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Carbohydrate-Deficient Transferrin in Serum: a New Marker of Potentially Harmful Alcohol Consumption Reviewed

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During the last 16 years an increasing number of studies have indicated a new diagnostic marker of alcohol abuse, unrelated to any of the conventional markers of alcoholism. This marker, now called carbohydrate-deficient transferrin, consists mainly of one or two isoforms of transferrin that are deficient in their terminal trisaccharides. Such isoforms have so far been detected by methods based on charge, i.e., isoelectric focusing, chromatofocusing, and anion-exchange chromatography of various designs combined with immunological detection techniques. This transferrin abnormality measures an accumulated effect of alcohol consumption, appearing after regular intake of 50–80 g of ethanol/day for at least one week and normalizing slowly during abstinence (half-life = about 15 days). To summarize all studies to date, ~2500 individuals have been examined, with a total clinical sensitivity of 82% and a specificity of 97%. False-positive results have only occasionally been reported: in a few patients with severe liver disease, usually primary biliary cirrhosis and chronic active hepatitis; in patients with genetic D variants of transferrin; and in patients with (and some carriers of) a recently identified inborn error of glycoprotein metabolism. The mechanism behind the transferrin abnormality is unknown but an acetaldehyde-mediated inhibition of glycosyl transfer has been suggested. Carbohydrate-deficient transferrin may thus offer a new possibility of diagnosing alcohol-related disorders. Its measurement is little affected by other conditions and, contrary to conventional markers of alcohol abuse, is apparently largely independent of concomitant liver disease.

Additional Keyphrases: *sialic acid · complex carbohydrate chains · carbohydrate-deficient glycoprotein syndrome · metabolism · acetaldehyde*

Alcohol abuse is very common all over the world. Even in Sweden, where alcohol consumption is relatively low, ~5% of the population, or 400 000 individuals, are considered to be alcohol abusers. The social cost to Swedish society for alcohol-related diseases is estimated as 100 billion Swedish crowns annually, according to a recent socioeconomic investigation (A. Jonsson, Sober Förlag, Sweden, 1991). Both psychiatric and disabling (sometimes lethal) neurological and other somatic complications are common among alcohol abusers. Fetal alcohol syndrome in children of alcohol-abusing women is a much-feared complication, known for thousands of years, causing mental retardation in one of 600 children born live (*1*). The magnitude of the problem of alcohol abuse has to be seen in light of three basic features of ethanol: it is easy to produce, it is a solvent, and it has sedative and euphorizing effects on the central nervous system, similar to those of anesthetics. These characteristics have made ethanol the most widely used centrally acting drug known. Some other basic facts about ethanol are also important: it is often consumed to high blood concentrations (1/1000 = 20 mmol/L); it has immediate and direct biochemical and biophysical effects on cell membranes, similar to those of anesthetics (*2, 3*); it has basic metabolic consequences through its oxidation (*4, 5*); it commonly produces simultaneous nutritional deficiencies, and it rapidly produces biological tolerance (*6, 7*).

The biological, clinical, and social effects of alcohol abuse have long made evident the need for objective and specific markers for alcohol-related diseases and for early detection of alcohol consumers at risk. Most alcohol-related disorders simulate diseases of other etiologies, which often result in time-consuming and expensive investigations when alcohol abuse is not evident to the physician or is denied by the patient. Alcohol abusers may exhibit several clinico-chemical alterations, some of which have been used as markers of alcoholism, e.g., γ-glutamyltransferase, aspartate aminotransferase, glutamate dehydrogenase, and mean corpuscular volume (see Table 2 for references). All of the markers available thus far have two drawbacks: either they are indicators of disease in a particular organ, with poor specificity for different etiological possibilities, or they are not sensitive enough to detect abuse before the stage of organic complications. In addition, the biological kinetics of these markers relative to duration and amount of alcohol intake or to abstinence is usually poorly defined or considered.

The ideal diagnostic marker would be a clinical or biochemical phenomenon that is specifically related to the presence or metabolism of ethanol, is dependent on the amount of ethanol consumed, is sensitive enough to detect consumption levels associated with somatic and psychiatric risk, and the kinetics of which during abstinence can be defined. Another type of marker, a preven-

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tative marker, would identify individuals at risk of developing alcohol abuse for genetic or other reasons. Although preventative markers have attracted considerable interest, e.g., monoamine oxidase B and alcohol and aldehyde dehydrogenase isoenzymes (see ref. *8* for a review), their clinical importance is as yet insufficiently known.

During the last decade a new diagnostic marker has been introduced that appears to fulfill important clinical criteria. It is apparently dependent on ethanol or its metabolism, it appears in serum after regular high alcohol intake, and it shows a high sensitivity and specificity. This marker is carbohydrate-deficient transferrin (CDT).

History

While investigating cerebrospinal fluid and serum proteins in patients with neurological diseases by means of the then-new method of isoelectric focusing, Stibler and Kjellin (*9*) reported abnormal microheterogeneity of transferrin in cerebrospinal fluid in patients with alcoholic cerebellar degeneration. In half of the patients, one of these components, at pI 5.7, was also observed in serum (*9*). In subsequent studies, the transferrin abnormality in serum was found with a high frequency in alcohol abusers; it disappeared after abstinence and was apparently specific for alcohol abuse (*10, 11*). The initial qualitative estimation of this transferrin change was followed by quantitative methods combining isoelectric focusing with immunofixation (*12, 13*) or zone immunoelectrophoresis (*14, 15*), and quantifying an isoform with pI 5.7 in relation either to total transferrin (*12, 14, 15*) or to the main isotransferrin with pI 5.4 (*13*). Both high sensitivity and high specificity for alcohol abuse were suggested by these early studies. However, methodological aspects became an early problem. Isoelectric focusing followed by immunological detection and quantification was too complex and time-consuming for routine clinical purposes. Later, two separation techniques were developed, based on anion-exchange chromatography on a micro scale (*16–18*) or chromatofocusing (*19, 20*) combined with radioimmunoassay. These techniques have permitted clinical evaluation of larger groups of patients in only a few years.

Definition

The normal microheterogeneity of serum transferrin on isoelectric focusing depends on variations in its amino acid composition and its iron and carbohydrate content. Amino acid variations are ordinarily seen in individuals with genetic variants of transferrin that are usually rare and readily recognized; variations in iron content can be overcome by iron saturation of the sample (*10, 21, 22*). The normally occurring isoforms of iron-saturated diferric transferrin depend on variations in carbohydrate content, especially the content of the strongly negatively charged sialic acid (*23, 24*). Transferrin usually contains two complex carbohydrate chains consisting of four different carbohydrates (*N*-acetylglucosamine, mannose, galactose, sialic acid), the exact content of which varies considerably (*25, 26*). Sialic acid is the only charged carbohydrate; it is always positioned terminally, and zero to six or more residues may be present (*26*), resulting in variations in isoelectric point of ~0.1 pH unit/residue (*22–24*). The normal main isoform of transferrin has a pI of 5.4 and four sialic acid residues, two on each carbohydrate chain (*23–26*). Minor isoforms with lower pI values (penta- and hexasialotransferrin) and higher pI values (tri- and disialotransferrin) are ordinarily present in decreasing amounts (*26–28*).

Components with high pI values, whose charge corresponds to disialo- (pI 5.7) and to a lesser degree mono- and asialotransferrin (pI 5.8 and 5.9, respectively), are present in the serum of alcohol-abusing patients (*9–12, 27, 28*) (Figure 1). Investigators learned early that the difference between alcoholic patients and controls disappeared after neuraminidase treatment of the sample (*11*); a decrease of sialic acid content in such samples was later confirmed quantitatively (*28*). Further studies of affinity-purified transferrin showed that the defect was more complex and also included the neutral carbohydrates galactose and *N*-acetylglucosamine (*27*), i.e., the carbohydrates constituting the terminal trisaccharide in transferrin. Because in alcohol-abusing patients some of the serum transferrin molecules apparently lacked two to four of their terminal trisaccharides (*28*), the term "carbohydrate-deficient transferrin," or CDT, was developed (*17*).

Clinical Experiences with CDT

For more than 10 years after the first report concerning abnormal transferrin in alcoholics in 1976 (*9*), isoelectric focusing was the predominant method used to demonstrate this phenomenon. Its presence was initially evaluated qualitatively (*9–11, 29, 30*) but this was later followed by quantitative measurement after immunological detection by various means (*12–15*). The inexactness of qualitative methods is clear, and quanti-

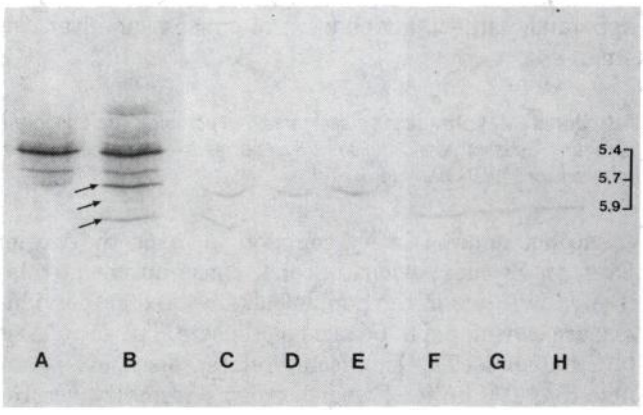

Fig. 1. Isoelectric focusing of serum transferrin

A and *B*, serum transferrin purified by immunoaffinity chromatography (*27*), from (*A*) a healthy control and (*B*) an alcohol-abusing patient. *C–E*, immunofixation of transferrin isoforms eluted after isocratic anion-exchange chromatography of iron-saturated serum at pH 5.65 (*17*) from alcohol-abusing patients. *F–H*, same as in *C–E* but after anion-exchange chromatography by ionic strength (*42*). Isoelectric points are given at the *right*. *Arrows* indicate abnormal transferrin isoforms

tative techniques based on isoelectric focusing have the disadvantage that quantification of isoforms requires the determination of a ratio of the isoform(s) under investigation in relation to total transferrin or to the main isoform, because variations in staining intensity and sample penetration into the gel may be considerable (*31*). A disadvantage with such ratios is that total transferrin has been found to be unrelated to CDT (*17, 32*), which may give rise to both false-positive and false-negative ratio values, owing to independent variations in total transferrin concentration. Despite the technical problems associated with these early methods and limited study populations, high figures for sensitivity and specificity for alcohol abuse were reported, varying from 81% to 100% for sensitivity and from 97% to 100% for specificity (*9–15, 29, 30*). Only two studies reported lower figures, but those also included abstaining alcohol abusers (*14, 29*). We had already shown in 1978 (*10*) that the transferrin abnormality depended on the amount of alcohol consumed and that it declined or normalized within 10 days of alcohol abstinence.

An improved quantitative method was clearly needed to permit studies of larger clinical populations and routine analyses. Two new approaches were presented by Stibler et al. (*16*) and by Storey et al. (*19*) in 1985, the former based on isocratic anion-exchange chromatography and the latter on chromatofocusing. In the original method of Stibler et al. (*16, 17*), iron-saturated whole serum was passed through a microcolumn, and all isotransferrins with pI values >pH 5.65 were eluted (Figure 1) and quantified by a subsequent transferrin radioimmunoassay. The method of Storey et al. (*19, 20*) required an initial partial isolation of serum transferrin followed by chromatofocusing on a Mono P column and collection of the isotransferrin with pI 5.7. The isotransferrin was later quantified with a transferrin radioimmunoassay (*18*). Although the latter assay gave good resolution, it involved several time-consuming steps, and only one of the abnormal transferrin components was measured. The procedure of Stibler et al. (*17*) required no purification, measured all of the abnormal isotransferrins, and provided quantitative results within half a day. The introduction of their CDT assay resulted in several investigations of large clinical populations within a few years (*16, 17, 32–41*). The characteristics of the CDT present in relation to the amount of alcohol consumed and to the duration of consumption and abstinence could be rather well defined, as could the sensitivity and specificity of this assay in relation to low alcohol consumers and to patients with various other diseases. Since 1986, 11 studies have been published involving use of the original CDT assay (*17, 32–41*) and two involving modifications of this method (*18, 42*). Altogether, >20 investigations of carbohydrate-deficient transferrin in alcoholics have been reported, originating from 11 different laboratories and based on five different methods. About 2500 individuals have been examined qualitatively or quantitatively, with an approximately equal distribution of alcohol abusers, healthy controls, and patients with non-alcohol-related diseases. The total sensitivity for alcohol abuse in the whole population investigated, irrespective of method, is calculated as 82%, the specificity 97%. Table 1 summarizes these studies. Table 2 shows the data for six other markers studied in comparison with CDT. When all published results are examined, 40 individuals with "false-positive" CDT values can be identified. The majority of these were patients with severe hepatic insufficiency, mainly due to cirrhosis caused by chronic active hepatitis or primary biliary cirrhosis (*29, 32, 34, 42*). However, the large majority of non-alcohol-consuming patients with liver diseases did not have this transferrin abnormality (*11, 17–20, 29, 30, 32–34, 40, 42*). One patient with rectal carcinoma and three with neuropsychiatric diseases have also been reported with slightly increased concentrations of CDT (*17*). Individuals with genetically rare D-variants of transferrin may also have increased results in the CDT assay, whereas the common genetic subtypes of transferrin C fall within normal limits (*39*). A large number of commonly used drugs do not interfere with the CDT values (*17*), nor does opiate therapy or abuse (*17, 34*). A special situation has appeared with a recently discovered inherited disorder of glycoprotein metabolism, carbohydrate-deficient glycoprotein (CDG) syndrome, which is associated with extremely high CDT values (*43, 44*); this disorder is discussed later. Known causes of false-positive CDT values are summarized in Table 3.

Clearly, therefore, CDT as a marker has high specificity, and other clear advantages over other available markers of alcohol abuse, particularly its low susceptibility to interference from liver disease. Actually, high CDT values have been found in alcohol abusers whether liver dysfunction is present or not (*17, 18, 29, 32, 33, 45*); moreover, CDT is not correlated with common chemical or morphological liver measures (*17, 32, 33*). This fact provides good potential for differentiating between alcohol-induced hepatopathy and liver disorders of other origin. As far as sensitivity is concerned, the duration and amount of alcohol ingestion and the duration of abstinence are crucial factors. All available data indicate that consumption of ≥50–80 g of ethanol/day for at least one week is required to produce this abnormality in between 81% and 94% of patients with alcohol-related symptoms (*11–13, 17, 20, 29, 30, 32, 33, 35, 36, 38, 40*) (Table 1). For a consumption >60 g/day, some studies have found a slight but significant correlation between amount consumed or blood alcohol concentration and CDT value (*17, 33, 35, 37, 42*), but others have not (*18, 32, 40*). This discrepancy may result from incomplete information regarding consumption or may suggest that CDT depends only indirectly on ethanol itself. It is of biomedical interest that increases in CDT values appear after ingestion of the mentioned quantity of ethanol, because regular intake of such amounts is considered to be necessary for the development of cirrhosis in men (*46*). CDT may therefore be a marker not only of "high" alcohol intake but also of drinking habits

Table 1. Summary of Clinical Studies of CDT as a Marker of Alcohol Abuse

Type of study	No. in groups	Method	Sensitivity, %	Specificity, %	Reference
Alcoholic cerebellar degeneration	10	IF, qual.	80	99	*9*
Neurological disease	28				
Controls	40				
Alcoholics	16	IF, qual.	94	—	*10*
Alcoholics	71	IF, qual.	64–81	99	*11*
Liver diseases	22				
Healthy controls	100				
Alcoholics	31	IF, quant. ratio	81	100	*12*
Healthy controls	28				
Alcoholics	20	IF, quant. ratio	95–100	92–97	*15*
Healthy controls	26				
Alcoholics	20	CF	85	100	*19*
Liver diseases	14				
Healthy controls	14				
Alcoholic liver diseases	41	IF, qual.	56	92	*29*
Nonalcoholic liver diseases	72		(including abstinent alcoholics)		
Alcoholics	77	CDT assay	91	99	*17*
Non-alcohol-related diseases	187				
Healthy controls	80				
Alcoholics	50	IF, quant. ratio	82	100	*13*
Healthy controls	25				
Alcoholics	20	CF, quant. ratio	90	100	*20*
Non-alcohol-related diseases	24				
Healthy controls	13				
Alcoholic liver diseases	15	CDT assay	87	100	*33*
Nonalcoholic liver diseases	87				
Alcoholics	100	CDT assay	91	99	*34*
Non-alcohol-related diseases	344				
Ex-alcoholics	30				
Healthy controls	155				
Alcoholics	107	CDT assay	81	91	*32*
Nonalcoholic liver diseases	64				
Healthy controls	18				
Female alcoholics	42	CDT assay	83	100	*35*
Healthy women	62				
Alcoholics	34	CDT assay	68	100	*36*
Healthy controls	35		(including abstinent alcoholics)		
Alcoholics	35	IF, quant.	52	82	*14*
Healthy controls	39		(including abstinent alcoholics)		
Alcoholics	160	CDT assay	>76	>90	*40*
Liver diseases	23		(10th and 90th percentiles)		
Healthy controls	50				
Alcoholics	37	IF, qual.	92	99	*30*
Alcoholic liver diseases	68				
Nonalcoholic liver diseases	47				
Non-alcohol-related diseases	38				
Alcoholics	36	CDT assay	83	100	*41*
Healthy controls	23				
Alcoholics	26	AE or CF, quant.	81–85	90–97	*18*
Non-alcohol-related diseases	21				
Healthy controls	16				
Alcoholics	78	CDT assay (modified)	94	98	*42*
Liver diseases	55				
Ex-alcoholics	20				
Healthy controls	71				

IF, isoelectric focusing; CF, chromatofocusing; CDT assay, isocratic micro anion-exchange chromatography on PBE-like gel; AE, isocratic anion-exchange chromatography on diethylaminoethyl-Sephacel; CDT assay (modified), micro anion-exchange chromatography by ionic strength; quant., quantitative; qual., qualitative evaluation.

Table 2. Sensitivity and Specificity of Six Biochemical Markers of Alcohol Abuse Compared with CDT Assayed by Anion-Exchange Chromatography

	Ref.[a]	CDT	GT	tASAT	mASAT	mASAT/tASAT	ALAT	GDH	MCV
Sensitivity, %	*17*	91	76	68	—	—	55	—	60
Specificity, %		99	—	—	—	—	—	—	—
Sensitivity, %	*18*	81–85	69	69	92	92	58	—	73
Specificity, %		90–97	59	68	52–70	52–70	57	—	76
Sensitivity, %	*32*	81	59	—	—	—	—	—	25
Specificity, %		91	50	—	—	—	—	—	95
Sensitivity, %	*33*	87	93	—	—	—	—	—	—
Specificity, %		100	5	50	—	—	26	—	—
Sensitivity, %	*35*	83	59	50	—	—	47	—	91
Specificity, %		100	—	—	—	—	—	—	—
Sensitivity, %	*40*	>76	88	—	—	—	—	81	—
Specificity, %		>90	9–90	—	—	—	—	30–92	—

[a] For information on clinical study groups, see Table 1.

GT, γ-glutamyltransferase; ASAT, aspartate aminotransferase (t = total, m = mitochondrial); ALAT, alanine aminotransferase; GDH, glutamate dehydrogenase; MCV, mean corpuscular volume.

of potential medical danger.

Numerous studies show that the transferrin abnormality normalizes during abstention (*10, 11, 17–19, 29, 30, 33, 35, 38, 42*). The CDT assay has determined the half-life of the marker to be about two weeks (*17, 35, 36, 38, 42*). The amount of ethanol consumed and the duration of abstinence before sampling are thus important factors to consider when evaluating clinical sensitivity and specificity and may explain the relatively low sensitivity in one study (*36*). The inability to discriminate between alcoholic and nonalcoholic liver disease in the work by Poupon et al. (*47*), who studied patients hospitalized for several weeks, appears to have the same reason. The negative results reported by Chapman et al. (*48*), however, seem mainly to have been due to technical failure to identify the isotransferrins correctly. Even when these factors have been taken into account, 6–19% false-negative results have been reported (*17, 32–35, 40–42*). The reason for this has not yet been clarified. As yet unknown genetic or metabolic factors may protect glycoproteins (e.g., transferrin) in some individuals from the action of ethanol or its metabolism.

The value of CDT for monitoring treatment of alcohol-related disorders has been studied in two investigations (*38, 49*). In one of these, unexplained increases of the CDT values were found in 10% of 72 patients during outpatient treatment for up to six months (*38*). In another study, of 15 patients who were followed weekly for three to 22 months, increases of the CDT values occurred only in connection with abuse relapses, which were verified either clinically or by measurement of 5-hydroxytryptophol in urine (*49*). Long-time abstaining former alcoholics have been found to have CDT values within the normal range (*34, 42*). Although there are not yet any explanations for increasing concentrations of CDT in "abstinent" alcoholic patients other than alcohol ingestion, as yet undefined metabolic causes cannot be excluded.

From a methodological point of view, the original CDT assay (*17*) had one disadvantage: correct separation of isotransferrins was dependent on a buffer of very low ionic strength with a stable pH of 5.65 ± 0.03. Because the stability of this buffer was not considered sufficient for routine use, a modification of the chromatography step was introduced. This modified CDT assay (*42*), which has now been in use for two years, is based on separation by a carefully adjusted ionic strength rather than by pH. For optimal separation without contamination by normal isoforms, the ionic strength was chosen such that mainly components with pI values >5.7 were measured, and only a small amount of the isotransferrin with pI 5.7 was included (Figure 1). This assay exhibited a satisfactory technical stability but gave lower absolute CDT values. Separation in disposable micro ion-exchange columns can be regarded as a compromise between requirements of utility for routine purposes and scientific demands for complete separation and recovery. However, the diagnostic sensitivity and specificity have been the same as with the original method, or even somewhat better (*42*) (Figure 2). Table 4 summarizes the characteristics of the modified CDT assay. A previously observed small sex-related difference in normal values for CDT (*39*) has become clearly significant with the modified assay. Values for healthy low-alcohol-consuming women are somewhat higher than for men of corresponding characteristics (*42*), indicating that the least glycosylated but minor isotransferrins (mono- and asialotransferrin) ordinarily are present in higher serum concentrations in women (Figure 2).

Table 3. Causes of "False-Positive" CDT Values[c]

	Reference
Isolated cases with hepatic insufficiency due to primary biliary cirrhosis, chronic active hepatitis, and drug hepatopathy	*32, 34, 42*
Genetic D-variants of transferrin	*39*
Patients with CDG syndrome (and 25% of the healthy carriers)	*43, 44*
Analytical causes	*17*

[c] Falsely positive diagnoses of alcohol-related diseases.

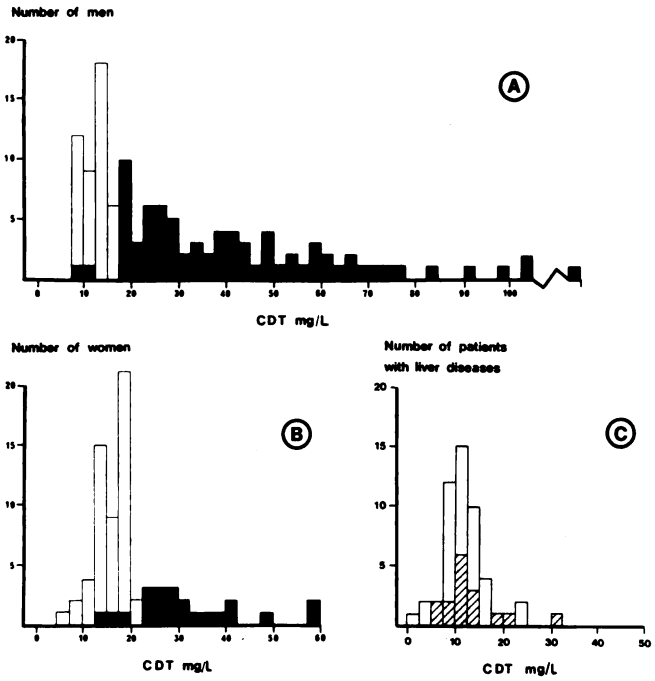

Fig. 2. Distribution of CDT values in the modified CDT assay in 251 individuals (*42*)

(*A*) 43 healthy men (alcohol consumption <40 g/day) (□), and 80 alcohol-abusing men (alcohol consumption >60 g/day) (■). (*B*) 51 healthy women (alcohol consumption <15 g/day) (□), and 22 alcohol-abusing women (alcohol consumption ≥60 g/day) (■). (*C*) 55 patients with non-alcohol-related liver diseases: □, men; ▨, women

Table 4. Characteristics of the Modified CDT Assay (*42*) in Relation to Alcohol Intake

Measures mainly isotransferrins with pI >5.7.
Total sensitivity = 93% (255 individuals).
Total specificity = 99% (255 individuals).
Values are increased for subjects taking ≥60 g of ethanol per day for more than seven days.
Values correlated to current alcohol intake (r = 0.38, P <0.01).
Values normalize during abstinence ($t_{1/2}$ = 15 ± 5 days).

Effects of Alcohol Abuse on Other Glycoconjugates

Ethanol exposure interferes with the metabolism of several glycoconjugates of various origins, ranging from proteoglycans of the bacterial cell envelope (*50*), gastric mucus glycoproteins (*51*), glycoproteins and glycolipids in rat and mouse brain (*52–56*), and rat liver glycoproteins (*57, 58*) to glycoconjugates in human erythrocyte membranes (*59, 60*). Several glycoproteins in human serum have been studied for changes similar to those of transferrin; so far, abnormal microheterogeneity of the kind seen in transferrin has been clearly observed only in α_1-antitrypsin (*29*). One possible reason for this might be that, in contrast to many other circulating glycoproteins, the secretion of transferrin from and its elimination by the liver are relatively independent of the carbohydrate content (*61, 62*).

Observed changes in membrane-bound glycoconjugates in the brain during experimental ethanol exposure have been implicated in the development of fetal alcohol syndrome (*52, 55*), and inhibition of secretion of secretory glycoproteins from the liver has been noted in the development of alcoholic liver disease (*63, 64*). Altered carbohydrate composition in erythrocyte membranes from alcoholics has been shown to be related to hemolysis (*65*) and to biological tolerance phenomena—such as changes in (Na^+K^+)ATPase activity and enzyme resistance to ethanol (*59*), as well as to biophysical tolerance (reduced membrane fluidization by ethanol) and impaired partitioning of ethanol into the membrane (*60, 66*). Chronic ethanol intake thus apparently has widespread effects on the metabolism and turnover of glycoconjugates, some of which may be important for biological complications of alcohol abuse. It is not yet known whether CDT itself has any biological effects. Sialic acid-free transferrin delivers iron to the liver more readily than does sialylated transferrin (*67*). However, transferrin with the more complex carbohydrate defect as is present in CDT (*27*) has not yet been studied in this or other functional respects.

Possible Mechanism(s)

Synthesis, glycosylation, secretion, elimination, and degradation of most serum glycoproteins take place in the liver and involve several intricate metabolic steps that require adequate precursor supplies, endomembrane transport, specific multiple glycosyltransferase systems, intact tubulin and receptor function, and lysosomal degradation (*68, 69*). Experimental ethanol exposure, specifically acetaldehyde, interferes with some of these events, e.g., causes morphological changes of the Golgi complex and impairs the secretory process by inhibiting tubulin polymerization (*63, 64*). Inhibition of glycosyltransferase activities has been observed both in rat brain and liver, and depends on blood alcohol concentration and duration of ethanol exposure (*52, 70–72*). Recently, altered elimination, lysosomal transport, and degradation of asialo-orosomucoid have been demonstrated in perfused liver of ethanol-fed rats (*73*). One difficulty in interpreting such results from experiments in rats is that, in two of the most commonly used models of chronic ethanol effects, no change of the transferrin microheterogeneity that is similar to that in alcohol-abusing humans has been observed (*74*).

Only a few studies of glycoprotein metabolism have been carried out in alcoholic humans. The only effects of alcohol abuse on glycoprotein metabolism in men demonstrated thus far are impaired tubulin polymerization, which is necessary for the secretory process (*64*), and reduced activities of three glycosyltransferases in serum (*45*). Furthermore, in humans, glycosyl transfer is inhibited by acetaldehyde but not by ethanol itself (*45*). Interference with transferrin protein synthesis appears improbable, because CDT concentration is unrelated to the concentration of total transferrin in serum (*17, 32*), which is closely associated with transferrin synthesis rate (*75*). Moreover, transferrin turnover may be either increased in steatosis or decreased in cirrhosis (*75*), whereas CDT values are increased in abusing patients with either condition (*17, 18, 29, 32, 33, 35*). Much work remains to be done to clarify the mechanism behind CDT, but apparently ethanol metabolism may interfere

with several steps in glycoconjugate turnover. The reactivity of acetaldehyde with several proteins (*76*), together with the observations made so far, make this metabolite an interesting candidate in such interactions.

Carbohydrate-Deficient Glycoprotein (CDG) Syndrome

A new inborn multisystemic syndrome with major nervous system involvement has recently been identified. Clinically, this disease is dominated by psychomotor retardation, olivoponto-cerebellar hypoplasia, peripheral neuropathy, and retinal pigmental degeneration accompanied by early hepatopathy, nephropathy, and abnormalities of cardiac, skeletal, gonodal, skin, and adipose tissue (*77*). The inheritance is autosomal and recessive and may be detected at birth (*77*), but patients as old as 47 years have been identified (*77*). Biochemically, this disorder is characterized by a complex carbohydrate deficiency in glycoproteins, which is most pronounced in transferrin (*43, 44*), resulting in extremely high CDT values in all patients (*44*). CDT concentrations are also increased in 25% of healthy carriers of CDG syndrome (*44*), which may be an infrequent cause of false-positive CDT measurements in the diagnosis of alcohol-related diseases. With an estimated gene frequency of 0.005, healthy carriers of CDG syndrome may be responsible for unexplained, permanently increased CDT values in one of 300–500 individuals. CDG syndrome appears to represent a new group of disorders of glycoprotein metabolism and is of considerable interest as a new clinical, biochemical, and genetic concept. The biochemical similarity between the acquired transferrin change in alcoholic patients and that in patients with the inborn CDG syndrome may provide clues to the understanding of the metabolic background of the effects of alcohol abuse on glycoprotein metabolism and its possible importance for the pathogenesis of organic complications of alcohol abuse.

Conclusions

Fifteen years after the initial observation of an abnormal isotransferrin pattern in CSF and serum in patients with alcoholic cerebellar degeneration, this phenomenon has been shown to be a very sensitive and specific marker of regular, potentially harmful, high alcohol consumption. Characterization of the abnormal transferrin isoforms has demonstrated that they are deficient to various degrees in their terminal trisaccharides, their appearance is related to the amount of ethanol ingested and the duration of consumption, their increases are reversible during abstinence, and they are independent of other commonly used chemical markers of alcohol abuse and of concomitant liver disease. Because of the small difference in charge between the normal and abnormal isotransferrins, the latter can so far be studied only by methods based on charge—either isoelectric focusing, chromatofocusing, or anion-exchange chromatography of various designs. In studies with new, quantitative, microchromatographic methods, sensitivities of 81–94% and specificities of 91–100% have been reported for current alcohol intake at definite risk levels (>60 g/day). False-positive values have been noted for certain genetic reasons and in isolated patients with severe hepatic failure. False-negative results have been obtained in connection with abstinence before sampling, consumption rates <60 g of ethanol per day, or alcohol intake for periods of less than one week. Negative results have been found in 6–19% of the patients, for reasons that await explanation.

Determination of CDT in serum thus appears to have the potential of filling a clinical void in the diagnosis of alcohol-related diseases. It is also of scientific interest for further studies of the biological action and effects of alcohol abuse on glycoprotein metabolism in a broader perspective.

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