Technicon RA-1000 random-access analyser, with a commercial enzymatic reagent ("Monotest Cholesterol-High performance, CHOD-PAP method"; Boehringer, Mannheim, F.R.G.). The method was standardized with "Precision Cholesterol" (Boehringer Mannheim, cat. no. 125504) containing 1.29 mmol of analyte per liter. The standard solution was diluted 11-fold with PEG-6000, 100 g/L. Instrument settings were as follows: Type 2; % Smp vol 60; Filter pos 4 w 500; Delay 9; % Rgt vol 70. The standard curve was linear from 3.5 to 0.14 mmol/L. Within-assay precision (CV, %) was 0.8 for total HDL Chol and 2.2 for cholesterol in the supernates.

The optimal concentrations of dextran sulfate and MgCl₂ were deduced from a "titration curve" that demonstrated in the middle-age normal population the following average values for cholesterol in the dextran sulfate/MgCl₂ precipitable fraction: 25.7 (SD = 3.7) % of total HDL Chol in males, 28.5 (SD = 3.4) % in females, and 26.8 (SD = 3.5) % in the whole group. Figure 1 shows the distribution of this fraction as the percentage of total HDL Chol in 263 male and 244 female subjects.

This different content of HDL subclasses demonstrated, in both sexes, biochemical patterns that are not age-dependent and not always well correlated with HDL Chol total concentrations (γ = -20.7 + 0.44x; r = 0.77). Therefore, we believe that the variations in HDL subclasses can assume significance in themselves, from both an epidemiological and a clinical point of view. We probably need to understand the causes of these changes and to examine closely the chemical-pathological correlations. For this purpose the percentage distribution of the HDL-precipitable fraction in HDL seems to us to be more important than information on its concentration alone (11), a view supported by our study.

References


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Decreased Apolipoprotein A-I and B Content in Plasma of Individuals with Sickie Cell Anemia

To the Editor:

Individuals with sickle cell anemia have decreased concentrations of cholesterol and phospholipid in their plasma (1, 2). Analysis of lipid content of specific plasma lipoproteins isolated by ultracentrifugation reveals a low value for both the low-density (LDL) and high-density (HDL) lipoprotein fractions. However, the concentration in plasma of apolipoproteins that are characteristic of these two lipoproteins is unknown. Therefore, we quantified apo B, the primary protein component of LDL, and apo A-I, one of the major proteins of HDL, in children with sickle cell anemia.

Our patient population consisted of 41 children (19 male, 22 female) with sickle cell anemia, as diagnosed by standard hematological studies and electrophoresis. The patients' ages ranged from one to 18 years; the mean age was 10. Control samples of plasma were obtained from 20 sex- and age-matched black children homozygous for hemoglobin A (10 male, 10 female). Blood samples were collected, after an overnight fast, into tubes containing EDTA as an anticoagulant. The p-values were calculated from the two-sample Student's t-test for two-tailed hypothesis. Plasma apo A-I concentrations were quantified by single radial immunodiffusion (3). The concentration of apo B in plasma was quantified by electroimmunoassay (4).

The mean concentration of apo A-I in plasma for children with sickle cell anemia was 110 (SD 23) mg/dL, significantly lower than the mean value of 130 (SD 16) mg/dL observed for controls of the same age group. The concentration of apo A-I in plasma from adult controls was 133 (SD 22) mg/dL. The mean concentration of apo B in the plasma of individuals with sickle cell anemia was also low, 78 (SD 15) mg/dL, compared with the mean for controls, 96 (SD 13) mg/dL. The apo B concentration in plasma of adult controls was 96 (SD 28) mg/dL.

Evidently, children with sickle cell anemia have a significantly decreased concentration of apo A-I and apo B in their plasma, which agrees with previously observed low concentrations of HDL and LDL lipid. The low apolipoprotein concentrations could result from a decreased rate of synthesis, an increased rate of catabolism of lipoprotein particles, or both.

Individuals with sickle cell anemia have a shortened erythrocyte lifetime (5). Erythrocyte lipids are in equilibrium with plasma lipids, so the rapid turnover of erythrocytes in children with sickle cell anemia must also be reflected in increased turnover of plasma lipids. This may be the major contributor to the decreased steady-state concentration of lipids and lipoprotein particles observed in the plasma of these individuals.

Apo A-I and B currently are believed to be synthesized mainly in the liver and the intestine. Individuals having sickle cell anemia commonly have hepatomegaly and jaundice as a result of increased hemolysis, or side effects of transfusion therapy such as hemolytic anemia, viral hepatitis, or cirrhosis (5). In most such individuals there is only a small decrease in hepatic function, presumably resulting in a slight decrease in apolipoprotein synthesis and contributing in a relatively minor way to the lower plasma apolipoprotein concentration.

References

Creatine Kinase MB Isoenzyme in Serum of Uremic Patients: Electrophoresis and Quantification with the Corning Models 720 and 760 Fluorometer/Densitometers

To the Editor:

In previous studies with electrophoresis (1, 2), investigators have found increased creatine kinase (EC 2.7.3.2; CK) MB isoenzyme activity in serum of uremic patients who are undergoing maintenance dialysis and who had no evidence of acute myocardial infarction at the time their serum was studied. These authors imply that increased proportions of CK-MB isoenzyme may not indicate cardiac disease, thereby decreasing the reliability of this laboratory test in this group of patients.

At our hospital, most uremic patients maintained by dialysis have diabetic nephropathy; many have co-existent coronary atherosclerosis, and not uncommonly they complain of atypical chest pain. Moreover, they frequently have markedly abnormal, baseline electrocardiographic patterns, which makes new ischemic changes difficult to detect. In such patients, CK-MB results assume increased importance, and the decreased reliability of electrophoresis results suggested by previous studies (1, 2) would, if true, cause problems.

In an effort to clarify this issue, we quantified CK-MB isoenzyme activity by electrophoresis of serum from uremic patients without evidence of acute myocardial infarction. Our goals were to (a) determine the prevalence of CK-MB in serum of patients with renal failure and (b) compare the amount of CK-MB as quantified by two fluorometer/densitometers—the Model 720 (Corning Medical, Medfield, MA), which we have used in this laboratory for the past five years, and the Corning Model 760, which we have recently acquired.

We studied 81 uremic patients on maintenance dialysis treatment, 35 of them being treated by hemodialysis, and 46 by peritoneal dialysis. The median duration of dialysis was 17.5 months (range, two to 228 months). The mean age of these patients (51 men and 30 women) was 53.1 years (range, 24–94 years). The most common cause of renal insufficiency in 58 patients (76%) was diabetes mellitus. The median value for serum urea nitrogen was 78.5 μg/L (range, 26–202 μg/L); for serum creatinine, 10.6 μg/L (range 3.1–22.2 μg/L). No patient had clinical or electrocardiographic evidence of acute myocardial infarction at the time we sampled their serum.

For controls we studied 20 outpatients (nine men, 11 women) without renal failure or known cardiac diseases. The mean age of the control patients was 50.3 years (range, 29–79 years). All had normal concentrations of urea nitrogen (reference interval, 5–24 μg/L) and serum creatinine (reference interval, up to 1.1 μg/L) at the time of study.

We determined total serum CK activity and CK-MB activity by the modified Rosalki method (3). To determine total CK, we used a Multistat III microcentrifugal analyzer (Instrumentation Laboratory, Lexington, MA). As we described elsewhere (4), we quantified CK-MB isoenzyme activity by electrophoresis on agarose gel, using a system and reagents supplied by Corning, and using both models of fluorometer/densitometers for the same serum samples. The Model 720 instrument is completely automatic. We used the Model 760 instrument in its manual mode, adjusting the baseline accordingly to the background fluorescence of each individual patient’s serum. To avoid quantifying non-CK fluorescent artifacts, discovered by viewing the gels before incubation with the CK-isoenzyme substrate set (Corning), we used the “suppess” option of the Model 760 instrument.

Although the median total CK activity of each group was virtually identical, the prevalence of CK-MB isoenzyme activity in serum was increased in the uremic patients as compared with the control group (Table 1). Moreover, the quantity of CK-MB measured in serum depended on which fluorometer/densitometer we used. If <5 U of CK-MB per liter of serum is considered to be within normal limits (5), then, according to results with the Model 720 instrument, half of the uremic patients had abnormally increased CK-MB activity in serum, triple the number indicated by the Model 760. There was no significant difference between the fluorometer/densitometers in values for the control group.

The difference in the amount of CK-MB activity quantified can be explained by the modes of operation of each instrument. The Model 720 instrument has both an automatic gain and an automatic zero: the maximum signal is amplified to produce full excursion of the pen and the minimum signal is considered zero. In serum with an intense signal (i.e., high total CK), all other fluorescence (including background fluorescence) is relatively minimized; when the signal is weak, both the signal and the background fluorescence are amplified. Because the sera from most of the uremic patients in this study had a low total CK (median, 64 U/L; reference interval; up to 155 U/L), the amplification of signal led to a baseline. The Model 720 instrument, by including the baseline elevation in its automatic integration of the CK-MB peak, falsely quantified an increase in CK-MB. The great advantage of the Model 760 instrument is that the baseline can be adjusted manually: the tracing and its automatic integration can be modified by the user according to the background fluorescence of an individual patient’s serum, which is prominent in uremic patients. Results for CK-MB obtained with the Model 760 instrument can be approximated from the Model 720 results by manually drawing the correct baseline on the tracing, cutting out (with scissors) the CK isoenzyme peaks, and weighing the corresponding pieces of paper.

Because the difference in isoenzyme as quantified by the two instruments is medically important, knowledge of which fluorometer/densitometer is being used by the laboratory is essential to correct clinical interpretation of CK-MB isoenzyme results determined by electrophoresis. We do not mean to imply that the diagnosis of acute myo-

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