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CARBOHYDRATE-DEFICIENT TRANSFERRIN IS ELEVATED IN CATABOLIC FEMALE PATIENTS

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Abstract — Serum carbohydrate-deficient transferrin (CDT) is currently widely used as a biochemical marker of alcohol misuse. However, various recent studies have questioned the diagnostic value of this parameter and reported low levels of both specificity and sensitivity, especially in women. Thus, we sought to identify sub-groups of female individuals in which CDT is elevated independently of alcohol consumption. Significantly increased CDT levels were found in catabolic disease states due to psychiatric disorders distinct from alcoholism. None of those patients reported frequent alcohol consumption. CDT therefore appears also to be increased by metabolic processes distinct from alcohol degradation. Possible biochemical mechanisms of this phenomenon are discussed. As a consequence of these findings, the measurement of CDT alone is not suitable to screen for alcohol misuse in catabolic subjects.

INTRODUCTION

Serum carbohydrate-deficient transferrin (CDT) has over the past 20 years been described as a biochemical marker for alcohol consumption (van Eijk *et al*., 1983; Stibler *et al*., 1988; Stibler, 1991). Human transferrin comprises at least six different isoforms, with respect to the number of sialic acid side chains: penta-, tetra-, tri-, di-, mono- and asialo-transferrin (Wong and Regoeczi, 1977). In subjects with high levels of alcohol consumption, isoforms with 0–3 sialo residues are increased, whereas the 4 and 5 sialo forms are decreased (Stibler *et al*., 1979). The former are designated as CDT. The exact mechanism by which alcohol intake elevates CDT is not yet exactly known and seems to be a multi-step process (Sillanaukee *et al*., 2001): enzymes which glycosylate transferrin are inhibited by ethanol metabolites (Xin *et al*., 1995), enhanced loss of sialic acid groups occurs (Ghosh *et al*., 1993), and receptor-mediated CDT uptake might be inhibited (Petren and Vesterberg, 1988). To date, several commercial tests are available for clinical use, and the measurement of CDT is commonly applied in clinical and forensic medicine (Wetterling and Kanitz, 1997).

Initially, very high levels of both sensitivity and specificity have been reported, so that CDT was considered to be the best biomarker of alcoholism available (Stibler *et al*., 1986; Gjerde *et al*., 1988; Kapur *et al*., 1989; Kwoh-Gain *et al*., 1990; Lesch *et al*., 1996; Burke *et al*., 1998; Reynaud *et al*., 1998). However, follow-up studies failed to reproduce these promising results; a large number of studies demonstrated that CDT is not superior to gamma-glutamyltranspeptidase (γ-GT) and mean corpuscular volume (MCV), which are not correlated to CDT (Helander *et al*., 1996), in the identification of alcohol misuse (Nilssen *et al*., 1992; Gronbaek *et al*., 1995; Aithal *et al*., 1998; Schmitt *et al*., 1998; Sillanaukee *et al*., 1998; Limin *et al*., 1999). This has been confirmed by two recent literature reviews (Salaspuro, 1999; Scouller *et al*., 2000). It also appeared that CDT has a worse predictive power in women compared to men; Schmitt *et al*. (1998) reported a sensitivity of 0% (at a specificity level of 95%) for females, and in a recent study CDT levels of peri-menopausal women were reported to have a sensitivity of 30% (van Pelt *et al*., 2000). Although some other studies found better values, it was consistently noted that CDT alone is not suitable as a biomarker of alcohol intake in women (Nystrom *et al*., 1992; Gronbaek *et al*., 1995; Yeastedt *et al*., 1998; Allen *et al*., 2000; Brathen *et al*., 2000). However, the cause for this gender-specific effect is not yet known and further investigations are needed to clarify this issue.

As the determination of CDT is more than twice as expensive as that of γ-GT or MCV, and, even more importantly, a false-positive test result might have important consequences for the patient, we sought to identify conditions in which CDT is elevated independently of alcohol intake. Most noteworthy, several variables distinct from gender have been shown to have an influence on the CDT level, e.g. age, smoking status, obesity (Sillanaukee *et al*., 1998; Whitfield *et al*., 1998), hypertension (Fagerberg *et al*., 1994*a*), serum iron (De Feo *et al*., 1999) and insulin levels (Fagerberg *et al*., 1994*b*). However, most of these do not increase the CDT concentration above the cut-off value in teetotallers (Whitfield *et al*., 1998). As we had the clinical impression that serum CDT concentration is increased in women reporting recent weight loss, we screened all female patients who were in a catabolic (negative metabolic) state when attending our department. Our hypothesis was that catabolism of various aetiologies might result in elevated CDT levels in female (psychiatric) patients.

SUBJECTS AND METHODS

Subjects

Female in-patients presenting at the admission ward of our department have been screened for elevated CDT levels if they reported a recent history of weight loss or showed clear clinical signs thereof. All of them suffered from psychiatric disorders distinct from alcoholism; only patients reliably denying any alcohol intake above one drink per week were included in the screening programme. Higher alcohol consumption was an exclusion criterion, as was a score of ≥1 in the CAGE questionnaire (Mayfield *et al*., 1974; Ewing, 1984).

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Alcohol abstinence was confirmed by relatives or professional staff, when available; three patients did not have any opportunity to obtain alcoholic beverages, due to immobilization or placement in a closed ward. Serum CDT and other parameters were determined within the first 4 days after admission. Eleven patients matched all the inclusion criteria described above; they were between 20 and 86 years old with a mean age of 45 years. The recruitment interval was 5 months.

The causes of weight loss were varied, as were the psychiatric diagnoses (cf. Table 2). Patient #1 could not care for adequate food intake due to long-lasting dementia, whereas patient #2 refused to cook and eat, because of a paranoid schizophrenia resulting in a delusion of impoverishment. Patient #3 suffered from chronic malnutrition and neglect due to paranoid schizophrenia, and #4 reported that she refused to eat because of erotomania. Patient #5 suffered from anorexia nervosa. Patient #6 suffered from a personality disorder exaggerated by an acute polymorph psychotic disorder; additionally, she had juvenile diabetes. Due to both psychiatric diseases, she had very low compliance with respect to insulin treatment resulting in repetitive hypo- as well as hyperglycaemias and ketoacidosis. Patient #7 had ideas that both food and beverages were poisoned due to hebephrenic schizophrenia. Patient #8 had an obsession that she was not allowed to eat, as she had delusions that she would get punished for this by a former nurse (she suffered from paranoid schizophrenia for over 30 years); patient #9 had a severe case of anorexia nervosa with a body mass index (BMI) of 10 kg/m^2. Patient #10 had an episode of schizoaffective disorder and lost 14 kg of weight due to maniform excitation, loss of appetite and altruistic overwork. Patient #11 had had anorexia nervosa for years, and concomitant chronic pancreatitis which explains her elevated γ-GT value (cf. Table 1).

For comparison, we investigated 24 female control subjects from the same ward and time interval, who have been screened routinely for excessive alcohol intake by the use of CDT; this group has been divided into abstinent/social drinkers [i.e. persons consuming alcohol not at all or socially, maximally twice a week, and not fulfilling any ICD-10 (World Health Organization, 1992) criterion for harmful alcohol use or alcohol dependence] and heavy drinkers (fulfilling the ICD-10 criteria for harmful alcohol use or alcohol dependence, drinking alcohol on a daily basis with frequent intoxications). The rationale for this division was an extensive open clinical interview including self-reported alcohol consumption, as well as information by relatives, when possible. One patient had to be excluded because of persisting uncertainties about her alcohol consumption (CDT value: 6.3%).

Determination of serum parameters

The concentration of CDT was performed in the routine laboratory of our department. As the assay method might substantially influence the sensitivity of the test (Sorvajarvi *et al*., 1996), we chose to utilize the %CDT method. By using this method, total transferrin levels are separately determined according to the protocol of the manufacturer. Therefore, changes in total transferrin concentrations have a smaller influence on the measured CDT value. The %CDT turbidimetric immunoassay by Bio-Rad was used (Bio-Rad Laboratories GmbH, Munich, Germany), which is based on the method by Axis Biochemicals. Briefly, serum transferrin is saturated by Fe^{3+} ions; as a result of this, transferrin isoforms are differentially charged, depending on the number of sialic acid groups. Thereafter, the samples are loaded onto an ion exchange column where transferrin isoforms are separated according to their different charges. The CDT isoforms are then detected turbidimetrically by the use of a polyclonal anti-human transferrin antibody. This test covers all six transferrin isoforms (penta-, tetra-, tri-, di-, mono- and a-sialo-transferrin) and reports CDT as a percentage of total transferrin. The assay has been calibrated by the manufacturer using a high-performance liquid chromatography (HPLC) method (r^2 = 0.98 of %CDT against HPLC). The cut-off value for elevated CDT was 6% according to the manufacturer's protocol.

All other parameters were determined in the same laboratory by established techniques for routine measurements, with a Vitros Chemistry Systems 750XRC apparatus. MCV was calculated as the quotient between the haematocrit and the number of erythrocytes as determined with a Sysmex K1000 apparatus. Reference ranges were as follows: MCV, 85–98 fl; γ-GT, ≤28 U/l; uric acid, 2.4–7.0 g/l; total cholesterol, 140–220 mg/l; total triglycerides, 74–172 mg/l.

RESULTS

Within 5 months, 11 female patients were admitted to our admission ward and reported weight loss in recent weeks, or showed clear clinical signs thereof and conceded decreased food intake in an open clinical interview. All of them consumed less than one alcoholic drink per week. We investigated whether CDT is elevated in these subjects, being in a catabolic state (exclusion criteria are given in the Subjects and methods section).

Of 11 patients, seven were found to have CDT levels above the cut-off value (6%). Thus, the sensitivity of elevated CDT concentrations for catabolic metabolism in abstinent patients was 63%. In all patients, other known markers for alcohol misuse have been determined for comparison. Weight as well as height have been measured and the body mass index (BMI) has been calculated using the formula BMI = weight (kg)/[height (m)]^2. Table 1 summarizes all investigated parameters. The mean ± SD serum CDT concentration of this group was 6.7 ± 1.8%; CDT concentrations and BMI, as well as other laboratory parameters, were not correlated.

During the same time-frame, the CDT value of 24 other female in-patients of the same ward has been determined routinely to screen for alcohol misuse. According to their self-reported history of alcohol consumption and the clinician's impression, this group has been divided into abstinent/social and heavy drinkers according to the rationale given in the Subjects and methods section. One patient has been excluded as her drinking status remained unclear; none of these patients reported recent weight loss. In Table 2, the CDT value, the main psychiatric diagnosis and the drinking status of those patients is presented. The mean ± SD CDT value of abstinent/social drinkers was 4.4 ± 1.0%, and for heavy drinkers 5.8 ± 2.2, respectively. The CDT values of the abstinent/social and the heavy drinkers did not differ significantly (P = 0.075, two-tailed Student's t-test). The CDT value had a sensitivity for heavy alcohol consumption of 33% (at a cut-off level of 6%). In abstinent/social drinkers, only one patient was found to

Table 1. Carbohydrate-deficient transferrin (CDT) concentrations in catabolic patients

	CDT (%)	γ-GT (U/l)	MCV (fl)	Uric acid (g/l)	Total cholesterol (mg/l)	Triglycerides (mg/l)	BMI (kg/m^2)
Reference value	<6	<28	85–96	2.4–7.0	140–220	74–172	20–25
Patient no.							
#1	2.9	11	112	2.3	171	78	21
#2	5.1	35	96	2.3	197	93	15
#3	5.7	7	80	4.1	181	96	15
#4	5.9	9	90	5.1	174	168	20
#5	6.5	14	89	2.6	161	83	14
#6	6.7	18	89	4.0	172	101	25
#7	7.0	9	68	2.0	184	80	20
#8	7.6	9	90	3.0	206	90	22
#9	7.9	12	94	5.0	136	63	10
#10	8.4	11	85	2.4	203	231	23
#11	9.6	98	97	4.0	187	149	14

In catabolic female patients, CDT and additional parameters have been determined as described in the Subjects and methods section. None of the patients reported a history of alcohol consumption; all of them had 0 points in the CAGE screening test.
γ-GT, gamma-glutamyltranspeptidase; MCV, mean corpuscular volume; BMI, body mass index.

Table 2. Carbohydrate-deficient transferrin (CDT) values of control psychiatric in-patients compared with the weight-loss group

Weight-loss group shown in Table 1			Control groups					
			Abstinent/social drinkers			Heavy drinkers		
No.	Main diagnosis	CDT	No.	Main diagnosis	CDT	No.	Main diagnosis	CDT
1	Dementia	2.9	1	Dementia	3.1	1	Factitious disorder	3.6
2	Hebephrenic schizophrenia	5.1	2	Personality disorder	3.3	2	Personality disorder	3.5
3	Paranoid schizophrenia	5.7	3	Depressive episode	3.4	3	Alcohol dependence	3.8
4	Catatonic schizophrenia	5.9	4	APPD	3.6	4	Alcohol dependence	3.9
5	Anorexia nervosa	6.5	5	Dementia	4.3	5	Personality disorder	4.3
6	APPD	6.7	6	Personality disorder	4.5	6	Alcohol dependence	4.5
7	Hebephrenic schizophrenia	7.0	7	Personality disorder	4.5	7	Alcohol dependence	5.6
8	Paranoid schizophrenia	7.6	8	Catatonic schizophrenia	4.6	8	APPD	5.7
9	Anorexia nervosa	7.9	9	Personality disorder	4.7	9	Alcohol dependence	7.6
10	APPD	8.4	10	Depressive episode	5.4	10	Alcohol dependence	8.4
11	Anorexia nervosa	9.6	11	APPD	6.7	11	Personality disorder	8.4
						12	Depressive episode	10.4

Psychiatric patients have been categorized as abstinent/social or heavy drinkers as described in the Subjects and methods section. CDT has been determined as a screening test for excessive alcohol intake and is given as % of total transferrin (reference point: <6%). The main psychiatric diagnosis is given according to ICD-10 criteria. Some patients were co-morbid with other psychiatric diseases distinct from substance misuse; in those cases, the leading diagnosis is given. For comparison, those patients in whom CDT has been investigated due to catabolism (weight-loss group; cf. Table. 1) are also summarized here; none of them reported alcohol consumption. APPD denotes acute polymorph psychotic disorder.

have an increased CDT concentration, thus the specificity of CDT was 91% when abstinent/social drinkers were the 'healthy' control group. Considering CDT as a marker for excessive alcohol intake, its specificity when calculated for the weight-loss group was as low as 36%, i.e. seven false-positive test results in 11 patients.

DISCUSSION

The determination of CDT is currently commonly applied to screen for alcohol misuse. However, there is an increasing body of evidence that this marker has lower levels of sensitivity and specificity than previously described (Salaspuro, 1999; Scouller *et al*., 2000). Additionally, a range of confounding variables has emerged (Whitfield *et al*., 1998) and it appears that this marker is much less valid in female, than in male, subjects (Allen *et al*., 2000). The specificity for women ranges from 57 to 100% with a mean of 87 ± 12% (calculated from the values given in Allen *et al*., 2000). Equally important is the corresponding level of sensitivity. In the present study, we found sensitivity levels of 33% for women. Consistent with this, CDT sensitivity levels for women from 19 to 86% have been reported (mean ± SD: 47 ± 18%; Allen *et al*., 2000). Thus, the diagnostic value of CDT for female subjects is fairly poor. The low level of sensitivity might easily cause a diagnostic bias when CDT is applied as a biomarker for alcohol consumption. A false-positive test result might have severe consequences for the patient, especially in forensic psychiatry. It is therefore of extreme importance to identify disease states in which CDT is elevated independently of alcohol consumption.

We present here cases of non-alcohol-drinking female patients in whom CDT was elevated due to a catabolic state. The underlying psychiatric diseases were predominantly anorexia nervosa and schizophrenia spectrum disorders.

Malnutrition in industrialized countries is becoming rare; however, it can be found frequently in psychiatric hospitals due to eating disorders, delusions or compulsions. Recently, a study by Agelink *et al*. (1999) suggested CDT as a highly specific parameter for alcohol misuse in schizophrenic patients. Considering the results of the present study, a catabolic state should be considered when CDT alone is found to be elevated. Notably, the BMI was not a marker for recent malnutrition (cf. Table 1). The overall constellation of laboratory parameters has to be taken into account; MCV, another alcoholism parameter, which has recently been suggested to be diagnostically equal to CDT (Schmitt *et al*., 1998; Allen *et al*., 2000), might also be elevated in malnutrition, due to decreased cobalamine or folic acid intake. When γ-GT is increased too, this could indicate increased alcohol intake; low uric acid, cholesterol and triglyceride values suggest malnutrition, but tend to be elevated in alcoholics (Burke *et al*., 1998). Thus, CDT proves to be useful only in combination with other routine laboratory parameters, as suggested by other groups (Nilssen *et al*., 1992; Aithal *et al*., 1998; Yeastedt *et al*., 1998; Allen *et al*., 2000; van Pelt *et al*., 2000). Nonetheless, serum parameters can only support a clinician's diagnosis of alcohol dependence, never substantiate it. Screening questionnaires, like the CAGE test, have proven to be useful (Aithal *et al*., 1998), and can be carried out quickly and at almost no cost, and should therefore be used first-line in the diagnostic hierarchy.

Why is CDT elevated in catabolic states? Glycosyltransferases, enzymes which are involved in the glycosylation of transferrin, are inhibited by the ethanol metabolite acetaldehyde (Ghosh *et al*., 1993; Xin *et al*., 1995). This results in decreased synthesis of fully glycosylated transferrin and contributes to the elevation of CDT in subjects with excessive alcohol intake. In catabolic states, ketones are formed, one of which is acetoacetate (Stryer, 1988). Acetoacetate and acetaldehyde bear structural similarities (Fig. 1). Therefore, it is possible that acetoacetate could also inhibit glycosyltransferases, resulting in an increase of CDT (Scheme 1). Interestingly, patient #6 had type I diabetes with repeating ketoacidosis, which supports this theory. A recent study found no influence of diabetes on CDT (Meerkerk *et al*., 1998), but usually diabetic patients attending a general practice (as in this report) are not expected to suffer from diabetic decompensations resulting in ketoacidosis. It would therefore be interesting to determine CDT in non-compliant patients suffering from juvenile diabetes, especially in correlation with ketones. In line with this suggestion, elevated CDT was found to be negatively correlated with fasting plasma insulin in hypertensive men (Fagerberg *et al*., 1994*b*). Although our hypothesis has yet to be proven, it provides a basis for future basic research, e.g. biochemical experiments investigating the possible inhibition of glycosyltransferases by acetoacetate. Additional mechanisms could of course include deficient availability of carbohydrates in catabolic states or other mechanisms not yet known.

Obviously, some uncertainties about both the exact drinking status and the precise nutritional history do exist in the present study due to the fact that we utilized a retrospective study design. According to these methodological problems, it would be fruitful to reproduce the findings presented in this pilot study by the use of a prospective study design with a greater number of both male and female patients by the use of a matched controls study design. A replication study investigating the possible CDT increase in patients suffering from anorexia nervosa is presently under way, and preliminary data indicate the validity of our findings. Taken together, we conclude that CDT is not suitable as a marker for alcohol intake in catabolic female patients; furthermore, when CDT is elevated, recent weight loss should be considered and CAGE-positivity, γ-GT, MCV, uric acid, cholesterol and triglycerides have also to be determined.

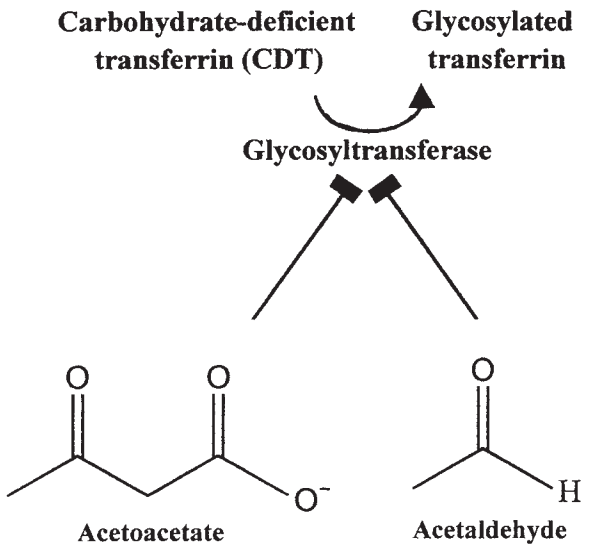

Fig. 1. Proposed mechanism for the elevation of carbohydrate-deficient transferrin (CDT) in catabolic states.

Transferrin is glycosylated by specific enzymes, called glycosyltransferases. These are inhibited by acetaldehyde (right), a metabolite of ethanol (Ghosh *et al*., 1993; Xin *et al*., 1995), resulting in an increase in CDT. The ketone acetoacetate (left), which is formed in catabolic states, might also inhibit the enzymes due to its structural similarity to acetaldehyde, resulting also in increased CDT. The formulae of both acetaldehyde and acetoacetate are displayed to point out their structural similarity.

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REFERENCES

Agelink, M. W., Ullrich, H., Lemmer, W., Dirkes-Kersting, A. and Zeit, T. (1999) Screening for concomitant alcohol abuse in schizophrenia: clinical significance of the Munich Alcoholism Test and laboratory tests. *European Addiction Research* **5**, 82–87.

Aithal, G. P., Thornes, H., Dwarakanath, A. D. and Tanner, A. R. (1998) Measurement of carbohydrate-deficient transferrin (CDT) in a general medical clinic: is this test useful in assessing alcohol consumption? *Alcohol and Alcoholism* **33**, 304–309.

Allen, J. P., Litten, R. Z., Fertig, J. B. and Sillanaukee, P. (2000) Carbohydrate-deficient transferrin, gamma-glutamyltransferase, and macrocytic volume as biomarkers of alcohol problems in women. *Alcoholism: Clinical and Experimental Research* **24**, 492–496.

Brathen, G., Bjerve, K. S., Brodtkorb, E. and Bovim, G. (2000) Validity of carbohydrate deficient transferrin and other markers as diagnostic aids in the detection of alcohol related seizures. *Journal of Neurology, Neurosurgery and Psychiatry* **68**, 342–348.

Burke, V., Puddey, I. B., Rakic, V., Swanson, N. R., Dimmitt, S. B., Beilin, L. J., Ching, S. and Beilby, J. P. (1998) Carbohydrate-deficient transferrin as a marker of change in alcohol intake in men drinking 20 to 60 g of alcohol per day. *Alcoholism: Clinical and Experimental Research* **22**, 1973–1980.

De Feo, T. M., Fargion, S., Duca, L., Mattioli, M., Cappellini, M. D., Sampietro, M., Cesana, B. M. and Fiorelli, G. (1999) Carbohydrate-deficient transferrin, a sensitive marker of chronic alcohol abuse, is highly influenced by body iron. *Hepatology* **29**, 658–663.

Ewing, J. R. (1984) Detecting alcoholism. The CAGE questionnaire. *Journal of the American Medical Association* **252**, 1905–1907.

Fagerberg, B., Agewall, S., Berglund, A., Wysocki, M., Lundberg, P.-A. and Lindstedt, G. (1994*a*) Is carbohydrate-deficient transferrin in serum useful for detecting excessive alcohol consumption in hypertensive patients? *Clinical Chemistry* **40**, 2057–2063.

Fagerberg, B., Agewall, S., Urbanavicius, V., Attvall, S., Lundberg, P. A. and Lindstedt, G. (1994*b*) Carbohydrate-deficient transferrin is associated with insulin sensitivity in hypertensive men. *Journal of Clinical Endocrinology and Metabolism* **79**, 712–715.

Ghosh, P., Okuh, C., Liu, Q. H. and Lakshman, M. R. (1993) Effects of chronic ethanol on enzymes regulating sialylation and desialylation of transferrin in rats. *Alcoholism: Clinical and Experimental Research* **17**, 576–579.

Gjerde, H., Johnsen, J., Bjorneboe, A., Bjorneboe, G.-E. and Mørland, J. (1988) A comparison of carbohydrate-deficient transferrin with other biological markers of excessive drinking. *Scandinavian Journal of Clinical Laboratory Investigation* **48**, 1–6.

Gronbaek, M., Henriksen, J. H. and Becker, U. (1995) Carbohydrate-deficient transferrin — a valid marker of alcoholism in population studies? Results from the Copenhagen City Heart Study. *Alcoholism: Clinical and Experimental Research* **19**, 457–461.

Helander, A., Carlsson, A. V. and Borg, S. (1996) Longitudinal comparison of carbohydrate-deficient transferrin and gamma-glutamyltransferase: complementary markers of excessive alcohol consumption. *Alcohol and Alcoholism* **31**, 101–107.

Kapur, A., Wild, G., Milford-Ward, A. and Triger, D. R. (1989) Carbohydrate-deficient transferrin: a marker for alcohol abuse. *British Medical Journal* **299**, 427–431.

Kwoh-Gain, I., Fletcher, M., Price, J., Powell, L. W. and Halliday, J. W. (1990) Desialylated transferrin and mitochondrial aspartate aminotransferase compared as laboratory markers of excessive alcohol consumption. *Clinical Chemistry* **36**, 841–845.

Lesch, O. M., Walter, H., Freitag, H., Heggli, D.-E., Leitner, A., Mader, R., Neumeister, A., Passweg, V., Pusch, H., Semler, B., Sundrehagen, E. and Kasper, S. (1996) Carbohydrate-deficient transferrin as a screening marker for drinking in a general hospital population. *Alcohol and Alcoholism* **31**, 249–256.

Limin, S., Jarvie, D. R., Chick, J. and Simpson, D. (1999) Limitations of CDT and GGT in detecting relapses in patients attending an alcohol problems clinic. *Scottish Medical Journal* **44**, 140–142.

Mayfield, D., McLeod, G. and Hall, P. (1974) The CAGE questionnaire: validation of a new alcoholism screening instrument. *American Journal of Psychiatry* **131**, 1121–1123.

Meerkerk, G. J., Njoo, K. H., Bongers, I. M., Trienekens, P. and van Oers, J. A. (1998) The specificity of the CDT assay in general practice: the influence of common chronic diseases and medication on the serum CDT concentration. *Alcoholism: Clinical and Experimental Research* **22**, 908–913.

Nilssen, O., Huseby, N. E., Hoyer, G., Brenn, T., Schirmer, H. and Forde, O. H. (1992) New alcohol markers — how useful are they in population studies: the Svalbard Study 1988–89. *Alcoholism: Clinical and Experimental Research* **16**, 82–86.

Nystrom, M., Perasalo, J. and Salaspuro, M. (1992) Carbohydrate-deficient transferrin (CDT) in serum as a possible indicator of heavy drinking in young university students. *Alcoholism: Clinical and Experimental Research* **16**, 93–97.

Petren, S. and Vesterberg, O. (1988) Concentration differences in isoforms of transferrin in blood from alcoholics during abuse and abstinence. *Clinica et Chimica Acta* **175**, 183–187.

Reynaud, M., Hourcade, F., Planche, F., Albuisson, E., Meunier, M. N. and Planche, R. (1998) Usefulness of carbohydrate-deficient transferrin in alcoholic patients with normal gamma-glutamyltranspeptidase. *Alcoholism: Clinical and Experimental Research* **22**, 615–618.

Salaspuro, M. (1999) Carbohydrate-deficient transferrin as compared to other markers of alcoholism: a systematic review. *Alcohol* **19**, 261–271.

Schmitt, U. M., Stieber, P., Jüngst, D., Bilzer, M., Wächtler, M., Heberger, S. and Seidel, D. (1998) Carbohydrate-deficient transferrin is not a useful marker in the detection of chronic alcohol abuse. *European Journal of Clinical Investigation* **28**, 615–621.

Scouller, K., Conigrave, K. M., Macaskill, P., Irwig, L. and Whitfield, J. B. (2000) Should we use carbohydrate-deficient transferrin instead of gamma-glutamyltransferase for detecting problem drinkers? A systematic review and meta-analysis. *Clinical Chemistry* **46**, 1894–1902.

Sillanaukee, P., Aalto, M. and Seppa, K. (1998) Carbohydrate-deficient transferrin and conventional alcohol markers as indicators for brief intervention among heavy-drinkers in primary health care. *Alcoholism: Clinical and Experimental Research* **22**, 892–896.

Sillanaukee, P., Strid, N., Allen, J. P. and Litten, R. Z. (2001) Possible reasons why heavy drinking increases carbohydrate-deficient transferrin. *Alcoholism: Clinical and Experimental Research* **25**, 34–40.

Sorvajarvi, K., Blake, J. E., Israel, Y. and Niemela, O. (1996) Sensitivity and specificity of carbohydrate-deficient transferrin as a marker of alcohol abuse are significantly influenced by alterations in serum transferrin: comparison of two methods. *Alcoholism: Clinical and Experimental Research* **20**, 449–454.

Stibler, H. (1991) Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clinical Chemistry* **37**, 2029–2037.

Stibler, H., Borg, S. and Allgulander, C. (1979) Clinical significance of abnormal heterogeneity of transferrin in relation to alcohol consumption. *Acta Medica Scandinavia* **206**, 275–281.

Stibler, H., Borg, S. and Joustra, M. (1986) Micro anion exchange chromatography of carbohydrate-deficient transferrin in serum related to alcohol consumption. *Alcoholism: Clinical and Experimental Research* **10**, 535–544.

Stibler, H., Borg, S., Joustra, M. and Hultcrantz, R. (1988) Carbohydrate-deficient transferrin (CDT) in serum as a marker of high alcohol consumption. In *Advances in the Biosciences*, Vol. 71, Nordmann, R., Ribiere, C. and Rouach, H. eds, pp. 353–357. Pergamon Press, Oxford.

Stryer, L. (1988) *Biochemistry*. W. H. Freeman, New York.

van Eijk, H., van Noort, W., Dubelaar, M. L. and van der Heul, C. (1983) The microheterogenity of human transferrins in biological fluids. *Clinica et Chimica Acta* **132**, 167–171.

van Pelt, J., Leusink, G. L., van Nierop, P. W. and Keyzer, J. J. (2000) Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. *Alcoholism: Clinical and Experimental Research* **24**, 176–179.

Wetterling, T. and Kanitz, R.-D. (1997) Der neue "Alkoholmarker" carbohydratdefizientes Transferrin (CDT). *Fortschritte der Neurologie und Psychiatrie* **65**, 337–346.

Whitfield, J. B., Fletcher, L. M., Murphy, T. L., Powell, L. W., Halliday, J., Heath, A. C. and Martin, N. G. (1998) Smoking, obesity, and hypertension alter the dose–response curve and the test sensitivity of carbohydrate-deficient transferrin as a marker of alcohol intake. *Clinical Chemistry* **44**, 2480–2489.

Wong, K. L. and Regoeczi, E. (1977) Some observations on the carbohydrate composition of purified transferrin. *International Journal of Peptide Research* **9**, 241–248.

World Health Organization (1992) *International Classification of Diseases*, 10th revision. World Health Organization, Geneva.

Xin, Y., Lasker, J. M. and Lieber, C. S. (1995) Serum carbohydrate-deficient transferrin: mechanism of increase after chronic alcohol intake. *Hepatology* **22**, 1462–1468.

Yeastedt, J., LaGrange, L. and Anton, R. F. (1998) Female alcoholic outpatients and female college students: a correlational study of self-reported alcohol consumption and carbohydrate-deficient transferrin levels. *Journal of Studies on Alcohol* **59**, 555–559.