Original Contribution

A Prospective Study of Plasma Ferritin Level and Incident Diabetes

The Atherosclerosis Risk in Communities (ARIC) Study

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The authors performed a case-cohort study nested within the Atherosclerosis Risk in Communities (ARIC) Study to determine the association between plasma ferritin level and risk of type 2 diabetes mellitus. Persons with incident cases of type 2 diabetes diagnosed over an average follow-up period of 7.9 years ($n = 599$) were compared with a random sample of the cohort ($n = 690$). After adjustment for age, gender, menopausal status, ethnicity, center, smoking, and alcohol intake, the hazard ratio for diabetes, comparing the fifth quintile of ferritin with the first quintile, was 1.74 (95% confidence interval: 1.14, 2.65; p-trend < 0.001). After further adjustment for body mass index and components of the metabolic syndrome, the hazard ratio was 0.81 (95% confidence interval: 0.49, 1.34; p-trend $= 0.87$). From a causal perspective, there are two alternative interpretations of these findings. Elevated iron stores, reflected in elevated plasma ferritin levels, may induce baseline metabolic abnormalities that ultimately result in diabetes. Alternatively, elevated ferritin may be just one of several metabolic abnormalities related to the underlying process that ultimately results in diabetes, rather than a causal factor for diabetes. Longitudinal studies with repeated measurements of glucose and iron metabolism parameters are needed to establish the role of iron stores and plasma ferritin in diabetes development.

cohort studies; diabetes mellitus, type 2; ferritins; incidence; iron

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence interval.

Impaired glucose metabolism and diabetes mellitus are common clinical manifestations of iron overload in patients with hemochromatosis (1). More recently, moderately elevated iron stores below the levels commonly associated with hemochromatosis have also been implicated in the etiology of diabetes (2–4). Levels of plasma ferritin, a biomarker of iron stores, are elevated in persons with prevalent diabetes as compared with nondiabetic controls (2, 5). Markers of iron status are also correlated with the prevalence of the metabolic syndrome (6, 7) and with measures of insulin

resistance, such as elevated glucose and insulin levels (6, 8–10). In addition, two prospective studies have identified an independent association between baseline elevations in iron stores and the incidence of diabetes (11, 12).

Elevated iron stores may induce diabetes through a variety of mechanisms, including oxidative damage to pancreatic beta cells, impairment of hepatic insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production (2–4). The confirmation of an increased risk of diabetes with moderately elevated ferritin levels could have clinical and public health consequences, since persons at high risk could be targeted for more intensive screening and preventive interventions.

Our objective in this study was to prospectively evaluate the association between plasma ferritin level and incidence of type 2 diabetes in the Atherosclerosis Risk in Communities (ARIC) Study. We hypothesized that an elevated level of ferritin, a biomarker of iron stores commonly used in epidemiologic studies, would predict an increased risk of diabetes independently of traditional risk factors.

MATERIALS AND METHODS

Study population

The ARIC Study is a population-based cohort study of 15,792 men and women aged 45–64 years designed to examine risk factors for clinical and subclinical atherosclerosis and their variation over time. Participants were recruited during 1987–1989 at four clinical centers in the United States: Forsyth County, North Carolina; Jackson, Mississippi; the northwestern suburbs of Minneapolis, Minnesota; and Washington County, Maryland (13). Three follow-up visits occurred during 1990–1992, 1993–1995, and 1996–1998, with follow-up rates of 93 percent, 86 percent, and 80 percent, respectively (14). Human-subject research review committees at the involved institutions approved the study, and all participants gave written consent.

A case-cohort study design was used to investigate the relation between baseline plasma ferritin level and incident type 2 diabetes in ARIC (15, 16). Case-cohort analyses compare incident cases of a disease appearing in a cohort over time with a random sample of the whole cohort. They are valid alternatives to nested case-control studies for efficient processing and analysis of cohort data. Prior to case-cohort sampling, we excluded participants with prevalent diabetes at baseline ($n = 2,018$), participants from minority groups with small numbers ($n = 95$), participants whose diabetes status could not be determined at at least one follow-up visit ($n = 879$), participants who did not give consent for use of stored blood specimens ($n = 7$), participants with missing values for anthropometric variables at baseline ($n = 12$), and participants for whom plasma samples were exhausted in previous nested studies of cardiovascular disease or were held in reserve ($n = 2,506$). The eligible sampling frame comprised 10,275 participants, including 1,155 incident cases of diabetes.

For the case-cohort design, we selected a random sample of 576 cases from the group of 1,155 incident diabetes cases and a random sample of 690 participants from the cohort (cohort random sample). Sampling was stratified on ethnicity, with sampling fractions of 72 percent and 39 percent for African-American and White diabetes cases and 15 percent and 5 percent for African-American and White cohort random-sample participants. The final case-cohort study included 599 diabetes cases (the 576 randomly selected cases plus 23 additional cases selected only in the cohort random sample) and the 690 participants in the cohort random sample (591 participants who did not develop diabetes during follow-up, 76 participants who developed diabetes and were also selected in the case group, and 23 participants who developed diabetes but were not selected in the case group). The average length of follow-up for participants in the cohort random sample was 7.9 years.

Measurements

Ferritin level was determined in plasma samples collected at the ARIC baseline examination and frozen at $-70°C$. Ferritin levels were measured in duplicate on a Hitachi autoanalyzer using an immunoturbidimetric assay (Roche Diagnostics, Indianapolis, Indiana). The reliability coefficient for measures in 35 subjects with blinded replicate samples collected at baseline was 0.85.

Other information gathered at baseline included data on demographic variables, educational level, self-reported smoking and alcohol intake, and parental history of diabetes (15, 16). Women were classified as postmenopausal if they had not menstruated in the past 2 years or if they had undergone bilateral oophorectomy. Women with incomplete or missing data on menopausal status ($n = 25$) were classified as postmenopausal if they were 50 years of age or older (the 90th percentile of age at onset of menopause among cohort random-sample women). Body mass index (weight (kg)/height (m)^2) was derived from measurements of height and weight. Hypertension was defined as a blood pressure greater than 140/90 mmHg or current use of antihypertensive medication. Other laboratory values included measurements of fasting glucose, insulin, triglycerides, and total and high density lipoprotein cholesterol (15, 16). In addition, a panel of six inflammation markers was measured (C-reactive protein, interleukin-6, orosomucoid (α_1-acid glycoprotein), sialic acid, white blood cell count, and fibrinogen). An inflammation score, composed of one additional point for each inflammation marker above the median level in the cohort random sample, was used as a summary measure. This score predicted incident diabetes better than individual inflammation markers in the ARIC Study (15). The results were unchanged when individual inflammation markers were used instead of the inflammation score (not shown).

Case definition

Incident cases of diabetes were defined by the appearance of any one of the following during follow-up: 1) a fasting (≥ 8 hours) glucose level ≥ 7.0 mmol/liter; 2) a nonfasting glucose level ≥ 11.1 mmol/liter; 3) use of diabetes medication; or 4) a self-reported physician diagnosis. The presence of diabetes was assessed at baseline and at each of the three follow-up visits. For persons whose status was ascertained

TABLE 1.   Baseline characteristics of participants in a case-cohort study of plasma ferritin levels and risk of type 2 diabetes mellitus, Atherosclerosis Risk in Communities Study, 1987–1989

	Diabetes cases (n = 599)		Cohort random sample (n = 690)		p value*
	Mean or %	SE† or 95% CI†	Mean or %	SE or 95% CI	
Age (years)	53.5	0.24	52.8	0.24	0.02
Gender (%)					0.005
Men	42.3	2.09	37.2	2.01	
Postmenopausal women	41.1	2.06	39.0	2.06	
Premenopausal women	16.6	1.56	23.8	1.82	
Race (% Whites)	66.1	—‡	80.6	—‡	<0.001
Current smoker (%)	21.3	1.74	22.1	1.74	0.82
Current alcohol drinker (%)	50.4	2.06	62.5	1.96	<0.001
Body mass index§ (kg/m^2)	30.8	0.24	27.2	0.19	<0.001
Waist circumference (cm)					
Men	104.4	0.74	97.4	0.66	<0.001
Women	104.5	0.80	93.6	0.72	<0.001
Hypertensive (%)	46.2	2.08	27.7	1.78	<0.001
High density lipoprotein cholesterol level (mmol/liter)	1.21	0.01	1.38	0.02	<0.001
Triglyceride level¶ (mmol/liter)	1.47	1.41, 1.53	1.19	1.14, 1.24	<0.001
Fasting glucose level (mmol/liter)	5.99	0.02	5.44	0.02	<0.001
Fasting insulin level¶ (pmol/liter)	98.9	93.8, 104.2	58.2	55.1, 61.4	<0.001
Inflammation score#	3.88	0.07	2.93	0.08	<0.001
Ferritin level¶ (ng/ml)	106.4	93.6, 120.9	87.5	78.5, 97.4	0.004

  * p values were calculated for the comparison between diabetes cases and noncases, taking into account the case-cohort design and the stratified sampling of cases and cohort random-sample participants, using the method of Lin (26).
  † SE, standard error; CI, confidence interval.
  ‡ Stratification variable.
  § Weight (kg)/height (m)^2.
  ¶ Geometric mean values.
  # The inflammation score was calculated by adding one point for each inflammation marker (C-reactive protein, interleukin-6, orosomucoid (α_1-acid glycoprotein), sialic acid, white blood cell count, and fibrinogen) above the median level in the cohort random sample.

only on the basis of fasting glucose level, the time of diabetes onset was estimated as the time at which glucose level reached 7.0 mmol/liter, determined by linear interpolation using information from the visit at which diabetes was ascertained and the previous visit. For persons who reported a doctor's diagnosis or medication use, the time to a glucose level of 7.0 mmol/liter was estimated using the fasting glucose value at the previous visit and the slope of change in glucose values estimated from all of the persons with diabetes who were unaware of their diabetes status (15, 16).

Statistical analysis

All analyses were weighted to account for the sampling fractions of diabetes cases and of participants in the cohort random sample. Plasma ferritin levels were right-skewed and thus were log-transformed prior to analysis. Cutoffs for quintiles of ferritin were based on the distribution in the cohort random sample. Determinants of ferritin levels among cohort random-sample participants were evaluated using linear models with log ferritin as the dependent variable.

Hazard ratios and 95 percent confidence intervals for the association of ferritin with incident diabetes were estimated with proportional hazards regression, where follow-up times were time to diabetes for incident cases and time to the last follow-up examination attended for those not diagnosed with diabetes. To account for the case-cohort design and for the stratified sampling of cases and cohort random-sample participants, we used the method of Lin (17) to fit weighted proportional hazards models using SUDAAN (18). Tests for trend across quintiles of ferritin were conducted by incorporating a variable with the median value of each quintile in regression models.

All proportional hazards models were stratified by sex and menopausal status. Stratified proportional hazards models allow for differences in background hazard functions

TABLE 2. Ratio of ferritin levels according to baseline characteristics in cohort random-sample participants, Atherosclerosis Risk in Communities Study, 1987–1989

	Ratio of ferritin levels*	95% confidence interval	p value
Age (per 10-year increase)	1.16	0.97, 1.40	0.11
Gender			<0.001
Postmenopausal women vs. men	0.70	0.56, 0.88	
Premenopausal women vs. men	0.24	0.18, 0.32	
Race (African Americans vs. Whites)	1.25	0.72, 2.16	0.43
Smoking			0.91
Former smokers vs. never smokers	0.95	0.76, 1.20	
Current smokers vs. never smokers	0.96	0.71, 1.30	
Alcohol drinking			0.06
Former drinkers vs. never drinkers	0.87	0.59, 1.30	
Current drinkers vs. never drinkers	1.26	0.97, 1.64	
Body mass index† (per 1-kg/m² increase)	1.00	0.98, 1.02	0.88
Waist circumference (per 5-cm increase)	1.00	0.96, 1.04	0.98
Hypertensive (yes vs. no)	1.24	1.01, 1.53	0.04
High density lipoprotein cholesterol level (per 1-mmol/liter increase)	0.97	0.76, 1.23	0.78
Triglyceride level (per twofold increase)	1.08	0.92, 1.26	0.36
Fasting glucose level (per 1-mmol/liter increase)	1.34	1.12, 1.60	0.001
Fasting insulin level (per twofold increase)	1.10	0.99, 1.21	0.07
Inflammation score‡ (≥3 vs. <3)	1.01	0.83, 1.24	0.89

* Age, gender, menopausal status, ethnicity, and center-adjusted ratios of geometric mean ferritin levels associated with differences in baseline characteristics. For instance, the ratio for age (1.16) is interpreted as a 16% increase in the geometric mean ferritin level associated with a 10-year increase in age (i.e., the predicted plasma ferritin level for a 10-year age increase in a participant with a baseline plasma ferritin level of 80 ng/ml would be 92.8 ng/ml—assuming constant menopausal status).

† Weight (kg)/height (m)².

‡ The inflammation score was calculated by adding one point for each inflammation marker (C-reactive protein, interleukin-6, orosomucoid (α_1-acid glycoprotein), sialic acid, white blood cell count, and fibrinogen) above the median level in the cohort random sample.

across stratification categories. In addition to sex and menopausal status, we initially adjusted for age, center, ethnicity, smoking, and alcohol drinking. Then we further adjusted for body mass index, metabolic syndrome components (waist circumference, hypertension, high density lipoprotein cholesterol, fasting triglycerides, and fasting glucose)

(19), fasting insulin level, and inflammation score. Levels of all of these covariates were determined at the baseline visit. Statistical analysis was performed using SUDAAN 9 software (RTI International, Research Triangle Park, North Carolina) (18).

RESULTS

Participants with diabetes were more likely to be older, male, and African-American, to have a higher body mass index, and to have higher levels of metabolic syndrome components and inflammation markers at baseline (table 1). Baseline plasma ferritin levels were significantly higher in diabetes cases than in noncases (geometric mean = 106.4 ng/ml vs. 82.7 ng/ml, a 28.7 percent difference (95 percent confidence interval (CI): 8.2, 53.0 percent; $p < 0.001$)).

In the cohort random sample, plasma ferritin levels were significantly higher in men (134.4 ng/ml) than in postmenopausal women (97.3 ng/ml) or premenopausal women (32.2 ng/ml). Plasma ferritin was also positively associated with the prevalence of hypertension and with fasting glucose and insulin levels (table 2).

After adjustment for age, center, ethnicity, gender, menopausal status, smoking, and alcohol intake (table 3), persons in the highest quintile of plasma ferritin level had an increased risk of diabetes compared with those in the lowest quintile (hazard ratio = 1.74, 95 percent CI: 1.14, 2.65), with a significant trend in risk across quintiles (p-trend < 0.001). This association was slightly attenuated after further adjustment for body mass index (hazard ratio = 1.51, 95 percent CI: 0.98, 2.31; p-trend = 0.002). After adjustment for metabolic syndrome components, however, the diabetes hazard ratio comparing the highest quintile of ferritin with the lowest was 0.81 (95 percent CI: 0.49, 1.34; p-trend = 0.87). Of the metabolic syndrome components, fasting glucose level had the largest impact on the risk estimates associated with plasma ferritin. Further adjusting for fasting insulin level or inflammation score did not materially change the estimates (table 3).

The hazard ratios associated with log ferritin introduced as a continuous variable in metabolic syndrome-adjusted models were below 1 for males, postmenopausal women, and premenopausal women, in spite of widely differing ferritin levels (figure 1). Furthermore, there were no consistent differences in the association between ferritin and diabetes in subgroups defined by age, gender, menopausal status, ethnicity, smoking, alcohol intake, body mass index, waist circumference, hypertension, high density lipoprotein cholesterol, triglycerides, fasting glucose, fasting insulin, or inflammation score (figure 1).

DISCUSSION

In this large nested case-cohort study, there was a moderate trend of increasing diabetes risk across quintiles of plasma ferritin level. However, this association disappeared after adjustment for components of the metabolic syndrome, indicating that plasma ferritin does not predict the risk of diabetes beyond established risk factors. From a causal

TABLE 3. Hazard ratio for incident type 2 diabetes mellitus according to quintile of baseline plasma ferritin level among case-cohort study participants, Atherosclerosis Risk in Communities Study, 1987–1990*

Quintile	Median ferritin level (ng/ml)	Model 1†		Model 2‡		Model 3§		Model 4¶	
		HR#	95% CI#	HR	95% CI	HR	95% CI	HR	95% CI
1	20.0	1.00		1.00		1.00		1.00	
2	59.2	0.87	0.58, 1.31	0.89	0.58, 1.37	0.78	0.48, 1.27	0.78	0.47, 1.29
3	106.6	0.88	0.58, 1.35	0.73	0.46, 1.16	0.71	0.44, 1.17	0.72	0.44, 1.18
4	173.1	1.18	0.77, 1.80	1.16	0.76, 1.78	0.83	0.52, 1.34	0.84	0.52, 1.36
5	354.5	1.74	1.14, 2.65	1.51	0.98, 2.31	0.81	0.49, 1.34	0.79	0.48, 1.32
p-trend		<0.001		0.002		0.87		0.78	

 * Data in all proportional hazards models were stratified by gender and menopausal status. Cutoff values for ferritin quintiles were 40.0 ng/ml, 81.7 ng/ml, 130.8 ng/ml, and 235.4 ng/ml.

 † Adjusted for age (continuous variable), center, ethnicity, smoking (never/former/current), and alcohol drinking (never/former/current).

 ‡ Further adjusted for body mass index (continuous variable).

 § Further adjusted for metabolic syndrome components: high density lipoprotein cholesterol (continuous variable), waist circumference (continuous variable), hypertension (yes/no), fasting glucose level (continuous variable), and fasting triglyceride level (continuous variable, log-transformed).

 ¶ Further adjusted for fasting insulin level (continuous variable, log-transformed) and inflammation score ($<3/\geq3$).

 # HR, hazard ratio; CI, confidence interval.

perspective, there are at least two possible interpretations of our findings. Elevated iron stores, reflected in elevated plasma ferritin levels, may increase baseline glucose levels and induce other metabolic abnormalities that ultimately result in diabetes. Alternatively, elevated plasma ferritin may be just one of several metabolic abnormalities related to the underlying process that ultimately results in diabetes, rather than a causal factor for diabetes development. Furthermore, while plasma ferritin levels reflect iron stores, ferritin levels are also determined by many other genetic and environmental factors. It is thus possible that the association between ferritin and diabetes may not reflect changes in iron stores.

Type 2 diabetes occurs in 25–75 percent of patients with hereditary hemochromatosis (1), a disorder of abnormal iron absorption resulting in the progressive accumulation of iron in the liver, heart, pancreas, and other organs. In hemochromatosis, the initial glucose abnormalities include insulin resistance and hyperinsulinemia, followed by impaired insulin secretion, which may be a result of iron deposition in pancreatic beta cells (4). The precise molecular mechanisms underlying the pathogenesis of iron-overload-related diabetes have not been identified. Iron overload may induce insulin resistance by reducing insulin extraction in the liver (20–23) or by reducing insulin synthesis and excretion by the pancreatic beta cells (4, 24). Iron-mediated oxidation of free fatty acids may also lead to reduced glucose utilization by muscle tissue, which would result in insulin resistance (9, 25).

Moderately elevated iron stores and plasma ferritin levels, below the levels of hemochromatosis, are also associated with insulin resistance, diabetes, and the metabolic syndrome in cross-sectional studies (2–6, 9, 10, 26). Our findings are consistent with these studies, as we observed strong associations between baseline plasma ferritin and some components of the metabolic syndrome, primarily fasting glucose and insulin levels. Cross-sectional studies, however,

cannot determine whether altered iron metabolism is a cause or a consequence of altered insulin metabolism, or whether both abnormalities result from a third, independent cause.

In a prospective study in Finnish men, Salonen et al. (11) identified an increased risk of diabetes among participants with higher ferritin levels, but the small size of the study (41 cases and 82 nondiabetic controls) complicates interpretation of the findings. A prospective case-control study nested within the Nurses' Health Study cohort (12) provided stronger evidence of a positive relation between plasma ferritin level and incident diabetes, in sharp contrast to our null findings in the ARIC cohort. The Nurses' Health Study enrolled middle-aged professional women, mostly White, while our population included a community-based sample of African-American and White men and women from four geographic locations. Ferritin levels among women in the two studies were similar (63.4 ng/ml in women the ARIC cohort random-sample participants vs. 71.5 ng/ml in the Nurses' Health Study control participants), suggesting that the discrepancies did not result from different levels of exposure. Furthermore, when we restricted the analyses to White women ($n = 364$; 156 diabetes cases and 208 non-cases), the hazard ratio for diabetes associated with a fivefold increase in ferritin level, adjusted for age, menopausal status, smoking, drinking, and body mass index, was 1.14 (95 percent CI: 0.85, 1.53). After further adjustment for metabolic syndrome components, the hazard ratio was 0.94 (95 percent CI: 0.70, 1.20).

In the ARIC Study, the association of ferritin with incident diabetes disappeared after adjustment for metabolic syndrome components. In the Nurses' Health Study, fasting samples were not available for all study participants, and it is uncertain how the risk estimates from this study would change after adjustment for baseline metabolic abnormalities, especially fasting glucose level. Investigators in the
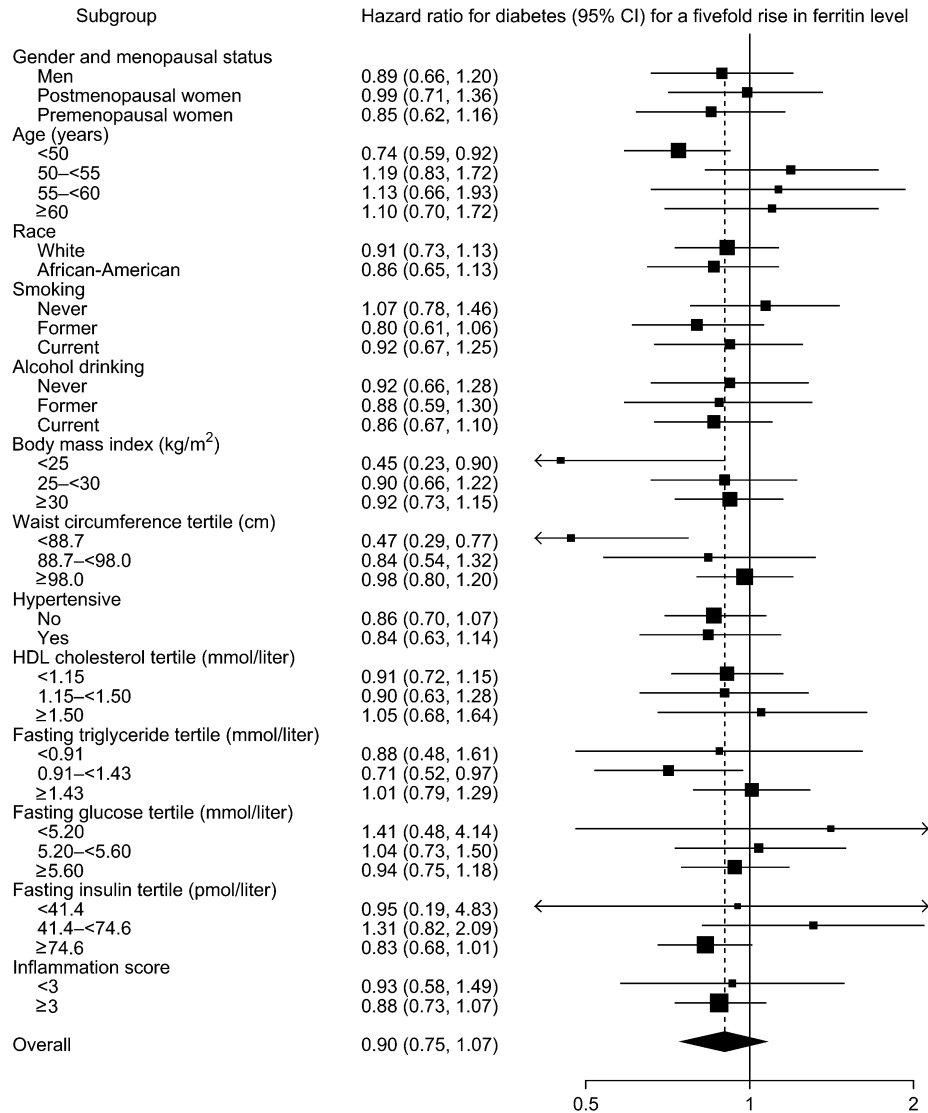
Subgroup	Hazard ratio for diabetes (95% CI) for a fivefold rise in ferritin level
Gender and menopausal status	
Men	0.89 (0.66, 1.20)
Postmenopausal women	0.99 (0.71, 1.36)
Premenopausal women	0.85 (0.62, 1.16)
Age (years)	
<50	0.74 (0.59, 0.92)
50–<55	1.19 (0.83, 1.72)
55–<60	1.13 (0.66, 1.93)
≥60	1.10 (0.70, 1.72)
Race	
White	0.91 (0.73, 1.13)
African-American	0.86 (0.65, 1.13)
Smoking	
Never	1.07 (0.78, 1.46)
Former	0.80 (0.61, 1.06)
Current	0.92 (0.67, 1.25)
Alcohol drinking	
Never	0.92 (0.66, 1.28)
Former	0.88 (0.59, 1.30)
Current	0.86 (0.67, 1.10)
Body mass index (kg/m^2)	
<25	0.45 (0.23, 0.90)
25–<30	0.90 (0.66, 1.22)
≥30	0.92 (0.73, 1.15)
Waist circumference tertile (cm)	
<88.7	0.47 (0.29, 0.77)
88.7–<98.0	0.84 (0.54, 1.32)
≥98.0	0.98 (0.80, 1.20)
Hypertensive	
No	0.86 (0.70, 1.07)
Yes	0.84 (0.63, 1.14)
HDL cholesterol tertile (mmol/liter)	
<1.15	0.91 (0.72, 1.15)
1.15–<1.50	0.90 (0.63, 1.28)
≥1.50	1.05 (0.68, 1.64)
Fasting triglyceride tertile (mmol/liter)	
<0.91	0.88 (0.48, 1.61)
0.91–<1.43	0.71 (0.52, 0.97)
≥1.43	1.01 (0.79, 1.29)
Fasting glucose tertile (mmol/liter)	
<5.20	1.41 (0.48, 4.14)
5.20–<5.60	1.04 (0.73, 1.50)
≥5.60	0.94 (0.75, 1.18)
Fasting insulin tertile (pmol/liter)	
<41.4	0.95 (0.19, 4.83)
41.4–<74.6	1.31 (0.82, 2.09)
≥74.6	0.83 (0.68, 1.01)
Inflammation score	
<3	0.93 (0.58, 1.49)
≥3	0.88 (0.73, 1.07)
Overall	0.90 (0.75, 1.07)

FIGURE 1. Hazard ratio for type 2 diabetes mellitus associated with a fivefold increase in plasma ferritin level for selected subgroups of case-cohort study participants, Atherosclerosis Risk in Communities Study, 1987–1990. All hazard ratio estimates were adjusted for the other variables shown in the figure. The squares are inversely proportional to the variance of the log hazard ratios. The horizontal lines show the 95% confidence intervals (CIs). HDL, high density lipoprotein.

ARIC Study and the Nurses' Health Study also used different methods to diagnose incident diabetes. In the Nurses' Health Study, diabetes diagnosis was based on self-report, while in ARIC, researchers used fasting glucose levels in addition to self-reports. However, when we restricted our analysis to the diabetes cases diagnosed on the basis of self-report (*n* = 251), the diabetes hazard ratios comparing the highest quintile of ferritin with the lowest in models 2 and 3 were 1.56 and 0.88, slightly higher than the corresponding hazard ratios for all cases but unlikely to explain the different findings in ARIC versus the Nurses' Health Study.

While an elevated plasma ferritin level may be a causal factor for diabetes, it is also possible that the moderately increased ferritin levels of diabetes patients are just a marker

for the metabolic alterations that ultimately result in diabetes, without a causal role in diabetes development. In fact, metabolic abnormalities may lead to increased ferritin levels through a variety of mechanisms. Insulin stimulates the expression of ferritin mRNA, an effect that is probably mediated through insulin-like growth factor receptors (27). In certain insulin-sensitive cells, such as adipocytes, receptors for transferrin, glucose, and insulin-like growth factor II colocalize in the cell membrane, and the presence of insulin results in the simultaneous translocation of all three proteins (28). Therefore, it has been hypothesized that insulin-mediated glucose transport may lead to increased transferrin receptors on the cell surface, resulting in increased uptake of extracellular iron (29).

Hyperinsulinemia may also up-regulate hypoxia-inducible transcription factor-1α (30). Hypoxia-inducible transcription factor-1α promotes the synthesis of growth factors, including erythropoietin (30), which may result in increased iron absorption. In addition, ferritin levels may be increased in response to oxidative stress (31–34), to nitric oxide signaling (34–36), and to several inflammatory cytokines (34, 37, 38)—three mechanisms that are involved in the pathogenesis of the metabolic syndrome (39–41). Finally, increased ferritin levels could be a reflection of liver involvement in insulin resistance (42, 43). Ferritin levels are associated with nonalcoholic fatty liver disease (42, 44) and with increased liver fibrosis (45), but the role of hepatic iron excess in the progression of metabolic abnormalities is uncertain. It is well established that iron overload is a cause of insulin resistance and diabetes and that plasma ferritin reflects body iron stores. However, the modest elevations in ferritin levels observed in the metabolic syndrome and diabetes may be the consequence or a marker, rather than the cause, of impending insulin resistance and may not reflect elevated body iron stores.

Our study had several strengths, including the presence of extensive baseline and follow-up data with which to diagnose cases of diabetes and adjust for potential confounders, a large sample size, and the use of a population-based sample from four separate geographic regions, with representation of both genders as well as Caucasians and African Americans. However, several limitations need to be addressed. First, although ferritin is a widely used marker of iron status in epidemiologic studies (1), the physiologic role of plasma ferritin (46) and its relation to the intracellular labile iron pool, which may be responsible for iron-related oxidative damage, is uncertain (47). Other biomarkers of iron, such as non-transferrin-bound iron, may be needed to evaluate the impact of iron on disease development (47). Second, the sampling frame for the present case-cohort study did not include the entire ARIC cohort. Rather, incident diabetes cases and random controls were selected after exclusion of participants whose plasma samples were either exhausted or held in reserve for studies of cardiovascular disease. This sampling strategy could have introduced a selection bias if the ratio of ferritin levels in the excluded diabetes cases compared with the excluded controls was different from that in those eligible for the sampling frame. This could have happened, for instance, if plasma ferritin was associated with cardiovascular disease incidence. However, the accumulated evidence indicates that ferritin levels are not associated with increased cardiovascular disease risk in prospective studies (48, 49). Furthermore, in the ARIC Study, increased ferritin levels did not predict carotid intima-media thickness, a marker of subclinical cardiovascular disease (50). It also seems unlikely that this type of selection bias could explain the change in risk estimates for ferritin after adjustment for metabolic syndrome components.

In summary, our results are consistent with previous research showing that elevated body iron stores confer a moderately increased risk of type 2 diabetes. This increased risk was explained by other metabolic alterations that comprise the insulin resistance–metabolic syndrome. Because of our study design, we cannot identify whether these findings reflect a causal role of elevated iron stores and plasma ferritin in diabetes risk that is mediated through elevated fasting glucose and other metabolic abnormalities, or whether elevated plasma ferritin is one more of the metabolic abnormalities appearing during diabetes development but not a causal factor for diabetes. Longitudinal studies with repeated measurements of glucose and iron metabolism parameters are needed to establish the causal role of iron stores and plasma ferritin levels in diabetes development.

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