The Effect of Alcohol Consumption on the Prevalence of Iron Overload, Iron Deficiency, and Iron Deficiency Anemia

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Background & Aims: **Our aim was to investigate the relationship between alcohol consumption and iron overload, iron deficiency, or iron deficiency anemia in the U.S. population.** ***Methods:*** **Adult participants of the Third National Health and Nutrition Examination Survey who did not consume alcohol (n = 8839) were compared with participants who consumed ≤1 (n = 4976), >1 to ≤2 (n = 1153), or >2 (n = 915) alcoholic drinks/day during the preceding 12 months. We examined the following markers of iron overload: elevated serum transferrin-iron saturation (TS) level (>45%, >50%, and >60%), elevated serum ferritin level (>300, >400, >500, and >600 ng/mL), and combinations of both elevated serum TS and ferritin levels. Iron deficiency was defined as the presence of at least 2 of the following: serum ferritin level <12 ng/mL, serum TS level <15%, and erythrocyte protoporphyrin level >1.24 μmol/L. Iron deficiency anemia was defined as the presence of both iron deficiency and anemia.** ***Results:*** **Compared with nondrinkers, the prevalence of all markers of iron overload was significantly elevated among those who consumed >2 alcoholic drinks/day after adjusting for potential confounders. Consumption of any amount of alcohol was associated with a 40% reduction in the risk of iron deficiency anemia.** ***Conclusions:*** **Consumption of up to 2 alcoholic drinks/day seems to be associated with reduced risk of iron deficiency and iron deficiency anemia without a concomitant increase in the risk of iron overload. Consumption of >2 alcoholic drinks/day is associated with a significant elevation in the risk of iron overload.**

The association between excessive alcohol consumption and iron overload in patients with chronic alcoholism or alcoholic cirrhosis has been well described.[1–8] In contrast, few studies have investigated the effects of mild or moderate alcohol consumption among the general population on serum markers of iron stores.[9,10] These studies have suggested that the mean levels of serum markers of iron stores (serum ferritin, transferrin-iron saturation [TS], and iron) are higher in persons who consume mild or moderate amounts of alcohol compared with nondrinkers. This finding raises the possibility that alcohol consumption may increase the prevalence of iron overload or decrease the prevalence of iron deficiency and iron deficiency anemia.

An association between alcohol consumption and iron overload would be clinically important because alcohol and iron are believed to have synergistic hepatotoxic effects.[11–17] Conversely, mild or moderate alcohol consumption may have a beneficial effect if it reduces the prevalence of iron deficiency and iron deficiency anemia, which remain common in the United States, especially among women.[18]

Our aim was to determine whether alcohol consumption in the U.S. population is associated with iron overload, iron deficiency, or iron deficiency anemia using data from the Third National Health and Nutrition Examination Survey (NHANES III).

Materials and Methods

Survey Design and Collection of Data

NHANES III was conducted by the National Center for Health Statistics between 1988 and 1994 to assess the health and nutritional status of the civilian, noninstitutionalized population of the United States.[19] It was a cross-sectional study of 33,994 persons at least 2 months of age from 89 randomly selected locations throughout the United States and involved household interviews, standardized physical examinations, and laboratory investigations.

Study Sample

Of 20,511 NHANES III participants aged 16 years or older, we excluded 3062 persons who were not tested for serum

Abbreviations used in this paper: CI, confidence interval; NHANES III, Third National Health and Nutrition Examination Survey; OR, odds ratio; TS, transferrin-iron saturation.

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iron, TS, ferritin, or erythrocyte protoporphyrin levels; 1052 persons with missing data regarding alcohol consumption; and 514 persons with missing data regarding body mass index, educational attainment, or menstrual history. This left 15,883 participants in this analysis.

Assessment of Alcohol Consumption

NHANES III reported the number of days during which alcohol was consumed over the preceding year and the average number of alcoholic drinks that were consumed on those days. An alcoholic drink was defined as a 12-oz beer, a 4-oz glass of wine, or 1 oz of liquor. Using this information, we calculated the average number of alcoholic drinks consumed per day during the preceding year and divided alcohol consumption into 4 categories: (1) none, which included participants who consumed no alcohol or fewer than 12 drinks in the past 12 months, (2) mild ($\leq$1 drink/day), (3) moderate ($>$1 to $\leq$2 drinks/day), and (4) heavy ($>$2 drinks/day).

Measurement of Hemoglobin Concentration and Serum Markers of Iron Stores

Details of the assay methods used in NHANES III for laboratory investigations have been described elsewhere.[20,21] Hemoglobin was measured using a Coulter S-Plus electronic counter (Coulter Electronics, Hialeah, FL). Serum iron level and total iron binding capacity were measured colorimetrically (Alpkem RFA analyzer, Clackamas, OR), and 1% thiourea was added to complex copper and prevent copper interference. TS level was calculated as the ratio of serum iron to total iron binding capacity. Free erythrocyte protoporphyrin level was measured via fluorescence extraction, and serum ferritin level was measured using the BioRad Quantimmune IRMA kit (BioRad Laboratories, Hercules, CA).

Assessment of Potential Confounding Variables

Previous studies have suggested that sex, menopausal status, body mass index, age, race/ethnicity, hepatitis C status, education, and poverty index may be associated with both alcohol consumption and the levels of serum markers of iron stores.[18,22,23] Therefore, these variables were selected a priori for inclusion in regression models as potential confounders. All of these variables except hepatitis C status were ascertained by structured subject interviews and physical examinations. Race/ethnicity was categorized into 4 groups: non-Hispanic white, non-Hispanic black, Mexican American, and other. There were too few participants aged 19 years or younger in the highest alcohol consumption group, so the first age category included participants 29 years of age or younger, followed by 10-year age categories. Educational attainment was classified according to whether or not a participant had graduated from high school. The poverty income ratio, a measure of poverty that is independent of calendar year, was computed as the ratio of self-reported family income to the poverty threshold according to each calendar year. Participants with a poverty income ratio $<$1.0 were considered to be below the poverty level. Body mass index was computed as the ratio of the measured weight in kilograms to the square of the measured height in meters and was modeled as a continuous variable. A second-generation enzyme immunoassay and a supplemental test were used to test for anti–hepatitis C virus antibodies. Samples that tested positive were subsequently tested for hepatitis C virus RNA by reverse-transcriptase polymerase chain reaction.[23,24] For this study, only participants with positive hepatitis C virus RNA were considered to be chronically infected with hepatitis C.

Table 1. Definition of Anemia by Age and Sex

Sex	Age (yr)	Level of hemoglobin below which participants were labeled as anemic (*g/dL*)
Women	16–19	$<$12.0
	20–49	$<$12.0
	50–69	$<$12.0
	70 or older	$<$11.8
Men	16–19	$<$13.6
	20–49	$<$13.7
	50–69	$<$13.3
	70 or older	$<$12.4

Definitions of Iron Overload, Iron Deficiency, and Iron Deficiency Anemia

Iron deficiency was defined as the presence of at least 2 of the following 3 criteria: serum ferritin level $<$12 ng/mL, TS level $<$15%, and erythrocyte protoporphyrin level $>$1.24 μmol/L. These values are at the fifth percentile of the distribution in a reference group of healthy individuals in the case of serum ferritin and erythrocyte protoporphyrin and at the 12th percentile in the case of TS, which has a greater known biologic variability.[18,25] These criteria have been used previously to define iron deficiency in the United States using NHANES III[18] or NHANES II data.[25] The basis of the use of multiple tests was the finding that the prevalence of anemia was substantially elevated only in populations who had abnormal iron status by 2 or 3 tests.[26,27] The use of these cutoffs for the diagnosis of iron deficiency is also supported by clinical studies that observed absent iron staining of bone marrow aspirates in 94%–96% of anemic persons with a serum ferritin level $<$12 ng/mL,[28–30] 50%–72% of anemic persons with a serum TS level $<$15%,[28] and 54% of anemic persons with a red cell protoporphyrin level $>$1.24 μmol/L.[29] We defined anemia as a level of hemoglobin concentration below the fifth percentile value of each age and sex group[18] (Table 1). Iron deficiency anemia was defined as the presence of both iron deficiency and anemia as previously defined.

There is no consensus on the threshold values of serum TS and/or serum ferritin that are considered diagnostic for iron overload, as indicated by the wide variety in previously used thresholds.[31,32] Therefore, we considered separately serum TS thresholds of $>$45%, $>$50%, or $>$60% and serum ferritin thresholds of $>$400 ng/mL in men and $>$300 ng/mL in women, $>$500 ng/mL in men and $>$400 ng/mL in women, or

>600 ng/mL in men and >500 ng/mL in women. In general, higher values of serum TS or ferritin are associated with a higher probability of tissue iron overload; serum ferritin, in particular, has a strong linear relationship with body iron content as measured by iron depletion.[33] In addition, we considered elevations in both serum TS (>45% or >50%) and serum ferritin values (>400 ng/mL in men and >300 ng/mL in women or >500 ng/mL in men and >400 ng/mL in women) because this reduces the likelihood of a false-positive diagnosis of iron overload due to elevation of serum ferritin level secondary to inflammatory conditions. Previous population-based studies have shown that 67%–100% of persons with similar elevations in serum TS and ferritin levels were subsequently confirmed to have iron overload based on data from liver biopsy or quantitative phlebotomy.[31,32]

Statistical Analysis

A multivariate linear regression model was used to compare the mean difference in the levels of serum iron, TS, ferritin, and erythrocyte protoporphyrin between the nondrinkers and the persons in the 3 alcohol consumption categories after adjusting for the potential confounders described previously. Two additional models that also included either the levels of serum folate or the levels of serum aspartate and alanine aminotransferase were used to determine whether folate deficiency or hepatic necroinflammation, respectively, could explain any potential associations between alcohol consumption and serum markers of iron stores. Multivariate logistic regression models were used to compare alcohol users with nonusers with respect to the prevalence of serum markers of iron overload, iron deficiency, or iron deficiency anemia.

Interaction terms between alcohol consumption category and sex, race/ethnicity, hepatitis C status, or age category were used in additional models to investigate whether any potential association between alcohol consumption and serum markers of iron stores was modified by these characteristics. Furthermore, the multivariate linear regressions were performed separately for men and women to look for any sex-specific differences in the association between alcohol consumption and serum markers of iron stores.

To increase the efficiency of the sampling process, NHANES III used a complex, multistage sampling design whereby potential participants were identified at 89 locations in the United States. This induces a correlation structure among the observations that cannot be treated as a simple random sample. In addition, because NHANES III involved increased rates of sampling for certain age and racial groups, sample weights are provided to reflect this and also to attempt to adjust for nonresponse bias (due to people refusing to participate) and noncoverage bias (due to people who do not live in households and therefore could not participate). We used Stata software version 7.0 (Stata Corp., College Station, TX) in our analyses to account for both the sampling and the weighting processes.

Results

Of the 15,883 participants, 8839 (56%) reported drinking no alcohol or fewer than 12 drinks over the past 12 months and 4976 (31%) reported mild, 1153 (7%) moderate, and 915 (6%) heavy alcohol consumption (Table 2). Increasing alcohol consumption was associated with hepatitis C infection, male sex, age 30–59 years, and abnormal values of serum aspartate and alanine aminotransferase. The proportion of participants whose income was below poverty level or who had education less than or equal to a high school diploma was greater among the nondrinkers and the heavy drinkers than among the mild and moderate drinkers. There was a greater prevalence of obesity (defined as body mass index ≥ 30 kg/m^2) in the nondrinkers (27%) than in the alcohol drinkers (18%–21%). Alcohol consumption did not vary appreciably by race/ethnicity.

Mean serum ferritin, TS, and iron levels were all found to increase with increasing alcohol consumption after adjusting for age, sex, menopausal status, body mass index, hepatitis C infection, education, and poverty index (Figure 1). The association between alcohol consumption and TS level was entirely attributed to elevated serum iron level. Levels of total iron binding capacity, which is the other component of TS, were similar in alcohol drinkers and nondrinkers (data not shown). Mean erythrocyte protoporphyrin level, which is high in iron-deficient states, was lower in alcohol drinkers compared with nondrinkers (Figure 1). None of these associations was appreciably changed by further adjusting for either the levels of serum aspartate and alanine aminotransferase (which are believed to reflect hepatic necroinflammation) or the levels of serum folate (data not shown). This finding suggests that the changes in serum ferritin, TS, iron, and erythrocyte protoporphyrin levels that we observed in association with alcohol consumption are not related to hepatic necroinflammation or serum folate deficiency. The adjusted mean differences between alcohol drinkers and nondrinkers in the levels of serum ferritin, TS, iron, and erythrocyte protoporphyrin were similar in subgroups defined by age, race/ethnicity, hepatitis C infection, and sex.

To evaluate the clinical significance of the observed associations between alcohol consumption and serum markers of iron stores, we investigated whether alcohol consumption was associated with iron overload (Table 3), iron deficiency (Table 4), or iron deficiency anemia. Compared with nondrinkers, the risk of elevated levels of serum ferritin, TS, or both was significantly increased

Table 2. Characteristics of 15,883 Participants Aged 16 Years or Older by Alcohol Consumption Category

	Alcohol consumption over the past 12 months							
	None		Mild		Moderate		Heavy	
	n	%	n	%	n	%	n	%
Total	8839	56	4976	31	1153	7	915	6
Positive hepatitis C RNA status	95	1.1	74	1.5	40	3.5	49	5.4
Sex/menopausal status								
Female, premenopausal	2495	33	1563	31	198	17	91	10
Female, postmenopausal	2862	32	679	14	107	9.3	33	4
Male	3032	34	2734	55	848	74	791	86
Race/ethnicity								
White (non-Hispanic)	3481	39	2281	46	499	43	334	37
Black (non-Hispanic)	2545	29	1217	24	315	27	252	28
Mexican American	2422	27	1301	26	306	27	303	33
Other	391	4	177	4	33	3	26	3
Age (*yr*)								
29 or younger	2028	23	1467	29	362	31	272	30
30–39	1319	15	1167	23	253	22	218	24
40–49	1156	13	807	16	202	18	172	19
50–59	952	11	526	11	114	9.9	103	11
60 or older	3384	38	1009	20	222	19	150	16
Body mass index ≥30 kg/m^2	2468	28	1068	21	209	18	194	21
Below poverty level[a]	3050	34	1193	24	310	27	295	32
Education ≤12th grade[b]	6894	78	3068	62	772	67	707	77
Aspartate aminotransferase level >40 U/L	296	3.4	189	3.9	75	6.6	125	14
Alanine aminotransferase level >40 U/L	318	3.7	277	5.7	95	8.4	138	15
Serum folate level <2 ng/mL	504	5.7	279	5.6	63	5.5	33	3.6

NOTE. Alcohol consumption was characterized as follows: none, no alcohol or fewer than 12 drinks in the past 12 months; mild, ≤1 drink/day; moderate, >1 to ≤2 drinks/day; heavy, >2 drinks/day.

[a]Calculated as the poverty income ratio based on self-report of family income, family size, and tables published annually by the U.S. Census Bureau. Persons with a poverty income ratio ≥1.0 were categorized as being at or above the poverty level.

[b]Only among participants older than 17 years.

only for the heavy alcohol drinkers, not for the mild or moderate alcohol drinkers (Table 3). The odds ratios (ORs) comparing heavy alcohol drinkers with nondrinkers were higher for higher thresholds of serum TS (for TS level >60%: OR, 3.1; 95% confidence interval [CI], 1.3–7.7; for TS level >50%: OR, 2.6; 95% CI, 1.5–4.4; for TS level >45%: OR, 1.9; 95% CI, 1.4–2.4) or ferritin (for ferritin level >600 ng/mL [men] or >500 ng/mL [women]: OR, 2.9; 95% CI, 1.6–5.2; for ferritin level >500 ng/mL [men] or >400 ng/mL [women]: OR, 2.6; 95% CI, 1.6–4.1; for ferritin level >400 ng/mL [men] or >300 ng/mL [women]: OR, 2.2; 95% CI, 1.6–3.0) and were even higher for outcomes that compared elevations in both serum TS and ferritin levels (ORs ranging from 3.7 to 4.8).

Using our definition of iron deficiency, the overall prevalence of this condition in the United States among persons aged 16 years or older was 5.3%. The prevalence of all markers of iron deficiency (low serum ferritin, low serum TS, or elevated erythrocyte protoporphyrin levels) decreased progressively with increasing alcohol consumption (Table 4). However, after adjusting for age, sex, menstruation, hepatitis C infection, education, and poverty index, all categories of alcohol consumption were associated with a similar 30%–50% reduction in the likelihood of iron deficiency compared with the no-alcohol category (Table 4).

The overall prevalence of iron deficiency anemia in the United States among persons aged 16 years or older was 1.9%. We compared the prevalence of iron deficiency anemia in nondrinkers (2.9%) with the prevalence in all drinkers combined (1.1%) because there were too few persons with iron deficiency anemia in the heavy alcohol consumption category and because, as previously described, the rates of iron deficiency were similar in all 3 alcohol consumption categories. Any amount of alcohol consumption was associated with a statistically significant 42% reduction in the risk of iron deficiency anemia (OR, 0.58; 95% CI, 0.4–0.8).

The associations described between alcohol consumption and serum markers of iron overload, iron deficiency, and iron deficiency anemia were not significantly different between different groups defined by sex, race/ethnicity, age category, or hepatitis C status.

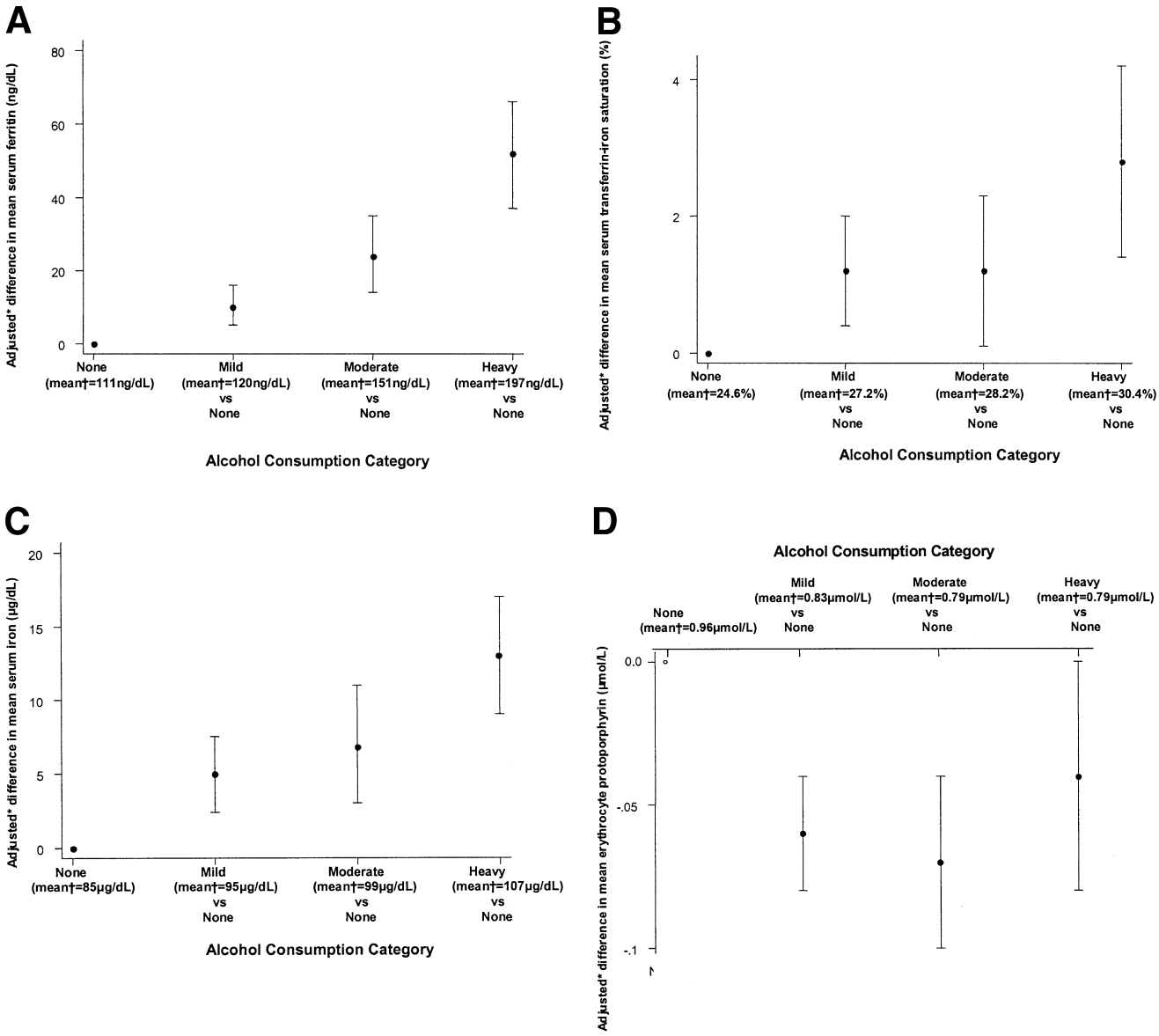

Figure 1. Adjusted difference in mean values of (*A*) serum ferritin, (*B*) serum TS, (*C*) serum iron, and (*D*) serum erythrocyte protoporphyrin between different alcohol consumption categories. In each graph, the *circle* represents the adjusted mean difference comparing persons in each category of alcohol consumption with the nondrinkers and the *vertical bar* represents the 95% CI. The adjusted mean difference is statistically significant if the 95% CI does not cross zero. Alcohol consumption was categorized as none (no alcohol consumption or fewer than 12 drinks in the past 12 months), mild (≤1 drink/day), moderate (>1 to ≤2 drinks/day), and heavy (>2 drinks/day). *Calculated from survey linear regression and adjusted for age, hepatitis C RNA status, body mass index, sex, menstruation, race/ethnicity, education, and poverty index. †The unadjusted means in each alcohol consumption category.

Discussion

In this population-based study, we observed that increasing alcohol consumption was associated with steadily increasing levels of serum ferritin, iron, and TS and decreased levels of erythrocyte protoporphyrin. The risks of iron deficiency and iron deficiency anemia were approximately 40% lower among persons who consumed any amount of alcohol compared with nondrinkers. However, among those who consumed more than 2 alcoholic drinks/day, there was also a significantly increased risk of iron overload as measured by the presence of elevated serum TS or ferritin or both TS and ferritin levels.

Many of the complications of severe alcohol abuse and alcoholic cirrhosis (e.g., alcoholic gastritis or esophagitis, Mallory–Weiss tears, gastroesophageal varices, and portal gastropathy) lead to gastrointestinal blood loss and hence iron deficiency and anemia. In contrast, in this population-based study, we found that alcohol consumption was actually associated with a decrease in the risk of

Table 3. Prevalence of Serum Markers of Iron Overload in Relation to Level of Alcohol Consumption

	Prevalence (%) SE				Adjusted[a] OR (95% CI)		
	None	Mild	Moderate	Heavy	Mild vs. none	Moderate vs. none	Heavy vs. none
Ferritin level >400 ng/mL (men) or >300 ng/mL (women) (n = 879)	4.6 (0.4)	3.9 (0.4)	4.3 (0.8)	9.9 (1.3)	1.1 (0.9–1.5)	1.1 (0.7–1.7)	2.2 (1.6–3.1)
Ferritin level >500 ng/mL (men) or >400 ng/mL (women) (n = 474)	2.2 (0.2)	1.8 (0.3)	2.2 (0.5)	5.5 (1.0)	1.1 (0.8–1.6)	1.2 (0.7–2.0)	2.6 (1.6–4.1)
Ferritin level >600 ng/mL (men) or >500 ng/mL (women) (n = 265)	1.1 (0.2)	1.1 (0.3)	1.4 (0.4)	3.1 (0.6)	1.4 (0.8–2.3)	1.5 (0.7–3.1)	2.9 (1.6–5.2)
TS level >45% (n = 880)	4.7 (0.4)	7.2 (0.5)	7.3 (1.2)	12.5 (1.7)	1.2 (0.9–1.6)	1.1 (0.7–1.7)	1.9 (1.4–2.4)
TS level >50% (n = 504)	2.3 (0.3)	4.4 (0.5)	4.3 (0.8)	7.8 (1.4)	1.6 (1.1–2.3)	1.4 (0.9–2.2)	2.6 (1.5–4.4)
TS level >60% (n = 162)	0.7 (0.2)	1.0 (0.2)	1.4 (0.4)	3.4 (0.9)	1.0 (0.5–1.9)	1.4 (0.6–3.3)	3.1 (1.3–7.7)
Ferritin level >400 ng/mL (men) or >300 ng/mL (women) and TS level >45% (n = 123)	0.4 (0.1)	0.5 (0.2)	0.5 (0.2)	1.6 (0.4)	1.9 (0.8–4.3)	1.7 (0.7–4.0)	3.7 (1.5–9.5)
Ferritin level >500 ng/mL (men) or >400 ng/mL (women) and TS level >45% (n = 67)	0.2 (0.08)	0.3 (0.1)	0.5 (0.2)	0.9 (0.4)[b]	1.5 (0.6–3.7)	2.2 (0.8–6.3)	4.0 (1.2–12.8)
Ferritin level >400 ng/mL (men) or >300 ng/mL (women) and TS level >50% (n = 80)	0.3 (0.1)	0.4 (0.2)	0.5 (0.2)	1.3 (0.4)	2.0 (0.7–5.7)	2.2 (0.9–5.8)	4.8 (1.6–14.5)

NOTE. Alcohol consumption was characterized as follows: none, no alcohol or fewer than 12 drinks in the past 12 months; mild, ≤1 drink/day; moderate, >1 to ≤2 drinks/day; heavy, >2 drinks/day.

[a]Calculated from survey logistic regression and adjusted for age, hepatitis C RNA status, body mass index, sex, menstruation, race/ethnicity, education, and poverty index.

[b]This estimate is potentially statistically unreliable because the reported proportion is small relative to the population size.[53]

iron deficiency and iron deficiency anemia. This discrepancy is probably explained by the fact that only a very small proportion of alcohol drinkers in the general population develop complications that lead to blood and iron loss. Instead, in most alcohol drinkers, alcohol consumption is associated with an increase in iron stores and a reduction in the prevalence of iron deficiency and iron deficiency anemia.

Our finding that consumption of more than 2 alcoholic drinks per day is associated with increased risk of iron overload may have important implications with regard to the risk of developing hepatic fibrosis and cirrhosis among alcohol drinkers. Both iron overload and heavy alcohol consumption are associated with oxidative stress in the liver.[13–17] In fact, it has been suggested that the combination of both agents may synergistically ex-

Table 4. Prevalence of Iron Deficiency in Relation to Level of Alcohol Consumption

	Prevalence (%) (SE)				Adjusted[a] OR (95% CI)		
	None	Mild	Moderate	Heavy	Mild vs. none	Moderate vs. none	Heavy vs. none
Ferritin level <12 ng/mL	6.7 (0.5)	4.5 (0.4)	2.7 (0.6)	1.0 (0.4)[b]	0.72 (0.53–0.96)	0.67 (0.4–1.1)	0.41 (0.18–0.97)
TS level <15%	17.6 (0.6)	12.4 (0.8)	10.1 (1.3)	7.2 (1.4)	0.85 (0.7–1.0)	0.87 (0.64–1.2)	0.74 (0.48–1.13)
Erythrocyte protoporphyrin level >1.24 μmol/L	12.5 (0.7)	5.6 (0.5)	5.6 (0.9)	3.8 (0.8)	0.61 (0.48–0.79)	0.80 (0.53–1.21)	0.62 (0.38–1.03)
Iron deficiency based on 2 or more abnormal values[c]	8.2 (0.5)	3.9 (0.4)	3.2 (0.6)	1.6 (0.5)	0.61 (0.46–0.81)	0.71 (0.44–1.15)	0.50 (0.26–0.98)

NOTE. Alcohol consumption was characterized as follows: none, no alcohol or fewer than 12 drinks in the past 12 months; mild, ≤1 drink/day; moderate, >1 to ≤2 drinks/day; heavy, >2 drinks/day.

[a]Calculated from survey logistic regression and adjusted for age, hepatitis C RNA status, body mass index, sex, menstruation, race/ethnicity, education, and poverty index.

[b]This estimate is potentially statistically unreliable because the reported proportion is small relative to the population size.[53]

[c]Abnormal values in at least 2 of the following 3 indicators of iron deficiency: ferritin, TS, and erythrocyte protoporphyrin.

acerbate oxidative injury and fibrogenesis in the liver.[11,12] Of the persons who consumed more than 2 alcoholic drinks per day, 3.1% had elevated serum ferritin levels (>600 ng/mL in men and >500 ng/mL in women), 1.4% had elevated serum TS levels (>60%), and 1.3% had elevations in both serum TS (>50%) and serum ferritin levels (>400 ng/mL in men and >300 ng/mL in women). We speculate that this latter group may have a relatively high risk of developing hepatic fibrosis and cirrhosis due to the potential synergistic hepatotoxic effects of iron and alcohol.

We tested 2 hypotheses that attempt to explain the increases in serum markers of iron stores observed among alcohol users: (1) that alcohol leads to hepatic necroinflammation, which in turn leads to release of iron and ferritin from hepatocytes, and (2) that alcohol causes folic acid deficiency, which induces a megaloblastic anemia and stimulates plasma iron turnover.[34] Our findings suggest that it is unlikely that either hepatic necroinflammation (as measured by serum aminotransferase levels) or reduced serum folate levels are important mechanisms of alcohol-mediated iron overload because adjusting for serum aminotransferase and folate levels had no effect on the observed associations between alcohol consumption and iron overload. Another hypothesis suggests that alcohol-related iron overload is caused at least in part by the iron present in certain alcoholic beverages such as red wine.[35,36] We were unable to test this hypothesis because alcohol consumption was not subdivided according to beverage type in NHANES III. However, even if this was true, this cannot be the only mechanism because iron overload has been shown in alcoholic patients who consumed beverages containing only trace amounts of iron, such as whiskey and gin.[35] Moreover, consumption of beer, which has a lower iron content than wine, has been reported to be associated with higher levels of serum ferritin than consumption of wine.[9] It has been postulated that alcohol consumption affects iron regulation based on the observation that alcohol causes a reduction in the number of carbohydrate groups that make up the 2 glycan chains of transferrin, the main iron-transport protein.[37,38] However, the mechanism by which the presence of carbohydrate-deficient transferrin may lead to iron overload is unclear. A possible mechanism for alcohol-related hepatic iron overload has recently been suggested by the demonstration that transferrin receptor expression was up-regulated in hepatocytes of habitual alcohol drinkers.[39] Finally, a frequently proposed mechanism by which chronic alcohol consumption may increase body iron stores is through enhanced absorption of dietary iron,[9,40–46] perhaps mediated through passive, non–carrier-mediated paracellular transfer.[47]

It is possible that the elevations in serum markers of iron stores that we observed among alcohol users do not reflect a true elevation in body iron stores. In particular, serum ferritin is an acute-phase reactant and may be elevated in acute and chronic inflammatory conditions. However, there is no reason to suspect that these conditions are more prevalent in alcohol drinkers than nondrinkers, which would be necessary for such inflammatory conditions to be a source of uncontrolled confounding. It is also possible that alcohol consumption itself provokes changes in acute-phase proteins, of which ferritin is one; however, 2 recent studies found no association between alcohol consumption and several other acute-phase proteins, including C-reactive protein, soluble E-selectin, factor VIIIc, von Willebrand factor, fibrinogen, α_1-antichymotrypsin, and ceruloplasmin.[48,49] Moreover, the acute-phase response results in a decrease in serum iron levels.[50] Our finding that alcohol was associated with changes suggestive of increased iron stores in all serum iron markers that we investigated (serum ferritin, iron, TS, and erythrocyte protoporphyrin) suggests that these changes are likely to reflect increased body iron stores.

The main limitation of this study is that we defined iron overload and iron deficiency based entirely on serum markers of iron stores. In the case of iron deficiency, iron staining of bone marrow aspirate is considered the gold standard for diagnosis. Iron overload is best determined either by histologic or quantitative measurement of iron in tissue biopsy samples (e.g., liver biopsies) or from the quantity of phlebotomized blood required to induce iron depletion. None of these techniques is applicable to population-based studies of healthy volunteers. Instead, we used the best available serum markers of iron stores using cutoffs that identified populations at very high probability of iron overload or iron deficiency.[51,52] The robustness of our results is supported by the fact that alcohol consumption was associated with a decreased prevalence of all markers of iron deficiency (low serum ferritin level, low serum TS level, high erythrocyte protoporphyrin level, or combinations of 2 or more of these markers) and heavy alcohol consumption was associated with an increased prevalence of all serum markers of iron overload (high ferritin level, high TS level, or combinations of these 2 markers). Furthermore, the ORs comparing heavy alcohol drinkers with nondrinkers were numerically higher for outcomes that were more specific for iron overload (i.e., higher thresholds of TS or ferritin compared with lower thresholds, or combination of ele-

vations in both serum TS and ferritin levels compared with elevations in just one of these markers).

Estimation of alcohol consumption in NHANES III was based entirely on self-reporting and may have been subject to underreporting. If NHANES III participants underreported their alcohol consumption, then the ORs that we describe are likely to be overestimates of the excess risk associated with each category of alcohol consumption. At the same time, to the extent that some people who drink small amounts of alcohol report their intake as none, it is possible that all of the ORs that we describe are systematically low. It is impossible to predict the net direction of these sources of bias.

In addition, our study is limited by the lack of data on mutations in the hemochromatosis gene (*HFE*), which can cause iron overload. The absence of data on *HFE* mutations is unlikely to have given rise to uncontrolled confounding because there is no reason to suspect that the prevalence of *HFE* mutations is different in alcohol users compared with nonusers. On the other hand, we could not test the hypothesis that alcohol consumption may be associated with greater elevations in serum markers of iron stores in persons who carry *HFE* mutations than in persons who do not. Such an interaction is unlikely to be present in our data because it would be expected to lead to greater elevations in serum iron markers in white persons compared with black persons, because *HFE* mutations are found predominantly in white persons. The fact that we found no significant difference between black persons and white persons in the degree of iron overload associated with alcohol consumption makes this interaction unlikely.

In summary, consumption of any amount of alcohol was associated with a reduction in the risk of iron deficiency and iron deficiency anemia in the U.S. population. However, consumption of more than 2 drinks/day was associated with a significantly increased risk of iron overload, which may have important implications for the development of hepatic fibrosis and cirrhosis.

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