Association between iron deficiency and HbA1c levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999-2006

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**Objective:** Iron deficiency has been reported to elevate hemoglobin A1c (HbA1c) levels apart from glycemia. We examined the influence of iron deficiency on HbA1c distribution among adults without diabetes.

**Research Design and Methods:** Participants included adults without self-reported diabetes or chronic kidney disease in NHANES 1999-2006 who were aged ≥ 18 years of age and had complete blood counts, iron studies, and HbA1c levels. Iron deficiency was defined as at least 2 abnormalities including: free erythrocyte protoporphyrin > 70 ug/dl red blood cells, transferrin saturation < 16%, or serum ferritin < 15 ug/L. Anemia was defined as hemoglobin < 13.5 g/dl in men and < 12.0 g/dl in women.

**Results:** Among women (n=6,666), 13.7% had iron deficiency and 4.0% had iron deficiency anemia. While 316 women with iron deficiency had Hba1c > =5.5%, only 32 women with iron deficiency had Hba1c > =6.5%. Among men (n=3,869), only 13 had iron deficiency and an HbA1c > =5.5% and only 1 had iron deficiency and an HbA1c > 6.5%. Among women, iron deficiency was associated with a greater odds of HbA1c > = 5.5% (OR 1.39, 95% CI, 1.11, 1.73) after adjustment for age, race/ethnicity, and waist circumference, but not with a greater odds of HbA1c > = 6.5% (OR 0.79, 95% CI 0.33, 1.85).

**Conclusions:** Iron deficiency is common among women and is associated with shifts in HbA1c distribution from an HbA1c < 5.5% to > 5.5%. Further research is needed to examine whether iron deficiency is associated with shifts at higher HbA1c levels.
Hemoglobin A1c (HbA1c) is formed by the glycation of the terminal valine of the β-chain of hemoglobin. It is used commonly as a screening test for diabetes in clinical practice. HbA1c may be less susceptible than other measures of glycemia to temporary fluctuations due to diet, physical activity, or illness as well as differences in local testing standards; as a result, an expert committee has recently endorsed an HbA1c ≥ 6.5% as diagnostic for diabetes.

Previous studies have reported that depletion of iron stores may alter the glycation rate of hemoglobin and elevate HbA1c concentrations, independent of glycemia. Iron deficiency may be present without associated anemia. While iron deficiency is the most common nutritional deficiency, the clinical relevance of iron deficiency upon the use of HbA1c as a screening test for diabetes has not been studied. Reproductive-age women are particularly vulnerable to iron deficiency, reflecting iron loss through menstruation and pregnancy. In the Third National Health and Nutrition Examination (NHANES) 1988-1994 and later NHANES waves, greater than 11% of women had iron deficiency.

Using a recent population-based sample of U.S. adults, we examined the distribution of HbA1c by iron deficiency status among adults without known diabetes. We hypothesized that adults with iron deficiency would be more likely to have elevated HbA1c levels, even after consideration of fasting plasma glucose. We also hypothesized that any differences would persist after adjustment for other factors associated with HbA1c and iron deficiency including age, race/ethnicity, and waist circumference.

**RESEARCH DESIGN AND METHODS**

**Study Population.** We used data from the NHANES 1999-2006 conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) to assess the health and nutritional status of the U.S. population. The NHANES 1999-2006 included a nationally representative probability sample of the U.S. civilian noninstitutionalized population, identified through a complex multistage cluster sampling design. During a household interview, participants provided information on sociodemographics and health status, and physicians and healthcare technicians conducted a standard examination on sampled subjects within 4 weeks of the interview. For the purposes of this analysis, we included NHANES 1999-2006 participants aged 18 years of age and older who had a complete blood count, iron studies, and HbA1c levels. We excluded persons with known self-reported diabetes (n=1,029) and pregnant women (n=1,144). Conditions such as renal disease that shorten erythrocyte survival may proportionately decrease HbA1c levels and are also associated with iron deficiency, but the degree of renal impairment at which anemia occurs is unclear. Therefore, we also excluded participants with chronic kidney disease (n=1,266), defined as a glomerular filtration rate (GFR) < 60 ml/min/1.73 m² or GFR from 60 to 90 ml/min/1.73 m² with microalbuminuria from the primary analysis.

**Main Outcome Measures.** HbA1c measurements for NHANES 1999-2004 were performed by the Diabetes Diagnostic Laboratory at the University of Missouri-Columbia using Primus CLC330 and Primus CLC 385 (Primus Corporation, Kansas City, MO). HbA1c measurements in NHANES 2005-2006 were performed by the Diabetes Laboratory at the University of Minnesota using Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Francisco, CA). Both assays use a High Performance
Liquid Chromatography (HPLC) system and were standardized to the Diabetes Control and Complications Trial reference method. Collection procedures were similar from 1999-2004 and between 2005-2006, intraassay variation was < 3% for both assays, and assays correlated at 0.98, with similar values for the range of HbA1c examined in this analysis.(9-11) Therefore, for the purposes of this analysis, we combine the results from the 2 assays.

According to analyses of 1999-2004 NHANES data, an HbA1c of 5.5% has a sensitivity of 89% and a specificity of 80% when compared with fasting glucose, and an HbA1c of 6.1% has a sensitivity of 67% and a specificity of 98% when compared to fasting glucose levels ≥ 126 mg/dl to detect diabetes.(12) In 2009, an expert committee endorsed an HbA1c as being diagnostic of diabetes.(1) For the purposes of this report, HbA1c was categorized in two ways. Due to the distribution of HbA1c values (Table 1), we examined a cutpoint of < 5.5% vs. ≥ 5.5%. Due to the expert committee recommendation, we examined a cutpoint of < 6.5% vs. ≥ 6.5%.

**Independent Variables.** Iron status may be assessed through several laboratory tests. We used the definition applied previously in the Third NHANES and the NHANES 1999-2000 for iron deficiency, i.e. any two of the following three indices: erythrocyte protoporphyrin levels > 70 ug/dl red blood cells; ferritin < 15 ug/L, and transferrin saturation levels < 16%.(3) The presence of anemia was defined as a hemoglobin level of < 12.0 g/dl for women and < 13.5 g/dl for men.(3)

We controlled for several factors would could have served as confounders due to their associations with both iron deficiency and HbA1c, including age,(13; 14) race,(13; 14) and obesity, particularly visceral adiposity.(14; 15) In the NHANES 1998-2006, race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Mexican-American, and other race. For the total estimates of iron deficiency, all racial/ethnic groups were combined. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and waist circumference is reported in centimeters. In women, we also controlled for self-report of parity and hysterectomy status as these may be associated with iron deficiency(16) and glucose tolerance.(17; 18)

**Statistical Analyses.** We performed analyses using SAS for data management and SUDAAN to account for unequal probabilities of selection, planned oversampling, and the complex sample design of the NHANES.(5) All analyses were stratified by gender, as anemia cutpoints for hemoglobin levels differ by sex. We compared categorical variables by iron deficiency status using chi-square tests and continuous variables using ANOVA. We constructed several types of multivariable models. First, we compared the examined the distribution of HbA1c between participants with and without iron deficiency. Ninety-seven percent of participants had HbA1c levels < 5.5 or 5.5-6.0 (Table 1); therefore, we used 5.5 as a cutpoint in one analysis and 6.5 as a cutpoint in another analysis. We used multiple logistic regression models to describe the odds of having an HbA1c levels > 5.5 vs. < 5.5 or > 6.5 vs. < 6.5 by iron deficiency status, before and after adjustment for age, race/ethnicity, waist circumference, and among women, parity (as a continuous variable) and hysterectomy status. Models using BMI instead of waist circumference were also constructed with similar results, so only the results using waist circumference are shown. We also calculated the predicted prevalence of an elevated HbA1c level according to iron deficiency status, and differences between prevalences, before and after adjustment for the covariates mentioned above.
We conducted several sensitivity analyses. First, we used multiple linear regression to calculate the adjusted prevalence of mean HbA1c levels by iron deficiency status before and after adjustment for the above factors. Due to the skewed HbA1c distribution, we log-transformed HbA1c levels but obtained similar results, and therefore for ease of interpretation present the analysis without log-transformation. To determine if the association between iron deficiency and HbA1c was independent of glycemia, we examined the subpopulation of men and women in our sample who also underwent a fasting glucose (n=2796 women and n=1680 men). Approximately one half of NHANES participants were sampled to attend the morning session. These participants were instructed to fast at least nine hours prior to the appointment time. Fasting plasma glucose values are available for those adults who attended the morning examination and were fasting 8 or more hours. In this subpopulation, we included fasting glucose levels as a continuous covariate when examining the association between iron deficiency and HbA1c levels. Due to the small number of male participants with both elevated Hba1c levels as well as iron deficiency, we performed sensitivity analyses only among women participants. In the third sensitivity analysis, we also included adults with GFR from 60 to 90 ml/min/1.73 m² with microalbuminuria, resulting in an increase in sample size from 2993 women and 1799 men to 3033 women and 2044 men. Finally, we examined the subpopulation of adults with anemia to determine if any associations were more pronounced in the subgroup of women with iron deficiency anemia, but we did not find this (results not shown).

RESULTS
Unadjusted characteristics of men and women with and without iron deficiency are shown in Table 1. Among women (n=6,666), 13.7% (n=1150) had iron deficiency, and 30% of iron deficient women also had anemia. Among men (n=3,869), 1.6% (n=75) had iron deficiency, and 33% of iron deficient men also had anemia. Among women, 316 participants with iron deficiency had an HbA1c ≥ 5.5%; 32 participants with iron deficiency had an HbA1c ≥ 6.5%. Among men, 13 participants with iron deficiency had an HbA1c ≥ 5.5, and only one male participant with iron deficiency had an HbA1c ≥ 6.5%.

Characteristics of iron deficient adults differed among men and women. Women with iron deficiency tended to be younger than 50 years of age, and were more likely to be African-American or Hispanic, obese, and to have a greater waist circumference, and were less likely to have had a hysterectomy. Men with iron deficiency tended to be older than 50 years of age and to have greater waist circumference. In the subpopulation of participants who had fasting glucose levels, iron deficient women had slightly lower fasting glucose compared to non-iron deficient women, but similar proportions had fasting glucose < 126 mg/dl. Iron deficient men had similar mean fasting glucose to non-iron deficient men, although no iron deficient men had a fasting glucose < 126 mg/dl. Among women, unadjusted HbA1c mean levels did not differ between iron deficient and non-iron deficient adults; HbA1c distributions were primarily shifted from < 5.5% to 5.5%-5.9%. Among men, unadjusted mean HbA1c levels were higher in iron deficient men compared to iron sufficient men.

The odds of having an HbA1c ≥ 5.5% by iron deficiency status are shown in Table 2. Among women, iron deficiency was associated with increased odds of an HbA1c ≥ 5.5% before and after adjustment for age and race/ethnicity, waist circumference, parity and hysterectomy. Among men with and without iron deficiency, the odds of having an
HbA1c $\geq 5.5\%$ did not reach statistical significance after adjustment for covariates (Table 2). The odds of having an HbA1c $\geq 6.5$ by iron deficiency status for women is also shown in Table 2, with no statistically significant association, although the number of iron deficient women with HbA1c $\geq 6.5\%$ was small. Since only one male had iron deficiency as well as an HbA1c $\geq 6.5$, multivariate regression was not performed.

Table 3 illustrates the predicted prevalence of women with an elevated HbA1c by iron status, before and after adjustment for covariates. The difference in the predicted prevalence of an HbA1c $\geq 5.5\%$ between women with and without iron deficiency was small, although statistically significant. There was no significant difference in the predicted prevalence of an HbA1c $\geq 6.5\%$ between women with and without iron deficiency, although again the number of women with iron deficiency and an HbA1c $\geq 6.5\%$ was small.

**Sensitivity Analyses.** While mean HbA1c levels differed between women with and without iron deficiency, differences were small. The mean HbA1c among iron deficient and non-iron deficient women was 5.33% and 5.27% after adjustment for age and race/ethnicity ($p=0.002$), 5.31% and 5.27% after further adjustment for waist circumference ($p=0.059$), and 5.32% and 5.27% after further adjustment for parity and hysterectomy ($p=0.022$). The mean HbA1c among iron deficient men and non-iron deficient men was 5.38% and 5.29% after adjustment for age and race/ethnicity ($p=0.22$) and 5.29% and 5.29% after further adjustment for waist circumference ($p=0.022$).

When we examined only women with a fasting glucose level and included fasting glucose as an adjuster, the odds of having an HbA1c $\geq 5.5\%$ remained significant after adjustment for age, race/ethnicity, waist circumference, parity and hysterectomy, and fasting glucose ($p<0.05$) Mean HbA1c levels were also significantly different after adjustment for these factors ($p<0.001$). When we included adults with mild renal impairment, the odds of having an HbA1c $\geq 5.5\%$ with iron deficiency no longer remained significantly decreased after adjustment for age, race/ethnicity, waist circumference, parity, hysterectomy, as well as fasting glucose among women ($p<0.01$). The association was still not significant when we used the cutpoint of 6.5% ($p<0.01$). Among men, the odds of having an HbA1c $\geq 5.5\%$ with iron deficiency remained non-significant (results not shown).

**CONCLUSIONS**

The optimal screening strategies for diabetes in terms of sensitivity, specificity, and cost may vary between different populations based on demographics and other risk factors for diabetes. We found that iron deficiency, a common condition among reproductive-age women, was associated with shifts in HbA1c distribution to higher levels, but this shift occurred primarily between < 5.5 to 5.5-6.0. Although we did not find an association between iron deficiency and shifts in HbA1c between < 6.5% and $\geq 6.5\%$, few women and men had both iron deficiency and HbA1c elevations $\geq 6.5\%$, and therefore conclusions regarding iron deficiency and the higher cutpoint are limited.

Previous studies of the influence of iron deficiency and glucose control have documented the high prevalence of iron deficiency in pregnancy (19) and the association with erythrocyte indices.(20) In a premenopausal non-pregnant population, Koga and colleagues found that red cell counts and HbA1c were associated in premenopausal women with otherwise normal glucose tolerance.(20) Hashimoto and colleagues found that A1c levels were significantly increased in the 3rd trimester compared to earlier in pregnancy, but serum glycated albumin did not change; A1c was
negatively correlated with serum ferritin and transferrin saturation, suggesting that A1c was influenced by iron stores rather than glucose control. Furthermore, replacement with iron is associated with decreases in HbA1c, independent of glucose changes. Coban and colleagues found that among non-diabetic adults with iron deficiency anemia, the mean HbA1c was 7.4%±0.3% before treatment to 6.2%±0.6% after treatment. Similarly, Tarim and colleagues found that HbA1c in iron deficient patients decreased from 7.6%±2.6% to 6.2%±1.4% after iron therapy (p<0.05), despite similar glucose levels. We did not find such large shifts in HbA1c associated with iron deficiency, either due to the population-based nature of the sample or differences in HbA1c assays. In addition, we did not examine pregnant patients, and the previous studies of non-pregnant patients may have included some adults with undiagnosed diabetes, as suggested by the HbA1c levels. In this respect, our results are similar to a subanalysis of the Diabetes Control and Complications Trial, in which comparison of HbA1c and glucose associations were similar between premenopausal women and men, suggesting that iron deficiency might not be influential in larger samples, although actually iron measurements were not available in that study.

When we examined only women who underwent a fasting glucose and included fasting glucose as an adjuster, iron deficiency was still associated with a greater mean level of HbA1c after adjustment as well as a greater odds of having an HbA1c ≥ 5.5%. When we excluded women likely to have undiagnosed diabetes by fasting glucose, iron deficiency was still associated with a higher mean level of HbA1c after adjustment, but the increased odds of having an HbA1c ≤ 5.5% was no longer significant. When we included adults with renal impairment, the association between iron deficiency and HbA1c was attenuated. This is consistent with the observation that factors contributing to shorter erythrocyte half-life such as renal disease may lower the range of HbA1c values and reduce the strength of the association between HbA1c and factors such as iron deficiency.

The strengths of our report include its population-based sampling frame, size, and standardized HbA1c measurements that accounted for factors that might alter HbA1c measures such as hemoglobinopathies. Our study has several limitations. Iron studies may be affected by inflammation, and we have limited ability to assess such inflammation. Whereas previous studies have not found that adjustment for C-reactive protein affected estimates of iron deficiency, it is possible that adults more prone to glucose intolerance and higher HbA1c levels were also prone to inflammation that was not detected. However, inflammation would be expected to raise ferritin levels so adults with iron deficiency would be less likely to be diagnosed with iron deficiency, thus biasing estimates of association between HbA1c and iron deficiency to the null, and we used a low cut-off for ferritin, 15 mg/dl. We were also unable to account for other factors that might affect red cell production, including malignancies and aplastic anemia. These factors might act as effect modifiers by decreasing red cell half-life and thus artificially lower HbA1c, thus reducing the magnitude of the association, and might also act as confounders through influencing iron resorption, although we expect that these conditions were probably uncommon and would bias any associations to the null. As with any observational study, residual confounding from measurement error may account for the observed associations and multiple testing may have contributed to chance positive findings.

In conclusion, we found that iron deficiency was common among women, this iron
deficiency was not necessarily accompanied with anemia, and iron deficiency shifted the HbA1c slightly upwards independent of fasting glucose level. However, the shift occurred at the lower end of the HbA1c spectrum and we were unable to conclude whether iron deficiency affected HbA1c distributions at a higher cutpoint of < 6.5 vs. >=6.5, a new recommended diagnostic cutpoint.(1) Similar relationships were observed in men, although the proportion of men with iron deficiency was fairly low, prohibiting more definitive conclusions. Although younger populations are generally at low risk for diabetes compared to older populations, the incidence and prevalence of diabetes are increasing among younger women and pregnant women with the obesity epidemic as well as advancing maternal age.(24; 25) Research needs to be done to confirm that iron deficiency does not affect HbA1c readings in the population with known diabetes as well as at higher diagnostic cutpoints than 5.5%.

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| Characteristics                          | Women                         | Men                          | p-value † | Women                         | Men                          | p-value † |
|-----------------------------------------|-------------------------------|------------------------------|-----------|-------------------------------|------------------------------|-----------|
| Sample size, N (%)                     | 1150 (13.7)                  | 5516 (86.3)                  | <0.001    | 75 (1.6)                      | 3794 (98.4)                  | <0.001    |
| Age, %                                  |                               |                              |           |                               |                              |           |
| < 50 years                              | 88.7                         | 69.4                         | <0.001    | 63.7                         | 71.2                         | 0.216     |
| ≥ 50 years                              | 11.3                         | 30.6                         |           | 36.3                         | 28.9                         |           |
| Race/ethnicity, %                       |                               |                              |           |                               |                              |           |
| Non-Hispanic White                      | 56.7                         | 72.4                         | <0.001    | 75.0                         | 71.8                         | 0.876     |
| African-American                       | 18.2                         | 10.9                         |           | 9.8                          | 9.7                          |           |
| Hispanic                                | 20.7                         | 12.3                         |           | 11.5                         | 15.0                         |           |
| Other                                   | 4.4                          | 4.4                          |           | 3.7                          | 3.6                          |           |
| Body mass index, %                      |                               |                              |           |                               |                              |           |
| < 25 kg/m²                              | 35.7                         | 44.1                         | <0.001    | 28.0                         | 36.4                         | 0.225     |
| 25-<30 kg/m²                            | 24.1                         | 26.6                         |           | 35.2                         | 40.4                         |           |
| ≥ 30 kg/m²                              | 40.3                         | 29.3                         |           | 36.8                         | 23.3                         |           |
| Waist circumference (cm), mean (SE)     | 94.2 (0.8)                   | 91.1 (0.4)                   | <0.001    | 107.8 (3.3)                   | 97.0 (0.3)                   | 0.002     |
| Parity (women only), %                  |                               |                              |           |                               |                              |           |
| 0                                       | 24.3                         | 27.3                         | 0.157     | 33.3                         | 3.3                          | <0.001    |
| 1                                       | 15.9                         | 16.5                         |           | 22.5                         | 37.9                         |           |
| 2                                       | 25.6                         | 27.3                         |           | 7.3                          | 0.0                          | 2.0       |
| 3                                       | 18.7                         | 17.3                         |           | 0.6                          | 0.0                          | 0.6       |
| 4                                       | 9.1                          | 6.3                          |           | 0.4                          | 0.6                          | 0.4       |
| 5 or more births                       | 6.4                          | 5.3                          |           | 0.3                          | 0.4                          | 0.3       |
| Hysterectomy (women only), %            | 5.6                          | 17.6                         | <0.001    | 5.6                          | 17.6                         | <0.001    |
| Anemia, %                               | 29.5                         | 2.3                          | <0.001    | 33.3                         | 3.3                          | <0.001    |
| Hemoglobin A1c, mean (SE)               | 5.31 (0.02)                  | 5.27 (0.01)                  | 0.127     | 5.43 (0.06)                   | 5.29 (0.02)                  | 0.035     |
| Hemoglobin A1c, %                       |                               |                              |           |                               |                              |           |
| < 5.4                                   | 73.6                         | 76.5                         | 0.366     | 54.7                         | 73.9                         | <0.001    |
| 5.5-6.0                                 | 23.9                         | 20.5                         |           | 37.9                         | 22.5                         |           |
| 6.1-6.4                                 | 1.5                          | 1.7                          |           | 7.3                          | 2.0                          |           |
| 6.5-6.9                                 | 0.4                          | 0.6                          |           | 0.0                          | 0.6                          |           |
| 7.0-7.9                                 | 0.3                          | 0.3                          |           | 0.0                          | 0.4                          |           |
| 8.0-8.9                                 | 0.2                          | 0.1                          |           | 0.1                          | 0.2                          |           |
| > 9.0                                   | 0.3                          | 0.3                          |           | 0.0                          | 0.4                          |           |
| Fasting glucose (mg/dl), mean (SE)      | n=2993 women, n=1799 men     | 92.4 (0.8)                   | 0.034     | 101.5 (2.3)                   | 98.8 (0.6)                   | 0.269     |
| Fasting glucose < 126 mg/dl, %          | n=2993 women, n=1799 men     | 99.2                         | 98.5      | 100.0                         | 97.3                         | <0.001    |

Data are given as weighted percentage or mean (standard error) adjusted for complex survey design. Percentages may not total 100 due to rounding.

† P-value was determined by design-corrected $\chi^2$ test or t-test.
Table 2. Odds ratios (95% confidence interval) for iron deficiency predicting high HbA1c among adults aged 18 years and older, NHANES 1999-2006. The referent group is adults who are iron sufficient; an odds ratio > 1 indicates that an iron deficient adult has greater odds of having an elevated HbA1c than an iron sufficient adult, and an odds ratio < 1 indicates that an iron deficient adult has a lower odds of having an elevated HbA1c than an iron sufficient adult.

|                      | Women * |                      | Men † |
|----------------------|---------|----------------------|-------|
|                      | HbA1c ≥ 5.5% | HbA1c ≥ 6.5% | HbA1c ≥ 5.5% |
|                      | N=6666  | N=6666  | N=3869  |
| Unadjusted           | 1.17 (0.95, 1.43) | 0.82 (0.37, 1.80) | 2.34 (1.26, 4.33) |
| Adjusted for age, race/ethnicity | 1.47 (1.19, 1.83) | 0.90 (0.42, 1.94) | 2.03 (0.81, 5.08) |
| Adjusted for age, race/ethnicity, waist circumference | 1.39 (1.11, 1.73) | 0.79 (0.33, 1.85) | 1.40 (0.69, 2.87) |
| Adjusted for age, race/ethnicity, waist circumference, parity, hysterectomy | 1.33 (1.05, 1.67) | 0.78 (0.32, 1.90) | N/A |

* 316 female participants with iron deficiency had a measured HbA1c ≥ 5.5%; 32 female participants with iron deficiency had a measured HbA1c ≥ 6.5%.
† 13 male participants with iron deficiency had a measured HbA1c ≥ 5.5%; one male participant with iron deficiency had a measured HbA1c ≥ 6.5%.
Table 3. Predicted prevalence (standard error) of elevated among adults aged 18 years and older with and without iron deficiency. Percentages illustrated.

|                                      | Predicted prevalence of women with an HbA1c $\geq 5.5\%^*$ | Difference in predicted prevalence of HbA1c $\geq 5.5\%$ between iron deficient and iron sufficient women | Predicted percentage of men with an HbA1c $\geq 5.5\%^*$ | Difference in predicted prevalence of HbA1c $\geq 5.5\%$ between iron deficient and iron sufficient men |
|--------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
|                                      | Iron deficient N=1150                                       |                                                                                                         | Iron deficient N=75                                        |                                                                                                         |
|                                      | 26.4 (1.8)                                                 | 2.8 (1.9)                                                                                                | 45.3 (7.5)                                               | 19.1 (7.6)‡                                                                                                |
|                                      | Iron sufficient N=5516                                     |                                                                                                         | Iron sufficient N=3794                                   |                                                                                                         |
|                                      | 23.5 (0.9)                                                 |                                                                                                         | 26.2 (1.3)                                               |                                                                                                         |
|                                      | Adjusted for age, race/ethnicity                           | 29.4 (1.8)                                                 | 6.3 (1.8)‡                                               | 39.5 (9.2)                                                                                               |
|                                      |                                                            | 23.1 (0.9)                                                 |                                                                                                         | 26.2 (1.3)                                               | 13.2 (9.3)                                                                                                |
|                                      | Adjusted for above, and waist circumference                | 27.9 (1.7)                                                 | 4.8 (1.7)‡                                               |                                                                                                         | 31.8 (6.0)                                                                                               |
|                                      |                                                            | 23.1 (0.9)                                                 |                                                                                                         | 26.1 (1.3)                                               | 5.7 (6.2)                                                                                                |
|                                      | Adjusted for above, and parity, hysterectomy               | 27.4 (1.7)                                                 | 4.1 (1.7)‡                                               |                                                                                                         |                                                                                                         |
|                                      |                                                            | 23.3 (1.0)                                                 |                                                                                                         |                                                                                                         |                                                                                                         |

|                                      | Predicted prevalence of women with an HbA1c $\geq 6.5\%^†$ | Difference in predicted prevalence of HbA1c $\geq 6.5\%$ between iron deficient and iron sufficient women |
|                                      | Iron deficient N=1150                                       |                                                                                                         | Iron sufficient N=5516                                   |                                                                                                         |
|                                      | 1.1 (0.4)                                                  | 0.2 (0.4)                                                                                                | 1.3 (0.2)                                                | 0.1 (0.4)                                                                                               |
|                                      | Iron sufficient N=5516                                     |                                                                                                         |                                                          |                                                                                                         |
|                                      | 1.3 (0.2)                                                  |                                                                                                         |                                                          |                                                                                                         |
|                                      | Adjusted for age, race/ethnicity                           | 1.1 (0.4)                                                  | 0.1 (0.4)                                                |                                                                                                         | 1.3 (0.2)                                                                                               |
|                                      |                                                            | 1.3 (0.2)                                                  |                                                                                                         | 0.3 (0.4)                                                 | 0.3 (0.4)                                                                                                |
|                                      | Adjusted for above, and waist circumference                | 1.0 (0.4)                                                  | 0.3 (0.4)                                                |                                                                                                         | 1.3 (0.2)                                                                                               |
|                                      |                                                            | 1.3 (0.2)                                                  |                                                                                                         | 0.3 (0.4)                                                 |                                                                                                         |
|                                      | Adjusted for above, and parity, hysterectomy               | 1.0 (0.4)                                                  | 0.3 (0.4)                                                |                                                                                                         | 1.3 (0.2)                                                                                               |
|                                      |                                                            | 1.3 (0.2)                                                  |                                                                                                         | 0.3 (0.4)                                                 |                                                                                                         |

$^*$316 female participants with iron deficiency and 1478 without iron deficiency had a measured HbA1c $\geq 5.5\%$, 13 male participants with iron deficiency and 1178 without iron deficiency had a measured HbA1c $\geq 5.5$.

$^†$32 female participants with iron deficiency and 102 without iron deficiency had a measured HbA1c $\geq 6.5\%$.

$‡p$-value $< 0.05$