

## Retrospective Study

## Association of insulin resistance with serum ferritin and aminotransferases-iron hypothesis

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## Abstract

**AIM:** To investigate the relationship of iron indices with diabetes mellitus (DM) in those without hemochromatosis.

**METHODS:** This cross-sectional study examined data collected during the Third National Health and Nutrition Examination Survey (NHANES III). Only those who fasted properly and were not anemic with transferrin saturation < 45% were included ( $n = 6849$ ). Insulin sensitivity and beta cell function were calculated from fasting glucose and insulin concentrations. Indices of iron metabolism were examined in the presence or absence of DM. We examined the relationship of insulin sensitivity and beta cell function with serum ferritin concentration. The influence of C-reactive protein and liver enzymes was also investigated.

**RESULTS:** Serum ferritin concentration was significantly higher in diabetic subjects ( $P = 0.0001$  to  $< 0.000001$ ). The difference remained significant after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement ( $P = 0.03$  to  $< 0.000001$ ). In those who did not take insulin, serum ferritin concentration was negatively associated with insulin sensitivity ( $P = 0.05$  to  $0.00001$ ), but not with beta cell function. The alanine aminotransferase was correlated with serum ferritin concentration ( $P = 0.02$  to  $< 0.000001$ ) but not with insulin sensitivity, suggesting the role of the liver in iron-associated insulin resistance.

**CONCLUSION:** As most of diabetes is type 2 diabetes and insulin resistance is a cardinal feature of type 2 diabetes, disordered iron metabolism could play a role in the pathogenesis of insulin resistance and type 2 diabetes through its effect on liver function.

**Key words:** Diabetes mellitus; Insulin sensitivity; Beta cell function; Ferritin; Liver

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**Core tip:** Hemochromatosis and excess iron load has been implicated to play a role in the pathogenesis of diabetes mellitus. Serum ferritin concentration was significantly higher in diabetic subjects. Serum ferritin concentration was negatively associated with insulin sensitivity, but not with beta cell function. The association of alanine aminotransferase correlated with serum ferritin concentration, but not insulin sensitivity, suggesting the role of the liver in iron-associated insulin resistance. Disordered iron metabolism could play a role in the pathogenesis of insulin resistance and type 2 diabetes mellitus through its effect on liver function.

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## INTRODUCTION

Diabetes mellitus (DM) is a common manifestation (53%-80%) of hereditary hemochromatosis<sup>[1]</sup>, which is an autosomal recessive disorder caused by mutations in a gene designated HFE (OMIM: 235200). A mutation, C282Y, was detected in 83% of the patients, while it was only found in 3.2% of control chromosomes<sup>[2]</sup>. However, the allelic frequencies of C282Y mutation were similar between diabetic and control groups (6.3% vs 5.5%) in a large population from the United Kingdom<sup>[3]</sup>. Meta-analysis of published studies showed no evidence for over-representation of this allele in patients with type 2 diabetes<sup>[3]</sup>. Therefore, the C282Y mutation does not play a role in the pathogenesis of type 2 diabetes. Nevertheless, the role of iron metabolism in the pathogenesis of diabetes in the general population has been suggested in many cross-sectional studies<sup>[4-7]</sup>. Furthermore, a nested case-control study suggested a potential interaction between the HFE genotypes and heme iron in relation to the risk of type 2 diabetes<sup>[8]</sup>.

In hereditary hemochromatosis, both insulin resistance and impaired insulin secretion have been suggested to play a role in its pathogenesis<sup>[9]</sup>. The role of insulin resistance in patients with secondary hemochromatosis from thalassemia major has been reported, while an additional defect in beta cell secretion cannot be excluded<sup>[10]</sup>. The association of serum ferritin concentration and insulin resistance has been reported in various liver diseases<sup>[11,12]</sup>. Furthermore, the underlying mechanism of iron-associated abnormal glucose homeostasis in the general population is not well understood.

To examine the role of iron in the pathogenesis of diabetes, we investigated the iron indices and the relative influence of an inflammatory marker and liver enzymes on glucose homeostasis in a nationally representative survey, third National Health and Nutrition Examination Survey (NHANES III).

## MATERIALS AND METHODS

### Ethics statement

The National Center for Health Statistics of the Centers for Disease Control and Prevention conducted the NHANES III in the United States from 1988 through 1994. This survey was designed to assess the health and nutrition status of a large representative sample in the United States. The survey and data collection was approved by the NHANES Institutional Review Board and documented consent was obtained from participants. Analysis of de-identified data from the survey is exempt from the federal regulations for the protection of human research participants. Only de-identified data from the survey was used in this study.

**Study design and study sample**

Detailed descriptions of the survey and the analytical methods of various assays have been published<sup>[13]</sup> and are also available at its website (<http://www.cdc.gov/nchs/about/major/nhanes/datalink.htm#NHANESIII>).

Race and ethnicity were self-reported by the participants. NHANES III was designed to provide reliable information from three major racial/ethnic groups: Non-Hispanic whites (NHW), non-Hispanic blacks (NHB), and Hispanics. The 4<sup>th</sup> group was excluded from this analysis for its small sample size and for encompassing a heterogeneous racial/ethnic group. There were 15021 subjects who had serum ferritin, fasting glucose and insulin concentration measured. Proper fasting is required to define diabetes status and to calculate insulin sensitivity and beta cell function from the fasting samples<sup>[14,15]</sup>. Only those who fasted for  $\geq 8$  h and  $\leq 16$  h were included ( $n = 7701$ ). We excluded 180 subjects with hemoglobin  $< 11$  g/dL, which is frequently associated with iron deficiency and falsely low HbA<sub>1c</sub>. Since hemochromatosis is an established cause of diabetes, those with transferrin saturation  $\geq 45$  were also excluded<sup>[16]</sup> ( $n = 672$ ), which identified 98% of iron-overloaded subjects<sup>[17]</sup>.

**Ascertainment of DM**

Diabetes was defined as a fasting glucose concentration  $\geq 126$  mg/dL (7.0 mmol/L) or a 2-h postchallenged glucose concentration  $\geq 200$  mg/dL (11.1 mmol/L)<sup>[14]</sup>. Without the 2-h postchallenged glucose concentration, the diagnosis of diabetes is frequently missed in those with elevated 2-h postchallenged glucose concentrations and normal fasting glucose concentrations<sup>[18]</sup>. However, only 3010 subjects had 2-h postchallenged glucose concentration measured. Their HbA<sub>1c</sub> was very well correlated with 2-h postchallenged glucose concentration ( $r = 0.7558$ ,  $P < 0.000001$ ) and 2-h postchallenged glucose concentration of 200 mg/dL (11.1 mmol/L) was equivalent to HbA<sub>1c</sub> of 6.3%. Therefore, we also defined diabetes in those with HbA<sub>1c</sub>  $\geq 6.3\%$ .

**Calculation of beta cell function and insulin sensitivity**

Beta cell function (%B) and insulin sensitivity (%S) were calculated based on the homeostasis model assessment (HOMA)<sup>[15,19]</sup>.

$$\%B = (20 \times \text{fasting insulin concentration in mU/L}) / (\text{fasting glucose concentration in mmol/L} - 3.5).$$

$$\%S = 22.5 / (\text{fasting insulin concentration in mU/L} \times \text{fasting glucose concentration in mmol/L}).$$

Those with fasting glucose concentration  $< 3.5$  mmol/L were excluded from analysis, since they had negative %B ( $n = 9$ ). %B and %S obtained from the HOMA had been shown to correlate very well with the measured beta cell function and insulin sensitivity from various methods<sup>[15,20-22]</sup>. A quantitative insulin

sensitivity check index (QUICKI), which had been shown to correlate with the measured insulin sensitivity by hyperinsulinemic euglycemic clamp very well<sup>[23]</sup>, was also used.

$$\text{QUICKI} = 1 / [\log_{10}(\text{fasting insulin concentration in mU/L}) + \log_{10}(\text{fasting glucose concentration in mg/dL})].$$

All of these methods have been validated in both non-diabetic subjects and diabetic subjects who did not take insulin<sup>[21-23]</sup>. Those who took insulin were excluded from these analyses ( $n = 51$ ).

**Statistical analysis**

General descriptive variables were expressed as means  $\pm$  SD. Since gender and ethnicity could potentially affect both iron metabolism and glucose homeostasis, the data were analyzed separately by gender and ethnic groups. Continuous variables were compared using two-tail Student *t* test between two groups or Analysis of Variance for more than two groups. Continuous data were expressed as means with 95%CI. Analysis of variance was used to examine the influence of covariates [age and body mass index (BMI)] on continuous variables between two groups. Least square regression analysis was used to investigate the relationship between two continuous variables. The influence of covariates (age, BMI, alcohol consumption, and mineral/iron intake) was also accounted for least square regression analysis. To further assess the association of serum ferritin concentration with estimated beta function and insulin sensitivity indices as well as the association of liver aminotransferases and C-reactive protein (CRP) with serum ferritin concentration and estimated insulin sensitivity indices, we also examine the trend across the quintile of serum ferritin concentration, liver aminotransferases and CRP. The comparisons were also adjusted for age, BMI, alcohol consumption, and mineral/iron intake. All the analyses were conducted in SYSTAT 11, Systat Software, Inc., Point Richmond, California, United States. A *P* value less than 0.05 was considered significant.

**RESULTS****Study populations**

The clinical features of the studied subjects were shown by gender and ethnic groups in Table 1. Based on previously published upper reference ranges<sup>[24]</sup>, in male participants, 8.2% had elevated aspartate aminotransferase (AST  $> 37$  U/L) and 9.6% had elevated alanine aminotransferase (ALT  $> 40$  U/L) and in female participants, 7.7% had elevated AST ( $> 37$  U/L) and 7.1% had elevated ALT ( $> 37$  U/L).

**Comparison of indices of iron metabolism in the presence or absence of diabetes**

Iron, total iron binding capacity (TIBC), transferrin saturation, and serum ferritin concentration were

**Table 1 Clinical features of studied subjects**

	Non-Hispanic whites		Non-Hispanic blacks		Hispanics	
	Male	Female	Male	Female	Male	Female
<i>n</i>	1373	1602	896	1057	957	964
Age (yr)	55 ± 19	54 ± 20	43 ± 17	42 ± 16	42 ± 17	41 ± 17
Systolic blood pressure (mmHg)	130 ± 18	125 ± 21	128 ± 18	122 ± 20	125 ± 17	120 ± 20
Diastolic blood pressure (mmHg)	76 ± 10	72 ± 9	79 ± 11	74 ± 11	76 ± 10	71 ± 10
Body mass index (kg/m <sup>2</sup> )	26.75 ± 4.63	26.39 ± 5.80	26.74 ± 5.34	29.07 ± 7.22	27.10 ± 4.64	28.26 ± 5.97
Transferrin saturation (%)	27.93 ± 8.39	24.58 ± 8.78	26.22 ± 8.11	21.75 ± 8.51	28.23 ± 8.59	22.95 ± 8.98
Ferritin (mcg/L)	176 ± 142	95 ± 104	215 ± 170	93 ± 111	169 ± 142	67 ± 95
Aspartate aminotransferase (U/L)	22 ± 8	19 ± 8	25 ± 17	22 ± 8	27 ± 16	23 ± 19
Alanine aminotransferase (U/L)	18 ± 11	14 ± 10	19 ± 13	18 ± 11	28 ± 24	21 ± 23
Gamma glutamyl transferase (U/L)	35 ± 55	21 ± 20	46 ± 59	35 ± 55	46 ± 53	30 ± 31
C-reactive protein (mg/dL)	0.43 ± 0.76	0.45 ± 0.62	0.48 ± 0.79	0.53 ± 0.65	0.44 ± 0.84	0.53 ± 0.80

Data presented mean ± SD.

**Table 2 Comparison of serum indices of iron by the presence or absence of diabetes mellitus**

	Non-Hispanic whites				Non-Hispanic blacks				Hispanics			
	Male		Female		Male		Female		Male		Female	
	DM	Non-DM	DM	Non-DM	DM	Non-DM	DM	Non-DM	DM	Non-DM	DM	Non-DM
<i>n</i>	166	1207	137	1465	98	798	106	951	115	842	132	832
Iron (mcg/dL)												
Mean	93	95	83	87	82	87	70	75	90	98	82	84
(95%CI)	(89, 97)	(94, 97)	(78, 88)	(86, 89)	(77, 87)	(85, 89)	(64, 75)	(74, 77)	(84, 97)	(96, 100)	(76, 87)	(82, 86)
<i>P</i> <sup>1</sup>	NS		NS		NS		NS		0.01		NS	
<i>P</i> <sup>2</sup>	NS		NS		NS		NS		NS		NS	
<i>P</i> <sup>3</sup>	NS		NS		NS		NS		NS		NS	
Total iron binding capacity (mcg/dL)												
Mean	351	344	359	360	333	334	342	353	353	354	362	377
(95%CI)	(343, 359)	(341, 346)	(350, 368)	(357, 363)	(322, 344)	(330, 337)	(331, 352)	(349, 356)	(343, 363)	(351, 358)	(351, 373)	(372, 381)
<i>P</i> <sup>1</sup>	NS		NS		NS		NS		NS		0.01	
<i>P</i> <sup>2</sup>	0.002		NS		NS		NS		NS		NS	
<i>P</i> <sup>3</sup>	NS		NS		NS		NS		NS		NS	
Transferrin saturation (%)												
Mean	27	28	23	25	25	26	21	22	26	28	23	23
(95%CI)	(26, 28)	(28, 29)	(22, 25)	(24, 25)	(23, 26)	(26, 27)	(19, 22)	(21, 22)	(24, 28)	(27, 28)	(22, 25)	(22, 23)
<i>P</i> <sup>1</sup>	NS		NS		NS		NS		0.03		NS	
<i>P</i> <sup>2</sup>	NS		NS		NS		NS		NS		NS	
<i>P</i> <sup>3</sup>	NS		NS		NS		NS		NS		NS	
Ferritin (mcg/L)												
Mean	228	169	152	84	282	206	167	86	230	150	138	56
(95%CI)	(200, 256)	(161, 177)	(129, 175)	(79, 89)	(237, 327)	(195, 218)	(139, 195)	(79, 92)	(190, 270)	(142, 159)	(104, 171)	(52, 60)
<i>P</i> <sup>1</sup>	< 0.000001		< 0.000001		0.00003		< 0.000001		< 0.000001		< 0.000001	
<i>P</i> <sup>2</sup>	0.0001		0.000003		0.02		0.0001		0.00001		< 0.000001	
<i>P</i> <sup>3</sup>	0.0002		0.000003		0.03		0.0001		0.000004		< 0.000001	

Data presented mean with 95%CI. To convert values for iron and total iron binding capacity to mol/L, multiply by 0.1791. <sup>1</sup>*P* values for unadjusted comparison; <sup>2</sup>*P* values for comparison after adjustment for age and body mass index; <sup>3</sup>*P* values for comparison after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement. NS: Not significant (*P* > 0.05); DM: Diabetes mellitus; NDM: Non-diabetic mellitus.

compared between those with or without diabetes (Table 2). No consistent results were observed for iron, TIBC, and transferrin saturation, while serum ferritin concentration was markedly higher in diabetic than in non-diabetic subjects in all six groups. Diabetic subjects were older than non-diabetic subjects by 14-16 years (*P* < 0.000001) and were also more obese than non-diabetic subjects per BMI by 2.40-4.81 kg/m<sup>2</sup> (*P* < 0.000001). The difference in ferritin concentration between diabetic and non-diabetic subjects remained significant after adjustment of age, BMI, alcohol intake, and mineral/iron supplement. Thus, diabetes was

associated with elevated serum ferritin concentration.

### **Association of serum ferritin concentration with beta cell function and insulin sensitivity**

Diabetes results from an imbalance between beta cell function and insulin sensitivity. Thus, serum ferritin concentration could potentially be associated with either beta cell function, insulin sensitivity, or both. No association between %B and serum ferritin concentration was found after adjustment for both age and BMI (Table 3). In contrast, serum ferritin concentration was negatively associated with %S and



**Table 3** Estimated beta cell function and insulin sensitivity indices by quintile of serum ferritin concentrations

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P <sup>1</sup>	P <sup>2</sup>	P <sup>3</sup>
Beta cell function by the homeostasis model assessment (%B) <sup>4</sup>								
Non-Hispanic white males	103 (96, 110)	98 (92, 105)	97 (90, 104)	106 (94, 118)	113 (102, 124)	NS	NS	NS
Non-Hispanic white females	117 (110, 124)	115 (108, 122)	109 (103, 114)	111 (104, 119)	110 (102, 118)	NS	NS	NS
Non-Hispanic black males	113 (104, 122)	118 (107, 130)	112 (103, 120)	144 (107, 181)	117 (106, 129)	NS	NS	NS
Non-Hispanic black females	177 (156, 197)	178 (161, 196)	151 (137, 164)	135 (121, 150)	135 (123, 147)	0.000001	NS	NS
Hispanic males	104 (91, 118)	112 (101, 124)	106 (97, 114)	123 (106, 141)	128 (116, 140)	0.004	NS	NS
Hispanic females	152 (140, 165)	150 (135, 164)	140 (129, 150)	136 (124, 147)	130 (119, 141)	0.003	NS	NS
Insulin sensitivity by the homeostasis model assessment (%S) <sup>5</sup>								
Non-Hispanic white males	0.503 (0.472, 0.535)	0.549 (0.513, 0.585)	0.518 (0.484, 0.553)	0.494 (0.463, 0.525)	0.421 (0.388, 0.455)	0.00005	NS	0.05
Non-Hispanic white females	0.643 (0.610, 0.677)	0.603 (0.570, 0.637)	0.574 (0.539, 0.609)	0.555 (0.519, 0.591)	0.455 (0.420, 0.491)	< 0.000001	0.04	0.02
Non-Hispanic black males	0.562 (0.516, 0.608)	0.553 (0.504, 0.601)	0.559 (0.512, 0.605)	0.555 (0.498, 0.611)	0.48 (0.426, 0.535)	0.04	NS	0.05
Non-Hispanic black females	0.510 (0.476, 0.545)	0.479 (0.437, 0.522)	0.453 (0.421, 0.485)	0.458 (0.422, 0.495)	0.379 (0.343, 0.414)	0.000001	0.02	0.007
Hispanic males	0.538 (0.495, 0.580)	0.533 (0.489, 0.578)	0.527 (0.472, 0.583)	0.524 (0.460, 0.588)	0.338 (0.307, 0.370)	< 0.000001	0.01	0.007
Hispanic females	0.523 (0.482, 0.563)	0.535 (0.484, 0.587)	0.464 (0.426, 0.502)	0.444 (0.403, 0.485)	0.321 (0.289, 0.352)	< 0.000001	0.005	0.003
Insulin sensitivity by the simple QUICKI <sup>6</sup>								
Non-Hispanic white males	0.339 (0.336, 0.342)	0.343 (0.340, 0.346)	0.340 (0.336, 0.343)	0.337 (0.334, 0.341)	0.327 (0.323, 0.331)	< 0.000001	0.008	0.002
Non-Hispanic white females	0.352 (0.349, 0.355)	0.348 (0.345, 0.351)	0.345 (0.342, 0.348)	0.343 (0.340, 0.346)	0.33 (0.327, 0.334)	< 0.000001	0.00005	0.00001
Non-Hispanic black males	0.344 (0.339, 0.348)	0.342 (0.338, 0.347)	0.343 (0.339, 0.348)	0.341 (0.335, 0.346)	0.333 (0.328, 0.338)	0.003	0.03	0.01
Non-Hispanic black females	0.340 (0.336, 0.343)	0.335 (0.331, 0.339)	0.333 (0.329, 0.337)	0.333 (0.329, 0.337)	0.322 (0.317, 0.326)	< 0.000001	0.0006	0.0001
Hispanic males	0.341 (0.336, 0.345)	0.34 (0.336, 0.345)	0.339 (0.335, 0.344)	0.337 (0.332, 0.342)	0.318 (0.314, 0.322)	< 0.000001	0.0001	0.00001
Hispanic females	0.340 (0.336, 0.344)	0.340 (0.336, 0.345)	0.334 (0.330, 0.338)	0.330 (0.326, 0.335)	0.315 (0.310, 0.319)	< 0.000001	0.00003	0.00002

Data presented mean with 95%CI. <sup>1</sup>P values for trend, unadjusted; <sup>2</sup>P values for trend, after adjustment for age and body mass index; <sup>3</sup>P values for trend, after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement; <sup>4</sup>%B = (20 × fasting insulin concentration in mU/L) / (fasting glucose concentration in mmol/L - 3.5). Those with negative %B were excluded from analysis; <sup>5</sup>%S = 22.5/(fasting insulin concentration in mU/L × fasting glucose concentration in mmol/L); <sup>6</sup>Quicki = 1/[log(fasting glucose concentration in mg/dL) + log(fasting insulin concentration in mU/L)]. NS: Not significant; QUICKI: Quantitative insulin sensitivity check index.

QUICKI in all six groups. This relationship persisted after adjustment for age, BMI, alcohol consumption, and mineral/iron supplement. Therefore, we concluded that ferritin concentration was negatively associated with insulin sensitivity.

#### **Role of inflammation in association between serum ferritin concentration and insulin sensitivity**

In addition to reflection of the body iron store, serum ferritin is also an acute reactant. To explore the role of inflammation on the observed correlation, we examined the relationship of these indices with a marker of inflammation, CRP. No consistent association of CRP with either %S or QUICKI was observed (Table 4). A positive association between CRP and serum ferritin concentration was observed in NHW (both males and females), non-Hispanic black females, and Hispanic

females, but not in non-Hispanic black. The associations remained unchanged after adjustment for age, BMI, alcohol intake, and mineral/iron supplement. Therefore, inflammation could not provide a uniform explanation for the underlying mechanism of the observed correlation between serum ferritin concentration and insulin sensitivity.

#### **Role of the liver in association between serum ferritin concentration and insulin sensitivity**

Elevated liver enzymes could result from iron deposition in the liver. To examine the role of the liver in the association of serum ferritin concentration with insulin sensitivity, we investigated the association of liver enzymes with serum ferritin concentration and insulin sensitivity. We focused on AST or SGOT, gamma glutamyl transpeptidase (GGT), and ALT or SGPT.

**Table 4 Serum ferritin concentration and estimated insulin sensitivity indices by quintile of inflammatory marker - C-reactive protein**

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P <sup>1</sup>	P <sup>2</sup>	P <sup>3</sup>
Serum ferritin concentration (mcg/L)								
Non-Hispanic white males	142 (130, 155)	157 (144, 170)	179 (164, 195)	201 (180, 222)	201 (181, 221)	< 0.000001	0.000002	0.000005
Non-Hispanic white females	19 (18, 20)	49 (48, 50)	120 (116, 125)	152 (134, 171)	108 (95, 120)	0.000003	0.00003	0.00003
Non-Hispanic black males	186 (163, 209)	187 (167, 207)	267 (236, 298)	227 (202, 253)	254 (281, 228)	NS	NS	NS
Non-Hispanic black females	90 (74, 106)	79 (66, 91)	77 (65, 89)	102 (87, 118)	115 (98, 132)	0.002	0.0009	0.001
Hispanic males	150 (134, 166)	157 (135, 179)	161 (137, 185)	157 (136, 178)	172 (154, 191)	NS	NS	NS
Hispanic females	52 (44, 60)	56 (48, 64)	63 (53, 72)	72 (60, 84)	92 (68, 115)	0.00001	0.007	0.006
Insulin sensitivity by the homeostasis model assessment (%S) <sup>4</sup>								
Non-Hispanic white males	0.541 (0.505, 0.578)	0.531 (0.500, 0.562)	0.546 (0.509, 0.582)	0.459 (0.425, 0.494)	0.412 (0.383, 0.440)	< 0.000001	0.05	0.03
Non-Hispanic white females	0.684 (0.651, 0.718)	0.627 (0.592, 0.661)	0.596 (0.558, 0.634)	0.494 (0.461, 0.526)	0.422 (0.392, 0.452)	< 0.000001	0.004	0.004
Non-Hispanic black males	0.562 (0.517, 0.607)	0.572 (0.526, 0.618)	0.561 (0.499, 0.623)	0.549 (0.488, 0.611)	0.478 (0.426, 0.530)	0.05	NS	NS
Non-Hispanic black females	0.524 (0.489, 0.558)	0.524 (0.487, 0.560)	0.497 (0.464, 0.530)	0.404 (0.369, 0.438)	0.327 (0.288, 0.365)	< 0.000001	0.004	0.002
Hispanic males	0.558 (0.507, 0.608)	0.484 (0.438, 0.530)	0.525 (0.472, 0.578)	0.513 (0.460, 0.566)	0.372 (0.334, 0.411)	0.00001	NS	NS
Hispanic females	0.538 (0.495, 0.582)	0.53 (0.481, 0.579)	0.471 (0.430, 0.513)	0.392 (0.355, 0.429)	0.353 (0.319, 0.387)	< 0.000001	NS	NS
Insulin sensitivity by the simple QUICKI <sup>5</sup>								
Non-Hispanic white males	0.342 (0.339, 0.346)	0.342 (0.339, 0.345)	0.342 (0.339, 0.346)	0.332 (0.328, 0.336)	0.328 (0.325, 0.331)	< 0.000001	0.01	0.008
Non-Hispanic white females	0.356 (0.354, 0.359)	0.351 (0.348, 0.354)	0.347 (0.343, 0.350)	0.336 (0.333, 0.340)	0.328 (0.324, 0.331)	< 0.000001	0.004	0.005
Non-Hispanic black males	0.345 (0.341, 0.349)	0.345 (0.340, 0.349)	0.341 (0.336, 0.346)	0.34 (0.334, 0.345)	0.332 (0.327, 0.338)	0.01	NS	NS
Non-Hispanic black females	0.341 (0.337, 0.345)	0.341 (0.337, 0.345)	0.339 (0.335, 0.342)	0.327 (0.323, 0.331)	0.314 (0.310, 0.319)	< 0.000001	0.000002	0.000001
Hispanic males	0.342 (0.338, 0.347)	0.335 (0.331, 0.340)	0.338 (0.333, 0.343)	0.338 (0.333, 0.342)	0.321 (0.317, 0.326)	< 0.000001	NS	NS
Hispanic females	0.342 (0.338, 0.346)	0.34 (0.336, 0.344)	0.334 (0.329, 0.338)	0.325 (0.321, 0.329)	0.319 (0.314, 0.323)	< 0.000001	0.02	0.03

Data presented mean with 95%CI. <sup>1</sup>P values for trend, unadjusted; <sup>2</sup>P values for trend, after adjustment for age and body mass index; <sup>3</sup>P values for trend, after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement; <sup>4</sup>%S = 22.5/(fasting insulin concentration in mU/L × fasting glucose concentration in mmol/L); <sup>5</sup>Quicki = 1/[log(fasting glucose concentration in mg/dL) + log(fasting insulin concentration in mU/L)]. NS: Not significant; QUICKI: Quantitative insulin sensitivity check index.

The association of AST with %S and QUICKI was only noted in Hispanic males, but not in other 5 groups (Table 5). Adjustment for age, BMI, alcohol consumption, and mineral/iron supplement had no impact on the results. In contrast, a very close association was noted between AST and ferritin concentration in all 6 groups. Since AST is present in many tissues, including heart, skeletal muscle, kidney, and brain, we could not exclude the role of the liver based on no association between AST and insulin sensitivity in some groups.

GGT is a very sensitive indicator of hepatobiliary diseases and is found predominately throughout the hepatobiliary system, but also in other tissues. It was negatively associated with %S and QUICKI in all 6 groups (Table 6). The association remained after adjustment for age, BMI, alcohol consumption, and mineral/iron supplement. It was positively associated with serum ferritin concentration after adjustment for

age and BMI. Therefore, the relationship of GGT with insulin sensitivity and serum ferritin concentration could provide a mechanistic insight of the liver in the association between insulin sensitivity and serum ferritin concentration.

The primary source of ALT is the liver. It was negatively associated with both %S and QUICKI in all six groups, and remained highly significant regardless of the adjustment for age, BMI, alcohol consumption, and mineral/iron supplement (Table 7). This relationship indicated an association of insulin resistance and liver diseases. Serum ferritin concentration was positively associated with ALT and also this association remained significant regardless of adjustment for age, BMI, alcohol consumption, and mineral/iron supplement. Since ALT is an indicator of liver diseases, a positive association between ALT and serum ferritin concentration suggests that increased iron deposition

**Table 5** Serum ferritin concentration and estimated insulin sensitivity indices by quintile of aspartate aminotransferase

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P <sup>1</sup>	P <sup>2</sup>	P <sup>3</sup>
Serum ferritin concentration(mcg/L)								
Non-Hispanic white males	148 (134, 162)	154 (140, 167)	180 (165, 196)	170 (155, 186)	228 (205, 251)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	58 (51, 65)	61 (55, 68)	85 (76, 94)	102 (91, 112)	140 (122, 158)	0.0004	0.02	0.02
Non-Hispanic black males	175 (155, 196)	203 (178, 228)	197 (173, 222)	227 (203, 251)	267 (236, 298)	0.05	0.003	0.004
Non-Hispanic black females	84 (71, 96)	77 (66, 88)	80 (67, 92)	95 (81, 109)	127 (106, 149)	0.00001	0.004	0.005
Hispanic males	139 (124, 153)	134 (118, 151)	149 (134, 163)	155 (132, 177)	223 (194, 251)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	47 (39, 56)	49 (42, 55)	59 (50, 68)	79 (57, 101)	101 (86, 115)	< 0.000001	0.00001	0.00002
Insulin sensitivity by the homeostasis model assessment (%S) <sup>4</sup>								
Non-Hispanic white males	0.491 (0.457, 0.526)	0.517 (0.484, 0.550)	0.497 (0.466, 0.528)	0.506 (0.471, 0.541)	0.478 (0.441, 0.514)	NS	NS	NS
Non-Hispanic white females	0.575 (0.544, 0.607)	0.597 (0.559, 0.630)	0.591 (0.554, 0.628)	0.555 (0.521, 0.588)	0.506 (0.470, 0.543)	0.02	NS	NS
Non-Hispanic black males	0.512 (0.465, 0.559)	0.531 (0.479, 0.584)	0.528 (0.486, 0.569)	0.557 (0.508, 0.605)	0.561 (0.499, 0.623)	NS	NS	NS
Non-Hispanic black females	0.447 (0.410, 0.484)	0.452 (0.418, 0.487)	0.472 (0.437, 0.506)	0.461 (0.424, 0.499)	0.443 (0.401, 0.485)	NS	NS	NS
Hispanic males	0.501 (0.460, 0.542)	0.55 (0.495, 0.604)	0.524 (0.473, 0.574)	0.477 (0.424, 0.530)	0.404 (0.359, 0.449)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	0.472 (0.430, 0.514)	0.48 (0.443, 0.516)	0.468 (0.430, 0.507)	0.49 (0.442, 0.539)	0.374 (0.330, 0.418)	0.006	NS	NS
Insulin sensitivity by the simple QUICKI <sup>5</sup>								
Non-Hispanic white males	0.337 (0.333, 0.340)	0.34 (0.337, 0.343)	0.338 (0.335, 0.341)	0.338 (0.334, 0.342)	0.334 (0.330, 0.338)	NS	NS	NS
Non-Hispanic white females	0.346 (0.343, 0.349)	0.347 (0.344, 0.350)	0.347 (0.343, 0.350)	0.343 (0.340, 0.346)	0.336 (0.332, 0.340)	0.009	NS	NS
Non-Hispanic black males	0.337 (0.332, 0.342)	0.339 (0.334, 0.344)	0.341 (0.337, 0.345)	0.343 (0.338, 0.348)	0.3417 (0.336, 0.346)	NS	NS	NS
Non-Hispanic black females	0.331 (0.327, 0.335)	0.332 (0.328, 0.336)	0.335 (0.332, 0.339)	0.333 (0.329, 0.337)	0.33 (0.326, 0.334)	NS	NS	NS
Hispanic males	0.337 (0.333, 0.342)	0.342 (0.337, 0.346)	0.338 (0.333, 0.343)	0.333 (0.328, 0.338)	0.325 (0.320, 0.329)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	0.334 (0.329, 0.338)	0.336 (0.332, 0.340)	0.334 (0.330, 0.338)	0.335 (0.331, 0.340)	0.32 (0.315, 0.325)	0.0001	NS	NS

Data presented mean with 95%CI. <sup>1</sup>P values for trend, unadjusted; <sup>2</sup>P values for trend, after adjustment for age and body mass index; <sup>3</sup>P values for trend, after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement; <sup>4</sup>%S = 22.5/(fasting insulin concentration in mU/L × fasting glucose concentration in mmol/L); <sup>5</sup>Quicki = 1/[log(fasting glucose concentration in mg/dL) + log(fasting insulin concentration in mU/L)]. NS: Not significant; QUICKI: Quantitative insulin sensitivity check index.

in the liver is associated with liver dysfunction. Furthermore, a positive association between ALT and serum ferritin concentration and a negative association between ALT and insulin sensitivity suggests a negative association of serum ferritin concentration with insulin sensitivity as we had observed.

## DISCUSSION

To examine the role of iron metabolism in diabetes, the indices of iron metabolism were compared in patients with and without diabetes. We found that subjects with diabetes had a higher serum ferritin concentration than those without diabetes. To explore the underlying pathophysiology, we observed that serum ferritin concentration was negatively associated with insulin sensitivity (%S and QUICKI), but not with beta cell function. Therefore, a high serum ferritin concentration

is associated with insulin resistance and is a risk factor for DM.

Since ferritin contains the second largest pool of iron in the body next to hemoglobin<sup>[16]</sup>, serum ferritin concentration closely correlates with total body iron stores, mainly in the liver<sup>[26]</sup>. In this study, serum ferritin concentration is negatively associated with insulin sensitivity, suggesting an association of insulin resistance with total body iron stores. However, it is well known that ferritin is also an acute phase reactant<sup>[26]</sup>. To further explore this issue, we examined the correlation of CRP, an inflammatory marker<sup>[27]</sup>, with insulin sensitivity and serum ferritin concentration. Without a consistent result across 3 ethnic/racial groups and both genders, we concluded that in this study, no consistent relationship of CRP with either insulin sensitivity or serum ferritin concentration was observed in all 6 groups. Furthermore, in this population only

**Table 6** Serum ferritin concentration and estimated insulin sensitivity indices by quintile of gamma glutamyl transferase

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P <sup>1</sup>	P <sup>2</sup>	P <sup>3</sup>
Serum ferritin concentration (mcg/L)								
Non-Hispanic white males	141 (125, 157)	146 (131, 161)	174 (156, 191)	179 (162, 196)	227 (204, 250)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	55 (48, 62)	70 (61, 78)	95 (81, 110)	92 (77, 107)	137 (121, 154)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic black males	165 (144, 187)	191 (163, 219)	221 (195, 247)	220 (194, 246)	298 (263, 333)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic black females	60 (45, 74)	79 (68, 91)	84 (67, 100)	97 (83, 112)	141 (119, 163)	< 0.000001	< 0.000001	< 0.000001
Hispanic males	105 (93, 117)	137 (121, 153)	152 (132, 172)	173 (144, 202)	240 (208, 272)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	38 (30, 47)	38 (32, 44)	59 (47, 70)	82 (70, 93)	109 (90, 129)	< 0.000001	< 0.000001	< 0.000001
Insulin sensitivity by the homeostasis model assessment (%S) <sup>4</sup>								
Non-Hispanic white males	0.622 (0.584, 0.659)	0.521 (0.486, 0.556)	0.5061 (0.465, 0.546)	0.422 (0.387, 0.458)	0.402 (0.367, 0.436)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	0.732 (0.695, 0.769)	0.648 (0.609, 0.686)	0.587 (0.547, 0.626)	0.48 (0.444, 0.516)	0.397 (0.361, 0.433)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic black males	0.607 (0.560, 0.654)	0.588 (0.522, 0.653)	0.537 (0.491, 0.583)	0.489 (0.429, 0.548)	0.462 (0.404, 0.520)	0.0001	NS	0.05
Non-Hispanic black females	0.554 (0.515, 0.594)	0.454 (0.419, 0.489)	0.45 (0.409, 0.491)	0.407 (0.372, 0.442)	0.383 (0.332, 0.434)	< 0.000001	0.0002	0.00002
Hispanic males	0.67 (0.625, 0.715)	0.562 (0.486, 0.638)	0.423 (0.383, 0.463)	0.439 (0.376, 0.501)	0.359 (0.299, 0.419)	< 0.000001	0.00005	0.000005
Hispanic females	0.623 (0.569, 0.677)	0.494 (0.448, 0.539)	0.424 (0.381, 0.466)	0.338 (0.301, 0.375)	0.343 (0.298, 0.389)	< 0.000001	0.000002	0.000003
Insulin sensitivity by the simple QUICKI <sup>5</sup>								
Non-Hispanic white males	0.351 (0.348, 0.354)	0.341 (0.338, 0.344)	0.339 (0.335, 0.342)	0.328 (0.324, 0.332)	0.325 (0.321, 0.330)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	0.361 (0.358, 0.364)	0.353 (0.350, 0.356)	0.347 (0.344, 0.350)	0.335 (0.332, 0.339)	0.323 (0.319, 0.327)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic black males	0.349 (0.345, 0.353)	0.344 (0.338, 0.350)	0.342 (0.337, 0.346)	0.334 (0.328, 0.339)	0.331 (0.325, 0.337)	< 0.000001	0.01	0.004
Non-Hispanic black females	0.345 (0.341, 0.348)	0.334 (0.330, 0.338)	0.332 (0.328, 0.337)	0.327 (0.323, 0.332)	0.321 (0.315, 0.326)	< 0.000001	< 0.000001	< 0.000001
Hispanic males	0.356 (0.352, 0.360)	0.341 (0.335, 0.347)	0.330 (0.325, 0.334)	0.329 (0.323, 0.334)	0.318 (0.312, 0.324)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	0.351 (0.346, 0.355)	0.338 (0.334, 0.342)	0.329 (0.325, 0.334)	0.318 (0.313, 0.323)	0.316 (0.311, 0.322)	< 0.000001	< 0.000001	< 0.000001

Data presented mean with 95%CI. <sup>1</sup>P values for trend, unadjusted; <sup>2</sup>P values for trend, after adjustment for age and body mass index; <sup>3</sup>P values for trend, after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement; <sup>4</sup>%S = 22.5/(fasting insulin concentration in mU/L × fasting glucose concentration in mmol/L); <sup>5</sup>Quicki = 1/[log(fasting glucose concentration in mg/dL) + log(fasting insulin concentration in mU/L)]. NS: Not significant; QUICKI: Quantitative insulin sensitivity check index.

1.28% (range: 0.64% in Hispanic males to 1.95% in non-Hispanic black males) of the participants had an elevated CRP  $\geq 3$  mg/L, which is the 90<sup>th</sup> percentile for healthy young adults<sup>[28]</sup>. After exclusion of those with a CRP  $\geq 3$  mg/L, association of CRP with insulin sensitivity was only observed in NHW females ( $P = 0.006$  for %S and  $P = 0.00002$  for QUICKI) and NHB females ( $P = 0.03$  for %S and  $P = 0.0001$  for QUICKI) and correlation of CRP with ferritin concentration was only observed in NHW females ( $P = 0.005$ ), NHB females ( $P = 0.001$ ), and MA females ( $P = 0.01$ ), after adjustment for age and BMI. Therefore, it is very unlikely that inflammation is the underlying mechanism for the observed negative association between serum ferritin concentration and insulin sensitivity.

Next we examined the role of the liver on the observed association between insulin sensitivity and serum ferritin concentration. Among these three liver

enzymes, AST is the least specific liver maker and ALT is the most liver specific marker. All three liver enzymes were correlated with serum ferritin concentration very well, suggesting a closed association between elevated serum ferritin and liver dysfunction. However, we observed the different strengths of the correlation of insulin sensitivity across three liver enzymes. Among them, AST is the least specific for the liver diseases, the association between AST and insulin sensitivity only observed in Hispanic male group. In contrast, a negative association of ALT and GGT with insulin sensitivity was observed in all 6 groups. The negative correlation of liver enzymes with insulin sensitivity indicates the role of hepatic dysfunction in insulin resistance. Therefore, these observations imply the role of iron-associated elevated ALT and GGT in the pathogenesis of insulin resistance. The role of the liver in the pathogenesis of DM is well-established<sup>[29,30]</sup>. Furthermore, the relation-



**Table 7 Serum ferritin concentration and estimated insulin sensitivity indices by quintile of alanine aminotransferase**

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P <sup>1</sup>	P <sup>2</sup>	P <sup>3</sup>
Serum ferritin concentration (mcg/L)								
Non-Hispanic white males	147 (132, 162)	169 (153, 185)	168 (152, 183)	177 (159, 194)	220 (200, 241)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	71 (63, 79)	71 (61, 82)	86 (77, 95)	95 (84, 107)	123 (107, 139)	0.05	0.02	0.02
Non-Hispanic black males	168 (147, 189)	187 (165, 210)	201 (177, 225)	247 (219, 275)	267 (238, 296)	0.01	0.00005	0.00005
Non-Hispanic black females	80 (67, 92)	79 (68, 89)	87 (73, 100)	98 (81, 114)	120 (100, 140)	0.00003	0.000004	0.000005
Hispanic males	128 (113, 143)	128 (116, 140)	142 (126, 157)	172 (144, 200)	229 (206, 253)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	42 (35, 49)	70 (48, 93)	57 (49, 65)	64 (55, 73)	101 (87, 116)	< 0.000001	0.000009	0.000009
Insulin sensitivity by the homeostasis model assessment (%S) <sup>4</sup>								
Non-Hispanic white males	0.551 (0.517, 0.584)	0.544 (0.511, 0.578)	0.512 (0.476, 0.549)	0.483 (0.448, 0.518)	0.399 (0.370, 0.429)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	0.634 (0.602, 0.666)	0.628 (0.591, 0.664)	0.583 (0.546, 0.622)	0.55 (0.519, 0.582)	0.429 (0.395, 0.463)	0.00002	0.004	0.002
Non-Hispanic black males	0.63 (0.576, 0.684)	0.576 (0.522, 0.630)	0.564 (0.514, 0.615)	0.478 (0.423, 0.524)	0.44 (0.397, 0.482)	0.00002	0.003	0.003
Non-Hispanic black females	0.527 (0.490, 0.563)	0.498 (0.462, 0.534)	0.462 (0.424, 0.500)	0.427 (0.389, 0.465)	0.363 (0.330, 0.396)	< 0.000001	< 0.000001	< 0.000001
Hispanic males	0.635 (0.569, 0.700)	0.57 (0.525, 0.615)	0.48 (0.430, 0.530)	0.448 (0.410, 0.486)	0.324 (0.295, 0.352)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	0.55 (0.509, 0.591)	0.528 (0.484, 0.571)	0.468 (0.427, 0.509)	0.409 (0.365, 0.452)	0.331 (0.295, 0.368)	< 0.000001	0.000003	0.000002
Insulin sensitivity by the simple QUICKI <sup>5</sup>								
Non-Hispanic white males	0.344 (0.341, 0.347)	0.343 (0.339, 0.346)	0.339 (0.335, 0.342)	0.335 (0.332, 0.339)	0.326 (0.322, 0.329)	< 0.000001	< 0.000001	< 0.000001
Non-Hispanic white females	0.352 (0.349, 0.355)	0.35 (0.347, 0.345)	0.346 (0.342, 0.349)	0.343 (0.340, 0.346)	0.327 (0.324, 0.331)	0.000001	0.0005	0.0002
Non-Hispanic black males	0.349 (0.344, 0.354)	0.344 (0.339, 0.349)	0.343 (0.338, 0.348)	0.335 (0.330, 0.339)	0.33 (0.326, 0.335)	0.00005	0.01	0.009
Non-Hispanic black females	0.341 (0.337, 0.345)	0.338 (0.334, 0.342)	0.333 (0.329, 0.337)	0.33 (0.326, 0.334)	0.321 (0.316, 0.325)	< 0.000001	< 0.000001	< 0.000001
Hispanic males	0.348 (0.343, 0.353)	0.345 (0.341, 0.349)	0.333 (0.328, 0.338)	0.332 (0.328, 0.336)	0.317 (0.313, 0.321)	< 0.000001	< 0.000001	< 0.000001
Hispanic females	0.343 (0.339, 0.347)	0.34 (0.336, 0.344)	0.334 (0.330, 0.338)	0.327 (0.323, 0.331)	0.315 (0.311, 0.320)	< 0.000001	< 0.000001	< 0.000001

Data presented mean with 95%CI. <sup>1</sup>P values for trend, unadjusted; <sup>2</sup>P values for trend, after adjustment for age and body mass index; <sup>3</sup>P values for trend, after adjustment for age, body mass index, alcohol consumption, and mineral/iron supplement; <sup>4</sup>%S = 22.5/(fasting insulin concentration in mU/L × fasting glucose concentration in mmol/L); <sup>5</sup>Quicki = 1/[log(fasting glucose concentration in mg/dL) + log(fasting insulin concentration in mU/L)]. NS: Not significant; QUICKI: Quantitative insulin sensitivity check index.

ship of elevated ALT concentration with diabetes and insulin resistance has been reported<sup>[31]</sup>. In Pima Indians, elevated ALT was associated with hepatic insulin resistance but not with whole body insulin resistance or beta cell function<sup>[32]</sup>. Insulin sensitivity obtained from the HOMA as used in this study, has been demonstrated to be correlated with hepatic insulin sensitivity<sup>[33]</sup>. Thus from the observations in this study, iron could play a role in hepatic insulin resistance.

The role of serum ferritin concentration in diabetes<sup>[4]</sup> has been examined in this population. Elevated ferritin concentration has been reported to be associated with an increased risk of diabetes, but the role of inflammation could not be excluded<sup>[4]</sup>. Elevated serum ferritin concentration also has been reported to be associated with insulin resistance<sup>[34]</sup>. In the present study, we confirmed the association of serum ferritin concentration with DM and insulin sensitivity assessed by both %S

and QUICKI. Although the association of inflammation and insulin resistance has been demonstrated in this population<sup>[35]</sup>, we demonstrated that the inflammatory hypothesis is not likely the underlying mechanism of the reported associations in this study. In addition, from the observed associations of ALT and GGT with serum ferritin concentration and insulin sensitivity, we provided the evidence suggesting that the role of liver in the pathogenesis of iron-associated insulin resistance.

Iron-induced oxidant stress has proposed to play a key role in iron-mediated tissue damage<sup>[36-38]</sup>. Although the molecular events of iron-mediated tissue damage have not been fully elucidated, mitochondria are the targets of iron-mediated damage and iron may be preferentially toxic to cells with high mitochondrial activity<sup>[39]</sup>, such as hepatocytes and pancreatic beta cells. Impaired mitochondrial activity has been observed in the insulin-resistant offspring of patients with type

2 diabetes<sup>[40]</sup> and mitochondrial defect can also lead to the metabolic syndrome<sup>[41]</sup>. Therefore, iron-induced oxidative stress with mitochondrial dysfunction could be one of the underlying mechanisms in these metabolic disorders.

Because of the cross-sectional nature of the study, a temporal relationship and the biological basis of the association between serum ferritin concentration and these metabolic disorders could not be established. However, our observations have some bearing on the plausible mechanisms. Furthermore, the caustic role of iron in these processes is suggested by interventional studies. In patients with clinical evidence of non-alcoholic fatty liver disease, quantitative phlebotomy induced iron depletion to a level of near-iron deficiency results in a 40%-55% improvement of both fasting and glucose-stimulated plasma insulin concentrations and near-normalization of ALT<sup>[42]</sup>. Quantitative phlebotomy also leads to improvement in insulin sensitivity in a group of subjects with high-ferritin type 2 diabetes<sup>[43]</sup>. Therefore, iron could be the culprit of these conditions.

The current sample set did provide enough information to distinguish type 1 and type 2 diabetes. However, as 95% of diabetes is type 2 diabetes and insulin resistance is a cardinal feature of type 2 diabetes, the current study is most applicable to type 2 diabetes. Our observations are consistent with the published results<sup>[44-46]</sup> with some new clinical implications. In this study, even without clinical evidence of iron overload, iron could be associated with liver damage and insulin resistance. A clinical trial of quantitative phlebotomy in the subjects with elevated ferritin concentration is warranted to test this hypothesis. Once it is demonstrated, quantitative phlebotomy could be recommended for those patients with DM or insulin resistance, who also have elevated serum ferritin concentration. Although the underlying molecular mechanism of the association remains to be elucidated, our observations imply that the liver could play a role in iron-associated insulin resistance.

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## COMMENTS

### Background

Liver has been noted to play a role in the pathogenesis of type 2 diabetes. Hemochromatosis and excess iron load has been implicated to play a role in the pathogenesis of type 2 diabetes. Iron metabolism could play a role in the pathogenesis of type 2 diabetes.

### Research frontiers

Diabetes mellitus is a common manifestation of hemochromatosis. Although the common mutation of *HFE* is not associated with type 2 diabetes, the iron metabolism could play a role in the pathogenesis of diabetes. In hemochromatosis, both insulin resistance and impaired insulin secretion have

been suggested to play a role in its pathogenesis. However, the underlying mechanism of iron-associated abnormal glucose homeostasis in the general population is not well understood.

## Innovations and breakthroughs

In this study, the authors found that subjects with diabetes had a higher serum ferritin concentration than those without diabetes. The authors also observed that serum ferritin concentration was negatively associated with insulin sensitivity (%S and QUICKI), but not with beta cell function. Therefore, a high serum ferritin concentration is associated with insulin resistance and is a risk factor for diabetes mellitus. The authors further demonstrated that the inflammatory hypothesis is not likely the underlying mechanism of the reported associations in this study. In addition, from the observed associations of alanine aminotransferase (ALT) and gamma glutamyl transpeptidase with serum ferritin concentration and insulin sensitivity, the authors provided the evidence suggesting that the role of liver in the pathogenesis of iron-associated insulin resistance. Iron-induced oxidant stress has proposed to play a key role in iron-mediated tissue damage and iron-induced oxidative stress with mitochondrial dysfunction could be one of the underlying mechanisms in these metabolic disorders.

## Applications

As in patients with clinical evidence of non-alcoholic fatty liver disease, quantitative phlebotomy induced iron depletion to a level of near-iron deficiency results in a 40%-55% improvement of both fasting and glucose-stimulated plasma insulin concentrations and near-normalization of ALT and quantitative phlebotomy also leads to improvement in insulin sensitivity in a group of subjects with high-ferritin type 2 diabetes, a clinical trial of quantitative phlebotomy in the subjects with elevated ferritin concentration is warranted to test this hypothesis. Once it is demonstrated, quantitative phlebotomy could be recommended for those patients with diabetes mellitus or insulin resistance, who also have elevated serum ferritin concentration.

## Terminology

Ferritin: Ferritin is a protein in the body that binds to iron; most of the iron stored in the body is bound to ferritin. In humans, it acts as a buffer against iron deficiency and iron overload. Serum ferritin concentration closely correlates with total body iron stores, mainly in the liver.

## Peer-review

This is an interesting study. The authors have evaluated the relationship of iron with diabetes mellitus (DM) and concluded that disordered iron metabolism could play a role in the pathogenesis of insulin resistance and DM through its effect on liver function.

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