

Iron Intake and the Risk of Type 2 Diabetes in Women

A prospective cohort study

SWAPNIL RAJPATHAK, MD, DRPH^{1,2}
JING MA, MD, PHD³
JOANN MANSON, MD, DRPH^{2,3,4}

WALTER C. WILLETT, MD, DRPH^{1,2,3}
FRANK B. HU, MD, PHD^{1,2,3}

OBJECTIVE — Epidemiological studies suggest that high body iron stores are associated with insulin resistance and type 2 diabetes. The aim of this study was to evaluate the association between dietary intake of iron and the risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS — We conducted a prospective cohort study within the Nurses' Health Study. We followed 85,031 healthy women aged 34–59 years from 1980 to 2000. Dietary data were collected every 4 years, and data on medical history and lifestyle factors were updated biennially.

RESULTS — During the 20 years of follow-up, we documented 4,599 incident cases of type 2 diabetes. We found no association between total, dietary, supplemental, or nonheme iron and the risk of type 2 diabetes. However, heme iron intake (derived from animal products) was positively associated with risk; relative risks (RRs) across increasing quintiles of cumulative intake were 1.00, 1.08 (95% CI 0.97–1.19), 1.20 (1.09–1.33), 1.27 (1.14–1.41), and 1.28 (1.14–1.45) ($P_{\text{trend}} < 0.0001$) after controlling for age, BMI, and other nondietary and dietary risk factors. In addition, when we modeled heme iron in seven categories, the multivariate RR comparing women who consumed ≥ 2.25 mg/day and those with intake < 0.75 mg/day was 1.52 (1.22–1.88). The association between heme iron and the risk of diabetes was significant in both overweight and lean women.

CONCLUSIONS — This large cohort study suggests that higher heme iron intake is associated with a significantly increased risk of type 2 diabetes.

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Diet and lifestyle play a major role in the prevention of type 2 diabetes. The quality and quantity of macronutrients (especially fat and carbohydrates) are known to have an impact on the risk of type 2 diabetes; however, the role of micronutrients is not well established (1). Several studies have suggested a possible role of minerals such as magnesium (2,3), chromium (4,5), calcium (6), and iron (7) in insulin resistance or diabetes.

Iron is a transitional metal and a potential catalyst in many cellular reactions that produce reactive oxygen species. Such reactions contribute to tissue damage and increase oxidative stress, thereby potentially altering the risk of type 2 diabetes (8). Several studies have suggested a possible link between high body iron stores and metabolic parameters (9–13) (serum insulin and glucose) as well as hypertension (9,14), dyslipidemia (9,15,16), and obesity (9,17). In addition,

epidemiological studies have reported an association between high iron stores and increased risk of cardiovascular disease (18), metabolic syndrome (10,12,19), gestational diabetes (20,21), and type 2 diabetes (22–27). The major source of body iron is derived from the diet. Dietary iron exists as either heme (derived from meat and meat products) or nonheme iron. In two recent prospective cohort studies (28,29), intake of total or nonheme iron was not associated with the risk of type 2 diabetes, but heme iron was associated with elevated risk. The aim of this study was to evaluate the association between iron intake and the risk of type 2 diabetes in the Nurses' Health Study (NHS). The large sample size allowed us to evaluate this risk with respect to different sources of iron (heme, nonheme, supplemental, and dietary) separately.

RESEARCH DESIGN AND METHODS

The NHS cohort was established in 1976, when 121,700 registered nurses aged 30–55 years from 11 U.S. states responded to a mailed questionnaire on risk factors for cancer and cardiovascular disease. This study was approved by the human research committee of the Brigham and Women's Hospital. Every 2 years, follow-up questionnaires are sent to update information on potential risk factors and health outcomes. In 1980, a 61-item food frequency questionnaire (FFQ) designed to assess dietary intake was added. In 1984, 1986, 1990, 1994, and 1998, an expanded (131-item) FFQ was used. The reproducibility and validity of the dietary data have been reported in detail elsewhere (30–32). Women without FFQ data at baseline or those with unreasonably high ($> 3,500$ kcal/day) or low (< 500 kcal/day) intakes and those who had left > 10 items blank were excluded from the analyses. In this study, we also excluded participants with a history of cancer (other than nonmelanoma skin cancer), cardiovascular disease (angina, coronary bypass or angioplasty, myocardial infarction, or stroke), and diabetes at baseline. Therefore, we con-

From the ¹Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts; ²Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the ³Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; and the ⁴Department of Preventive Medicine, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Swapnil Rajpathak, Departments of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY 11375. E-mail: srajpath@post.harvard.edu.

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Abbreviations: FFQ, food frequency questionnaire; NHS, Nurses' Health Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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ducted these analyses among 85,031 women.

Assessment of nutrient intake

The semiquantitative FFQs provide information on the average frequency of consumption of selected foods and beverages during the preceding year. The food composition database used to calculate the nutrient values is based primarily on U.S. Department of Agriculture data (33) supplemented with manufacturers' data. In addition to multivitamin supplements, participants also reported the use of any specific iron supplements including their dose. Total iron intake was calculated as the sum of dietary and all supplemental intakes. All nutrient intakes (except supplemental iron and alcohol) were energy adjusted by the residual method (34). The Pearson correlation coefficient for total iron intake estimated from FFQs and four 1-week diet records (3 months apart) in a validation study among a subset of 150 women was 0.55 (31). In our previous analysis, heme iron and supplemental intake were significant predictors of body iron stores measured by serum ferritin (35).

In addition to total, dietary, and supplemental iron, we estimated the intake of heme iron, which is found only in animal products. We calculated nonheme iron (derived from fortified cereals, plant-based foods, and supplements) as the difference between total and heme iron intake.

Assessment of nondietary factors

In the NHS, information on body weight, smoking, postmenopausal hormone use, and physical activity is updated every 2 years. We have previously evaluated the reproducibility and validity of these self-reported measures including body weight in a subset of the cohort participants with technician-assessed measurements (36). Self-reported weight and the average of two technician measures were highly correlated (Pearson's correlation coefficient = 0.96). Women provided information regarding family history of diabetes in their first-degree relatives in 1982 and 1988. During the 20-year follow-up, participants reported physical activity (hours per week) in 1980, 1982, 1986, 1988, 1992, 1996, and 1998. We estimated the physical activity level of an individual by using the cumulative average number of hours per week spent in moderate or vigorous activity (brisk walk-

ing, vigorous sports, jogging, cycling, and heavy gardening and housework).

Assessment of diabetes

If a woman self-reported a diagnosis of type 2 diabetes on any of the 2-year follow-up questionnaires, we mailed a supplementary questionnaire regarding her symptoms, diagnostic testing, and current treatment. Diagnosis was confirmed if at least one of the following criteria was reported: 1) at least one of the classic symptoms (polydipsia, polyuria, polyphagia, weight loss, or coma) in addition to a fasting plasma glucose level of ≥ 140 mg/dl (7.8 mmol/l) or a random level of ≥ 200 mg/dl (11.1 mmol/l); 2) at least two elevated plasma glucose concentrations on different occasions (fasting ≥ 140 mg/dl [7.8 mmol/l], random ≥ 200 mg/dl [11.1 mmol/l]) or random ≥ 200 mg/dl (11.1 mmol/l) after at least 2 h of oral glucose tolerance testing in the absence of symptoms; or 3) treatment with oral drugs for hyperglycemia or with insulin. Because most of cases of diabetes were diagnosed before 1997, our diagnostic criteria correspond to those of the National Diabetes Data Group (37). Women with gestational or type 1 diabetes were not included in this study. A study in a subsample of women demonstrated a high validity of the supplemental questionnaire in the confirmation of diabetes diagnosis (38). A random sample of 84 women was selected from among those confirmed to have type 2 diabetes based on a supplemental questionnaire. Medical records were obtained for 62 of these women and a physician blinded to the questionnaire data reviewed the records. We confirmed diagnosis of type 2 diabetes in 61 (98%) of the 62 women.

Statistical analysis

All statistical analyses were performed with SAS software (SAS Institute, Cary, NC). Participants contributed follow-up time from the date of questionnaire return in 1980 to the date of diagnosis of type 2 diabetes, death, or 1 June 2000, whichever came first. For a more accurate measure of long-term diet, we calculated the cumulative average intake for each nutrient using FFQ data provided until the beginning of each 2-year follow-up interval (39). We stopped updating diet when a woman reported a diagnosis of cardiovascular disease, hypertension, hypercholesterolemia, or cancer during follow-up because diagnosis of these conditions may lead to changes in diet.

We divided participants into five categories (quintiles) according to their cumulative iron intake. Incidence rates for type 2 diabetes were calculated by dividing the number of cases by the person-years of follow-up for each quintile of iron intake. Relative risks (RRs) of type 2 diabetes were calculated as an incidence rate ratio by dividing the rate in each quintile by the rate in the lowest quintile of intake. We used the Cox proportional hazards model (40) to calculate RRs for diabetes adjusted for potential confounders including age, BMI, family history of diabetes (first-degree relative), smoking, alcohol intake, postmenopausal hormone use, use of multivitamin supplements, and physical activity. Further, we adjusted for dietary factors including trans fat, the polyunsaturated-to-saturated fat ratio, cereal fiber, total calories, whole grains, fruits and vegetables, red meat, caffeine, glycemic load, and magnesium. We evaluated the proportional hazard assumption by conducting likelihood ratio tests comparing models with and without interaction terms between exposure categories and follow-up time. None of these tests was statistically significant, i.e., there was no violation of the proportional hazards assumption. Tests of linear trend across quintiles of iron intake were conducted by assigning the median value for each quintile and fitting this continuous variable in the model.

RESULTS— During the 20-year follow-up period (1980–2000; 1,578,982 person-years), we documented 4,599 incident cases of diabetes. Women in the highest quintile of total iron intake at baseline were more physically active and were less likely to smoke or to consume alcohol or to have hypertension or elevated cholesterol compared with women in the lower quintiles (Table 1). High iron intake was also associated with higher intake of cereal fiber, fruits and vegetables, whole grains, magnesium, and multivitamin supplements. For most of these factors, we found an opposite trend with quintiles of heme iron intake at baseline. In addition, higher intake of heme iron was associated with higher intake of fat (total and saturated), red meat, and protein and with lower intake of carbohydrates and glycemic load.

In our study population, intake of total and dietary iron at baseline correlated highly with that of nonheme iron, because most iron in the diet is in this form. As the results for nonheme iron were sim-

Table 1—Age-standardized characteristics by quintiles (Q) of total iron intake at baseline

Characteristic	Total iron			Heme iron		
	Q1	Q3	Q5	Q1	Q3	Q5
Median intake (mg/day)	8.0	11.0	24.0	0.8	1.5	2.3
Mean age (years)	45.6	45.9	45.9	46.2	45.6	46.0
Mean physical activity (h/week)*	3.5	4.0	4.2	4.1	4.0	3.7
Family history of diabetes (%)	16.2	18.9	18.0	16.4	18.2	18.3
Mean BMI (kg/m ²)†	24.2	24.2	23.7	23.4	24.1	24.8
Current smokers (%)	19.6	13.9	10.6	12.3	14.0	17.4
Postmenopausal hormone use (%)	6.7	6.0	6.4	6.1	5.8	8.0
Hypertension (%)	7.3	5.4	4.1	4.4	5.0	8.0
High cholesterol (%)	2.3	1.9	1.6	1.8	1.7	2.6
Mean alcohol intake (g/day)	7.9	6.7	5.7	6.7	6.5	6.0
Mean total calories (kcal/day)	1,532	1,573	1,548	1,576	1,546	1,570
Calories from carbohydrates (%)	40.1	37.9	39.5	45.9	39.1	31.6
Calories from total fat (%)	38.3	39.4	38.2	34.0	38.6	44.4
Calories from protein (%)	17.7	19.5	19.5	17.0	19.1	21.0
Calories from trans fat (%)	2.2	2.3	2.2	2.1	2.2	2.4
Calories from saturated fat (%)	15.7	16.0	15.3	13.1	15.5	17.9
Calories from polyunsaturated fat (%)	5.2	5.3	5.2	5.4	5.3	5.1
Polyunsaturated-to-saturated ratio	0.35	0.36	0.36	0.42	0.35	0.29
Mean cereal fiber intake (g/day)	1.8	2.6	2.8	3.0	2.7	1.9
Magnesium intake (mg/day)	272	298	302	308	293	277
Caffeine intake (mg/day)	391	417	378	371	402	416
Mean glycemic load	90.7	84.6	85.5	100.6	86.3	69.3
Intake of total meat (servings/day)	1.5	1.8	1.6	1.1	1.7	2.3
Intake of red meat (servings/day)	1.2	1.5	1.3	0.9	1.4	1.9
Intake of chicken/turkey (servings/day)	0.22	0.27	0.26	0.21	0.26	0.29
Intake of fish meat (servings/day)	0.14	0.17	0.18	0.15	0.17	0.18
Intake of fruits and vegetables (servings/day)	3.5	4.1	4.1	4.3	4.0	3.7
Whole grain intake (g/day)‡	10.0	13.3	17.0	17.2	13.4	12.0
Multivitamin supplement users (%)	25.3	24.6	60.3	37.9	33.9	31.4
Iron supplement users (%)	2.0	1.0	20.1	7.1	5.6	3.9

*Moderate or vigorous activity. †BMI is calculated as weight in kilograms divided by the square of the height in meters. ‡Whole-grain intake and iron supplement users are based on 1984 data.

ilar to those for total iron, we did not include them separately in the article.

Both total and dietary iron were inversely associated with the risk of type 2 diabetes in the models adjusted for age, BMI, and nondietary factors (Table 2). However, this association disappeared when we also adjusted for dietary factors. Although this confounding was not attributable to any one covariate in particular, the important factors were glycemic load, caffeine, red meat, and cereal fiber. Excluding women who reported current use of iron supplements (4.2%) did not change the results when we evaluated dietary iron. In addition, we did not find any significant association between supplemental iron and the risk of diabetes.

Heme iron intake was associated with an elevated risk of diabetes (Table 3). The age-adjusted RR between extreme quintiles of cumulative heme iron intake was

1.87 (95% CI 1.69–2.07; $P_{\text{trend}} < 0.0001$), which was attenuated to 1.24 (1.12–1.37; $P_{\text{trend}} < 0.0001$) when we added BMI in the model. When we adjusted for other nondietary and dietary factors, this RR was 1.28 (1.14–1.45; $P_{\text{trend}} < 0.0001$). This association remained statistically significant even after adjustment of red meat intake (1.20 [1.05–1.37]; $P_{\text{trend}} = 0.003$). When we modeled heme iron as a continuous variable, the multivariate RR for every 1-mg increase in intake was 1.23 (1.13–1.34). In a model in which heme iron was used in seven categories, the RR comparing women who consumed ≥ 2.25 mg/day and those whose consumed < 0.75 mg/day was 1.52 (1.22–1.88). In a separate multivariate model with all forms (heme, dietary nonheme, and supplemental) of cumulative iron intake mutually adjusted, only the RR between extreme quintiles of heme iron was significant

(1.16 [1.01–1.33]; $P_{\text{trend}}: 0.01$). In addition, we conducted analyses for heme iron using different methods of updating the exposure and obtained similar results. When we used the simple update method with 4 years of latency (e.g., diet in 1986 was used to predict the risk between 1990 and 1994), the results were slightly stronger (Table 3). However, using only baseline heme yielded nonsignificant association (RR between extreme quintiles 1.08 [0.97–1.21]; $P_{\text{trend}} 0.07$), suggesting the advantage of using repeated measures for dietary assessment.

Given that red meat is the main source of heme iron in the diet and its intake is positively associated with the diabetes risk in this cohort of women (41), it may confound the association between heme iron and the risk of diabetes. When we added red meat in the model, the estimates for heme iron remained significant. However, the simultaneous control

Table 2—RRs and 95% CIs for type 2 diabetes during 20 years of follow-up by quintiles (Q) of iron intake in women

	Q1	Q2	Q3	Q4	Q5	<i>P</i> _{trend} *
Total iron						
Intake (mg/day)	8.0 (2.0–9.0)	9.6 (9.0–10.3)	11.0 (10.3–12.4)	14.0 (12.4–17.4)	24.0 (17.4–400)	
<i>n</i>	675	1,103	1,028	956	837	
Person-years	260,205	372,142	317,664	314,337	314,635	
Age adjusted	1	1.05 (0.95–1.16)	0.98 (0.88–1.08)	0.83 (0.75–0.92)	0.75 (0.67–0.83)	<0.0001
Age and BMI adjusted	1	0.97 (0.88–1.07)	0.91 (0.83–1.01)	0.85 (0.77–0.94)	0.82 (0.73–0.91)	<0.0001
Nondietary factors adjusted†	1	0.97 (0.88–1.07)	0.89 (0.81–0.99)	0.76 (0.74–0.93)	0.74 (0.72–0.92)	0.0006
Dietary and nondietary factors adjusted‡	1	1.05 (0.95–1.16)	1.05 (0.94–1.17)	1.03 (0.92–1.16)	1.02 (0.90–1.15)	0.78
Dietary iron§						
Intake (mg/day)	8.0 (2.0–9.0)	9.2 (9.0–10.0)	10.3 (10.0 to –11.0)	11.5 (11.0–12.3)	14.0 (12.3–87.0)	
<i>n</i>	798	916	960	939	791	
Person-years	298,521	291,825	306,210	300,464	294,837	
Age adjusted	1	1.05 (0.96–1.16)	0.99 (0.90–1.08)	0.95 (0.87–1.05)	0.76 (0.69–0.84)	<0.0001
Age and BMI adjusted	1	0.98 (0.89–1.08)	0.91 (0.83–1.00)	0.88 (0.80–0.97)	0.79 (0.71–0.87)	<0.0001
Nondietary factors adjusted†	1	0.98 (0.89–1.08)	0.91 (0.82–0.99)	0.89 (0.80–0.98)	0.78 (0.70–0.86)	<0.0001
Dietary and nondietary factors adjusted‡	1	1.06 (0.96–1.17)	1.03 (0.93–1.14)	1.07 (0.96–1.19)	1.02 (0.91–1.15)	0.90
Supplemental¶						
Intake (mg/day)	0	3.4 (0.2–5.5)	8.4 (5.5–10.4)	12.6 (10.4–15.9)	22.0 (15.9–391.7)	
<i>n</i>	3,104	413	330	422	330	
Person-years	1,071,670	133,012	123,760	124,333	126,207	
Age adjusted	1	0.81 (0.73–0.90)	0.74 (0.66–0.83)	0.90 (0.80–1.01)	0.74 (0.65–0.84)	<0.0001
Age and BMI adjusted	1	0.88 (0.80–0.98)	0.81 (0.72–0.92)	1.01 (0.89–1.14)	0.88 (0.77–0.99)	0.08
Nondietary factors adjusted†	1	0.90 (0.80–1.00)	0.85 (0.75–0.96)	1.04 (0.91–1.18)	0.90 (0.79–1.04)	0.19
Dietary and nondietary factors adjusted‡	1	0.92 (0.82–1.03)	0.85 (0.75–0.96)	1.08 (0.95–1.23)	0.96 (0.84–1.10)	0.67

Data for intake are median (range). **P* value for test for linear trend conducted across increasing categories of iron intake using medians of intake as continuous the variable. †Nondietary factors include age (5-year categories), BMI (10 categories), family history of diabetes (yes/no), smoking status (never, past, and current smoking of 1–14, 15–24, and ≥25 cigarettes/day), alcohol intake (0, 0–4.9, 5.0–9.9, 10.0–14.9, and ≥15 g/day), quintiles of physical activity (h/week), postmenopausal hormone use (premenopausal, current, past, and never user), and use of multivitamin supplements. ‡Dietary factors include intake of calories (kcal/day), cereal fiber (g/day), magnesium (mg/day), polyunsaturated-to-saturated fat ratio, glycemic load, caffeine (mg/day), and trans fat (% energy) (all quintiles). §Iron supplement users were excluded in these analyses. ¶The reference group included nonusers of iron supplements and follow-up started from 1984 when data on use were first reported. The dietary factors for this analysis also included whole grains (g/day), fruits and vegetables (servings/day), and red meat intake (servings/day).

for red meat may not be appropriate given that heme iron is mainly derived from red meat but suggests that the association for heme iron is not fully explained by red meat intake. To address this issue further, we conducted analysis by subdividing heme iron into red meat heme and heme derived from other sources (fish and poultry). Both of these sources were independently associated with an elevated risk of diabetes in the multivariate models, suggesting that the association of heme iron and diabetes is unlikely to be confounded by the intake of red meat.

Body iron may be associated with obesity, menopausal status, alcohol (42), and intake of phytates, which impair heme iron absorption (43). Therefore, we conducted multivariate analyses within strata of these factors but did not find any

significant effect modification by these factors.

CONCLUSIONS— In this prospective cohort study, we found no association between total, dietary, supplemental, or nonheme iron intake and the risk of diabetes. However, heme iron intake was positively associated with this risk, and this association was not entirely explained by red meat intake.

The extraordinary capacity of the human body to accumulate iron is seen in hereditary hemochromatosis (44). In this genetic disorder, iron deposition occurs in most tissues including the liver, heart, musculoskeletal system, and pancreas (resulting in secondary diabetes). High body iron stores less extreme than those in hemochromatosis have been linked to

insulin resistance (10). Iron is a strong prooxidant that catalyzes the formation of hydroxyl radicals, and the increase in oxidative stress may be associated with the risk of diabetes (45). Some researchers have also suggested that high iron levels impede insulin extraction in the liver, leading to peripheral hyperinsulinemia (46,47). Another potential mechanism is direct iron deposition in the pancreatic β -cells that can impair insulin secretion (48). In addition, the hemochromatosis C282Y and H63D mutations in the *HFE* gene (chromosome 6) that are associated with high iron stores may also play a role in the development of diabetes (49,50).

Serum ferritin is now proposed to be a component of the metabolic syndrome (49). Interestingly, Moirand et al. (51) described a new non-HLA-linked iron over-

Table 3—RRs and 95% CI for type 2 diabetes during 20 years of follow-up by quintiles (Q) of heme intake in women

	Q1	Q2	Q3	Q4	Q5	P_{trend}^*
Cumulative average						
Intake (mg/day)	0.8 (0–1.0)	1.1 (1.0–1.2)	1.3 (1.2 to –1.4)	1.5 (1.4 to –1.7)	1.9 (1.7–7.0)	
<i>n</i>	658	886	985	1,064	1,006	
Person-years	304,896	324,506	307,698	314,470	327,412	
Age adjusted	1	1.25 (1.12–1.39)	1.50 (1.36–1.67)	1.73 (1.57–1.91)	1.87 (1.69–2.07)	<0.0001
Age and BMI adjusted	1	1.05 (0.95–1.17)	1.17 (1.05–1.28)	1.21 (1.10–1.34)	1.24 (1.12–1.37)	<0.0001
Nondietary factors adjusted†	1	1.05 (0.95–1.16)	1.15 (1.04–1.27)	1.20 (1.08–1.33)	1.23 (1.11–1.36)	<0.0001
Dietary and nondietary factors adjusted‡	1	1.08 (0.97–1.19)	1.20 (1.09–1.33)	1.27 (1.14–1.41)	1.28 (1.14–1.45)	<0.0001
Simple update with 4-year latency§						
Intake (mg/day)	0.6 (0–0.7)	0.9 (0.8–1.0)	1.1 (1.1–1.3)	1.4 (1.3–1.6)	2.0 (1.7–11.6)	
<i>n</i>	517	962	727	1,054	872	
Person-years	234,216	367,213	241,608	373,625	359,555	
Age adjusted	1	1.28 (1.15–1.43)	1.59 (1.42–1.78)	1.69 (1.52–1.88)	1.87 (1.67–2.09)	<0.0001
Age and BMI adjusted	1	1.11 (1.00–1.23)	1.25 (1.12–1.40)	1.26 (1.13–1.40)	1.30 (1.16–1.45)	<0.0001
Nondietary factors adjusted	1	1.13 (1.01–1.25)	1.27 (1.14–1.42)	1.27 (1.14–1.42)	1.31 (1.17–1.47)	<0.0001
Dietary and nondietary factors adjusted	1	1.15 (1.03–1.28)	1.30 (1.16–1.46)	1.31 (1.17–1.47)	1.35 (1.19–1.54)	0.0001

Data for intake are median (range). * P value for test for linear trend conducted across increasing categories of iron intake using medians of intake as continuous variable. †Nondietary factors include age (5-year categories), BMI (10 categories), family history of diabetes (yes/no), smoking status (never, past, and current smoking of 1–14, 15–24, and ≥ 25 cigarettes/day), alcohol intake (0, 0–4.9, 5.0–9.9, 10.0–14.9, and ≥ 15 g/day), quintiles of physical activity (h/week), postmenopausal hormone use (premenopausal, current, past, and never user), and use of multivitamin supplements. ‡Dietary factors include intake of calories (kcal/day), cereal fiber (g/day), whole grains (g/day), fruits and vegetables (servings/day), magnesium (mg/day), polyunsaturated-to-saturated fat ratio, glycemic load, caffeine (mg/day), and trans fat (% energy) (all quintiles). §For example, diet in 1986 was used to predict the disease risk between 1990 and 1994.

load syndrome, which suggests a relation between iron excess and metabolic disorders. This condition of insulin resistance-associated hepatic iron overload is characterized by the presence of hyperferritinemia in addition to the entities of metabolic syndrome. In a cross-sectional analysis using National Health and Nutrition Examination Survey (NHANES) data, the indexes of body fat distribution including waist-to-hip ratio, were positively associated with serum ferritin levels in Mexican-American men aged between 20 and 49 years (23). Further, several clinical trials of venesection therapy showed beneficial effects on the signs and symptoms of metabolic syndrome (52–54). In a clinical trial among 28 diabetic participants randomly assigned to undergo blood letting (three times, 500 ml, 2 weeks apart), the phlebotomy group had improved insulin sensitivity at 4 and 12 months compared with the observation group (53).

Although several cross-sectional and case-control studies have linked elevated iron indexes with diabetes (23,25–27), similar data from prospective studies are limited. Salonen et al. (55) first reported an association between serum ferritin lev-

els and incidence of diabetes in a group of Finnish men (RR between extreme quartiles 2.4 [95%CI 1.03–5.50]; $P_{\text{trend}} = 0.04$). In a recent nested case-control study (22) (698 case patients and 716 control subjects), we reported an elevated risk of diabetes in women with high ferritin levels (RR between quintile 5 vs. 1 2.68 [1.75–4.11]; $P_{\text{trend}} < 0.001$) and those with a low transferrin receptor-to-ferritin ratio (RR between quintile 1 vs. 5 2.44 [1.61–3.71]; $P_{\text{trend}} = 0.01$).

Because dietary intake of iron is an important determinant of body iron stores (56), higher intake may be associated with an elevated risk of diabetes. It is useful to evaluate dietary iron as intake can be readily modified. In a sample of 620 healthy postmenopausal women in this cohort, higher intakes of heme and supplemental iron were associated with higher serum ferritin levels (35). However, the specificity of serum ferritin as a marker of body iron store is controversial because it is affected by other factors including chronic inflammation (16,57).

In our study, heme iron was associated with a higher risk of incident diabetes. This form of iron is more bioavailable because of its higher absorption indepen-

dent of body iron status, unlike the absorption of nonheme iron, which is well regulated (56,58). Therefore, it is probable that a chronically high intake of heme iron can lead to high body iron stores and thus may elevate the risk of diabetes. Some reports have suggested that alcohol affects iron metabolism by some unknown mechanisms that involve the iron-binding proteins (42). Lee et al. (29) found a stronger positive association between heme iron and risk of diabetes among women who consumed alcohol compared with nondrinkers. However, we found no significant effect modification by alcohol.

Supplemental iron has also been associated with higher serum ferritin levels in a subset of these women (35). However, we found no association of supplemental iron and the risk of diabetes. In this cohort, current use of iron supplements was relatively rare (<5%). It is possible that women who used iron supplements may have had a much lower baseline iron status.

Because of the observational nature of our study, we cannot completely exclude the possibility that the association was due to residual confounding by unmea-

sured factors. However, the large sample size, prospective design with repeated dietary assessments, high rate of follow-up, and information on a multitude of covariates minimize major sources of such bias. Because BMI is the strongest predictor of diabetes, we controlled for it in 10 categories. Modeling BMI as a continuous variable did not alter our results. Another potential concern is the measurement error associated with dietary assessment; however, this error is most likely to bias our results toward the null. In addition, by using repeated dietary measurements with a validated FFQ to account for dietary changes, we reduced the extent of this error (39).

In summary, we found no association between total, dietary, supplemental, and nonheme iron and the risk of diabetes. However, heme iron intake was positively associated with the risk of diabetes independent of BMI and other risk factors for diabetes.

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References

- Jiang R, Schulze MB, Li T, Rifai N, Stampfer MJ, Rimm EB, Hu FB: Non-HDL cholesterol and apolipoprotein B predict cardiovascular disease events among men with type 2 diabetes. *Diabetes Care* 27:1991–1997, 2004
- Paolisso G, Barbagallo M: Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. *Am J Hypertens* 10:346–355, 1997
- Lopez-Ridaura R, Willett WC, Rimm EB, Liu S, Stampfer MJ, Manson JE, Hu FB: Magnesium intake and risk of type 2 diabetes in men and women. *Diabetes Care* 27:134–140, 2004
- Cefalu WT, Hu FB: Role of chromium in human health and in diabetes. *Diabetes Care* 27:2741–2751, 2004
- Rajpathak S, Rimm EB, Li T, Morris JS, Stampfer MJ, Willett WC, Hu FB: Lower toenail chromium in men with diabetes and cardiovascular disease compared with healthy men. *Diabetes Care* 27:2211–2216, 2004
- Sanchez M, de la Sierra A, Coca A, Poch E, Giner V, Urbano-Marquez A: Oral calcium supplementation reduces intraplatelet free calcium concentration and insulin resistance in essential hypertensive patients. *Hypertension* 29:531–536, 1997
- Fernandez-Real JM, Lopez-Bermejo A, Ricart W: Cross-talk between iron metabolism and diabetes. *Diabetes* 51:2348–2354, 2002
- Opara EC: Role of oxidative stress in the etiology of type 2 diabetes and the effect of antioxidant supplementation on glycemic control. *J Investig Med* 52:19–23, 2004
- Ramakrishnan U, Kuklina E, Stein AD: Iron stores and cardiovascular disease risk factors in women of reproductive age in the United States. *Am J Clin Nutr* 76:1256–1260, 2002
- Sheu WH, Chen YT, Lee WJ, Wang CW, Lin LY: A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. *Clin Endocrinol (Oxf)* 58:380–385, 2003
- Tuomainen TP, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T, Kaplan GA, Salonen JT: Body iron stores are associated with serum insulin and blood glucose concentrations: population study in 1,013 eastern Finnish men. *Diabetes Care* 20:426–428, 1997
- Hua NW, Stoohs RA, Facchini FS: Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. *Br J Nutr* 86:515–519, 2001
- Tilbrook L: Cross talk between iron metabolism and diabetes. *Ann Clin Biochem* 41(Pt. 3):255, 2004
- Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, Salvioni A, Mariani R, Mancina G: Increased serum ferritin is common in men with essential hypertension. *J Hypertens* 20:1513–1518, 2002
- Halle M, Konig D, Berg A, Keul J, Baumstark MW: Relationship of serum ferritin concentrations with metabolic cardiovascular risk factors in men without evidence for coronary artery disease. *Atherosclerosis* 128:235–240, 1997
- Williams MJ, Poulton R, Williams S: Relationship of serum ferritin with cardiovascular risk factors and inflammation in young men and women. *Atherosclerosis* 165:179–184, 2002
- Gillum RF: Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men: the Third National Health and Nutrition Examination Survey. *Int J Obes Relat Metab Disord* 25:639–645, 2001
- Alpert PT: New and emerging theories of cardiovascular disease: infection and elevated iron. *Biol Res Nurs* 6:3–10, 2004
- Jehn M, Clark JM, Guallar E: Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 27:2422–2428, 2004
- Lao TT, Tam KF: Maternal serum ferritin and gestational impaired glucose tolerance. *Diabetes Care* 20:1368–1369, 1997
- Lao TT, Chan PL, Tam KF: Gestational diabetes mellitus in the last trimester: a feature of maternal iron excess? *Diabet Med* 18:218–223, 2001
- Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA* 291:711–717, 2004
- Ford ES, Cogswell ME: Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 22:1978–1983, 1999
- Hernandez C, Genesca J, Ignasi Esteban J, Garcia L, Simo R: Relationship between iron stores and diabetes mellitus in patients infected by hepatitis C virus: a case-control study. *Med Clin (Barc)* 115:21–22, 2000
- Hughes K, Choo M, Kuperan P, Ong CN, Aw TC: Cardiovascular risk factors in non-insulin-dependent diabetics compared to non-diabetic controls: a population-based survey among Asians in Singapore. *Atherosclerosis* 136:25–31, 1998
- Thomas MC, MacIsaac RJ, Tsalamandris C, Jerums G: Elevated iron indices in patients with diabetes. *Diabet Med* 21:798–802, 2004
- Kaye TB, Guay AT, Simonson DC: Non-insulin-dependent diabetes mellitus and elevated serum ferritin level. *J Diabetes Complications* 7:246–249, 1993
- Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB: Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. *Am J Clin Nutr* 79:70–75, 2004
- Lee DH, Folsom AR, Jacobs DR Jr: Dietary iron intake and type 2 diabetes incidence in postmenopausal women: the Iowa Women's Health Study. *Diabetologia* 47:185–194, 2004
- Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE: Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 122:51–65, 1985
- Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE: The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 127:188–199, 1988
- Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML: Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. *J Am Diet Assoc* 87:43–47, 1987
- Agricultural Research Service: *USDA Nutrient Database for Standard Reference*. Release 10. Washington, DC, U.S. Dept. of Agriculture, 1995
- Willett W, Stampfer MJ: Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 124:17–27, 1986
- Liu JM, Hankinson SE, Stampfer MJ, Rifai N, Willett WC, Ma J: Body iron stores and their determinants in healthy postmenopausal US women. *Am J Clin Nutr* 78:

- 1160–1167, 2003
36. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC: Validity of self-reported waist and hip circumferences in men and women. *Epidemiology* 1:466–473, 1990
 37. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 28:1039–1057, 1979
 38. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, Speizer FE: Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. *Lancet* 338:774–778, 1991
 39. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC: Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol* 149:531–540, 1999
 40. Cox D, Oakes D: *Analysis of Survival Data*. London, Chapman and Hall, 1984
 41. Fung TT, Schulze M, Manson JE, Willett WC, Hu FB: Dietary patterns, meat intake, and the risk of type 2 diabetes in women. *Arch Intern Med* 164:2235–2240, 2004
 42. Fletcher LM, Halliday JW, Powell LW: Interrelationships of alcohol and iron in liver disease with particular reference to the iron-binding proteins, ferritin and transferrin. *J Gastroenterol Hepatol* 14: 202–214, 1999
 43. Zijp IM, Korver O, Tijburg LB: Effect of tea and other dietary factors on iron absorption. *Crit Rev Food Sci Nutr* 40:371–398, 2000
 44. Powell LW: Diagnosis of hemochromatosis. *Semin Gastrointest Dis* 13:80–88, 2002
 45. Wolff SP: Diabetes mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Br Med Bull* 49:642–652, 1993
 46. Ferrannini E: Insulin resistance, iron, and the liver. *Lancet* 355:2181–2182, 2000
 47. Niederau C, Berger M, Stremmel W, Starke A, Strohmeyer G, Ebert R, Siegel E, Creutzfeldt W: Hyperinsulinaemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? *Diabetologia* 26:441–444, 1984
 48. Wilson JG, Lindquist JH, Grambow SC, Crook ED, Maher JF: Potential role of increased iron stores in diabetes. *Am J Med Sci* 325:332–339, 2003
 49. Fernandez-Real JM, Vendrell J, Baiget M, Gimferrer E, Ricart W: C282Y and H63D mutations of the hemochromatosis candidate gene in type 2 diabetes. *Diabetes Care* 22:525–526, 1999
 50. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, Zhu G, Duffy DL, Martin NG: Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. *Am J Hum Genet* 66:1246–1258, 2000
 51. Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y: A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 349:95–97, 1997
 52. Guillygomarc'h A, Mendler MH, Moirand R, Laine F, Quentin V, David V, Brissot P, Deugnier Y: Venesection therapy of insulin resistance-associated hepatic iron overload. *J Hepatol* 35:344–349, 2001
 53. Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Hernandez-Aguado I, Ricart W: Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and β -cell function. *Diabetes* 51: 1000–1004, 2002
 54. Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Lopez-Bermejo A, Ricart W: Blood letting in high-ferritin type 2 diabetes: effects on vascular reactivity. *Diabetes Care* 25:2249–2255, 2002
 55. Salonen JT, Tuomainen TP, Nyyssonen K, Lakka HM, Punnonen K: Relation between iron stores and non-insulin dependent diabetes in men: case-control study. *BMJ* 317:727, 1998
 56. Cook JD: Adaptation in iron metabolism. *Am J Clin Nutr* 51:301–308, 1990
 57. Torti SV, Torti FM: Iron and ferritin in inflammation and cancer. *Adv Inorg Biochem* 10:119–137, 1994
 58. Monsen ER: Iron nutrition and absorption: dietary factors which impact iron bioavailability. *J Am Diet Assoc* 88:786–790, 1988