Long-term risk of type 2 diabetes in relation to habitual iron intake in women with a history of gestational diabetes: a prospective cohort study1,2

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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 ≥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)9 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 β 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 β 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

1Supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH (contract HHSN275201000020C). The Nurses’ Health Study II was funded by research grants DK58845, CA50385, P30 DK46200, and U1M CA176726 from the NIH. DKT was supported by a mentored fellowship from the American Diabetes Association (7-12-MN-34) and a training grant from the NIH (1K01DK103720-01).

2Supplemental Table 1 and Supplemental Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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1Abbreviations used: FFQ, food-frequency questionnaire; GDM, gestational diabetes mellitus; NHSSII, Nurses’ Health Study II; T2DM, type 2 diabetes mellitus.

Received February 4, 2015. Accepted for publication November 10, 2015. First published online January 13, 2016; doi: 10.3945/ajcn.115.108712.
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 NHSII 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). 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 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/m²) 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 ≥7.0 mmol/L or random plasma glucose concentration ≥11.1 mmol/L); 2) no symptoms reported but ≥2 elevated plasma glucose concentrations on more than one occasion (fasting concentration ≥7.0 mmol/L; random concentration ≥11.1 mmol/L, or 2-h oral-glucose-tolerance test concentration ≥11.1 mmol/L); or 3) treatment with insulin or an oral hypoglycemic agent. Before 1998, a fasting plasma glucose concentration ≥7.8 mmol/L.
was used instead of one ≥7.0 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 supplement intake (0, 0.1–29.9, or ≥30.0 mg/d). The covariates in the adjusted models were age (mo), parity (1, 2, 3, or ≥4), BMI (<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 (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 ≥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 ≥40 y), family history of diabetes (yes or no), smoking (never or ever), obesity (BMI <30 or ≥30 kg/m²), and time since the first GDM pregnancy (<10 or ≥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 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 ≥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...
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 with T2DM risk observed in this high-risk population was consistent with findings from a meta-analysis of prospective cohort studies conducted in the general population (22). The magnitude of the association between 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.

Tests for a significant linear trend across quartiles (total iron intake) or categories (supplemental iron intake) were carried out by assigning median supplemental iron, an adjustment for intakes of dietary iron was also performed. HRs (95% CIs) were estimated with the use of Cox proportional hazards models. Tests for a significant linear trend across quartiles in our study population is in general agreement with findings in postmenopausal women in the Iowa Women’s Health Study (16).

Additional studies are needed to confirm the association of dietary nonheme iron intake and diabetes risk. The inverse association of dietary nonheme iron intake with T2DM risk observed in this study. 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>
<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), and vitamin C (quartiles). Other measures 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), and vitamin C (quartiles). For the analysis of supplemental iron, an adjustment for dietary iron was also performed. HRs (95% CIs) were estimated with the use of Cox proportional hazards models. Tests for a significant linear trend across quartiles (total iron intake) or categories (supplemental iron intake) were carried out by assigning median values of each quartile or category of iron intake as a continuous variable. GDM, gestational diabetes mellitus; Q, quartile; T2DM, type 2 diabetes mellitus.

2All values are medians.

3HR; 95% CI in parentheses (all such values).

4In 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), and vitamin C (quartiles). Other measures 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), and vitamin C (quartiles). For the analysis of supplemental iron, an adjustment for dietary iron was also performed. HRs (95% CIs) were estimated with the use of Cox proportional hazards models. Tests for a significant linear trend across quartiles (total iron intake) or categories (supplemental iron intake) were carried out by assigning median values of each quartile or category of iron intake as a continuous variable. GDM, gestational diabetes mellitus; Q, quartile; T2DM, type 2 diabetes mellitus.
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 \( \beta \) cells through elevated oxidative stress, leading to \( \beta \) 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 glucoseogenic 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 intake 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.

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