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Iron Depletion by Phlebotomy Improves Insulin Resistance in Patients With Nonalcoholic Fatty Liver Disease and Hyperferritinemia: Evidence from a Case-Control Study

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OBJECTIVES: Hyperferritinemia is frequently observed in nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome characterized by hepatic insulin resistance and considered high cardiovascular risk. Iron depletion by phlebotomy has been reported to decrease insulin resistance in NAFLD in small, uncontrolled studies. Aims of this study were to define the relationship between ferritin and iron stores in patients with NAFLD, the effect of iron depletion on insulin resistance, and whether basal ferritin levels influence treatment outcome.

METHODS: Subjects were included if ferritin and/or ALT were persistently elevated after 4 months of standard therapy. Sixty-four phlebotomized subjects were matched 1:1 for age, sex, ferritin, obesity, and ALT levels with patients who underwent lifestyle modifications only. Insulin resistance was evaluated by insulin levels, determined by RIA and the HOMA-R index, at baseline and after 8 months.

RESULTS: Baseline ferritin levels were associated with body iron stores ($P < 0.0001$). Iron depletion produced a significantly larger decrease in insulin resistance ($P = 0.0016$ for insulin, $P = 0.0042$ for HOMA-R) compared with nutritional counseling alone, independent of changes in BMI, baseline HOMA-R, and the presence of the metabolic syndrome. Iron depletion was more effective in reducing HOMA-R in patients in the top two tertiles of ferritin concentrations ($P < 0.05$ *vs* controls), and in carriers of the mutations in the *HFE* gene of hereditary hemochromatosis ($P < 0.05$ *vs* noncarriers).

CONCLUSIONS: Given that phlebotomy reduces insulin resistance, which is associated with liver tissue damage, future studies should evaluate the effect of iron depletion on liver histology and cardiovascular end points.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) affects 20% of the U.S. population (1), and confers a higher risk of type 2 diabetes and cardiovascular diseases (2–4). The disease is characterized by hepatic insulin resistance and is now considered the hepatic expression of the metabolic syndrome (5–7). Although a hypocaloric diet and exercise have proven beneficial (8), a proportion of patients displays high liver enzymes and insulin resistance despite lifestyle modifications and pharmacological therapy with insulin sensitizing agents (9), evaluated in a few relatively small studies (10–12).

Hyperferritinemia associated with mild hepatic iron accumulation is observed in about one-third of NAFLD cases (13–15), and a condition known as insulin resistance-associated hepatic iron overload (IR-HIO) has been described (16). In IR-HIO, iron overload occurs in both hepatocytes and, unlike in hereditary hemochromatosis, sinusoidal cells (17), reflecting both acquired and genetic factors (18).

Higher iron stores are being investigated as risk factors for cardiovascular disease (19). Evidence is accumulating in ethnically different populations that serum ferritin and body iron excess are risk factors for the metabolic syndrome (20–22), and an association among ferritin, iron intake, and diabetes has been reported in Chinese women (23). A reciprocal influence between iron metabolism and insulin has been suggested.

Insulin stimulates ferritin synthesis and facilitates iron uptake (24), and conversely, iron influences insulin signaling (25), reduces the hepatic extraction and the metabolism of

insulin, leading to peripheral hyperinsulinemia (26), and may increase the cellular oxidative stress, inhibiting the internalization and the actions of insulin (27).

Following this last hypothesis (24), a few studies have evaluated the effect of iron depletion by phlebotomy on glucose metabolism. Phlebotomy treatment was well tolerated (28), and was reported to improve insulin resistance in a small number of patients without iron overload affected by NAFLD and either impaired (29) or normal (30) glucose tolerance. In addition, iron depletion ameliorated metabolic control in subjects with type 2 diabetes associated with hyperferritinemia (31). However, these studies lacked adequate controls and, due to the limited sample size, they did not assess the effect of basal iron status and *HFE* genotype on metabolic outcomes.

The aim of this study was to analyze the relationship between basal ferritin and body iron stores in patients with NAFLD, the effect of iron depletion by phlebotomy on insulin resistance, evaluated by the HOMA-R index (32), and to define whether basal iron stores affect treatment outcome.

METHODS

Patients

One hundred twenty-eight patients, out of a larger series of 347 NAFLD patients referred to three liver units of Northern Italy between September 2003 and September 2005, were included in this study. Because we previously showed in patients with NAFLD that ALT and serum ferritin levels evaluated at the time of biopsy were independent predictors of fibrosis and more severe liver damage (14, 18), patients were considered eligible in the presence of hyperferritinemia and/or increased liver enzymes (ALT), which persisted abnormal after 4 months of a hypocaloric diet and aerobic exercise with/without pharmacological therapy (vitamin E or other antioxidants). The diagnosis of NAFLD was based on histology (N = 105, 82%) and/or on ultrasonographic findings, in the presence of insulin resistance and/or dyslipidemia. In all cases, alcohol intake was <20 g/day, confirmed by at least one family member, and Wilson's disease, alpha1-antitrypsin deficiency, and autoimmune hepatitis were also ruled out. We excluded patients with hereditary hemochromatosis (*HFE* C282Y +/+), hypobetalipoproteinemia, overt diabetes requiring pharmacological treatment, cardiopathy, and hematological and inflammatory diseases.

Study Design

The study design is depicted in Figure 1. According to an agreed protocol, all NAFLD cases initially receive counseling for nutrition and physical activity after a diagnostic screening, and specific treatments are delayed by 4–6 months to explore the beneficial effects of lifestyle modifications. Counseling is provided by an expert nutritionist; subjects are advised to follow a hypocaloric (in the presence of overweight or obesity), low-lipid diet, to avoid refined carbohydrates, and to increase fiber intake. They are also advised to practice aerobic physical exercise (at least 20 min, three times per week).

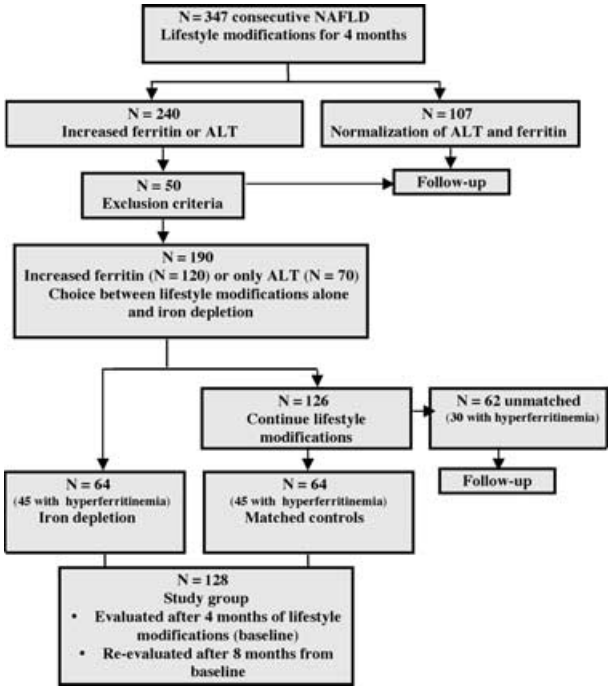

Figure 1. Study design.

Follow-up visits for weight measurement and counseling are scheduled monthly for obese subjects.

After 4 months, we offered phlebotomy treatment until iron depletion to the 190 patients with persistently elevated ALT or ferritin levels who did not meet the exclusion criteria (N = 50). Excluded were patients with pharmacologically treated diabetes (N = 22), C282Y +/+ hereditary hemochromatosis (N = 12), ischemic heart disease or other cardiopathy (N = 8), hypobetalipoproteinemia (N = 3), anemia (N = 3), and rheumatological diseases (N = 2). The protocol was approved by the Senior Staff Committee of our institutions, a board comparable to an Institutional Review Board. All patients gave written informed consent to data collection; 64 consented also to phlebotomy and were iron depleted. Iron depletion was achieved by removing 350 cc of blood every 10–15 days according to baseline hemoglobin values and venesection tolerance, until ferritin was <80 ng/mL in the presence of normal or low transferrin saturation. Weekly phlebotomies were allowed for carriers of the C282Y *HFE* mutation. Maintenance phlebotomies (as many as required) were then instituted to keep iron stores depleted (ferritin <100 ng/mL and transferrin saturation ≤35%). Before starting treatment, all patients underwent ECG and echocardiography (in the presence of hyperglycemia or hypertension), which were normal. For each iron-depleted patient, the best possible match for sex, age (±5 yr), serum ferritin (<300, 300–600, >600 ng/mL), presence of obesity (body mass index [BMI] > or < 30 kg/m^2), and ALT levels > or < 1.5 upper normal values (UNV) was chosen among the 126 patients with NAFLD resistant to 4 months of lifestyle modification who did not accept phlebotomy. Both groups continued diet, exercise, and

Table 1. Demographic, Anthropometric, and Clinical Features of 64 Patients With NAFLD Submitted to Phlebotomy and 64 Matched Controls

	No Phlebotomy	Phlebotomy
M/F (%)	53/11 (83/17)	53/11 (83/17)
Age (yr)	50.6 ± 12.8	49.3 ± 11.6
BMI (<25 kg/m^2)	27.3 ± 3.7	26.8 ± 2.8
Abdominal circumference M (<102/88 cm M/F)	99.7 ± 10.8	96.8 ± 10.4
Hypertension (%)	23 (36)	27 (42)
LDL (<100 mg/dL)	137.9 ± 42 (85)	139.3 ± 44 (89)
HDL (>55/65 M/F mg/dL)	47.6 ± 14	42.1 ± 12
Triglycerides (<160 mg/dL)	138.2 ± 73	136 ± 71
Uric acid (<6/5.6 mg/dL M/F)	5.7 ± 1.2	5.5 ± 1.2
Glucose (<100 mg/dL)	93.8 ± 17	98.4 ± 16
Fasting insulin (<15 mIU/L)	14.9 ± 8	19.9 ± 11
HOMA-R (<2.7)	3.51 ± 2.3*	4.81 ± 3*
Metabolic syndrome	18 (29)	26 (41)
AST (<40 IU/L)	33.4 ± 17	33.4 ± 18
ALT (<42 IU/L)	52.1 ± 33	57.9 ± 44
GGT (IU/L)	56.2 ± 67	47.9 ± 49
Ferritin† (<320/240 M/F ng/mL)	387 {197–606}	438 {212–628}
Transferrin saturation %	34.9 ± 12	39.2 ± 15.6
HFE mutation status‡		
C282Y +	3/52* (6)	11/54* (20)
H63D +	15/52 (29)	19/54 (39)
Presence of fibrosis§	12 (19)	12 (19)

Note that all subjects had counseling for lifestyle modifications as standard treatment.
Normal values are in parentheses.
(): % abnormal values.
{}: Interquartile range.
* $P < 0.05$.
†Median value.
‡Available for 96 patients from the Milano center who agreed to undergo genotypic analysis.
§In 102 patients submitted to biopsy.

any ancillary pharmacological therapy during the study period. Liver enzymes, HOMA-R index, and serum lipids were evaluated at baseline (after 4 months of diet and exercise) and re-evaluated after 8 months in all subjects. Baseline clinical features of patients and controls are shown in Table 1. The two groups were similar for sex distribution, age, body weight, lipid parameters, glucose levels, and liver enzymes. Phlebotomized subjects had a significantly higher HOMA-R index and a higher prevalence of the C282Y *HFE* heterozygous mutation compared with controls.

Assays

Glucose and lipid parameters were determined by standard laboratory techniques. Serum insulin was determined by radio-immune assay (Biochem Immunosystems, Bologna, Italy), and insulin resistance was estimated by the homeostatic metabolic assessment insulin resistance index (HOMA-R) (32). All analyses were performed in duplicate. Hepatic iron stores were determined by atomic absorption spectrometry, and the amount of iron mobilized by quantitative phlebotomy (28). *HFE* genotype was determined from DNA obtained from peripheral blood by restriction analysis, as previously described (33).

The prevalence of the metabolic syndrome was assessed in the whole series according to the criteria proposed by the National Cholesterol Education Program – Adult Treatment Panel III (34).

Statistical Analysis

Data are expressed as mean ± standard deviation except for serum ferritin for which the median and interquartile range are shown, and differences between groups were tested for significance by unpaired Student's *t*-test (2-tailed) and correlated by Pearson's correlation. Logistic regression analysis was performed to adjust the association between the treatment group and the study outcome (decrease in insulin resistance) for confounding factors. Results were considered significant when $P < 0.05$ (2-tailed). The Bonferroni's method was used to correct for multiple comparisons the significance level of treatment effect on the prespecified study end points (changes in HOMA-R, insulin, serum ferritin, ALT levels, HDL cholesterol, and triglycerides) and the correlations between the considered variables (all the variables tested). Analyses were carried out with JMP 5.1 statistical analysis software (SAS Institute Inc., Cary, NC).

RESULTS

Effect of Iron Depletion on Hematological Parameters, Iron Status, and Body Weight

To reach iron depletion, patients submitted to venesection removed 2.46 ± 1.48 g of iron in the course of 9.3 ± 6.8 (median 9, range 3–28) phlebotomies. Iron removed was significantly correlated with the hepatic iron concentration as evaluated by atomic absorption spectrometry and/or biomagnetic liver susceptometry (SQUID) (performed in 20 subjects submitted to iron depletion, $R^2 = 0.7$, $P < 0.0001$). Hemoglobin values dropped from 15.1 ± 1.1 g/L (range 12.3–17.6) to 13.8 ± 1 (range 11–15.7, evaluated 3 days after the last venesection) ($P < 0.0001$). Values in the lowest range were observed in two subjects with a beta-thalassemia trait. No adverse events were observed during treatment; in particular, no cases had syncope, angina, cardiovascular events, increase in blood urea, and creatinine levels. Mild dizziness and easy fatigability were reported for less than 24 h after the first phlebotomy in six cases.

As expected, phlebotomy to iron depletion resulted in a significant decrease in serum ferritin and transferrin saturation values when compared with continuing standard therapy alone (Table 2), which *per se* produced a modest decrease in ferritin levels.

Iron depletion did not influence body weight loss during treatment, and did not further ameliorate dyslipidemia, as evaluated by fasting LDL cholesterol, HDL cholesterol, and triglyceride concentrations compared with lifestyle modification alone; it also did not further improve liver enzymes and systolic and diastolic arterial pressure values (evaluated at least 2 wk after the last venesection).

Table 2. Effect of Therapy on Metabolic Features and Liver Enzymes

	No Phlebotomy		Phlebotomy		
	Variation*	Value at 1 Yr	Variation*	Value at 1 Yr	P §
BMI (kg/m^2)	−1.4 ± 1.4	25.8 ± 3.7†	−0.9 ± 1.3	25.9 ± 3.0†	0.1
Ferritin (ng/mL)	−59 {−10; −168}	308†‡ {194–454}	−363 {−158; −577}	52†‡ {27–96}	<0.0008
Transferrin sat %	+0.9 ± 12.3	35.8 ± 13.2	−15.1 ± 15.5	25.0 ± 8.7†	<0.0008
Glucose (mg/dL)	−2.4 ± 16.4	91.2 ± 15	−5.9 ± 13.4	92.5 ± 11†	ns
Fasting insulin (mIU/L)	+0.52 ± 8.2	15.4 ± 9.9	−6.23 ± 11.5	13.7 ± 8.3†	0.0016
HOMA-R	+0.04 ± 2.7	3.55 ± 2.8	−1.69 ± 2.9	3.12 ± 1.9†	0.0042
Triglycerides (mg/dL)	−25.1 ± 47	110.4 ± 40†	−30.6 ± 54	105.3 ± 64†	ns
LDL (mg/dL)	−19.0 ± 37	123.5 ± 29†	−20.8 ± 31	119.9 ± 32†	ns
HDL (mg/dL)	+5.6 ± 9.8	53.2 ± 15.2†	+8.1 ± 13.4	49.9 ± 15†	ns
ALT (IU/L)	−17.3 ± 29	34.8 ± 17†	−23.8 ± 43	34.3 ± 27†	ns

*Variation stands for final minus basal level of the considered variables.
†$P < 0.05$ *versus* baseline values.
‡$P < 0.05$ for values at the end of the study.
§Corrected P for variation between patients treated with behavioral therapy alone or in combination with phlebotomy untill iron depletion; between final values in phlebotomized and nonphlebotomized subjects.

Effect of Iron Depletion on Insulin Resistance and Glucose Metabolism

At the end of treatment, serum glucose and insulin levels and the HOMA-R index were significantly decreased in iron-depleted subjects but not in controls (Table 2). Consequently, the changes in insulin and HOMA-R values were significantly larger in phlebotomized patients ($P = 0.0042$). No significant differences in ALT changes were observed between the two treatment groups.

To analyze whether the effect of iron depletion on insulin resistance was influenced by baseline HOMA-R, the effect of phlebotomy was analyzed separately in patients with baseline values higher and lower than 3.4 (median value of the entire population).

Iron-depleted patients had a larger decrease HOMA-R after therapy compared with controls both when starting from high HOMA-R (−2.61 ± 3.5 *vs* −0.56 ± 3.8, $P = 0.03$) and from low HOMA-R (−0.51 ± 1.1 *vs* +0.51 ± 1.4, $P = 0.002$). Iron depletion was also associated with a larger decrease in insulin values in both groups (high HOMA-R: −9.7 ± 14 *vs* −1.8 ± 9.9, $P = 0.009$; low HOMA-R: −1.7 ± 4 *vs* +2.3 ± 6, $P = 0.002$).

The decrease in HOMA-R was larger in phlebotomized *versus* not phlebotomized subjects, both in subjects with (−1.89 ± 2.2 *vs* +0.51 ± 3.5, $P = 0.006$) and without (−1.55 ± 3.3 *vs* −0.2 ± 2.4, $P = 0.02$) the metabolic syndrome, and similar differences were observed in insulin concentrations (not reported in detail).

To analyze whether the biochemical alterations at baseline influenced the outcome, the effect of phlebotomy on insulin resistance was analyzed separately in patients with both increased ferritin and ALT (N = 52), increased ferritin alone (N = 38), and increased ALT alone (N = 38). The decrease in HOMA-R remained significantly higher in phlebotomized *versus* nonphlebotomized subjects with both increased ferritin and ALT (−2.67 ± 3.2 *vs* −0.48 ± 4.1, $P = 0.03$), increased ferritin alone (−1.1 ± 1.8 *vs* 0.59 ± 1.4, $P = 0.008$), and increased ALT alone (−1.33 ± 3.1 *vs* +0.11 ± 1.8, $P = 0.04$). Similar differences were observed in insulin concentrations (not reported in detail).

Correlation Between Variation in Iron Status and Metabolic Parameters

In the whole group of patients, BMI changes during the treatment period were positively correlated with changes in insulin, HOMA-R ($R^2 = 0.05$, $P = 0.072$), AST ($R^2 = 0.06$, $P = 0.048$), ALT ($R^2 = 0.08$, $P = 0.008$), and GGT ($R^2 = 0.13$, $P < 0.0008$).

Transferrin saturation varied during treatment in correlation with ferritin changes in phlebotomized and nonphlebotomized subjects ($R^2 = 0.06$, $P = 0.3$ and $R^2 = 0.13$, $P = 0.024$, respectively). In the overall series, the changes in transferrin saturation and ferritin levels correlated with changes in insulin and HOMA-R (transferrin saturation: $R^2 = 0.1$, $P = 0.003$ with HOMA-R and $R^2 = 0.12$, $P < 0.0008$ with insulin; ferritin: $R^2 = 0.17$, $P < 0.0008$ with HOMA-R and $R^2 = 0.19$, $P < 0.0008$ with insulin).

Effect of Therapy According to Basal Ferritin Values and HFE *Status*

We next analyzed the effect of adding phlebotomy to standard therapy in patients subdivided according to baseline ferritin tertiles (Fig. 2). The amount of iron removed to reach depletion in phlebotomized subjects significantly increased with increasing ferritin tertile, suggesting that basal ferritin at least partially reflects iron stores (I tertile 1.74 ± 0.9 g, II tertile 2.26 ± 1 g, III tertile 3.42 ± 1.2 g, $P < 0.0001$). Despite BMI loss being more marked in controls, in patients submitted to phlebotomy we observed a larger decrease in insulin and HOMA-R in the second and third ferritin tertiles. Phlebotomized patients included in the third ferritin tertile also had a larger decrease in ALT *versus* nonphlebotomized patients. The percentage decrease in HOMA-R values according to the ferritin tertile is shown in Figure 3.

Patients carrying *HFE* mutations showed a more marked improvement in insulin and HOMA-R, but not in BMI and

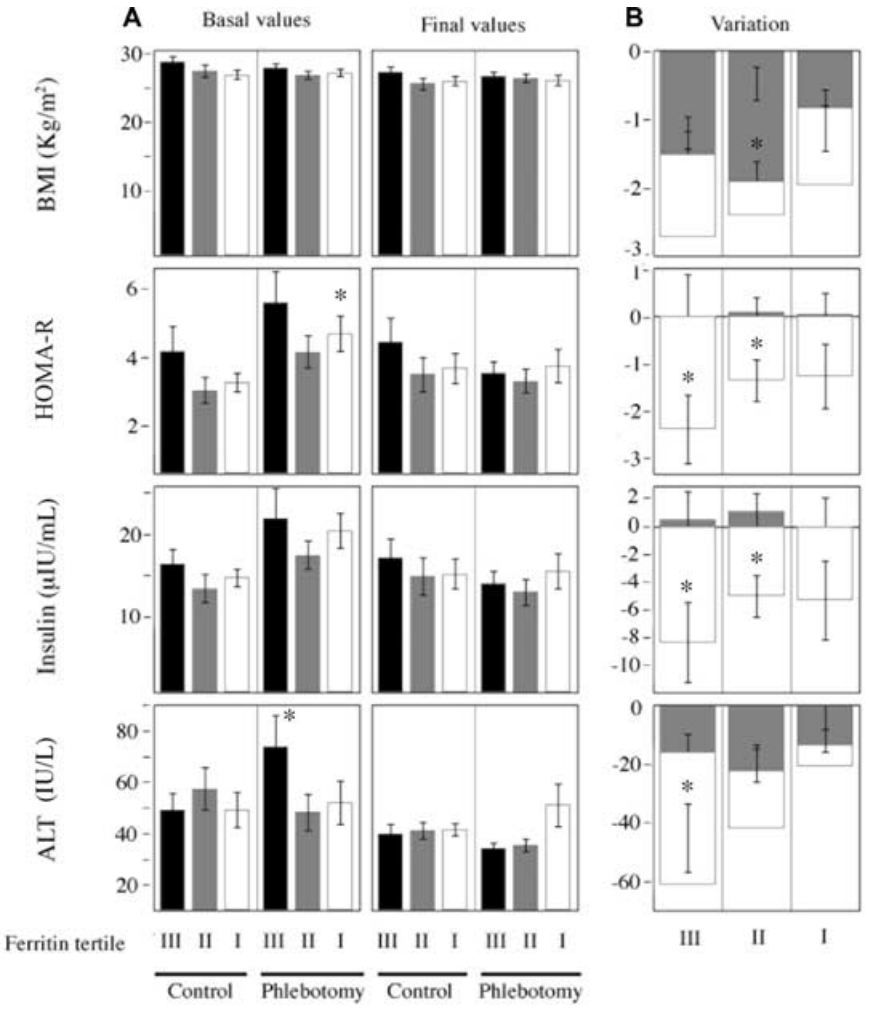

Figure 2. Effect of therapy on BMI, HOMA-R, insulin, and ALT levels according to basal ferritin tertiles. (*A*) Basal and final values. (*B*) Variation—white bars: phlebotomized subjects, grey bars: nonphlebotomized subjects. Data are shown as mean ± SE. $^{*}P < 0.05$ *versus* controls.

ALT values *versus* noncarriers, when submitted to iron depletion (Table 3). However, phlebotomy was still associated with a larger improvement in HOMA-R (-1.47 ± 2.7 *vs*–0.06 ± 2.8, $P = 0.01$) and insulin levels (-5.3 ± 10.8 *vs*+0.02 ± 7.2 mIU/L, $P = 0.006$) also in patients not carrying the C282Y *HFE* mutation.

At logistic regression analysis considering age (yr), sex (M/F), the presence of the metabolic syndrome, the changes in BMI (kg/m^2), triglycerides (mg/dL), ALT levels (UI/L), ferritin tertile, the presence of *HFE* mutations, and the percentage change in HOMA-R values as independent variables, and the type of treatment as the dependent variable, only the percentage change in HOMA-R value was significantly associated with phlebotomy treatment ($P = 0.0005$).

DISCUSSION

In this article, we analyzed the effect of adding iron depletion to standard lifestyle modifications on insulin resistance in patients with NAFLD, and observed a significantly larger improvement in insulin levels and the HOMA-R index in iron-depleted patients *versus* controls. These results confirm previous findings in smaller uncontrolled series of NAFLD (29, 30, 35), in patients with type 2 diabetes (31), and with insulin resistance related to hepatic iron overload (36). The effect of iron depletion was independent of baseline HOMA-R index and the presence of the metabolic syndrome, thus excluding higher insulin resistance in phlebotomized subjects as a likely explanation of these findings. Since insulin resistance is a key pathogenic feature of NASH, diabetes, and atherosclerotic disease, the observed improvement in the metabolic picture could translate into a clinically significant benefit in the long term.

The study has strengths. Data were derived from a large, well-defined cohort. Although observational in design, the careful matching with a control group was the basis to define the beneficial effects of phlebotomy independent of lifestyle modifications.

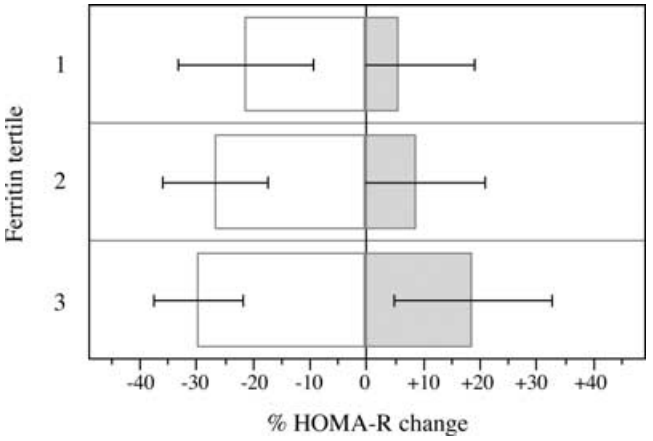

Figure 3. Effect of iron depletion on the percentage decrease in HOMA-R values according to ferritin tertiles. White bars: phlebotomized subjects, grey bars: nonphlebotomized subjects. Data are shown as mean ± SE. *P* was <0.05 for HOMA-R change between phlebotomized and nonphlebotomized subjects in each ferritin tertile.

Unlike previous studies (35, 37), we did not observe any further improvement in liver enzymes and lipid levels in the overall series of iron-depleted subjects *versus* controls. It is worth noting that controls were mostly compliant with lifestyle modifications (38) and had a lower prevalence of both impaired glucose tolerance and severe iron overload, and the clinical observation was relatively short.

Interestingly, the variations in insulin and HOMA-R observed during treatment were correlated with those of ferritin and transferrin saturation, and the correlation with transferrin saturation was also almost significant in nonphlebotomized subjects, underscoring the strict association between iron status and insulin resistance in NAFLD. As to the mechanisms underlying the beneficial effect of iron depletion on insulin resistance, preliminary experimental data suggest that iron status influences hepatic insulin signaling and glucose uptake (25), as also reported in adipose tissue through a mechanism independent of fatty acids (39). This is a crucial issue, because excessive free fatty acid flux may be both a cause and an effect of insulin resistance. It is however worth noting that despite lipid levels and liver enzymes ameliorated with weight loss in iron-depleted subjects submitted to counseling (25), this was not accompanied by a significant decrease in insulin resistance during the study period.

Table 3. Effect of Therapy on Liver Necrosis Indices and Metabolic Features According to the Presence of the C282Y and the H63D *HFE* Mutations in 106 Patients

	HFE Mutations	
	Present (N = 45)	Absent (N = 61)
BMI (kg/m^2)	−0.9 ± 0.5	−0.4 ± 0.3
ALT (U/L)	+13.2 ± 22	+6.8 ± 9
Insulin (μU/mL)	+7.0 ± 6.2*	+2.4 ± 2.9
HOMA-R	+1.9 ± 1.4*	+0.69 ± 1.3
Iron removed (g)†	1.7 ± 2.1	1.0 ± 1.5

Data are given as changes in the variable in patients submitted to phlebotomy minus the changes in matched patients undergoing behavioral therapy alone. Positive values indicate an advantage for iron depletion; negative values indicate an advantage for lifestyle modification alone.
*$P < 0.05$.
†Values were determined in phlebotomized patients.

We next analyzed whether basal iron status could predict treatment outcome. As iron depletion was previously reported to improve insulin sensitivity in healthy subjects (40, 41) and in patients with NAFLD without iron overload (29), we also included in this study subjects with normal ferritin levels. Our results support previous findings linking hyperferritinemia with mild hepatic iron overload in NAFLD (13, 15, 33), and indicate that the benefit of iron depletion compared with standard therapy is higher with increasing ferritin levels, and in subjects carrying *HFE* mutations, even in the absence of overt signs of iron overload. The beneficial effect of iron depletion extends to ALT levels in the higher ferritin tertile. However, it should be noted that the percentage decrease in HOMA-R was similar among ferritin tertiles, and iron depletion was associated with the percentage decrease in HOMA-R independently of confounding factors.

Interestingly, the amount of iron removed to reach depletion increased with increasing ferritin tertiles, supporting the view that hyperferritinemia also reflects mild iron overload in the metabolic syndrome, and suggesting a possible role of inherited factors in determining susceptibility to a specific therapy for NAFLD (*i.e.*, correction of insulin resistance), which should be assessed in future studies.

Because the degree of hepatic insulin resistance and hyperinsulinemia are associated with cardiovascular damage and liver disease in NAFLD (3, 4, 42), these results suggest that iron depletion by phlebotomy could favorably influence the natural history of patients with NAFLD and normal iron stores, a hypothesis supported by the recent evidence that iron depletion improves endothelial function (19, 43).

The major limit of the study is the lack of a hard histological outcome to support a beneficial effect of iron depletion. Moreover, the prevalence of subjects positive for the C282Y *HFE* mutation was moderately higher in patients submitted to phlebotomy because of the better compliance of these subjects to the protocol. However, iron depletion was associated with a decrease in insulin resistance even after excluding from the analysis subjects positive for the C282Y mutation, and in familial hemochromatosis glucose intolerance is not related to insulin resistance, but to decreased insulin release (44). Large randomized studies, considering histology and cardiovascular disease as final outcomes, are nonetheless required before this therapy can be proposed outside the research setting.

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STUDY HIGHLIGHTS

What Is Current Knowledge

- Hyperferritinemia is frequently observed in nonalcoholic fatty liver disease (NAFLD).
- Iron overload is possibly a risk factor for metabolic diseases.
- Iron depletion by phlebotomy improved insulin resistance in small uncontrolled studies.

What Is New Here

- Ferritin levels reflect iron stores in NAFLD.
- Iron depletion improves insulin resistance more effectively than lifestyle changes alone.
- The effect is higher in patients with hyperferritinemia and carrying *HFE* mutations.

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CONFLICT OF INTEREST

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