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Research article

# Dietary haem-iron and iron supplementation in the third trimester are associated with gestational diabetes mellitus risk: cross-sectional analysis of a prospective study

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ABSTRACT

Gestational diabetes mellitus (GDM) poses significant short- and long-term risks to both mother and foetus. Understanding how maternal iron intake from foods and supplements influences GDM risk is crucial. This study assessed the relationship between iron intake and GDM risk through a cross-sectional analysis of 632 Malaysian pregnant women attending antenatal check-ups. Dietary iron intake was measured using a semi-quantitative food frequency questionnaire that included 135 commonly consumed Malaysian foods and beverages. Multivariate logistic analysis examined the associations between total iron, dietary haem-iron and non-haem-iron, iron supplements, and GDM risk. Logistic models with interaction terms assessed the interaction between iron intake and third trimester haemoglobin (Hb) levels on GDM risk. Results showed that in the third trimester, total iron intake, particularly from haem-iron and iron supplements, was significantly associated with increased GDM risk. High intakes of total iron and haem-iron were linked to higher GDM risk in women with high Hb levels, while iron supplements were associated with higher GDM risk in women with low Hb levels.

## 1. Introduction

Gestational Diabetes Mellitus (GDM), a form of hyperglycaemia that develops during pregnancy, is becoming more frequent as obesity and Type 2 Diabetes Mellitus (T2DM) become more prevalent. In 2021, there were 21.1 million (16.7%) live births to women with some form of hyperglycaemia in pregnancy. Of these, 80.3% were due to GDM [1]. A meta-analysis of GDM prevalence showed that the pooled GDM prevalence was around 10%, with low-to-middle-income countries (LMICs) having higher estimate than high-income countries (HICs) [1]. Vietnam and Singapore have the highest GDM prevalence in Asia, followed by China and Malaysia [2]However, a recent meta-analysis of 57 studies reported that the prevalence of GDM standardized to a universal Oral Glucose Tolerance Test (OGTT) strategy, International Association of the Diabetes and Pregnancy Study Groups (IADPGS) criteria and women of 25-30 years of age was 14.0% with high-income countries having the highest prevalence (14.2%) as compared to low-income

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(12.7%) and middle-income (9.2%) countries [3]. In Malaysia, the National Health and Morbidity Survey showed that about 13.5% of women aged 15 to 49 were diagnosed with hyperglycaemia during pregnancy in 2016 [4]and the proportion increased to 27.1% in 2022. The 6th Report of National Obstetrics Registry indicated that the overall prevalence of GDM in 9 tertiary hospitals in Malaysia increased from 7.9% in 2016 to 21.4% in 2020 [5].

Dietary iron intake contributes significantly to iron stores in the body [6]. Iron in the diet can be found as haem (derived mostly from meat and animal products) or non-haem (derived from plant sources) iron with haem iron being more bioavailable than non-haem iron [7]. Previous studies on iron intake and T2DM/GDM risk found that high dietary iron intake, particularly haem iron from meat, as well as supplemental iron, were associated with an elevated T2DM or GDM risk [8]. However, none of these investigations showed that higher dietary non-haem iron was significantly related to elevated body iron stores or an increased T2DM or GDM risk. These findings are supported by two systematic reviews that reported high dietary haem iron but not non-haem iron increased GDM risk [9,10]. The relationships between dietary haem iron and non-haem iron intakes with T2DM or GDM risk may be explained by their differences in bioavailability and their ability to raise body iron [8].

The evidence on dietary iron and risk of GDM was based mainly on studies in Western countries [8–11], where the average consumption of meat (including red meat, white meat and processed meats) and fortified cereals is substantially higher than in many Asian countries [12]. As a result, the generalizability of these findings to Southeast Asian populations, including Malaysia, remains limited. Over the past decade, Malaysians have steadily increased their meat intake, with 58% of meat intake came from red meat [13]. Both national and local studies [14–16] in Malaysia showed that the average protein intake of adults in recent years exceeded the recommendation (10-15% of energy intake) [17]. Similarly, prospective cohort studies among pregnant women indicated high protein intake, with one study showing that most women (88.9 – 90.3%) had 10 – 20% of energy intake from protein and at least 75% of the recommended protein intake (74.6 – 86.5%) before and during pregnancy [18,19].

Iron supplementation is the most widely used strategy to address iron deficiency in pregnant women [20]. Several studies have indicated that routine iron supplementation (30 – 40 mg iron) during pregnancy may be related with a greater GDM risk [9,21]. In Malaysia, iron supplements are routinely prescribed to all pregnant women as part of standard antenatal care [22], whereby the recommended dose of prophylactic iron supplement in pregnancy is 400 mg ferrous fumarate per week, while iron deficiency anaemia (IDA) is treated with 400 mg ferrous fumarate per day or 200 mg twice a day. However, iron supplements are typically prescribed in a universal manner regardless of the woman's baseline iron status, haemoglobin concentration, or dietary iron intake. Given that excessive iron intake may promote oxidative stress, and impair insulin sensitivity, this practice warrants critical re-evaluation, especially in a setting where anaemia remains a significant public health concern.

Despite widespread antenatal iron supplementation and increasing red meat consumption, there is a lack of studies in Malaysia specifically examining the relationship between various forms of iron intake (total iron, haem iron, non-haem iron, and iron supplementation) and GDM risk. Given that GDM poses significant short-term and long-term risks to the health of both mother and foetus, it is critical to comprehend the extent to which maternal iron intake from foods and supplements may raise GDM risk. Moreover, as GDM prevalence continues to rise in Malaysia, generating population-specific evidence is essential to inform more tailored dietary and supplementation guidelines in pregnancy. This study aimed to determine the associations between dietary iron and iron supplements with GDM risk as well as to examine the interactions between iron intake with haemoglobin level in the third trimester on GDM risk.

## 2. Materials and methods

### 2.1. Study area

This cross-sectional analysis was part of a prospective study that examined the contribution of iron from different sources during pregnancy to the aetiology of GDM among women with varying iron status. Pregnant women attending routine antenatal care were recruited from two maternal and child health (MCH) clinics in the Hulu Langat district of Selangor, an urbanized state located on the west coast of Peninsular Malaysia. The study was conducted from December 2018 to December 2020. The inclusion criteria were Malaysian pregnant women between the ages of 25 and 45, those who were less than 14 weeks gestation at the time of enrolment, singleton pregnancy, body mass index (BMI) $< 40$ kg/m$^2$, and no medical conditions during pregnancy (e.g. pre-eclampsia gestational, diabetes mellitus) or long-term health issues (e.g., renal disease, heart disease). The exclusion criteria were women with multiple pregnancies, became pregnant using advanced reproductive technology, BMI $\geq$40 kg/m$^2$, unable to complete oral glucose tolerance test (OGTT), diagnosed with hyperglycaemia in early pregnancy ($<$14th gestational weeks), and had diabetes that required medication outside of pregnancy or other medical problems (e.g. hypertension, Hepatitis B/C, HIV positive, renal disease, thalassemia).

### 2.2. Measurements

An interviewer-administered questionnaire, conducted in Bahasa Malaysia to ensure comprehension among participants, was used to collect data on socio-demographic, obstetrical, and third trimester dietary intake, and iron supplementation (for more details, please refer to supplementary information). The socio-demographic variables included age, ethnicity, education level, occupation status, and monthly household income. Obstetrical variables included gravidity, parity, history of GDM, and family history of diabetes mellitus. Women were also measured for anthropometric and biochemical parameters.

#### 2.2.1. Dietary intake and iron supplementation in the third trimester

A semi-quantitative food frequency questionnaire (SFFQ) that consisted of 135 foods and beverages (with standard serving size)

commonly consumed by Malaysians was used to assess dietary intake [23]. Women were requested to recall the usual frequency and portion size of intake for each food item. The United States Department of Agriculture food database was used to estimate energy and nutrient intakes [24].

Iron of each food item was estimated using the following formula: iron intake = frequency of consumption x serving size x iron content of the food. The dietary haem iron content was estimated by multiplying the total iron content of all meat items by 0.4 [25,26]. Dietary non-haem iron was calculated as the sum of total iron in non-meat items and 0.6 of total iron in meats [25,26]. Dietary iron intake was defined as the sum of dietary haem iron and dietary non-haem iron.

Respondents were asked to self-report their multivitamin and iron supplement usage and dosage. Based on the guideline, weekly oral iron and folic acid supplementation with 400 mg ferrous fumarate and 5 mg folic acid are recommended for non-anaemic pregnant women (Hb $\geq$ 11 g/dL). Daily oral iron (400 mg of ferrous fumarate) and folic acid (5 mg folic) supplementation are recommended for pregnant women with Hb $<$ 11.0 g/dL [22]. Total iron intake was calculated as the sum of dietary iron and iron supplements.

#### 2.2.2. Anthropometric information

A stadiometer was used to measure height at the first prenatal visit (<12 weeks). Pre-pregnancy body mass index (kg/m$^2$) was calculated using self-reported pre-pregnancy weight and measured height, with pre-pregnancy weight (kg) divided by height square (m$^2$). Using the World Health Organization criteria, pre-pregnancy BMI was categorised as underweight (<18.5 kg/m$^2$), normal weight (18.5–24.9 kg/m$^2$), overweight (25.0–29.9 kg/m$^2$), or obese (30.0 kg/m$^2$) [27].

#### 2.2.3. Biochemical measurements

Haemoglobin (Hb) was used as a biomarker of iron status. A 2 ml of venous blood sample was collected by clinic staff at the first prenatal visit (mean gestational age: 10.73 $\pm$ 0.34 weeks) and third trimester (mean gestational age: 29.24 $\pm$ 1.88 weeks) to obtain haemoglobin level. Haemoglobin level was analysed using automated blood cell counter techniques.

The diagnosis of GDM was based on a standard two-point 75 g oral glucose tolerance test (OGTT) at 28 – 32 weeks of gestation. Blood samples were collected and sent to the clinical laboratory of MCH clinics for analysis of fasting plasma glucose and 2-hr plasma glucose. GDM was diagnosed by one or more of the following values from the 75g OGTT: fasting plasma glucose $\geq$5.1 mmol/L, and/or 2 h plasma glucose $\geq$7.8 mmol/L [28].

### 2.3. Statistical analysis

#### 2.3.1. Sample size calculation

The sample size for this study was determined based on the requirements for a cohort study [29,30]. Helin et al. (2012) reported that pregnant women in the highest total daily iron intake group had an adjusted odds ratio (OR) of 2.35 for GDM [31]. Based on this, an OR of 5.5, a 95% confidence level, and a 5% precision level, was applied to the sample size calculation. A minimum of 408 pregnant women were required as study subjects. To account for a 20% anticipated dropout rate, the required sample size was adjusted to 490 women.

To ensure that the final sample size was achieved, about 695 women were screened (Fig. 1). Of these, 63 were excluded due to abnormal glycaemia at screening. Consequently, 632 eligible and consenting women were enrolled and included in the final analysis, yielding a participation rate of approximately 91.0%. All included participants had complete data for key variables. Therefore, no imputation for missing data was necessary.

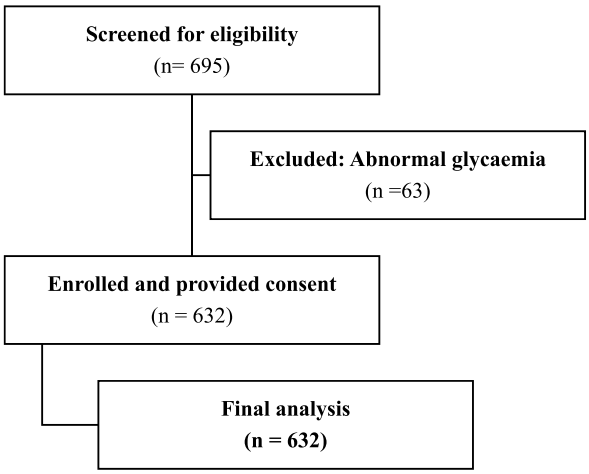


**Fig. 1.** Participant flow diagram.

**Table 1**
Characteristics of women by GDM status (N = 632).

| Characteristics | Non-GDM (n = 479) | GDM (n = 153) | $t/\chi^2$ | p-value |
|---|---|---|---|---|
| | n (%)/Mean ± SD | | | |
| Age (years) | 29.79 ± 4.28 | 31.63 ± 4.91 | −4.15 | 0.01* |
| ≤30 | 284 (59.3) | 68 (44.4) | 10.36 | 0.001** |
| >30 | 195 (40.7) | 85 (55.6) | | |
| Ethnicity | | | | |
| Malay | 48 (93.5) | 138 (90.2) | 2.81 | 0.25 |
| Chinese | 10 (2.1) | 3 (2.0) | | |
| Indian and others | 21 (4.4) | 12 (7.8) | | |
| Education level (years) | 14.49 ± 2.77 | 14.27 ± 2.60 | 0.88 | 0.38 |
| Secondary and lower | 95 (19.8) | 35 (22.9) | 0.67 | 0.72 |
| STPM/Matric/Diploma/Certificate | 141 (29.4) | 44 (28.8) | | |
| Tertiary and above | 243 (50.8) | 74 (48.3) | | |
| Occupation status | | | | |
| Unemployed | 150 (31.3) | 47 (30.7) | 0.02 | 0.89 |
| Employed | 329 (68.7) | 106 (69.3) | | |
| Monthly household income (RM) | | | | |
| <2000 | 14 (2.9) | 8 (5.2) | 1.92 | 0.38 |
| 2001 – 5999 | 317 (66.2) | 97 (63.4) | | |
| ≥6000 | 148 (30.9) | 48 (31.4) | | |
| Household size | 3.30 ± 1.49 | 3.59 ± 1.58 | −2.08 | 0.04* |
| **Obstetrical information** | | | | |
| Gravidity | 2.17 ± 0.06 | 2.45 ± 0.13 | −1.99 | 0.05 |
| Parity | 0.98 ± 0.05 | 1.14 ± 0.10 | −1.37 | 0.17 |
| Medical history of GDM (n = 346) | | | | |
| No | 28 (10.9) | 29 (33.0) | 23.30 | 0.001** |
| Yes | 230 (89.1) | 59 (67.0) | | |
| Family history of diabetes mellitus (T2DM) | | | | |
| No | 172 (35.9) | 65 (42.5) | 2.14 | 0.15 |
| Yes | 307 (64.1) | 88 (57.5) | | |
| **Anthropometric measurements** | | | | |
| Height (cm) | 156.36 ± 5.39 | 156.51 ± 6.13 | −0.28 | 0.77 |
| Pre-pregnancy weight (kg) | 57.99 ± 12.67 | 62.49 ± 14.31 | −3.70 | 0.001** |
| Pre-pregnancy BMI ($kg/m^2$) | 23.70 ± 4.97 | 25.45 ± 5.38 | −3.71 | 0.001** |
| Underweight (<18.5) | 66 (13.8) | 11 (7.2) | 20.20 | 0.001** |
| Normal (18.5-24.9) | 259 (54.1) | 65 (42.5) | | |
| Overweight (25.0-29.9) | 93 (19.4) | 54 (35.3) | | |
| Obese (≥30.0) | 61 (12.7) | 23 (15.0) | | |
| **Biochemical measurements at third trimester** | | | | |
| Oral glucose tolerance test (OGTT) | | | | |
| Gestational weeks at OGTT performed | 29.37 ± 1.88 | 28.84 ± 1.82 | 3.12 | 0.02* |
| Fasting plasma glucose (FPG) (mmol/L) | 4.27 ± 0.32 | 5.24 ± 1.05 | −11.15 | 0.001** |
| 2-h plasma glucose (2hPG)(mmol/L) | 5.92 ± 0.94 | 8.25 ± 2.41 | −11.70 | 0.001** |
| Haemoglobin (Hb) (g/dL) | 11.24 ± 0.99 | 11.83 ± 1.19 | −6.06 | 0.001** |
| **Dietary intake in the third trimester** | | | | |
| Gestational weeks | 29.37 ± 1.88 | 28.84 ± 1.82 | 3.12 | 0.02* |
| Energy (kcal/day) | 1675 ± 691.28 | 1950 ± 819.04 | −3.75 | 0.001** |
| Carbohydrate (% energy) | 51.95 ± 8.34 | 53.10 ± 9.79 | −1.31 | 0.16 |
| Protein (% energy) | 33.81 ± 7.14 | 33.69 ± 7.20 | 0.19 | 0.85 |
| Fat (% energy) | 16.55 ± 5.46 | 16.74 ± 5.20 | −0.37 | 0.71 |
| Total iron (mg)[b,c] | 12.05 (8.76, 16.05) | 15.14 (10.08, 30.52) | 21.65 | 0.001** |
| Dietary iron (mg)[b,c] | 11.49 (8.60, 14.53) | 13.25 (9.75, 17.84) | 12.37 | 0.001** |
| Haem-iron (mg)[b,c] | 1.04 (0.78, 1.39) | 1.13 (0.88, 1.72) | 12.36 | 0.001** |
| Non-haem iron (mg)[b,c] | 10.31 (7.82, 13.03) | 11.63 (8.74, 15.50) | 12.42 | 0.001** |
| Iron supplementation (mg)[b,c] | 66.00 (60.00, 115.00) | 90.50 (66.00, 115.00) | 0.56 | 0.45 |
| Iron supplementation | | | | |
| No | 422 (88.1) | 119 (77.8) | 10.03 | 0.01* |
| Yes | 57 (11.9) | 34 (22.2) | | |
| Dosage (elemental iron intake) (n = 91) | | | | |
| 30 – 60 mg | 6 (10.5) | 5 (14.7) | - | 0.74[a] |
| >60 mg | 51 (89.5) | 29 (85.3) | | |

Note. Total iron intake = sum of dietary iron and iron supplementation. Dietary iron intake = sum of non-haem and haem iron.
*p < 0.05, **p < 0.001.
† Gestational weeks at the first prenatal visit: 10.73 ± 0.34 weeks.
‡ GDM according to MOH criteria, either of both FPG ≥5.1 mmoL/l or 2hPG ≥7.8 mmol/L.
[a] Fisher Extract test.
[b] Median (25th percentile, 75th percentile).
[c] Kruskal–Wallis test.

### 2.4. Statistical test

Statistical Package for Social Science (SPSS Version 26) was employed for data analysis. Descriptive statistics including frequencies, percentages, mean, and standard deviations were used to describe data. Independent samples *t*-test and Chi-Square were used to determine the differences in maternal characteristics, dietary iron intake (total, haem and non-haem iron), and iron supplementation between non-GDM and GDM women. Fisher's Exact Test was applied only for the variable "Dosage (elemental iron intake) due to small cell counts. Logistic regression was used to determine the associations between iron intake and GDM risk, as well as with interaction terms of Hb level in the third trimester. Stratified logistic regression analysis was conducted to determine the associations between iron intakes and GDM risk by different Hb levels in the third trimester. Hb status was divided into three groups: low (Hb $< 11.0$ g/dL), normal (Hb 11.0 – 12.4 g/dL), and high (Hb $\geq 12.5$ g/dL), with normal Hb was defined as the reference group. A significant level for statistical analysis was set at $p < 0.05$.

## 3. Results

Socio-demographic and obstetrical characteristics exhibited no significant differences between GDM and non-GDM, with the exceptions of age ($t = -4.15$, $p < 0.05$), medical history of GDM ($\chi^2 = 23.30$, $p < 0.05$), and household size ($t = 2.08$, $p < 0.05$) (Table 1). GDM women had significantly higher mean pre-pregnancy weight (62.49 ± 14.31 kg) and BMI (25.45 ± 5.38 kg/m$^2$) compared to non-GDM women (pre-pregnancy weight = 57.99 ± 12.67 kg; BMI = 23.70 ± 4.97 kg/m$^2$). Furthermore, GDM women exhibited significantly higher Hb level in the third trimester (11.83 ± 1.19 g/dL) compared to non-GDM women (11.24 ± 0.99 g/dL). Overall, there were significant differences in energy and iron intakes (total iron, dietary iron, haem-iron and non-haem iron) in third trimester between non-GDM and GDM women. In this cohort, dietary haem iron constituted approximately 9% of the total dietary iron intake (with non-haem iron accounting for the remaining 91%). Moreover, a significantly higher percentage of GDM women reported taking iron supplements than non-GDM women (22.2% vs 11.9%).

Table 2 illustrates the associations between dietary iron, iron supplements in the third trimester, and GDM risk. The total iron intake during this period was significantly and positively associated with GDM risk (AOR = 1.01, 95% CI = 1.01 – 1.03). Among the types of iron, only haem-iron and iron supplements showed significant associations with GDM risk. Interaction analysis indicated significant interactions between Hb level in the third trimester and the intakes of total iron (AOR = 1.01, 95% CI = 1.01 – 1.03), haem iron (AOR = 1.02, 95% CI = 1.01 – 1.04) and iron supplements (AOR = 1.02, 95% CI = 1.01 – 1.03) in relation to GDM risk.

Table 3 reveals that while significant associations between higher intakes of total iron and haem iron with GDM risk were observed among women with high Hb levels in the third trimester, the significant association between iron supplementation and GDM risk was only found among women with low Hb level in the third trimester.

## 4. Discussion

A growing body of evidence suggests that dietary iron intake during pregnancy, particularly haem-iron, is significantly and positively associated to GDM risk [31,32]. Iron is known for its strong pro-oxidant characteristic and its ability to produce reactive oxygen species such as hydroxyl radicals. Iron overload can increase β-cell oxidative stress, leading to insulin resistance and impaired glucose metabolism [33,34]. This association might be explained by several potential mechanisms involving multiple tissues and organs. For instance, excess iron accumulation can induce oxidative stress, causing cell damage and apoptosis, which can reduce insulin secretion (3). In adipocytes, excessive iron can decrease insulin-induced glucose transport, while in the muscles, it may shift

**Table 2**
The main and interaction effects of the associations between dietary iron and iron supplements at the third trimester with gestational diabetes mellitus (GDM) risk.

| | GDM |
|---|---|
| | AOR [95% CI] |
| Total iron (mg/day)[a] | 1.01 [1.01 – 1.03]* |
| Dietary iron (mg/day)[a] | 1.02 [0.96 – 1.07] |
| Haem-iron (mg/day)[a] | 1.26 [1.03 – 1.54]* |
| Non-haem iron (mg/day)[a] | 0.98 [0.94 – 1.06] |
| Iron supplementation (mg/day)[a] | 1.02 [1.01 – 1.03]* |
| **Interaction terms** [a] | **Stratified AOR [95% CI]** |
| Total iron x Hb level at third trimester | 1.01 [1.01 – 1.03]* |
| Dietary iron x Hb level at third trimester | 1.04 [0.99 – 1.08] |
| Haem-iron x Hb level at third trimester | 1.02 [1.01 – 1.04]* |
| Non-haem iron x Hb level at third trimester | 1.01 [0.98 – 1.07] |
| Iron supplementation x Hb level at third trimester | 1.02 [1.01 – 1.03]* |

Note. Non-GDM as reference group.
*$p < 0.05$.
[a] Adjusted by age, parity, pre-pregnancy BMI, gestational week at dietary intake assessment, total energy intake, and Hb at first prenatal visit.

**Table 3**
Associations between iron intake and GDM risk stratified by Hb levels in the third trimester.

| | Hb categories in the third trimester | | |
|---|---|---|---|
| | Hb < 11.0 g/dL (n= 193) | Hb 11.0–12.4 g/dL (n= 342) | Hb ≥ 12.5 g/dL (n= 97) |
| | AOR [95% CI] | AOR [95% CI] | AOR [95% CI] |
| Total iron | 1.02 [0.99 – 1.03] | 0.99 [0.97 – 1.01] | 1.01 [1.01 – 1.03]* |
| Dietary iron | 1.02 [0.94 – 1.10] | 1.04 [0.92 – 1.10] | 1.11 [0.93 – 1.33] |
| Haem-iron | 1.17 [0.76 – 1.80] | 1.19 [0.91 – 1.56] | 2.18 [1.04 – 4.59]* |
| Non-haem iron | 1.02 [0.93 – 1.11] | 0.98 [0.89 – 1.09] | 1.05 [0.86 – 1.27] |
| Iron supplementation | 1.03 [1.01 – 1.04]* | 1.01 [0.99 – 1.02] | 0.99 [0.98 – 1.01] |

Adjusted by age, parity, pre-pregnancy BMI, gestational week at dietary intake assessment, total energy intake, and Hb level at the first prenatal visit.
*$p < 0.05$.

metabolism from glucose to fatty acid oxidation (6, 7). High iron stores in the liver may induce insulin resistance by impairing insulin signalling and reducing the liver's ability to response to insulin (4, 5).

In this study, only women with Hb levels ≥12.5 g/dL in the third trimester showed a significant association between haem-iron intake and GDM risk. Both elevated Hb levels and higher haem-iron intakes could lead to the accumulation of oxidative stress, which can cause insulin resistance and impaired glucose metabolism. Qiu et al. (2011) showed that women with high haem-iron intake of ≥1.12 mg/day had a significantly increased risk of GDM compared to those with a low haem iron intake of <0.48 mg/day [32]. The threshold for high haem iron intake observed in Qiu et al. (2011) closely matches the value (>1.18 mg/day) found to be significantly associated with GDM risk in the present study. These findings suggest that the risk of GDM may significantly increase when haem iron intake reaches 1.12 – 1.18 mg/day, which is equivalent to approximately 20 – 28 g of cooked shellfish or 90 – 100 g of cooked lean beef. However, future studies are warranted to confirm this cut-off value for at-risk haem iron, as the relatively small sample size in the present study could limit the conclusions drawn from its subgroup analysis.

The present study found that iron supplements were associated with an increased risk of GDM, with a significant association observed only among women with low Hb levels in the third trimester (Hb < 11.0 g/dL). These findings should be interpreted cautiously, as the rate of iron supplement usage was low, with approximately 14.4% of women in the total sample and 10.9% among those with Hb < 11.0 g/dL reporting the use of iron-containing supplements. Further subgroup analysis of women with Hb < 11.0 g/dL showed that the mean daily iron supplement doses ranged from 30 mg to 247 mg of elemental iron, with 19% had >120 mg of elemental iron per day or the therapeutic recommended dose for the treatment of anaemia [22,35]. Additionally, a significantly higher percentage of GDM women in this group had family history of diabetes mellitus (DM) compared to those in the normal and high Hb groups ($p < 0.05$). The significant association between iron supplements and GDM risk among women with low Hb level in the third trimester became stronger (crude OR = 1.01 vs. adjustment of family history of DM AOR = 1.05) after adjusting for family history of DM. These findings suggest that the impact of high iron supplementation on the risk of GDM may, at least in part, be contributed to by a family history of DM. Therefore, it is believed that there might be other important unknown and unmeasured factors affecting the association among this group of women. Whether this association is simply due to the relatively small number of cases of GDM diagnosed in women with Hb < 11.0 g/dL who are taking iron supplements or truly reflects the complex and multifactorial nature of GDM risk remain to be elucidated.

Non-haem iron intake appeared to be negatively associated with GDM risk, which was consistent with previous studies, although the association was not statistically significant. Qiu et al. (2011) examined the relationships between dietary haem-iron and non-haem iron with the GDM risk and found that dietary non-haem iron was inversely link with the risk of developing GDM, but this relationship did not reach statistical significance [32]. Similarly, the Iowa Women's Health Study revealed that dietary non-haem iron was inversely linked with the risk of T2DM in postmenopausal women [25]. In this cohort, almost all (91%) of total dietary iron was from non-haem sources, and about 69.4% of women with Hb levels ≥11.0 g/dL. Therefore, despite the low absorption of non-haem iron, the absolute amount of available non-haem iron may be comparable to that of available haem iron. The plausible explanation for the different results between haem and non-haem is that haem iron and non-haem iron have different absorption rates. While haem iron accounts for around 40% of total iron absorption, with no notable inhibitors other than calcium, the absorption rate of non-haem iron varies from 2% to 20% and it is regulated by several other dietary components [36]. Furthermore, non-haem iron is predominantly found in plant-based foods such as whole grains, nuts, seeds, legumes, foods containing high levels of vitamins and minerals that offer many health benefits [37]. As a result, the extent of their associations with GDM may vary.

There are several limitations to this study. The study sample may not be representative of pregnant women in the general population of Malaysia, as the majority were Malay and had tertiary education and above. Although the SFFQ was validated, food album and household measures were employed to help the respondents to better estimate the amounts they consumed to minimize reporting bias. The accuracy of iron intake estimations might be limited, as the iron content of foods and beverages was based on the USDA food database or product packaging [38]. However, it is unlikely that the iron content of iron-fortified foods on the market would differ significantly from that of the USDA food database. The estimation of iron intake from supplements was based on label values, which may reduce the possibility of over-estimation or under-estimation.

## 5. Conclusions

This cross-sectional analysis has revealed significant positive associations between total iron intakes, encompassing both dietary haem iron and iron supplements, during the early third trimester with GDM risk. Notably, the significant relationship between haem iron and GDM risk was discerned exclusively among women with high Hb levels (Hb $\geq$ 12.5 g/dL), whereas the relationship between iron supplements and GDM risk was found in women with low Hb levels (Hb $<$ 11.0 g/dL). Given the preliminary nature of these findings, further investigation is imperative to validate these associations, elucidate the underlying mechanism, and establish precise thresholds for different iron sources to identify high-risk groups for GDM development. These efforts will contribute significantly to our understanding of GDM risk factors and inform strategies for its prevention and management.

## CRediT authorship contribution statement

**Heng Yaw Yong:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Conceptualization. **Zalilah Mohd Shariff:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Su Peng Loh:** Writing – review & editing, Methodology. **Barakatun Nisak Mohd Yusof:** Writing – review & editing, Methodology. **Nor Baizura Md Yusop:** Writing – review & editing, Methodology.

## Ethical statement

Ethical approval for this study was obtained from the Medical Research Ethics Committee, Ministry of Health Malaysia (Ethics Approval Number: NMRR-18-2606-43752; Approval Date: 6 December 2018). Permission to conduct the study was also granted by the Selangor State Health Department and the Hulu Langat District Health Office. All participants provided written informed consent prior to enrolment.

## Data availability statement

Data used for this study can be accessed upon request from the corresponding author (zalilahms@upm.edu.my).

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2026.e44683.

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