <70%[31] or that had a high proportion of overweight/obese participants.[15,16] Exclusion of these studies decreased heterogeneity from 96% to 49% but did not change the direction of the pooled analysis (mean $\text{difference}_{\text{pooled}}$ 13.5 pmol/L, 95% CI 8.8, 18.2, I^2: 49%, test for overall effect P<0.00001) (**Figure 2b**).

Results from pooled analyses of serum iron concentrations reveal that women with GDM had higher concentrations of serum iron concentrations (mean $\text{difference}_{\text{pooled}}$ 208.2 mcg/L, 95% CI 152.4, 264.0, I^2: 89%) (**Figure 3)**. For serum iron concentrations, attempts to decrease heterogeneity between pooled analyses by excluding studies with low quality assessment

scores or by conducting separate pooled analyses by timing of blood sampling (<20 vs. ≥20 weeks gestation) relatively little change in the high I^2 measure. When studies with quality scores ≤60% were excluded, the mean difference for pooled analyses for serum iron concentrations was 224.2 mcg/L (95% CI 165.8, 282.6, I^2: 90%). The mean difference in pooled analyses was 159.9 mcg/L (95% CI 60.3, 259.5, I^2: 95%) for studies that collected blood samples before 20 weeks gestation and 257.9 mcg/L (95% CI 185.8, 330.1, I^2: 90%) for studies that collected blood samples ≥20 weeks gestation.

Of the case-control studies that assessed iron biomarkers other than serum ferritin and serum iron, 3 studies found no differences between GDM and non-GDM women in the concentrations of transferrin,[31,35,36] transferrin saturation,[36] or total iron-binding capacity (TIBC),[35] and 3 studies found unadjusted associations between iron biomarkers (i.e. transferrin, transferrin saturation levels or TIBC) and GDM indicative of excess iron (**Table 3c**).[12,37,38] The study by Derbent et al. found that ferritin, serum iron and hepcidin levels measured at 24- 28 weeks of gestation were significantly higher among GDM women.[34] The study also found that body mass index was higher in women with GDM women and was closely correlated with hepcidin levels; however, CRP and white blood cells (WBC) were not correlated to hepicidin levels. Hepcidin was not correlated to ferritin or serum iron and was positively correlated to parameters of glucose metabolism (fasting blood glucose, fasting insulin level and glucose value response to glucose challenge test).

DISCUSSION

A better understanding of whether elevated iron increases risk of GDM is needed to identify high risk pregnancies that could benefit from early intervention. This study reports on the association between maternal iron status, iron intake (dietary and supplemental) and the risk

of GDM. Results from our in-house study indicate that elevated ferritin concentrations are associated with increased risk of developing GDM. We did not find an association between sTfR concentrations and GDM. Serum levels of this soluble form of TfR are directly proportional to the tissue TfR concentration and have been proposed as a novel marker of iron status that is not affected by the presence of inflammation.[39] However, there is contrary evidence which shows that sTfR concentrations may in fact be impacted by inflammation.[40] Studies have reported lower sTfR concentrations in patients with inflammation associated with cancer,[41] malaria,[42] and HIV.[43] To strengthen the results from our in-house study, we performed a comprehensive systematic review of the literature and incorporated results from our in-house study with other biomarker studies examining the association between iron and GDM.

Results from our meta-analysis of iron biomarker studies found that higher ferritin and serum iron concentrations were associated with GDM. The ferritin results are consistent with those from our in-house study. The higher serum iron concentrations among women with GDM suggest that excess iron is associated with GDM. Serum iron levels increase during iron overload and decrease during infection and inflammation because iron is trapped inside macrophages.[44] Pooled analyses found a large amount of heterogeneity that was not explained after sub-group analyses were performed by study quality or timing of blood sampling. One possible explanation for the heterogeneity is the diurnal variation; serum iron concentrations vary considerably among individuals within a single day. While pooled analyses showed high heterogeneity, the pooled result was consistent and in the same direction across sub-group analyses.

Only one study examined hepcidin, the key regulator of iron homeostasis and found that GDM women had significantly higher hepcidin levels.[34] These findings suggest hepcidin

synthesis was increased as a response to increased iron rather than inflammation.[40] Derbent et al. also found that inflammatory biomarkers, CRP and white blood cells, were not correlated to hepicidin levels and that hepcidin was positively correlated to parameters of glucose metabolism (fasting blood glucose, fasting insulin level and glucose value response to glucose challenge test). This was the only study to examine maternal serum hepcidin values in women with GDM, therefore additional studies in other study populations are needed to replicate these findings. Furthermore, the study shared many of the limitations of other biomarkers studies, including: non-representative study populations; restricted to single clinic or hospital settings; small sample sizes, not adjusting for confounders and measuring iron biomarkers at the time of GDM diagnosis.

To further our examination of the association between iron and GDM, we also included all study designs in our systematic review and identified two iron supplementation trials and three dietary iron studies. The two trials of iron supplementation in early pregnancy found no association with GDM. Possible explanations for their null findings include short exposure times to iron supplements, exposure to low doses of supplemental iron, and lack of data on inflammation or dietary iron intakes. The results from two high quality dietary iron intake studies support an increased risk of GDM among pregnant women consuming high levels dietary heme but not non-heme iron intake. It has been proposed that under certain conditions, such as inflammation, ingestion of excess iron in certain forms (i.e. processed meats) may be hazardous.[3,5] Dietary advanced glycation endproducts (AGEs) which are present in large amounts of processed red meats and in high-fat animal products have been associated with development of diabetes [5] and increased inflammatory plasma cytokines in diabetic subjects. [45] The link between AGEs and diabetes may explain why these and other

general population studies[3-5] show high dietary intakes of red meat and heme iron (animal sources) are associated with risk of diabetes but not with total dietary iron or non-heme iron.

Strengths of this study include an in-house study and a comprehensive systematic review on the broader research question. Strengths of our in-house study include a large sample size, measurement of biomarkers in early pregnancy and prior to GDM, measurement of iron biomarker in addition to ferritin as well as an inflammatory biomarker and adjustment for a range of important confounders. Limitations include lack of data on maternal anemia, iron supplement use, maternal diet, and insulin and glucose concentrations. Strengths of the systematic review include an extensive and systematic literature search, use of explicit inclusion criteria, a standardised approach for extracting data and assessment of study quality by at least two of the authors. Limitations include restricting our search strategy to published studies and those in the English language.

In summary, a review of the literature to date indicates that increased risk of GDM does not result from short exposures to iron supplements during pregnancy but is associated with higher intakes of dietary heme iron during the preconceptional and early pregnancy period. Evidence from biomarker studies suggests that elevated iron biomarkers in GDM women reflect a response to excess iron rather than inflammation. Further studies are warranted that better characterise iron's role in the pathophysiological pathways that lead to GDM, that measure and compare multiple iron biomarkers in combination with dietary and supplemental sources of iron, and that identify high-risk populations for intervention studies.

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Contribution to authorship

AZK, CLR, NN conceived and designed the study; AZK, AA, PN, CLR, JM, KP, VT, NN acquired data; AZK was responsible for the integrity of data and statistical analysis; AZK drafted the manuscript; and all authors approved the manuscript and critically reviewed the manuscript for important intellectual content.

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Declaration of Competing Interests

None of the authors have a conflict of interest to declare.

REFERENCES

1. Fernández-Real J, López-Bermejo, A., Ricart, W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348-54.

2. Swinkels D, Janssen, MC., Bergmans, J., et al. Hereditary hemochromatosis: genetic complexity and new diagnostic approaches. Clin Chem 2006;52:950-68.

3. Bao W, Rong, Y., Rong, S., et al. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. BMC Med 2012;10:119-29.

4. Kunutsor S, Apekey, TA., Walley, J., et al. Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. Diabetes Metab Res Rev 2013;29:308-18.

5. White D, Collinson, A. Red meat, dietary heme iron, and risk of type 2 diabetes: The involvement of advanced lipoxidation endproducts. Adv Nutr 2013;4:403-11.

6. Liu Q, Sun, L., Tan, Y., et al. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. Curr Med Chem 2009;16:113–29.

7. Casanueva E, Viteri, FE. Iron and oxidative stress in pregnancy. J Nutr 2003;133:1700-8.

8. Simcox J, McClain, DA. Iron and diabetes risk. Cell Metab 2013;17:329-41.

9. Bowers K, Yeung, E., Williams, MA., Qi, L., Tobias, DK., Hu, FB., Zhang, C. A prospective study of prepregnancy dietary iron intake and risk for gestational diabetes mellitus. Diabetes Care 2011;34:1557-63.

10. Chan K. et al. Iron supplement in pregnancy and development of gestational diabetes—a randomised placebo-controlled trial. BJOG 2009;116:789-97.

11. Helin A, Kinnunen, TI., Raitanen, J., Ahonen, S., Virtanen, SM., Luoto, R. . Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study. BMJ Open 2012;doi: 10.1136/bmjopen-2012-001730.

12. Afkhami-Ardekani M, Rashidi, M. Iron status in women with and without gestational diabetes mellitus. J Diabetes Complications 2009;23:194-8.

13. Amiri F, Basirat, Z., Omidvar, S. et al. Comparison of the serum iron, ferritin levels and total iron-binding capacity between pregnant women with and without gestational diabetes. J Nat Sci Biol Med 2013;4:302-5.

14. Behboudi-Gandevani S, Safary, K., Moghaddam-Banaem, L. et al. The relationship between maternal serum iron and zinc levels and their nutritional intakes in early pregnancy with gestational diabetes. Biol Trace Elem Res 2013;1547:7-13.

15. Chen X, Scholl, TO., Stein, TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: The Camden study. Diabetes Care 2006;29:1077-82.

16. Sharifi F, Ziaee, A., Feizi, A. et al. . Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. Diabetes Metab Syndr Obes 2010;1:413-9.

17. Soubasi V, Petridou, S., Sarafidis, K. et al. Association of increased maternal ferritin levels with gestational diabetes and intra-uterine growth retardation. Diabetes Metab 2010;36:58-63.

18. National Centre for Classification in Health. Australian Coding Standards for ICD-10-AM and ACHI, 5th edn. Sydney: National Centre for Classification in Health, University of Sydney. 2004.

19. Roberts C, Cameron, CA., Bell, JC et al. Measuring maternal morbidity in routinely collected health data: development and validation of a maternal morbidity outcome indicator. Med Care 2008;46:786–94.

20. Taylor L, Travis, S., Pym, M., Olive, E., Henderson-Smart, DJ. How useful are hospital morbidity data for monitoring conditions occurring in the perinatal period? . Aust N Z J Obstet Gynaecol 2005;45:36-41.

21. Lawrence G, Dinh, I., Taylor, L. The centre for health record linkage: a new resource for health services research and evaluation. HIM J 2008;37:60-2.

22. Bell J, Ford, JB., Cameron, CA., Roberts, CL. The accuracy of population health data for monitoring trends and outcomes among women with diabetes in pregnancy. Diabetes Res Clin Pract 2008;81:105-9.

23. Lain S, Hadfield, RM., Raynes-Greenow, CH., Ford, JB., Mealing, NM., Algert, CS. Quality of data in perinatal population health databases: a systematic review. Med Care 2002;50:e7-20.

24. AIHW: Holdenson Z, Catanzariti L, Phillips G & Waters AM 2003. A picture of diabetes in overseas-born Australians. Bulletin No. 9. AIHW Cat. No. AUS 38. Canberra: AIHW.

25. Brown M, Lindheimer, MD., de Swiet, M., Van Assche, A., Moutquin, JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 2001 2001;20:IX–XIV.

26. Liberati A, Altman, DG., Tetzlaff, J., Mulrow, C., Gøtzsche, PC., et al. . The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLoS Med 2009;6:e1000100. doi:10.1371/journal.pmed.

27. Stroup D, Berlin, JA., Morton, SC., Olkin, I., Williamson, GD., Rennie, D., Moher, D., Becker, BJ., Sipe, TA., Thacker, SB. Meta-analysis of observational studies in

epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008-12.

28. Hróbjartsson A, Boutron, I., Turner, L., Altman, DG., Moher, D. Assessing risk of bias in randomised clinical trials included in Cochrane Reviews: the why is easy, the how is a challenge Cochrane Database of Systematic Reviews 2013;4:ED000058. dx.doi.org/10.1002/14651858.ED000058.

29. Wells G, Shea, B., O'Connell, D., et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Website accessed on June 12, 2014 2011;http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

30. Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.0.2 [updated September 2009]. The Cochrane Collaboration, 2009. Available from www.cochrane-handbook.org.

31. Gungor E, Danisman, N., Mollamahmutoglu, L. Maternal serum ferritin and hemoglobin values in patients with gestational diabetes mellitus. Saudi Medical Journal 2007;28:478-80.

32. Ouladsahebmadarek E, Sayyah-Melli, M., Taghavi, S., Abbasalizadeh, S., Seyedhejazie, M The effect of supplemental iron elimination on pregnancy outcome. Pakistan Journal of Medical Sciences 2011;27:641-5.

33. Qiu C. et al. A prospective study of maternal serum C-reactive protein concentrations and risk of gestational diabetes mellitus. Paediatr Perinat Epidemiol 2004;18:377-84.

34. Derbent A, Simavli, SA., Kaygusuz, I. et al. . Serum hepcidin is associated with parameters of glucose metabolism in women with gestational diabetes mellitus. J Matern Fetal Neonatal Med 2013;26:1112-5.

35. Yeniel A, Ergenoglu, AM., Sanhal, CY., Sahin, C., Ulukus, M., Oztekin, K. Does high maternal fi rst trimester iron status have an eff ect on the 50 g oral glucose test? Journal of Obstetrics and Gynaecology 2012;32:332–4.

36. Lao T, Tam, K. Maternal serum ferritin and gestational impaired glucose tolerance. Diabetes Care 1997;20:1368-69.

37. Kaygusuz I, Gumus, II., Yilmaz, S., Simavli, S., Uysal, S., Derbent, AU et al Serum levels of visfatin and possible interaction with iron parameters in gestational diabetes mellitus. Gynecol Obstet Invest 2013;75:203-9.

38. Lao T, Chan, PL., Tam, KF. Gestational diabetes mellitus in the last trimester: a feature of maternal iron excess? . Diabet Med 2001;18:218-23.

39. Chang J, Bird, R., Clague, A., Carter, A Clinical utility of serum soluble transferrin receptor levels and comparison with bone marrow iron stores as an index for iron-deficient erythropoiesis in a heterogeneous group of patients. Pathology 2007;39:349–53.

40. Thurnham D, McCabe, GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization. Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva, World Health Organization, 2012.

41. Feelders R, Vreugdenhil, G., Eggermont, AM., Kuiper-Kramer, PA., et al. Regulation of iron metabolism in the acute-phase response: interferon-g and tumor necrosis factor-a induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest 1998;28:520–7.

42. Beesley R, Filteau, S., Tomkins, A., Doherty, T., Ayles, H., et al Impact of acute malaria on plasma concentrations of transferrin receptors. Trans R Soc Trop Med Hyg 2000 94:295-8.

43. Rawat R, Stoltzfus, RJ., Ntozini, R., Mutasa, K., Iliff, PJ., Humphrey, JH. Influence of inflammation as measured by alpha-1-acid glycoprotein on iron status indicators among HIV-positive postpartum Zimbabwean women. Eur J Clin Nutr 2009;63:787-93.

44. Johnson E, Wessling-Resnick, M. Iron metabolism and the innate immune response to infection. Microbes Infect 2012;14:207-16.

45. Vlassara H, Cai, W., Crandall, J., et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci 2002;99:15596–601.

46. Akhlaghi F, Bagheri, SM., Rajabi, O. A comparative study of relationship between micronutrients and gestational diabetes. SRN Obstet Gynecol 2012;doi: 10.5402/2012/470419.

47. Al-Saleh E, Nandakumaran, M., Al-Shammari, M., Al-Harouny, A Maternal-fetal status of copper, iron, molybdenum, selenium and zinc in patients with gestational diabetes. Journal of Maternal-Fetal and Neonatal Medicine 2004;16:15-21.

48. Bar J, Hod, M., Maymon, R., Moroz, C. Placental isoferritin in gestational diabetes mellitus. Acta Obstetricia et Gynecologica Scandinavica 1998;77:504-7.

Table 1

Maternal and pregnancy characteristics and biochemical indices among women with and without gestational diabetes mellitus (GDM).

	Gestational diabetes mellitus (GDM)		
	Yes N=129	**No N=3, 647**	
	N (%)	**N (%)**	**P-value***
Maternal characteristics			
Maternal age, years			
<25	4 (3.1)	260 (7.8)	0.004
25-34	70 (54.7)	2094 (62.7)	
≥35	54 (42.2)	988 (29.6)	
Country of birth identified as high risk for GDM[¥]	32 (25.0)	574 (16.1)	0.007
Maternal weight quintiles (kg)			
<55	23 (18.9)	521 (16.8)	0.05
55-60	24 (19.7)	652 (21.0)	
61-67	21 (17.2)	669 (21.6)	
68-76	18 (14.8)	649 (20.9)	
≥77	36 (29.5)	609 (19.7)	
Smoking during pregnancy	8 (6.2)	192 (5.7)	0.70
Socioeconomic disadvantage quintiles			
1 (most disadvantage)	36 (27.9)	685 (19.4)	0.09
2	16 (12.4)	591 (16.7)	
3	24 (18.6)	708 (20.0)	
4	21 (16.3)	761 (21.5)	
5 (least disadvantage)	32 (24.8)	788 (22.3)	
Pregnancy characteristics			
Nulliparous	63 (48.8)	1747 (51.8)	0.51
Gestational age at time of serum sampling, weeks			
9-10	8 (8.4)	322 (14.6)	0.06
11	44 (46.3)	785 (35.6)	
12-14	43 (45.3)	1096 (49.8)	
Hypertensive disorders of pregnancy	14 (10.9)	187 (5.1)	0.009
Biochemical indices			
Serum ferritin (µg/L), median (25^{th}, 75^{th} centile)	32.8 (17.3, 55.3)	24.8 (14.1, 42.2)	0.001
Iron deficient (serum ferritin <12 µg/L)	12 (9.3)	725 (19.9)	0.003
Transferrin receptor (TfR; nmol/L), median (25^{th}, 75^{th} centile)	15.7 (12.9, 19.3)	15.1 (12.2, 18.6)	0.11
C-reactive protein (CRP; mg/L) >90^{th} centile	19 (14.8)	338 (9.5)	0.05

***** Chi-square test and Fisher's exact test for small cell sizes.

[¥] Countries classified as high risk included Oceania, Southern and Eastern Europe, Middle East and North Africa, South-East Asia, Southern and Central Asia.[9]

Table 2
Univariate and multivariate logistic regression analysis from the in-house study examining first trimester maternal iron biomarkers and subsequent risk of developing gestational diabetes mellitus (GDM).

Maternal iron biomarker	GDM	Non-GDM	Unadjusted odds ratio (OR) and 95% CI intervals	Adjusted odds ratio (OR) and 95% CI intervals[a]
Ferritin, µg/L	129	3, 647	1.60 (1.28, 1.99)	1.41 (1.11, 1.78)
Ferritin tertiles				
tertile 1 (<17 µg/L)	31	1, 172	0.89 (0.55, 1.44)	1.10 (0.67, 1.81)
tertile 2 (17-34 µg/L)	38	1, 279	Reference	Reference
tertile 3 (≥35 µg/L)	60	1, 196	1.69 (1.12, 2.55)	1.60 (1.03, 2.49)
Ferritin quartile				
>75th percentile (≥43 µg/L)	45	886	1.67 (1.15, 2.42)	1.39 (0.93, 2.06)
Ferritin quintile				
>80th percentile (≥48 µg/L)	39	712	1.79 (1.22, 2.62)	1.43 (0.95, 2.16)
Transferrin receptor (nmol/L)	129	3, 647	1.02 (0.99, 1.05)	1.00 (0.97, 1.03)

[a] Adjusted for age, country of birth, parity, maternal weight, smoking during pregnancy, hypertensive disorders in pregnancy and C-reactive protein concentrations.

Table 3a
Characteristics of studies identified in the systematic review examining association between iron supplement use in pregnancy and gestational diabetes mellitus (GDM).

Study, country, quality score	Study design	Study population	Intervention group	Control group	Definition of gestational diabetes mellitus	General finding
Chan, 2009[10] Hong Kong; 92%	Randomised placebo controlled trial	1164 women with singleton pregnancies ≤16 weeks gestation without anemia, hemoglobinopathies or pre-existing diabetes.	565 women randomized to receive 300 mg ferrous sulphate tablet daily (60 mg of elemental iron).	599 women randomized to receive placebo tablet daily containing starch and lactose.	Glucose testing. 75-g OGTT at 28–30 weeks gestation and OGTT at 36 weeks (ADA criteria).	No difference in GDM in study (n=56, 11%) vs. control (n=60, 11.3%) groups (p=0.86).
Ouladsaheb madarek, 2011[32] Iran; 58%	Double-blind-randomized clinical trial	960 women with singleton pregnancies in first trimester without anemia and have not taken iron supplement in last month.	480 women randomized to receive daily 30 mg of elemental iron and multivitamin (contents not reported).	480 women randomized to receive placebo and one multivitamin tablet daily.	Recorded on a questionnaire.	No difference in GDM in study (n=2, 0.5%) vs. control (n=3, 0.8%) groups (p=0.67).

Table 3b
Characteristics of studies identified in the systematic review examining dietary iron intakes in pregnant women and gestational diabetes mellitus (GDM).

Study, country, quality score	**Study design**	**Study population**	**Measurement of dietary iron intake**	**Categories for comparing dietary iron intake**	**Definition of gestational diabetes mellitus**	**General finding**
Bowers, 2011[9] USA; 72%	Prospective cohort (Nurses' Health Study)	13,475/ 116,671 women with singleton pregnancy	Total iron, heme, non-heme and supplemental iron.	Women in lowest quintile vs. women with intakes in 2nd, 3rd, 4th and 5th quintiles.	Self-reported in biennial questionnaire.	Difference in GDM for dietary heme iron intake but not for non-heme iron intake.
Helin, 2012[11] Finland; 95%	Prospective cohort (based on a cluster-RCT where intervention and usual care groups were combined)	399/2271 pregnant women	Total daily iron intake during pregnancy, and hemoglobin in early pregnancy.	Women in lowest 80th percentile vs. women in highest 20th percentile.	Glucose testing. 75g OGTT at 26-28 weeks (ADA criteria).	No significance difference in total iron intake.
Qiu, 2011[33] USA; 83%	Prospective cohort (Omega Study)	3158/4000 pregnant women	Pre-conceptional and early pregnancy heme and nonheme iron intake.	Women in lowest quartile vs. women in 2nd, 3rd, and 4th quartiles.	Glucose testing. 50g 1h OGCT at 24-28 weeks; those who failed had 100g 3h OGTT ~1-2 weeks later (ADA criteria).	Difference in GDM for dietary heme iron intake but not for non-heme iron intake.

Table 3c
Characteristics of studies identified in the systematic review examining serum iron biomarkers in pregnant women and gestational diabetes mellitus (GDM).

Study, country, quality score	Study design	Study population	Serum iron biomarkers	Selection and matching of controls	Definition of gestational diabetes mellitus	General finding
Afkhami-Ardekani, 2009[12] Iran; 73%	Case-control	34 GDM cases 34 non-GDM controls	Ferritin, serum iron, transferrin saturation, total iron binding capacity.	Matched by age, parity, and BMI.	Glucose testing. 100-g oral glucose load (ADA criteria).	Unadjusted serum ferritin, serum iron, and transferrin saturation levels were significantly higher and TIBC was significantly lower in the GDM group.
Akhlaghi, 2012[46] Iran; 53%	Case-control	30 GDM cases 30 non-GDM controls	Serum iron.	Matched but not stated on what characteristics.	Glucose testing. Second OGTT (ADA criteria).	Unadjusted serum iron levels are significantly lower in the GDM group.
Al-Saleh, 2004[47] Kuwait; 60%	Case-control	15 GDM cases 15 non-GDM controls	Serum iron.	Randomly selected.	Hospital record.	Unadjusted serum iron levels are significantly lower in the GDM group.
Bar, 1998[48] Israel; 70%	Case-control	28 GDM cases 146 non-GDM controls	Placental isoferritin (PLF).	Not reported.	Glucose testing. High level of fasting plasma glucose (>105 mg/dl) or abnormal OGTT (ADA criteria).	Unadjusted serum placental isoferritin levels are significantly lower in the second and third trimester of pregnancy in the GDM group.
Behboudi-Gandevani, 2013[14] Iran; 73%	Prospective cohort (nested case-control)	72 GDM cases 961 non-GDM controls	Serum iron.	Not reported.	Glucose testing. 50 g GCT and abnormal 3-h 100g OGTT (Carpenter and Coustan criteria).	Adjusted serum iron levels (continuous variable) are significantly higher in the GDM group (AOR: 1.01, 95% CI: 1.00, 1.01).
Chen, 2006[15]	Prospective	35 GDM cases	Ferritin.	Randomly selected	Glucose testing.	Adjusted ferritin levels (3rd tertile

USA; 70%	cohort (nested case-control)	137 non-GDM controls		among non-GDM women in each tertile of serum ferritin concentration.	50-g OGCT and OGGT. (Carpenter/Coustan conversion as recommended ADA).	vs tertiles 1 and 2) are not significantly different in the GDM group (AOR: 1.88, 95% CI: 0.81, 4.36).
Derbent, 2013[34] Turkey; 80%	Case-control	30 GDM cases 72 non-GDM controls	Ferritin, serum iron, transferrin, hepcidin.	Matched by gestational week.	Glucose testing. 50g GCT and 100g OGTT (Carpenter and Coustan modification of the NDDG criteria).	Unadjusted serum ferritin, serum iron, and hepcidin levels are significantly higher in the GDM group. Transferrin levels are not significantly different in the GDM group.
Gungor, 2007[31] Turkey; 53%	Case-control	56 GDM cases and 56 non-GDM controls	Ferritin, transferrin.	Not reported.	Glucose testing. 50g glucose load screening test and 3-h OGTT (Carpenter and Coustan OGTT criteria).	Unadjusted ferritin and transferrin levels are not significantly different in GDM group.
Kaygusuz, 2013[37] Turkey; 73%	Case-control	30 GDM cases and 28 non-GDM controls	Ferritin, serum iron, transferrin saturation, TIBC	Not reported.	Glucose testing. 50g GCT and 100g OGTT (Carpenter and Coustan modification of the NDDG criteria).	Unadjusted serum ferritin, serum iron, and transferrin saturation levels were significantly higher and TIBC was significantly lower in the GDM group.
Lao, 1997[36] China; 73%	Case-control	60 GDM cases 60 non-GDM controls	Ferritin, serum iron, transferrin, transferrin saturation.	Matched on exact parity and maternal age (± 1y).	Glucose testing. 75g OGTT (WHO criteria).	Unadjusted serum ferritin levels are significantly higher in the GDM group. Serum iron, transferrin and transferrin saturation levels are not significantly different in the GDM group.

Lao, 2001[38] China; 73%	Case-control	97 GDM cases 194 non-GDM controls	Ferritin, serum iron, transferrin, transferrin saturation.	Matched on parity, two controls per case.	Glucose testing. Abnormal 75g OGTT (WHO criteria).	Unadjusted serum ferritin, serum iron and levels are significantly higher and transferrin levels significantly lower in the GDM group.
Sharifi, 2010[16] Iran; 83%	Case-control	64 GDM cases 64 non-GDM controls	Ferritin.	Matched on age.	Glucose testing. 50g glucose challenge and 3h OGTT (Carpenter Coustan criteria).	Adjusted serum ferritin levels (>75th percentile) are significantly higher in the GDM group (AOR: 5.1, 95% CI: 1.0-38).
Yeniel, 2012[35] Turkey; 77%	Case-control	29 GDM cases 94 non-GDM controls	Ferritin, serum iron transferrin, TIBC.	Matched on age, BMI, gravidity, and parity	Glucose testing. OGTT = 1h serum glucose and 50g glucose load (Carpenter and Coustan criteria).	Unadjusted serum ferritin, serum iron, transferrin and TIBC are not significantly different in the GDM group.

TABLE 4
Maternal serum iron biomarkers presented as the mean (standard deviation) in women with and without gestational diabetes mellitus (GDM) among studies identified in the systematic review and the in-house study.

Study, country	Cases (N)	Controls (N)	Gestational week at sampling	Maternal serum iron biomarker	Unit	Biomarker level in cases Mean (SD)	Biomarker level in controls Mean (SD)	P-value
Afkhami-Ardekani, 2009 (Iran)	34	34	24–28	Ferritin Serum iron Transferrin saturation Total iron binding capacity (TIBC)	pmol/L µg/l % µg/dl	164.8 (71.3) 1000.4 (220.9) 26.5 (5.9) 383.1 (30.6)	93.4 (63.6) 568.5 (230.3) 12.8 (5.7) 457.8 (58.2)	<0.001 <0.001 <0.001 <0.001
Akhlaghi, 2012 (Iran)	30	30	24–28	Serum iron	µg/mL	73.3 (NR) [1]	85.5 (NR) [1]	<0.05
Al-Saleh, 2004 (Kuwait)	15	15	At delivery	Serum iron	µg/l	2061.6 (262.9)	2020.1 (266.0)	NS[1]
Bar, 1998 (Israel)	28	146	20–24 30–34	Serum placental isoferritin (PLF) Serum placental isoferritin (PLF)	U/ml	9.4 (13.8) 3.7 (10.0)	21.5 (26.6) 19 (31.4)	<0.001 <0.0001
Behboudi-Gandevani, 2013 (Iran)	72	961	14–20	Serum iron	µg/dl	143.8 (48.7)	112.5 (69.4)	<0.0001
Chen, 2006 (USA)	35	137	15.6 (±0.1)	Ferritin	pmol/L	141 (17)	86 (9)	<0.01
Derbent, 2013 (Turkey)	30	72	24-28	Ferritin Serum iron Transferrin Hepcidin	pmol/L µg/l mg/L ng/mL	29.7 (30.1) 635.0 (335.0) 384 (69) 15.3 (9.6)	18.9 (21.1) 490.0 (372.0) 361 (58) 9.5 (3.9)	0.008 0.014 NS[1] 0.002
Gungor, 2007 (Turkey)	56	56	28-30	Ferritin Transferrin	pmol/L µmol/L	38.6 (30.4) 63.5 (18.8)	36.5 (34.3) 61.4 (16.6)	NS[41] NS
Kaygusuz, 2013 (Turkey)	30	28	24-28	Ferritin Serum iron Transferrin saturation Total iron binding capacity (TIBC)	pmol/L µmol/L % µmol/L	15.5 (8.2)[2] 86.5 (84.5)[2] 17.9 (20.4) 381.5 (102.5)	7.5 (9.8)[2] 58.0 (39.3)[2] 12.3 (7.6) 448.0 (95.9)	<0.001 0.04 <0.01 0.02

Lao, 1997 (China)	60	60	28-30	Ferritin	pmol/L	56.5 (33.2)	41.0 (42.24)	0.0001
				Serum iron	µg/l	821.0 (329.0)	737.2 (394.8)	NS[1]
				Transferrin	µmol/L	67.7 (7.7)	69.4 (7.7)	NS
				Transferrin saturation	%	22.1 (9.4)	19.5 (10.6)	NS
Lao, 2001 (China)	97	194	28-30	Ferritin	pmol/L	47.4 (NR) [1]	22.5 (NR) [1]	<0.0001
				Serum iron	µmol/L	14.9 (NR)	12.6 (NR)	0.0073
				Transferrin	µmol/L	69.4 (NR)	74.7 (NR)	<0.0001
				Transferrin saturation	%	22.0 (NR)	17.2 (NR)	0.0004
Sharifi, 2010 (Iran)	64	64	24-28	Ferritin	pmol/L	112.3 (28.4)	65.0 (16.9)	0.001
Yeniel, 2012 (Turkey)	29	94	12	Ferritin	pmol/L	74.6 (126)	50.4 (63.7)	NS[1]
				Serum iron	µg/l	790.0 (390.0)	956.0 (516.0)	NS
				Transferrin	µg/dL	305.3 (60.3)	297.6 (78.1)	NS
				Total iron binding capacity (TIBC)	µg/dL	361.3 (71.0)	361.5 (74.5)	NS
Khambalia, 2014[a] (Australia)	129	3, 647	9-14	Ferritin	pmol/L	43.2 (34.8)	32.9 (29.3)	0.0001
				Transferrin receptor (TfR)	nmol/L	16.5 (5.1)	15.9 (5.7)	0.11

[1]Abbreviations: NS represents non-significant; NR represents not reported.

[2]Values presented are median (interquartile range).

Legend Page for Figures

Figure 1
Flowchart of selection procedure.

Figure 2a
Forest plot of all studies reporting on serum ferritin concentrations (µg/L) among women with and without gestational diabetes mellitus.

Figure 2b
Forest plot of select studies reporting on serum ferritin concentrations (µg/L) among women with and without gestational diabetes mellitus.

Figure 3
Forest plot summarising mean differences in serum iron concentrations (mcg/L) among women with and without gestational diabetes mellitus.

Figure 1
Flowchart of selection procedure.

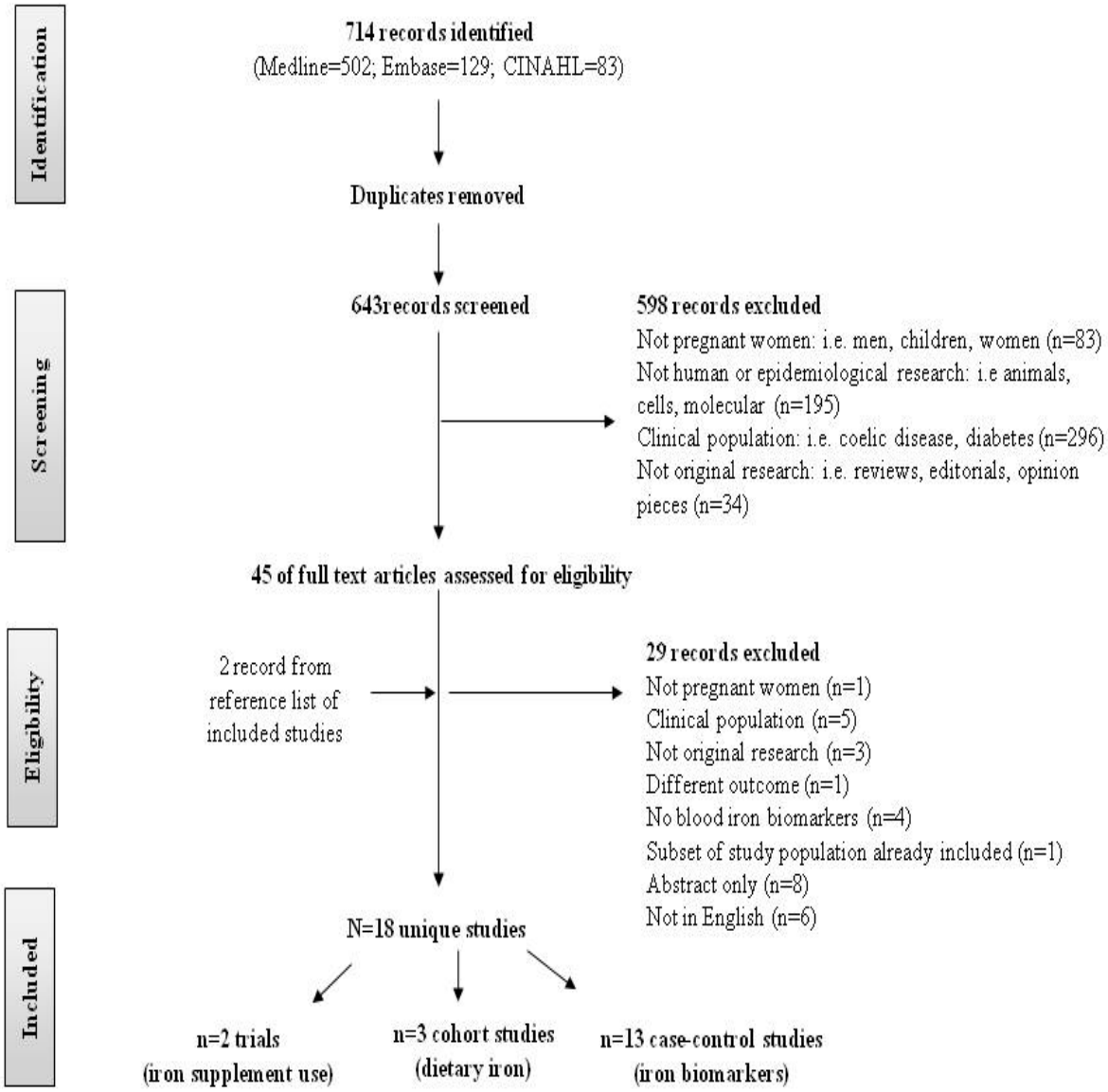


Figure 2a
Forest plot of all studies reporting on serum ferritin concentrations (µg/L) among women with and without gestational diabetes mellitus.

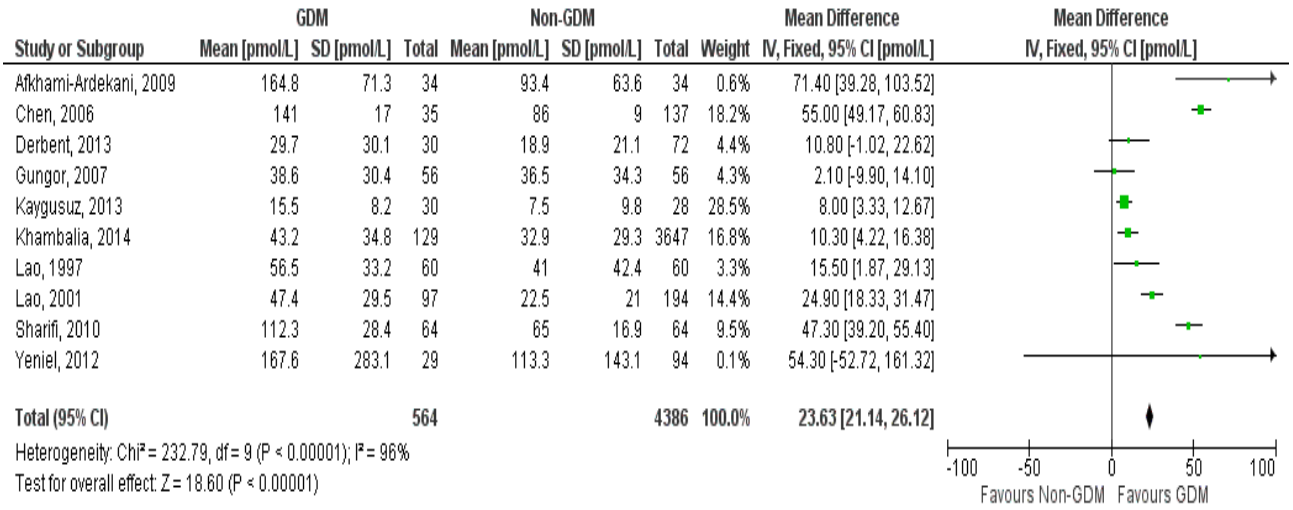


Figure 2b
Forest plot of select studies reporting on serum ferritin concentrations (µg/L) among women with and without gestational diabetes mellitus.

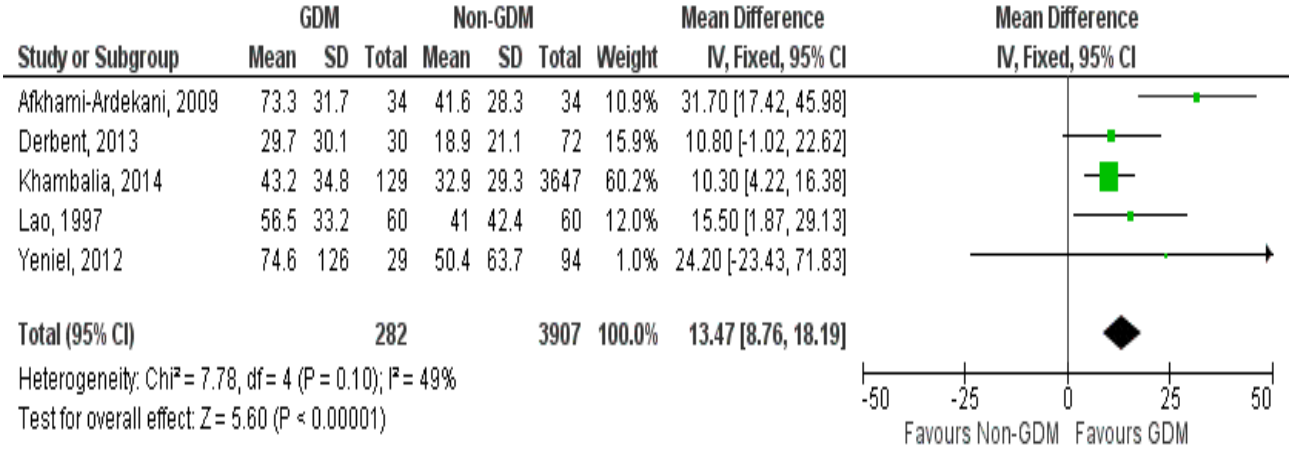
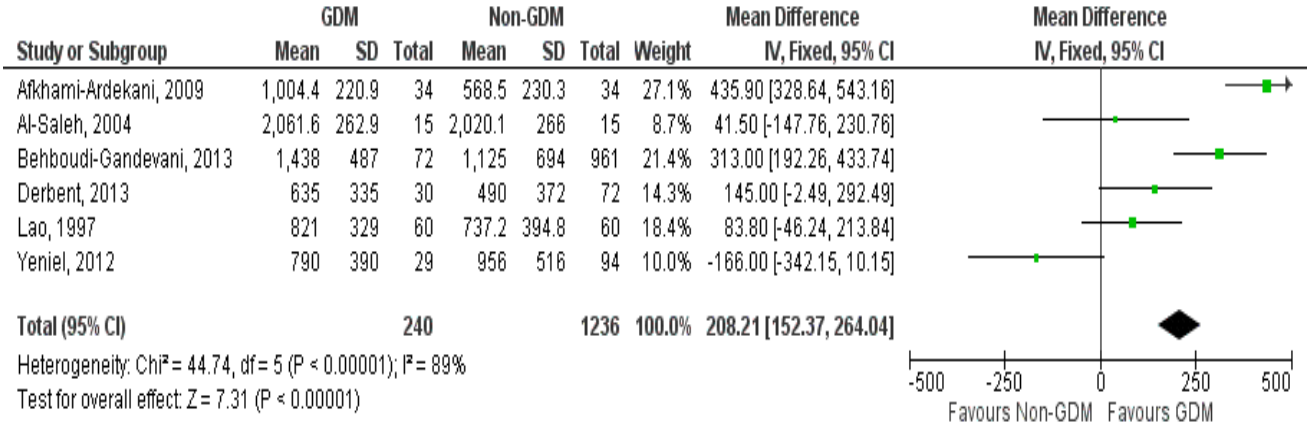


Figure 3
Forest plot summarising mean differences in serum iron concentrations (mcg/L) among women with and without gestational diabetes mellitus.



Supplementary Table 1
Search strategies in electronic databases.

Database	Search number, terms (records identified)
Ovid MEDLINE	1 exp *Diabetes, Gestational/ (5246) 2 exp *diabetes Mellitus/ (243380) 3 ((Maternal or Gestational or pregnan*) adj3 diabet*).tw. (12728) 4 exp *Hyperglycemia/ (14425) 5 hyperglyc*.tw. (38285) 6 exp *Blood Glucose/ (37240) 7 glucose.tw. (304606) 8 exp *Insulin Resistance/ (33280) 9 exp *Insulin/ (76806) 10 insulin.tw. (251694) 11 ((elevate* or raise* or high*) adj3 glucose).tw. (22401) 12 exp *Iron/ (42794) 13 iron.tw. (112699) 14 exp *Iron-Binding Proteins/ (41322) 15 hepcidin.tw. (1774) 16 ferritin.tw. (19025) 17 transferrin.tw. (23287) 18 exp *Iron Compounds/ (22902) 19 1 or 2 or 3 (248983) 20 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (487709) 21 19 and 20 (108289) 22 12 or 13 or 14 or 15 or 16 or 17 or 18 (175931) 23 21 and 22 (502)
Embase	1 transferrin:ab,ti (27,596) 2 ferritin:ab,ti (25,411) 3 hepcidin:ab,ti (3,104) 4 iron:ab,ti (148,846) 5 'iron compounds' (784) 6 'iron binding proteins' (384) 7 transferrin:ab,ti OR ferritin:ab,ti OR hepcidin:ab,ti OR iron:ab,ti OR 'iron compounds' OR 'iron binding proteins'(174,323) 8 'gestational diabetes mellitus'/exp OR 'gestational diabetes mellitus'/de (19,395) 9 diabet*:ab,ti (548,577) 10 'gestational diabetes mellitus'/exp OR 'gestational diabetes mellitus'/de AND diabet*:ab,ti (15,960) 11 transferrin:ab,ti OR ferritin:ab,ti OR hepcidin:ab,ti OR iron:ab,ti OR 'iron compounds' OR 'iron binding proteins' AND ('gestational diabetes mellitus'/exp OR 'gestational diabetes mellitus'/de) AND diabet*:ab,ti (129)
CINAHL	1 (MM "Diabetes Mellitus, Gestational") (1,662) 2 (MH "Pregnancy in Diabetes+") OR (MH "Diabetes Mellitus, Gestational") (3,239) 3 TI (diabet*) (47,611) 4 S1 OR S2 OR S3 (48,653) 5 (MM "Preganacy+") (0) 6 (MM "Pregnancy*") (19,571) 7 (MM "Pregnancy Complications*") (757)

	8 (MM "Pregnancy, High Risk") (757) 9 TI (pregnan*) (23,681) 10 5 OR 6 OR 7 OR 8 OR 9 (32,733) 11 TI (hyperglyc#emia) (998) 12 (MM "Blood Glucose") (3,711) 13 TI (glucose) (6,444) 14 (MM "insulin Resistance+") (5,723) 15 (MM "Insulin+") (6,172) 16 TI (insulin) (8,899) 17 11 OR 12 OR 13 OR 14 OR 15 OR 16 (20,966) 18 10 AND 17 (502) 19 4 OR 18 (48,790) 20 (MM "iron") (1,555) 21 TI (iron) (2,778) 22 TI (hepcidin) (83) 23 TI (ferritin) (198) 24 TI (transferrin) (117) 25 (MM "iron compounds+") (861) 26 20 OR 21 OR 22 OR 23 OR 24 OR 25 (3,457) 27 19 AND 26 (83)