Body Iron Stores in Relation to Risk of Type 2 Diabetes in Apparently Healthy Women

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EXCESSIVE IRON STORES CAN CAUSE type 2 diabetes among patients with hemochromatosis.[1] However, it is not clear whether moderately elevated iron stores predict the risk of developing type 2 diabetes among healthy individuals. Iron is a catalyst in the formation of hydroxyl radicals, which are powerful prooxidants that attack cellular membrane lipids, proteins, and nucleic acids.[2-4] It has been hypothesized that formation of hydroxyl radicals catalyzed by iron contributes initially to insulin resistance and subsequently to decreased insulin secretion and then to the development of type 2 diabetes.[5-7] Findings on the association between serum ferritin concentration and insulin resistance or type 2 diabetes risk from cross-sectional or case-control studies have been inconsistent. Several of these studies observed positive associations[7-12]; however, serum ferritin concentrations may reflect systemic inflammation coexisting with diabetes rather than high iron stores because blood samples are collected after the diagnosis of diabetes. Also, the directionality of the associations cannot be established based on retrospective or cross-sectional data.

One small prospective nested case-control study from Finland (41 cases and 82 controls, blood samples were collected prior to diabetes diagnosis) has shown a direct association between iron stores, as measured by the ratio of serum transferrin receptor concentration to serum ferritin concentration, and the incidence of diabetes in men.[13] To our knowledge, there are no other prospective studies relating iron stores to incident type 2 diabetes in a healthy population. To test the hypothesis that higher iron stores might predict development of type 2 diabetes, we conducted a large prospective nested case-control study to evaluate biomarkers reflecting iron stores, including plasma ferritin concentration and the ratio of the concentrations of transfer-

Context Type 2 diabetes is a common manifestation of hemochromatosis, a disease of iron overload. However, it is not clear whether higher iron stores predict the development of type 2 diabetes in a healthy population.

Objective To examine plasma ferritin concentration and the ratio of the concentrations of transferrin receptors to ferritin in relation to risk of type 2 diabetes.

Design, Setting, and Participants Prospective nested case-control study within the Nurses' Health Study cohort. Of the 32 826 women who provided blood samples during 1989-1990 and were free of diagnosed diabetes, cardiovascular disease, and cancer, 698 developed diabetes during 10 years of follow-up. The controls (n=716) were matched to cases on age, race, and fasting status; and on body mass index (BMI) for cases in the top BMI decile.

Main Outcome Measure Incident cases of type 2 diabetes.

Results Among cases, the mean (SD) concentration of ferritin was significantly higher (109 [105] vs 71.5 [68.7] ng/mL for controls; $P<.001$ for difference) and the mean (SD) ratio of transferrin receptors to ferritin was significantly lower (102 [205] vs 141 [340], respectively; $P=.01$). In conditional logistic regression stratified on the matching factors and controlled for BMI and other diabetes risk factors, the multivariate relative risks [RRs] of incident type 2 diabetes across increasing quintiles of ferritin were 1.00, 1.09 (95% confidence interval [CI], 0.70-1.70), 1.26 (95% CI, 0.82-1.95), 1.30 (95% CI, 0.83-2.04), and 2.68 (95% CI, 1.75-4.11) ($P<.001$ for trend). The RRs across increasing quintiles of transferrin receptors to ferritin ratio were 2.44 (95% CI, 1.61-3.71), 1.00 (95% CI, 0.64-1.56), 1.13 (95% CI, 0.73-1.74), 0.99 (95% CI, 0.64-1.53), and 1.00 ($P=.01$ for trend). Further adjustment for an inflammatory marker (C-reactive protein) did not change the results appreciably. The associations persisted within strata defined by levels of BMI, menopausal status, alcohol consumption, and C-reactive protein.

Conclusion Higher iron stores (reflected by an elevated ferritin concentration and a lower ratio of transferrin receptors to ferritin) are associated with an increased risk of type 2 diabetes in healthy women independent of known diabetes risk factors.

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rin receptors to ferritin in relation to the development of type 2 diabetes in apparently healthy middle-aged women enrolled in the Nurses' Health Study.

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METHODS

Study Population

This study had a case-control design, and was nested in the Nurses' Health Study, a prospective investigation initiated in 1976 that was designed to study the etiological characteristics of heart disease, cancer, and other major diseases in 121 700 female registered nurses aged 30 to 55 years at baseline.[14] During 1989-1990, 32 826 women free of diagnosed diabetes, cardiovascular disease, and cancer provided blood samples. By 2000, 698 had developed definite type 2 diabetes. For each woman who developed type 2 diabetes, a control individual was chosen at random among women free of self-reported diabetes at the time the case individual reported her event. The controls were matched to the cases on age (within 1 year), race, and fasting status at blood draw. Fasting was defined as 8 hours or longer since last meal prior to sample collection. For diabetic cases in the top 10% of body mass index (BMI) (very obese cases), another control individual was chosen who was further matched on BMI (if available) to better control for obesity. Body mass index was calculated as weight in kilograms divided by the square height in meters. The final study group included 698 cases and 716 controls. This study was approved by the human subjects committee at Brigham and Women's Hospital, Boston, Mass.

Ascertainment of Diabetes

Diabetes incidence was identified by self-report on biennial follow-up questionnaires and confirmed by a validated supplementary questionnaire regarding diabetes symptoms, diagnostic tests, and treatments. Based on the diagnostic criteria proposed by the National Diabetes Data Group,[15] a diagnosis of diabetes (prior to 1998) was established when at least 1 of following criteria was reported on the supplementary questionnaire: (1) one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, or coma) plus a fasting plasma glucose concentration of 140 mg/dL (7.77 mmol/L) or higher or a random plasma glucose concentration of 200 mg/dL (11.1 mmol/L) or higher; or (2) at least 2 elevated plasma glucose concentrations on different occasions (fasting $\geq$140 mg/dL [$\geq$7.77 mmol/L] and/or random $\geq$200 mg/dL [$\geq$11.1 mmol/L] and/or $\geq$200 mg/dL [$\geq$11.1 mmol/L] after $\geq$2 hours with oral glucose tolerance testing) in the absence of symptoms; or (3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agents). These criteria were changed in 1997[16]; the fasting glucose concentration of 126 mg/dL (6.99 mmol/L) or higher was considered diagnostic for cases after 1998. We excluded women with type 1 diabetes and women classified as having gestational diabetes only. A validation study in a subsample of the Nurses' Health Study demonstrated that our supplementary questionnaire is highly reliable in confirming diabetes diagnosis.[17] Among a random sample of 84 women classified by our criteria as having type 2 diabetes according to the information reported on the supplementary questionnaire, medical records were available for 62. An endocrinologist blinded to the information reported on the questionnaire reviewed the records. The diagnosis of type 2 diabetes was confirmed in 61 (98%) of the 62 women.

Laboratory Procedures

We sent a phlebotomy kit (including sodium heparin blood tubes, needles, a tourniquet, etc) and instructions to women willing to provide blood specimens in 1989-1990. Blood specimens were returned by overnight mail in a frozen water bottle and on arrival were centrifuged and stored in liquid nitrogen until laboratory analysis. Ninety-seven percent of samples arrived within 26 hours of phlebotomy. Quality-control samples were routinely frozen along with study samples to monitor for plasma changes due to long-term storage and to monitor for changes in assay variability. Previous work has documented the long-term stability of plasma samples collected and stored under this protocol.[18] Frozen plasma aliquots from cases and controls were selected for simultaneous analysis in 2002 and were analyzed in randomly ordered case-control pairs to reduce systematic bias and interassay variation.

Concentrations of ferritin and transferrin receptors were measured by a particle-enhanced immunoturbidimetric assay using the Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, Ind). C-reactive protein (CRP) concentrations were measured via a highly sensitive latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, Del). Insulin concentrations were measured using a double antibody system with less than 0.2% cross-reactivity between insulin and its precursors (Linco Research, St Louis, Mo). Hemoglobin A_{1c} was measured by immunoassay (Hitachi 911 Analyzer). The coefficients of variation for each analyte were: ferritin, 3.75%; transferrin receptors, 8.4%; CRP, 3.8%; fasting insulin, 9.5%; and hemoglobin A_{1c}, 3.8%.

Assessment of Lifestyle Factors

The participants provided information on family history of diabetes in first-degree relatives in 1982 and 1988. They provided information on their body weight, cigarette smoking, and physical activity, menopausal status, and use or nonuse of postmenopausal hormone therapy every 2 years since 1976. The correlation coefficient between self-reported weight and measured weight was 0.96.[18] Physical activity (metabolic equivalent hours per week) was based on the reported time spent on various activities, weighting each activity by its intensity level.[19]

Diet was assessed in 1980, 1984, 1986, and 1990 by using semiquantitative food frequency questionnaires (SFFQs). The SFFQ in 1980 included 61 food items and was revised and expanded to about twice the number of foods in later years. A full description of the SFFQ and the reproducibility and validity of the dietary questionnaires have been previously published.[20,21] We used the cumulative average of dietary intake (from all available dietary questionnaires up to the start of this study) because it reduces within-

subject variation and best represents long-term diet, and has been shown to be a stronger predictor of type 2 diabetes than the baseline diet in a previous study of our cohort.[22] The calculation of cumulative average of dietary intake was previously reported.[23]

Statistical Analysis

We first calculated mean (SDs), medians, and proportions of potential diabetes risk factors for the cases and the controls at baseline. t and χ^2 Tests were used for comparisons of the means and the proportions. We divided the distributions of the markers of iron stores in the controls into quintiles; quintile-specific relative risks (RRs) of diabetes were estimated from conditional logistic regression models stratified on matching factors (age, race, and fasting status). In multivariate models, we adjusted for conventional diabetes risk factors including BMI, family history of diabetes, physical activity, smoking status, alcohol use, menopausal status, and dietary variables.[22] We also adjusted for a sensitive biomarker of inflammation (CRP). Tests for trend were conducted using the median values for each quintile of ferritin or the ratio of transferrin receptors to ferritin as a continuous variable in the regression models. Tests for interaction were performed using likelihood ratio tests by comparing 2 nested models, one with the main effects only and the other with both the main effects and interaction terms. In addition, we used restricted cubic spline regressions with 4 knots[24] to model the associations between ferritin and the ratio of transferrin receptors to ferritin (as continuous variables) and risk of type 2 diabetes. All P values were 2-sided. $P \leq .05$ was considered statistically significant. All analyses were performed using SAS statistical software (Version 8.12, SAS Institute Inc, Cary, NC).

RESULTS

The distributions of potential risk factors for type 2 diabetes in cases (n=698) and healthy controls (n=716) are presented in TABLE 1. Overall, women who subsequently developed diabetes during follow-up were heavier, more likely to have a family history of diabetes, less likely to exercise and consume alcohol, and had higher plasma concentrations of CRP, fasting insulin, and hemoglobin A_{1c} at baseline. In addition, diabetic women tended to have higher baseline average intake of heme iron, transfat, red and processed meats, total calories, and lower intake of cereal fiber and magnesium. The correlation between ferritin and CRP was 0.14, and the correlation between the ratio of transferrin receptors to ferritin and CRP was –0.12.

At baseline, the mean (SD) ferritin concentration was significantly higher (109 [105] vs 71.5 [68.7] ng/mL; $P<.001$ for the difference) and the mean (SD) ratio of transferrin receptors to ferritin was significantly lower (102 [205] vs 141 [340]; $P=.01$ for the difference) in the cases than in the healthy controls (Table 1). In conditional logistic regression analyses stratified on matching factors (age, race, and fasting status), the RRs across increasing quintiles of ferritin were 1.00, 1.19 (95% confidence interval [CI], 0.81-1.75), 1.53 (95% CI, 1.06-2.23),

Table 1. Risk Factors for Type 2 Diabetes at Baseline (1989-1990)

Risk Factor	Mean (SD) [Median]*		P Value for Difference of Means
	Case (n = 698)	Control (n = 716)	
Demographic			
Race, No. (%)			
White	635 (94.4)	651 (94.6)	.83
Nonwhite	38 (5.4)	37 (5.4)	.83
Family history of diabetes, No. (%)	323 (46.3)	149 (20.8)	<.001
Current smoker, No. (%)	99 (14.2)	97 (13.6)	.73
Postmenopausal, No. (%)	459 (65.8)	467 (65.2)	.83
Age, y	56.5 (6.9) [57.0]*	56.4 (6.9) [57.0]	.80
Clinical			
Body mass index†	30.3 (5.7) [29.3]	26.2 (6.1) [24.5]	<.001
Physical activity, MET in h/wk	12.1 (14.8) [6.6]	15.9 (28.0) [9.5]	.002
Alcohol consumption, g/d	3.7 (7.1) [1.1]	6.6 (9.2) [2.8]	<.001
Ferritin, ng/mL	109 (105) [79.7]	71.5 (68.7) [53.1]	<.001
Ratio of transferring receptors to ferritin‡	102 (205) [41.8]	141 (340) [54.6]	.01
C-reactive protein, mg/dL	0.53 (0.54) [0.37]	0.28 (0.34) [0.16]	<.001
Fasting insulin, uU/mL§	13.9 (8.9) [12.0]	9.4 (6.2) [8.2]	<.001
Hemoglobin A_{1c}, %‖	6.4 (1.2) [6.1]	5.6 (0.3) [5.6]	<.001
Dietary			
Cereal fiber, g/d	3.9 (1.6) [3.6]	4.1 (1.7) [3.8]	.01
Magnesium, mg/d	293 (55.3) [289]	300 (59.2) [296]	.02
Total iron, mg/d	15.6 (8.7) [12.9]	15.6 (8.1) [12.9]	.96
Use of iron supplements, No. (%)	20 (2.9)	29 (4.1)	.22
Heme iron, mg/d	1.26 (0.34) [1.25]	1.19 (0.34) [1.18]	<.001
Ratio of polyunsaturated fat to saturated fat	0.50 (0.12) [0.49]	0.52 (0.15) [0.49]	.10
Trans-fat, % energy	1.80 (0.46) [1.76]	1.75 (0.47) [1.73]	.04
Red and processed meats, servings/d¶	1.20 (0.57) [1.12]	1.11 (0.57) [1.06]	.01
Total energy, cal/d	1777 (474) [1705]	1729 (438) [1701]	.05
Glycemic load	139 (20.9) [138]	138 (23.8) [137]	.45

Abbreviation: MET, metabolic equivalent.
*Unless otherwise indicated.
†Calculated as the weight in kilograms divided by the square of height in meters.
‡For this analysis, there were 695 cases and 714 controls.
§For this analysis, there were 412 cases and 361 controls. To convert insulin to pmol/L multiply by 6.945.
‖For this analysis, there were 391 cases and 374 controls.
¶Composite of beef, pork, or lamb as a main dish or mixed dish, hamburgers, hot dogs, bacon, and processed meats.

Table 2. Relative Risks of Type 2 Diabetes

	Quintile*					P Value for Trend
	1	2	3	4	5	
Ferritin						
Concentration, ng/mL	<21.1	21.1-41.1	41.2-69.8	69.9-107.1	≥107.2	NA
No. of cases	92	101	129	112	264	NA
No. of controls	144	143	143	143	143	NA
RR (95% CI)†	1.00	1.19 (0.81-1.75)	1.53 (1.06-2.23)	1.38 (0.94-2.03)	3.20 (2.22-4.62)	<.001
BMI-adjusted RR (95% CI)‡	1.00	1.16 (0.77-1.75)	1.27 (0.85-1.89)	1.17 (0.78-1.78)	2.61 (1.78-3.85)	<.001
Multivariate RR (95% CI)§	1.00	1.09 (0.70-1.70)	1.26 (0.82-1.95)	1.30 (0.83-2.04)	2.68 (1.75-4.11)	<.001
Additional adjustment for C-reactive protein	1.00	1.09 (0.69-1.74)	1.21 (0.77-1.91)	1.30 (0.81-2.07)	2.61 (1.68-4.07)	<.001
Transferrin receptors and ferritin						
Ratio	<26.7	26.7-42.4	42.5-71.5	71.6-149.3	≥149.4	NA
No. of cases	248	104	129	109	105	NA
No. of controls	142	143	143	143	143	NA
RR (95% CI)†	2.59 (1.82-3.68)	1.04 (0.72-1.52)	1.31 (0.91-1.88)	1.11 (0.76-1.61)	1.00	<.001
BMI-adjusted RR (95% CI)‡	2.27 (1.56-3.30)	1.00 (0.66-1.49)	1.07 (0.72-1.59)	1.06 (0.71-1.57)	1.00	.007
Multivariate RR (95% CI)§	2.44 (1.61-3.71)	1.00 (0.64-1.56)	1.13 (0.73-1.74)	0.99 (0.64-1.53)	1.00	.01
Additional adjustment for C-reactive protein	2.40 (1.55-3.71)	0.89 (0.56-1.41)	1.10 (0.70-1.72)	0.97 (0.62-1.54)	1.00	.02

Abbreviations: BMI, body mass index; CI, confidence interval; NA, not applicable; RR, relative risk.
*In controls.
†Conditional analysis matched on age, race, and fasting status.
‡Conditional analysis matched on age, race, and fasting status and controlled for BMI (continuous variable).
§Conditional analysis matched on age, race, and fasting status and controlled for BMI, family history of diabetes in a first-degree relative, physical activity, cigarette smoking status, alcohol consumption, menopausal status, glycemic load, intake of total energy, cereal fiber, magnesium, and *trans*-fat, and ratio of polyunsaturated fat to saturated fat.

Figure 1. Relative Risks of Type 2 Diabetes According to Ferritin Concentration and the Ratio of Transferrin Receptors to Ferritin
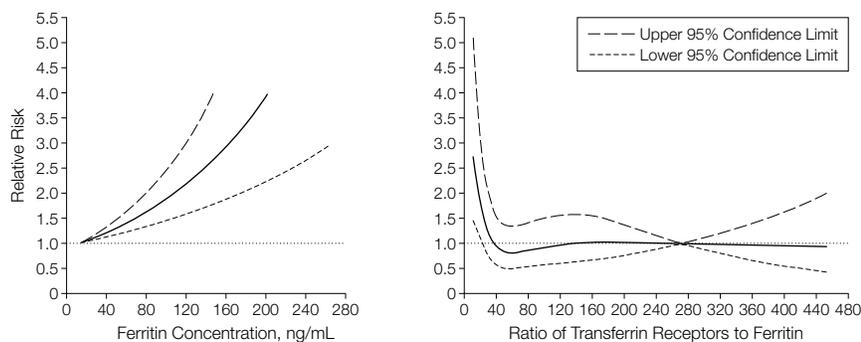
Women with the lowest and highest 5% of ferritin concentration and ratio of transferrin receptors to ferritin were excluded. Dashed lines are 95% confidence intervals. Relative risks were estimated using conditional logistic regression modeling matched for age, race, and fasting status, and controlled for body mass index, family history of diabetes in a first-degree relative, physical activity, cigarette smoking status, alcohol consumption, menopausal status, glycemic load, intake of total energy, cereal fiber, magnesium, and transfat, ratio of polyunsaturated fat to saturated fat, and C-reactive protein.

1.38 (95% CI, 0.94-2.03), and 3.20 (95% CI, 2.22-4.62) (P≤.001 for linear trend) (TABLE 2). The RRs across increasing quintiles of the ratio of transferrin receptors to ferritin were 2.59 (95% CI, 1.82-3.68), 1.04 (95% CI, 0.72-1.52), 1.31 (95% CI, 0.91-1.88), 1.11 (95% CI, 0.76-1.61), and 1.00

(P<.001 for linear trend). These RRs were modestly attenuated after adjusting for BMI, but remained statistically significant. Additional adjustment for other diabetes risk factors including family history of diabetes, physical activity, smoking status, alcohol use, menopausal status, and diet did not

change the results appreciably. Because ferritin concentration reflects both the storage of iron and acute-phase inflammation, we further adjusted for CRP to reduce potential confounding by inflammation. The associations between ferritin concentration and ratio of transferrin receptors to ferritin with diabetes risk remained virtually unchanged (Table 2). Exclusion of 7 individuals with exceptionally elevated ferritin concentrations (≥500 ng/mL) or iron supplement users did not change the results.

In the subset of women who provided waist circumference measurements (420 cases and 515 controls), we adjusted for both BMI and waist circumference (as continuous variables) in the multivariate conditional logistic regression models. The results did not change appreciably. The RR was 2.40 (95% CI, 1.34-4.28) comparing the highest with the lowest quintile of ferritin (P<.001 for trend) and was 2.41 (95% CI, 1.37-4.26) comparing the lowest with the highest quintiles of the ratio of transferrin receptors to ferritin (P=.12 for trend).

To eliminate potential bias due to undiagnosed diabetes in the control group, we excluded control women with hemoglobin A_{1c} levels higher than 6.5% and repeated the multivariate analysis. The RR was 2.63 (95% CI, 1.69-4.11) comparing women in the highest with the lowest quintile of ferritin ($P<.001$ for trend) and was 2.43 (95% CI, 1.57-3.75) comparing women in the lowest with the highest quintile of ratio of transferrin receptors to ferritin ($P=.02$ for trend).

We also used restricted cubic spline regressions with 4 knots to model the associations continuously. The regression splines demonstrated a linear relationship between ferritin and the risk of type 2 diabetes ($P=.29$ for curvature; **FIGURE 1**). However, there is a possible threshold effect for the ratio of transferrin receptors to ferritin of approximately 50 on diabetes risk ($P\leq.001$ for curvature).

To assess whether the associations between ferritin concentration and ratio of transferrin receptors to ferritin and risk of diabetes were modified by CRP concentrations, we examined the joint associations of ferritin and CRP (**FIGURE 2**) and the ratio of transferrin receptors to ferritin and CRP. In the joint analyses, the associations of the markers of iron storage and CRP with diabetes risk tended to be independent ($P=.25$ for interaction between ferritin and CRP; $P=.35$ for interaction between the ratio of transferrin receptors to ferritin and CRP). Overall, women with the highest concentrations of ferritin and CRP or with the lowest ratio of transferrin receptors to ferritin and the highest concentrations of CRP had the highest diabetes risk (Figure 2).

Because menstruation causes iron loss and alcohol consumption can accelerate the effects of iron overload, we further conducted multivariate analyses within strata defined by levels of BMI (≤25, 25-29.9, and ≥30), menopausal status (premenopausal and postmenopausal), and alcohol consumption (<5 g/d or ≥5 g/d). We observed that the associations between ferritin concentration and ratio of transferrin receptors to ferritin and risk of type 2 diabetes persisted in all subgroups (**TABLE 3**). We found no apparent modification in the relationships with these factors ($P>.05$ for all interaction tests).

COMMENT

In this prospective nested case-control study of middle-aged women, body iron stores reflected by a higher ferritin concentration and a lower ratio of transferrin receptors to ferritin were associated with a significantly increased incidence of type 2 diabetes after adjustment for obesity and other diabetes risk factors. A possible threshold effect of the ratio of transferrin receptors to ferritin on diabetes risk was suggested by regression splines. The associations persisted in all subgroup analyses according to BMI, menopausal status, and alcohol consumption. These data provide evidence that increased total body iron stores are an independent risk factor for type 2 diabetes in this healthy population.

Iron is a transitional metal that can catalyze the conversion of poorly reactive free radicals into highly active free radicals. It has been suggested that formation of hydroxyl radicals catalyzed by iron may play a role in the development of diabetes because the highly active radicals can attack cell membrane lipids, proteins, and DNA and cause tissue damage.[3-6] Studies have shown that iron deposition in muscle decreases glucose uptake because of muscle damage,[25] while iron accumulation interferes with hepatic insulin extraction[26] and affects insulin synthesis and secretion in the pancreas.[27] Iron excess seems to contribute initially to insulin resistance and subsequently to decreased insulin secretion.[27]

One concern of this study is that the ferritin concentration is not an entirely specific marker for iron storage and may reflect other mechanisms, especially subclinical systemic inflammation related to insulin resistance and risk of type 2 diabetes.[1] We tried to minimize the potential confounding by inflammation in several ways. First, we

Figure 2. Relative Risks of Type 2 Diabetes According to Joint Classification of Ferritin and C-Reactive Protein Concentration and Ratio of Transferrin Receptors to Ferritin and C-Reactive Protein Concentrations
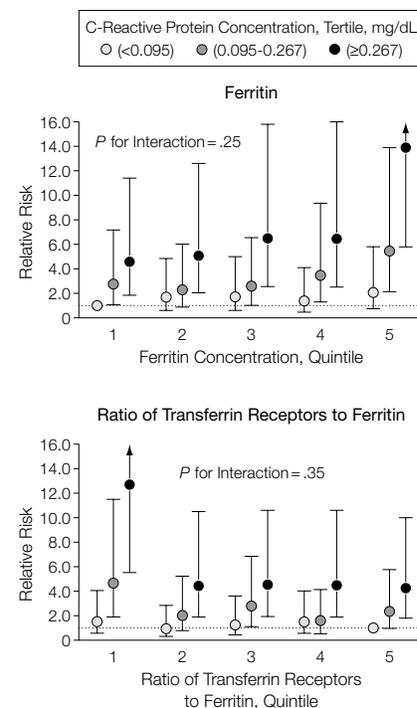
Relative risks were estimated using conditional logistic regression modeling matched for age, race, and fasting status, and controlled for body mass index, family history of diabetes in a first-degree relative, physical activity, cigarette smoking status, alcohol consumption, menopausal status, glycemic load, intake of total energy, cereal fiber, magnesium, and *trans*-fat, ratio of polyunsaturated fat to saturated fat, and C-reactive protein. The quintiles of ferritin concentration and the ratio of transferrin receptors to ferritin are consistent with those in Table 2 and Table 3. Error bars indicate 95% confidence intervals.

conducted a prospective nested case-control study in which all blood samples were collected before the disease outcome developed; therefore, the incident cases of diabetes that developed during the follow-up would be unlikely to affect the ferritin concentrations at baseline. We also excluded women with diagnosed diabetes, cardiovascular disease, and cancer at baseline. Second, we controlled for CRP in the multivariate models, although the correlation between ferritin and CRP was small ($r=0.14$). This statistical control did not attenuate associations of iron markers and risk of diabetes.

Another potential concern is residual confounding by obesity because obesity is an important determinant of type 2 diabetes. In our study, along with matching on BMI for the most obese cases, we controlled for BMI using a continuous variable. In an additional analysis, we controlled for both BMI and waist circumference among women who provided waist circumference measurements. The associations for markers of iron stores did not substantially change. Although we cannot rule out the possibility of residual confounding by other diabetes risk factors, it is unlikely that they can explain the observed strong associations. Because our controls were not uniformly screened for glucose intolerance, some cases of diabetes may have been undiagnosed. However, when the analyses were restricted to women with hemoglobin A_{1c} levels of less than 6.5%, the results did not change, suggesting that a bias due to undiagnosed diabe-

tes is unlikely. It also should be noted that the diagnostic criteria for type 2 diabetes in this study were changed after 1998 (a lower fasting glucose threshold of 126 mg/dL [6.99 mmol/L] was considered the diagnostic cut point compared with that before 1998). If the new criteria were used for diagnosing cases before 1998, some women classified as not having diabetes would have been diagnosed as having diabetes. But, inclusion of those with diabetes into the group without diabetes would tend to weaken the observed association.

Our results are consistent with the findings from a small prospective nested case-control study in Finland.[13] In that study (41 cases and 82 controls), men in the lowest quarter for the ratio of transferrin receptors to ferritin were 2.4 times more likely to develop diabetes than men in the highest quarter. To our knowledge, no other study has evaluated the associations between biomarkers of iron stores and diabetes inci-

dence in a healthy population. Cross-sectional or case-control studies have produced mixed findings about the difference in serum ferritin concentration between diabetic patients and nondiabetic individuals. Several of these studies observed positive associations between serum ferritin concentrations and insulin resistance or risk of diabetes.[7-12] However, serum ferritin concentration in cross-sectional and case-control studies may reflect systemic inflammation associated with diabetes rather than high iron storage.

Type 2 diabetes is an established, common complication of hemochromatosis, a genetic defect in the regulation of iron absorption. Individuals who have homozygous hereditary hemochromatosis absorb more iron than normal. Excess iron accumulation in patients with hemochromatosis often results in clinical manifestation of type 2 diabetes (53%-82% of patients with hemochromatosis develop diabetes[1]), which pro-

Table 3. Relative Risks of Type 2 Diabetes Stratified by Body Mass Index, Menopausal Status, and Alcohol Consumption*

	Quintile†					P Value for Trend
	1	2	3	4	5	
Ferritin concentration, ng/mL	<21.1	21.1-41.1	41.2-69.8	69.9-107.1	≥107.2	NA
Body mass index‡						
<25	1.00	1.34 (0.59-3.04)	1.29 (0.57-2.90)	1.61 (0.69-3.74)	3.00 (1.36-6.58)	.003
25-29.9	1.00	1.29 (0.57-2.92)	1.81 (0.82-4.02)	1.44 (0.65-3.17)	2.40 (1.15-5.01)	.02
≥30	1.00	0.78 (0.34-1.77)	0.96 (0.44-2.09)	1.06 (0.48-2.34)	2.67 (1.23-5.80)	.001
Menopausal status						
Premenopausal	1.00	1.63 (0.80-3.33)	1.48 (0.71-3.10)	1.86 (0.77-4.47)	3.08 (1.11-8.53)	.03
Postmenopausal	1.00	0.70 (0.35-1.36)	0.97 (0.52-1.83)	0.94 (0.50-1.77)	2.17 (1.20-3.93)	<.001
Alcohol, g/d						
<5	1.00	0.90 (0.53-1.54)	1.08 (0.65-1.81)	1.16 (0.68-2.00)	2.25 (1.35-3.76)	<.001
≥5	1.00	1.69 (0.64-4.49)	1.85 (0.69-4.94)	1.61 (0.63-4.13)	3.31 (1.36-8.05)	.006
Ratio of transferrin receptors to ferritin	<26.7	26.7-42.4	42.5-71.5	71.6-149.3	≥149.4	NA
Body mass index‡						
<25	2.74 (1.27-5.91)	0.99 (0.43-2.30)	1.23 (0.54-2.76)	1.79 (0.80-3.98)	1.00	.14
25-29.9	2.18 (1.05-4.52)	1.15 (0.53-2.53)	2.20 (1.00-4.87)	1.01 (0.45-2.26)	1.00	.07
≥30	2.86 (1.27-6.45)	0.74 (0.32-1.69)	0.62 (0.29-1.33)	0.54 (0.24-1.18)	1.00	.30
Menopausal status						
Premenopausal	3.71 (1.25-11.0)	1.18 (0.51-2.73)	1.05 (0.50-2.23)	1.36 (0.67-2.76)	1.00	.21
Postmenopausal	2.04 (1.16-3.60)	0.79 (0.43-1.47)	1.04 (0.57-1.91)	0.67 (0.35-1.29)	1.00	.05
Alcohol, g/d						
<5	1.97 (1.18-3.30)	0.77 (0.45-1.32)	0.90 (0.53-1.50)	0.80 (0.47-1.36)	1.00	.30
≥5	3.51 (1.49-8.29)	1.37 (0.53-3.55)	2.00 (0.79-5.06)	1.88 (0.71-4.94)	1.00	.02

Abbreviations: CI, confidence interval; NA, not applicable; RR, relative risk.
*Conditional analysis matched on age, race, and fasting status and controlled for body mass index, family history of diabetes in a first-degree relative, physical activity, cigarette smoking status, alcohol consumption, menopausal status, glycemic load, intake of total energy, cereal fiber, magnesium, transfat, ratio of polyunsaturated fat to saturated fat, and C-reactive protein. The variable used for stratification was not included in the model.
†Values expressed as RR (95% CI) unless otherwise indicated.
‡Calculated as the weight in kilograms divided by the square of height in meters.

vides clinical evidence that excess iron stores are strongly associated with development of type 2 diabetes. Iron reduction therapy in individuals with hereditary hemochromatosis and transfusional iron overload is associated with improved glucose tolerance and reduced incidence of secondary diabetes.[27] Trials of iron reduction therapy in type 2 diabetes have shown some promising results but are inconclusive.[27-29]

There has been considerable interest in the possibility that excess iron stores may contribute to the pathogenesis of cardiovascular disease. The cumulative epidemiological evidence has been inconsistent, but most studies do not support the iron and cardiovascular disease hypothesis.[30,31] However, most studies have important limitations including short follow-up time and small numbers of cases and few have included women. Although diabetes and cardiovascular disease share many risk factors and pathophysiological pathways, the primary mechanisms for type 2 diabetes involve insulin resistance and beta-cell dysfunction, both of which can be directly affected by high iron storage. Excess iron is usually stored in the liver, muscle, and pancreas and may cause organ-specific oxidative damage leading to insulin resistance and eventually beta-cell failure. This may not be the case for cardiovascular disease because cardiomyopathy due to iron deposition in the heart, but not ischemic heart disease, is often seen in late-stage hemochromatosis patients. The fact that type 2 diabetes is a common complication in patients with hemochromatosis and iron reduction therapy can improve glucose tolerance provide clinical evidence that excess iron storage may directly contribute to the development of type 2 diabetes. Our study provides support for the hypothesis that higher iron stores may also contribute to the origin of type 2 diabetes in a generally healthy population.

In summary, an elevated ferritin concentration and a low ratio of transferrin receptors to ferritin were associated with an increased incidence of type 2 diabetes in apparently healthy middle-aged women independent of known diabetes risk factors. This finding may have important implications for the prevention of type 2 diabetes because elevated ferritin concentration and lower concentration in the ratio of tranferrin receptors to ferritin in healthy populations may help to identify a high-risk population for type 2 diabetes who may benefit from further evaluation and interventions (lifestyle or therapeutic).

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