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Qualitative and Quantitative Analysis of Urinary Lipids in the Nephrotic Syndrome *

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Abstract. A qualitative and quantitative analysis of urinary lipids in the nephrotic syndrome is presented. The following lipids were identified in the urine of patients with the nephrotic syndrome: free cholesterol, cholesterol esters, triglycerides, free fatty acids, and phospholipids. Glass paper chromatography identified the cholesterol esters as palmitate, oleate, linoleate, and arachidonate, and identified the phospholipids as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine.

Urinary lipid excretion was much greater in patients with the nephrotic syndrome than in patients with chronic renal disease and minimal proteinuria, or in patients with hyperlipidemia from other causes.

Urinary lipid excretion varied widely among the 13 patients with the nephrotic syndrome studied, and no quantitative correlation with serum lipid levels was observed. However, qualitatively at least, the proportion of cholesterol esters excreted in the urine was similar to the proportion of these esters in plasma. A good correlation was found between lipid excretion and glomerular permeability. Furthermore, during steroid therapy urinary lipid excretion decreased concomitant with a decrease in proteinuria. All these observations support the idea that lipiduria in the nephrotic syndrome is related to protein loss and that most of the lipid in the urine enters the glomerular filtrate in the form of lipoproteins.

Introduction

Lipiduria is a commonly emphasized component of the nephrotic syndrome (1). The term usually refers to the birefringent and/or anisotropic crystals found in the urinary sediment of patients with the nephrotic syndrome. These anisotropic bodies have the typical "Maltese cross" appearance when viewed with a polarizing microscope. Cholesterol esters have been identified by Zimmer et al. (2) as the major component of these elements. However, the qualitative and quantitative pattern of lipids excreted in the urine of patients with the nephrotic syndrome has not been elucidated. Our study was designed to clarify the nature of the lipids excreted in the urine of patients with the nephrotic syndrome as well as to gain insight into the mechanisms of urinary lipid excretion.

Methods

24-hr urine specimens were collected on 32 different occasions from five normal subjects, 13 patients with the nephrotic syndrome, four patients with hyperlipidemia

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TABLE I

Clinical data in patients studied

Patients	Sex	Age	Plasma proteins		Serum lipids			C_{cr}	Histological or clinical diagnosis
			Alb	Glob	Chol	Pl	Tg		
		yr	*g/100 ml*			*mg/100 ml*		*ml/min*	
Normal subjects									
M.Y.	M	19	3.6	2.2	147	235	84	83	—
L.C.	M	17	3.8	2.5	164	242	78	90	—
S.A.	F	65	3.8	3.4	133	236	59	77	—
L.J.	M	40	3.2	3.5	194	283	90	92	—
M.E.A.	F	64	3.4	2.7	167	270	79	—	—
Nephrotic syndrome									
J.H.R.	M	26	2.1	2.2	286	302	205	23	Membranous GN
J.G.O.	M	14	1.8	2.0	570	610	278	8	Membranous GN
M.I.O.	F	38	2.5	2.6	440	450	—	61	Focal GN
W.R.	M	9½	2.3	2.1	642	616	185	47	—
E.N.	M	13	1.6	2.2	412	460	189	69	—
H.A.	M	31	2.0	2.0	440	447	158	52	Membranous GN
B.L.	M	24	2.1	2.2	370	374	286	52	Chronic GN
E.A.	F	32	2.2	1.6	400	443	338	103	Proliferative GN
C.M.	F	24	1.1	3.2	330	380	276	37	Chronic GN
J.R.R.	M	59	1.7	2.1	405	415	—	21	Chronic GN
C.A.	F	15	2.0	3.1	326	374	185	21	Chronic GN
M.G.C.	F	54	2.2	1.9	390	410	—	33	—
A.R.	F	8	1.9	2.7	340	432	—	32	Minimal change
Hyperlipidemia from other causes									
D.S.	F	58	2.0	4.6	800	1180	246	—	Biliary cirrhosis
N.G.	F	27	3.9	2.2	390	454	146	—	Familial hyperlipidemia
R.V.	M	32	4.4	2.4	347	—	—	—	Familial hyperlipidemia
M.P.	F	53	3.6	2.1	480	630	162	—	Hypothyroidism
Chronic renal disease									
J.O.	M	38	3.5	2.6	196	306	74	73	Chronic GN
A.P.	M	44	3.7	2.8	232	368	90	16	Chronic GN
G.B.	F	21	3.9	2.9	130	233	61	9	Chronic pyelonephritis

Alb, albumin; glob, globulin; chol, cholesterol; Pl, phospholipids; Tg, triglycerides; C_{cr}, creatinine clearance; GN, glomerulonephritis.

from other causes, and three patients with chronic renal disease and minimal proteinuria. Fasting blood samples were obtained the same day as the urine collections for the determination of total cholesterol, phospholipids, triglycerides, creatinine, albumin, and globulin. Among the 13 patients with the nephrotic syndrome studied there were seven males and six females; their ages ranged from 8 to 59 yr with a mean of 28.2 yr. Renal biopsies suitable for histological diagnosis were obtained in nine of the 13 patients. The clinical data of the patients studied are presented in Table I.

In four patients with the nephrotic syndrome, samples of urine and blood were obtained on admission and after 4 wk of glucocorticoid therapy. Duplicate aliquots of 100 ml of each 24 hr urine specimen were dialyzed against 20 g of polyethylene glycol ("Carbowax," Union Carbide Corporation, New York, N. Y.) previously moistened and placed in dialysis tubing (3). The dialysis was carried out until the urine volume was reduced to 5 or 10 ml. This concentrated urine aliquot was extracted with a 2:1 (v/v) chloroform-methanol mixture in the proportion of 20 volumes of solvent to 1 volume of urine. The extract was then filtered and subsequently washed with dilute sulfuric acid (0.03 N). The resulting purified chloroform layer was evaporated to dryness at temperatures ranging from 45° to 50°C and finally it was reconstituted with chloroform to a volume of 10 ml. Concentrated aliquots from the chloroform extract were analyzed in duplicate for total cholesterol, lipid phosphorus, triglycerides, and cholesterol esters.

Total cholesterol was estimated by the ferric chloride method of Zlatkis et al. (4). Phospholipids were estimated in duplicate 1 ml aliquots to which 0.15 ml of concentrated sulfuric acid was added. This mixture was placed in a sand bath at 280°C for 20 min. After the addition of two drops of peroxide, the mixture was placed in the same sand bath for an additional 10 min. After cooling to room temperature, the lipid phosphorus was determined by the method of Fiske and Subbarow (5). The qualitative determination of lipids and the quantification of triglycerides in the urine were performed using thin layer chromatography. Chromato-

plates were prepared with silica gel G using a Stahl applicator (6). Duplicate aliquots of 2.5 ml of the chloroform extract were evaporated to dryness and reconstituted with 0.5 ml of hexane. 20 μl were applied in duplicate to each chromatoplate and run using a solvent system of hexane–ethyl ether–acetic acid, 40:10:0.5 (v/v). Standards for free cholesterol, cholesterol esters, triglycerides, and free fatty acids were run simultaneously. After developing and drying, the chromatogram was exposed to iodine vapors which revealed the different spots. For the determination of triglycerides, the corresponding spots (identified by a triolein standard run simultaneously) were scraped off and dissolved in 3 ml of methanol. This eluate was evaporated to dryness and redissolved in 3 ml of a mixture of ethanol–ethyl ether, 3:1 (v/v). Triglycerides were finally determined by the esterification of fatty acids with hydroxylamine using the method of Stern and Shapiro (7). Cholesterol esters were separated by glass paper chromatography (8). For this procedure duplicate 2-ml samples of the chloroform extract were evaporated to dryness and reconstituted with 0.5 ml of a mixture of isopropylic ether–ethanol, 1:2 (v/v). Thereafter, duplicate 10-μl samples of this extract were run using a solvent system of isooctane–isopropyl acetate, 50:0.05 (v/v). After development, the chromatogram was dried, sprayed with concentrated sulfuric acid, and placed in the oven at 180°C for 5 min. Densitometries of the charred spots of standard and unknown samples were compared using a specially designed densitometer (9).

Urinary phospholipids were identified by glass paper chromatography using 20 μl of a concentrated sample (5:1). Samples were run in duplicate with a solvent system of isopropyl ether–methanol–basic solvent, 25:3:1 (v/v). The basic solvent was made up of ammonia and water, 2:5 (v/v). The development of the chromatograms was similar to that described for the identification of cholesterol esters.

Blood samples were treated the same way as urine samples, with the exception of the concentrating procedure. Creatinine was determined by the method of Bonsnes and Taussky (10). Total protein was quantitated by the micro-Kjeldahl method of Folin and Farmer (11). The respective concentrations of albumin and globulin were determined by paper electrophoresis. Creatinine clearances were calculated with standard formulae (12).

TABLE II

Percentage distribution of cholesterol esters in serum and urine of four patients with the nephrotic syndrome

Cholesterol esters	Per cent of total	
	Serum	Urine
Palmitate	15.2 (±3.1)	19.5 (±1.7)
Oleate	28.8 (±1.3)	27.7 (±1.1)
Linoleate	42.6 (±2.2)	39.3 (±2.8)
Arachidonate	13.3 (±1.7)	13.5 (±3.0)

Values are means (±SD).

TABLE III

Urinary lipid and albumin excretion in patients studied

Patients	Cholesterol	Phospholipids	Triglycerides	Urinary albumin
		mg/24 hr		*g/24 hr*
Normal subjects				
M.Y.	1.2	10.3	N.D.	None
L.C.	3.3	13.3	N.D.	None
S.A.	3.8	7.0	N.D.	None
L.J.	2.6	9.2	N.D.	None
M.E.A.	2.7	7.6	N.D.	None
Mean	2.7	9.5		
Nephrotic syndrome				
J.H.R.	5.1	8.0	8.8	—
J.G.O.	37.3	25.0	6.4	5.9
M.I.O.	46.2	84.0	45.2	11.3
W.R.1	42.0	100.0	7.6	8.2
W.R.2	2.6	9.0	—	1.1
E.N.1	149.1	122.6	2.4	—
E.N.2	5.8	14.4	—	1.2
H.A.	15.7	25.6	2.4	7.5
B.L.1	61.0	112.7	54.4	9.5
B.L.2	26.2	20.8	—	3.2
E.A.	101.4	181.4	15.2	15.7
C.M.1	84.3	91.9	48.4	5.7
C.M.2	39.1	35.3	—	3.7
J.R.R.	14.4	12.5	33.2	—
C.A.	6.8	13.8	32.0	—
M.G.L.	45.9	61.3	—	6.6
A.R.	64.8	53.8	—	11.3
Mean	51.8	68.7	23.3	7.0
Hyperlipidemia				
D.S.	20.4	23.6	1.2	None
N.C.	10.0	11.8	N.D.	None
R.V.	7.8	10.6	N.D.	None
M.P.	10.2	14.2	N.D.	None
Mean	12.1	17.5	—	—
Chronic renal disease				
A.P.	7.4	14.4	N.D.	0.35
J.O.	5.7	15.0	N.D.	0.32
G.B.	6.9	15.2	N.D.	0.15
Mean	6.7	15.2	—	0.27

Only initial values for lipid excretion before therapy were used for the calculation of means.
N.D., none detectable; —, analysis not carried out.

Results

Qualitative lipid pattern. The following lipids were identified in the serum and urine of patients with the nephrotic syndrome: free cholesterol, cholesterol esters, triglycerides, free fatty acids, and phospholipids. Glass paper chromatography identified the cholesterol esters as palmitate, oleate, linoleate, and arachidonate, and identified the phospholipids as phosphatidylcholine (lecithin), phosphatidylethanolamine (cephalin), and phosphatidylserine. These same lipids were present in the serum of normal subjects, but, with the ex-

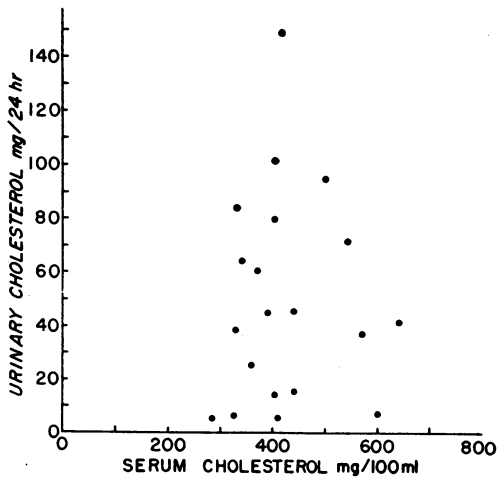

FIG. 1. URINARY CHOLESTEROL EXCRETION AND PLASMA CHOLESTEROL LEVELS IN SUBJECTS WITH THE NEPHROTIC SYNDROME.

ception of cholesterol esters, were not present in the urine of normal individuals.

Quantitative lipid pattern. Table II depicts the percentage distribution of the individual cholesterol esters in the serum and urine of four patients with the nephrotic syndrome. Linoleate comprised 43% of the total serum cholesterol esters and arachidonate the smallest per cent as is the case in normal subjects. The linoleate to oleate ratio or L/O ratio was approximately 1.5 in these nephrotic patients, a ratio similar to that seen in normal subjects. The proportions of the individual urinary cholesterol esters were similar to those in serum. Table III shows the values of total cholesterol, phospholipids, and triglycerides excreted in the urine in 24 hr in five normal subjects, four patients with hyperlipidemia not due to nephrotic syndrome, three patients with renal disease and minimal proteinuria (less than 500 mg/24 hr), and 13 patients with the nephrotic syndrome. In the normal subjects urinary cholesterol excretion ranged from 1.2 to 3.8 mg/24 hr with a mean of 2.7. Others have reported similar values (13, 14). The mean 24 hr cholesterol excretion was 12.1 mg in the hyperlipidemic patients, 6.7 mg in the patients with renal disease, and 51.8 mg in patients with the nephrotic syndrome.

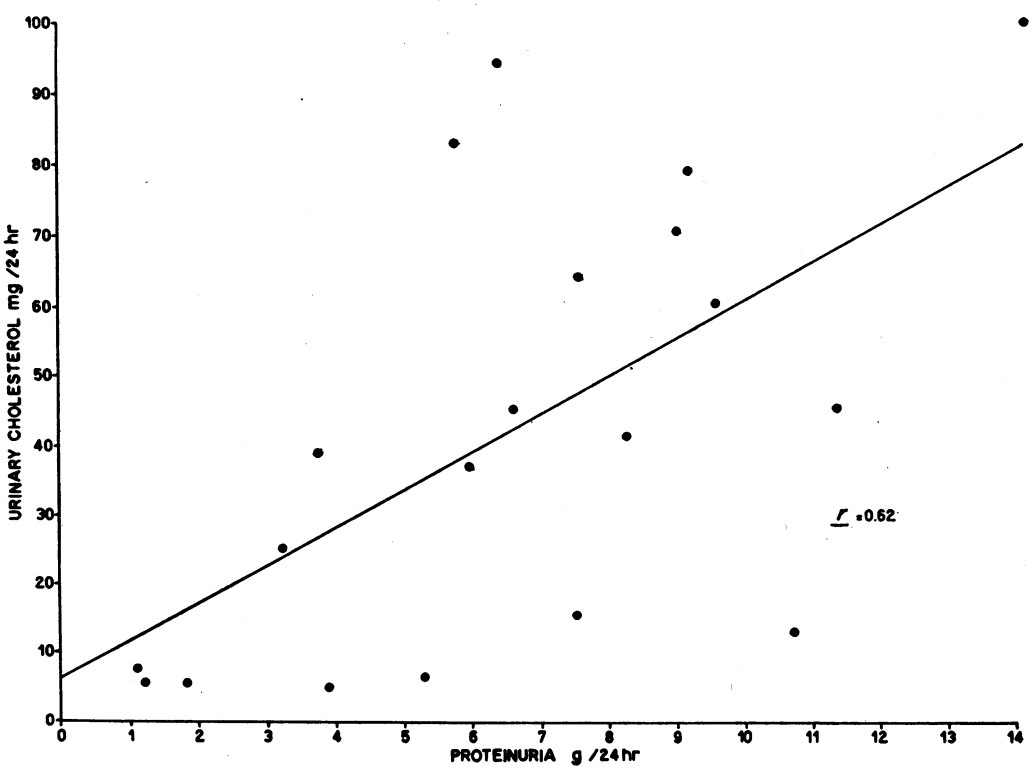

FIG. 2. URINARY CHOLESTEROL EXCRETION AND PROTEINURIA IN PATIENTS WITH THE NEPHROTIC SYNDROME. The regression line was calculated by the method of least squares.

The mean urinary excretion for phospholipids was 9.5 mg/24 hr in the normal subjects, 17.5 mg/24 hr in the hyperlipidemic patients, 15.2 mg/24 hr in the patients with chronic renal disease, and 68.7 mg/24 hr in the subjects with the nephrotic syndrome.

Triglycerides were not detected in the urine of patients with chronic renal disease or in normal subjects. Only one of the patients with hyperlipidemia not due to the nephrotic syndrome had triglycerides in detectable amounts in the urine. The 24 hr excretion of triglycerides in this patient was 1.2 mg. The mean 24 hr urinary triglyceride excretion in 11 patients with nephrotic syndrome was 23.3 mg.

Proteinuria and urinary lipid excretion. To determine whether or not a correlation existed between urinary cholesterol excretion and plasma cholesterol levels in subjects with the nephrotic syndrome, we plotted both values as shown in Fig. 1. Included are 20 observations in 13 patients with the nephrotic syndrome. No correlation was found between urinary cholesterol excretion and blood levels. The urinary excretion of phospholipids and triglycerides also varied considerably among different patients and no correlation with blood levels was observed.

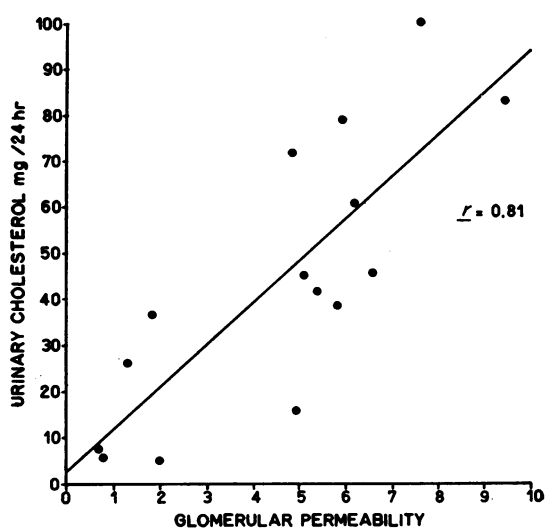

FIG. 3. URINARY CHOLESTEROL EXCRETION PLOTTED AS A FUNCTION OF "GLOMERULAR PERMEABILITY." Glomerular permeability was calculated using the data of albumin excretion, plasma albumin levels and creatinine clearance (15).

TABLE IV

Serum and urinary findings in four patients with the nephrotic syndrome before and after steroid therapy

	Before therapy	After therapy
Serum cholesterol, *mg/100 ml*	414 (±70.1)	390 (±67.5)
Urine cholesterol, *mg/24 hr*	84.1 (±23.4)	19.7 (± 8.0)
Proteinuria, *g/24 hr*	6.8 (± 2.1)	2.2 (± 0.7)

Determinations were performed before and after 4 wk of prednisone administration (80 mg daily). Values in parentheses are SE of the means.

In Fig. 2 the 24 hr cholesterol excretion is plotted on the ordinate and proteinuria on the abscissa. Despite a considerable dispersion of the values, the correlation coefficient was 0.62. In general, 24 hr urinary cholesterol excretion tended to increase with a greater 24 hr excretion.

In Fig. 3, 24 hr urinary cholesterol excretion is plotted on the ordinate and "glomerular permeability" on the abscissa. Glomerular permeability was calculated as described by Schreiner using the data of albumin excretion, plasma albumin levels, and creatinine clearance (15). A good correlation was observed between glomerular permeability and urinary cholesterol excretion. The correlation coefficient was 0.81. If the regression line, calculated by the method of least squares, was extrapolated to a glomerular permeability of 0, the calculated 24 hr urinary cholesterol excretion would be 2.5 mg, a figure similar to the mean of 2.7 mg of cholesterol excreted in the five normal subjects studied.

Effect of glucocorticoid therapy. Table IV shows the serum cholesterol levels, 24 hr urinary cholesterol, and protein excretions in four patients with the nephrotic syndrome before and after 4 wk of glucocorticoid therapy (80 mg of prednisone daily). A marked decrease in urinary cholesterol occurred. Mean values were 84.1 mg/24 hr before therapy and 19.7 mg/24 hr after therapy. There was practically no change in serum cholesterol levels; mean values before and after therapy were 414 and 393 mg/100 ml. In these four patients proteinuria decreased markedly from a mean of 6.8 g/24 hr to a mean of 2.2 g/24 hr after glucocorticoid administration.

Discussion

Anisotropic lipid material has been observed in the urine sediment of patients with the nephrotic

syndrome since the early 1900's. These glomerular anisotropic bodies have the well-known Maltese cross appearance when viewed with a polarizing microscope. Zimmer and his associates (2) pointed out that this anisotropic lipid material is apparently composed largely of cholesterol ester with a smaller proportion of free cholesterol. However, no attention has been given to the possibility of urinary excretion of lipids other than cholesterol esters and free cholesterol in nephrotic subjects. In our patients both thin layer chromatography and glass paper chromatography were employed, as previously described in Results, to show the presence of other lipids in the urine of patients with the nephrotic syndrome. The percentage distribution of urinary cholesterol esters was similar to the distribution of these same esters in plasma.

Several previous studies of the quantitative excretion of urinary cholesterol in various disease states have been made (13, 14, 16). The range of normal in these studies was 0.15–4.2 mg/24 hr, a range very similar to the urinary excretion of cholesterol by our normal subjects (mean 2.7 mg). In our study, urinary lipid excretion was much greater in patients with the nephrotic syndrome than in patients with chronic renal disease and minimal proteinuria, or in patients with hyperlipidemia from other causes. Urinary lipid excretion varied widely among the 13 patients with the nephrotic syndrome studied, and no quantitative correlation with serum lipid levels was observed.

More than 30 yr ago Bing and Starup (17) reported a parallelism between protein and cholesterol excretion in a nephrotic patient studied over an 8 hr period whose cholesterol excretion was varied by cholesterol feeding. Neuman et al. (18), in a study of 100 patients whose proteinuria was associated with a variety of diseases, showed a correlation between the degree of proteinuria and the number of fatty elements in the urine as determined by Sudan staining. Within our nephrotic group, four patients were studied before and after 4 wk of glucocorticoid therapy. In each patient, concomitant with a decrease in proteinuria there was a marked decrease in urinary cholesterol excretion, despite the fact that serum cholesterol levels did not change appreciably. When urinary cholesterol excretion was plotted against protein excretion, a moderately good correlation was observed. If correction is allowed for plasma and albumin levels and glomerular filtration rate, the excretion of albumin in the final urine offers a rough estimate of glomerular permeability (15). When the glomerular permeability, calculated as an albumin clearance corrected/100 ml of glomerular filtration rate, was plotted against urinary cholesterol excretion, a good correlation ($r = 0.82$) was obtained. All these observations support the idea that lipiduria in the nephrotic syndrome is related to protein loss and suggest that most of the urinary lipid enters the glomerular filtrate in the form of lipoprotein. Schrade et al. (19) demonstrated that lipoprotein is lost through damaged glomeruli in patients with proteinuria of any etiology. This lipoprotein could be partially reabsorbed by the tubule. The possibility that some of the lipid appearing in the urine is obtained from the blood through tubular secretion is unlikely but not entirely excluded. However, experiments with amphibians indicated that glomerular damage is necessary before cholesterol can enter the urine (20); and in dogs with dietary-induced hyperlipidemia no urinary cholesterol excretion could be detected until a renal lesion (presumably glomerular) had been produced with uranium nitrate (21). In human beings the hypercholesterolemic state alone is not sufficient to produce abnormal amounts of urinary lipids. In only one of our patients with hyperlipidemia not due to the nephrotic syndrome was there an appreciable amount of urinary lipids. In this patient (D.S.) with biliary tract obstruction, the possibility that some of the lipid material in the urine could represent biliary acids was not completely excluded. It appears, therefore, that an increased glomerular permeability to lipoprotein is probably necessary before appreciable amounts of lipid appear in the urine.

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