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Measuring Carbohydrate-Deficient Transferrin by Direct Immunoassay: Factors Affecting Diagnostic Sensitivity for Excessive Alcohol Intake

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BACKGROUND: Carbohydrate-deficient transferrin (CDT) is a marker of alcohol intake that is used for detecting or monitoring alcohol-use disorders. The introduction of a new direct immunoassay for CDT justifies reevaluation of test performance and reexamination of factors affecting test diagnostic sensitivity and specificity.

METHODS: Individuals enrolled in twin/family studies of alcohol use and dependence provided blood samples and information on recent alcohol use. Serum CDT concentration was measured in 2 088 people with the N Latex CDT (Dade Behring) method, and CDT percentage (CDT%) was calculated as the proportion of the total transferrin concentration measured with Roche reagents.

RESULTS: Diagnostic sensitivity was low, both for comparisons of men who reported an alcohol intake of >28 drinks/week vs those who consumed ≤28 drinks/week (28% sensitivity) and for women who consumed >14 drinks/week vs those who consumed ≤14 drinks/week (18% sensitivity), at cutoff values that yielded a 95% specificity. Body mass index, variables associated with metabolic syndrome, and smoking had notable effects on the probability of an abnormal CDT result with excessive alcohol use. Diagnostic sensitivity was greater in men of normal weight (43%) than in obese men (10%) and greater in male smokers (38%) than in male nonsmokers (21%). In women, diagnostic sensitivities were ≤20%, even for those of normal weight and for smokers.

CONCLUSIONS: CDT is a poor marker of excessive alcohol intake in both women and men who are overweight or obese. It is also less useful in nonsmokers than in smokers. The diagnostic performance of the direct immunoassay and the effects of obesity and smoking are similar to those reported with previous anion-exchange immunoassay methods.

Changes in the glycan structure of plasma transferrin associated with excessive alcohol use were first described by Stibler and colleagues in 1979 *(1)*. Since then, a substantial number of reports on the measurement of isoforms of carbohydrate-deficient transferrin (CDT)[4] as a marker of alcohol intake have been published [reviewed in *(2–5)*]. The advantages of this marker are that the concentration remains increased for a long period after the cessation of drinking (unlike ethanol metabolites) and it is comparatively specific for patients with liver disease (unlike γ-glutamyltransferase). Despite initial reports of very high diagnostic sensitivity and specificity *(1, 6)*, which were probably inflated by spectrum bias, both false-negative and false-positive results have been found with this marker. In 2000, Scouller et al. *(3)* reviewed test-performance data and potential causes of variation and conducted a metaanalysis that showed that commonly used methods produced diagnostic odds ratios of approximately 12, corresponding to a 40% sensitivity at a 95% specificity, but with wide confidence intervals. Since that time, additional reports and reviews have described similarly intermediate estimates of test performance *(7–12)*.

Despite the clinical and economic significance of alcohol abuse and dependence, the use of CDT as a marker has not become widespread. This fact can be attributed in part to the lack of a fast, automated, and reliable method and in part to substantial false-nega-

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[4] Nonstandard abbreviations: CDT, carbohydrate-deficient transferrin; BMI, body mass index; CDT%, CDT concentration as a percentage of the total transferrin concentration; AUC, area under the ROC curve.

tive rates for the assays. CDT testing is likely to become more widely used with the recent development of both an HPLC method (a potential reference method) *(13)* and an automated direct immunoassay *(14)*. Until now, the methods have required separate isoform-separation and transferrin-quantification steps, such as isoelectric focusing and immunofixation, or anion-exchange column chromatography and immunoassay. Another important feature is that the more widely used and commercially available anion-exchange/immunoassay methods have varied in their degree of inclusion of disialotransferrin and trisialotransferrin [see *(4)*]. Disialotransferrin is believed to be the most appropriate target analyte for CDT methods *(15)* and should be included, whereas trisialotransferrin is probably not related to alcohol consumption *(16, 17)* and should be excluded. Because there is evidence that CDT test performance is method related *(3, 18–21)*, a reevaluation of CDT diagnostic sensitivity and specificity and the factors affecting them becomes necessary when a method based on a new analytical principle is introduced. The direct immunoassay (N Latex CDT; Dade Behring, now Siemens Healthcare Diagnostics) has been evaluated for analytical performance and comparability with HPLC assay *(14)* but has not yet been evaluated for diagnostic performance.

Several patient characteristics that affect both diagnostic sensitivity and specificity have mainly been documented for the anion-exchange methods. These characteristics include the effects of obesity and associated metabolic characteristics *(22–27)* and iron status *(28, 29)* on the relationship between alcohol use and CDT concentration. There is a need to confirm and extend previously published information on CDT measurement, both specifically for this method and more generally to characterize the biological and metabolic factors that affect the dose–response relationship between alcohol intake and CDT concentration.

We used the new immunoassay method to evaluate the sensitivity and specificity of CDT as a marker for hazardous drinking in a community-based sample and assessed the effects of body mass index (BMI), HDL and LDL cholesterol, triglycerides, ferritin, smoking, and alcohol dependence on the reference interval in nondrinkers or light drinkers, the alcohol–CDT dose–response curve, and test sensitivity. Because of our interest in butyrylcholinesterase as a marker of metabolic syndrome *(30)*, we also included the results of this measurement in the data analysis.

Materials and Methods

Participants were recruited for studies on the genetics of alcohol and nicotine dependence and on the biological consequences of alcohol dependence. The core group consisted of twins who had participated in our previous studies on alcohol consumption *(31, 32)* and was extended to include spouses, parents, siblings, and adult children. All individuals gave informed consent to participate, and the studies were approved by the appropriate ethics review committees.

Blood was collected between 2001 and 2005 from 9031 people (3998 men and 5033 women) of ages 18–92 years. The participants lived throughout Australia, and blood samples were sent for processing to the Queensland Institute of Medical Research in Brisbane via courier for next-day delivery. After centrifugation, the sera obtained from tubes without anticoagulant were stored at −70 °C. Aliquots sent to Sydney for analysis were transported on dry ice and also stored at −70 °C until analysis.

Participants provided information about their alcohol use and symptoms associated with alcohol dependence as part of a telephone interview and at the time of blood collection completed a 7-day retrospective diary of alcohol use and the use of tobacco products. Lifetime alcohol dependence was assessed with the DSM-IIIR criteria of the American Psychiatric Association *(33)*. Alcohol and smoking data were compiled from the self-report diaries by summing the number of alcoholic drinks or the number of times tobacco products were used over the 7-day period. Anyone who used any tobacco products, including snuff and chewing tobacco, was characterized as a "smoker," but practically all tobacco use (98%) was as cigarettes. BMI was calculated from self-reports of weight and height. Safe or desirable drinking limits were taken from the 2001 recommendations of the Australian National Health and Medical Research Council to establish safe or desirable drinking limits, which were ≤28 standard drinks (each containing 10 g of ethanol) per week for men and ≤14 for women *(34)*. Study participants who reported alcohol consumption greater than these limits are referred to in this report as hazardous drinkers. CDT was measured in all available samples from study participants who reported alcohol intake greater than these limits (n = 1173; 634 men and 539 women) and was measured in 915 people (367 men and 548 women) who reported lower or no alcohol intake.

Serum samples were used for all biochemical tests. Between May 2006 and July 2007, we used the N Latex CDT method (Dade Behring/Siemens Healthcare Diagnostics) on a Dade BN-II nephelometric analyzer to measure CDT concentration. The mean between-batch imprecision (CV) was 8.0% for low-control sera (CDT concentration, 55 mg/L) and 4.5% for high controls (CDT concentration, 159 mg/L). Other biochemical results were obtained with a Roche 917 analyzer. Because total transferrin had already been measured

before the CDT assays, we did not reassay it with the Dade reagents. CDT as a percentage of total transferrin (CDT%) was calculated from the CDT concentration obtained with the Dade method and the total transferrin concentration measured with the Roche assay. The comparability of the transferrin results obtained with the Dade and Roche assays was assessed by comparing the means and distributions from our data with those published for the previous evaluation of the N latex CDT assay *(14)*, and from external quality-assurance data. Statistical analyses were performed with SPSS 15.0 (SPSS).

Overall details about the study participants and details of the CDT measurements are given in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol54/issue7. One participant with a total transferrin concentration of 0.47 g/L was excluded from the data analysis; this 59-year-old man had reported consuming 71 drinks in the preceding week (CDT, 53.9 mg/L; CDT%, 11.47%).

Results

REFERENCE INTERVALS: CDT CONCENTRATION AND CDT%

CDT reference intervals were obtained from participants who reported no alcohol use or consumption of ≤7 drinks in the preceding week (1 drink/day). This analysis yielded a 95% central range (2.5th–97.5th percentiles) of 1.04%–2.27% (25.4–55.7 mg/L) for men and 1.03%–1.90% (26.1–63.8 mg/L) for women. The difference between the sexes in the mean CDT% (SE) for this group just reached statistical significance [men, 1.515% (0.023%); women, 1.460% (0.012%); $P = 0.035$].

After adjusting for sex and alcohol intake, we found total transferrin concentration to be correlated with both CDT concentration ($r = 0.264$; $P < 0.001$) and CDT% ($r = -0.153$; $P < 0.001$). Comparison of CDT and CDT% ROC curves revealed mean (SE) areas under the curve (AUCs) of 0.691 (0.012) and 0.719 (0.012), respectively.

THE ALCOHOL–CDT DOSE–RESPONSE CURVE

The effects of reported alcohol intake on mean CDT%, the range of results, and the proportion of people with CDT% results >2.0% are summarized in Fig. 1. Fig. 2 summarizes the comparisons of alcohol–CDT% relationships for men vs women, for men of normal weight vs overweight and obese men, and for smoking vs nonsmoking men. The mean CDT% increased with a self-reported alcohol intake of >7 drinks/week and continued to increase in men across the entire range of alcohol intakes encountered in this population sample. For women, however, the mean CDT% reached its highest value in the group reporting 29–35 drinks/week and did not change significantly with higher reported intakes. The increase in CDT% with increasing alcohol intake was greater in men of normal weight (BMI ≤25 kg/m^2) than in those who were overweight or obese and was greater in male smokers than in male nonsmokers.

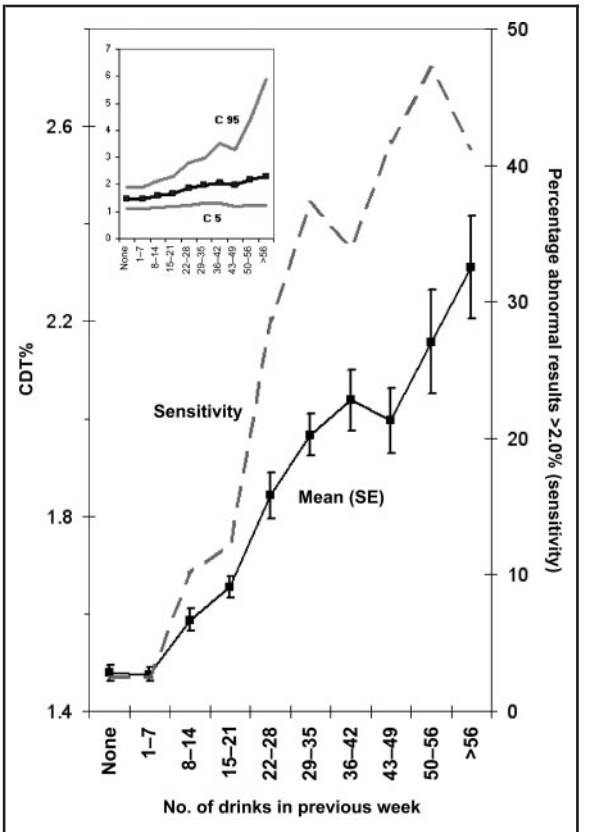

Fig. 1. Effects of reported alcohol intake on the mean, range, and test sensitivity of CDT as a percentage of total transferrin.

The *x* axis shows the reported number of alcoholic drinks in the week preceding blood collection. The continuous line and error bars represent the CDT% means and SEs for each drinking category (left *y* axis), and the interrupted line shows the proportion of CDT% values >2.0% (sensitivity, right *y* axis). The inset shows that the distribution of CDT% values [shown as the 5th (C5) and 95th percentiles (C95)] widens as alcohol intake increases.

OTHER EFFECTS ON CDT CONCENTRATION

Table 2 in the online Data Supplement summarizes the relationships between CDT% and other variables, first for the participants in the control group who consumed ≤7 drinks/week and, second, for the study participants whose alcohol consumption exceeded the

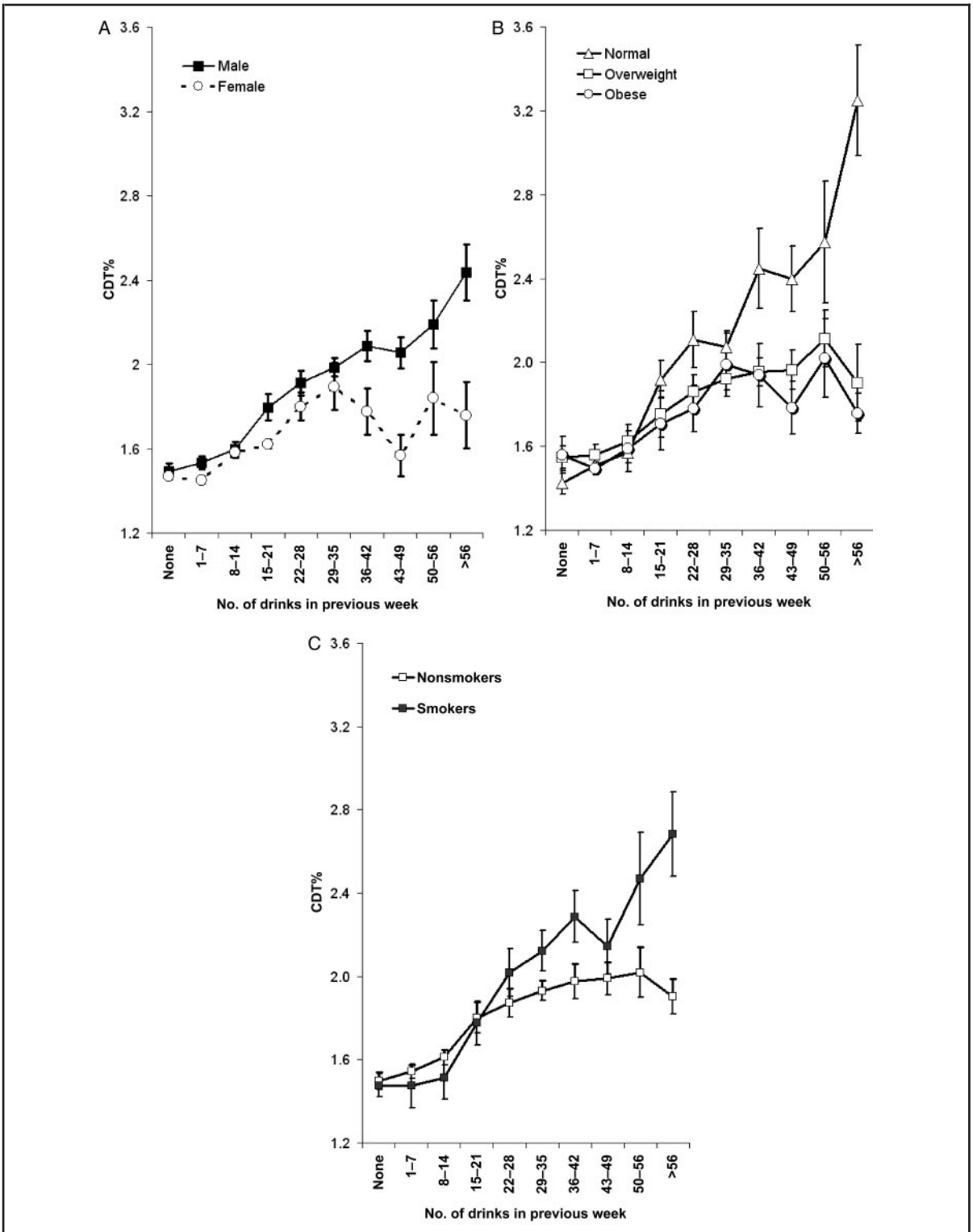

Fig. 2. Effects of sex (A), BMI in men (B), and smoking in men (C) on the dose–response curve for self-reported alcohol intake and CDT%.

Table 1. ROC analysis of CDT% by participant characteristics.[a]

	Male		Female	
	AUC	Sensitivity at 95% specificity	AUC	Sensitivity at 95% specificity
All	0.714 (0.017)	0.28	0.631 (0.017)	0.18
BMI				
Normal (≤25 kg/m^2)	0.786 (0.026)	0.43	0.670 (0.023)	0.19
Overweight (25.01–30 kg/m^2)	0.695 (0.024)	0.27	0.656 (0.031)	0.20
Obese (>30 kg/m^2)	0.627 (0.045)	0.10	0.483 (0.041)	0.11
Smoking				
Nonsmoker	0.684 (0.021)	0.21	0.631 (0.020)	0.18
Smoker	0.766 (0.030)	0.38	0.594 (0.036)	0.18
GGT (All participants with CDT results)	0.658 (0.018)	0.16	0.606 (0.017)	0.09

[a] The contrasted groups (controls and cases) were people who reported consuming ≤28 drinks in the previous week vs >28 drinks for men and ≤14 drinks in the previous week vs >14 drinks for women. Results for γ-glutamyltransferase (GGT) are provided for the same individuals for comparison. AUC data are presented as the estimate (SE).

sex-specific limits of 28 and 14 drinks/week. The tested variables are associated with obesity, metabolic syndrome, cardiovascular risk, and iron status, which have been shown to affect or correlate with CDT concentration. In the control group, CDT% was significantly correlated with triglyceride and C-reactive protein concentrations in men and women. Among the hazardous drinkers, CDT% showed significant univariate correlations in both sexes with alcohol intake, transferrin, BMI, HDL cholesterol, LDL cholesterol, triglycerides, butyrylcholinesterase, and smoking status. We assessed independent factors associated with CDT% in the at-risk drinkers with multiple regression and logistic regression (see Table 3 in the online Data Supplement). In this group, CDT% and the probability of an abnormal result were independently associated with transferrin concentration, HDL cholesterol, and smoking but notably were not associated with the number of drinks consumed. BMI showed independent effects only in men.

TEST SENSITIVITY IN HAZARDOUS DRINKERS AND ROC CURVE ANALYSIS

Test sensitivity and the AUC were assessed for a number of scenarios (Table 1). The most stringent test is to include all study participants with valid CDT results (i.e., excluding only the man with the low total transferrin concentration) and to divide the participants into 2 groups of individuals who reported alcohol intakes less than or greater than the threshold (28 drinks for men and 14 drinks for women). The AUCs for this test were 0.714 for men and 0.631 for women. Equivalent results obtained for γ-glutamyltransferase showed a lower AUC and sensitivity, particularly in men.

In a less demanding comparison, contrasting a control group of people who reported consuming ≤7 drinks/week against clearly excessive drinkers who reported consuming twice the alcohol in the recommended limits (i.e., 56 drinks/week for men, 28 for women), CDT AUCs were 0.781 (0.027) for men and 0.687 (0.032) for women. The observed sensitivities were 0.43 and 0.35, respectively, at cutoff limits (2.02% and 1.83%) that yielded 95% specificities.

After identifying or confirming the characteristics that affect the probability of an abnormal CDT% result in self-reported at-risk drinkers, we examined test sensitivity and ROC curves in participants classified according to BMI and smoking status. Although these variables were not the most powerful predictors of an abnormal CDT concentration in the logistic regression analysis, they are more suitable for the practical interpretation of test results. These results are summarized in Table 1 and illustrated in Fig. 1 in the online Data Supplement. AUCs were higher in men than in women and decreased with increasing BMI. In men, but not in women, AUCs were higher in smokers than in nonsmokers.

Discussion

We found the direct immunoassay method to have a test sensitivity within the range reported for anion-exchange methods, and its application and usefulness are similarly dependent on other patient characteristics, in addition to alcohol intake.

The mean CDT% and the probability of a CDT% result >2.0% increased with the reported alcohol intake, even at low intake levels (8–14 drinks/week,

Fig. 1). In men, the mean CDT% continued to increase with increasing intake up to our highest category (Fig. 2A). In women, however, the mean CDT% was highest in the 29–35 drinks/week category and did not increase further with higher reported intakes; the apparently lower values in the next 2 intake categories do not constitute a statistically significant difference. These dose–response curves are consistent with those found with anion-exchange/immunoassay methods *(24, 35)*, but we were able to extend our observations to higher alcohol-intake categories, where the male and female results diverge. The reasons for this divergence are not clear.

The observed test sensitivity with a cutoff value that yields a 95% specificity was 0.28 (i.e., 28%) in men and 0.18 (18%) in women. At first sight these figures appear disappointing but result from simply dividing participants into those who reported consuming ≤28 drinks/week and those who consumed >28 drinks/week (for men; 14 drinks/week for women). Thus, a man who reported consuming 26 drinks/week would be classified with the control group, and another man who consumed 30 drinks/week would be classified in the case group, although the reported difference in their alcohol intakes is small. However, contrasting groups of participants with more extreme differences in alcohol intake, such as those reporting ≤1 drink/day and high-risk drinkers (>8 drinks/day for men or >4 drinks/day for women), increased test sensitivity only to 43% for men and 35% for women. Evidently, the test will detect only between a third and a half of these drinkers. Whatever contrast is considered, the diagnostic sensitivity is lower for women than for men. This finding is partly due to the lower level of alcohol intake considered hazardous for women, but women also have lower CDT% values than men at alcohol intakes of >35 drinks/week. Together, these factors limit the usefulness of CDT% for women.

These sensitivity estimates are in accord with a metaanalysis of results obtained up to 1998 *(3)*, which estimated diagnostic odds ratios for the major categories of CDT methods. The analysis implied sensitivities of 39% and 36% for the modified CDTect and Axis %CDT methods, respectively, at 95% sensitivity. Older methods, such as isoelectric focusing, produced better results than anion exchange, even in studies that reported paired comparisons in which the same samples were analyzed with both methods.

Previous studies have shown that several physiological or metabolic variables affect the probability of an abnormal CDT result in at-risk or dependent drinkers. In summary, these reports indicated that sensitivity tends to be lower in women *(27, 36–38)*; in people with obesity, insulin resistance, characteristics associated with the metabolic syndrome, dyslipidemia, or hypertension *(22–26, 37, 39)*; in conditions with iron overload *(28)*; and in nonsmokers *(24, 27)*. Some, but not all, of these limitations previously found to affect CDT concentration in studies that used anion-exchange column methods also apply to the direct immunoassay method. CDT% was significantly associated or correlated in the hazardous drinking groups with BMI, HDL and LDL cholesterol, triglycerides, butyrylcholinesterase, smoking, and transferrin (see Table 2 in the online Data Supplement). A multiple regression or logistic regression analysis of men revealed that BMI, HDL cholesterol, butyrylcholinesterase, transferrin, ferritin, and age were independent predictors of CDT% or the probability of an abnormal CDT% value (see Table 3 in the online Data Supplement), and HDL cholesterol, butyrylcholinesterase, transferrin, and smoking showed independent effects for women. This finding reinforces the view that CDT variation, or at least the CDT response to alcohol intake, is a metabolic phenomenon and that either the synthesis of the glycans or the receptor-mediated process of CDT removal from the circulation is associated with aspects of lipid metabolism, insulin resistance, and metabolic syndrome.

On the other hand, the previously reported effect of current or previous alcohol dependence *(24)* was not found, and the reported number of drinks had no independent effect on CDT% when only the men or women who consumed alcohol at levels above the recommended limits were considered.

The ROC curves (see Fig. 1 in the online Data Supplement) for comparing test performance by sex, degree of obesity, and smoking show the practical implications of these results. Clearly, CDT testing is most likely to produce a reliable and interpretable answer in a male smoker with a nonpathologic BMI. The biological reasons for this finding are still unclear; there is only a modest amount of experimental information on the effects of alcohol on transferrin glycan synthesis, on transferrin isoform turnover, and on the effects of iron status. There are no data on the interactions with obesity-related factors. The effect of smoking may be due to the overlap between smoking and alcohol dependence, and hence to a higher degree of unreported alcohol use, but the issue of underreporting cannot be tested with our data.

It will be important to determine whether HPLC or capillary electrophoresis methods give diagnostic results that are better than those for immunoassays. The initial evaluation of the N Latex CDT method *(14)* showed a good correlation of results with those for HPLC measurement across a wide range of values, which suggests that the diagnostic performance of HPLC measurement will suffer from similar limitations. Another recent study that used HPLC measurement *(40)* examined the effects of multiple participant

characteristics, including sex, BMI, and smoking, on transferrin isoforms, but the emphasis was on the effects on the reference range and test specificity rather than on sensitivity.

Our study is subject to some limitations. The main practical one is that we have not included alcohol-dependent patients from treatment or detoxification facilities. The test sensitivities in such patients categorized by sex, obesity, and smoking status remain to be determined. Another limitation is that our study participants were related and therefore not genetically independent; this design feature means that the statistical significance (but not the magnitude) of the described effects may be slightly overestimated. Because our main focus was on the effects on estimated test sensitivity, we consider this limitation to be minor. The transfer of blood samples from multiple sites to a central laboratory imposed delays on serum separation, but the means and distributions of our results do not seem to have been affected by this delay. The storage times before measurement varied for total transferrin and CDT measurements, but both transferrin species are stable for long periods. CDT is stable for up to 8 years at −20 °C *(41)*, and transferrin is stable for more than a year at −70 °C *(42)*. Finally, the calculation of CDT% from CDT and transferrin concentrations may be affected by our use of an independent method for measuring total transferrin. We can compare our CDT% ranges for men and women who reported low alcohol intakes (1.04%–2.27% and 1.03%–1.90%, respectively) with those reported by Delanghe et al. *(14)*, who used the Dade assay for total transferrin. These investigators reported an upper limit (97.5th percentile) for CDT% of 2.35% for both sexes among "apparently healthy adults" who consumed ≤2 drinks/day. Delanghe et al. reported transferrin concentrations of 2.0–3.8 g/L, whereas we obtained values of 2.00–3.44 g/L for men and 2.12–3.98 g/L for women (all 2.5th–97.5th percentiles). An evaluation of the external quality-assurance program data for transferrin (16 samples with target transferrin concentrations of 1.47–3.62 g/L) suggests that transferrin results obtained with the Roche 917 method may be 4%–5% higher than nephelometric results obtained with the Dade method (1.53 g/L vs 1.47 g/L, respectively, at the low end of the range and 3.70 g/L vs 3.54 g/L at the high end). Overall, any differences caused by the use of the Roche method for total transferrin are small and in any case will not affect our conclusions about the ROC curves and the factors that affect them.

In conclusion, we found that the sensitivity of the N Latex CDT test for detecting risky but not extreme drinking patterns in the general population is similar to the sensitivities of previously described methods and that some easily ascertained patient characteristics (sex, BMI, smoking) affect the usefulness of the test. False negatives are more likely in women, in nonsmokers, and in people with a higher BMI. This fact should be taken into account in deciding whether to use CDT measurement for any individual patient and in interpreting the result.

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