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Metabolism of low density lipoproteins in nephrotic dyslipidemia: Comparison of hypercholesterolemia alone and combined hyperlipidemia

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Metabolism of low density lipoproteins in nephrotic dyslipidemia: comparison of hypercholesteremia alone and combined hyperlipidemia. High levels of low-density lipoprotein cholesterol (LDL) (hypercholesterolemia) are commonly present in the nephrotic syndrome. Another pattern of dyslipidemia in nephrotic patients is an elevation of both cholesterol and triglyceride levels (combined hyperlipidemia). It has been postulated that the underlying cause of nephrotic dyslipidemia is an hepatic overproduction of apolipoprotein B (apo B)-containing lipoproteins. To examine this hypothesis, the metabolism of LDL-apo B was compared between nephrotic patients with hypercholesterolemia and with combined hyperlipidemia. Thirteen patients (7 with hypercholesterolemia, and 6 with combined hyperlipidemia) underwent measurements of turnover rates of autologous LDL apo B. The results were compared to normolipidemic controls and to patients with primary combined hyperlipidemia previously studied in our laboratory. Nephrotic patients with hypercholesterolemia generally had: (a) lower fractional catabolic rates of LDL apo B than normolipidemic healthy individuals; (b) LDL particles enriched in cholesterol; but (c) no overproduction of LDL apo B. In contrast, patients with combined hyperlipidemia were found to have: (a) high fractional catabolic rates for LDL apo B compared to normolipidemic controls; (b) cholesterol-poor LDL particles; and (c) markedly elevated production rates for LDL. Also, for the group as a whole, there was a positive correlation between plasma triglyceride levels and fractional catabolic rates. These data indicate that the metabolism of LDL is strikingly different between the two forms of nephrotic dyslipidemia. Although there may be common mechanisms contributing to LDL levels in nephrotic patients, there also appears to be a divergence of mechanisms depending on whether hypertriglyceridemia is associated with hypercholesterolemia.

Dyslipidemia is a common feature of the nephrotic syndrome. Either of two types of dyslipidemia are observed: elevated serum cholesterol alone (hypercholesterolemia), and simultaneous elevation of serum cholesterol and triglyceride (combined hyperlipidemia). Some workers have postulated that the dyslipidemia of the nephrotic syndrome is caused primarily by increased hepatic synthesis and secretion of lipoproteins containing apolipoprotein B [very low density (VLDL) and low density lipoproteins (LDL)] [1–4]. Also, it has been proposed that hypoalbuminemia induces the oversynthesis of lipoproteins [5–7]. These observations led to the lipoprotein "overproduction" hypothesis to explain nephrotic dyslipidemia. In simple terms, it is commonly believed that elevation of plasma lipids accompanying the nephrotic syndrome results from an increased hepatic secretion of apo B-containing lipoproteins in response to hypoalbuminemia.

More recently, however, this hypothesis has been challenged along several lines. First, hypercholesterolemia has been observed in normoalbuminemic patients with nephrotic syndrome, and treatment of these patients with angiotensin I converting enzyme inhibitors reduces protein excretion and lowers plasma cholesterol levels [8]. This finding suggests that proteinuria is more directly linked to dyslipidemia, and its effect is not necessarily mediated through hypoalbuminemia. And second, that overproduction of lipoproteins is the major cause of dyslipidemia is open to question because of the findings of reduced catabolism of apo B-containing lipoproteins in experimental animals [9–11] and in some patients [12–14] with the nephrotic syndrome. Thus, two mechanisms might contribute to nephrotic dyslipidemia: (a) an overproduction of apo-B containing lipoproteins, and (b) an impaired catabolism of these lipoproteins. In addition, the reasons for the occurrence of two different patterns of dyslipidemia—hypercholesterolemia alone and combined hyperlipidemia—remain unexplained. It is apparent then that important issues remain to be resolved about the mechanisms underlying nephrotic dyslipidemia.

The current study was designed to determine whether nephrotic patients with hypercholesterolemia and combined hyperlipidemia have similar or dissimilar patterns of LDL metabolism. Specifically, it was asked whether overproduction or decreased clearance of LDL predominates in each of these two patterns of dyslipidemia. In addition, we asked whether other factors (such as obesity, proteinuria, or hypoalbuminemia), might contribute to increases in LDL cholesterol that are typical of many patients with nephrotic dyslipidemia.

Methods

Patients

Thirteen patients (7 women, and 6 men) were recruited for study from the renal clinics at Parkland Memorial Hospital, and the Veterans Affairs Medical Center at Dallas, Texas. All subjects had nephrotic syndrome due to primary glomerular disease. The time since onset varied from three months to 20 years. Selection

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criteria included proteinuria greater than 3.0 g per day, hypoalbuminemia (serum albumin less than or equal to 3.5 g per dl), creatinine clearance greater than or equal to 30 ml per minute per 1.73 m^2, and serum cholesterol greater than 240 mg per dl. Patients were excluded if they had a history of hypothyroidism or diabetes mellitus, chronic hepatic disease, hematocrit less than 35%, chronic estrogen, corticosteroid or immunosuppressive therapy, known allergies to iodine or to hypolipidemic drugs. Other exclusion criteria were family history of hypercholesterolemia and previous treatment with hypolipidemic agents. Patients selected for study had a diagnosis of dyslipidemia confirmed by repeated measurement of serum and triglycerides on two separate occasions. Hypercholesterolemia was defined as a total cholesterol greater than 240 mg/dl and a total triglyceride less than 250 mg/dl [15]. Combined hyperlipidemia was defined as a total cholesterol greater than or equal to 240 mg and triglycerides greater than or equal to 250 mg/dl. Lipid measurements were made after a 12 hour fast, and samples were collected at least one week apart. The study, a control phase of a drug trial was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas. All subjects gave informed written consent to participate in the study.

LDL kinetic data from selected control groups of previously published studies from our laboratory were employed for comparison with nephrotic patients [16–18]. The groups consisted of: (1) 19 young men [mean age 25 ± 1 years, body mass = 102 ± 2% SEM of desirable weight], (2) 16 middle-aged men [mean age = 55 ± 2 years, body mass index (BMI) = 24.6 ± 1.1 kg/m^2], (3) 13 post-menopausal women (mean age = 57 ± 2 years, BMI = 26.0 ± 0.4 kg/m^2), and (4) 11 middle-aged men (mean age = 52 ± 1 years, ideal body mass = 144 ± 2%) with primary combined hyperlipidemia. The groups of young and middle-aged men, and postmenopausal women were normolipidemic. The same methods for study of lipoprotein metabolism were employed in previous studies and in the current one. All the measurements for the control groups and the patients with nephrotic syndrome were made in the same laboratory without a change in procedure.

Diets

The study diet consisted of 40% of total calories as fat (18% saturates, 17% monounsaturates, and 5% polyunsaturates), 15% protein, 45% carbohydrate and 400 mg of dietary cholesterol per day. Dietary counseling was provided by the dietician throughout the study. Weights of the patients were monitored regularly, and caloric adjustments were done as needed to maintain a constant body weight.

Measurement of plasma lipids and lipoprotein cholesterol

Total cholesterol and triglyceride levels in plasma were measured enzymatically [19, 20]. High-density lipoprotein (HDL) cholesterol was measured after polyanion precipitation of apo B-containing lipoproteins with heparin-manganese. LDL-cholesterol was determined after ultracentrifugal isolation of the fraction d < 1.019 g per ml (VLDL + IDL) from plasma samples, and determination of cholesterol in the VLDL + intermediate density lipoprotein (IDL) and the plasma infranatant (d > 1.019 g per ml). LDL cholesterol was calculated as the difference between total plasma cholesterol and the sum of VLDL + IDL + HDL cholesterol [17].

Measurement of the turnover rates of LDL-apo B

Each patient had a plasmapheresis done one week before the turnover test. The plasma was used to isolate autologous LDL as detailed previously [21]. Briefly, LDL was isolated by preparative ultracentrifugation at a density of 1.019 to 1.063 g per ml. The lipoprotein was washed once at its native density, and an aliquot was dialyzed against 150 mM NaCl, 0.01% disodium ethylenediamine tetraacetate (EDTA), pH 7.4. LDL apolipoprotein (2 to 3 mg of protein) was iodinated with ^{131}I using the iodine monochloride method [21, 22]. Unbound radioactive iodine was removed from LDL by dialysis. The lipoprotein was mixed with 5% human serum albumin and unlabeled autologous LDL, millipore filtered through a 22 μm pyrogen-free filter. The injection mixture was tested for pyrogens with a limulus test.

The patients were admitted to the General Clinical Research Center to start the turnover test. Approximately 20 μCi of autologous ^{131}I-LDL was injected intravenously after a 12 hour fast. Blood samples were collected at 0, 5, 10, 20, 30, 60, 120, 180, and 240 minutes after injection, and at 8, 12, 24 hours, followed by a daily blood drawing for 13 more days. Plasma samples were aliquoted for counting, and a plot of fraction of injected dose as a function of time was constructed. This plot was used subsequently for estimation of the fractional catabolic rates using a two-pool mammillary model with the Simulation and Modeling Program, version 30 [23]. Plasma volumes were estimated by isotopic dilution using the 10 minute sample for these calculations. Levels of LDL-apo B were measured as detailed below. The pool size of LDL apo B was calculated as the product of LDL apo B concentration and plasma volume. Input rates were estimated as the product of the pool size and the fractional catabolic rate.

Measurement of LDL-apo B concentrations

During the last week of the turnover, additional blood was drawn on five consecutive days to isolate LDL by preparative ultracentrifugation [24]. The content of total cholesterol and apolipoprotein was measured and the ratio of LDL-cholesterol-to-apolipoprotein was calculated. The absolute concentration of apo-B was estimated by dividing the absolute concentration of LDL-cholesterol by the ratio [21].

Renal function measurements

Urinary and plasma creatinine were measured by the Jaffe reaction and urinary protein was measured by the pyrogallol red method (Smith, Kline Laboratories, Dallas, Texas, USA). Urine protein excretion rate was normalized for urinary creatinine excretion and expressed as the ratio of protein to creatinine. Creatinine clearance was expressed in ml/min/1.73 m^2.

Statistical analysis

Data are summarized as means ± standard deviation. Analysis of variance (ANOVA) was used to compare control groups to hypercholesterolemic subjects, and to subjects with combined hyperlipidemia. Comparisons of parameter means between the hypercholesterolemic group and subjects with combined hyperlipidemia also were done by ANOVA. If the Bonferroni equality was used for multiplicity of testing, a P value (alpha) would be considered statistically significant at $P < 0.05/5$ or 0.01 because

Table 1. Clinical characteristics of patients

Patient	Renal disease	Age/ gender *years*	Body mass index *kg/m²*	Serum creatinine *mg/dl*	Serum albumin *g/dl*	Creatinine clearance *ml/min/1.73 m²*	Urine protein/ creatinine ratio
Hypercholesterolemia							
1	MGN	65/F	35.1	1.9	2.7	39	4.1
2	FS	28/F	30.7	1.7	2.6	34	5.6
3	FS	48/F	37.2	1.6	3.5	73	1.9
4	NIL	39/F	40.0	0.8	3.4	129	7.0
5	FS	27/F	35.0	1.4	2.4	80	7.8
6	MGN	68/M	43.2	1.2	2.8	61	5.4
7	FS	23/M	29.8	1.6	1.8	105	6.7
Mean		43	35.9	1.4	2.7	74	5.5
± SE		± 7	± 1.8	± 0.1	± 0.2	± 13	± 0.8
Combined hyperlipidemia							
8	FS	18/F	23.1	0.7	1.0	99	14.3
9	FS	40/F	25.9	1.7	3	44	2.5
10	UNK	61/M	31.9	2.5	2.5	41	3.8
11	MGN	44/M	27.6	2.3	3.2	59	4.7
12	MGN	44/M	28.1	1.5	2.8	91	6.8
13	FS	21/M	22.7	1.6	1.0	66	16.0
Mean		38	26.6	1.7	2.3	67	8.0
± SE		± 7	± 1.4	± 0.3	± 0.4	± 10	± 2.3
P		0.642	0.002	0.406	0.422	0.642	0.341

Abbreviations are MGN, membranous glomerulonephritis; FS, focal sclerosis; NIL, Nil disease; UNK, unknown disease.

five comparisons were made. Finally, Pearson's correlation coefficients were calculated for regression analysis of plasma triglyceride levels versus fractional catabolic rates for LDL after combining the data for both groups: hypercholesterolemia and combined hyperlipidemia.

Results

Comparison of clinical presentations of the two groups

The clinical characteristics of the patients are compared in Table 1. Seven patients had hypercholesterolemia, and six had combined hyperlipidemia. The mean ages for the two groups were similar. Patients with hypercholesterolemia alone had a higher average body mass index than those with combined hyperlipidemia ($P = 0.002$; Table 1). There were no differences in levels of serum creatinine, or albumin between the two groups of patients. Creatinine clearances and degree of proteinuria were similar in both groups. Among the hypercholesterolemic group, four patients (Nos. 2, 3, 5, and 7) had history of focal sclerosis, two (Nos. 1, and 6) had membranous glomerulonephritis, and one (No. 10) had Nil disease. Among the patients with combined hyperlipidemia, three had focal sclerosis, two had membranous glomerulonephritis, and patient No. 10 did not have a renal biopsy to determine the etiology of the nephrotic syndrome. In addition to elevated plasma cholesterol and obesity, other risk factors for coronary artery disease included hypertension (9 patients) and smoking (one patient). Two patients had history of coronary artery disease. Patient No. 1 had documented history of myocardial infarction, and patient No. 10 had coronary artery bypass surgery three years before entering the study. None of the patients were taking ACE inhibitors. The patients with hypertension were taking anti-hypertensive medications, but only patient No. 10 was taking a beta-adrenergic blocker. All patients continued their baseline medications and health habits during the time of the study.

Table 2. Plasma total lipids

Patient	Total cholesterol	Triglycerides	HDL cholesterol
	mg/dl		
Hypercholesterolemia			
1	282	104	50
2	258	192	39
3	280	141	75
4	222	100	60
5	289	79	64
6	286	216	39
7	212	123	47
Mean	261	136	53
± SE	± 12	± 19	± 5
Combined hyperlipidemia			
8	458	1021	23
9	268	390	33
10	275	617	22
11	404	323	33
12	281	733	27
13	578	336	26
Mean	377	570	27
± SE	± 51	± 12	± 2
P	0.037	0.002	0.001

Patients with combined hyperlipidemia had significantly higher levels of total cholesterol ($P < 0.037$), and triglyceride ($P < 0.002$), and lower levels of HDL cholesterol ($P < 0.001$) than did the patients with hypercholesterolemia (Table 2). However, there were no significant differences in the severity of the nephrotic syndrome between these two groups (Table 1). Although all patients with hypercholesterolemia had total cholesterol exceeding 240 mg/dl at the time of recruitment, the levels in two patients

Table 3. Kinetic parameters of LDL apo B

Patient	LDL cholesterol	LDL apo B	LDL cholesterol/ apo B	LDL apo B pool size	Fractional catabolic rate	LDL apo B input rate
	mg/dl		*ratio*	*mg*	*pools/day*	*mg/day/kg*
Hypercholesterolemia						
1	197	107	1.84	3093	0.211	6.5
2	187	120	1.56	3156	0.332	13.3
3	184	104	1.77	2458	0.375	12.9
4	146	73	2.00	3180	0.282	9.3
5	202	119	1.70	4931	0.254	13.6
6	196	122	1.61	4624	0.352	15.0
7	139	72	1.93	2560	0.296	9.6
Mean	179	102	1.77	3429	0.300	11.8
± SE	± 10	± 8	± 0.06	± 366	± 0.022	± 1.1
Combined hyperlipidemia						
8	172	150	1.15	4313	0.978	66.0
9	178	116	1.53	3059	0.330	17.2
10	120	104	1.15	3695	0.449	21.0
11	213	150	1.42	5437	0.429	29.0
12	111	98	1.13	3685	0.680	30.0
13	343	210	1.63	5112	0.214	20.1
Mean	189	138	1.34	4217	0.513	30.5
± SE	± 34	± 17	± 0.09	± 374	± 0.112	± 7.4
P	0.768	0.069	0.002	0 .162	0.069	0.02

(Nos. 4 and 7) were in the range of 200 to 240 mg/dl at the time of the lipoprotein turnover study (Table 2).

LDL metabolism

Nephrotic hypercholesterolemia vs. nephrotic combined hyperlipidemia. This comparison is shown in Table 3. There was no difference in average LDL-cholesterol levels between the two groups. However, LDL-apo B levels were somewhat higher in the group with combined hyperlipidemia. The ratio of LDL-cholesterol/apo B was lower in those with combined hyperlipidemia, indicating that they carried less cholesterol per LDL particle than did patients with hypercholesterolemia. There was a trend towards higher fractional catabolic rates (FCRs) in patients with combined hyperlipidemia. The input rates of LDL apo B were definitely higher than in hypercholesterolemia.

Nephrotic hypercholesterolemia versus normolipidemic controls. Levels of lipids, lipoproteins, and LDL kinetic parameters for control patients are in Table 4; a statistical comparison of the parameters with those of the two nephrotic groups are given in Table 5. Patients with nephrotic hypercholesterolemia had significantly higher levels of LDL cholesterol ($P < 0.0001$) than those of all the control groups (young, middle-aged men, and postmenopausal women). Levels of apo B also were significantly higher than young men and postmenopausal women ($P < 0.0001$). Further, the ratios of LDL cholesterol-to-apo B were significantly higher in nephrotic patients than in middle-aged men ($P < 0.001$) with a trend for significance for the other groups. Furthermore, there was a trend towards lower FCRs of LDL apo B in hypercholesterolemic nephrotic patients compared to control groups. In contrast, there were no differences in input rates for LDL apo B between the hypercholesterolemic patients and controls.

Table 4. Levels of plasma lipids, lipoproteins, and LDL kinetics of control groups

Parameter	Young men	Middle-aged men	Post-menopausal women	Primary combined hyperlipidemia
Number	19	16	13	11
Concentration *md/dl*				
Total cholesterol	158 ± 7	202 ± 4	211 ± 7	262 ± 4
Triglycerides	88 ± 7	138 ± 2	125 ± 20	492 ± 26
HDL-cholesterol	42 ± 3	38 ± 2	53 ± 3	25 ± 0.3
LDL-cholesterol	108 ± 7	129 ± 4	128 ± 4	130 ± 5
LDL apo B	70 ± 4	91 ± 3	80 ± 2	98 ± 4
LDL-chol/apo B (ratio)	1.54 ± 0.09	1.42 ± 0.03	1.59 ± 0.04	133 ± 0.03
Kinetic parameters				
LDL apo B fractional catabolic rate *(pools/day)*	0.36 ± 0.02	0.31 ± 0.01	0.35 ± 0.01	0.58 ± 0.02
LDL apo B input rate *(mg/day/kg)*	10.1 ± 0.5	12.8 ± 0.5	11.1 ± 0.6	22.0 ± 0.6

Nephrotic combined hyperlipidemia versus controls. Levels of LDL cholesterol and LDL apo B were significantly higher ($P < 0.001$) in nephrotic patients with combined hyperlipidemia compared to normolipidemic controls and to patients with primary combined hyperlipidemia. The ratios of LDL cholesterol-to-apo B tended to be lower in the nephrotic patients than in normolipidemic controls, but not compared to patients with primary combined hyperlipidemia. Further, FCRs for LDL apo B were significantly higher ($P < 0.001$) in nephrotic patients than in normolipidemic controls, but FCRs were similar to those in

Table 5. Alpha values for analysis of variance

	Hypercholesterolemia			
	vs. Young men	vs. Middle-aged men	vs. Postmenopausal women	vs. Primary combined hyperlipidemia
LDL-cholesterol	0.0001	0.0001	0.0001	0.0001
LDL apo B	0.0001	>0.1	0.0002	>0.1
LDL-cholesterol/ apo B	0.0334	0.0001	0.0977	0.0001
LDL apo B FCR	0.0347	>0.1	0.0977	0.0001
LDL apo B input rate	>0.1	>0.1	>0.1	0.0001
	Combined hyperlipidemia			
	vs. Young men	vs. Middle-aged men	vs. Postmenopausal women	vs. Primary combined hyperlipidemia
LDL-cholesterol	0.0001	0.0001	0.0001	0.0001
LDL apo B	0.0001	0.0001	0.0001	0.0001
LDL-cholesterol/ apo B	0.0977	0.0977	0.0977	>0.1
LDL-apo B FCR	0.0001	0.0001	0.0001	>0.1
LDL apo B input rate	0.0001	0.0001	0.0001	0.0001

patients with primary combined hyperlipidemia. LDL-apo B input rates in the groups with nephrotic combined hyperlipidemia were significantly higher ($P < 0.001$) than in both normolipidemic controls and patients with primary combined hyperlipidemia.

Relation of plasma triglycerides to fractional catabolic rate of LDL. The levels of plasma triglyceride correlated positively with the fractional catabolic rate of LDL for all patients ($N = 13$; Fig. 1). The correlation coefficient was 0.90, $P = 0.0001$. Three patients (Nos. 8, 10, 12) with combined hyperlipidemia had triglyceride levels above 600 mg/dl, and they also had the highest FCRs for LDL.

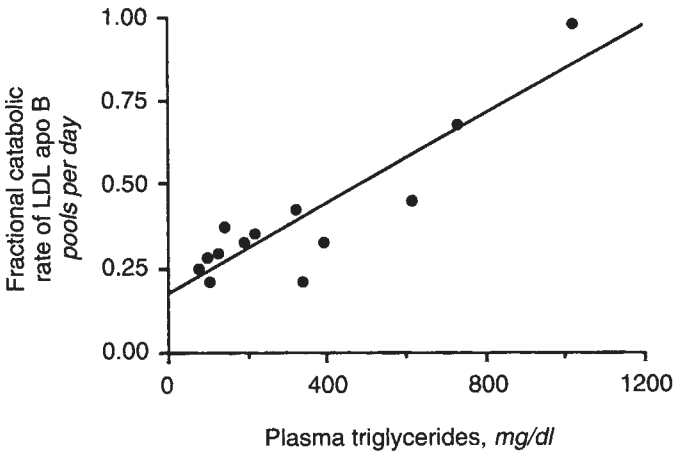

Fig. 1. *Plot of fractional catabolic rate (pools per day) of LDL apo B as a function of plasma triglyceride concentrations (mg/dl).* The linear regression analysis of plasma triglyceride levels vs. fractional catabolic rate of LDL apo B for all of the thirteen patients with nephrotic syndrome showed a significantly high correlation.

Discussion

For many years it has been postulated that the primary mechanism for hyperlipidemia in the nephrotic syndrome is an hepatic overproduction of apo B-containing lipoproteins. Studies in laboratory animals [9–11] and in tissue culture [5, 6] have suggested that low levels of serum albumin (or oncotic pressure) lead to increased secretion of lipoproteins by liver cells. In more recent years however, there is some evidence from animal studies showing that other mechanisms may contribute to the dyslipidemia of the nephrotic syndrome [9, 10, 25–27].

A limited number of investigations on the metabolism of apo B-containing lipoproteins have been carried out in human patients with nephrotic syndrome in the attempt to determine the causes of hyperlipidemia. Primary emphasis has been given to understanding causes of hypercholesterolemia (elevated LDL cholesterol). In several previous reports [12–14, 28], mixed patterns of LDL-apo B metabolism have been observed in nephrotic patients with hypercholesterolemia, and no single pattern has emerged as being predominant. For example, Vega and Grundy [21] reported that some nephrotic patients had an increased input (overproduction) of LDL, whereas other patients had a reduced clearance of this lipoprotein. In another study, Joven et al [13] concluded that increased production of LDL was mainly responsible for the hypercholesterolemia. Still, careful examination of their data reveals that input rates were not markedly elevated, and some of their patients also had reduced fractional clearance for LDL. Warwick et al [12, 14, 28] in three different reports indicated a dual mechanism for nephrotic hypercholesterolemia; some of their patients had overproduction of LDL, whereas others had a delayed clearance of LDL. An important point to make is that in these studies no attempt was made to correlate the metabolism of LDL with triglyceride levels, that is, patients with normotriglyceridemia and hypertriglyceridemia were not distinguished. Our previous studies [29–31] in patients with primary hypertriglyceridemia, however, have shown that LDL kinetics differ markedly depending on whether triglyceride levels are elevated. For this reason, the current study was designed to compare and contrast turnover rates of LDL in nephrotic patients with hypercholesterolemia and those with combined hyperlipidemia.

Hypercholesterolemia

In the current patients diagnosed as hypercholesterolemic, LDL-cholesterol levels were significantly higher than all control groups. Three mechanisms might account for increased LDL-cholesterol concentrations: overproduction of LDL particles, defective clearance of LDL, and an enhanced cholesterol content of LDL particles. Either of the former should be accompanied by increased LDL-apo B concentrations. Indeed, a high LDL-apo B level was present in the nephrotic patients which implicated one or the other, or both, of the first two mechanisms. Input rates for LDL-apo B were not found to be significantly higher in nephrotic patients compared to controls. However, input data for hypercholesterolemic subjects were normalized for total body weight, and since our nephrotic patients in this category were on the average more obese than controls, absolute input rates for LDL may have been somewhat higher in the nephrotic patients than in controls. Previous investigations [32, 33] have shown that obesity itself increases absolute input rates for LDL-apo B, but when input rates are normalized for total body weight, values are similar to those of normal-weight subjects. Therefore, if the nephrotic

syndrome had independently, raised input rates for LDL-apo B, values should have been elevated *after* normalization for body weight. Since such an increase was not found, it seems unlikely that the nephrotic syndrome *per se* contributed importantly to any enhanced input of LDL apo B in our hypercholesterolemic patients.

In contrast, fractional clearance of LDL apo B tended to be lower than in controls, especially as compared to young adult men and postmenopausal women. This finding suggests the presence of a relative defect in clearance of LDL apo B. If so, patients with the nephrotic syndrome may have had some down-regulation of LDL-receptor activity. This effect could be secondary to an increased synthesis of cholesterol, which has been reported in animals with experimental nephrosis [26, 27].

Another feature of LDL metabolism in our hypercholesterolemic patients was the presence of LDL particles that were overly enriched with cholesterol. LDL-cholesterol/apo B ratios averaged higher than those of control patients. Since each LDL particle contains only one molecule of apo B, an increase in the cholesterol/apo B ratio signifies an abnormally high cholesterol content of individual LDL particles. Our data indicate that a high LDL-cholesterol/apo B ratio contributed importantly to the hypercholesterolemia in many of nephrotic patients. The mechanism for this high ratio is not entirely clear. Those patients having higher ratios tended to have lower FCRs for LDL; consequently, a delay in clearance of LDL may have allowed more time for cholesterol ester transfer protein (CETP) to transfer more cholesterol ester to LDL particles. In addition, it has been reported that plasma levels of CETP are high in nephrotic patients [34, 35]. An elevation of CETP could cause still more enrichment of LDL particles with cholesterol esters. Thus, in our nephrotic patients having only hypercholesterolemia, a relatively low fractional clearance rate for LDL plus cholesterol enrichment of LDL particles appeared to contribute more to high LDL-cholesterol levels than did overproduction of LDL particles.

Combined hyperlipidemia

This condition denotes increased concentrations of both cholesterol and triglycerides. The nephrotic syndrome classically is characterized by hypercholesterolemia, but elevated triglycerides commonly are present as well. The mechanisms responsible for high triglyceride levels are not well understood. However, we previously reported that hypertriglyceridemia in nephrotic humans is due mainly to defective catabolism of VLDL triglycerides [21]. This finding has been confirmed by Warwick et al [14]. From studies in rats, Davies et al [9] proposed that urinary loss of a liporegulatory substance in the urine, independent of albumin, causes defective clearance of triglyceride-rich lipoproteins. Furukawa et al [10] likewise reported that experimental nephrosis in rats is associated with defective catabolism of VLDL triglycerides; this defect appeared to be due to resistance of VLDL to the action of lipoprotein lipase. Apo C-II, which is known to activate lipoprotein lipase, is lost in the urine of nephrotic patients [36], but since only small amounts are required for apo C-II's cofactor action, this mechanism probably does not explain the decreased catabolism of triglyceride-rich lipoproteins. Other factors therefore appear to be responsible.

Our patients with the nephrotic syndrome and combined hyperlipidemia, like those with hypercholesterolemia, had high levels of LDL cholesterol and LDL apo B. However, in contrast to the hypercholesterolemic patients, those with combined hyperlipidemia had strikingly elevated input rates for LDL apo B. An increased input of LDL apo B is consistent with studies in experimental animals in which an increased secretion of apo B-containing lipoproteins has been identified [1–3]. Nonetheless an alternate mechanism for a high input of LDL apo B can be envisioned. This is a reduced uptake of LDL precursors (VLDL and IDL) by the liver. By this mechanism, more of these LDL precursors would have the chance to be converted to LDL. Our kinetic studies do not directly differentiate between the alternative explanations for a high input of LDL apo B, but an important clue to a mechanism may be forthcoming by examining FCRs for LDL.

The nephrotic patients with combined hyperlipidemia had relatively high FCRs for LDL apo B. Theoretically, an increased hepatic secretion of apo B-containing lipoproteins would not be expected to produce the high FCRs for LDL observed in our patients. Instead, hepatic overproduction of lipoproteins should cause "overloading" of receptor-mediated clearance of lipoproteins, including LDL receptors, by LDL precursors; if so, FCRs for LDL should be relatively low. Instead, our patients with the highest input rates for LDL apo B generally had the highest FCRs for LDL. Since this response was the opposite of what would be expected from hepatic overproduction of apo B-containing lipoproteins, it seems unlikely that the high inputs of LDL can be explained primarily by this mechanism.

As indicated above, another mechanism for increased input into the LDL-apo B compartment could be a reduced hepatic intake of LDL precursors by the liver. In this case, more of these precursors (VLDL and IDL) would be converted to LDL. Since fewer precursors would be removed by the liver, more hepatic LDL receptors should be available for uptake of LDL, and hence, FCRs for LDL would be higher. This could account for the relatively high FCRs for LDL observed in nephrotic patients with combined hyperlipidemia. In fact, the fewer the number of LDL precursors removed by LDL receptors, the greater would be the number of lipoprotein particles converted to LDL and the higher would be FCRs for LDL. This pattern in fact was observed in our patients, and the phenomenon supports this mechanism as an explanation for their high input rates for LDL. Previous studies have shown a similar pattern of LDL kinetics in patients with primary hypertriglyceridemia, as well as in those with primary combined hyperlipidemia (Table 4). There appears to be something about the hypertriglyceridemic state that favors the conversion of LDL precursors to LDL, rather than direct uptake of these precursors by the liver. The precise mechanisms for this redistribution of fates of LDL precursors are unclear.

An important question is whether a redistribution in catabolic pathways for LDL precursors contributes to higher LDL-apo B levels. Certainly, there is an increased input of LDL apo B that should raise the levels. On the other hand, more LDL receptors are left unused by precursors and thus are available for LDL uptake. Consequently, the high FCRs for LDL could correct the imbalance in LDL levels caused by an increased input rate for LDL apo B. This appears to be the case for many patients with primary hypertriglyceridemia who do not have elevated LDL-apo B concentrations [29–31]. Thus, inspite of relatively high FCRs for LDL, the high LDL apo-B levels in patients with combined hyperlipidemia of the nephrotic syndrome still could be the result of a decrease in the expression of LDL receptors, with perhaps a contribution from increased hepatic secretion of apo B-containing

lipoproteins. Nonetheless, it is clear that the kinetics of LDL-apo B are quite different between the two forms of nephrotic dyslipidemia: hypercholesterolemia and combined hyperlipidemia. It also is apparent that kinetic data from these two forms of dyslipidemia should not be combined in any attempt to understand the mechanisms of elevated LDL-apo B concentrations in the nephrotic syndrome.

Conclusions

This study shows that LDL-apo B kinetics are strikingly different between nephrotic patients with hypercholesterolemia alone and those with combined hyperlipidemia. However, both forms are characterized by high levels of LDL apo B and LDL cholesterol. In patients with hypercholesterolemia, elevated LDL apo B concentrations appear to be due more to a decrease in fractional clearance of LDL apo B than to an overproduction of LDL particles. In these patients LDL-cholesterol levels are further enhanced by increases in LDL-cholesterol/apo B ratios. Patients with combined hyperlipidemia also have high levels of LDL apo B, but in contrast to those with hypercholesterolemia, LDL-cholesterol/apo B ratios tend to be low. Thus, in patients with combined hyperlipidemia, LDL-apo B concentrations usually are disproportionately high, compared to LDL-cholesterol levels, whereas the opposite is true for those with hypercholesterolemia. Another striking difference between the two forms of dyslipidemia is that input rates for LDL apo B are high in patients with combined hyperlipidemia, and yet, LDL-apo B levels remain only moderately elevated because of corresponding high FCRs for LDL. Since high input rates and high FCRs for LDL appear to be linked in these patients, it is not certain that the former are primarily responsible for the high LDL apo B levels. Instead, it is certainly possible that a reduced expression of LDL receptors still contributes to elevated LDL apo B in these patients. We must note that some degree of enhanced hepatic secretion of apo B-containing lipoproteins, as reported in some animal models, could not be excluded by our isotopic methods. However, the present study points to additional mechanisms besides overproduction of apo B-containing lipoproteins for augmenting LDL-apo B and LDL-cholesterol levels in the nephrotic syndrome, and these other mechanisms need further investigation.

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