METABOLIC SYNDROME AND RELATED DISORDERS
Volume 13, Number 2, 2015

Pp. 57–63
DOI: 10.1089/met.2014.0106

ORIGINAL ARTICLES

Serum Ferritin, Insulin Resistance, and Metabolic Syndrome: Clinical and Laboratory Associations in 769 Non-Hispanic Whites Without Diabetes Mellitus in the HEIRS Study

Ronald T. Acton, PhD,[1] J. Clayborn Barton, BS,[2] and James C. Barton, MD[2,3]

Abstract

Background: In some reports, serum ferritin (SF) has been associated with insulin resistance and metabolic syndrome.
Methods: We studied non-Hispanic whites without diabetes mellitus in a postscreening examination. Participants included cases [*HFE* C282Y homozygosity; and transferrin saturation (TS) >50% and SF >300 μg/L (males) and TS >45% and SF >200 μg/dL (females), regardless of *HFE* genotype] and controls [*HFE* wild-type (wt/wt) and TS/SF 25th–75th percentiles]. We excluded participants with overnight fasts <8 hr, cirrhosis, hepatitis B or C, pregnancy, or missing data. Observations were age, sex, C282Y homozygosity, body mass index (BMI), systolic and diastolic blood pressures (SBP, DBP), lymphocytes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), TS, SF, and glucose/insulin. Insulin resistance was defined as homeostasis model assessment of insulin resistance (HOMA-IR) 4th quartile (≥2.70).
Results: A total of 407 women and 362 men (mean age 54 years) included 188 C282Y homozygotes and 371 wt/wt. Significant trends across HOMA-IR quartiles included age, male sex, BMI, SBP, DBP, lymphocytes, ALT, CRP >0.5 mg/dL (positive), and TS (negative). Multiple regression on HOMA-IR revealed significant associations with male sex, BMI, SBP, lymphocytes, ALT, CRP >0.5 mg/dL (positive), and DBP and SF (negative). Logistic regression on HOMA-IR 4th quartile revealed significant positive associations with age, male sex, BMI, and lymphocytes. Metabolic syndrome occurred in 53 participants (6.9%). Logistic regression on metabolic syndrome revealed these odds ratios: HOMA-IR 4th quartile [9.1 (4.8, 17.3)] and CRP >0.5 mg/dL [2.9 (1.6, 5.4)].
Conclusions: Age, male sex, BMI, and lymphocytes were positively associated with HOMA-IR after correction for other factors. HOMA-IR 4th quartile and CRP >0.5 mg/dL predicted metabolic syndrome.

Introduction

Serum ferritin (SF) has been positively associated with insulin resistance and the metabolic syndrome in whites without diabetes mellitus.[1–4] SF levels are increased by elevated body iron stores, a major cause of which is hemochromatosis.[5–7] In western Europeans, hemochromatosis due to polymorphisms of the *HFE* gene (chromosome 6p21.3) is common. There are two common *HFE* polymorphisms in whites: C282Y (exon 4; c.845G→A) the frequency of which is 0.0683 [95% confidence interval (CI) 0.0666, 0.0699]; and H63D (exon 2; c.187C→G), the frequency of which is 0.1532 (0.1508, 0.1556).[7] Hemochromatosis due to C282Y homozygosity occurs in 0.3%–0.6% (3–6/1000) of persons of northwestern European descent[7,8] and accounts for ~90% of hemochromatosis in these people.[7,8] In 44,082 US white participants in the Hemochromatosis and Iron Overload Screening (HEIRS) Study, the prevalence of hemochromatosis associated with *HFE* C282Y homozygosity was 0.44% (1/227).[7] SF >300 μg/L was observed in 88% of untreated male C282Y homozygotes and >200 μg/L in 57% of female untreated C282Y homozygotes. Geometric mean SF levels among male or female participants with the C282Y/H63D, H63D/H63D, C282Y/+, and H63D/+ genotypes were not significantly different than the levels among male or female participants,

[1]Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama.
[2]Southern Iron Disorders Center, Birmingham, Birmingham, Alabama.
[3]Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama.

respectively, with the wild-type *HFE* genotype.[7] SF is also increased in association with inflammation (including common liver disorders)[9–14] and neoplasms.[15,16]

The aim of this study was to determine the relationships of SF to insulin resistance (determined by homeostasis model assessment of insulin resistance, HOMA-IR)[17] and the metabolic syndrome[18] in a cohort enriched with persons who had hemochromatosis, iron overload, and hyperferritinemia. We evaluated observations in 769 US non-Hispanic white adults without diabetes who attended the postscreening clinical examination (CE) of the HEIRS Study.[19] We compared characteristics of 490 cases (*HFE* C282Y homozygotes and other participants with high-iron phenotypes) and 279 controls [*HFE* wild-type (wt/wt) and normal iron phenotypes] using univariable methods. We evaluated age, sex, C282Y homozygosity, body mass index (BMI), systolic and diastolic blood pressures (SBP, DBP), levels of blood neutrophils and lymphocytes, serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities; and serum levels of C-reactive protein (CRP), transferrin saturation (TS), and SF across HOMA-IR quartiles. Using logistic regressions, we determined significant associations of HOMA-IR 4th quartile and metabolic syndrome with available independent variables. The present results are discussed in the context of previous reports of SF, insulin resistance, and metabolic syndrome in persons without diabetes mellitus.

Methods

Subjects

The National Institutes of Health HEIRS Study (January, 2000–January, 2006) evaluated the prevalence, genetic and environmental determinants, and potential clinical, personal, and societal impacts of hemochromatosis and iron overload in a multiethnic, primary care-based sample of 101,168 adults enrolled over a 2-year period at four field centers in the United States and one in Canada. This study was conducted in accordance with the principles of the Declaration of Helsinki. Participants, at least 25 years of age and able to give informed consent, were recruited from a health maintenance organization, diagnostic blood collection centers, and public and private primary care offices in ambulatory clinics associated with the field centers.[20] Initial screening of participants included genotyping for the *HFE* C282Y and H63D alleles and phenotyping that included serum iron and unsaturated iron-binding capacity and calculated TS and SF levels.[20]

Clinical examination

The study protocol was approved by the institutional review board of each field center and informed consent was obtained for screening and for a CE.[19] Use of HOMA-IR to quantify insulin resistance and its relationship to diabetes mellitus in HEIRS Study CE participants was planned and approved prospectively by the study investigators. CE invitations were extended to all *HFE* C282Y homozygotes (regardless of iron phenotype) and all other participants whose TS and SF values exceeded study thresholds (TS $>50\%$ and SF $>300\,\mu g/L$ for men; TS $>45\%$ and SF $>200\,\mu g/L$ for women), regardless of *HFE* genotype (cases).[19,20] Participants without C282Y or H63D *HFE* alleles (*i.e.*, *HFE* wt/wt) and TS and SF levels between the 25th and 75th percentile of sex-specific distributions at initial screening were frequency-matched for age and sex to cases studied at each field center and invited to undergo CE (controls). Participants eligible for the CE were informed of their initial screening genotype, TS, and SF. The median interval between initial screening and CE participation was 8 months. The CE included a questionnaire addressing medical history and medications completed by the participant and a focused physical examination performed by a HEIRS Study physician that were designed to document symptoms and clinical conditions associated with hemochromatosis and iron overload.[21–24]

At CE, a morning fasting blood sample was obtained to confirm initial screening *HFE* genotype,[8,25] and to perform complete blood count (Beckman Coulter GenS, Beckman/Coulter, Fullerton, CA), measurements of serum ALT and AST activities, serum CRP, serum glucose (Hitachi 9/11 Analyzer, Roche Applied Science, Madison, WI), serum insulin (DPC IMMULITE Analyzer, Diagnostic Products, Los Angeles, CA), and TS and SF (Hitachi 9/11 Analyzer, Roche Applied Science, Madison, WI).[20,26] Using control specimens that represented normal ranges of SF, the total coefficient of variation (CV) for this device was 5.82%–6.78%. For higher-range SF standards, the total CV was 5.98%–8.24%.[27] In participants with elevated ALT activities, reflex testing for hepatitis B surface antigen and hepatitis C antibody was performed (VITROS ECi, Ortho-Clinical Diagnostics Incorporated, Raritan, NJ). Initial screening *HFE* genotypes of each participant were verified after their CE participation. All testing was performed at the HEIRS Study Central Laboratory (Fairview-University Medical Center Clinical Laboratory, University of Minnesota, Fairview, MN).

Participant exclusions

The initial dataset consisted of observations on 2319 CE participants. Of these, 953 reported that they were non-Hispanic whites who did not have diabetes (initial screening and CE) and whose medication list did not include antidiabetes drugs. We excluded 59 participants because they fasted less than 8 hr before their CE blood specimens were drawn for glucose and insulin measurements. We excluded 42 subjects with evidence of chronic hepatitis B ($n=2$) or chronic hepatitis C ($n=40$) at CE. Five participants were excluded because they reported a medical history or diagnosis of cirrhosis at either initial screening or CE. We excluded nine women who reported that they were pregnant or possibly pregnant. We excluded other participants because some of their data were missing: *HFE* genotype (1); BMI (2); SBP/DBP (4); leukocyte or leukocyte differential counts (58); and serum glucose level (4).

Definition of HOMA-IR

Insulin resistance was estimated using HOMA-IR {[serum glucose (mg/dL) $\times$ serum insulin (mIU/L)] $\div$ 405}.[17] HOMA-IR values were divided as quartiles, yielding corresponding HOMA-IR ranges. Participants in the 4th quartile (HOMA-IR ≥ 2.70) were defined as having insulin resistance.

Definition of metabolic syndrome

Metabolic syndrome was defined as concurrence of each of these three attributes: BMI $\geq 30\,kg/m^2$; SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg; and fasting serum glucose ≥ 100 mg/dL.[18,28]

We used BMI instead of a central obesity measure.[18] We grouped positivity for these three attributes into a dichotomous metabolic syndrome variable.

Statistics

There were complete observations in 769 participants. Distributions of SBP, DBP, and TS values in all participants and in participants grouped by HOMA-IR quartiles were normal (d'Agostino's test). We used natural log (ln) transformation to normalize other data (age, sex, leukocytes, lymphocytes, neutrophils, ALT, AST, and SF). Each mean ln-transformed datum was converted to an anti-ln (95% CI) for display. Dichotomous variables included sex, case, *HFE* C282Y homozygosity, and CRP >0.5 mg/dL. Proportions of participants with *HFE* genotype wt/wt were not included due to the close relationship of this category with the dichotomous control category.

Multiple linear regression on HOMA-IR was performed to identify significant associations among available independent variables. In exploratory analyses, the significance associated with leukocytes was almost entirely attributable to lymphocytes and not to neutrophils. Thus, we used only lymphocytes for some final multiple and logistic regression analyses. We performed logistic regression on HOMA-IR values in the 4th quartile (dichotomous) and metabolic syndrome using available independent variables.

Analyses were performed with SAS (v. 9.1, SAS Institute Inc., Cary, NC), Excel 2000® (Microsoft Corp., Redmond, WA), and GB-Stat® (v. 10.0, Dynamic Microsystems, Inc., Silver Spring, MD). Descriptive data are displayed as enumerations, percentages, mean ± 1 standard deviation (SD), or mean (95% CI). Means of normally distributed and normalized data were compared using the Student *t*-test (two-tailed). Proportions were compared using the Pearson chi-squared test or Fisher exact test, as appropriate. We computed the Pearson correlation coefficient and value of *P* for linear regressions of the independent variable HOMA-IR quartiles 1–4 versus the respective dichotomous or continuous variables as a measure of trend. Odds ratios (OR) and 95% CI are displayed for some logistic regressions. We defined nominal values of $P < 0.05$ to be significant, although Bonferroni corrections were applied to control the type I error rate at 0.05 for 15 or 16 separate comparisons of continuous and dichotomous data, as appropriate.

Results

Comparisons of case and control participants

Nominal values of *P* are displayed in Table 1. After Bonferroni corrections (significant $P < 0.0031$), the proportions of men, proportion of *HFE* C282Y homozygotes (by definition), and mean BMI, SBP, TS, and SF were higher in cases than controls (Table 1).

Table 1. Characteristics of 769 HEIRS Study Cases and Controls[a,b]

Characteristic	*Cases (490)*	*Controls (279)*	*Value of P*[c]
Mean age, years (SD)	54 (52, 60)	54 (53, 55)	0.6099
Men, %	51.8 (254)	38.7 (108)	0.0005
HFE C282Y homozygosity	38.4% (188)	0	<0.0001
HFE wt/wt, %[d]	18.6 (91)	100.0 (279)	<0.0001
Mean BMI, kg/m^2	28.4 (27.9, 28.9)	26.4 (25.9, 27.0)	<0.0001
Mean SBP, mm Hg	127 ± 17	122 ± 17	0.0012
Mean DBP, mm Hg	78 ± 10	76 ± 10	0.0607
Mean leukocytes × 10^3/μL	5.5 (5.4, 5.7)	5.7 (5.5, 5.9)	0.2931
Mean lymphocytes × 10^3/μL	1.5 (1.5, 1.6)	1.5 (1.5, 1.6)	0.4644
Mean neutrophils × 10^3/μL	3.2 (3.1, 3.4)	3.4 (3.2, 3.5)	0.1650
Mean ALT, IU/L	23 (22, 24)	21 (20, 22)	0.0734
Mean AST, IU/L	24 (23, 25)	23 (22, 24)	0.1127
CRP >0.5 mg/dL, % (n)	28.4 (139)	28.7 (80)	0.8471
Mean TS, %	56 ± 22	29 ± 9	<0.0001
Mean SF, μg/L	326 (299, 356)	80 (73, 87)	<0.0001
Mean HOMA-IR	1.92 (1.80, 2.05)	1.68 (1.57, 1.81)	0.0068

Results are displayed as range, mean ± 1 standard deviation (SD), mean [95% confidence interval (CI)], or as percentage (*n*).

[a]Cases were defined as all C282Y homozygotes (regardless of iron phenotype) and participants with other *HFE* genotypes whose initial screening TS and SF values exceeded study thresholds (see Methods). Cases included 211 participants with high TS/SF phenotypes who had *HFE* genotypes other than C282Y/C282Y or wild-type (wt/wt). Controls were derived from a group of participants who had *HFE* genotype wt/wt and who also had TS and SF levels between the 25th and 75th percentiles of sex-specific distributions.

[b]Reference ranges for blood cell analytes included: white blood cells 4.0–11.0 × 10^3/μL, absolute neutrophils 1.6–8.3 × 10^3/μL, and absolute lymphocytes 0.8–5.3 × 10^3 μL. Reference ranges for liver-related analytes included: ALT 0–31 IU/L (females) and 0–40 IU/L (males), AST 0–31 IU/L (females) and 0–37 IU/L (males), and CRP 0–0.5 mg/dL. ALT activity <4 IU/L was imputed as 3 IU/L. CRP <0.3 mg/dL was imputed as 0.2 mg/dL. Elevated CRP levels were defined as >0.5 mg/dL. Reference ranges for iron-related analytes included: TS 15%–50%, SF 20–300 μg/L (males), SF 10–120 μg/L (females 15–45 years) and 10–300 μg/L (females >45 years), serum total iron-binding capacity 228–428 μg/dL, and serum iron concentration 45–160 μg/dL (males) and 30–160 μg/dL (females). TS <15% was imputed as 7.5%. The reference ranges for serum glucose and serum insulin were 60–115 mg/dL and 0–20 mIU/L, respectively.

[c]These are nominal values of *P*. Bonferroni correction for 16 comparisons yielded a revised *P* for significance of <0.0031.

[d]Other *HFE* genotypes in 490 cases included: C282Y/H63D compound heterozygosity 10.0% (49), C282Y heterozygosity 11.2% (55), H63D homozygosity 5.9% (29), H63D heterozygosity 15.9% (78), and wt/wt 18.6% (91). By definition, *HFE* wt/wt category comprises all genotypes that do not include either C282Y or H63D.

HEIRS Study, Hemochromatosis and Iron Overload Screening Study; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, serum alanine aminotransferase activity; AST, serum aspartate aminotransferase activity; CRP, C-reactive protein; TS, transferrin saturation; SF, serum ferritin; HOMA-IR, homeostasis model assessment for insulin resistance.

Participants with elevated SF at CE

In 72 men with *HFE* C282Y homozygosity, 50 (69.4%) had SF >300 μg/L. In 116 women with C282Y homozygosity, 60 (56.6%) had SF >200 μg/L. An additional 302 participants without *HFE* C282Y homozygosity were classified as cases based on initial screening TS/SF. In 182 men, 145 (79.7%) had SF >300 μg/L. Among 120 women, 93 (77.5%) had SF >200 μg/L. In 108 control men, one (0.3%) had SF >300 μg/L. In 171 control women, two (1.2%) had SF >200 μg/L. Altogether, 196 men and 155 women (45.6% of 769 participants) had SF greater than the corresponding upper reference limits.

Participants with subnormal SF at CE

Subnormal SF levels were defined as SF <20 μg/L (males); SF <10 μg/L (females 15–45 years), and <10 μg/L (females >45 years). Among 188 C282Y homozygotes, seven women (6.6%) had SF <10 μg/L and two men (2.8%) had SF <20 μg/L. Subnormal SF levels were not observed in any of the 302 case participants without *HFE* C282Y homozygosity. Among controls, eight women (4.7%) had SF <10 μg/L and one man (0.9%) had SF <20 μg/L. Altogether, three men and 15 women (2.3%) of the 769 participants had SF levels below the corresponding lower reference limits.

Comparison of 1st and 4th HOMA-IR quartile participant characteristics

Nominal values of P are displayed in Table 2. After Bonferroni corrections (significant at $P<0.0033$), differences in these variables were observed: Age, male sex, proportion of *HFE* C282Y homozygotes, BMI, SBP, DBP, leukocytes, lymphocytes, ALT, AST, and CRP >0.5 mg/dL (Table 2).

Trends of values across the four HOMA-IR quartiles

Nominal values of P are displayed in Table 2. After Bonferroni corrections (significant at $P<0.0033$), differences in these variables were observed: Age, male sex, BMI, SBP, DBP, leukocytes, lymphocytes, ALT, and CRP >0.5 mg/dL. A significant negative trend was observed with TS (Table 2).

Regressions on HOMA-IR values

Multiple regression on HOMA-IR values revealed these positive associations: Male sex ($P=0.0119$), BMI ($P=0.0003$), SBP ($P<0.0001$), lymphocytes ($P=0.0002$), ALT ($P<0.0001$), and CRP >0.5 mg/dL ($P=0.0153$). We identified these negative associations: DBP ($P=0.0005$) and SF ($P=0.0178$). Significance of ANOVA for this regression was $P<0.0001$. This model accounted for 9.2% of the variance of HOMA-IR values.

Regression on HOMA-IR 4th quartile category

Logistic regression on HOMA-IR 4th quartile (dichotomous) revealed these positive associations: Age [$P<0.0001$, OR 1.0 (95% CI: 1.0, 1.1)], male sex [$P=0.0003$; 2.1 (1.4, 3.1)], BMI [$P<0.0001$; 1.2 (1.2 1.3)], and lymphocytes [$P=0.0025$; 1.7 (1.2, 2.4)]. Significance of this regression was $P<0.0001$. This model accounted for 21.8% of the variance in HOMA-IR 4th quartile.

Metabolic syndrome and HOMA-IR

Metabolic syndrome occurred in 53 participants (6.9%). Insulin resistance (HOMA-IR 4th quartile) occurred in 192 participants (25.0%). Univariable analysis revealed no significant difference between the mean SF in participants with and without insulin resistance (Table 3). Similarly, there was no significant difference between the mean SF in participants with and without metabolic syndrome (Table 3). Logistic regression on metabolic syndrome revealed two positive associations: HOMA-IR 4th quartile [$P<0.0001$; OR 7.9 (4.0, 15.8)], and CRP >0.5 mg/dL [$P=0.0005$; OR 3.6 (1.8, 7.1)]. Significance of this regression was $P<0.0001$. This model accounted for 21.5% of the variance of metabolic syndrome.

Discussion

We selected HEIRS Study white participants who attended a postscreening CE, were characterized by *HFE* genotyping and iron phenotyping, and were grouped as cases and controls in accordance with the HEIRS Study design.[19] The National Health and Nutrition Examination Survey (NHANES) III studied nondiabetic US adults of diverse race/ethnicity without regard to possible hemochromatosis or iron overload.[4] The HOMA-IR quartile values in the present paper were similar to those from NHANES III.[4] Positive trends of age, BMI, SBP, DBP, CRP, and SF by HOMA-IR quartiles in the present study were similar to those of the NHANES III study.[4] The HEIRS Study CE design would account in part for the higher mean SF values across all HOMA-IR quartiles than those described in the NHANES III report.[4] We observed a significant trend of percentage of men across HOMA-IR quartiles, whereas NHANES III investigators did not.[4] The NHANES III study did not evaluate TS.[4]

Mean SF in the present study was higher in participants in the fourth than the first HOMA-IR quartile, but this difference was not significant. The trend of mean SF across all HOMA-IR quartiles was upward, but this difference was also nonsignificant. These results differ from those of a previous study in which participants were chosen without regard to *HFE* genotype or iron phenotype for references.[4] Consistent with our observations on SF and HOMA-IR, our logistic regressions revealed that neither SF, *HFE* homozygosity, nor the case subgroup variable was a positive predictor of HOMA-IR 4th quartile or metabolic syndrome. Yeap et al.[29] observed that SF was not associated with insulin resistance in 3922 nondiabetic community-dwelling men and women in Australia who did not have *HFE* genotypes C282Y/C282Y or C282Y/H63D.[29] Taken together, these observations demonstrate that SF is not a significant predictor of insulin resistance in cohorts of whites that are enriched with or depleted of persons with hemochromatosis genotypes.

Other investigators reached different conclusions regarding SF and its relationship to insulin resistance in persons without diabetes. In 76 Spanish adults, log SF was an independent predictor of insulin sensitivity in a multivariate analysis.[2] In 1013 middle-aged Finnish men, the mean concentration of fasting serum insulin was significantly higher in the fifth quintile of SF than in the first quintile.[1] In 538 nondiabetic German adults, there was a positive association of SF with 2-hr glucose concentrations and a negative association with insulin sensitivity.[3] In 6044 adult NHANES III participants, insulin resistance increased across

Table 2. Characteristics of 769 Non-Hispanic Whites Without Reports of Diabetes Mellitus[a]

Characteristic	*HOMA-IR quartiles*				*Value of* P *(quartile 1 vs. 4)*[b]	*Value of* P *(correlation coefficient) for trend*[c]
	1 (n = *191)*	*2* (n = *194)*	*3* (n = *192)*	*4* (n = *192)*		
HOMA-IR, range	≤ 1.26	1.27–1.82	1.83–2.69	≥ 2.70	—	—
Mean age, years	51 [49, 53]	53 [51, 56]	54 [52, 56]	58 [55, 59]	< 0.0001	< 0.0001 (0.1421)
Male, % (*n*)	35.6 (68)	46.9 (91)	52.1 (100)	53.6 (103)	0.0004	0.0002 (0.1325)
Cases, % (*n*)[d]	60.2 (115)	58.2 (113)	64.6 (124)	71.9 (138)	0.0159	0.0076 (0.0961)
HFE C282Y homozygosity, % (*n*)	15.2 (29)	24.7 (48)	27.1 (52)	30.7 (59)	0.0003	0.6965 (0.0141)
Mean BMI, kg/m^2	24.5 [23.9, 25.0]	26.5 [26.0, 27.1]	28.4 [27.8, 29.0]	31.7 [30.2, 32.6]	< 0.0001	< 0.0001 (0.5003)
Mean SBP, mm Hg	119 ± 18	124 ± 17	127 ± 17	129 ± 15	< 0.0001	< 0.0001 (0.2033)
Mean DBP, mm Hg	74 ± 10	78 ± 10	78 ± 11	79 ± 9	< 0.0001	< 0.0001 (0.1674)
Mean leukocytes × 10^3/μL	5.2 [5.0, 5.5]	5.5 [5.3, 5.8]	5.6 [5.4, 5.9]	6.0 [5.9, 6.2]	< 0.0001	0.0004 (0.1273)
Mean lymphocytes × 10^3/μL	1.4 [1.4, 1.5]	1.5 [1.4, 1.6]	1.5 [1.4, 1.6]	1.6 [1.6, 1.7]	< 0.0001	< 0.0001 (0.1560)
Mean neutrophils × 10^3/μL	3.1 [3.1, 3.3]	3.2 [3.0, 3.5]	3.4 [3.1, 3.6]	3.5 [3.2, 3.7]	0.0188	0.0832 (0.0625)
Mean ALT, IU/L	18 [17, 19]	20 [18, 21]	23 [22, 25]	29 [27, 31]	< 0.0001	< 0.0001 (0.2382)
Mean AST, IU/L	22 [21, 23]	22 [21, 24]	23 [22, 25]	26 [24, 28]	0.0001	0.0902 (0.0124)
Mean TS, %	50 ± 24	46 ± 22	44 ± 20	43 ± 22	0.0036	0.0019 (-0.1121)
Mean SF, μg/L	183 [158, 212]	175 [149, 205]	214 [182, 251]	215 [182, 253]	0.1607	0.0212 (0.0831)
CRP > 0.5 mg/dL, % (n)	20.4 (39)	22.2 (43)	31.8 (61)	30.7 (59)	< 0.0001	< 0.0001 (0.1661)

HFE wt/wt category comprises all genotypes including neither C282Y nor H63D. Results are displayed as range, mean ± 1 standard deviation (SD), mean (95% confidence interval [CI]) or percentage (*n*).

[a]Reference ranges for blood cell analytes included: white blood cells 4.0–11.0 × 10^3/μL, absolute neutrophils 1.6–8.3 × 10^3/μL, and absolute lymphocytes 0.8–5.3 × 10^3 μL. Reference ranges for liver-related analytes included: ALT 0–31 IU/L (females) and 0–40 IU/L (males), AST 0–31 IU/L (females) and 0–37 IU/L (males), and CRP 0–0.5 mg/dL. ALT activity < 4 IU/L was imputed as 3 IU/L. CRP < 0.3 mg/dL was imputed as 0.2 mg/dL. Elevated CRP levels were defined as > 0.5 mg/dL. Reference ranges for iron-related analytes included: TS 15%–50%, SF 20–300 μg/L (males), SF 10–120 μg/L (females 15–45 years) and 10–300 μg/L (females > 45 years), serum total iron-binding capacity 228–428 μg/dL, and serum iron concentration 45–160 μg/dL (males) and 30–160 μg/dL (females). TS < 15% was imputed as 7.5%. The reference ranges for serum glucose and serum insulin were 60–115 mg/dL and 0–20 mIU/L, respectively.

[b]These are nominal values of *P*. Bonferroni correction for 15 comparisons yielded a revised *P* for significance of < 0.0033.

[c]These are nominal values represent *P* (Pearson correlation coefficient) for linear regressions of the respective dichotomous or continuous variables by the independent variable HOMA-IR quartiles 1–4. Bonferroni correction for 15 comparisons yielded a revised *P* for significance of < 0.0033.

[d]Cases were defined as all C282Y homozygotes (regardless of initial screening iron phenotype) and participants with other *HFE* genotypes whose initial screening TS and SF values exceeded study thresholds (see Methods). Controls were derived from a group of participants who had neither *HFE* C282Y nor H63D [defined as *HFE* genotype wild-type (wt/wt)] and who also had TS and SF levels between the 25th and 75th percentiles of sex-specific distributions.

HOMA-IR, homeostasis model assessment for insulin resistance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, serum alanine aminotransferase activity; AST, serum aspartate aminotransferase activity; TS, transferrin saturation; SF, serum ferritin. CRP, C-reactive protein.

Table 3. Serum Ferritin in HOMA-IR 4th Quartile and Metabolic Syndrome

Characteristic	*No. of subjects*	*Mean SF, μg/L (95% CI)*	*Value of* P
HOMA-IR 4th quartile	192	215 (67, 685)	—
HOMA-IR, quartiles 1–3	577	191 (63, 577)	0.2019
Metabolic syndrome	53	246 (86, 685)	—
No metabolic syndrome	716	192 (63, 592)	0.1106

HOMA-IR, homeostasis model assessment of insulin resistance; CI, confidence interval.

quartiles of SF for men and postmenopausal women after adjustment for other factors and exclusion of participants with elevated TS and SF values that suggested hemochromatosis.[30] Brudevold et al. demonstrated that hyperferritinemia was associated with insulin resistance in a cohort of 40 Norwegian patients with fatty liver whose liver biopsies did not demonstrate increased iron.[31] Thus, dissimilar clinical variables and methods of analysis could account for some differences observed across the present and previous studies.

The prevalence of metabolic syndrome in the present cohort was 7%, whereas the overall prevalence of the metabolic syndrome in 11,512 nondiabetic European adults in a meta-analysis of 11 studies was 15%.[32] SF was not significantly associated with the metabolic syndrome in a multiple regression analysis in the present cohort. In contrast, SF was positively associated with the presence of the metabolic syndrome in a meta-analysis of 15 studies of Caucasian, Asian, and mixed race/ethnicity populations that did not exclude persons with diabetes.[33]

We propose that SF was not a significant predictor of HOMA-IR 4th quartile or metabolic syndrome in our analyses in part because hyperferritinemia in the present participants was due predominantly to increased iron storage, not to inflammation, neoplasms, or other causes. SF is a mixture of iron-rich ferritin and apoferritin.[34,35] Iron-rich SF is in equilibrium with body iron stores.[36,37] The iron composition of SF is increased in hemochromatosis and other iron overload disorders, consistent with the function of ferritin as an iron storage protein.[5,34] Because the liver is a major iron storage organ, the iron content of SF in non-iron liver disorders associated with hepatocellular necrosis is also increased, presumably due to the release of intracellular iron-rich ferritin into the blood.[5,34,35] Ferritin released into the blood as an acute-phase reactant due to inflammation, anemia of chronic disease, or malignancy is typically apoferritin and has low iron content,[5,34,35] especially ferritin molecules released from cells or tissues that do not typically store iron.[9,11,15,16] Interleukin-1 and ethanol enhance the production and secretion of apoferritin.[12,13]

Uncertainties of the present work include the possibility that some participants had undiagnosed diabetes. None of the accepted measures of body iron stores was available for our analyses.[38] SF is commonly used as a surrogate measure of iron stores,[5] although within-person variation of SF is great.[39] The linear correlation of SF with iron removed by phlebotomy to achieve iron depletion in whites with hemochromatosis is positive and significant, but the Pearson correlation coefficient is not particularly high.[6] Similar findings have been reported in other ethnic populations regardless of *HFE* genotype.[40] These observations indicate that SF includes ferritin molecules released into blood due to non-iron causes, even in persons with hemochromatosis or iron overload phenotypes.

We conclude that age, male sex, BMI, and blood lymphocyte counts, but not SF, were positively associated with HOMA-IR after correction for other factors in the present cohort of 769 non-Hispanic whites without diagnoses of diabetes mellitus. HOMA-IR 4th quartile and CRP >0.5 mg/dL predicted metabolic syndrome.

Acknowledgments

The HEIRS Study (January 2000–January 2006) was supported by the National Heart, Lung, and Blood Institute (NHLBI), in conjunction with the National Human Genome Research Institute. Individual investigators, grants, and contracts have been acknowledged in detail previously.[7,19] The authors also acknowledge the HEIRS Study participants and partial support of the present work by Southern Iron Disorders Center.

Author contributions: R.T.A. conceived the study, performed statistics, and drafted the manuscript; Ja.C.B. conceived the study, evaluated patients, performed statistics, and drafted the manuscript; J.Cl.B. performed statistics and drafted the manuscript. All authors approved the manuscript in its final form.

Author Disclosure Statement

No conflicting financial interests exist.

References

1. Tuomainen TP, Nyyssönen K, Salonen R, et al. Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. *Diabetes Care* 1997;20:426–428.
2. Fernandez-Real JM, Ricart-Engel W, Arroyo E, et al. Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care* 1998;21:62–68.
3. Haap M, Fritsche A, Mensing HJ, et al. Association of high serum ferritin concentration with glucose intolerance and insulin resistance in healthy people. *Ann Intern Med* 2003; 139:869–871.
4. Chen J, Wildman RP, Hamm LL, et al. Association between inflammation and insulin resistance in U.S. nondiabetic adults: Results from the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004;27: 2960–2965.
5. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med* 1974;290:1213–1216.
6. Beutler E, Felitti V, Ho NJ, et al. Relationship of body iron stores to levels of serum ferritin, serum iron, unsaturated iron binding capacity and transferrin saturation in patients with iron storage disease. *Acta Haematol* 2002;107:145–149.
7. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 2005;352:1769–1778.
8. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399–408.
9. Reissman KR, Dietrich MR. On the presence of ferritin in the peripheral blood of patients with hepatocellular disease. *J Clin Invest* 1956;35:588–595.

10. Birgegård G, Hällgren R, Killander A, et al. Serum ferritin during infection. A longitudinal study. *Scand J Haematol* 1978;21:333–340.
11. Rothwell RS, Davis P. Relationship between serum ferritin, anemia, and disease activity in acute and chronic rheumatoid arthritis. *Rheumatol Int* 1981;1:65–67.
12. Moirand R, Lescoat G, Delamaire D, et al. Increase in glycosylated and nonglycosylated serum ferritin in chronic alcoholism and their evolution during alcohol withdrawal. *Alcohol Clin Exp Res* 1991;15:963–969.
13. Pinero DJ, Hu J, Cook BM, et al. Interleukin-1beta increases binding of the iron regulatory protein and the synthesis of ferritin by increasing the labile iron pool. *Biochim Biophys Acta* 2000;1497:279–288.
14. Adams PC, Barton JC. A diagnostic approach to hyperferritinemia with a non-elevated transferrin saturation. *J Hepatol* 2011;55:453–458.
15. Matzner Y, Konijn AM, Hershko C. Serum ferritin in hematologic malignancies. *Am J Hematol* 1980;9:13–22.
16. Jacobs A. Serum ferritin and malignant tumours. *Med Oncol Tumor Pharmacother* 1984;1:149–156.
17. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419.
18. Laaksonen DE, Lakka HM, Niskanen LK, et al. Metabolic syndrome and development of diabetes mellitus: Application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. *Am J Epidemiol* 2002;156:1070–1077.
19. McLaren GD, McLaren CE, Adams PC, et al. Clinical manifestations of hemochromatosis in *HFE* C282Y homozygotes identified by screening. *Can J Gastroenterol* 2008;22:923–930.
20. McLaren CE, Barton JC, Adams PC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) study design for an evaluation of 100,000 primary care-based adults. *Am J Med Sci* 2003;325:53–62.
21. Witte DL, Crosby WH, Edwards CQ, et al. Practice guideline development task force of the College of American Pathologists. Hereditary hemochromatosis. *Clin Chim Acta* 1996;245:139–200.
22. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. *J Hepatol* 2000;33:485–504.
23. Tavill AS. Diagnosis and management of hemochromatosis. *Hepatology* 2001;33:1321–1328.
24. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. *N Engl J Med* 2004;350:2383–2397.
25. Jeffrey GP, Chakrabarti S, Hegele RA, et al. Polymorphism in intron 4 of *HFE* may cause overestimation of C282Y homozygote prevalence in haemochromatosis. *Nat Genet* 1999;22:325–326.
26. Adams PC, Reboussin DM, Leiendecker-Foster C, et al. Comparison of the unsaturated iron-binding capacity with transferrin saturation as a screening test to detect C282Y homozygotes for hemochromatosis in 101,168 participants in the hemochromatosis and iron overload screening (HEIRS) study. *Clin Chem* 2005;51:1048–1052.
27. van Straalen JP, Leyte A, Weber JA, et al. Evaluation of the Hitachi 911 for routine urine analysis and for measurement of various special serum analytes. *Eur J Clin Chem Clin Biochem* 1995;33:315–322.
28. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care* 2005;28:2745–2749.
29. Yeap BB, Divitini ML, Gunton JE, et al. Higher ferritin levels, but not serum iron or transferrin saturation, are associated with type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis. *Clin Endocrinol (Oxf)* 2014;DOI: 10.1111/cen.12529.
30. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 2004;27:2422–2428.
31. Brudevold R, Hole T, Hammerstrom J. Hyperferritinemia is associated with insulin resistance and fatty liver in patients without iron overload. *PLoS One* 2008;3:e3547.
32. Hu G, Qiao Q, Tuomilehto J, et al. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med* 2004;164:1066–1076.
33. Abril-Ulloa V, Flores-Mateo G, Sola-Alberich R, et al. Ferritin levels and risk of metabolic syndrome: meta-analysis of observational studies. *BMC Public Healt* 2014;14:483.
34. Herbert V, Jayatilleke E, Shaw S, et al. Serum ferritin iron, a new test, measures human body iron stores unconfounded by inflammation. *Stem Cells* 1997;15:291–296.
35. Nielsen P, Günther U, Dürken M, et al. Serum ferritin iron in iron overload and liver damage: Correlation to body iron stores and diagnostic relevance. *J Lab Clin Med* 2000;135:413–418.
36. Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. *Blood* 1976;48:449–455.
37. Alfrey CP. Serum ferritin assay. *CRC Crit Rev Clin Lab Sci* 1978;9:179–208.
38. Adams PC, Barton JC. How I treat hemochromatosis. *Blood* 2010;116:317–325.
39. Lacher DA, Barletta J, Hughes JP. Biological variation of hematology tests based on the 1999–2002 National Health and Nutrition Examination Survey. *Natl Health Stat Report* 20121-10.
40. Gordeuk VR, Reboussin DM, McLaren CE, et al. Serum ferritin concentrations and body iron stores in a multicenter, multiethnic primary-care population. *Am J Hematol* 2008;83:618–626.

Address correspondence to:
Dr. Ronald T. Acton, PhD
2022 Brookwood Medical Center Drive, Suite 626,
Birmingham, AL 35209

E-mail: rtakma@bellsouth.net