Iron Deficiency in Patients With Nonalcoholic Fatty Liver Disease Is Associated With Obesity, Female Gender, and Low Serum Hepcidin

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BACKGROUND & AIMS: **Iron deficiency is often observed in obese individuals. The iron regulatory hormone hepcidin is regulated by iron and cytokines interleukin (IL) 6 and IL1β. We examine the relationship between obesity, circulating levels of hepcidin, and IL6 and IL1β, and other risk factors in patients with nonalcoholic fatty liver disease (NAFLD) with iron deficiency.**

METHODS: **We collected data on 675 adult subjects (>18 years old) enrolled in the Nonalcoholic Steatohepatitis Clinical Research Network. Subjects with transferrin saturation <20% were categorized as iron deficient, whereas those with transferrin saturation ≥20% were classified as iron normal. We assessed clinical, demographic, anthropometric, laboratory, dietary, and histologic data from patients, and serum levels of hepcidin and cytokines IL6 and IL1β. Univariate and multivariate analysis were used to identify risk factors for iron deficiency.**

RESULTS: **One-third of patients (231 of 675; 34%) were iron deficient. Obesity, diabetes, and metabolic syndrome were more common in subjects with iron deficiency ($P < .01$), compared with those that were iron normal. Serum levels of hepcidin were significantly lower in subjects with iron deficiency (61 ± 45 vs 81 ± 51 ng/mL; $P < .0001$). Iron deficiency was significantly associated with female gender, obesity, increased body mass index and waist circumference, presence of diabetes, lower alcohol consumption, black or American Indian/Alaska Native race ($P \leq .018$), and increased levels of IL6 and IL1β (6.6 vs 4.8 for iron normal, $P \leq .0001$; and 0.45 vs 0.32 for iron normal, $P \leq .005$).**

CONCLUSIONS: **Iron deficiency is prevalent in patients with NAFLD and associated with female gender, increased body mass index, and nonwhite race. Serum levels of hepcidin were lower in iron-deficient subjects, reflecting an appropriate physiologic response to decreased circulating levels of iron, rather than a primary cause of iron deficiency in the setting of obesity and NAFLD.**

Keywords: NASH CRN; BMI; NAFLD; Nutrition; Ferroportin; Inflammation.

Obesity and iron deficiency (ID) are considered the two most common nutritional disorders worldwide.[1] The association between obesity and iron status was first described by Wenzel and coworkers[2] in 1962, who noted that obese adolescents had lower serum iron compared with nonobese adolescents. A diet rich in carbohydrates and fats and poor in nutrients, such as iron, combined with a greater iron requirement in obese individuals may play a role.[3] However, Menzie and coworkers[4] evaluated the role of dietary factors in obese ID individuals and did not find an association between iron intake and ID. More recently, research has focused on the role of systemic, obesity-related, low-grade inflammation leading to ID by increased hepcidin expression.[5–7]

In response to increased iron stores, hepcidin, the iron regulatory hormone, binds and internalizes the cellular iron export protein ferroportin, thus downregulating iron efflux from the enterocyte, macrophage, and hepatocyte.[8] Conversely, decreased or deficient iron stores downregulate hepcidin to restore iron balance to

Abbreviations used in this paper: AI/AN, American Indian/Alaska Native; ALT, alanine transaminase; BMI, body mass index; DM, diabetes mellitus; ID, iron deficiency; IL, interleukin; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NASH CRN, NASH Clinical Research Network; TS, transferrin saturation.

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appropriate physiologic levels. Hepcidin expression is also increased in chronic inflammation, by inflammatory cytokines interleukin (IL) 6 and IL1β by signal transducers and activators of transcription 3.[9] Hepcidin is predominantly expressed in the liver, but also in subcutaneous and visceral adipose tissue, albeit at such a low level it may not contribute to systemic hepcidin levels.[6,10] Thus, the impact of obesity-induced hepcidin upregulation and the relationship between liver versus adipose-derived hepcidin and iron regulation in the setting of obesity is not well understood.

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the United States, with an estimated prevalence of 30% among US adults, and is associated with obesity, type 2 diabetes mellitus (DM), and metabolic syndrome (MS).[11] Up to a third of all patients with NAFLD progress to the more severe form called nonalcoholic steatohepatitis (NASH), characterized by hepatocellular ballooning, inflammation, and variable fibrosis.[12] We have previously shown, using a cutoff of serum ferritin 1.5 times the upper limit of normal, that there is an inverse relationship between body mass index (BMI) and serum iron studies in patients with NAFLD (ie, subjects with low transferrin saturation [TS] and low serum ferritin had significantly higher BMI).[13] We have also previously shown that subjects with hepatic iron staining had higher levels of serum hepcidin.[14] The goal of this study was to examine the relationship between circulating hepcidin level, obesity-induced systemic inflammation, and ID and to identify the prevalence of and associated risk factors for ID in patients with NAFLD.

Subjects and Methods

Subjects

A total of 675 adult (age >18 years) subjects enrolled in NASH Clinical Research Network (CRN) studies between October 2004 and February 2008, with biopsy-proved NAFLD (defined as >5% steatosis) and serum iron studies within 6 months of the liver biopsy were studied. The NASH CRN Database and PIVENS Trial inclusion/exclusion criteria have been reported elsewhere.[15,16] Demographic information including age, gender, ethnicity, and race and a detailed medical history including comorbidities, such as history of DM, hypertension, and hyperlipidemia, and menstrual history in women were obtained from patient interviews during screening. Dietary consumption of iron, vitamin C, tea and coffee, and supplemental vitamin C were determined from the Block 98 food frequency questionnaire (NutritionQuest, Berkeley, CA); alcohol consumption was determined from the Alcohol Use Disorders Identification Test Consumption questionnaires.[17] A complete physical examination including measurement of weight, height, and waist and hip circumference was obtained. Subjects with a BMI of $\geq$30 were defined as obese. ID was defined as TS (serum iron/total iron binding capacity) <20%, indicative of ID.[18,19] We also investigated the presence of ID anemia in our cohort using the criteria of serum ferritin <30, TS <20, and hemoglobin <12 in females and <13 in males; only 15 subjects met this criteria and therefore we did not analyze this subset separately. The prevalence of MS in this cohort was defined using the World Health Organization criteria. All subjects gave written informed consent and the study was approved by the institutional review board at each local site of the NASH CRN.

Serologic Data

Clinical laboratory data including hematologic, hepatic, and metabolic, lipid, and serum iron assessments were analyzed for subjects with values collected within 6 months of the liver biopsy. Serum hepcidin levels, available in 558 subjects, were determined by enzyme-linked immunosorbent assay (Intrinsic LifeSciences, San Diego, CA).[20] The lower limit of detection in this assay is 5 ng/mL. Eight subject values were below this limit and a value of 5 ng was imputed for the analysis. Plasma IL6 and IL1β levels, available in 371 and 242 subjects, respectively, were determined using Luminex technology and the human cytokine LINCOplex kit (Catalog number HCYTO-60K; Millipore, St. Charles, MO). The lower limit of detection for the assays was 0.79 and 0.19 pg/mL, respectively. Two subject IL6 values were below this limit and a value of 0.79 pg/mL was imputed for the analysis. Fifty-two IL1β values were below this limit including 13 ID subjects. A value of 0.19 pg/mL was imputed for the analysis.

HFE Genotyping

We examined the relationship between ID and the presence of mutations in the hemochromatosis gene *HFE*, which influence hepcidin production. Genotyping for the two common *HFE* mutations C282Y (rs1800562) and H63D (rs1799945) was performed using a real-time genotyping assay as previously described.[14] *HFE* genotyping data were available in 500 subjects.

Liver Histology

All patients underwent a liver biopsy, which was stained for hematoxylin-eosin, Masson trichrome, and Perls' iron stain. Histologic features of NAFLD and iron accumulation were assessed by the pathology committee of the NASH CRN in a centralized consensus review format, as previously described.[21] NAFLD activity score (range, 1–8) was tabulated by summing scores for steatosis, lobular inflammation, and ballooning degeneration.

Statistical Analysis

Data were compared using the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. Univariate logistic and stepwise forward multivariate logistic regression ($P < .20$ was used as a cutoff for incorporation into the model) were used to identify independent predictors for ID. Models were also created to identify risk factors in each gender. Differences in histologic features, such as fibrosis stage, steatosis, and lobular inflammation grade, were analyzed using ordinal regression. All analyses were performed using STATA (version 12; College Station, TX). Nominal, two-sided P values were used and were considered to be statistically significant if $P < .05$. No adjustments for multiple comparisons were made.

Results

Patient Characteristics

A total of 675 subjects (mean age, 48 ± 12 years) with biopsy-proved NAFLD (defined as >5% steatosis) and serum iron assessments within 6 months of their liver biopsy were evaluated in the present study. A total of 34% (231 subjects) were ID and 66% (444 subjects) were iron normal (TS ≥20%). Overall, most subjects in this study cohort were white (84%), obese (70%), and female (63%). Patient characteristics including clinical, demographic, racial, and specific dietary/behavioral factors thought to effect iron absorption, such as dietary iron, vitamin C, caffeine, and alcohol consumption, are summarized in Table 1. Subjects with ID were significantly more likely to be female, obese, and with greater waist circumference and BMI compared with iron-normal subjects. ID subjects were also more likely to have DM and MS. When we analyzed the presence of ID according to race, we found that the prevalence of ID was significantly increased among those with either black or American Indian/Alaska Native (AI/AN) race. Lower alcohol consumption was the only dietary factor associated with ID. There were no differences in the presence of the C282Y or H63D *HFE* hemochromatosis gene mutations between ID and iron-normal groups.

The proportion of subjects that were ID compared with iron normal, analyzed according to gender and obesity status, is shown in Figure 1. Women were much more likely to be ID compared with men (43% vs 20%; $P < .001$). Additionally, a greater proportion of women with ID were obese compared with men (35% vs 14%; $P < .001$). A higher percentage of women with regular periods were ID compared with postmenopausal women, although this difference was not statistically significant (51% vs 40%, respectively; $P = .15$). Obesity was more prevalent among ID women with regular periods compared with ID women without periods (46% vs 29%; $P = .042$).

Table 1. Patient Characteristics

Characteristic	Iron deficient	Iron normal	*P* value[a]
Number	231 (34)	444 (66)	
Age (*y*)	47.6 ± 11.7	48.0 ± 12.2	.96
Female	181 (78)	243 (55)	<.001
BMI (kg/m^2)	35.7 ± 6.9	33.1 ± 5.9	<.001
Obese (≥30 BMI)	182 (79)	291 (66)	<.001
Waist circumference	111 ± 15	107 ± 13	.008
DM	82 (36)	97 (22)	<.001
MS	167 (74)	283 (64)	.01
Race			
White	193 (84)	377 (85)	.65
Black	15 (6.5)	5 (1.1)	<.001
Asian	7 (3.0)	26 (5.9)	.13
AI/AN	22 (9.5)	21 (4.7)	.02
Native Hawaiian or Pacific Islander	0 (0)	8 (1.8)	.06
Other	21 (9.1)	37 (8.3)	.63
Ethnicity			.63
Non-Hispanic	203 (88)	384 (86)	
Hispanic	28 (12)	60 (14)	
No. of alcoholic drinks per week	0.36 ± 1.0	0.7 ± 1.5	.007
No. of coffee or tea drinks per day	7.8 ± 9.7	7.0 ± 8.8	.45
Dietary iron consumed (*mg/day*)	14.0 ± 9.1	14.1 ± 8.0	.61
Dietary vitamin C (*mg/day*)	109 ± 87	103 ± 76	.45
Supplemental vitamin C (*mg/day*)	163 ± 351	157 ± 355	.79
Presence of C282Y HFE mutations	19 (15)	40 (17)	.77
Presence of H63D HFE mutations	41 (27)	93 (32)	.33

NOTE. Values are n (%) or mean ± standard deviation.
[a]*P* values from Fisher exact test for categorical variables or Wilcoxon rank sum test for continuous variables.

Differences in Laboratory Tests Between Iron-Deficient and Iron-Normal Subjects

Significant differences in routine clinical laboratory assessments, serum iron studies, and proinflammatory cytokines IL6 and IL1β between the two groups of patients are shown in Table 2. ID subjects had lower alanine transaminase (ALT) and total and direct bilirubin levels, but higher ceruloplasmin and hemoglobin A_{1c} levels, aspartate aminotransferase/ALT ratios, and platelet counts. ID subjects had significantly lower hemoglobin, serum iron, ferritin, and TS and higher total iron-binding capacity, consistent with an iron-depleted state. Plasma levels of IL6 and IL1β were significantly increased in ID subjects compared with iron-normal subjects ($P \leq .0001$; $P \leq .008$; Table 2), suggesting systemic inflammation may be greater in ID compared with iron-normal subjects.

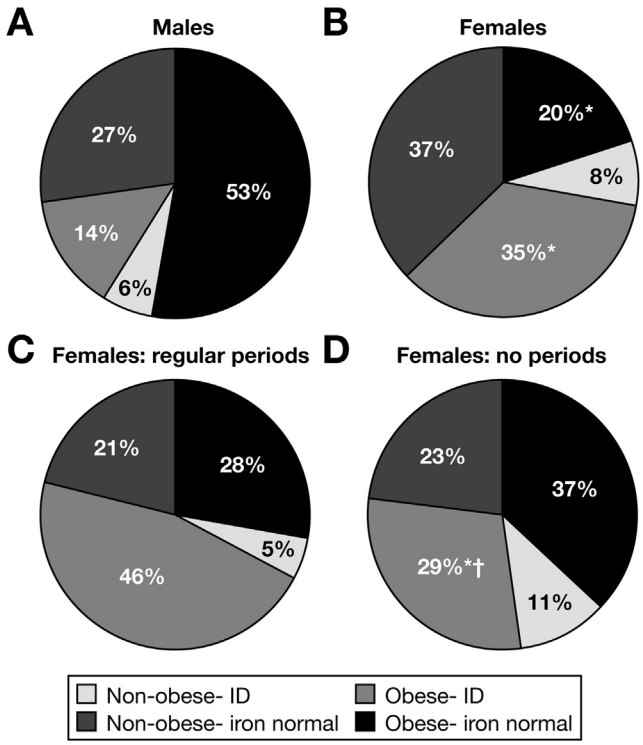

Figure 1. Proportion of subjects with ID and obesity according to (*A*) male gender, (*B*) female gender, (*C*) females with regular periods, and (*D*) females with no periods. The percentage of the total is labeled for each pattern. *$P < .001$ compared with males; †$P < .05$ compared with subjects with regular periods (Fisher exact test).

Relationship of Circulating Hepcidin Levels and Factors Associated With Iron Deficiency

Figure 2*A* shows the relative mean serum hepcidin level in ID subjects compared with iron-normal subjects stratified according to gender, menstrual status, or the presence of obesity, diabetes, and MS. Serum hepcidin levels were lower in ID subjects compared with iron-normal subjects ($P < .05$), except in males. Women who reported having regular periods showed the largest difference in serum hepcidin (−39%; 41 vs 67 ng/mL; $P = .03$) between ID and iron-normal subjects, respectively, and the lowest mean levels among all groups, reflecting the effect of blood loss on circulating hepcidin levels.

We compared serum hepcidin levels in obese versus nonobese ID patients, stratified according to gender and menstrual status (Figure 2*B*). In each group, nonobese ID subjects had 13%–14% lower serum hepcidin levels compared with obese ID subjects. We also observed that obese ID women with regular periods had significantly lower serum hepcidin levels compared with obese ID women with no periods (42 vs 64 ng/mL, respectively; $P = .006$), suggesting iron loss effectively reduces serum hepcidin even in the setting of obesity.

Table 2. Laboratory Value Differences Among Iron-Deficient and Normal Patients

Variable[a]	Iron deficient	Iron normal	*P* value[b]
ALT (*U/L*)	58 (36–80)	67 (44–101)	.0004
AST/ALT	0.81 (0.66–1.0)	0.70 (0.54–0.91)	<.0001
Direct bilirubin (*mg/dL*)	0.1 (0.1–0.1)	0.1 (0.1–0.2)	.0001
Total bilirubin (*mg/dL*)	0.5 (0.4–0.8)	0.7 (0.5–1.0)	<.0001
Hemoglobin A_{1c} (%)	5.8 (5.4–6.4)	5.6 (5.3–6.2)	.002
Platelets (*K/cmm*)	259 (204–308)	235 (195–274)	.0001
Ceruloplasmin (*mg/dL*)	31 (27–38)	28 (23–35)	.008
Hemoglobin (*g/dL*)	13.9 (13.0–14.7)	14.6 (13.7–15.6)	<.0001
Serum iron (*μg/dL*)	59 (50–70)	103 (86–123)	<.0001
Total iron-binding capacity (*μg/dL*)	399 (363–437)	347 (309–389)	<.0001
TS (iron/TIBC)	0.16 (0.12–0.18)	0.28 (0.24–0.36)	<.0001
Serum ferritin (*ng/mL*)	92 (47–155)	219 (103–361)	<.0001
IL6	6.6 (4.4–10.3)	4.8 (3.1–7.9)	.0001
IL1*β*	0.45 (0.25–0.83)	0.32 (0.19–0.59)	.009

AST, aspartate aminotransferase; TIBC, total iron-binding capacity.
[a]Values are medians (interquartile range).
[b]*P* values from Wilcoxon rank sum test.

Histologic Differences Between Groups

No significant difference in mean grade of steatosis (range, 0–3; 1.7 ± 0.9 vs 1.8 ± 0.9), lobular inflammation (range, 0–3; 1.6 ± 0.7 vs 1.6 ± 0.7), portal inflammation (range, 0–2; 1.1 ± 0.6 vs 1.0 ± 0.6), ballooning (range, 0–2; 1.1 ± 0.9 vs 1.1 ± 0.9), fibrosis (range, 0–4; 1.5 ± 1.3 vs 1.6 ± 1.3), or NAFLD activity score index (range, 0–8; 4.4 ± 1.7 vs 4.5 ± 1.6) was noted between the ID and iron-normal subjects, respectively.

Factors Associated With the Presence of Iron Deficiency

Logistic regression analysis was performed to investigate potential risk factors for ID in this population (Table 3). ID was associated with female gender, obesity, increased BMI and waist circumference, presence of DM or MS, fewer alcoholic drinks consumed per week, and black and AI/AN race in univariate analysis. To investigate the relationship between the combined effects of these factors on ID, stepwise multivariate logistic regression modeling was performed with ID as the dependent variable and each of the variables significantly associated with ID on univariate analysis as independent variables; a $P < .20$ was used as a cutoff for incorporation into the model. Overall, female gender, increased BMI, black and AI/AN race, and decreased ALT and serum hepcidin were independently associated with ID. In addition, multivariate regression analysis was performed in men and women as two separate groups. BMI, ALT, serum hepcidin, and AI/AN race were independently associated with ID in women, whereas in men only waist circumference was independently associated with ID. Potential factors that could explain the ID phenotype in the black and AI/AN race are shown in Table 4. The only significant difference between black

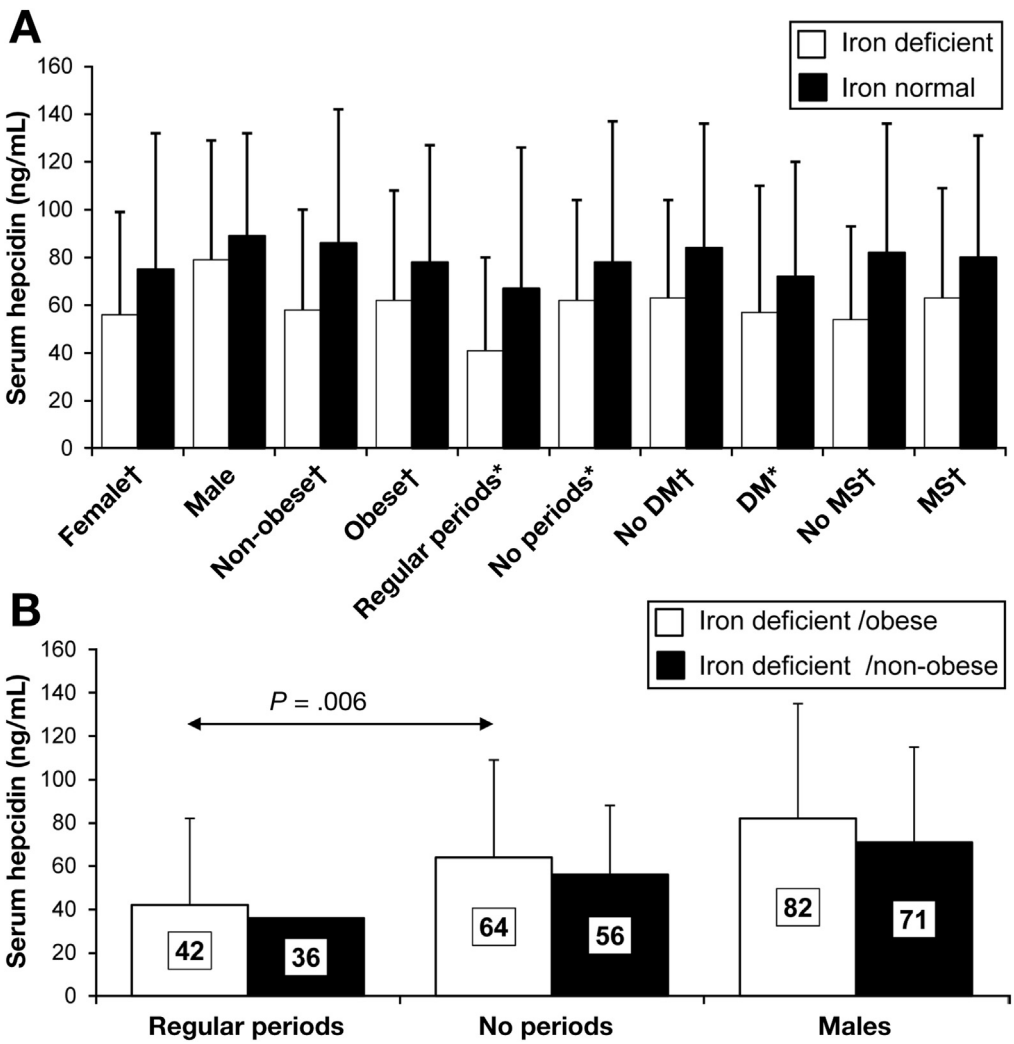

Figure 2. (*A*) Comparison of the mean serum hepcidin values of ID vs iron-normal NAFLD subjects according to gender, menstrual status, presence of obesity, diabetes, and MS. $^{\dagger}P < .001$; $^{*}P < .05$ (Wilcoxon rank sum test). (*B*) Comparison of the mean serum hepcidin values of ID NAFLD subjects according to gender, presence of obesity, and menarche. Standard deviations are indicated by the error bars.

subjects was that none of the ID black subjects took supplemental vitamin C, which increases intestinal iron absorption ($P = .02$). AI/AN ID subjects had increased BMI and waist circumference and were more likely to be obese compared with the AI/AN iron-normal subjects ($P < .05$).

Discussion

ID is common in obese children and adults; the cause of this association is not entirely clear and has been linked to several factors.[2–7] In the present study we found that one-third of adult NAFLD subjects were ID as defined by TS <20%, and that female gender, obesity (BMI ≥30), type 2 diabetes, and MS were more common in this cohort. We also found that alcohol consumption is protective against ID, consistent with our previous data from a population-based study.[22] Alcohol has been shown to decrease hepcidin expression, which could lead to increased iron absorption and recycling.[23] Both in vitro and human studies have described that alcohol suppressed hepcidin transcription in the liver and may protect from ID.[24] However, alcohol intake was unlikely to be a major factor on hepcidin levels in this study, given that excessive alcohol consumption was an exclusion criterion for all NASH CRN studies.[15,16] There were no differences in the presence of C282Y or H63D *HFE* hemochromatosis gene mutations between ID and iron-normal groups, which is in agreement with our previous report in a related cohort showing that heterozygous C282Y or H63D subjects did not have statistically different levels of serum iron parameters compared with wild-type subjects.[14] No other dietary factor was associated with ID, including amount of dietary iron or supplemental and dietary vitamin C or caffeine consumption. In addition, we found that ID was associated with black or AI/AN race. When each gender was considered separately, in women only BMI and AI/AN race, and only waist circumference in men, were independent predictors of ID on multivariate logistic regression. Obesity was more prevalent among the ID women with regular menses compared with ID women with amenorrhea. This was an interesting observation with no ready explanation and we speculate that amenorrhea secondary to polycystic ovarian syndrome may be contributing because polycystic ovarian syndrome is associated with increased insulin resistance.

Table 3. Independent Predictors of ID on Univariate[a] and Multivariate[b] Logistic Regression Modeling

	OR	95% CI	*P* value
Univariate analysis			
Female gender	2.99	2.08–4.31	<.001
Obesity	1.99	1.37–2.90	<.001
Diabetes present	1.97	1.39–2.80	<.001
BMI (*kg/m²*)	1.07	1.04–1.09	<.001
Hepcidin (*ng/mL*)	0.99	0.99–1.00	<.001
ALT (*U/L*)	0.99	0.99–1.00	<.001
Black race	6.10	2.19–17.00	.001
Waist circumference	1.02	1.01–1.03	.001
Alcohol consumption	0.79	0.66–0.93	.005
MS	1.59	1.11–2.27	.011
AI/AN	2.12	1.14–3.94	.018
Model 1 (all subjects)			
Female gender	2.23	1.43–3.48	<.001
BMI (*kg/m²*)	1.07	1.03–1.10	<.001
AI/AN	3.44	1.60–7.41	.002
Hepcidin (*ng/mL*)	0.99	0.99–1.00	.003
ALT (*U/L*)	0.99	0.99–1.00	.005
Black race	3.54	1.12–10.81	.027
Model 2 (female subjects)			
BMI mean (*kg/m²*)	1.06	1.02–1.10	.002
Hepcidin (*ng/mL*)	0.99	0.99–1.00	.006
ALT (*U/L*)	0.99	0.99–1.00	.009
AI/AN	3.45	1.26– 9.42	.016
Model 3 (male subjects)			
Waist circumference	1.04	1.01–1.07	.005

[a]Univariate logistic regression modeling was performed using presence of ID as the dependent variable.
[b]Stepwise multivariate logistic regression modeling was performed with ID as the dependent variable and each of the variables significantly associated with ID in univariate analysis; $P < .20$ was used as a cutoff for incorporation into the model.

Table 4. Potential Factors Associated With ID in Black or AI/AN Race

	Iron deficient	Iron normal	*P* value[a]
Black race			
Number	15 (75)	5 (25)	
Female gender	13 (87)	5 (100)	1.0
Obesity	15 (100)	4 (80)	.25
Diabetes present	8 (53)	3 (60)	1.0
BMI (*kg/m²*)	38.8 ± 6.4	34.4 ± 5.8	.089
Waist circumference	114 ± 17	107 ± 14	.52
No. of alcoholic drinks per week	0.1 ± 0.17	0 ± 0	.21
Dietary iron consumed (*mg/day*)	11.3 ± 8.5	14.1 ± 8.3	.38
Dietary vitamin C (*mg/day*)	134 ± 157	108 ± 58	.51
Supplemental vitamin C (*mg/day*)	0 ± 0	140 ± 269	**.02**
AI/AN			
Number	22 (51)	21 (49)	
Female gender	16 (73)	12 (57)	.35
Obesity	19 (86)	12 (57)	**.045**
Diabetes present	8 (36)	4 (19)	.31
BMI (*kg/m²*)	36.6 ± 4.3	32.3 ± 6.0	**.009**
Waist circumference	113 ± 13	103 ± 12	**.016**
No. of alcoholic drinks per week	0.14 ± 0.18	0.63 ± 1.9	.98
Dietary iron consumed (*mg/day*)	13.0 ± 6.2	15.4 ± 6.3	.24
Dietary vitamin C (*mg/day*)	130 ± 153	117 ± 60	.65
Supplemental vitamin C (*mg/day*)	118 ± 263	65 ± 154	.38

NOTE. Values are n (%) or mean ± standard deviation. Values in bold indicate statistical significance.
[a]*P* values from Fisher exact test for categorical variables or Wilcoxon rank sum test for continuous variables.

We found that overall, serum hepcidin was lower in ID subjects compared with iron-normal subjects, despite increased cytokine levels suggesting the presence of chronic inflammation, which would have been expected to increase serum hepcidin. These findings are in agreement with our previous report that subjects with hepatic iron staining had higher levels of serum hepcidin than subjects without stainable hepatic iron.[14] Although it is possible that obesity-induced inflammation-mediated hepcidin upregulation would contribute to the onset of ID among obese people, the decreased circulating hepcidin we observed in our ID subjects in comparison with the iron-normal subjects reflects an appropriate physiologic response to current body iron stores; when the body iron stores are low, hepcidin production is decreased, resulting in increased intestinal iron absorption and macrophage iron release. These data suggest that body iron stores rather than chronic inflammation are the primary signal mediating hepcidin levels in our ID NAFLD subjects.

Our data are in agreement with several animal studies suggesting ID, induced by either phlebotomy treatment[25,26] or dietary insufficiency,[26,27] causes down-regulation of hepcidin even in the presence of experimentally induced inflammation. In an elegant study by Theurl and coworkers,[26] these authors investigated hepcidin signaling in rat models of anemia of chronic disease (high hepcidin) and anemia of chronic disease with true ID (anemia of chronic disease/ID; low hepcidin levels) induced by either phlebotomy treatment or dietary iron insufficiency. Despite similarly high levels of signal transducers and activators of transcription 3, the inflammatory pathway hepcidin transactivator, in both models, the anemia of chronic disease/ID rats had decreased hepcidin transcription. This effect was mediated by inhibition of bone morphogenic protein-6 expression and reduced phosphorylation and trafficking of SMAD1/5/8. Taken together, these and other data and our results indicate that hepcidin regulation in the presence of multiple divergent signals in vivo may be determined by the most urgent physiologic needs and strength of the signal within the organism rather than a set hierarchy of hepcidin regulatory signals.[7,26,28]

Body iron stores and increased serum ferritin levels have been associated with more severe NAFLD.[13,21] In this study we did not observe any significant difference in histologic features of NASH between ID and iron-normal NAFLD patients. However, subjects with ID had

significantly lower ALT levels even after adjusting for age, gender, BMI, DM, and NAFLD activity score (data not shown).

An interesting observation in the current study was that ID was more prevalent among subjects self-described as black or AI/AN race. When we further investigated the presence of additional risk factors for ID in these subjects we found that AI/AN ID subjects had increased BMI and waist circumference and were more likely to be obese compared with the AI/AN iron-normal subjects. We also found that none of the black ID subjects took supplemental vitamin C, compared with mean consumption of 140 $\pm$ 269 mg/day in black iron-normal subjects. However, we interpret these findings with caution given the small number of subjects with these racial backgrounds.

Obesity and NAFLD are inflammatory conditions; we cannot distinguish the source of inflammation and whether there was an independent effect of obesity on hepcidin level, because all patients in the NASH CRN database by definition had NAFLD. Studies have shown that NAFLD patients have significantly more colorectal neoplasia and early colorectal cancer compared with those without NAFLD.[29] We did not have data available on screening for colorectal neoplasia; thus, the potential of underlying gastrointestinal bleed from undetected colorectal neoplasia may potentially be a cause for ID.

In conclusion, our study shows that ID is common in NAFLD patients. Circulating serum hepcidin levels in NAFLD patients with ID are low, reflecting an appropriate physiologic response of hepcidin signaling to ID. Obesity and female gender are likely the most important risk factors of ID in NAFLD subjects; however, alcohol consumption, presence of diabetes, and potentially racial background may also be contributing factors. We conclude that increased hepcidin as a result of obesity-induced systemic inflammation may initially contribute to ID, but after ID is established hepcidin is appropriately downregulated.

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A list of the members of the Nonalcoholic Steatohepatitis Clinical Research Network can be found in the Appendix to this article.

Conflicts of interest
The authors disclose no conflicts.

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Appendix

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