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Effects of Alcohol Consumption on Indices of Iron Stores and of Iron Stores on Alcohol Intake Markers

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Background: Alcohol increases body iron stores. Alcohol and iron may increase oxidative stress and the risk of alcohol-related liver disease. The relationship between low or "safe" levels of alcohol use and indices of body iron stores, and the factors that affect the alcohol-iron relationship, have not been fully characterized. Other aspects of the biological response to alcohol use have been reported to depend on iron status.

Methods: We have measured serum iron, transferrin, and ferritin as indices of iron stores in 3375 adult twin subjects recruited through the Australian Twin Registry. Information on alcohol use and dependence and smoking was obtained from questionnaires and interviews.

Results: Serum iron and ferritin increased progressively across classes of alcohol intake. The effects of beer consumption were greater than those of wine or spirits. Ferritin concentration was significantly higher in subjects who had ever been alcohol dependent. There was no evidence of interactions between *HFE* genotype or body mass index and alcohol. Alcohol intake–adjusted carbohydrate-deficient transferrin was increased in women in the lowest quartile of ferritin results, whereas adjusted γ-glutamyltransferase, aspartate aminotransferase, and alanine aminotransferase values were increased in subjects with high ferritin.

Conclusions: Alcohol intake at low level increases ferritin and, by inference, body iron stores. This may be either beneficial or harmful, depending on circumstances. The response of biological markers of alcohol intake can be affected by body iron stores; this has implications for test sensitivity and specificity and for variation in biological responses to alcohol use.

Key Words: Iron, Ferritin, Dependence, Biological Markers.

IRON STORES ARE known to be increased in alcoholics and heavy drinkers (Chick et al., 1987; Irving et al., 1988; Kristenson et al., 1981; Leggett et al., 1990; Moirand et al. 1991). Increases in indices of iron stores, such as serum ferritin, have also been described in subjects drinking small amounts of alcohol compared with teetotalers (Milman and Kirchhoff, 1996; Robinson et al., 1998). Several authors have suggested that iron accumulation is one of the mechanisms involved in alcoholic liver disease (Ishii et al., 1997; Nordmann, 1994; Nordmann et al., 1987; Shaw and Jayatilleke, 1992; Tsukamoto et al., 1995; Wisniewska-Knypl and Wronska-Nofer, 1994). Mortality from alcoholic cirrhosis is greater in subjects with a higher hepatic iron content (Ganne-Carrie et al., 2000).

Conditions such as porphyria cutanea tarda, hepatocellular carcinoma, and hepatitis C may be promoted or exacerbated by high hepatic iron content, alcohol, or both (Bonkovsky et al., 1996; Elder, 1998; Pagliaro et al., 1999; Sampietro et al., 1999; Turlin et al., 1995). There is evidence that both iron and alcohol can initiate the formation of free radicals and produce oxidative stress within the liver (Fernandez-Checa et al., 1998; Ishii et al., 1997; Niemela, 1999). The relationships between alcohol intake and iron stores are therefore of interest, both at the high end of the alcohol-intake spectrum and among the general population.

Because most studies have concentrated on experimental animals or on extremes of alcohol intake in humans, we have investigated three questions: first, how far "normal" levels of alcohol intake in the population affect indices of iron stores (and, by inference, hepatic iron); second, whether there are alcohol-genotype interactions in relation to polymorphisms in the *HFE* gene (Feder et al., 1996) or alcohol-smoking, alcohol–body mass index (BMI), or alcohol-dependence interactions [as we have previously reported for carbohydrate-deficient transferrin (CDT); Whitfield et al., 1998]; and third, whether iron stores affect the relationship between alcohol intake and biological markers of alcohol intake or its effects, including CDT, γ-glutamyl

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Table 1. Results of Model Testing for Effects of Current Alcohol Intake, Lifetime Alcohol Dependence, and Excessive Drinking Within the Past Year, Taking Account of Relatedness of Twin Pairs by Using the Mx Program

Model	Variables included (at baseline) or added (in subsequent steps)	Compared against model	Iron		Transferrin		Saturation		Ferritin	
			$\Delta\chi^2$	p	$\Delta\chi^2$	p	$\Delta\chi^2$	p	$\Delta\chi^2$	p
Baseline	age, sex, BMI, collection time, *HFE* C282Y and H63D polymorphisms	N/A								
1	alcohol intake in previous week	baseline	36.18	$<10^{-8}$	12.79	$<10^{-3}$	13.61	$<10^{-3}$	23.42	$<10^{-5}$
2	lifetime alcohol dependence	1	0	NS	0.02	NS	0	NS	6.29	0.012
3	reported excessive drinking in past year	2	1.61	NS	0.05	NS	0.93	NS	0.02	NS
4	lifetime alcohol dependence	baseline	2.76	NS	0.36	NS	0.68	NS	12.31	$<10^{-3}$
5	lifetime alcohol dependence but no reported excessive drinking in past year	baseline	1.62	NS	0.02	NS	0.68	NS	3.45	NS
6	lifetime alcohol dependence with reported excessive drinking in past year	baseline	0.87	NS	0.38	NS	0.07	NS	7.47	0.006

The Baseline model (Whitfield et al., 2000a) includes adjustment for effects of sex, age, body mass index, and collection time. At each subsequent step (1–3), an additional variable was added to the model and the change in goodness-of-fit to the data was assessed by the χ^2 test. In steps 4–6, effects of lifetime alcohol dependence with or without recent excessive drinking were tested against the baseline model (i.e., without adjustment for reported current alcohol intake).

N/A, not available; NS, not significant.

transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

SUBJECTS AND METHODS

Subjects

Subjects were adult twins from a volunteer twin registry (Australian NHMRC Twin Registry), born between 1902 and 1964. In 1992 and 1993, telephone interviews were conducted with 1879 men and 3659 women by using the Semi-Structured Assessment for Genetics of Alcoholism diagnostic interview (Bucholz et al., 1994). This interview allows diagnosis of alcohol dependence according to the DSM-III-R criteria of the American Psychiatric Association (Heath et al., 1997). From 1993 to 1996, blood was collected from 1134 of the men and 2241 of the women. Serum and buffy coat were separated from blood samples and stored at −70°C until analyzed.

Immediately before blood collection, subjects filled in a table that asked how many drinks containing alcohol (10 g) they had taken on each of the preceding 7 days, divided into beer, wine, spirits, fortified wine, or "other." The numbers of drinks were summed to obtain a total for the past week. This total showed a good correlation with a weekly drinking estimate based on habitual quantity and frequency of alcohol use previously provided by the subjects during the telephone interview ($r = 0.78$ for men and 0.75 for women on log-transformed estimates). Height and weight were measured and BMI was calculated.

Because information on smoking was not gathered at the time of blood collection, it was taken from self-reports on smoking status in an earlier survey in 1988 (when smoking status was ascertained for 5538 subjects from this cohort) and in a smaller but near-contemporary one from 1993 to 1996 (for 1573 subjects). On each occasion, subjects categorized themselves as never having smoked, as being an ex-smoker, or as being a current smoker. For the 1432 subjects from whom there was smoking information on both occasions, there was good agreement ($\kappa = 0.74$, Spearman rank correlation = 0.88). Further information on the validation of the smoking data are given by Whitfield et al. (2000b).

Methods

Serum iron, transferrin, and ferritin were measured with Roche methods on a Hitachi 917 (Hitachi, Ltd., Tokyo, Japan) analyzer. The percentage of iron saturation of transferrin was calculated as 100 × (iron in μmol/liter)/(transferrin in g/liter × 25). Plasma GGT, AST, and ALT were measured by Boehringer methods on a Hitachi 747 analyzer. These tests were performed on all available samples for the 3375 subjects. CDT was measured by ion-exchange chromatography and radioimmunoassay by using a Pharmacia (Piscataway, NJ) method (CDTect radioimmunoassay) on 1400 samples, from 539 men and 861 women aged between 29 and 92 years. Because of the skewed alcohol intake frequency distribution, samples were prioritized for CDT determination if they were from subjects who had ever met DSM-III-R alcohol dependence criteria or who were drinking more than four drinks a day (men) or two drinks a day (women). Samples were also analyzed from co-twins of such subjects and from randomly selected never-dependent subjects who reported nonhazardous or no alcohol consumption.

HFE genotypes for C282Y and H63D were determined by polymerase chain reaction and allele-specific oligonucleotide hybridization (Whitfield et al., 2000a). C282Y homozygotes were confirmed by polymerase chain reaction and restriction fragment length polymorphism analysis with modified primers and the restriction enzyme *Rsa*I, as described by Jeffrey et al. (1999).

Because the subjects were twins (and therefore observations are not statistically independent), the significance levels and the standard errors (but not the means or correlations themselves) from conventional tests are biased in a nonconservative direction for any variables with significant familial resemblance. In the figures, the standard errors have been calculated without adjustment for this nonindependence. The maximum error that can arise in this way, if all subjects were monozygotic pairs of twins and the traits were 100% heritable, would be to halve the effective number of subjects and increase the standard errors by a factor of $\sqrt{2}$ or 40%. In practice, the effect will be less than this.

This problem was overcome through use of the program Mx (Neale et al., 1999), which allows simultaneous modeling of fixed effects on means and random effects on covariates of related measurements. In the analysis of data by using Mx, for example in Table 1, variables potentially affecting the dependent variables (iron, transferrin, saturation, and ferritin) are added to the model in a series of steps, and the improvement in fit between the model and the data is tested at each step. In Table 1, the variables shown in previous work (Whitfield et al., 2000a) to affect serum iron, transferrin, saturation, and ferritin have been included at the first step (baseline model). Variables related to alcohol use or dependence have been added progressively and tested for significance at each step.

RESULTS

Effects of Alcohol Intake on Iron Store Indices

Results of tests for effects of alcohol intake in the previous week, lifetime history of DSM-III-R alcohol depen-

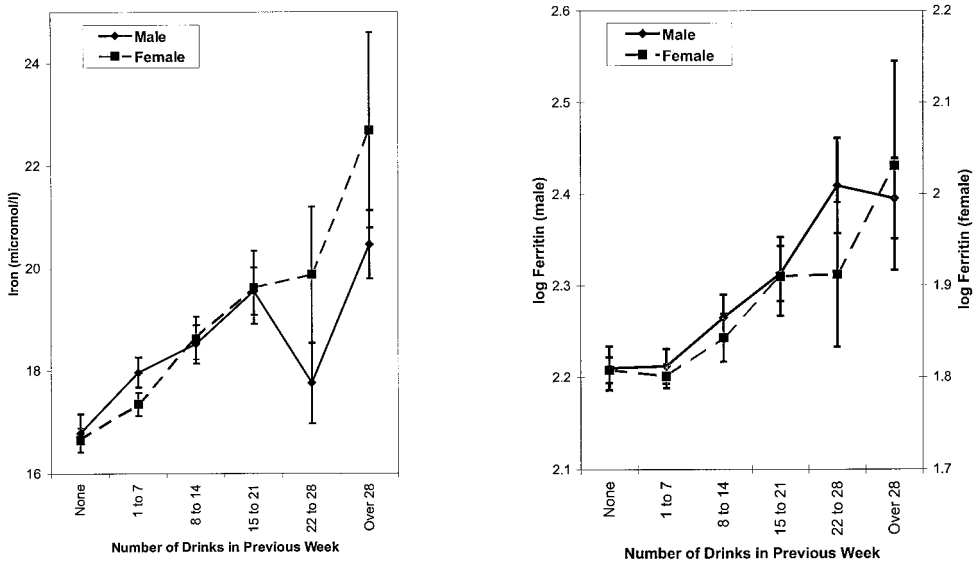

Fig. 1. Effect of reported alcohol intake in the previous week on serum iron (left) and ferritin (right): note that ferritin values are plotted against logarithmic scales. Iron results are adjusted for effects of age and collection time, and ferritin results are adjusted for effects of age.

Table 2. Differential Effects of Beverage Type on Indices of Iron Stores in Men and Women

Variable	Type of drink	Men	Women
Ferritin (log)	beer	0.15***	0.07**
	wine	0.05	−0.02
	spirits	0.05	−0.00
Iron	beer	0.12***	0.07**
	wine	0.09**	0.11***
	spirits	0.04	0.05*
Saturation	beer	0.09**	0.08***
	wine	0.04	0.08***
	spirits	0.04	0.05*
Transferrin	beer	0.06	−0.02
	wine	0.06	0.03
	spirits	0.01	−0.03

Values shown are β, the standardized regression coefficients from multiple regression with $\log_{10}$ (number of drinks of beer in previous week +1), $\log_{10}$ (number of drinks of wine in previous week +1), and $\log_{10}$ (number of drinks of spirits in previous week +1).

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. All others, $p > 0.05$.

dence, and excessive drinking on any occasion within the past year, after adjusting for sex, age, blood collection time, BMI, and *HFE* genotype, are shown in Table 1. Examination of the mean values by alcohol intake group showed that ferritin, iron, and saturation all increased with increasing alcohol intake in both men and women. Transferrin tended to increase (but not significantly) in women. Dose-response curves for the effects of self-reported alcohol consumption on iron and ferritin are shown graphically in Fig. 1. These curves for men and women are essentially parallel, so that after sex correction many of the further analyses could be done on the combined group including both male and female data.

Effects of beverage type on markers of iron stores are shown in Table 2. There was a significant effect of beer intake, but not wine or spirits intake, on serum ferritin in both men and women. For iron or saturation, both wine and spirits showed similar effects to beer. None of the beverage types had any significant effect on serum transferrin.

We next tested for a possible interaction between alcohol intake and other factors that might affect the response of iron stores to alcohol. Previous work with CDT on these subjects showed that the CDT response to alcohol became greater with decreasing BMI (Whitfield et al., 1998). Subjects were therefore grouped by BMI (above and below 25), and the alcohol-iron and alcohol-ferritin dose-response curves were calculated for each group (after correction for sex, age, and collection time). As previously reported (Whitfield et al., 2000a), high BMI was associated with increased ferritin and decreased iron, but the high-BMI and low-BMI curves were essentially parallel, and no BMI-alcohol interaction effect was seen (data not shown). Similarly, the effects of *HFE* C282Y and H63D polymorphisms on the alcohol-iron and alcohol-ferritin dose-response curves were simply to displace them upward without apparent changes in the slopes, indicating that these alleles act additively with drinking habits in affecting iron stores.

Again by analogy with our previous CDT study, effects of smoking status (current versus never or ex-) and of lifetime alcohol dependence history (DSM-III-R) on the relationship between current alcohol intake and iron or ferritin were checked. Smoking had no detectable effect (data not shown), but alcohol dependence history had a significant and unexpected effect on ferritin. Subjects who had ever been alcohol dependent had higher ferritin values, for any level of current alcohol intake, than never-dependent subjects. This was not the case for iron or for transferrin saturation with iron. These results are shown in Fig. 2 and were confirmed by the Mx analysis for effects of alcohol dependence (Table 1). This dependence effect was found only for ferritin, but attempts to distinguish between alcohol-dependent and never-dependent groups by calcu-

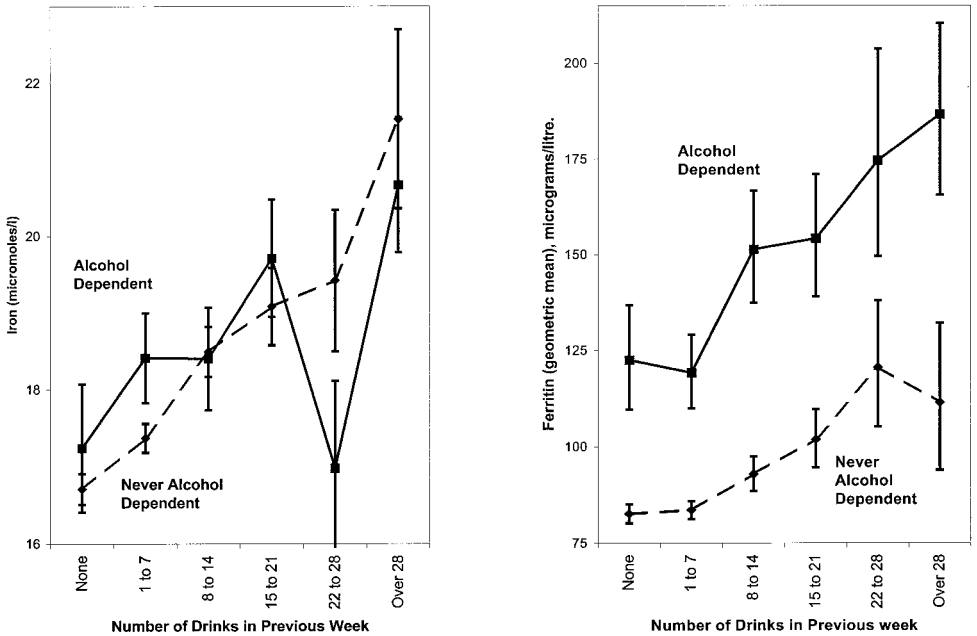

Fig. 2. Effect of lifetime alcohol dependence diagnosis (presence or absence of lifetime alcohol dependence by DSM-III-R criteria) on the alcohol-iron (left) and alcohol-ferritin (right) dose-response curves. Iron results are adjusted for effects of age, sex, BMI, and collection time, and ferritin results are adjusted for effects of age, sex, and BMI.

lating ferritin/iron or ferritin/saturation ratios gave no greater separation.

Further analysis of ferritin results in the alcohol-dependent group checked for effects of recent or current excessive alcohol use, by using the subjects' responses to a Semi-Structured Assessment for Genetics of Alcoholism question about maximum consumption on any day in the past year. Men were classified as having alcohol dependence with continuing excessive alcohol intake if their maximum reported number of drinks on any day in the previous year was greater than 10 and they were lifetime DSM-III-R positive. For women, the equivalent cutoff point was greater than five drinks on any day. The expectation was that ferritin levels might decrease if excessive alcohol consumption ceased, so that the group reporting a high past-year maximum intake would have higher ferritin values. This was not supported; when alcohol intake in the previous week and lifetime alcohol dependence diagnosis were entered in the model first, there was no additional effect of excessive intake in the past year. When alcohol intake in the previous week was omitted, both lifetime alcohol dependence and excessive consumption within the past year were significant for ferritin, but it seems that in this model the past-year maximum was acting as a surrogate for recent alcohol intake (Table 1).

Effects of Iron Status on Alcohol Intake Markers

There were significant correlations between CDT and transferrin concentrations ($R = 0.34$, $p < 0.001$) and between CDT and ferritin ($R = -0.35$, $p < 0.001$) in women. In men, the correlation with ferritin was not significant, and the correlation with transferrin ($R = 0.11$, $p < 0.01$) was considerably less. Therefore, men and women were divided into quartiles of ferritin, and the relationships between alcohol intake (in the previous week) and CDT were examined. Consistent with the hypothesis that iron deficiency is associated with increased CDT, it seemed that the lowest quartile of subjects for ferritin had higher alcohol-CDT dose-response curves, but the other three quartiles were similar to one another. Therefore, subjects were divided into two groups, lowest-quartile ferritin and other, and also by sex, for further analysis. Results are shown in Fig. 3. Lowest-quartile ferritin was associated with higher CDT, for any level of alcohol intake, in women but not in men. However, it should be noted that ferritin levels were substantially lower, for any sex-specific quartile, in women than men; the 25th, 50th, and 75th centiles were 114, 204, and 319 μg/liter for men and 36, 68, and 123 μg/liter for women.

There were highly significant positive correlations between ferritin and GGT, AST, and ALT in both men and women. More detailed examination of these relationships is shown in Table 3; after controlling for alcohol intake, increasing ferritin concentration was significantly associated with decreasing CDT and with increasing GGT, AST, and ALT. All of these markers of alcohol intake were strongly influenced by serum ferritin and, by inference, by body iron stores. In women, the effect of alcohol intake on GGT, AST, and ALT was nonsignificant or comparatively weak, possibly because of the narrower range of alcohol intake among the women.

Further analysis showed that variation in ferritin ac-

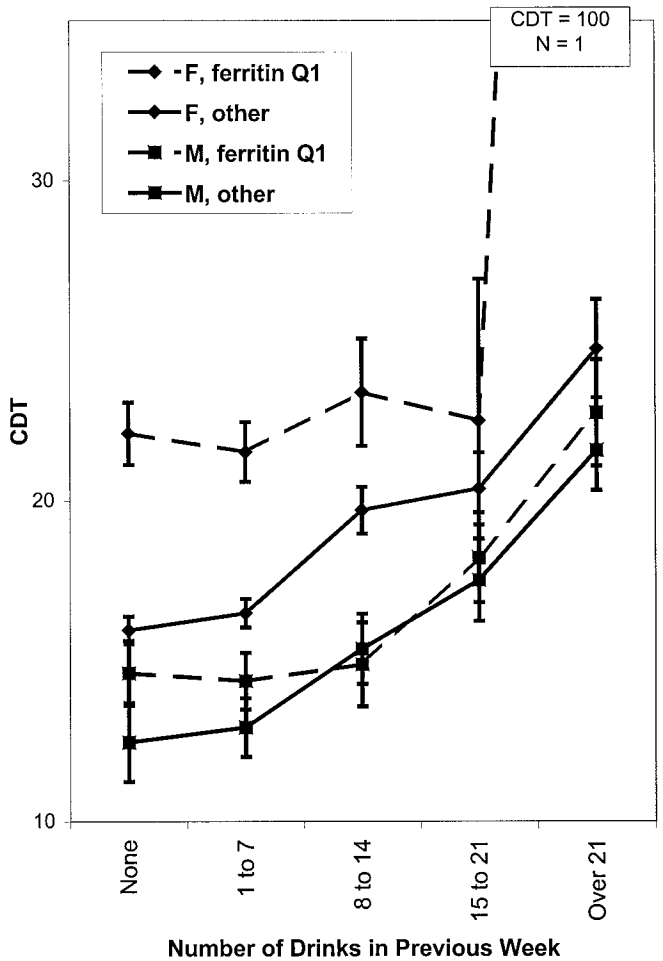

Fig. 3. Effect of serum ferritin concentration on the alcohol-CDT dose-response curve in men (M) and women (F), contrasting results from subjects in the lowest quartile (Q1) of ferritin (less than 114 μg/liter for men and 36 μg/liter for women) against those for subjects in the upper three quartiles (Other).

Table 3. Effects of Iron Stores (Ferritin) and Alcohol (Intake in Previous Week) on Alcohol Intake Markers

Variable	CDT	GGT (log)	AST (log)	ALT (log)
Male				
Ferritin	−0.09*	0.18***	0.09**	0.18***
Alcohol	0.35***	0.25***	0.15***	0.12***
Female				
Ferritin	−0.37***	0.29***	0.17***	0.20***
Alcohol	0.27***	0.03	−0.02	−0.06*

Values shown are β, the standardized partial regression coefficients for the four dependent variables CDT, GGT (log), AST (log), and ALT (log). Because of sex differences in alcohol intake and iron stores, results are shown separately for men and women.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. All others, $p > 0.05$.

counts for the inverse correlation between CDT and GGT in women (correlation, $-0.09, p = 0.013$; partial correlation adjusting for alcohol intake, $-0.10, p = 0.003$; partial correlation adjusting for alcohol intake and ferritin, -0.01, $p = 0.805$). This was not the case in men (correlation, $-0.00, p = 0.998$; partial correlation adjusting for alcohol intake, $-0.10, p = 0.019$; partial correlation adjusting for alcohol intake and ferritin, $-0.09, p = 0.044$).

DISCUSSION

Alcohol Intake: Effects on Markers of Iron Status

Our results confirm that there are substantial effects of current alcohol intake on iron status, as measured by these tests, and they show that even moderate alcohol use has a detectable effect. Under some circumstances, such an effect might be beneficial, whereas in others it is more likely to be harmful. Particularly in men, who are less likely to be iron deficient and who consume more alcohol, on average, than women, the synergistic effects of alcohol and iron may lead to or exacerbate liver damage.

However, we have measured indices of iron stores (serum iron and ferritin concentrations) rather than measuring iron stores directly. In general, serum ferritin is closely related to iron stores. A strong linear relationship between serum ferritin and body iron content has been demonstrated by iron depletion in human volunteers (Skikne et al., 1990). In the more specific context of this paper, it is important to consider whether there are features of alcohol consumption that might alter the relationship between ferritin and hepatic or total body iron.

A number of conditions apart from iron overload are known to increase serum ferritin, including acute liver injury, inflammation or infection, and malignant disease. If (and only if) any of these occur with increased frequency or severity in drinkers than in nondrinkers, and in heavier than in lighter drinkers, an association between ferritin and alcohol intake—not due to alcohol's increasing iron stores—would be seen.

The subjects in this study were recruited through a volunteer twin registry and made a special trip to a blood collection center as part of the protocol. Their mean age at the time of blood collection was 44 years, and it is most unlikely that any significant number was suffering from cancers sufficiently advanced to produce increased serum ferritin. Minor degrees of infection or inflammation sufficient to increase the ferritin results may have been present in some subjects, but for this to affect the conclusion that alcohol increases iron stores, the infection or inflammation would have to be more common among the heavier drinkers. Inspection of Fig. 1 shows that increases in serum iron and ferritin (compared with abstainers) were apparent in subjects taking only two to three drinks per day. Epidemiological studies on markers of inflammation (Cushman et al., 1999; Mulholland et al., 1999) do not support the idea that alcohol intake at this level provokes changes in acute phase proteins, so the changes in ferritin are probably unrelated to infection or inflammation.

Moreover, the acute phase response that occurs during inflammation or infection produces a decrease in serum iron as well as an increase in serum ferritin (Konijn, 1994). Our finding that both iron and ferritin increase with increasing alcohol use (Fig. 1) indicates that the two markers are reflecting a true effect of alcohol use on iron stores.

Many tests have been applied over the years to the

assessment of iron status. Accumulation of iron in the liver is best demonstrated by histological or chemical techniques applied to biopsy samples, and total body iron can be assessed by iron depletion. Iron content of the liver can also be measured with physical techniques, including magnetic resonance and superconducting quantum interference device biomagnetometry. None of these techniques is applicable in studies involving large numbers of volunteer subjects. As mentioned previously, serum ferritin shows an excellent correlation with body iron, assessed from iron depletion. There is a highly significant, but imperfect, correlation between serum ferritin and liver iron measured by biomagnetometry, with *R* values around 0.5 (in patients with secondary iron overload) reported by Nielsen et al. (1995, 2000).

Serum transferrin receptor is another test for iron status, particularly in the differential diagnosis of anemias. Although mean serum transferrin receptor is decreased in patients with iron overload (Khumalo et al., 1998; Looker et al., 1999), both these reports showed a high degree of overlap between the ranges found in healthy and iron-overloaded subjects. The iron depletion study of Skikne et al. (1990) included multiple measurements of serum transferrin receptor as iron stores decreased, and they concluded that "serum ferritin is the most sensitive index of iron status when there are residual iron stores, whereas the serum receptor is more sensitive when there is functional iron deficiency." For the purposes of this study, measurement of transferrin receptor would have contributed little.

Factors Affecting the Alcohol-Iron Dose-Response Curve

A number of factors were tested to determine whether they affected the relationship between alcohol use and iron store markers. In general, beer had a larger effect on indices of iron stores than wines or spirits, particularly for ferritin (the major indicator of body iron stores). The fact that beer consumption was more strongly associated with increased serum ferritin may also increase the risk of excessive iron stores in men, who were more likely to consume beer as their main alcoholic beverage.

The differential effect of beer, compared with wine or spirits, on iron stores is not easily explained. Because alcohol increases iron absorption by making the intestine more permeable and iron uptake increases through passive and unregulated transfer (Duane et al., 1992), it would be reasonable for a higher-iron alcoholic beverage to have a greater effect on iron stores. However, the iron content of beer in Australia is reported to be lower than that of either red or white wine and similar to that of spirits (English and Lewis, 1991). The difference may be due to the larger volume of beer that will be consumed for each 10-g "standard drink" of alcohol.

In addition to the substantial effects of reported alcohol consumption within the past week on serum iron and ferritin, there was a significant effect of lifetime alcohol dependence status on ferritin (Fig. 2). Subjects who had ever been dependent had higher ferritin values, suggesting an accumulation of iron from past excessive alcohol intake. It was not possible to demonstrate any effect of excessive drinking within the past year on ferritin values, and this implies that alcohol-induced iron overload may persist for a considerable time. The effect of alcohol dependence history was nontrivial; the ferritin values in lifetime-dependent subjects were about 50% more than those for never-dependent subjects, whereas the difference between subjects taking one drink per day or less and those taking four drinks per day was approximately 40% (Fig. 2). However, alcohol dependence history made no difference to serum iron results, even though the mean iron value for four-drinks-per-day subjects was approximately 25% more than that for subjects reporting no drinking in the previous week.

In considering the effects of lifetime alcohol dependence, over and above those of recent alcohol intake, the findings of Dawson (2000) may be relevant. She showed, in a large study of alcohol intake and all-cause mortality, that the relationship between mortality and alcohol consumption differed according to alcohol dependence status, with higher death rates (after adjusting for relevant covariates) in the dependent subjects. Therefore, we should consider the possibility that a period of alcohol dependence and associated intense alcohol use causes some long-term change in metabolic state that thereafter affects mortality, iron stores, and possibly other alcohol intake markers, including CDT (Whitfield et al., 1998).

We have previously reported (Whitfield et al., 2000a) that both BMI and *HFE* genotype have significant effects on iron status in these subjects. Our current results show that alcohol, BMI, and *HFE* genotype have additive effects on iron stores, rather than any alcohol-BMI or alcohol-genotype interactions. Our results do not, however, rule out long-term synergistic effects of alcohol and obesity on iron-related oxidative damage to the liver or other organs.

The smoking status of the subjects was not verified at the time of blood collection but was derived from data collected previously. As discussed in a previous report (Whitfield et al., 2000b), we believe that the assignment of smoking status is valid. In fact, no effect of smoking on the relationships between iron markers and alcohol use was found, but it is possible that a small effect could have remained undetected.

Iron's Effects on Biological Markers of Alcohol Intake

Biochemical tests such as CDT or GGT are useful but imperfect indicators of alcohol intake, and information on patient or subject characteristics that affect their response to alcohol has the potential to improve test interpretation. We found interactions between iron and the marker responses to alcohol intake in opposite directions for CDT and the liver function tests (AST, ALT, and GGT). These

interactions have a number of implications: first for test performance (sensitivity and perhaps specificity), second for the role of CDT in iron delivery in iron deficiency, and third for the role of iron stores in increasing liver function test results and possibly affecting liver disease or other negative outcomes.

It has been known for some time (De Feo et al., 1999; Jensen et al., 1994; Stauber et al., 1996) that iron deficiency has the effect of increasing serum CDT concentration. If unrecognized, the iron deficiency may cause false-positive results. Because iron stores are lower in women than men, this problem with CDT interpretation is more likely to affect women. This is consistent with our finding (Table 3) that CDT was strongly negatively correlated with ferritin in women but much less so in men, and with the results in Fig. 3. Indeed, the higher CDT values found in control (nonalcoholic) women than control men may be due to lower iron stores, and the decrease in the CDT reference range for women, which occurs around the age of 50 years (Whitfield et al., 1998), might be due to increasing iron retention when menstrual blood loss ceases.

Iron deficiency will also increase total transferrin; this complicates the relationships among CDT, iron deficiency, and transferrin concentration (Sorvajarvi et al., 1996). Future studies of the relative diagnostic performance of CDT concentration against the use of CDT as a percentage of total transferrin should consider the effects of iron and transferrin status.

The relationships between CDT and iron overload are still uncertain. Some experiments (Beguin et al., 1988; Regoeczi et al., 1984; Rudolph et al. 1986) have shown that iron is transferred into liver cells from asialotransferrin more rapidly than from normal transferrin, probably because asialotransferrin is internalized not only by the transferrin receptor but also by the asialoglycoprotein receptor. Moreover, the mechanisms that down-regulate iron uptake through the transferrin receptor when cellular iron stores are increased may not apply to the nonspecific asialoglycoprotein-mediated iron uptake. Therefore, increased CDT, as a result of excessive alcohol use, might lead to increased hepatic iron.

The concept that the biological response to alcohol intake may depend on iron status, discussed previously in relation to CDT, also applies to other markers. Many studies have shown that serum activity of the enzymes GGT, AST, and ALT varies with alcohol intake (e.g., Nagaya et al., 1999; Whitehead et al., 1978, 1996; Whitfield and Martin, 1985), among other factors. There is heterogeneity in this response; some people have higher serum enzyme activities than others for similar alcohol intakes. This heterogeneity probably has multiple causes, and variation in iron status, as assessed by serum ferritin (Table 3), is one. GGT, AST, and ALT were highly significantly and positively correlated with ferritin values in both men and women, even after statistical adjustment for alcohol intake.

In women, with a narrower range of alcohol consumption but a wider range of ferritin values, the enzymes' correlations with ferritin were stronger than for men. Indeed, we may speculate that the lower serum GGT values in women and the lesser increase in response to alcohol intake might be due to their lower iron stores.

It is not entirely clear why high ferritin is associated with higher GGT, AST, and ALT. Although it is well established that each of these variables increases with increasing alcohol consumption, data on correlations between ferritin and the enzyme values from community samples are sparse. In subjects receiving treatment for alcohol dependence (Lundin et al., 1981; Meyer et al., 1984; Moirand et al., 1991), significant enzyme-ferritin correlations have generally been found, and values for ferritin and GGT declined in parallel with abstinence. In community-based samples of men (Chick et al., 1987) and women (Nystrom et al., 1988), ferritin was correlated with GGT, but in a study in which subjects were selected for study on the basis of abnormal GGT (Kristenson et al., 1981), no GGT-ferritin correlation was found. The enzyme-ferritin correlations may be due to an indirect association; because iron overload is associated with liver damage, higher values of liver function tests are to be expected in subjects with high ferritin. A more direct association is also possible for GGT if iron-catalyzed free radical generation leads to hepatocyte glutathione depletion and GGT induction.

The associations between ferritin, on the one hand, and GGT, AST, or ALT, on the other, are independent of alcohol intake, but their interpretation is subject to the caveats discussed previously about ferritin as a marker of iron stores. If ferritin is a good measure of iron status, then iron overload is associated with some degree of liver abnormality; if high ferritin is an indication of an acute phase response, then the causes of such acute phase responses are also affecting the liver. If high serum ferritin is due to release from damaged liver cells, in parallel with release of AST and ALT but independent of iron status, then the reasons for such liver damage in our essentially healthy subjects are unknown. Overall, the most probable explanation is that serum ferritin does reflect iron stores, and high iron stores are associated with (initially minor) liver damage and abnormal serum enzyme results. The relationships between hazardous alcohol consumption, iron status, and liver damage deserve further study by using additional measures of iron stores.

CONCLUSIONS

Chronic alcohol consumption increases serum markers of iron stores, even when alcohol intake is not in the range known to be harmful. Beer has a greater effect on the main marker of cellular iron stores, ferritin, than equivalent amounts of alcohol taken as wine or spirits. Ferritin is also affected by past alcohol intake; alcohol dependence (inevitably involving a period of high alcohol consumption) has

a medium- to long-term result of increased iron stores independent of current alcohol use.

Second, the biological effects of alcohol are affected by iron status. Iron deficiency has substantial effects on CDT in women; this suggests that a reinterpretation of sex and age effects on CDT may be needed. Iron stores affect the liver function tests or alcohol markers GGT, AST, and ALT, but in the opposite direction from CDT; iron increases these enzymes, and the effect is not due to their common dependence on alcohol intake. This is consistent with the hypothesis that hepatic iron overload is a poor prognostic factor in alcohol-related liver disease.

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