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Changes in Serum Carbohydrate-Deficient Transferrin and Gammaglutamyl Transferase After Moderate Wine Consumption in Healthy Males

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Serum carbohydrate-deficient transferrin (CDT) concentrations and gammaglutamyl transferase (GGT) activities were measured in the fasting serum of healthy male subjects before and after 4 weeks consumption each day of 375 ml wine or 500 ml grape juice. After wine consumption, serum CDT concentrations rose in 38 of 48 individual test procedures, and the mean ± SEM increased from 17.8 ± 0.86 u/l to 20.9 ± 1.14 u/l (t_o = 4.66; P <0.001). Serum GGT activity rose in 35 of these test procedures, and the mean ± SEM increased from 19.6 ± 1.40 u/l to 22.3 ± 1.79 u/l (t_o = 3.58; P <0.001). When wine consumption was followed by 2 weeks of abstinence from alcohol, significant reductions in both CDT and GGT were noted, virtually reaching baseline levels. No significant change in either index occurred after 4 weeks of consuming grape juice. The correlation between CDT and GGT was rather low, suggesting that their responses to alcohol occur by different mechanisms. The results indicate that the response of CDT to alcohol dose is continuous, and that even moderate consumption can cause significant elevations in a healthy population. J. Clin. Lab. Anal. 12:92–97, 1998.

Key words: gammaglutamyl transferase; transferrin (carbohydrate deficient); alcoholic beverages; wine

INTRODUCTION

An abnormal high-isoelectric-point component of serum transferrin, a glycoprotein synthesized in the liver, was first identified in the serum of alcoholic patients by Stibler and colleagues (1). The protein was found to contain less sialic acid, galactose, and N-acetylglucosamine than normal transferrin (2,3) and hence was designated carbohydrate-deficient transferrin (CDT). This abnormal component appears to be due to the toxic effect of alcohol or its metabolites on the hepatic synthesis of transferrin (4,5). It was first proposed as a marker for chronic abusive alcohol consumption in 1979 (6).

Over the next 15 years, many investigations were reported concerning the utility of CDT as a marker of alcohol consumption among hospitalized alcoholics (7–13). Experience with the test was broadly favourable. Sensitivities ~80% at a specificity of 90% were usually achieved. It was shown to be effective in identifying alcohol abusers from three ethnic groups (14). Among females, CDT was initially described as being elevated in most alcohol abusers (15), but subsequent authors have not been able to confirm this finding (13, 16–18). Normal CDT values were found in patients with non-alcoholic liver disease (19), but elevated concentrations occurred in patients with early alcoholic liver disease, and the levels declined in proportion to the morphologic severity of the disease (20). CDT concentrations in alcoholic abusers fell quite rapidly after abstinence (21, 22), and rose once again following relapse (23,24).

Beyond special reference centres, experience with CDT assays in screening for alcohol abuse has been rather disappointing. Increased values of CDT were found in only 26% of alcohol abusers in well-population screening (25), in 22% of male students who were heavy drinkers (26), in 31% of young male soldiers admitted to an alcohol-treatment unit (27), and in 44% of hypertensive patients whose alcohol consumption was excessive (28). A limitation of all the above studies is that alcohol consumption was assessed retrospectively by a questionnaire. There have been relatively few studies in which alcohol was administered to healthy subjects at a fixed dose with the objective of determining its direct effect upon CDT (29). The present investigation was undertaken to address this issue and to compare CDT with the activity of serum gammaglutamyl transferase (GGT), another marker that has been used to identify alcohol abuse in both hospitalized and free-living populations (see Ref. 30 for Review).

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MATERIALS AND METHODS

Subjects

Twenty-six healthy males, ages 25–50 years participated in this study. They were accustomed to drinking moderate amounts of alcohol in a range of 7–21 drinks (12g alcohol/drink) per week. All gave informed consent to participate in this study which was approved by the Human Experimentation Committee of the University of Toronto. Screening by extensive clinical and laboratory procedures was as previously described (31). Apart from the periods of wine consumption, no alcohol was permitted, and all agreed to maintain a constant diet that was monitored each week by a nutritional analysis. They also adhered to a constant exercise schedule and refrained from any form of medication.

Experimental Protocol

After 2 weeks of abstinence, each volunteer consumed one of the following beverages each day for a period of 4 weeks: 500 ml grape juice; 375 ml white wine; 375 ml red wine. The alcohol content of both wines was 12% by volume. No other form of alcohol was permitted during this period. Fasting blood was collected on the day that beverage consumption began and again on the morning immediately after cessation. After clotting, the serum was separated by centrifugation and aliquots were stored at –70°C until analysis, when all of the samples from a given subject were assayed in the same batch. Twenty-four subjects completed the schedule for red wine and 24 for white wine consumption on separate occasions. Twenty completed the grape juice schedule. Sixteen subjects who completed 4 weeks consumption of wine abstained from all alcohol for the next 14 days after which a fasting blood sample was taken to evaluate the effects of alcohol withdrawal. Blood pressure, body weight and exercise remained constant throughout the study periods and the estimated intake of the various food groups varied by <10% during the 4-week periods of beverage consumption.

Assays

Serum CDT was analyzed in duplicate for each sample by a double-antibody radioimmunoassay (CDTect™, Kabi Pharmacia Diagnostics, Uppsala, Sweden). Separation of transferrin isoforms by anion exchange following saturation of the patient's specimen with iron, and measurement of CDT in the eluate by RIA were carried out as described in the manufacturer's assay procedure. Briefly, 50 µl of sample was mixed with 200 µl of ferric citrate solution and 1,000 µl of elution buffer; 500 µl of this mixture was applied in duplicate to equilibrated anion exchange columns, 50 µl of the eluate was taken together with 50 µl of ^{125}I-labelled transferrin, 50 µl of rabbit antihuman transferrin, and 2 ml of solid-phase secondary antibody immunoabsorbant suspension and incubated at room temperature for 60 min. The tubes were centrifuged at 1,500×g for 10 min, the supernatant was decanted and the radioactivity present in the pellet was measured by a γ-counter. A calibration curve was generated using supplied CDTect™ standards by a logit-log program and results were recorded as u/l. The assay analytical precision was within the manufacturer's specifications of 7.4–9.1% and 4.6–9.6% for within and between assay CV respectively.

GGT activity was measured at 30°C using an Unimate 3 GGT plus kit (Lot #NO431; Hoffman-La Roche Limited, Mississauga, Ont.) automated on the COBAS MIRA™S. The within assay CV% was <5% at 88 u/l GGT and <10% at 13 u/l GGT.

Statistics

Data for GGT and CDT before beverage consumption were compared with the corresponding results in the same subject after consumption (or the latter with the results after 2 weeks of abstinence) using student's paired t-test. Results are presented as mean ± SEM. Correlation and regression analyses between GGT and CDT were also performed under conditions to be described (Table 1). All calculations were carried out with the SAS Statistical Software Package (SAS Inst., Cary, NC, USA).

RESULTS

Effect of Grape Juice

Twenty subjects completed this protocol. The mean GGT activity declined over this 4-week period of abstinence from 18.1 ± 0.84 u/l to 17.7 ± 0.95 u/l, decreasing in 12 subjects, increasing in seven, and remaining unchanged in one. In all subjects, GGT activity was <38 u/l (the manufacturer's upper reference interval for males at 30°C) at both testing periods.

The mean CDT concentration fell from 17.2 ± 1.32 u/l to 15.8 ± 0.93 u/l during the 4 weeks of grape juice consumption. The value declined in 13 subjects and rose in seven. The manufacturer's upper reference interval of 20 u/l was exceeded in three subjects before (15%) and in one subject (5%) after grape juice consumption. Taking both GGT and CDT together, in only two subjects (10%) were both increased, and in seven (35%) both were decreased. However, none of the changes in both indices described above were statistically significant.

TABLE 1. Values for Correlation Coefficient (r), Regression Coefficient (R), and Intercept of Regression Equation (C) Defining Statistical Relationship Between GGT and CDT as Determined by Least-squares Fit of Data for Both Assays Under Different Beverage Regimens

	A. Grape juice	B. Wine consumption	C. Wine withdrawal	D. B plus C
r	–0.137	0.252	0.198	0.280
R[a]	–0.176 ± 0.033	0.153 ± 0.006	0.139 ± 0.068	0.142 ± 0.075
C[a]	17.8 ± 0.44	15.8 ± 0.10	14.9 ± 1.48	16.2 ± 1.77

[a]Data include SE for R and C.

Effect of Wine Consumption

Twenty-four subjects completed the protocols for red and white wine. This provided 48 sets of data allowing us to compare GGT and CDT values before and after 4 weeks consumption of wine. Analysis of the data for red wine and white wine separately revealed no significant difference between the two beverage groups with respect to the baseline and post-beverage values. The results were therefore pooled for analysis.

The mean serum GGT activity increased from 19.6 ± 1.40 u/l to 22.3 ± 1.79 u/l (t_o = 3.58; P <0.001). In 35 instances the value was increased, in 11 decreased, and in two the activity remained unchanged (Fig. 1A). The reference interval of 38 u/l was exceeded by one subject (2.1%) prior to and in five (10.4%) subsequent to the period of wine consumption.

For CDT, the mean concentration increased from 17.8 ± 0.86 u/l to 20.9 ± 1.14 u/l (t_o = 4.66; P <0.001). In 38 instances, the value was increased and in 10 it was decreased (Fig. 1B). The initial concentration exceeded the reference interval of 20 u/l in 12 instances (25%) and the postbeverage value was >20 u/l in 16 instances (33%).

Interestingly, both GGT and CDT increased after wine consumption on 27 (56%) of occasions, CDT alone on 11 (22.9%) occasions, and GGT alone on 8 (16.7%).

Effect of Wine Withdrawal

For 16 of the subjects, data were available to compare the GGT and CDT values on termination of the 4 weeks of wine consumption with those assayed 2 weeks later, duringwhich time they abstained from all forms of alcohol. The mean serum GGT activity in these subjects fell from 17.8 ± 2.01 u/l to 14.5 ± 1.09 u/l (t_o = 2.72; P <0.02). The value decreased in 11, increased in four, and remained unchanged in one subject (Fig. 2A). None of these values exceeded the upper reference interval for this assay.

With serum CDT concentration, the mean value decreased from 18.6 ± 1.24 u/l to 16.4 ± 0.88 u/l (t_o = 3.71; P <0.005), falling in 12, rising in two, and remaining unchanged in a further two subjects (Fig. 2B). The upper reference interval was exceeded in five subjects (31%) prior to wine withdrawal and in only one subject (6%) after 2 weeks of abstinence.

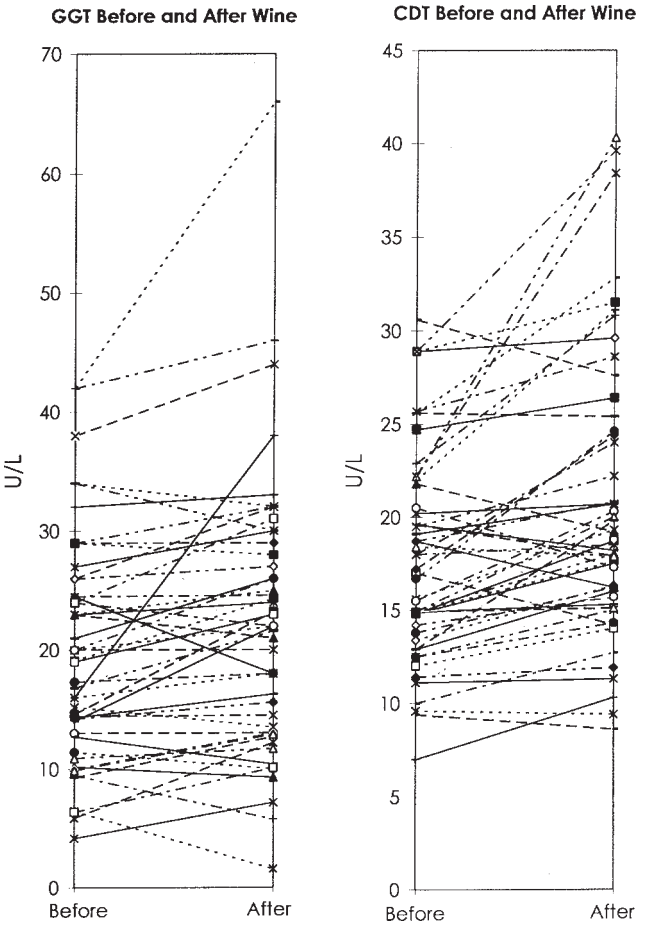

Fig. 1. Values for serum CDT (A, *left*) and GGT (B, *right*) in fasting blood of 24 subjects before and 4 weeks after two periods of wine consumption (375 ml/day). Data as u/l for both analytes. Each line represents a single subject.

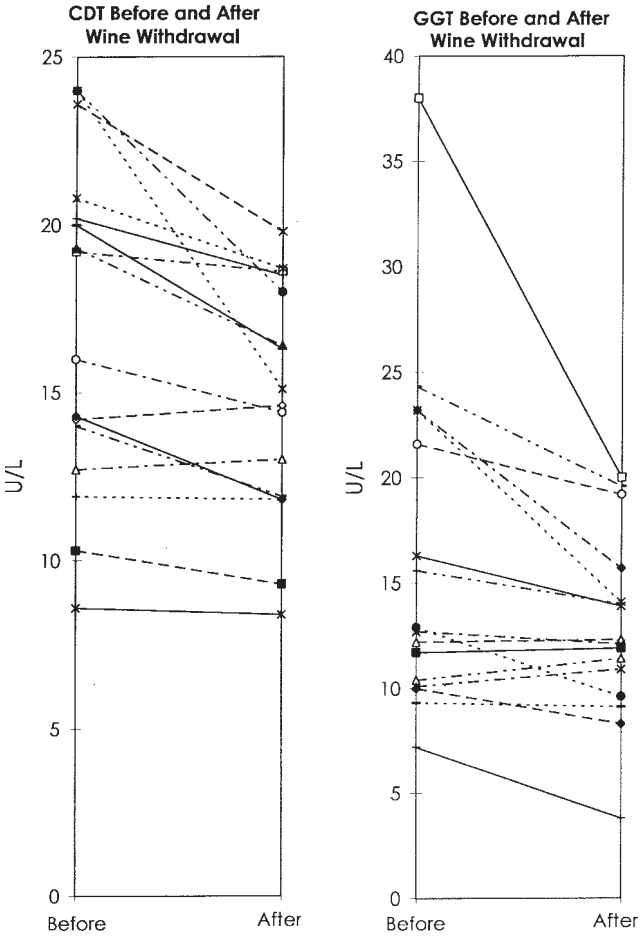

Fig. 2. Values for serum CDT (A, *left*) and GGT (B, *right*) in fasting blood of 16 subjects at the end of 4 weeks of wine consumption (initial value) and again after two weeks of abstinence (final value). Data as u/l for both analytes. Each line represents a single subject.

Relationship of GGT to CDT

The linear regression equation and the value of the correlation coefficient (r) relating GGT to CDT were derived for the following data sets: A, before and after grape juice consumption; B, before and after wine consumption; C, before and after wine withdrawal, and D, pooled data of B and C. The results are presented in Table 1. For A, there was a weakly and nonsignificant negative correlation (r = –0.176), and a negative regression coefficient suggesting that under basal conditions GGT and CDT are not closely associated. For B, the correlation and regression coefficients were both positive (r = 0.189; $P < 0.01$), but the association was rather weak in accordance with the notion that although they are both increased in response to alcohol consumption, the mechanisms responsible for these increases are different. This is supported by the results of C, where once again a weak correlation was observed (r = 0.198; $P < 0.05$). Finally, in D, the correlation and regressioncoefficients were somewhat more robust (r = 0.240; $P < 0.005$) but not particularly impressive, confirming the idea of a rather indirect relationship between GGT and CDT.

DISCUSSION

In their perceptive review, Allen and colleagues (32) highlighted some of the issues that remained to be resolved before CDT could be accepted as a routine procedure to diagnose alcohol abuse. They emphasized the unreliability of retrospective self-reporting of alcohol intake; the lack of information about the interval of time between the last drinking episode and venepuncture; the comparison of extreme groups only (abstinent and abusive), with inadequate knowledge of how serum CDT levels respond across the full range of drinking behaviors; paucity of controlled prospective studies in which the effects of a defined level of alcohol consumption could be ascertained; and the need to establish more precisely the period of abstinence necessary for CDT levels to decline.

Our data indicate that plasma CDT concentrations rise significantly in a population of normal males consuming a moderate fixed amount of alcohol over a 4-week period. Similar changes were noted for another biochemical index of alcohol abuse, serum GGT activity, although the correlation between the two indices was not strong and was consistent with the elevations being the result of different biochemical mechanisms. No significant change was observed during a 4-week period of abstinence accompanied by consumption of grape juice.

The response to alcohol on an individual basis was quite variable, with increases in CDT occurring in 79% and of GGT in 73% of instances. Most subjects underwent two periods of exposure to alcohol, once with red wine and once with white wine. For both CDT and GGT, the percentage of subjects manifesting the same response (increase or decrease) on both occasions approximated 65%, and in 35% an increase on the first occasion was followed by a decrease on the second, or *vice versa*. Whereas the effect of moderate alcohol consumption upon the population as a whole could be readily discerned, it was not possible to identify with certainty those subjects in whom the increase in CDT concentration was due to alcohol and exceeded the limits of biological variation for that subject. As an approach to overcoming this limitation, we calculated that the percentage change (increase or decrease) during grape juice consumption (mean $\pm$ SD) was 15.9 $\pm$ 6.4%. An increase >29% would therefore be due to chance on <2.5% occasions. By this criterion, an increase in plasma CDT likely to be due to alcohol occurred on 14 of the 48 occasions (29%).

We did not find the manufacturer's recommended upper reference value of 20 u/l for CDT to be appropriate for our subjects, since it was exceeded on 18% of the blood samples drawn before alcohol or before and after grape juice consumption. It is not clear whether this represents a population-dependent difference, since each laboratory is encouraged by the manufacturers to establish their own reference values. Alternately, an anomaly in the standard used for calibration could account for our findings. It should be emphasized that all samples from a given subject were analyzed in the same batch; the paired t-test in which the subject's own baseline sample is used for statistical evaluation would obviate any systematic bias introduced by the calibration process. As an alternative approach, we calculated a mean (SD) of 16.4 $\pm$ 3.6 u/l when all of the CDT values other than those taken after 4 weeks of alcohol consumption were pooled. Using an upper reference limit of 24 u/l (mean $\pm$ 2 SD), 12% of the postalcohol values were "abnormal."

Previous authors have examined the effects of defined alcohol intake upon small groups of healthy subjects with variable findings. Stibler and colleagues (6) reported elevatedCDT in three of eight subjects 11 days after consuming 0.6 g alcohol/kg body weight, but subsequently failed to find a significant increase in CDT in subjects consuming 0.3–0.9g of ethanol/kg body weight for 10 days (3). Increased CDT levels were noted in three healthy subjects consuming alcohol from 20–80g/day over 3 weeks (33). More recently, a significant increase in CDT was observed in 10 healthy males 3 weeks after consuming 60g alcohol per day, although the upper reference value was exceeded in only two (29).

In our investigation, subjects consumed 35 g alcohol per day, averaging 0.48 g/kg (range 0.42–0.61). This range was unavoidable given our desire to keep the protocol simple to ensure compliance; the instruction to consume one half bottle of wine per day is easily understood. Although this decision must inevitably have contributed to the variance of our data, there was no evidence for a significant inverse relationship between CDT response to wine and body weight in our subjects. Our results comprise the largest body of data on normal subjects consuming moderate amounts of alcohol. They clearly demonstrate a significant increase in plasma CDT concentrations under these circumstances

and strongly support the notion that the response of CDT to alcohol dose is continuous. There is unlikely to be a threshold below which the effects of alcohol are not observed. This implies that any decision level used to segregate alcohol abusers from social drinkers needs to be based upon a population that includes those whose consumption is just below the proposed cut-off level.

The reduction in CDT (and in GGT) 2 weeks after alcohol consumption ceased to levels that were not significantly different from baseline values suggests a more rapid clearance than has been observed by previous investigators (see Ref. 32 for review). However, these data were generally obtained in alcohol abusers (with or without associated hepatic damage). It is conceivable that in such persons the mechanisms responsible for CDT clearance are impaired and indeed such impairment may contribute to their elevated plasma CDT levels.

Finally, the availability of simpler assays for CDT and their inter-laboratory commutability deserve mention. Recently, a nephelometric method (34) and a dry blood spot method (35) have been described. These new techniques should enlarge the scale of application of CDT assays in the evaluation of alcohol consumption. Since most of the previous reports describing comparisons between different methods for CDT determination have noted good agreement (36, 37), it is likely that the present findings will prove to be reproducible by these newer simpler procedures.

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