Relation of hyperlipidemia in serum and loss of high density lipoproteins in urine in the nephrotic syndrome

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Summary

The mechanism leading to hyperlipidemia in the nephrotic syndrome is not fully understood but may be related in part to loss of high density lipoproteins in the urine of patients with nephrosis. To prove this hypothesis, we compared serum lipoprotein profiles with the excretion of high density lipoproteins in urine in 19 nephrotic patients. Serum cholesterol ranged from 19–152 (median value 45) mg/dl in very low density lipoproteins (VLDL), from 130–443 (median 186) mg/dl in low density lipoproteins (LDL) and from 19–64 (median 33) mg/dl in high density lipoproteins (HDL). Hyperlipoproteinemia was found in 17 patients, which was classified as phenotype IIa (Fredrickson) in 2, as phenotype IIb in 9 and as phenotype IV in 6 subjects. Two patients showed normal lipoprotein patterns. VLDL- and LDL-cholesterol were not found in detectable amounts in urine, whereas HDL-cholesterol was measured in low concentrations from 0.1–8.3 mg/24 h in all samples. There was no correlation between serum HDL-cholesterol and urinary HDL-cholesterol, but a positive correlation between serum LDL-cholesterol and urinary HDL-cholesterol ($r = +0.54$, $p < 0.05$). However, the total amount of the daily urinary loss of HDL ($< 1\%$ of total plasma HDL) seems not to be sufficient to explain hyperlipoproteinemia in the nephrotic syndrome.

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Introduction

The mechanisms causing hyperlipidemia in the nephrotic syndrome are complex and include both altered catabolism and enhanced synthesis of lipids [1–6]. In these patients an elevated excretion of urinary lipids has long been recognized [7,8] and it was suggested that loss of high density lipoproteins in urine might be related to hyperlipoproteinemia in serum [9,10].

Although HDL was identified in urine of nephrotic patients [11], quantitative measurements have not yet been done. We, therefore, compared serum lipoprotein patterns in patients with untreated nephrotic syndrome with the excretion of high density lipoproteins in urine. Quantitation of urinary HDL was performed by cholesterol measurements in HDL fractions after ultrafiltration and gel chromatography of the urinary samples. Although our results show a positive correlation between serum LDL-cholesterol and urinary HDL-cholesterol the amount of the daily urinary loss of HDL seems not to be sufficient to explain the hyperlipidemia in patients with nephrotic syndrome.

Materials and methods

Patients

Nineteen patients (age 17 to 69 yr) with nephrotic syndrome of various origins were included in the study (Table I). Diagnoses were established by the laboratory finding of proteinuria (> 2 g/24 h) as well as renal sonography, intravenous urography, cystoscopy and histological examination of biopsies. Five patients suffered from diabetic nephropathy, 4 had perimembranous, 3 epimembranous, 2 mesangial proliferative, 1 membranous-proliferative and 1 proliferative glomerulonephritis. Two renal biopsies showed focal glomerulosclerosis and 1 minimal glomerular lesions.

In each patient the 24 h-urine and blood specimens after fasting overnight were collected at the same time. No steroids were applied and no restrictive diets were kept during the collection period.

Chemical assays

Total serum albumin was measured with a Technicon SMA 12/60 Multiple Analyzer. Total serum cholesterol and triglyceride determinations were performed enzymatically with commercial test kits (Boehringer, Mannheim, FRG) [12,13]. Urinary protein was determined with the Biuret reagent [14]. Total cholesterol in urine was analyzed by gas-liquid chromatography using a Perkin Elmer chromatograph (model 900) with flame ionization detectors and a 180 cm $\times$ 2 mm, 1% XE 60 column, column temperature 220°C, as described previously [15].

Separation of serum lipoproteins

Serum lipoproteins were separated at $d = 1.006$ g/ml by preparative ultracentrifugation (105 000 $\times$ g, 22 h, 4°C) [16]. VLDL was removed by slicing the tube. In the infranate, LDL and HDL were further separated by precipitation with sodium phosphotungstate and magnesium chloride [17].

TABLE I

Serum albumin and lipids in 19 nephrotic patients

Patients	Age	Sex	Serum albumin (g/dl)	Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)	VLDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
1. B.R.	69	f	3.7	197	261	45	135	26
2. B.M.	34	m	3.5	249	253	71	186	36
3. B.H.	59	m	2.6	464	570	103	183	38
4. D.A.	55	m	3.0	316	88	25	230	37
5. F.J.	47	m	1.8	693	630	152	418	40
6. H.W.	50	m	2.6	284	234	61	163	29
7. H.F.	42	m	3.7	284	326	69	218	21
8. H.A.	40	m	4.1	232	213	41	144	56
9. H.J.	69	f	4.0	245	349	61	150	21
10. K.A.	39	m	2.7	403	242	68	306	30
11. K.Z.	44	m	2.5	340	264	63	244	19
12. L.S.	52	f	1.7	485	465	135	300	50
13. L.A.	22	m	4.7	221	137	33	141	33
14. M.M.	21	f	3.3	191	153	28	130	32
15. N.F.	62	m	2.4	422	327	75	331	38
16. R.M.	37	m	3.2	209	143	21	151	37
17. S.J.	50	m	3.2	197	253	37	154	23
18. S.E.	69	f	3.0	198	161	19	191	19
19. W.C.	17	f	2.1	543	113	44	443	64
Range			1.7–4.7	191–693	88–630	19–152	130–443	19–64
Median value			3.0	284	253	45	186	33
Normal value			3.7–4.8	< 230	< 200	< 30	<180	> m 35/f 40

Isolation of urinary high density lipoproteins

Within 1 h after the collection the 24 h-urine samples were passed through a Selecta filter no. 1117 1/2 (Schleicher Schüll, Dassel, FRG) in order to remove cellular debris of 5 to 10 μm in diameter. Then, NaCl was added up to a final concentration of 0.56 mol/l to precipitate the Tamm-Horsefall mucoprotein [18], which was removed by centrifugation for 10 min at 8000 rpm. Before the supernatant (90% of the volume) was concentrated by ultrafiltration at 4°C to a final volume of 3 to 5 ml in a stirred cell (Amicon, Witten, FRG; models 402 and 52) with PM 10 membranes, 0.2 g sodium azide (NaN_3) and 0.05 mmol phenylmethanesulfonyl fluoride (PMSF) in 100% isopropanol were added per 1000 ml of urine to avoid bacterial and enzymatic degradation. Aliquots of 2 to 3 ml of the concentrated urine were used for gel chromatography on a 100 × 2.5 cm Sephadex G-100 column. Elution was performed with 0.15 mol/l NaCl, 0.1 g/l Na_2-EDTA, 0.2 g/l NaN_3 and 0.05 mmol/l PMSF at pH 7.0 [19]. Usually 70 to 80 8-ml fractions were collected. The absorbance at 280 nm was recorded with a spectrophotometer (Beckman, Fullerton, CA, model 24) and total cholesterol was measured by gas-liquid chromatography. The cholesterol containing fractions were pooled and

concentrated to 3 to 5 ml. After rechromatography on a 120 × 1.8 cm Biogel A 5 m column about 50 8-ml fractions were collected. The absorbance at 280 nm and the cholesterol concentrations were plotted graphically. The elution pattern revealed 2 or 3 peaks, which were pooled and concentrated each to 0.5 to 2 ml.

Recovery studies for cholesterol showed a total loss between 30 and 50% at the end of the fractionation.

Immunodiffusion

Ouchterlony immunodiffusion was performed in 15 g/l bactoagar (0.02 mol/l sodium diethylbarbiturate, 0.03 mol/l sodium acetate), pH 8.6. The glass plates were incubated for 48 h at 20°C. The staining was done with azokarmine B (Sigma, München, FRG). We tested against antisera against α_1- and β-lipoproteins (Behring, Marburg, FRG) identical to apoHDL and apoLDL, respectively.

Statistical analysis

Using ranges and median values the statistical analysis of our data was carried out applying the t-test, the Spearman rank correlation coefficient and the analysis of the regression line [20].

Results

Serum albumin, lipids and lipoproteins

Single values of serum albumin, total cholesterol, triglycerides, VLDL-, LDL- and HDL-cholesterol of 19 patients with nephrotic syndrome are listed in Table I. In 14 out of 19 serum samples hypoalbuminemia was found. Only 4 patients had normal serum cholesterol and triglyceride concentrations, one of which showed a regular cholesterol distribution on the three main lipoprotein classes. In the remaining 18 patients at least one lipid parameter was abnormal. In 10 patients we found decreased plasma HDL-cholesterol levels and in 11 elevated plasma LDL-cholesterol concentrations.

Urinary protein, total cholesterol and HDL-cholesterol

The total urinary protein excretion varied from 2.0 to 24.5 g/24 h (median value 4.9 g/24 h) and total urinary cholesterol concentrations from 3.1 mg to 68.7 mg/24 h (median value 20.0 mg/24 h) (Table II).

For isolation of urinary HDL lower molecular proteins were separated from urinary lipids by gel filtration on a Sephadex G-100 column. The cholesterol containing void volume fractions were pooled, concentrated and fractionated subsequently on the Biogel A 5m column. In 13 patients an elution profile with two main peaks was seen (Fig. 1a). The first peak represented urinary cholesterol associated with membrane proteolipids which have been characterized previously [15]. The second peak was identified as HDL by immunodiffusion. In the remaining 6 patients the elution pattern revealed 3 peaks (Fig. 1b). The middle peak did contain LDL in only one urine sample and contained larger HDL aggregates in the others. Single values of urinary HDL-cholesterol were given in Table II.

TABLE II

Urinary protein, cholesterol and HDL-cholesterol excretion in nephrotic patients

Patients	Protein (g/24 h)	Cholesterol (mg/24 h)	HDL-cholesterol (mg/24 h)
1. B.R.	3.2	19.8	2.9
2. B.M.	24.5	57.9	1.7
3. B.H.	7.0	20.0	1.0
4. D.A.	6.9	12.2	0.7
5. F.J.	3.3	10.2	4.1
6. H.W.	4.1	5.9	0.1
7. H.F.	3.0	4.5	0.7
8. H.A.	3.1	6.2	0.1
9. H.J.	2.5	6.4	0.3
10. K.A.	4.9	24.6	7.6
11. K.Z.	5.1	9.8	3.1
12. L.S.	3.3	42.9	8.3
13. L.A.	8.2	68.7	0.2
14. M.M.	2.0	3.1	0.4
15. N.F.	21.0	42.8	5.0
16. R.M.	23.2	5.0	1.0
17. S.J.	18.3	38.0	1.4
18. S.E.	3.8	21.4	2.2
19. W.C.	7.7	21.5	0.9
Range	2.0–24.5	3.1–68.7	0.1–8.3
Median value	4.9	20.0	1.0

Serum-urine correlations

According to the statistical analysis the urinary total protein correlated significantly with urinary total cholesterol ($r = +0.76$, $p < 0.01$) (Fig. 2a). There was an
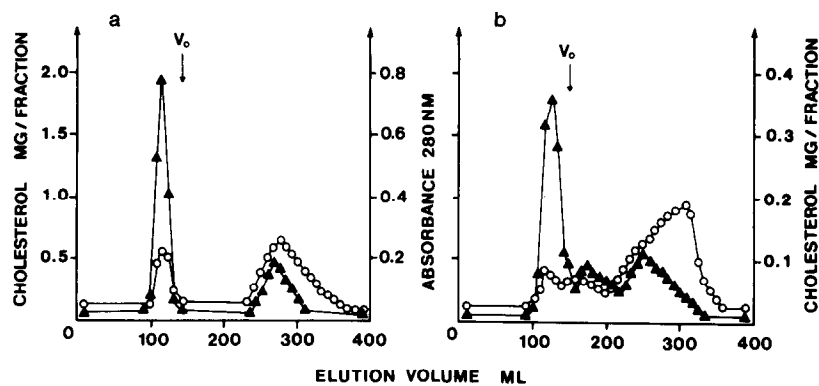

Fig. 1. a. Gel chromatographic elution pattern of a concentrated urinary sample on Biogel A 5m (two peaks). b. Gel chromatographic elution pattern of a concentrated urinary sample on Biogel A 5m (3 peaks). Column size 120×1.8 cm. Individual points represent total cholesterol concentrations (▲——▲) and the absorbance at 280 nm (O——O) measured in each collected fraction. V_o, void volume.

inverse correlation between serum albumin and serum total cholesterol ($r = -0.78$, $p < 0.01$) (Fig. 2b) and a positive correlation between urinary HDL-cholesterol and serum LDL-cholesterol ($r = +0.54$, $p < 0.05$) (Fig. 2c). Urinary total protein and serum albumin ($r = -0.25$), urinary total cholesterol and plasma total cholesterol ($r = +0.01$), urinary HDL-cholesterol and plasma HDL-cholesterol ($r = +0.08$)
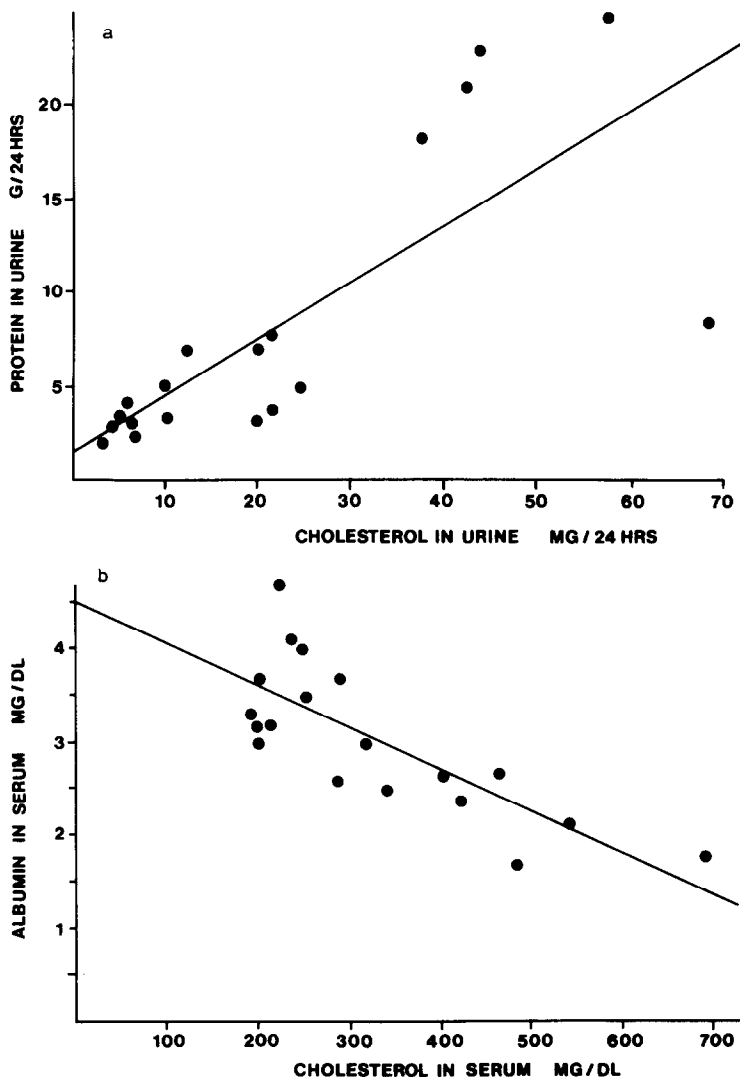

Fig. 2. a. Correlation between urinary protein and urinary total cholesterol ($r = +0.76$, $p < 0.01$), $y = 0.29x + 1.58$. b. Correlation between serum albumin and serum total cholesterol ($r = -0.78$, $p < 0.01$), $y = -0.0045x + 4.5$. c. Correlation between urinary HDL-cholesterol and serum LDL-cholesterol ($r = +0.54$, $p < 0.05$), $y = 0.016x - 1$.
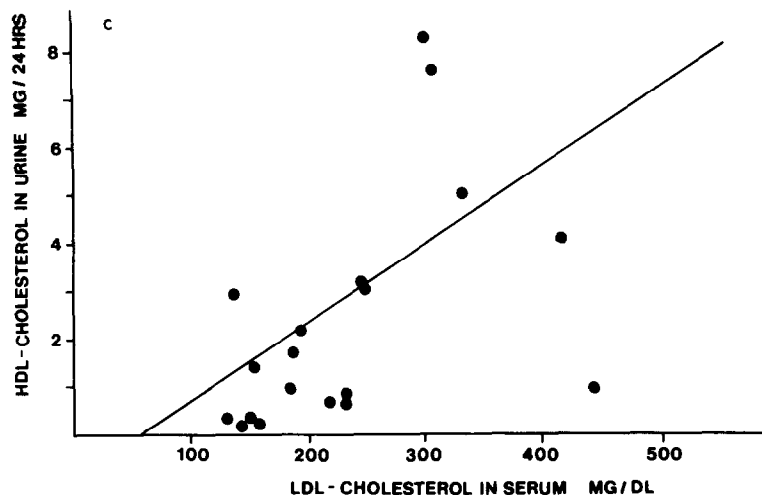
Fig. 2 (continued).

and plasma HDL-cholesterol and plasma LDL-cholesterol ($r = +0.48$) did not correlate significantly.

Discussion

Abnormal serum lipid and lipoprotein patterns are commonly found in the nephrotic syndrome [1,3]. However, the etiology of hyperlipoproteinemia has not yet been clarified completely.

Our data confirmed altered serum lipoprotein concentrations in 17 and hypoalbuminemia in 14 of the 19 patients. Normal plasma cholesterol and triglyceride levels and a regular cholesterol distribution on the 3 main lipoprotein classes were found in one 17-yr-old man with a mild form of nephrotic syndrome, which had been diagnosed only 3 mth ago, another patient showed a normal lipoprotein pattern. These findings are in variance with Chopra and coworkers [6], who reported normal serum lipoprotein profiles in 16 out of 33 patients. This discrepancy can be explained by the fact that in contrast to them only patients without steroid treatment were included in our study. Nine patients showed hyperlipoproteinemia phenotype IIb, 6 had phenotype IV and 2 phenotype IIa. The predominance of phenotype II and IV is confirmed by other investigators [3,6], although all phenotype of hyperlipoproteinemias can occur in the nephrotic syndrome. The presence of renal failure, special diets, the degree of hypoalbuminemia, age, sex and general condition of the patients or the etiology of nephrosis could be responsible for this heterogeneity [1,3].

Only few studies exist which take the urinary lipid and protein excretion into account [8,11]. Using double immunodiffusion and lipoprotein-electrophoresis Kashyap et al found HDL in the urine of 9 nephrotic patients investigated. LDL

was detected in only two of these patients with the nephrotic syndrome [11]. In our patients proteinuria up to 24.5 g/24 h and cholesteroluria up to 68.7 mg/24 h were observed. After gel filtration of the concentrated urinary samples, besides the membrane proteolipids, which were eluted in the void volume fractions, HDL was regularly identified in the lower molecular weight peak fractions. Lipid recovery studies in single urinary specimens showed a total loss of urinary cholesterol between 30 and 50%. Especially during the two ultrafiltration steps a considerable loss of lipids occurred most likely due to adherence of lipoproteins to the PM 10 membrane or to direct passage of free cholesterol molecules as a consequence of delipidation. Filtration using the Selecta filter 1117 1/2 and gel chromatography did not remove more than 5% of total cholesterol.

The lipid–protein correlations in our study were in good accordance to the results published by other authors [2,8,21,22]. Urinary total protein and urinary total cholesterol correlated positively ($r = +0.76$, $p < 0.01$). There was a significant inverse correlation between albumin and total cholesterol in serum ($r = -0.78$, $p < 0.01$). Apple et al reported similar results recently and suggested that albumin concentration and oncotic pressure might be closely related to the signal for enhanced hepatic production of lipoprotein lipids [21]. In discrepancy to Gherardi et al we found no reciprocal relation between serum HDL cholesterol and LDL-cholesterol [3], but a positive correlation ($r = +0.54$, $p < 0.05$) between serum LDL-cholesterol and urinary HDL-cholesterol. It has been speculated that the selective increase in lipoprotein-cholesterol fractions other than HDL cholesterol may relate in part to loss of HDL in the urine of patients with nephrosis [9,10]. HDL serves as a flexible reservoir in plasma for the physiologically important apo C II molecules. These are activators of the lipoprotein lipase and influence the clearance of circulating triglycerides. Although LDL-cholesterol in serum and HDL-cholesterol in urine correlated positively in our study, the causative relationship of these findings is questionable. Our results demonstrate no inverse correlation between HDL-cholesterol in serum and urine. The absolute amount of HDL excreted in the 24 h urine is obviously too small to decrease the HDL concentrations in serum significantly. Therefore, our results are inconsistent with the hypothesis [23] that the urinary loss of HDL might relate to the hyperlipidemia of nephrotic syndrome.

Acknowledgements

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