a personal density scanning imager (PDSI; Molecular Dynamics). Concentrations of LDL-chol and LDL-apo B were measured by a Hitachi 7170 automated analyzer (Hitachi), using Cholestest® LDL, and Apo B reagent kits (Daichi Pure Chemical). These analyses were based on homogeneous enzymatic assay (8) and turbidimetric immunoassay (9) methods, respectively.

The LDL-chol and LDL-apo B concentrations as well as the LDL particle sizes as measured by GGE and LDL-chol/LDL-apo B ratios are summarized as the mean ± SD in Table 1. A significant difference in LDL particle size was demonstrated between hyperlipemic and control samples by both GGE and the LDL-chol/LDL-apo B ratio. When LDL particle sizes measured by GGE were plotted against those determined in the same blood samples as LDL-chol/LDL-apo B ratios (Fig. 1), LDL-chol/LDL-apo B ratios showed a weak negative correlation with LDL particle sizes measured by GGE in control samples (Fig. 1A) and a weak positive correlation in hyperlipemic samples (Fig. 1B). In control samples, LDL-chol/LDL-apo B ratios were 1.14–1.39, and LDL particle size measured by GGE was 21.38–25.41 nm. Among hyperlipemic samples, despite reports that the LDL-chol/LDL-apo B ratio reflects the size of small dense LDL, some samples contained normal-sized LDL particles as determined by GGE but showed low LDL-chol/LDL-apo B ratios. This result reflects the fact that measurement of LDL-chol by the homogeneous enzyme assay is affected by high concentrations of TGs, which leads to underestimation of the LDL-chol concentration (8, 10). LDL size estimated by the LDL-chol/LDL-apo B ratio showed more negative correlation with TG concentrations than that estimated by GGE (r = −0.68 vs. −0.27). TG concentrations in these two samples were particularly high (8260 and 11 430 mg/L; Fig. 1B, □). In contrast, some samples shown to contain small LDL particles by GGE showed high LDL-chol/LDL-apo B ratios. The LDL-chol, LDL-apo B, and TG concentrations (Fig. 1B, △), respectively, were 1550, 1360, and 1810 mg/L in one sample and 1660, 1320, and 1890 mg/L in the other. When the LDL-chol/LDL-apo B ratio is used to evaluate LDL particle size, the size in these samples will be misreported as normal.

The presence of small, dense LDL increases the risk of atherosclerotic cardiovascular disease beyond that associated with normal LDL (11). Given the importance of LDL particle size determination, it should be performed by GGE rather than rapidly estimated by the LDL-chol/LDL-apo B ratio.

References


Does Trisialo-Transferrin Provide Valuable Information for the Laboratory Diagnosis of Chronically Increased Alcohol Consumption by Determination of Carbohydrate-deficient Transferrin? Leif Dibbelt (Institute of Clinical Chemistry, Medical University, D-23538 Luebeck, Germany; fax 49-451-5004849)

Transferrin, the principal iron transporter in human serum, is a glycoprotein bearing asparagine-linked polysaccharides (1). Because of differences in the glycan structure, several transferrin isofoms can be found in normal serum, the most prominent one containing two biantenary complex-type glycans with a total number of four negatively charged terminal sialic acid residues (2, 3). It has been known for >20 years that chronically increased consumption of ethanol affects the glycosylation pattern of serum transferrin, producing a higher proportion of transferrin isofoms that lack terminal sialic acid residues and probably other parts of the glycan structure and thus are referred to as carbohydrate-deficient transferrin (CDT) (3–5). Originally, CDT was defined as the sum of transferrin isofoms containing two or fewer two sialic acid residues (corresponding to pI values 5.7), and this definition has likewise been applied to the evaluation of CDT as a sensitive and specific laboratory marker of alcoholism (4, 5). During the last few years, however, commercial kits were launched that measure an operationally defined CDT fraction that in addition to asialo-, monosialo-, and disialo-transferrin also includes at least in part the trisialo-transferrin isofom (6). The inclusion of trisialo-transferrin in the definition of CDT has been claimed to improve the detection of chronic alcohol consumption (7), but the diagnostic significance of the trisialo-transferrin concentration remains unclear (8). Therefore, I reexamined our data from routine determinations of CDT in human serum in search of a quantitative
relationship between the various isotransferrins resolved by our method.

In our laboratory, determination of absolute and relative CDT concentrations in serum samples is routinely performed by HPLC on Mono Q® anion exchanger (Amersham Pharmacia Biotech), using the method described by Jeppson et al. (9) with minor modifications (10). This method separates at least four isotransferrin fractions in normal human serum exhibiting pI values of 5.7 (disialo-transferrin), 5.6 (trisialo-transferrin), 5.4 (tetrasialo-transferrin), and 5.2 (pentasialo-transferrin). In serum from patients with chronically increased alcohol consumption, the portion of disialo-transferrin usually is increased and another peak at pI 5.9 (asialo-transferrin) appears in the chromatogram that is not detected in serum from healthy abstinent people. CDT is calculated as the sum of asialo and disialo-transferrin (monosialo-transferrin usually is not detected by HPLC because of its low concentration) and is reported in relative concentration units, i.e., as a percentage of total transferrin. Baseline integration is applied to the HPLC chromatograms instead of the valley-to-valley integration described by Jeppson et al. (9).

![Fig. 1. Comparison of relative concentrations of disialo- and trisialo-transferrin in human serum as derived from routine analysis of CDT by HPLC (n = 870).](image)

**Table 1. Correlation coefficients (r) obtained from statistical analysis of relative concentrations of the various isotransferrins separated by HPLC in sera exhibiting normal (top) and increased CDT concentrations attributable to high alcohol intake (bottom).**

<table>
<thead>
<tr>
<th></th>
<th>Disialo-Tf</th>
<th>Trisialo-Tf</th>
<th>Tetrasialo-Tf</th>
<th>≥Pentasialo-Tf</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDT &lt;2.4% (n = 706)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disialo-Tf</td>
<td>0.806</td>
<td>0.194</td>
<td>-0.234</td>
<td>-0.039</td>
</tr>
<tr>
<td>Trisialo-Tf</td>
<td>-0.032</td>
<td>-0.038</td>
<td>-0.320</td>
<td>-0.248</td>
</tr>
<tr>
<td>Tetrasialo-Tf</td>
<td>-0.722</td>
<td>-0.027</td>
<td>-0.354</td>
<td>-0.821</td>
</tr>
<tr>
<td>≥Pentasialo-Tf</td>
<td>-0.180</td>
<td>-0.148</td>
<td>-0.327</td>
<td></td>
</tr>
<tr>
<td><strong>CDT ≥2.4%, heavy drinking (n = 116)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asialo-Tf</td>
<td>0.00–7.58c</td>
<td>2.47–19.7c</td>
<td>1.58–8.88c</td>
<td>55.3–81.1c</td>
</tr>
<tr>
<td></td>
<td>0.00–0.20–2.70d</td>
<td>2.62–4.99–17.1d</td>
<td>2.43–4.56–7.26d</td>
<td>61.7–73.4–78.0d</td>
</tr>
<tr>
<td></td>
<td>0.69 ± 1.13e</td>
<td>6.52 ± 4.07e</td>
<td>4.78 ± 1.42e</td>
<td>72.1 ± 5.28e</td>
</tr>
</tbody>
</table>

a TF, transferrin.
b Pentasialo- and hexasialo-transferrin.
c Range of values, %.
d 5th-50th-95th percentiles, %.
e Mean ± SD, %.
and Renner and Kanitz (10); with this modification our local reference range extends to 2.4% CDT, which is significantly higher than the cutoff values reported in the literature for valley-to-valley integration (8, 9, 12). Statistical analysis of data was performed using SPSS for Windows, release 6.1.

Between September 1, 1997 and August 31, 1998, our laboratory received 887 patient samples for routine analysis of CDT. Requests for these CDT determinations were all made for laboratory confirmation of suspected alcoholism or follow-up of its therapy; they came mainly from the clinics of psychiatry (40%), neurology (18%), internal medicine (18%), and anesthesiology (9%), whereas the remaining 15% stemmed from ~10 different institutions. Results of 17 samples indicated a heterozygous expression of genetic transferrin variants (16 allelic variants of the B type and 1 allelic D variant) that prevented reliable calculation of CDT. When the latter results were excluded, there remained 870 chromatograms that had been routinely evaluated for CDT. In 706 samples, the relative CDT concentration was at or below the upper limit of our reference range, whereas 164 samples exhibited increased CDT concentrations (>2.4%). For at least 116 of the latter samples, the increased CDT concentration could be retrospectively explained by heavy drinking according to information given by the patients or by markedly increased blood ethanol concentrations measured on patients’ admissions.

In addition to the asialo- and disialo-transferrin concentrations routinely used for calculation of the CDT concentration, I also extracted the relative concentrations of triasialo- and tetrasialo-transferrin as well as the total concentration of isotransferrins with higher numbers of terminal sialic acid residues (i.e., pentasialo- and hexasialo-transferrin) from all chromatograms. The relative disialo- and trisialo-transferrin concentrations measured in the complete series of samples are shown in Fig. 1. From this comparison, it clearly can be seen that high disialo-transferrin concentrations are not accompanied in general by high trisialo-transferrin concentrations. When the results were grouped according to either normal (≤2.4%) or increased (>2.4%) CDT concentration attributable to heavy drinking, I obtained the relative concentration ranges; the 5th, 50th, and 95th percentiles; and the means ± SD for the various isotransferrin fractions, as summarized in Table 1. Whereas the disialo-transferrin concentrations obviously differed significantly between the two groups (range, 0.00–2.43% vs 2.47–19.7%), the corresponding trisialo-transferrin concentrations were almost identical (range, 1.16–13.0% vs 1.58–8.88%). Statistical analysis revealed a rather low correlation (r = 0.194) between the concentrations of disialo- and trisialo-transferrin in samples exhibiting normal CDT values, but no correlation (r = –0.038) in samples showing increased CDT concentrations attributable to alcohol intake (Table 1). In the group exhibiting normal CDT values, correlation was highest between tetrasialo- and pentasialo-/hexasialo-transferrin (r = –0.821), indicating that highly sialylated isotransferrins are preferably produced at the expense of the tetrasialo-transferrin fraction. In the group of samples with increased CDT values attributable to heavy drinking, strong correlations were obtained for disialo- and asialo-transferrin (r = 0.806), disialo- and tetrasialo-transferrin (r = –0.845), and asialo- and tetrasialo-transferrin (r = –0.722). These observations agree well with the assumption that it is not the terminal sialylation of otherwise complete glycan moieties that is impaired by chronically increased intake of alcohol but rather the formation of one or both of the entire glycan chains (3, 13).

It is still unknown which physiological or pathological mechanisms regulate the portion of trisialo-transferrin in relation to other isotransferrins. Our data, however, clearly demonstrate that increased relative concentrations of disialo- and asialo-transferrin attributable to increased consumption of alcohol are not associated in general with increased trisialo-transferrin concentrations. Because trisialo-transferrin is obviously of no diagnostic value, I strongly recommend not including this isotransferrin in the CDT fraction measured for laboratory diagnosis of alcoholism.

I am most grateful to R. Albrecht-Groos, K. Blöcker, A. Blunk, A. Böltd, B. Brede, B. Ermert, M. Gierke, B. Gütschow, R. Hesemeyer, H. Hinz, A. Niemeier, and K. Stratmann for expert work in the routine HPLC analysis of CDT.

References