J. Clin. Chem. Clin. Biochem.
Vol. 17, 1979, pp. 773–775

Cholesterol in High-Density Lipoproteins: A Comparison Between Dextran Sulfate-Magnesium Chloride Precipitation and Preparative Ultracentrifugation

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(Received March 17/August 3, 1979)

Summary: The cholesterol in high density lipoproteins (HDL) has been determined in 140 serum samples after dextran sulfate-magnesium chloride precipitation of the apolipoprotein B-containing lipoproteins. The values correlated well with simultaneously measured values from preparative ultracentrifugation (slope 0.98, r = 0.93). Furthermore, there was a significant correlation in 60 specimen between the dextran sulfate-$MgCl_2$ and heparin-$MnCl_2$-precipitation method.

Dextran sulfate-$MgCl_2$ precipitation allows a precise and reproducible HDL-cholesterol determination (CV from 20 days 2.7 %).

Cholesterin in Lipoproteinen hoher Dichte: Ein Vergleich zwischen Dextransulfat-Magnesiumchloridpräzipitation und präparativer Ultrazentrifugation

Zusammenfassung: Das Cholesterin in Lipoproteinen hoher Dichte (HDL) wurde in 140 Proben nach Dextransulfat-Magnesiumchlorid-Fällung der Apolipoprotein B-haltigen Lipoproteine bestimmt. Die gemessenen Werte korrelierten gut mit den gleichzeitig bestimmten Werten von der präparativen Ultrazentrifuge (Anstieg der Korrelationsgerade 0,98, r = 0,93). Es bestand ebenfalls eine signifikante Korrelation in 60 Proben zwischen der Dextransulfat-$MgCl_2$- und Heparin-$MnCl_2$-Methode (1,03, r = 0,95).

Mit Dextransulfat-$MgCl_2$ ist eine präzise und reproduzierbare HDL-Cholesterinbestimmung möglich (VK von 20 Tagen 2,7 %).

Introduction

HDL[1]) cholesterol concentration is inversely associated with the incidence of atherosclerotic coronary heart disease (1–4). Common methods for measuring HDL cholesterol are based on polyanion precipitation of the lipoproteins that contain apolipoprotein B. Most of the investigations were performed with heparin-manganese chloride, and the resulting values showed a good correlation with ultracentrifugal HDL cholesterol values (5,6,7). Other methods, using dextran sulfate, or sodium phosphotungstate and magnesium chloride, may be simpler (8,9). Furthermore, the use of $MnCl_2$ has been criticized because it results in the production of complexes (10).

To our knowledge there are two reports concerning the high precision and specificity of the dextran sulfate method (8,9). The correlation with HDL cholesterol values after preparative ultracentrifugation has not been reported. We therefore determined HDL cholesterol

[1] VLDL =very low density lipoproteins, LDL = low density lipoproteins, HDL = high density lipoproteins.

0340-076X/79/0017-0773$2.00

Tab. 1. Linear regression analysis: HDL cholesterol, ultracentrifugation versus dextran-sulfate-$MgCl_2$ precipitation and dextran sulfate-$MgCl_2$ precipitation versus heparin-$MnCl_2$ precipitation. X $p < 0.001$.

	n	Cholesterol (mmol/l) $\bar{x} \pm s_{\bar{x}}$	Linear regression analysis Slope	y-intercept	r ± SD
HDL-cholesterol	140		0.98	2.86	0.93 ± 0.03[X]
Ultracentrifugation		1.19 ± 0.03			
Dextran sulfate-$MgCl_2$ precipitation		1.14 ± 0.03			
HDL-cholesterol	60		1.03	–0.389	0.95 ± 0.05[X]
Heparin-$MnCl_2$ precipitation		1.19 ± 0.05			
Dextran sulfate-$MgCl_2$ precipitation		1.16 ± 0.05			

simultaneously with these two methods, and compared the results with those obtained with the heparin-$MnCl_2$ precipitation method.

Material and Methods

Serum samples were obtained from 140 adults (mean age ± SEM 49 ± 3 years) after an overnight fast. Most of the subjects (n=112) were hyperlipoproteinemic outpatients. Values for serum total cholesterol ranged from 3.04 to 20.54 mmol/l and for serum triglycerides from 0.94 to 71.78 mmol/l. The fractionation of serum lipoproteins by preparative ultracentrifugation at densities $d < 1.006$ g/ml (VLDL separation) and $d < 1.063$ g/ml (LDL separation, HDL determination in the bottom fraction) was performed for all samples as described earlier (11,12). The recovery of lipoprotein lipids in VLDL, LDL and HDL was always within 100 ± 10 % of total serum cholesterol.

For the separation of HDL by dextran sulfate-$MgCl_2$ precipitation the method of *Kostner* (8) was used: 1 ml of serum was mixed with 50 µl of $MgCl_2$ solution (2 mol/l) and 50 µl of a dextran sulfate 500 solution (20 g/l, Pharmacia, Uppsala, Sweden), allowed to stand for 5 minutes, and centrifuged (5000 *g*, 30 min). Cholesterol was determined in the supernate. A correction factor of 1.1 was used to correct the dilution introduced by the precipitating solutions. Lipemic sera with serum triglycerides > 20 mmol/l were diluted twofold, and those with serum triglycerides > 40 mmol/l were diluted fourfold. By immunochemical techniques we ascertained that lipoproteins that contain apolipoprotein B were precipitated quantitatively up to a serum triglyceride concentration of 80 mmol/l.

A second precipitating method with heparin-$MnCl_2$ for the HDL separation was simultaneously used for 60 specimens. It was performed as described in the Lipid Research Clinic Program (13).

Cholesterol was measured enzymatically (14). The intra-assay precision of the cholesterol determination was examined with various volumes of the control serum Precilip (Boehringer, Mannheim, West-Germany). The intra-assay and inter-assay precision of the precipitation method was calculated from 20 determinations of a serum pool. The data are mean values ± SEM. After linear regression analysis with calculation of the slope and the y-intercept the significance of the correlation coefficients (r ± SD) was calculated by the t-test.

Results

As shown in table 1, there was a significant correlation between the HDL cholesterol values obtained by preparative ultracentrifugation and by dextran sulfate-$MgCl_2$ precipitation. The slope of the linear regression analysis was 0.98. The comparison between the dextran-$MgCl_2$ and, furthermore, the heparin-$MnCl_2$ precipitation in 60 specimens showed a significant correlation, with a slope of 1.03.

The intra-assay coefficient of variation (CV) for the dextran-$MgCl_2$ method was 2.3 %. The inter-assay CV of 20 separate days was 2.7 %.

Discussion

The dextran sulfate-$MgCl_2$ technique with subsequent enzymatic cholesterol assay is highly reproducible; it allows a satisfactory quantification of HDL cholesterol and compares well with preparative ultracentrifugation. In agreement with *Kostner* (8) we could demonstrate no apolipoprotein B immunoreactivity in the supernatant; this shows that apolipoprotein B-containing lipoproteins are completely precipitated. Lipemic sera must be diluted as proposed for other precipitation methods (5,7). For comparable cholesterol concentrations *Finley* et al. (9) found a slightly better CV (1.7 %, n = 30) for intra-assay precision.

There was a good correlation between dextran sulfate-$MgCl_2$ and heparin-$MnCl_2$ precipitation for HDL separation. Both methods give comparable results. The heparin-$MnCl_2$ method must be performed at 4 °C, while the dextran sulfate-$MgCl_2$ precipitation can be applied at room temperatures.

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