

# Smoking, obesity, and hypertension alter the dose–response curve and test sensitivity of carbohydrate-deficient transferrin as a marker of alcohol intake

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Serum carbohydrate-deficient transferrin (CDT) is a specific and comparatively sensitive marker of excessive alcohol use; however, reports of its sensitivity vary according to the population or patient groups studied and their average alcohol intake. We have characterized the dose–response curve between alcohol intake and CDT concentrations in a study of 1400 men and women from a community-based twin registry. Our results show that mean CDT increases with increasing reported alcohol consumption even within the range of alcohol use considered to be nonhazardous. We found significant effects of sex, age, smoking, previous alcohol dependence, body mass index, and diastolic hypertension on the alcohol–CDT dose–response curve. These variables either affect test sensitivity or require adjustment of reference intervals. The results also provide insight into the physiological and biochemical factors that affect CDT concentration.

Sustained high alcohol intake leads to increased serum concentrations of isoforms of transferrin with low sialic acid content [carbohydrate-deficient transferrin (CDT)<sup>4</sup>] (1). These isoforms can be measured by isoelectric focus-

ing with immunofixation or Western blotting (2), by liquid chromatography (3), or by ion-exchange chromatography followed by immunoassay of eluted transferrin (4–6).

CDT is the best available marker of excessive alcohol intake (7, 8), and serum CDT concentrations are reported to exceed the established reference intervals at an intake of 60–80 g/day. However, recent experimental studies in which volunteers have consumed this amount of alcohol for comparatively short periods (up to 1 month) have only produced increased CDT concentrations in a few of the subjects (9). CDT is more sensitive than  $\gamma$ -glutamyltransferase (GGT) in most situations and is also more specific for alcohol than GGT (8). Despite early reports of near-100% sensitivity for detection of actively drinking alcoholics (10–12), the consensus now is that CDT sensitivity is ~70–90% for clinical alcohol dependence (7, 8) when the reference range is selected to give a specificity of 95%.

Although test sensitivity should be independent of the prevalence of the condition sought, the ability of a test to detect hazardous alcohol consumption depends on the population being studied and, in particular, on the average consumption of the subjects defined into the “abnormal” or “heavy drinking” category. The cutoff point chosen as the upper limit of the reference range will depend on the alcohol consumption of the reference group; these may be nondrinkers or people with presumed safe alcohol consumption. Depending on the shape of the alcohol-marker dose–response curve, there may be differences in the reference range (and hence the test sensitivity), depending on the choice of reference group.

Men who average four drinks or more per day, or who drink to intoxication once a week, are at risk of harm from their drinking; for women, guidelines recommend half the amount of alcohol that men drink (13). These values were

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<sup>4</sup> Nonstandard abbreviations: CDT, carbohydrate-deficient transferrin; GGT,  $\gamma$ -glutamyltransferase; DSM-III-R, *Diagnostic and Statistical Manual of the American Psychiatric Association*, 3rd ed., revised; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; and HDL-C, HDL-cholesterol.

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confirmed recently by metaanalysis of studies on the relationship between alcohol intake and mortality (14). Because early intervention may have beneficial effects (15), a test that would detect an average intake of 40 g/day or more is desirable. Several reports have indicated sensitivities of 55% (3), 29% (16), 22% (17), 13% (18), or 62% (19) for CDT in detecting this intake.

Because sensitivity is variable and clearly less than the ideal 100%, it is useful to investigate what factors alter the alcohol-marker dose-response curve and the test sensitivity so that result interpretation can be more soundly based. Such factors could include sex, age, and possibly race; for some markers, physiological variables such as obesity or blood pressure may be relevant.

We have measured CDT on 1400 subjects drawn from a general population twin sample of mainly healthy adults in Australia. We report here on the sensitivity and specificity of CDT in men and women and on the physiological or biochemical factors that influence CDT results at various amounts of alcohol intake. Analysis of our results from a genetic/environmental perspective will be covered in the future.

### Subjects and Methods

Subjects were adult twins from a volunteer twin register (Australian National Health and Medical Research Council Twin Registry), born between 1893 and 1964. In 1992 and 1993, telephone interviews were conducted with 1879 men and 3659 women (20), using the Semistructured Assessment for Genetics of Alcoholism diagnostic interview (21). This interview allowed diagnosis of alcohol dependence by the criteria given in the *Diagnostic and Statistical Manual of the American Psychiatric Association* (DSM-III-R).

In 1993–1996, blood was collected from 1134 of the men and 2241 of the women. Subjects gave informed consent, and the protocol was approved by appropriate institutional ethics review committees. Immediately before blood collection, subjects filled in a table asking how many drinks containing alcohol (10 g) they had taken on each of the preceding 7 days, divided into categories: beer, wine, spirits, fortified wine, or "other". The numbers of drinks were summed to obtain a total for the past week, and the number of drinks of grain-based (beer + spirits) and grape-based (wine + fortified wine) beverages were also calculated. Unless otherwise stated, all data analysis was done using the total number of drinks reported regardless of type. This total showed a good correlation with a weekly drinking estimate based on habitual quantity and frequency of alcohol use provided previously by the subjects during the telephone interview ( $r = 0.78$  for men and 0.75 for women, on log-transformed estimates).

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 1988–1990 survey (when smoking status was ascertained for 5538 subjects from this cohort) and in a smaller but near-contemporary 1993–1996

survey (for 1573 subjects). On each occasion, subjects categorized themselves as having never smoked, as being an ex-smoker, or as a current smoker. For the 1432 subjects for whom there was smoking information on both occasions, there was good agreement ( $\kappa = 0.744$ ; Spearman rank correlation = 0.883).

Systolic and diastolic blood pressures were measured on the occasion when blood was collected, with the subjects sitting, using an automated blood pressure recorder (Dynamap 845 Vital Signs Monitor; Critikon Inc.). The mean of two results taken at 1-min intervals was calculated. The body mass index (BMI) was calculated from self-reported weight and height as weight (kg)/[height (m)]<sup>2</sup>.

Serum was separated from blood and stored at  $-70^{\circ}\text{C}$  until analysis. CDT was measured on 1400 samples, from 539 men and 861 women, ages 29–92 years. The mean ages were 46.5 years for women and 44.8 years for men. 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 averaging more than four drinks per day (men) or two drinks per 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. Of the 1400 subjects in whom CDT was measured, 24% had at some time met the DSM-III-R criteria for alcohol dependence. The rate of lifetime alcohol dependence rose continuously with increases in reported recent drinking: from 11% in subjects reporting no alcohol use in the week before blood collection to 78% in the group reporting >28 drinks in that time.

CDT was measured by ion-exchange chromatography and RIA, using a Pharmacia method (CDTect RIA). This method measures asialo-, monosialo-, and disialotransferrin (22), and according to the manufacturer, gives reference ranges of up to 20 units/L for men and 26 units/L for women (1 unit is approximately equal to 1 mg of transferrin). Repeated measurement of a single sample on 10 different days gave a coefficient of variation (CV) of 6.7% (mean  $\pm$  SD, 20.05  $\pm$  1.34 units/L), whereas within-day duplicate estimates on 10 samples with CDT concentrations within the reference range and 10 high-concentration samples gave CVs of 9.0% (mean  $\pm$  SD, 11.85  $\pm$  1.07 units/L) and 5.8% (mean  $\pm$  SD, 33.05  $\pm$  1.91 units/L), respectively.

Plasma GGT, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urate, total cholesterol, and triglycerides were measured by Boehringer Mannheim methods on a Hitachi 747 analyzer. HDL-cholesterol (HDL-C) was measured by precipitation of non-HDL lipoproteins with dextran/MgSO<sub>4</sub> followed by enzymatic cholesterol assay. Apolipoproteins A-I, A-II, B, and E were measured by immunonephelometry, using a Behring nephelometer and Behring reagents.

Statistical analyses were performed by ANOVA or by

estimation of correlations and regression slopes, using BMDP Dynamic (BMDP Statistical Software Inc.). Because of the possibility of nonlinear relationships, the ANOVA approach was favored, with subjects divided into groups on the basis of number of drinks in the previous week. The categories used were as follows: None; 1 to 7; 8 to 14; 15 to 21; 22 to 28; and Over 28; the numbers of male and female subjects in each of these categories are included in Table 1.

Because the subjects were twins (and observations therefore are not genetically independent), the significance levels (but not the statistics themselves) were biased in a nonconservative direction for any variables with significant heritability. As an approximate correction for this problem, all tests of significance were repeated using one-half the number of degrees of freedom. This approach was conservative, overcorrecting for the twin nature of the sample. Because of the large number of subjects, all results quoted showed similar significance levels with either approach.

## Results

### REFERENCE RANGES

For men and women reporting up to seven drinks in the previous week (10 g ethanol/day) the CDT means  $\pm$  SD were  $13.2 \pm 4.4$  and  $17.8 \pm 7.4$  units/L, respectively. The nonparametric 95th centiles were 20 units/L (men) and 30 units/L (women). For those drinking up to seven drinks in the previous week, or 10 g ethanol/day, 5.5% of the men and 10.5% of the women had CDT results above the Pharmacia-recommended gender-related reference ranges of 20 and 26 units/L, respectively.

The mean CDT values in this reference group changed with age in women, but not men, as shown in Fig. 1. Therefore the 95th centiles for the CDT distribution were also determined for women <50 years and >50 years of age. These values were 32 and 23 units/L, respectively; however, although a difference in reference range could be shown, it was not possible to determine (because of the small numbers of women reporting >28 drinks/week) whether the use of age-specific reference ranges would improve the performance of the test.

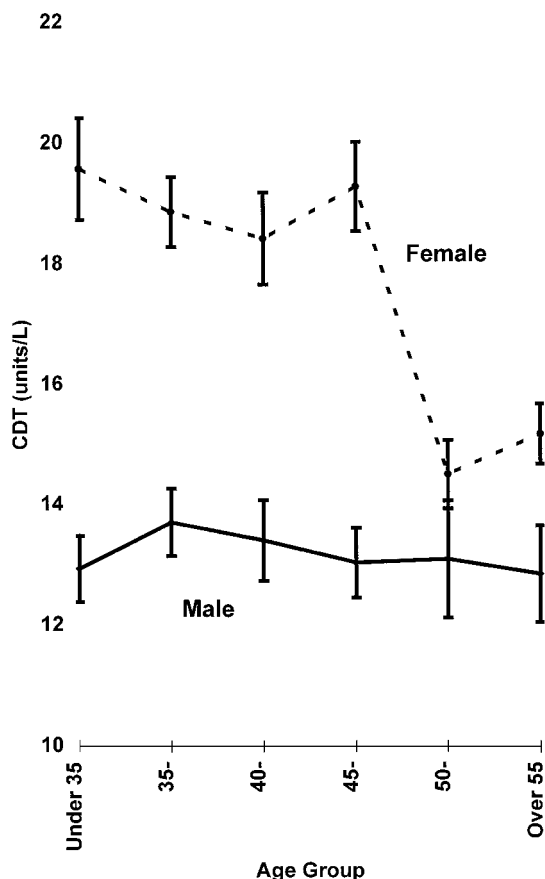


Fig. 1. Effects of sex and age on mean values of serum CDT in subjects reporting no alcohol use or up to seven drinks (equivalent to 70 g of ethanol) within the previous week.

Effects of age on CDT were significant in women ( $P < 0.0001$ ) but not in men. Error bars, mean  $\pm$  1 SE.

### ALCOHOL-CDT DOSE-RESPONSE CURVES AND TEST PERFORMANCE

The mean values for CDT in men and women, by alcohol consumption group, are shown in Table 1. The percentage of abnormal CDT results and, for comparison, the proportion of abnormal GGT results were calculated for male and female subjects grouped according to alcohol intake; these results are also shown in Table 1. The sensitivity and

**Table 1. Mean values of CDT by alcohol consumption group and sensitivity of CDT and GGT for detecting alcohol consumption above four drinks per day.<sup>a</sup>**

Number of drinks in previous week	Mean $\pm$ SD, women	Mean $\pm$ SD, men	% (n) CDT results		% (n) GGT results	
			>30 units/L, women	>20 units/L, men	>40 U/L, women	>65 U/L, men
None	17.6 $\pm$ 7.2	13.1 $\pm$ 4.4	5.5 (19 of 344)	2.9 (3 of 102)	6.3 (50 of 794)	6.1 (14 of 231)
1 to 7	17.9 $\pm$ 7.6	13.3 $\pm$ 4.5	5.1 (17 of 332)	7.1 (12 of 170)	5.6 (48 of 853)	4.5 (16 of 356)
8 to 14	20.5 $\pm$ 8.2	15.3 $\pm$ 8.9	12.6 (15 of 119)	13.2 (14 of 106)	7.7 (19 of 247)	7.1 (15 of 211)
15 to 21	20.6 $\pm$ 6.6	17.9 $\pm$ 11.4	6.8 (3 of 44)	19.5 (16 of 82)	2.5 (2 of 81)	6.4 (9 of 141)
22 to 28	28.2 $\pm$ 22.5	18.6 $\pm$ 11.8	20.0 (3 of 15)	28.6 (10 of 35)	19.2 (5 of 26)	15.2 (7 of 46)
Above 28	28.4 $\pm$ 17.8	23.4 $\pm$ 18.5	28.6 (2 of 7)	43.2 (19 of 44)	18.2 (2 of 11)	28.6 (18 of 63)

<sup>a</sup> The upper limits of the reference ranges (30 units/L for women and 20 units/L for men) were chosen to give a specificity of 95%.

specificity for GGT are calculated from results on all subjects who had GGT measured; results from only subjects with CDT results were similar.

#### EFFECTS OF AGE AND SEX ON THE ALCOHOL-CDT DOSE-RESPONSE CURVE

Mean CDT results were higher among women than men. Two-way ANOVA showed that alcohol intake ( $F_{5,1388} = 17.09$ ,  $P < 0.0001$ ) and sex ( $F_{1,1388} = 41.84$ ,  $P < 0.0001$ ) had significant effects, but the sex-intake interaction did not ( $F_{5,1388} = 1.07$ ,  $P = 0.374$ ). Much of the sex-based difference in CDT was attributable to higher values in women below the age of 50, as shown in Fig. 2. Analysis of results in women only showed that age (<50 vs  $\geq 50$ ), alcohol intake, and the interaction between them all had significant effects on CDT ( $F_{1,848} = 9.71$ ,  $P = 0.002$ ;  $F_{5,848} = 10.1$ ,  $P < 0.0001$ ; and  $F_{5,848} = 4.30$ ,  $P = 0.0007$ , respectively).

In light of these results, corrections for the sex-based difference were made for all further data analyses by subtracting 6 units/L from the CDT results for women <50 years of age and 2 units/L for women  $\geq 50$ . These were the average differences between men of all ages and

women in these two age groups, after allowing for differences in alcohol intake.

#### TYPE OF BEVERAGE

When the number of drinks of beer + spirits and of wine + sherry were summed and the mean CDT was plotted against the number of drinks of each type in the previous week, the curves for the grain-based and grape-based beverages were essentially parallel (Fig. 3).

#### EFFECTS OF SMOKING STATUS AND ALCOHOL DEPENDENCE HISTORY

There was a significant association between smoking status and mean CDT, which was found to be mainly attributable to the interaction between the effects of smoking and alcohol intake (using 1988–1990 smoking data, the association for smoking was  $F_{2,1322} = 23.48$ ,  $P < 0.0001$ ; and the smoking-intake interaction was  $F_{10,1322} = 3.43$ ,  $P = 0.0002$ ; using the smaller number of subjects for whom 1993–1996 smoking data were available, the association for smoking was  $F_{2,471} = 3.87$ ,  $P = 0.022$ ; and the smoking-intake interaction was  $F_{10,471} = 2.48$ ,  $P = 0.007$ ). The mean CDT values for subjects who

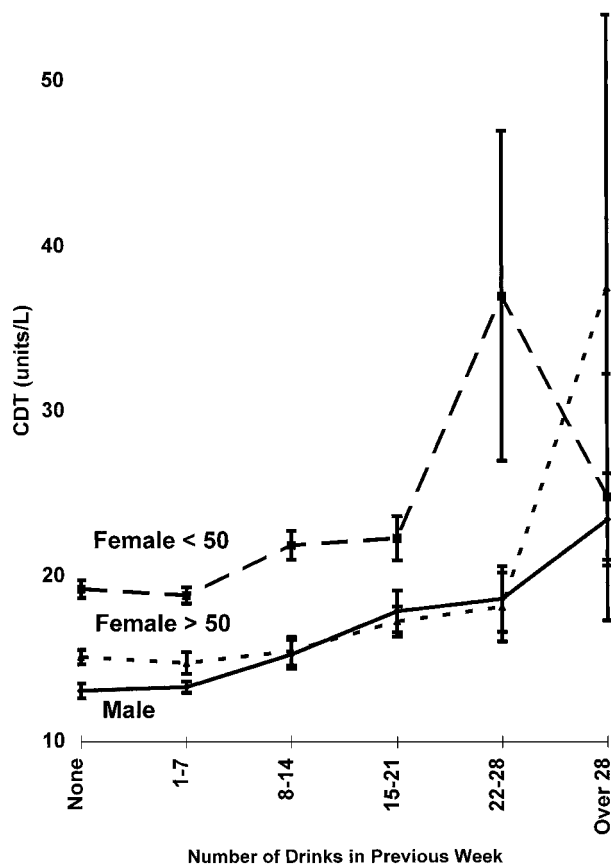


Fig. 2. Effect of sex and age (in women) on the alcohol-CDT dose-response curve.

Mean values differed significantly ( $P < 0.0001$ ) between men and all women, and also between women younger than and older than 50 years. *Female < 50*, women <50 years of age at the time of blood collection; *Female > 50*, women  $\geq 50$  years of age at the time of blood collection. *Error bars*, mean  $\pm 1$  SE. The number of drinks can be converted to grams of alcohol by multiplying by 10.

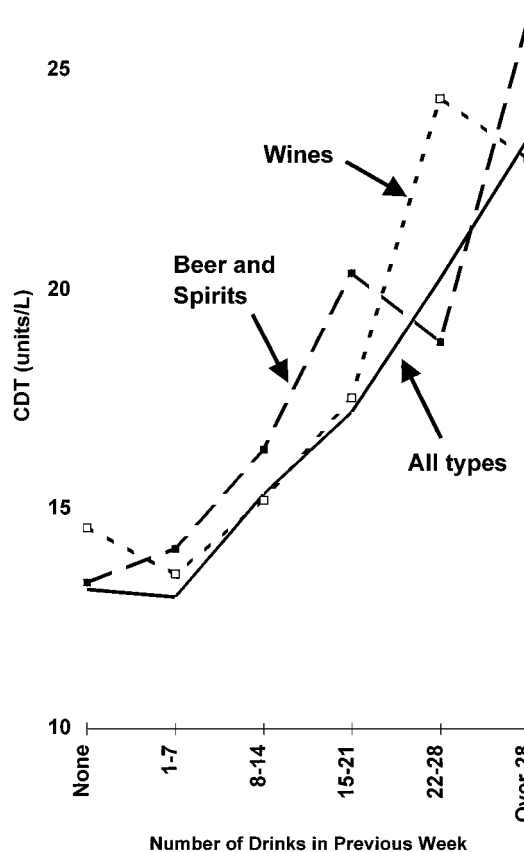


Fig. 3. Effect of type of beverage on the alcohol-CDT dose-response curve.

CDT values are sex-adjusted as described in the text. Wines includes fortified wines (e.g., sherry and port), and spirits includes brandy. The number of drinks can be converted to grams of alcohol by multiplying by 10.

had never smoked, ex-smokers, and current smokers (1988–1990 data), grouped by alcohol intake, are shown in Fig. 4. The test sensitivities for detection of weekly alcohol intake >28 drinks were 55% in current smokers and 20% in current nonsmokers (Fisher exact test,  $P = 0.124$ , two-tailed); for detection of weekly alcohol intake >21 drinks, the test sensitivities were 51% and 12%, respectively (Fisher exact test,  $P = 0.0015$ , two-tailed).

Because smoking status is associated significantly with alcohol dependence in these subjects, the effects of lifetime alcohol dependence history on the alcohol-CDT dose–response curve were investigated. For any reported frequency of drinking, subjects who met alcohol dependence diagnosis criteria had on average higher CDT values than never-dependent subjects (for alcohol dependence  $F_{1,1388} = 25.48$ ,  $P < 0.0001$ ; for weekly alcohol consumption  $F_{5,1388} = 13.88$ ,  $P < 0.0001$ ; for the interaction between dependence and intake  $F_{5,1388} = 2.01$ ,  $P = 0.075$ ; see Fig. 5).

Analysis of the effects of smoking in ever-dependent and never-dependent subjects led to the conclusion that

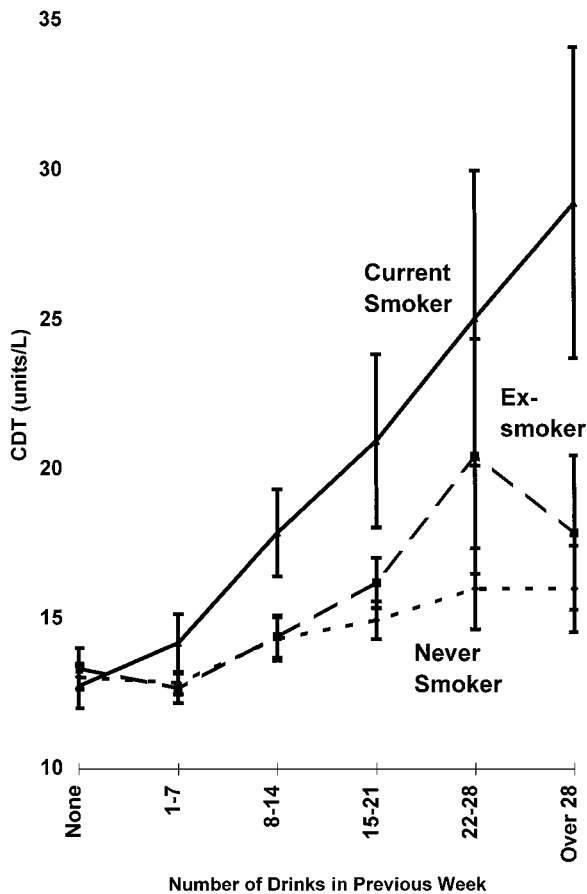


Fig. 4. Effect of smoking status (never smoker, ex-smoker, current smoker) on the alcohol-CDT dose–response curve.

CDT values are sex-adjusted as described in the text. There were significant differences in CDT between the smoking status groups ( $P < 0.0001$ ) and significant interaction effects of smoking status and alcohol intake ( $P = 0.0005$ ). Error bars, mean  $\pm$  1 SE. The number of drinks can be converted to grams of alcohol by multiplying by 10.

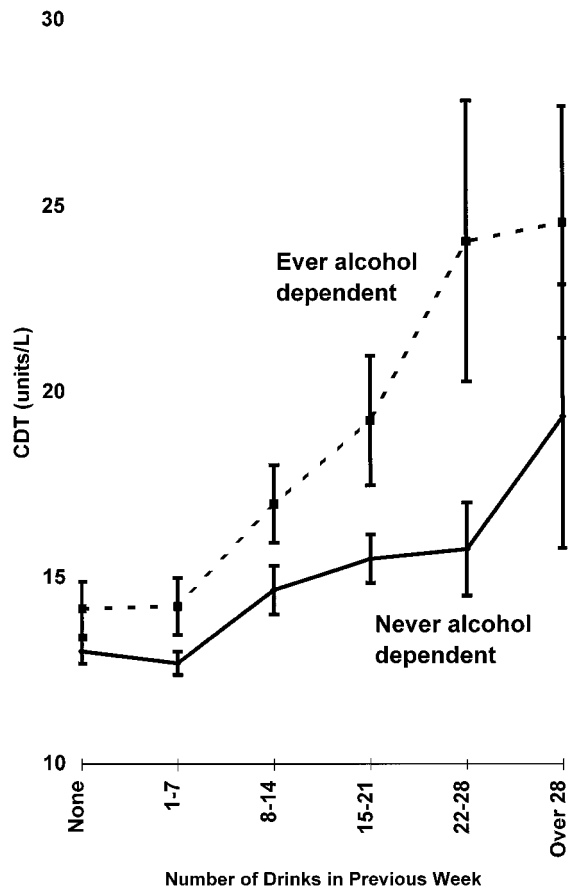


Fig. 5. Effect of alcohol dependence (DSM-III-R criteria, lifetime diagnosis) on the alcohol-CDT dose–response curve.

CDT values are sex-adjusted as described in the text. There were significant effects of alcohol dependence status ( $P < 0.0001$ ) and alcohol consumption ( $P < 0.0001$ ) on mean CDT. Error bars, mean  $\pm$  1 SE. The number of drinks can be converted to grams of alcohol by multiplying by 10.

smoking status had a much greater effect on CDT in subjects who had at some stage been alcohol-dependent (Fig. 6). In subjects who did not meet the criteria for lifetime DSM-III-R alcohol dependence, smoking had no significant effect on CDT ( $F_{2,1011} = 2.19$ ,  $P = 0.113$ ), whereas alcohol consumption did ( $F_{5,1011} = 5.40$ ,  $P = 0.0001$ ); however, there was some smoking-alcohol intake interaction effect ( $F_{10,1011} = 2.12$ ,  $P = 0.021$ ). Among the subjects who were or had been alcohol-dependent, smoking and drinking each had highly significant effects on CDT ( $F_{2,293} = 12.78$ ,  $P < 0.0001$ ; and  $F_{5,293} = 4.79$ ,  $P = 0.0003$ , respectively), again with a significant interaction effect ( $F_{10,293} = 2.23$ ,  $P = 0.016$ ).

CORRELATION WITH OTHER VARIABLES

The correlations for men and women, before allowing for effects of alcohol intake, are shown in Table 2. Because some or all of the correlations between CDT and other variables might be attributed to their common dependence on alcohol intake, partial correlations (adjusting for effects of alcohol intake) are also shown. The significant



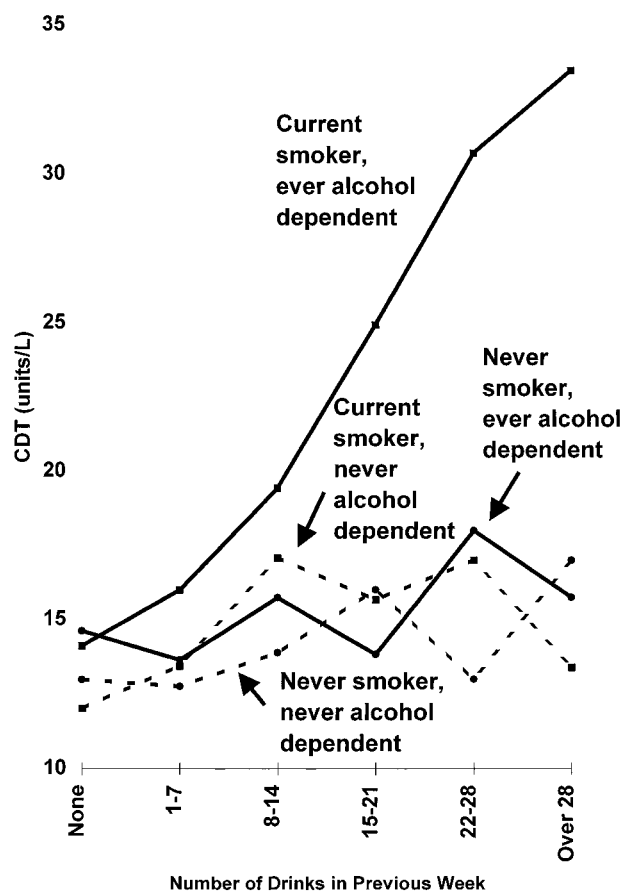


Fig. 6. Effects of smoking on the alcohol-CDT dose-response curve in subjects who had never met DSM-III-R alcohol dependence criteria (---) and subjects who had been alcohol-dependent (—).

Ex-smokers are omitted for clarity. CDT values are sex-adjusted as described in the text. Smoking had no significant effect on CDT in never-dependent subjects ( $P = 0.211$ ); however, it had a highly significant effect in currently or previously dependent subjects ( $P < 0.0001$ ). The number of drinks can be converted to grams of alcohol by multiplying by 10.

correlations between alcohol use and BMI, HDL-C, triglycerides, and lipoproteins were investigated further.

#### EFFECTS OF BMI, TRIGLYCERIDES, AND HDL-C

These three variables were correlated with CDT (see Table 2) and also with each other (BMI-triglycerides,  $r = 0.35$  for men and  $0.36$  for women; BMI-HDL-C,  $r = -0.29$  for men and  $-0.32$  for women; and triglycerides-HDL-C,  $r = -0.39$  for men and  $-0.40$  for women). Taking BMI as the independent variable and assuming that the correlations of triglycerides, HDL-C, and apolipoproteins with CDT were a consequence of BMI variation, we divided the subjects into five groups with increasing BMIs. The correlation of BMI with CDT was found to be mainly because of the interaction between alcohol intake, BMI, and CDT (see Fig. 7).

ANOVA showed that the effects of alcohol, BMI, and the interaction between the two were all highly significant: alcohol intake group,  $F_{4,1347} = 47.10$ ,  $P < 0.0001$ ; quintile of BMI,  $F_{4,1347} = 22.64$ ,  $P < 0.0001$ ; alcohol intake-

BMI interaction,  $F_{16,1347} = 4.09$ ,  $P < 0.0001$ . Similar results were obtained for the combined effects of alcohol intake and quintile of triglyceride concentration on CDT (alcohol intake group,  $F_{4,1349} = 39.10$ ,  $P < 0.0001$ ; quintile of triglycerides,  $F_{4,1349} = 12.85$ ,  $P < 0.0001$ ; alcohol intake-triglyceride interaction,  $F_{16,1349} = 2.58$ ,  $P = 0.0006$ ) and for alcohol intake and quintile of HDL-C (alcohol intake group,  $F_{4,1340} = 34.61$ ,  $P < 0.0001$ ; quintile of HDL-C,  $F_{4,1340} = 21.65$ ,  $P < 0.0001$ ; and alcohol intake-HDL-C interaction,  $F_{16,1340} = 5.78$ ,  $P < 0.0001$ ). Whereas for BMI and triglycerides low values (subjects in the first quintile) were associated with a greater CDT response to alcohol, the reverse was the case for HDL-C.

#### EFFECTS OF BLOOD PRESSURE

Subjects were divided according to their diastolic blood pressures into two groups, with blood pressures below and above 90 mmHg. Although the number of subjects with hypertension by this criterion was small (only 53 men and 37 women with diastolic blood pressure  $\geq 90$  mmHg), a significant interaction between alcohol and blood pressure effects on CDT could be shown: alcohol intake group,  $F_{5,689} = 5.19$ ,  $P = 0.0001$ ; diastolic blood pressure,  $F_{1,689} = 11.08$ ,  $P = 0.0009$ ; and alcohol intake-blood pressure interaction,  $F_{5,689} = 4.22$ ,  $P = 0.0009$ . These results are shown in Fig. 8.

#### Discussion

Although CDT is a sensitive and specific test for excessive drinking in alcohol-dependent subjects from hospital or clinic groups, it is less useful in detecting hazardous alcohol use in the general population (7, 8). Furthermore, little is known about the factors that determine whether any individual hazardous drinker will show an abnormal CDT result. The observed sensitivity of 30–40% to detect average drinking of more than four drinks per day ( $>40$  g ethanol/day) in this study is consistent with most previous reports (3, 16–19). We have identified a number of factors that influence mean CDT response to alcohol and test sensitivity. However, although our results cover the range of alcohol intake up to potentially harmful quantities, the nature of the population studied means that it is not possible to determine whether there are any effects on the sensitivity of CDT in detecting larger amounts, such as 60 or 80 g of alcohol per day.

Our results confirm that mean CDT increases with increasing reported alcohol consumption over the range from an average of one to an average of four drinks per day. No difference in mean CDT could be shown between people taking no alcohol and people taking up to seven drinks in the previous 7 days; beyond that point mean CDT increased. Despite persistent doubts about the accuracy of estimates of alcohol consumption, our results indicate that serum CDT may increase (within the reference range) with as little as two standard drinks (20 g of alcohol) per day. Therefore, chronic consumption of a small amount of alcohol has a measurable effect on mean

**Table 2. CDT correlations with alcohol markers and coronary heart disease risk factors in men and women.**

	Men, n	Men, <i>r</i>	Men, partial <i>r</i> <sup>a</sup>	Women, n	Women, <i>r</i>	Women, partial <i>r</i> <sup>a</sup>
GGT, log	512	0.000 <sup>b</sup>	-0.057 <sup>b</sup>	811	-0.088 <sup>c</sup>	-0.100 <sup>d</sup>
AST, log	512	0.048 <sup>b</sup>	0.030 <sup>b</sup>	811	-0.013 <sup>b</sup>	-0.009 <sup>b</sup>
ALT, log	512	-0.043 <sup>b</sup>	-0.061 <sup>b</sup>	810	-0.107 <sup>d</sup>	-0.100 <sup>d</sup>
Urate	475	-0.041 <sup>b</sup>	-0.111 <sup>c</sup>	738	-0.149 <sup>e</sup>	-0.159 <sup>e</sup>
Total cholesterol	538	-0.008 <sup>b</sup>	-0.052 <sup>b</sup>	860	-0.059 <sup>b</sup>	-0.041 <sup>b</sup>
Triglyceride, log	521	-0.145 <sup>d</sup>	-0.180 <sup>e</sup>	853	-0.216 <sup>e</sup>	-0.200 <sup>e</sup>
HDL-C	518	0.320 <sup>e</sup>	0.273 <sup>e</sup>	847	0.264 <sup>e</sup>	0.219 <sup>e</sup>
LDL-cholesterol, calculated	502	-0.050 <sup>b</sup>	-0.072 <sup>b</sup>	840	-0.089 <sup>c</sup>	-0.056 <sup>b</sup>
Apolipoprotein A-I	536	0.270 <sup>e</sup>	0.208 <sup>e</sup>	854	0.146 <sup>e</sup>	0.100 <sup>d</sup>
Apolipoprotein A-II	535	0.299 <sup>e</sup>	0.216 <sup>e</sup>	857	0.166 <sup>e</sup>	0.126 <sup>e</sup>
Apolipoprotein B	536	-0.072 <sup>b</sup>	-0.095 <sup>c</sup>	855	-0.114 <sup>d</sup>	-0.089 <sup>d</sup>
Apolipoprotein E	531	-0.112 <sup>c</sup>	-0.108 <sup>c</sup>	847	-0.137 <sup>e</sup>	-0.120 <sup>e</sup>
BMI	535	-0.107 <sup>c</sup>	-0.108 <sup>c</sup>	855	-0.215 <sup>e</sup>	-0.200 <sup>e</sup>
Blood pressure						
Systolic	301	0.072 <sup>b</sup>	0.062 <sup>b</sup>	400	-0.077 <sup>b</sup>	-0.057 <sup>b</sup>
Diastolic	301	-0.031 <sup>b</sup>	-0.054 <sup>b</sup>	400	0.023 <sup>b</sup>	0.024 <sup>b</sup>

<sup>a</sup> Partial correlations between CDT and other variables listed, correcting for reported alcohol intake.

<sup>b</sup> Not significant;  $P > 0.05$ .

<sup>c</sup>  $P < 0.05$ .

<sup>d</sup>  $P < 0.01$ .

<sup>e</sup>  $P < 0.001$ .

CDT, although short-term consumption of considerably larger amounts does not (9).

The sensitivity of CDT in detecting hazardous drinking is superior to that of GGT (Table 1). The sensitivity for GGT was calculated using all subjects for whom GGT had been measured; the proportion of subjects in each alcohol intake group showing increased GGT was similar, but with greater fluctuations, in the smaller number of subjects in whom both CDT and GGT results were available. This difference in sensitivity is as expected for a population-based group with few currently drinking alcoholics, although in clinically recruited alcoholics the sensitivity of GGT is not very different from that of CDT (19).

We have found that a number of readily assessable factors have significant effects, either on CDT concentrations in low-intake subjects, or on the dose-response curves for the effects of alcohol on CDT and by implication on the sensitivity of the test. The values encountered in the low alcohol intake group (up to seven drinks in the previous 7 days) differed between men and women and between women of different ages in a way consistent with a hormonal change at around the age of menopause. This is consistent with a previous report of higher CDT values in premenopausal women, although the exact cause of the CDT increase was not determined—no correlation with estradiol or progesterone concentrations was found (23).

Subjects who had ever been alcohol-dependent had higher CDT values than those who had not, even where they reported no alcohol consumption in the previous week. Smoking and hypertension had no discernible effects on basal CDT (in the low alcohol intake group); however, obesity and associated lipid disorders had minor effects. Therefore there might be advantages in setting

sex-, age-, and obesity-related reference ranges for CDT. Because of the comparatively small number of current hazardous drinkers among our subjects, we could not test whether this is useful in practice.

With respect to the alcohol-CDT dose-response curve, smoking had a substantial effect on the CDT response to alcohol intake. Although the information about smoking was not collected at the time of blood collection, the information about smoking collected on two occasions from >1400 subjects shows that smoking status is a stable characteristic and, in our view, justifies the use of the previously collected smoking information. Moreover, the same results were found (significant effect of smoking on CDT, and significant smoking-alcohol interaction effect) whichever set of smoking data was used.

Only a small increase in mean CDT with increasing alcohol intake could be shown in nonsmokers. Ex-smokers were closer to nonsmokers in this respect than to current smokers; therefore, the effect of smoking must be comparatively short-term. Further consideration of the characteristics of the group studied led to testing for effects of alcohol dependence history, and this also appeared to have a strong effect on CDT (even when current self-reported alcohol intake was included as a covariate).

As reported previously (24), smoking and excessive alcohol use are strongly associated, both in general and in this cohort of subjects. Several possible explanations of the effect of smoking and alcohol dependence on the alcohol-CDT dose-response curve can be proposed. Subjects who have ever met the alcohol dependence criteria might be substantially underreporting their alcohol intake. This would lead to the displaced curve seen in Fig. 4 and, because these subjects tend to be smokers, to higher CDT

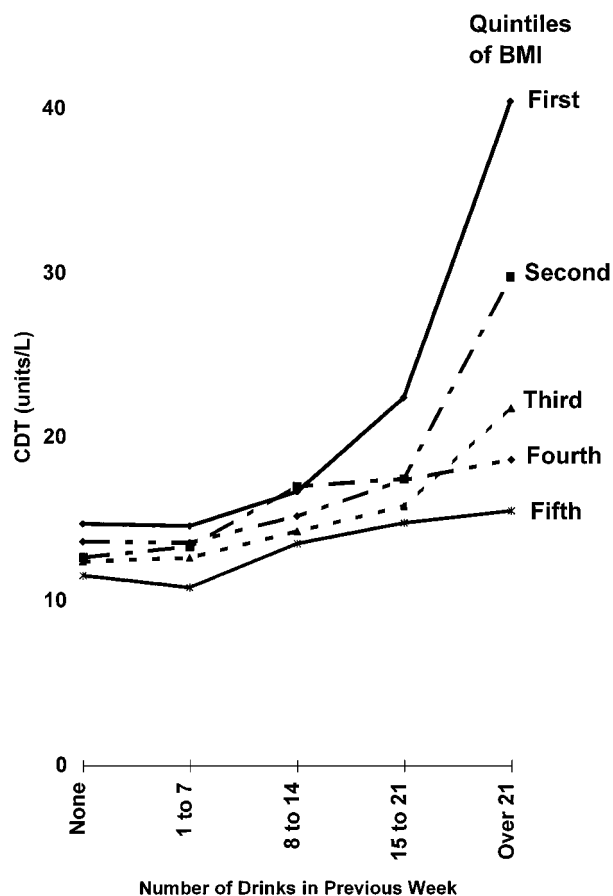


Fig. 7. Effect of BMI on the alcohol-CDT dose-response curve.

Subjects were divided into five groups representing quintiles of the BMI distribution. CDT values are sex-adjusted as described in the text. Alcohol intake, quintile of BMI, and alcohol-BMI interaction each had highly significant ( $P < 0.0001$ ) effects on CDT. The number of drinks can be converted to grams of alcohol by multiplying by 10.

values among smokers. However, this pattern of higher results in subjects who had ever been alcohol-dependent did not occur for other tests sensitive to alcohol intake (GGT, AST, ALT, urate, triglycerides, and HDL-C; data not shown); therefore, this explanation is probably incorrect.

It could also be that smoking changed the effect of alcohol on CDT, and the alcohol dependence effect would be secondary to that. However, the results shown in Fig. 6 reveal a more complex relationship. Smoking only has a significant effect in people who have at some stage been alcohol-dependent. This is an unexpected finding that suggests that some irreversible change occurs after a period of heavy alcohol intake associated with dependence; this persists into subsequent periods of nonhazardous drinking and is reinforced by smoking. Mechanisms that might lead to such an outcome are uncertain at present, but could involve hepatic microsomal enzyme induction.

The patterns of correlation between CDT and other variables, including other the alcohol intake markers and coronary heart disease risk factors shown in Table 2, suggest that the CDT response to alcohol is qualitatively

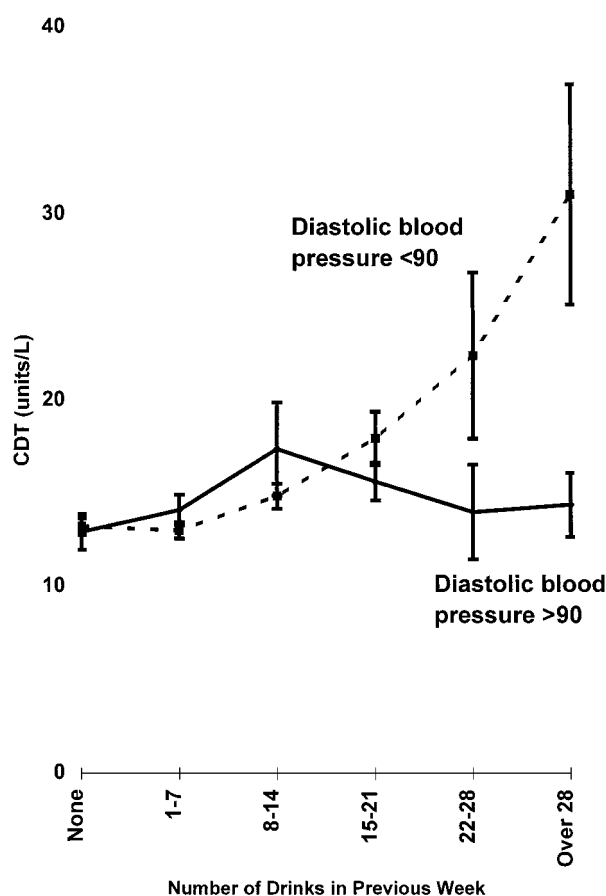


Fig. 8. Effect of hypertension (defined as diastolic blood pressure  $\geq 90$  mmHg) on the alcohol-CDT dose-response curve.

CDT values are sex-adjusted as described in the text. Alcohol intake, diastolic hypertension, and interaction each had significant effects on CDT (see text for details). *Diastolic blood pressure <90*, values below 90 mmHg; *Diastolic blood pressure >90*, values of 90 mmHg and above. Error bars, mean  $\pm 1$  SE. The number of drinks can be converted to grams of alcohol by multiplying by 10.

different from that of GGT, AST, and ALT. Increased CDT is associated with increases in HDL-C and apolipoproteins A-I and A-II, and with decreased triglycerides and apolipoproteins B and E. Correlations with the alcohol intake markers GGT, AST, ALT, or urate were nonsignificant or negative, particularly after compensating for their common correlation with alcohol intake, and this suggests that they are responding to independent, and possibly even mutually exclusive, processes. Some previous reports [Refs. (18, 25), and the references listed in (25)] have commented that CDT and GGT values in subjects with high alcohol intake are not associated with each other; however, in both cases the mechanisms of increase are unclear.

More detailed examination of our results also showed that a phenotype of high BMI, high triglycerides, and low HDL-C is associated with a decreased sensitivity of the CDT response to alcohol intake. These effects are probably caused by insulin resistance, as proposed previously by Fagerberg and co-workers (26, 27). Obesity is associated with high triglycerides, low HDL-C, and insulin



resistance (28). In this study we found that BMI, triglycerides, and HDL-C showed interactions with alcohol intake in influencing CDT results. Although we did not measure insulin concentrations, it seems probable that the BMI effects on the CDT response to alcohol intake are linked in some way with insulin resistance.

Fagerberg and co-workers (26, 27) also demonstrated that hypertensive subjects with insulin resistance (established by hyperinsulinemic euglycemic clamp studies) were less likely to have high CDT concentrations. However, Arndt et al. (29) found that patients with both kidney and pancreas transplants (who have hyperinsulinemia because of the connection of their pancreatic venous drainage to the systemic circulation) tended to have high CDT concentrations. Although these findings are to some extent contradictory, they do implicate insulin or insulin resistance in the setting of circulating CDT concentrations.

Although high blood pressure has some effect on CDT and its response to alcohol [consistent with one previous report (27)], it was less than the effect of BMI or obesity-related characteristics such as triglycerides or HDL-C. Furthermore, blood pressure data from all available subjects (normotensive and hypertensive) did not show significant correlation with CDT. The finding was that high blood pressure (or some characteristic associated with it) suppresses the CDT response to high alcohol consumption.

Most previous studies of CDT have concentrated on its use as a marker of heavy alcohol intake. Although our results have relevance to this, they also highlight a range of metabolic or endocrine associations of these isoforms of transferrin. The sex-based difference in CDT concentration, and in particular the presumably perimenopausal change in values for women at around the age of 50, suggest sex hormone effects. The associations between CDT response and alcohol and smoking, plasma lipids and lipoproteins, obesity, and hypertension indicate a link with insulin resistance or "syndrome X".

Explanations for these associations and correlations must ultimately be sought in the mechanisms for production and/or removal of CDT. Alcohol-induced increases in plasma CDT concentrations could in principle be caused by incomplete synthesis of the carbohydrate component of transferrin before release from the liver, by a greater rate of loss of terminal sialic acid groups in the circulation, or by a reduction in the receptor-based uptake of CDT by hepatocytes. It is not yet clear which mechanism is most important in producing an increased plasma CDT in response to alcohol; however, there is evidence (30) from both experimental animals and human studies that hepatic enzymes that synthesize the carbohydrate side chains of glycoproteins decrease, and those that remove sialic acid increase, in response to alcohol. In the context of our results, it is of interest to note that asialoglycoprotein receptor activity can be modulated in cell culture by mitogenic stimulation with epidermal growth factor and insulin (31) and also by variation in glucose concentration (32).

In conclusion, although precise mechanisms for these effects on CDT concentration are unknown at present, test sensitivity is affected in ways that may be clinically significant. Interpretation of test results, and decisions on test requesting, will be improved by identifying and understanding the factors that distinguish subjects who show an increased CDT after hazardous alcohol consumption from those who do not. Because there were few subjects with very high alcohol consumption in our sample, additional work is required on patients or people drinking more heavily to determine whether these factors affect the interpretation of CDT results at high alcohol intake, or whether the dose-response curves converge again at higher alcohol intake. With the data already collected, we hope to be able to define genetic or environmental factors that influence obesity, plasma lipids and lipoproteins, and CDT, and thereby to clarify the relationships between metabolic status and markers of alcohol intake.

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