Long-term risk of type 2 diabetes in relation to habitual iron intake in women with a history of gestational diabetes: a prospective cohort study

Wei Bao, Jorge E Chavarro, Deirdre K Tobias, Katherine Bowers, Shanshan Li, Frank B Hu, and Cuilin Zhang

ABSTRACT

Background: An iron overload may induce pancreatic islet damage and increase risk of diabetes. Women with a history of gestational diabetes mellitus (GDM) are at high risk of developing type 2 diabetes mellitus (T2DM) after pregnancy.

Objective: We aimed to examine the association of habitual iron intake with long-term risk of T2DM in this high-risk population.

Design: We included 3976 women with a history of GDM from the Nurses’ Health Study II cohort as part of the ongoing Diabetes & Women’s Health Study. The women were followed up through 2009. Iron intake was assessed with the use of a validated food-frequency questionnaire in 1991 and every 4 y thereafter. We used Cox proportional hazards models to estimate HRs and 95% CIs.

Results: We documented 641 incident T2DM cases during 57,683 person-years of observation. Adjusted HRs for T2DM for the highest quartile compared with the lowest quartile were 1.64 (95% CI: 1.20, 2.25; \( P \)-trend = 0.02) for total iron intake and 1.80 (95% CI: 1.18, 2.74; \( P \)-trend = 0.005) for dietary heme iron intake. In addition, women who consumed \( \geq 30.0 \) mg supplemental Fe/d, compared with nonusers, had an adjusted HR of 1.83 (95% CI: 1.25, 2.70; \( P \)-trend = 0.002).

Conclusion: In women with a history of GDM, greater intakes of total iron, dietary heme iron, and supplemental iron were associated with higher risk of T2DM. Am J Clin Nutr 2016;103:375–81.

Keywords: diet, gestational diabetes mellitus, iron, heme iron, type 2 diabetes mellitus

INTRODUCTION

The global number of type 2 diabetes mellitus (T2DM) cases has been rising at an alarming rate (1, 2). Women with a history of gestational diabetes mellitus (GDM), which is a common pregnancy complication that is defined as glucose intolerance with onset or first recognition during pregnancy (3), are at high risk of developing T2DM after pregnancy (4). Most GDM cases probably reflect chronic \( \beta \) cell dysfunction and insulin resistance, which may for the first time be detected during pregnancy but actually deteriorates over time through the life span (5). Therefore, compared with women with a normoglycemic pregnancy, women with a history of GDM may have a diminished defense capacity and be more susceptible to some adverse exposures that are related to the development of T2DM.

Iron is considered a double-edged sword for living systems (6). It is an essential micronutrient that plays a vital role in oxygen transport, electron transfer, gene-expression regulation, and cell growth and differentiation. Iron deficiency is the most common nutritional deficiency in the world (7). However, iron is potentially hazardous in excess, leading to oxidative stress because of its pro-oxidant properties (8). The pancreatic \( \beta \) cells are vulnerable to oxidative stress because their antioxidative defense mechanisms are particularly weak (9). Previous studies have shown that excess iron administration can induce diabetes in animals (10). Moreover, dietary iron restriction or iron chelation can protect against the development of diabetes or attenuate pathologic changes in diabetic animal models (11, 12). In humans, biomarkers of iron metabolism have been associated with adipocyte insulin resistance early in the pathogenesis of T2DM (13, 14).

Higher intake of dietary heme iron, which is found in meat and meat products, has been associated with greater risk of T2DM in the general population (15–22). Few studies have examined the...
associations of dietary nonheme iron and supplemental iron intakes with T2DM risk (16, 18). Dietary supplement use has steadily increased over time since the 1970s and has become common in contemporary women (23). It is estimated that 26% of US women aged 31–50 y are using iron-containing supplements (23). Women who use iron-containing supplements also have higher iron intake from food sources than nonusers do (24). As a result, ~7% of these iron supplement users have a total iron intake that exceeds the recommended Tolerable Upper Intake Level (45 mg total Fe/d) (24). Whether the elevated intake of total iron in contemporary women is associated with long-term risk of T2DM remains unclear. In this study, we aimed to comprehensively examine the associations of iron intake, including intakes of total iron, dietary heme and nonheme iron, and supplemental iron, with long-term risk of T2DM in women with a history of GDM, who are a high-risk population of T2DM.

METHODS

Study population

The study population was composed of women with a history of GDM in the Nurses’ Health Study II (NHSII) as part of the ongoing Diabetes & Women’s Health Study (25), which aims to identify determinants of the progression from GDM to T2DM. The NHSII, which was established in 1989, is an ongoing prospective cohort study of 116,430 female nurses aged 24–44 y at study initiation (26). The participants received a biennial questionnaire to update information on health-related behaviors and disease outcomes. Follow-up for each questionnaire cycle was >90%. This study was approved by the Partners Human Research Committee (Boston, MA) with participant consent implied by the return of the questionnaires.

Participants were eligible for inclusion if they reported prevalent GDM before 1991 or incident GDM from 1991 to 2001. GDM was last captured on the 2001 questionnaire in this cohort because the majority of NHSSII participants passed a reproductive age by then. In a previous validation study in a subgroup of this cohort, 94% of GDM self-reports were confirmed by medical records (26). In a random sample of parous women without GDM, 83% of them reported having a glucose-screening test during pregnancy, and 100% reported having frequent prenatal urine screenings, which indicated a high level of GDM surveillance in this cohort (26). Participants were excluded from the analysis if they reported chronic disease (T2DM, cardiovascular disease, or cancer) before their GDM pregnancy or before the return of their first post-GDM food-frequency questionnaire (FFQ), had a multiple-birth pregnancy, or returned no post-GDM FFQ.

Exposure assessment

Beginning in 1991 and every 4 y thereafter, participants reported their usual food intakes over the previous year with the use of a semiquantitative FFQ. Intakes of individual nutrients including iron were computed by multiplying the frequency of consumption of each unit of food by the nutrient content of the specified portions on the basis of food-composition data from USDA sources (27) and data from manufacturers. In addition to multivitamin supplements, participants also reported the use of any specific iron supplements including their doses. Total iron intake was calculated as the sum of dietary and all supplemental iron intakes. Iron from dietary sources included dietary heme iron (present in animal foods that originally contained hemoglobin and myoglobin, such as meat, poultry, and fish) and nonheme iron (present in both plant and animal products). The heme iron content was calculated by applying a factor of 0.4 to the total iron content of all items of meat, poultry, and fish (16, 28). The forms and preparations of specific iron supplements in the US pharmacy are diverse, but they are mainly nonheme iron in the forms of ferric and ferrous salts and in numerous formulations, e.g., amino acid chelates, carbonyl iron, polysaccharide iron complex, and combination products (29). The reproducibility and validity of the FFQ have been extensively documented elsewhere (30–32). The Pearson correlation coefficient for total iron intake between the FFQ and four 1-wk diet records was 0.55 in a similar cohort of US female nurses (31). Although dietary heme iron intake was not specifically evaluated in the previous validation study, the major dietary sources (i.e., meat, poultry, and fish) of heme iron intake have been evaluated. Pearson correlation coefficients between the FFQ and dietary records ranged from 0.38 for hamburger to 0.70 for bacon (32). In a previous study in Nurses’ Health Study participants, heme iron intake was significantly associated with elevated body iron stores (33), which was in line with findings from randomized controlled trials (34).

Covariate assessment

Information on age, weight, height, race-ethnicity, family history of diabetes, smoking status, age at first birth, use of oral contraceptives, and menopausal status was collected with the use of biennial questionnaires. Parity was defined as the number of pregnancies that last >6 mo. Self-reported weight was highly correlated with the measured weight ($r = 0.97$) in a previous validation study (35). BMI (in kg/m$^2$) was computed as weight divided by the square of height. Total physical activity was ascertained by the frequency of engaging in common recreational activities from which metabolic equivalent task–hours per week were derived. In a previous validation study in this cohort, the correlation between physical activity as reported in 1-wk recalls and that reported on the questionnaires was 0.79, and the correlation between physical activity reported in diaries and that reported on the questionnaires was 0.62 (36).

Ascertainment of outcome

Participants who reported physician-diagnosed T2DM in a biennial questionnaire were mailed an additional questionnaire regarding symptoms, diagnostic tests, and hypoglycemic therapy to confirm self-reported diagnoses. Confirmed diabetes cases were defined according to the American Diabetes Association criteria (37) as follows: 1) one or more classic symptoms (excessive thirst, polyuria, unintentional weight loss, or hunger) plus elevated glucose concentrations (fasting plasma glucose concentration $\geq$ 7.0 mmol/L or random plasma glucose concentration $\geq$ 11.1 mmol/L); 2) no symptoms reported but $\geq$ 2 elevated plasma glucose concentrations on more than one occasion (fasting concentration $\geq$ 7.0 mmol/L; random concentration $\geq$ 11.1 mmol/L, or 2-h oral-glucose-tolerance test concentration $\geq$ 11.1 mmol/L); or 3) treatment with insulin or an oral hypoglycemic agent. Before 1998, a fasting plasma glucose concentration $\geq$ 7.8 mmol/L.
was used instead of one \( \geq 7.0 \text{ mmol/L} \) for the diagnosis of diabetes according to the criteria of the National Diabetes Data Group (38). A subgroup validation study conducted in a similar cohort of US female nurses showed high accuracy (98%) for the comparison of our classification on the basis of the additional questionnaire with a medical records review (39).

**Statistical analysis**

Dietary variables including iron intake were energy adjusted with the use of the residual method (40) and updated as cumulative average intakes to reduce the within-subject variation and represent the long-term habitual diet after a GDM diagnosis (41). In this analysis, we defined the baseline as the questionnaire period in which participants first reported a GDM pregnancy. We computed the follow-up time from the date of the GDM diagnosis to the date of a T2DM diagnosis, death, or the return of the 2009 questionnaire, whichever came first. The updating of exposure ceased if a participant reported incident chronic diseases (cardiovascular disease or cancer). We carried forward missing exposure data from the most recent questionnaire for which data were captured.

We used Cox proportional hazards models to estimate the HRs and 95% CIs. We divided the participants into quartiles according to intakes of total iron, dietary heme iron, dietary nonheme iron, or 3 categories according to supplemental iron intake (0, 0.1–29.9, or \( \geq 30.0 \) mg/d). The covariates in the adjusted models were age (mo), parity (1, 2, 3, or \( \geq 4 \)), BMI (<23.0, 23.0–24.9, 25.0–26.9, 27.0–29.9, 30.0–34.9, or \( \geq 35.0 \)), age at first birth (12–24, 25–29, or \( \geq 30 \)), race-ethnicity (Caucasians, African American, Hispanic, Asian, or others), family history of diabetes (yes or no), oral contraceptive use (current, former, or never), menopausal status (premenopausal or postmenopausal), cigarette smoking (current, former, or never), alcohol intake (0, 0.1–4.9, 5.0–14.9, or \( \geq 15.0 \) g/d), physical activity (quartiles), the ratio of polyunsaturated fat intake to saturated fat intake (quartiles), and intakes of total energy (quartiles), saturated fat (quartiles), trans fat (quartiles), dietary cholesterol (quartiles), animal protein (quartiles), vegetable protein (quartiles), glycemic load (quartiles), cereal fiber (quartiles), calcium (quartiles), magnesium (quartiles), and vitamin C (quartiles). For the nutrients adjusted in the analysis, Pearson correlation coefficients between the FFQ and the dietary records ranged from 0.52 for saturated fat to 0.57 for dietary cholesterol in the previous validation study (30). Mutual adjustment was performed for dietary heme iron, dietary nonheme iron, and supplemental iron. For supplemental iron intake, we further analyzed the associations of T2DM with the use of specific iron supplements and iron-containing multivitamins. Tests for a significant linear trend across quartiles and categories were carried out by assigning median values of each quartile or category of iron intakes as a continuous variable.

We evaluated a potential effect modification by conducting stratified analyses according to age (<40 or \( \geq 40 \) y), family history of diabetes (yes or no), smoking (never or ever), obesity (BMI <30 or \( \geq 30 \) kg/m\(^2\)), and time since the first GDM pregnancy (<10 or \( \geq 10 \) y). We also conducted interaction tests via multiplicative interaction terms in multivariable models. To address the potential confounding by medical surveillance of T2DM, we conducted a sensitivity analysis that was restricted to subjects who reported at least one symptom of diabetes at the time of diagnosis. To minimize potential bias from undiagnosed T2DM before a GDM pregnancy, we conducted additional analyses in which we excluded women who reported T2DM in the questionnaire subsequent to reporting GDM (e.g., GDM was reported in 1991, and T2DM was reported in 1993).

All statistical analyses were performed with SAS software (version 9.3; SAS Institute). \( P < 0.05 \) was considered statistically significant.

**RESULTS**

We documented 641 incident T2DM cases from 3976 participants (16.1%) with a history of GDM, thereby contributing 57,683 person-years of observation. At baseline, participants in the higher quartile of total iron intake had lower BMI, were more physically active, and were less likely to be current smokers and to have a family history of diabetes. These subjects consumed more total calories from carbohydrates and protein and fewer total calories from fat. In addition, they consumed more dietary fiber, zinc, calcium, magnesium, potassium, vitamin E, vitamin C, folate, and whole grains and less alcohol and caffeine (Table 1). Baseline characteristics according to categories of intakes of dietary heme iron, nonheme iron, and supplemental iron in women with a history of GDM are shown in Supplemental Table 1.

We observed that intakes of total iron, dietary heme iron, and supplemental iron were positively associated with T2DM risk, whereas dietary nonheme iron intake was inversely associated with T2DM risk. For total iron, women with the highest quartile, compared with those with the lowest quartile, had 64% (HR: 1.64; 95% CI: 1.20, 2.25; \( P\)-trend = 0.02) higher risk of T2DM (Table 2). Adjusted HRs for T2DM for the comparison of the highest quartile with the lowest quartile were 1.80 (95% CI: 1.18, 2.74; \( P\)-trend = 0.005) for dietary heme iron intake and 0.71 (95% CI: 0.51, 1.00; \( P\)-trend = 0.06) for dietary nonheme iron intake (Table 3). In addition, women who consumed \( \geq 30.0 \) mg supplemental Fe/d, compared with nonusers, had an adjusted HR of 1.83 (95% CI: 1.25, 2.70; \( P\)-trend = 0.002) (Table 2). Compared with women who were taking neither an iron-containing multivitamin nor specific iron supplements, women who were taking an iron-containing multivitamin only had an adjusted HR of 1.50 (95% CI: 1.20, 1.89), and those who were taking specific iron supplements only or both an iron-containing multivitamin and specific iron supplements had an adjusted HR of 1.55 (95% CI: 1.14, 2.10) (Supplemental Figure 1).

The observed associations of total iron, dietary heme iron, and supplemental iron intakes with T2DM risk persisted in stratified analyses according to age, family history of diabetes, smoking, obesity status, and time since the first GDM pregnancy. To address potential screening bias by medical surveillance of T2DM, we conducted a sensitivity analysis in women who reported at least one symptom of diabetes at diagnosis and observed similar results to those for the entire cohort. In addition, to minimize potential bias from subclinical T2DM cases, we conducted additional sensitivity analyses by excluding women who reported T2DM in the subsequent questionnaire of reporting GDM, and the multivariate HRs of T2DM were not appreciably changed.

**DISCUSSION**

In a large prospective cohort study of women with a history of GDM, we showed that higher intakes of total iron, dietary heme iron, and supplemental iron were significantly associated with...
TABLE 1
Age-standardized baseline characteristics according to quartiles of total iron intake in women with a history of GDM\(^1\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile of total iron intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Participants, n</td>
<td>982</td>
</tr>
<tr>
<td>Age, y</td>
<td>37.5 ± 4.6(^2)</td>
</tr>
<tr>
<td>Family history of diabetes, %</td>
<td>29.0</td>
</tr>
<tr>
<td>Race, Caucasian, %</td>
<td>92.5</td>
</tr>
<tr>
<td>Current oral contraceptive use, %</td>
<td>8.4</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>14.4</td>
</tr>
<tr>
<td>Alcohol, g/d</td>
<td>2.7 ± 6.0</td>
</tr>
</tbody>
</table>

**Table Note:**
- At baseline: 27.1 ± 6.7, 27.4 ± 6.6, 26.7 ± 6.1, 26.1 ± 5.9 <0.001
- At age 18 y: 21.2 ± 3.4, 21.8 ± 3.7, 21.4 ± 3.2, 21.1 ± 3.2 <0.001
- Physical activity, MET-h/wk: 16.1 ± 23.6, 17.2 ± 24.4, 16.9 ± 19.9, 18.4 ± 23.2 <0.001
- Total calories, kcal/d: 1847 ± 585, 1928 ± 579, 1995 ± 568, 1851 ± 540 <0.001
- Carbohydrates, energy, %: 47.7 ± 7.5, 48.7 ± 7.2, 50.9 ± 7.2, 50.4 ± 7.8 <0.001
- Total protein, energy, %: 18.6 ± 3.5, 19.9 ± 3.4, 19.5 ± 3.2, 19.8 ± 3.6 <0.001
- Total fat, energy, %: 34.2 ± 5.7, 32.3 ± 5.6, 30.9 ± 5.7, 30.9 ± 5.9 <0.001
- SFAs, energy, %: 12.4 ± 2.4, 11.2 ± 2.3, 10.7 ± 2.4, 10.9 ± 2.5 <0.001
- MUFA energy, %: 13.1 ± 2.5, 12.4 ± 2.6, 11.8 ± 2.6, 11.8 ± 2.6 <0.001
- PUFA energy, %: 5.8 ± 1.6, 5.7 ± 1.4, 5.6 ± 1.3, 5.4 ± 1.5 <0.001
- trans Fat energy, %: 1.9 ± 0.7, 1.7 ± 0.6, 1.6 ± 0.6, 1.5 ± 0.6 <0.001
- Dietary cholesterol,\(^1\), mg/d: 250.1 ± 64.0, 256.6 ± 73.0, 240.5 ± 71.8, 244.5 ± 75.8 <0.001
- Total fiber,\(^2\), g/d: 15.6 ± 4.0, 18.5 ± 4.7, 19.9 ± 5.7, 19.3 ± 5.6 <0.001
- Cereal fiber,\(^2\), g/d: 4.3 ± 1.8, 5.5 ± 2.1, 6.5 ± 2.8, 6.3 ± 3.2 <0.001
- Fruit fiber,\(^1\), g/d: 2.9 ± 2.2, 3.2 ± 2.1, 3.5 ± 2.2, 3.6 ± 2.2 <0.001
- Vegetable fiber,\(^1\), g/d: 5.5 ± 2.4, 7.1 ± 3.1, 7.3 ± 3.7, 6.8 ± 3.4 <0.001
- Glycemic index\(^3\): 53.7 ± 3.5, 54.0 ± 3.2, 53.9 ± 3.4, 53.4 ± 3.6 <0.001
- Glycemic load\(^3\): 115.7 ± 21.8, 118.4 ± 20.3, 123.3 ± 21.1, 121.6 ± 22.5 <0.001
- Zinc,\(^3\), mg/d: 11.6 ± 4.8, 13.0 ± 7.4, 16.1 ± 9.5, 25.6 ± 16.3 <0.001
- Calcium,\(^3\), mg/d: 941.7 ± 409.7, 935.0 ± 399.6, 1042.3 ± 398.1, 1256.5 ± 500.3 <0.001
- Magnesium,\(^3\), mg/d: 271.5 ± 53.2, 302.6 ± 58.8, 338.0 ± 68.5, 363.9 ± 92.7 <0.001
- Potassium,\(^3\), mg/d: 2760.8 ± 356.0, 2976.2 ± 512.1, 3078.3 ± 578.8, 3096.3 ± 583.0 <0.001
- Vitamin E,\(^1\), mg/d: 17.7 ± 51.0, 21.9 ± 54.7, 24.8 ± 46.5, 44.0 ± 86.4 <0.001
- Vitamin C,\(^1\), mg/d: 167.1 ± 193.1, 207.4 ± 252.2, 248.8 ± 276.1, 340.3 ± 383.4 <0.001
- Folate,\(^4\), µg/d: 316.2 ± 163.2, 361.3 ± 163.5, 461.8 ± 163.3, 749.4 ± 350.5 <0.001
- Caffeine,\(^1\), mg/d: 221.7 ± 204.9, 233.4 ± 214.8, 208.4 ± 194.8, 199.0 ± 201.5 <0.001
- Red meat, servings/d: 1.1 ± 0.6, 1.1 ± 0.7, 1.0 ± 0.7, 0.9 ± 0.6 <0.001
- Poultry, servings/d: 0.4 ± 0.3, 0.5 ± 0.3, 0.5 ± 0.3, 0.5 ± 0.3 <0.001
- Fish, servings/d: 0.2 ± 0.2, 0.2 ± 0.2, 0.3 ± 0.2, 0.2 ± 0.2 <0.001
- Eggs, servings/d: 0.2 ± 0.2, 0.2 ± 0.2, 0.2 ± 0.2, 0.2 ± 0.2 <0.001
- Fruit, servings/d: 1.0 ± 0.9, 1.2 ± 0.9, 1.4 ± 1.1, 1.3 ± 0.9 <0.001
- Vegetables, servings/d: 2.8 ± 1.5, 3.8 ± 2.1, 4.1 ± 2.5, 3.5 ± 2.1 <0.001
- Nuts, servings/d: 0.3 ± 0.4, 0.3 ± 0.4, 0.3 ± 0.3, 0.5 ± 0.4 <0.001
- Legumes, servings/d: 0.3 ± 0.3, 0.4 ± 0.3, 0.5 ± 0.4, 0.4 ± 0.4 <0.001
- Whole grains, servings/d: 0.8 ± 0.8, 1.1 ± 1.0, 1.3 ± 1.1, 1.2 ± 1.1 <0.001
- Sugar-sweetened beverages, servings/d: 0.7 ± 1.1, 0.4 ± 0.8, 0.4 ± 0.8, 0.4 ± 0.8 <0.001

\(^1\)Baseline was defined as 1991 for prevalent GDM, and the year of the index pregnancy was used for incident GDM. All comparisons across quartiles of total iron intake were significant except for the following: age, race-ethnicity, current oral contraceptive use, and the consumption of eggs and nuts. GDM, gestational diabetes mellitus; MET-h, metabolic equivalent task hours.

\(^2\)Mean ± SD (all such values).

\(^3\)All values were energy adjusted.

greater risk of T2DM. These associations were independent of other major dietary and nondietary risk factors of T2DM.

To our knowledge, the current study is the first one to examine the association between iron intake and risk of T2DM in women with a history of GDM. The significant and positive associations of heme iron intake and T2DM risk in our study population seemed modestly stronger than in previous studies in middle-aged and elderly women (16–18). However, the significant and positive associations of T2DM with supplemental iron and total iron intakes observed in the current study are in contrast with those from previous studies in the general population in which null associations were reported (16–18). Note that our study population consumed an appreciably higher amount of total iron than did subjects in previous studies (16–18). Few studies have examined the relation between
Mutual adjustment was performed for dietary heme iron and nonheme iron intakes. Tests for a significant linear trend across quartiles were carried out by assigning median values of each quartile of iron intakes as a continuous variable. GDM, gestational diabetes mellitus; Q, quartile; T2DM, type 2 diabetes mellitus.

**TABLE 2**

<table>
<thead>
<tr>
<th>Iron intake</th>
<th>First Q or category</th>
<th>Second Q or category</th>
<th>Third Q or category</th>
<th>Fourth Q or category</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total iron intake, mg/d</td>
<td>11.6</td>
<td>14.9</td>
<td>21.1</td>
<td>37.2</td>
<td>—</td>
</tr>
<tr>
<td>T2DM cases, n</td>
<td>139</td>
<td>171</td>
<td>165</td>
<td>166</td>
<td>—</td>
</tr>
<tr>
<td>Person-years</td>
<td>14,431</td>
<td>14,436</td>
<td>14,405</td>
<td>14,411</td>
<td>—</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>1.00 (reference)</td>
<td>1.23 (0.96, 1.58)</td>
<td>1.05 (0.81, 1.35)</td>
<td>1.22 (0.95, 1.57)</td>
<td>0.28</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>1.00 (reference)</td>
<td>1.48 (1.11, 1.97)</td>
<td>1.33 (0.98, 1.80)</td>
<td>1.64 (1.20, 2.25)</td>
<td>0.02</td>
</tr>
<tr>
<td>Supplemental iron intake, mg/d</td>
<td>0</td>
<td>0.1–29.9</td>
<td>≥30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T2DM cases, n</td>
<td>252</td>
<td>340</td>
<td>49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Person-years</td>
<td>26,942</td>
<td>26,072</td>
<td>4669</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>1.00 (reference)</td>
<td>1.21 (1.01, 1.46)</td>
<td>1.50 (1.07, 2.10)</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>1.00 (reference)</td>
<td>1.47 (1.18, 1.84)</td>
<td>1.83 (1.25, 2.70)</td>
<td>—</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1In multivariate models, covariates included age (mo), parity (1, 2, 3, or ≥4), BMI [[(kg/m²) < 23.0, 23.0–24.9, 25.0–26.9, 27.0–29.9, 30.0–34.9, or ≥35.0], age at first birth (12–24, 25–29, or ≥30 y), race-ethnicity (Caucasian, African American, Hispanic, Asian, or other), family history of diabetes (yes or no), oral contraceptive use (current, former, or never), menopausal status (premenopausal or postmenopausal), cigarette smoking (never, former, or current), alcohol intake (0, 0.1–4.9, 5.0–14.9, or ≥15.0 g/d), physical activity (quartiles), the ratio of polyunsaturated fat intake to saturated fat intake (quartiles), and intakes of total energy (quartiles), saturated fat (quartiles), trans fat (quartiles), dietary cholesterol (quartiles), animal protein (quartiles), vegetable protein (quartiles), glycemic load (quartiles), cereal fiber (quartiles), calcium (quartiles), magnesium (quartiles), vitamin C (quartiles), and supplemental iron (0, 0.1–29.9, or ≥30 mg/d).

The association between hereditary hemochromatosis, which is an inherited iron-overload disorder, and risk of T2DM has long been recognized in humans (8). Moderately elevated body iron stores, as measured by ferritin, soluble transferrin receptor, and the soluble transferrin receptor:ferritin ratio, have also been linked to higher risk of T2DM in various populations (22). In contrast, a reduction in body iron stores through phlebotomy or iron-chelation therapy has been associated with an improved glucose tolerance and insulin sensitivity (42, 43). Several mechanisms may link iron overload to increased risk of T2DM.

**TABLE 3**

<table>
<thead>
<tr>
<th>Quartiles of dietary heme or nonheme iron intake</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median intake, mg/d</td>
<td>0.7</td>
<td>1.0</td>
<td>1.2</td>
<td>1.5</td>
<td>—</td>
</tr>
<tr>
<td>T2DM cases, n</td>
<td>124</td>
<td>136</td>
<td>169</td>
<td>212</td>
<td>—</td>
</tr>
<tr>
<td>Person-years</td>
<td>14,404</td>
<td>14,245</td>
<td>14,609</td>
<td>14,425</td>
<td>—</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>1.00 (reference)²</td>
<td>1.17 (0.89, 1.53)</td>
<td>1.57 (1.21, 2.04)</td>
<td>1.91 (1.49, 2.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>1.00 (reference)²</td>
<td>1.23 (0.89, 1.69)</td>
<td>1.45 (1.01, 2.08)</td>
<td>1.80 (1.18, 2.74)</td>
<td>0.005</td>
</tr>
<tr>
<td>Nonheme iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median intake, mg/d</td>
<td>9.0</td>
<td>11.1</td>
<td>13.0</td>
<td>16.6</td>
<td>—</td>
</tr>
<tr>
<td>T2DM cases, n</td>
<td>180</td>
<td>171</td>
<td>157</td>
<td>133</td>
<td>—</td>
</tr>
<tr>
<td>Person-years</td>
<td>13,930</td>
<td>14,652</td>
<td>14,540</td>
<td>14,561</td>
<td>—</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>1.00 (reference)²</td>
<td>0.81 (0.64, 1.03)</td>
<td>0.76 (0.60, 0.97)</td>
<td>0.72 (0.56, 0.93)</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>1.00 (reference)²</td>
<td>0.86 (0.66, 1.13)</td>
<td>0.76 (0.56, 1.03)</td>
<td>0.71 (0.51, 1.00)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1In multivariate models, covariates included age (mo), parity (1, 2, 3, or ≥4), BMI [[(kg/m²) < 23.0, 23.0–24.9, 25.0–26.9, 27.0–29.9, 30.0–34.9, or ≥35.0], age at first birth (12–24, 25–29, or ≥30 y), race-ethnicity (Caucasian, African American, Hispanic, Asian, or other), family history of diabetes (yes or no), oral contraceptive use (current, former, or never), menopausal status (premenopausal or postmenopausal), cigarette smoking (never, former, or current), alcohol intake (0, 0.1–4.9, 5.0–14.9, or ≥15.0 g/d), physical activity (quartiles), the ratio of polyunsaturated fat intake to saturated fat intake (quartiles), and intakes of total energy (quartiles), saturated fat (quartiles), trans fat (quartiles), dietary cholesterol (quartiles), animal protein (quartiles), vegetable protein (quartiles), glycemic load (quartiles), cereal fiber (quartiles), calcium (quartiles), magnesium (quartiles), vitamin C (quartiles), and supplemental iron (0, 0.1–29.9, or ≥30 mg/d).

2HR: 95% CI in parentheses (all such values).
First, iron is a powerful pro-oxidant and catalyst that promotes the formation of hydroxyl radicals, which are the most reactive form of reactive oxygen species (8). Excess iron can attack pancreatic β cells through elevated oxidative stress, leading to β cell apoptosis and a decrease in glucose-induced insulin secretion (44). Second, excess iron interferes with glucose use in muscle tissues that leads to a shift from glucose to fatty acid oxidation (45) and diminishes the insulin-induced glucose transport in adipocytes (46), which may impair insulin action and result in increased insulin resistance. In addition, excess iron also increases gluconeogenic substrate recycling to the liver, which may contribute to an elevated hepatic glucose production (45). Note that iron absorption is regulated by complex genetic regulatory mechanisms including homeostatic hormones such as hepcidin (47). Epidemiologic studies have suggested a possible association of hepcidin and its transcription modulator (e.g., the transmembrane protease serine 6 gene) with risk of T2DM (48), but the findings are still inconclusive (49). The role of hepcidin in mediating the association between iron intake and T2DM risk warrants investigation in future studies.

High intakes of total iron, dietary heme iron, and supplemental iron are significantly associated with elevated body iron stores (33, 34, 50). The divergent associations of dietary heme iron and nonheme iron intakes with T2DM risk may be explained, at least partly, by their differences in bioavailability and their ability to raise body iron stores. Heme iron has been estimated to contribute 10–15% of dietary total iron intake in meat-eating populations. However, because of its greater and more uniform absorption, heme iron could contribute 40% of the absorbed iron pool in the body (51). In contrast, nonheme iron absorption is usually much less efficient than heme iron is and can be inhibited by various factors, e.g., phytate, polyphenols, and calcium in foods (51). Higher dietary heme iron intake was significantly associated with elevated body iron stores in both the Framingham Heart Study and the Nurses’ Health Study (33, 50). However, neither of these studies showed that higher dietary nonheme iron intake was significantly associated with elevated body iron stores. Furthermore, the Framingham Heart Study (50) observed an inverse association between dietary nonheme iron intake and body iron stores although the association was NS ($P = 0.08$).

The strengths of the current study include the prospective cohort design with long-term follow-up, the high response rate of each questionnaire cycle, and detailed and repeated dietary assessments with the use of an extensively validated questionnaire. All study participants were registered nurses, which minimized the potential for confounding by educational attainment or differential access to health care. We acknowledge that our study has several potential limitations. First, misclassification of iron intakes was possible. Although a previous validation study observed a high correlation for total iron intake between the FFQ and four 1-wk diet records, the correlation for dietary iron intake was weaker (31). Because of the prospective design of this study, the random within-person error would have been nondifferential; therefore, our observed results may underestimate the true association. Moreover, the use of cumulative averages of dietary intakes for participants with multiple FFQs during the follow-up would reduce random error (41). Second, we did not have information on the clinical diagnosis of iron-deficiency anemia, which could be the cause for habitual consumption of iron supplements. However, there is evidence, although still limited, that has shown that iron-deficiency anemia is associated with lower risk of diabetes (8). Therefore, the positive association between supplemental iron intake and risk of T2DM cannot be attributed to the participants’ iron-deficient or anemic status. Whether the association between iron intake and T2DM differs by iron-deficient or anemic status warrants future investigation.

Third, our study population consisted mostly of US Caucasian women, and therefore, our findings may not be able to be directly generalized to other populations. However, the relative homogeneity of our study population reduced the potential confounding from socioeconomic variability. Fourth, screening bias may result if more health-conscious women regularly visited a physician, thereby increasing their chance of receiving a medical diagnosis. However, in our sensitivity analyses, similar results were seen when we restricted cases to symptomatic T2DM, which minimized concerns for this bias. Last, although we adjusted for known diabetes risk factors and a number of potential confounders, we could not completely rule out the possibility of residual confounding from unmeasured factors.

In conclusion, we observed that in women with a history of GDM, who are a high risk population for developing T2DM, higher intakes of total iron, dietary heme iron, and supplemental iron were significantly associated with greater risk of T2DM.

The authors’ responsibilities were as follows—WB: contributed to the design and analysis of the study and wrote the manuscript; WB and CZ: were the guarantors of this work and, as such, had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; JEC, KB, SL, and FBH: interpreted the results and reviewed and edited the manuscript; DKT: conducted the technical review and reviewed and edited the manuscript; and CZ: contributed to the design and analysis of the study and reviewed and edited the manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES


