High-Density Lipoprotein Cholesterol and Phospholipids, and Apoprotein A in Serum of Patients with Liver Disease

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Concentrations of apoprotein A in whole serum, and cholesterol and phospholipids concentrations in the high-density lipoprotein fraction of serum were measured after the precipitation of low-density and very-low-density lipoproteins with sodium phosphotungstate-Mg2+ in 23 patients with liver cirrhosis, 19 patients with extrahepatic biliary obstruction, and 20 healthy control subjects. Patients with cirrhosis and cholestasis showed approximately one-half as much cholesterol and apoprotein A in the nonprecipitable high-density lipoprotein fraction as normal subjects did. High-density lipoprotein phospholipids concentrations in those patients were normal or slightly increased, however, which is about double what one would expect from the apoprotein A and cholesterol content.

Additional Keyphrases: cirrhosis · cholestasis · enzymic methods

Since the last century it has been well known that liver diseases are associated with serum lipid disturbances. In recent years there has been a considerable increase in knowledge concerning the metabolism of lipoproteins in hepatic disease. Attention has mainly been focused on the changes in the high-density lipoproteins (HDL), and on the role played by the enzymes lecithin:cholesterol acyltransferase (LCAT; EC 2.3.1.43) and lipoprotein lipase (EC 3.1.1.34) in the transformation of the subfraction HDL1 to HDL2 and HDL3 (1-5). Determination of HDL-cholesterol and apoprotein A (apo A) concentrations in healthy subjects and in patients suffering from atheromatosis has been the topic of many studies. More recently, a method for quantification of phospholipids in the HDL after precipitation of low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) in healthy subjects has been described (6-8), and it has been suggested that measurement of HDL phospholipids might be of interest in patients with vascular risk in the same way as studies of HDL-cholesterol.

We report our studies of concentrations of apo A, cholesterol, and phospholipids in whole serum, and of cholesterol and phospholipids in HDL after precipitation of LDL and VLDL with sodium phosphotungstate-Mg2+, in patients with liver cirrhosis and cholestasis.

Materials and Methods

Subjects. The patients were 42 men with liver diseases, 19 at ages 54-67 years (mean ± SD, 60 ± 5) with extrahepatic biliary obstruction, and 23 at ages 40-67 years (56 ± 7) with liver cirrhosis. Diagnosis was based on clinical data, laboratory tests, endoscopic retrograde cholangiography, laparotomy findings, and percutaneous liver biopsies. Controls were 20 healthy men matched for age with the patients. Blood samples, taken the morning after an overnight fast, were kept cool on ice; serum was separated at 4 °C. Sera in which LCAT activity was to be measured were immediately frozen and kept at −16 °C. Aliquots of the same sera were stored at 4 °C for no more than five days.

Procedures. Serum cholesterol and serum phospholipids were determined by enzymic methods with reagents supplied in kit form by Carlo-Érba, Milan, Italy, and Wako Pure Chemical Ltd., Osaka, Japan, respectively. HDL-cholesterol and HDL phospholipids were measured by the same methods after precipitation of LDL and VLDL by sodium phosphotungstate-Mg2+ (9). Serum apo A was estimated by single radial immunodiffusion (M-Partigen Apo A; Behringwerke AG, Marburg, F.R.G.). Apo A and apoprotein B (apo B) (M-Partigen Apo B) were estimated in the supernate after LDL and VLDL were precipitated as described above. Serum LCAT activity was estimated after delipoproteinization by the simplified method described by Alcindor et al. (10), with
use of [7(N)-3H]cholesterol, 6–7 kCi/mol (Radiochemical Centre, Amersham, U.K.). Lipids were extracted from whole serum from 18 patients and nine controls and from the respective supernatant fraction by the method described by Folch et al. (11). Phospholipids were fractionated chromatographically on Silicagel F 254 plates (Merck, Darmstadt, F.R.G.). The solvent was chloroform/methanol/water (65/25/4 by vol). After staining the plates by spraying with ethanolic phosphomolybdic acid (100 g/L), we measured the intensity of the stained spots with a Vernon densitometer.

Student’s t-test was used in the statistical analysis of the results.

Results

Results of determinations of serum apo A, cholesterol, and phospholipids, and HDL-cholesterol and HDL-phospholipids are shown in Table 1. The correlation between concentrations of HDL-cholesterol (x) and HDL-phospholipid (y) was positive in control subjects (y = 182.4 ± 0.50x, r = 0.62, p < 0.005) and in patients with cholestasis (y = 170.8 ± 1.04x, r = 0.47, p < 0.005); there was no significant correlation in patients with liver cirrhosis (y = 278.7 ± 0.73x, r = 0.38). Apo A was not detected in the supernatant fraction in patients or in controls. The concentration of apo A in the supernatant fraction from controls and patients was >90% of that detected in whole serum. A good positive correlation between HDL-cholesterol (x) and apo A (y) concentrations was observed in patients with cirrhosis (y = 594.6 ± 0.21x, r = 0.95, p < 0.001), and to a lesser degree in patients with cholestasis (y = 288.9 ± 2.41x, r = 0.57, p < 0.005). Serum LCAT activity (mean ± SD) was 43.3 ± 17.6, 20.7 ± 12.1, and 16.9 ± 13.6 mmol/L per hour, respectively, in control subjects, patients with cholestasis, and patients with cirrhosis; the difference between both groups of patients and the controls were significant (p < 0.001). Lecithin/lysolecithin ratios obtained by chromatographic fractionation of phospholipids in whole serum and in the supernatant fraction after precipitation are shown in Table 2.

Discussion

The HDL-cholesterol concentrations (after precipitation of LDL and VLDL) in the serum of patients with liver disease were much less than we observed in patients with atheromatosis (12–14). The clinical implications of this for vascular risk are not yet known. Concentrations of apo A in serum are decreased in acute viral hepatitis, chronic liver disease (15), and cholestasis (16); our results in patients with cirrhosis and extrahepatic biliary obstruction agree with these previous studies. The positive correlation of decreased concentrations of apo A and HDL-cholesterol, the absence of apo B in the supernatant fractions, and the similarity of apo A concentrations in the supernates and in the whole serum specimens strongly suggests that the HDL fraction obtained after precipitation with sodium phosphotungstate-MgCl₂ in patients with liver disease contains proportions of apo A and cholesterol similar to those found in healthy subjects.

In the present study we confirm that in healthy subjects the correlation is good between cholesterol and phospholipids in the HDL fraction after precipitation of lipoproteins rich in apo B, as recently described by others (6–8). In addition, we found that HDL-phospholipids were not significantly decreased in patients with cholestasis and were slightly increased in patients with liver cirrhosis, showing that the phospholipid content of nonprecipitable HDL in patients with liver disease is approximately double what should be expected according its apo A and cholesterol content.

The finding of low serum LCAT activity in patients with liver disease is in agreement with previous studies (17–21), and probably accounts for the increased lecithin/lysolecithin ratio in our patients (20). Apparently, the difference in lecithin/lysolecithin ratio between subject groups is similar, whether based on examination of whole sera or nonprecipitable HDL. Furthermore, the lecithin/lysolecithin ratio in nonprecipitable HDL is less than in whole serum. This suggests that changes in LCAT activity may be more apparent in the HDL fraction than in whole serum.

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References

7. Weisweiler, P., Sperl, B., and Schwandr, P., Determination of

### Table 1. Concentrations of Apo A, HDL-Cholesterol, HDL-Phospholipids, Total Cholesterol, and Total Phospholipids (Mean ± SD) in Healthy Subjects and in Patients with Liver Disease

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 20)</th>
<th>Cholestasis patients (n = 19)</th>
<th>Cirrhosis patients (n = 25)</th>
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<tbody>
<tr>
<td>Conc., mg/L</td>
<td></td>
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<tr>
<td>Apo A</td>
<td>1727 ± 669</td>
<td>785 ± 403*</td>
<td>642 ± 349*</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>475 ± 164</td>
<td>206 ± 95*</td>
<td>287 ± 163*</td>
</tr>
<tr>
<td>HDL-phospholipids</td>
<td>419 ± 131</td>
<td>385 ± 212*</td>
<td>511 ± 206*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2118 ± 433 2224 ± 778</td>
<td>1984 ± 839</td>
<td></td>
</tr>
<tr>
<td>Total phospholipids</td>
<td>1249 ± 239 1942 ± 843* &amp; 1421 ± 772c</td>
<td></td>
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</tbody>
</table>

* Significantly different from controls, p < 0.001.  
+ Significantly different from controls, p < 0.002.  
& Significantly different from cholestasis patients, p < 0.05.

### Table 2. Lecithin/Lysolecithin Ratios in Whole Serum and Supernatant Fraction (Mean ± SD) in Healthy Subjects and in Patients with Liver Disease

<table>
<thead>
<tr>
<th></th>
<th>Lecithin/lysolecithin ratio</th>
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<tbody>
<tr>
<td></td>
<td>Whole serum</td>
</tr>
<tr>
<td>Controls (n = 9)</td>
<td>13.2 ± 6.9</td>
</tr>
<tr>
<td>Cholestasis (n = 10)</td>
<td>29.1 ± 19.9</td>
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<tr>
<td>Cirrhosis (n = 8)</td>
<td>39.4 ± 26.8</td>
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Rapid Assay for Amino Acids in Serum or Urine by Pre-Column Derivatization and Reversed-Phase Liquid Chromatography

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This method for estimating clinically important amino acids in serum or urine within 40 min involves o-phthalaldehyde/2-mercaptoethanol derivatization and reversed-phase "high-pressure" liquid chromatography. Homocysteic acid is an internal standard, and homoserine and norvaline are reference peaks. For all the amino acids estimated, the between-run coefficients of variation ranged from 2.0 to 13.5%, and the mean analytical recoveries from both serum and urine samples was 101%. Peak areas vary linearly with concentration up to 1500 μmol/L for all the amino acids assayed. The limit of detection for each amino acid was estimated to be 38 fmol.

The quantitative estimation of free amino acids by "high-pressure" liquid chromatography (HPLC) is potentially faster and more sensitive (1, 2) than the classical ion-exchange methods (3).

Pre-column o-phthalaldehyde/2-mercaptoethanol (OPA/MCE) derivatization is ideally suited to amino acid analysis by reversed-phase chromatography because the derivatizes produced are less polar than the free amino acids. Moreover, the formation of these amino acid derivatives from physiological fluids is rapid and can be performed in aqueous conditions. The problem of poor fluorescence intensity for the cystine derivative is overcome by pretreating the samples with iodoacetic acid (4).

We describe a rapid method for the quantitative estimation of clinically important free amino acids in serum or urine, by using fluorescence detection of OPA/MCE derivatives of the analytes, and their separation by gradient elution reversed-phase HPLC.

Materials and Methods

Instrumentation

The gradient HPLC system used was an Altex 420 (Altex Scientific Inc., Berkeley, CA 94710). Injections were made using a Rheodyne valve fitted with a 20-μL loop. The amino acid derivatives were detected with a Schoeffel FS 970 Fluorescence Detector (Kratos Inc., Westwood, NJ 07675), excitation wavelength 230 nm and emission cutoff filter at 418 nm. The data from the chromatography was processed by a SP4100 Computing Integrator (Spectra-Physics, Santa Clara, CA 95051). We used a 150 × 4.6 mm i.d. analytical column pre-packed with 5-μm diameter Ultrasphere ODS (Altex Scientific Inc.). The analytical column was fitted with a 70 × 2 mm i.d. pre-column packed with 25- to 37-mm diameter CO2:PELL ODS (Whatman Inc., Clifton, NJ 07014).

Reagents

All amino acids were obtained from the Sigma Chemical Co., St. Louis, MO 63178. Iodoacetic acid and 2-mercaptoethanol were purchased from Aldrich Chemical Co., Milwaukee, WI 53233. "Far UV-grade" acetonitrile was obtained from Fisons Scientific Apparatus, Loughborough, U.K. Unless stated, all other chemicals were analytical grade, obtained