Catabolism of Very Low Density Lipoproteins in Experimental Nephrosis

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Abstract. The effects of experimental nephrosis in rats, produced by puromycin aminonucleoside, include an elevation of plasma levels of all lipoprotein density classes and the appearance of high density lipoprotein (HDL) rich in apoprotein (apo) A-I and deficient in apo A-IV and apo E. The hyperlipoproteinemia is associated with an increase in hepatic synthesis of lipoproteins. The possible role of decreased very low density lipoprotein (VLDL were obtained from nonfasting animals by ultracentrifugation at *d* 1.006 and included chylomicrons) catabolism and its relationship to the apolipoprotein composition of nephrotic high density lipoproteins ($1.063 < d < 1.210$, or $1.072 < d < 1.210$ [HDL]) was explored. When ^{125}I-VLDL was injected, the faster plasma clearance of lower molecular weight apolipoprotein B (apo B_L) compared with that of higher molecular weight apo B_H which is seen in normal rats was not observed in nephrotic rats. Less labeled phospholipid, apo C, and apo E were transferred from VLDL to higher lipoprotein density classes. Heparin-releasable plasma lipoprotein lipase and hepatic lipase activities were decreased by 50% in nephrotic rats compared with pair-fed controls. Perfusion of livers with medium that contained heparin released 50% less lipase activity in nephrotic rats than in controls.

When heparin was injected intravenously, significant decreases in plasma levels of triglycerides and significant increases in levels of free fatty acids were observed in both groups of animals. In the nephrotic rats, 86% of the free fatty acids were in the lipoprotein fractions, as compared with 16% in the controls. Heparin treatment did not restore to normal the decreased apo B_L clearance in nephrotic rats but it produced an increased amount of apo A-IV and apo E in the plasma HDL. In vitro addition of partially pure lipoprotein lipase to whole serum from nephrotic rats significantly increased the content of apo E in HDL.

We conclude that the abnormal apoprotein composition of HDL in experimental nephrosis is the result of altered entry of apolipoproteins from triglyceride-rich lipoproteins, probably because of decreased lipolysis.

Introduction

The nephrotic syndrome is associated with increased plasma concentrations of lipoproteins and an increased hepatic lipoprotein synthesis rate (1, 2). A delay in triglyceride clearance due to decreased very low density lipoprotein (VLDL[1] were obtained from nonfasting animals by ultracentrifugation at *d* 1.006 and included chylomicrons) catabolism has been reported (3–5), which may contribute substantially to the hyperlipemia. We have previously reported studies of high density lipoprotein (HDL) catabolism in nephrotic rats, and have noted the deficiency of apoprotein (apo) A-IV and apo E in nephrotic HDL (6–8). The present investigation was undertaken to determine whether the abnormal apolipoprotein composition of nephrotic HDL could be related to changes in VLDL catabolism. Our previous studies of VLDL catabolism in normal rats (9) have shown that, of the two main forms of apo B in rat plasma, the smaller (apo B of lower molecular weight [apo B_L]) is removed at a more rapid rate than the larger (apo B of higher molecular weight [apo B_H]). For this differential catabolic rate to occur in the isolated perfused liver, prior exposure of the VLDL to lipase action is required

A portion of this work has been presented in abstract form (Garber, D. W., et al., 1983, *Fed. Proc.*, 42:2017).

Received for publication 16 August 1983 and in revised form 30 April 1984.

J. Clin. Invest.

0021-9738/84/10/1375/09 $1.00
Volume 74, October 1984, 1375–1383

1. *Abbreviations used in this paper:* apo, apoprotein; apo A-I, apo A-IV, apo B, apo C, apo C-II, apo E, apoproteins A-I, A-IV, B, C, C-II, and E; apo B_L, apo B of lower molecular weight; apo B_H, apo B of higher molecular weight; HDL, high density lipoproteins ($1.063 < d < 1.210$, or $1.072 < d < 1.210$); IDL, intermediate density lipoproteins ($1.006 < d < 1.020$); PAGE, polyacrylamide gel electrophoresis; VLDL, very low density lipoproteins that were obtained from nonfasting animals by ultracentrifugation at *d* 1.006 and included chylomicrons.

(10). In the present experiments, we have injected ^{125}I-VLDL and followed the disappearance of labeled apoproteins from plasma and have measured the concentrations of the remaining apoproteins in all of the lipoprotein density classes. We also measured heparin-releasable lipoprotein lipase and hepatic lipase activities. Our results provide evidence that decreased lipolysis of VLDL in nephrosis accounts for the abnormal apoprotein composition of HDL.

Methods

Induction of nephrosis. Male Fischer 344 rats that weighed between 200 and 250 g were injected intraperitoneally with 120 mg/kg of puromycin aminonucleoside (Sigma Chemical Co., St. Louis, MO). They were studied 7–9 d after injection, and only rats with accumulation of ascites fluid were used. Nephrosis was confirmed by measurements of levels of serum cholesterol and albumin. Rats were fed standard laboratory chow. Pair feeding was carried out by allowing control rats access to the same weight of chow that was eaten by the nephrotic rats the previous day.

Preparation of ^{125}I-VLDL and plasma lipoprotein fractionation. In the experiments in which heparin was injected in vivo, the VLDL ($d < 1.006$) was prepared from rats in which 10% sucrose was added to the drinking water. The lipoprotein was labeled with ^{125}I according to the procedure described by Bilheimer et al. (11) and contained 16% of the label in the protein moiety. All other ^{125}I-VLDL that was used in this study were obtained from rats fed a sucrose-casein special diet, labeled as previously described (9), and contained 72% of the label in the protein moiety. The difference in protein labeling may be related to the higher content of unsaturated fatty acids in ordinary chow diets and to a lower protein concentration that was used in the labeling procedure. Plasma lipoproteins were isolated by sequential density ultracentrifugation of densities of 1.006, 1.020, 1.063, and 1.210 as previously described (2), using a Ti-50 rotor in a Beckman L3-50 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) at 5°C. The top milliliter from each centrifuge tube was removed and dialyzed overnight against 0.15 M NaCl–0.002 M EDTA, pH 7.4, at 5°C, using dialysis tubing with an exclusion of 3,500 mol wt before analysis.

Apoprotein analysis. The measurement of apoprotein concentrations in isolated lipoproteins was carried out by SDS-polyacrylamide gel electrophoresis (PAGE), essentially according to Swaney et al. (12), as previously described (2). Radioassay of ^{125}I-labeled apoproteins was performed according to the method of Sparks and Marsh (13). Fractions of apoproteins that were obtained by treatment of whole plasma or isolated dialyzed lipoproteins were incubated 3 min at 100°C with an equal volume of a solution that contained 10% SDS, 10% glycerol, 10% 2-mercaptoethanol, and 0.1 M Tris (pH 7.0). The apoproteins were separated by SDS-agarose column chromatography (13), and the radioactivity was determined using a Beckman gamma spectrometer (Beckman Instruments Inc.) with a counting error of <5%. Lipoproteins were delipidated before analysis by the chloroform-methanol-ether procedure of Lux et al. (14).

Lipoprotein lipase and hepatic lipase measurement. Lipoprotein lipase and hepatic lipase activities were measured in the plasma of control and nephrotic rats that were obtained 10 min after the intravenous injection of heparin, by the method of Nilsson-Ehle and Schotz (15). The rats were anesthetized with Nembutal (60 mg/kg) and blood was obtained by abdominal aorta puncture 10 min after the injection of 1,000 U of sodium heparin (Liquaemin, Organon Inc., East Orange, NJ) per kilogram body weight. Postheparin plasma was isolated by centrifugation. In separate experiments, hepatic lipase activity was measured in the presence or absence of 1 M NaCl. After cannulation of the portal vein and the vena cava, blood was removed by perfusion with 15 ml of medium that contained 0.136 M NaCl and 0.02 M sodium phosphate, pH 7.4. Livers were then perfused with 30 ml of medium that contained 40 U of heparin per milliliter. The perfusates were collected and assayed for lipase activity after centrifugation to remove any cells that may have been present.

In the experiments in which incubation with lipase was carried out in vitro, lipoprotein lipase was obtained from the medium in which a rat adipocyte cell line had been cultured, and to which heparin was added before collection of the culture medium (16).

Lipid and protein analyses. Plasma levels of triglyceride were measured by the method of VanHandel and Zilversmit (17) after lipid extraction by the method of Folch et al. (18). Phospholipids were measured by the Bartlett method (19) and cholesterol by the method of Zlatkis et al. (20). Free fatty acids were measured by the method of Duncombe (21). Protein measurements were carried out in the presence of 0.4% SDS by the method of Lowry et al. (22) using bovine serum albumin as a standard. Plasma albumin was measured by the method of Doumas et al. (23).

Results

Nephrosis was confirmed in puromycin aminonucleoside-injected rats by the presence of plasma cholesterol levels > 250 mg/dl. Plasma albumin concentrations averaged 3.41±0.17 g/dl ($n = 7$) in controls and 1.46±0.07 g/dl ($n = 7$) in nephrotics.

The first series of experiments were designed to answer the question of whether or not there was a decrease in the removal of total apo B, or of the individual B apoproteins in nephrotic rats. At 3 min after the injection of ^{125}I-VLDL, the distribution of label among apoproteins in whole serum of nephrotics was not different from that of the injected material (data not shown). In controls, the apo B_L component of plasma was less than that of nephrotics at 3 min. By 30 min, the ratio of labeled B_L to B_H decreased from 4.0 to 1.4 in control rats, which was in agreement with our earlier observations (9), while in the nephrotics the labeling ratio was unchanged. The injection of heparin did not produce a further decrease in the apo B_L/apo B_H labeling ratio in either controls or nephrotics (Table I).

Significant changes in the lipid concentrations in plasma were seen after heparin injection in both groups of animals as shown in Table II. Triglyceride levels were decreased by 58% (in paired experiments) in the nephrotic rats while in the controls they decreased by 77%. No significant changes in plasma phospholipid or cholesterol levels were observed. Total free fatty acid levels were not different from normal, but after heparin injection they rose in both groups of animals, with the nephrotic rats showing a greater increase (Table II). Table III presents the distribution of free fatty acids, which were measured 30 min after heparin injection, in the lipoprotein density classes and in albumin (the $d > 1.21$ fraction). 86% of the free fatty acids were associated with the lipoproteins rather

*Table I. Distribution of Radioactivity in Whole Plasma after Injection of ^{125}I-VLDL**

Type of rat	Number of rats	Time after injection	Percentage of total apoprotein label					Ratio, apo B_L/apo B_H
			Apo B_H	Apo B_L	Apo E	Apo C	I–	
		min						
Normal	8	3	1.13±0.059	4.42±0.201‡	4.31±0.180	74.8±0.740	15.6±0.460	3.96±0.151
Nephrotic	8	3	1.23±0.089	5.54±0.216	4.43±0.103	72.7±1.14	16.1±1.02	4.68±0.377
Normal	2	30	1.10	2.01	5.72	79.8	11.9	1.40
Normal plus heparin	4	30	1.29±0.064	2.83±0.769‡	4.75±0.362	80.5±2.29‡	12.2±0.753‡	2.17±0.552‡
Nephrotic	4	30	1.37±0.102	6.23±0.076	4.46±0.172	74.0±0.343	13.9±0.551	4.61±0.306
Nephrotic plus heparin	4	30	1.33±0.093	5.23±0.415	4.30±0.553	72.5±3.81	16.6±2.87	3.94±0.208

* Rats were injected intravenously under nembutal anesthesia with 3×10^7 dpm of ^{125}I-VLDL. ‡ Significantly different from the mean values for nephrotic rats at 3 min ($P < 0.05$).

than albumin in the nephrotic plasma, which was a situation opposite to that seen in the controls.

When the plasma was separated into individual density classes after the injection of ^{125}I-VLDL, as shown in Table IV, significant shifts of labeled lipid from VLDL to all of the higher density ranges were seen in normal rats when heparin was injected, with the most striking changes seen in the intermediate density lipoprotein ($1.006 < d < 1.020$ [IDL]) and low density lipoprotein ($1.020 < d < 1.063$ [LDL]) density fractions. Since ~85% of the label was in phospholipid in the original VLDL, and no changes in total phospholipid concentration were seen (Table II), these shifts in density were probably due to particle changes that resulted from triglyceride hydrolysis. In the nephrotic rats, heparin produced a much smaller decline in VLDL-associated lipid label with shifts into the higher density classes.

Table V shows the effects of heparin injection on the labeled apolipoprotein distribution in control and nephrotic rats. The amount of labeled apo C that was associated with HDL increased from 8 to 27% of total labeled apo C after injection of ^{125}I-VLDL into control rats that were not given heparin. This increase was somewhat less (to 20%) in the nephrotic rats. After heparin, <10% of labeled apo C remained with VLDL in the normals, while in the nephrotics, more than half was still in VLDL. In the control animals, there was a noticeable shift of apo B_H and apo B_L out of VLDL and into the IDL density range which was greatly increased by heparin, and a further appearance of large amounts of label in LDL. In the nephrotics, these changes were greatly diminished. No apo B_H and only 4% of apo B_L appeared in LDL even after heparin. For apo E, 9% appeared in HDL at 30 min in normals, and this amount was increased fourfold after heparin.

Table II. Effect of Injection of Heparin on Lipid Levels in Normal and Nephrotic Rats

	Cholesterol	Phospholipid	Triglyceride	Free fatty acids
	mg/dl	*mg/dl*	*mg/dl*	*mM*
Type of rat				
Normal	46±2.8 (10)	83±6.1 (10)	92±18 (5)	0.26±0.094 (4)
Normal plus heparin	47±1.0 (3)	88±1.4 (3)	21±10 (4)*	0.68±0.011 (6)*
Nephrotic	327±16 (10)	494±50 (10)	708±110 (8)	0.27±0.019 (4)
Nephrotic plus heparin	261±26 (4)	453±27 (4)	388±42 (4)‡	1.41±0.350 (4)*

The numbers in parentheses represent the number of rats. * Significantly different from the mean values for uninjected rats ($P < 0.001$). ‡ In four rats, a paired comparison of triglyceride levels in plasma at 3 and at 30 min after injection of heparin indicated a mean decrease of 58±13.1% ($P < 0.05$).

Table III. Free Fatty Acid Distribution in Lipoproteins of Rat Plasma

Type of rat	Percentage of total free fatty acid 30 min after injection of heparin				
	VLDL	IDL	LDL	HDL	$d > 1.21$
Control (4)	0.97±0.97	6.6±1.75	7.3±2.48	11.5±2.18	73.6±1.90
Nephrotic (4)	25.4±5.67*	5.0±0.41	14.1±0.43‡	41.4±2.62*	14.2±3.02*

The numbers in parentheses represent the number of animals. * Significantly different from the mean of the controls ($P < 0.001$). ‡ Significantly different from the mean of the controls ($P < 0.05$).

Similar changes occurred for apo E in the nephrotics, but they were not as pronounced (Table V).

Since heparin injection increased the amount of labeled apo E that appeared in nephrotic rat HDL, we also examined the apoprotein composition of the HDL by SDS-PAGE. As indicated in Table VI and Fig. 1, an increase in the content of apo A-IV, as well as apo E, was seen. Because the concentration of apo E that was present in the $d < 1.063$ fraction in nephrotic plasma was high, we were able to test the assumption that the appearance of apo E in nephrotic HDL after heparin injection could be duplicated in vitro by the addition of lipoprotein lipase to whole serum. The results of this experiment are given in Table VII and Fig. 1, where it can be seen that the amounts of both apo E and apo C increased in HDL and that these apoproteins stayed with HDL after recentrifugation.

The lipoprotein lipase and hepatic lipase activities released into the plasma after the injection of heparin were also

Table IV. Effect of Injection of Heparin on the Distribution of Labeled Lipid in Plasma 30 Min after Injection of ^{125}I-VLDL

	Percentage of total lipid label in				
	VLDL	IDL	LDL	HDL	$d > 1.21$
^{125}I-VLDL* (1)	94.1	2.9	0.6	0.5	1.9
Normal rats (2)	60.1	5.9	4.9	17.2	11.9
Normal rats plus heparin (4)	37.0	13.3	9.7	26.9	13.0
Nephrotic rats (4)	94.0	1.8	0.5	3.1	0.5
Nephrotic rats plus heparin (4)	86.7	3.2	2.8	6.4	0.8

The numbers in parentheses represent the number of individual samples which were pooled before ultracentrifugation. In whole plasma, 85±3% of the total lipid label was in phospholipid, as determined by thin-layer chromatographic analysis (42).

* Samples of ^{125}I-VLDL (uninjected) were added to whole rat plasma and subjected to sequential density ultracentrifugation as described in Methods.

*Table V. Effect of the Injection of Heparin on the Distribution of Individual Apolipoproteins in Four Density Classes of Plasma Lipoproteins 30 Min after Injection of ^{125}I-VLDL**

	Percentage of total apoprotein label in			
	VLDL	IDL	LDL	HDL
Apo B_H				
^{125}I-VLDL	93.6	6.4	—	—
Normal rats (2)	86.1	13.9	—	—
Normal rats plus heparin (4)	17.2	51.7	31.1	—
Nephrotic rats (4)	93.8	6.2	—	—
Nephrotic rats plus heparin (4)	91.3	8.7	—	—
Apo B_L				
^{125}I-VLDL	96.4	3.6	—	—
Normal rats (2)	91.0	9.0	—	—
Normal rats plus heparin (4)	13.5	40.9	45.6	—
Nephrotic rats (4)	96.5	3.5	—	—
Nephrotic rats plus heparin (4)	91.6	4.5	3.9	—
Apo C				
^{125}I-VLDL	89.5	1.4	1.1	8.1
Normal rats (2)	69.4	1.3	2.6	26.6
Normal rats plus heparin (4)	7.2	3.4	5.1	84.3
Nephrotic rats (4)	75.9	1.9	2.1	20.0
Nephrotic rats plus heparin (4)	51.8	2.6	4.0	41.5
Apo E				
^{125}I-VLDL	92.3	7.7	—	—
Normal rats (2)	86.2	4.6	—	9.2
Normal rats plus heparin (4)	39.3	9.8	13.7	37.2
Nephrotic rats (4)	90.2	4.3	—	5.5
Nephrotic rats plus heparin (4)	67.7	3.0	3.2	26.1

* Apolipoproteins from each density fraction were separated on SDS-agarose columns as described in Methods. The numbers in parentheses represent the number of rats. The value of ^{125}I-VLDL represents the sample of the original material added to normal rat plasma and carried through the density class isolation procedure.

*Table VI. Effect of Heparin Injection on the Apoprotein Composition of HDL**

	Percentage of total apolipoprotein‡			
	Apo A-IV	Apo E	Apo A-I	Apo C
Normal rats	10.4	5.6	67.6	16.4
Nephrotic rats	—	—	88.6	11.4
Nephrotic rats plus heparin (a)	1.9	3.0	84.8	10.3
Nephrotic rats plus heparin (b)	3.1	2.8	78.0	16.1

* Lipoproteins were separated as described in Methods and the 1.071 < d < 1.210 density fraction was isolated. Pooled plasma from 20 rats fed the casein-sucrose diet constituted the normal rat sample. Four nephrotic rats, pooled in pairs (a and b), were used after heparin injection.

‡ SDS-PAGE was carried out in the isolated HDL as described in Methods, and the percentage composition for each apoprotein was determined by densitometric scanning of gels stained with Coomassie Blue (2). A correction was made for the effect of differential dye binding by individual apoproteins as follows. A standard of apo HDL in 1% SDS-gel buffer was prepared and analyzed by densitometric scanning of stained gels. The same standard was also analyzed by high pressure liquid chromatography on a 7.5 × 600-mm TSK-3000 column in the same SDS buffer. Assuming that the absorbancy of each apoprotein was the same at 280 nM, the percentage of composition of the standard was calculated. The ratio of the relative amounts of each apolipoprotein as determined by high performance liquid chromatography to that determined by dye binding was 0.57, 0.64, 1.16, and 1.35, respectively, for apo A-IV, apo E, apo A-I, and the C apoproteins. Using these ratios, the percentage of composition as measured by dye binding was corrected, and the corrected values were recalculated as a percentage of the total apolipoprotein.

measured (Table VIII). Compared with pair-fed controls, a 68% decrease in lipoprotein lipase activity and a 41% decrease in hepatic (salt resistant) lipase was found in two sets of experiments. Direct measurement of heparin-releasable lipase activity, either in pair-fed or ad lib. fed rats in the perfused liver gave consistent results in three sets of experiments, which indicated a 50% decline in lipase activity in nephrotic liver (Table IX).

Discussion

The present experiments provide several sets of observations which indicate that VLDL lipolysis is relatively decreased in experimental nephrosis, thereby magnifying the effects of increased hepatic VLDL synthesis on plasma levels. Gutman and Shafrir (24) previously reported decreased adipose tissue lipase in experimental nephrosis. Although there have been reports of decreased heparin-releasable lipoprotein lipase activity in nephrosis (25), earlier work of Rosenman and Byers (26)

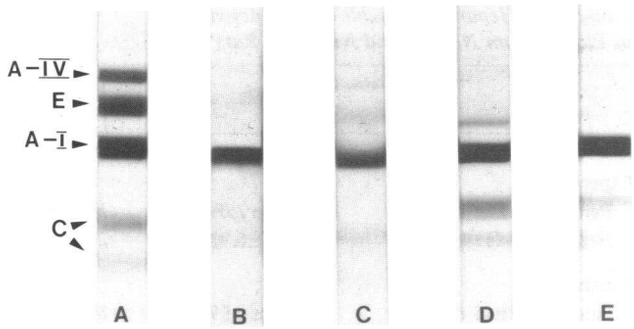

Figure 1. Coomassie-Blue stained SDS-PAGE of rat HDL (1.072 < d < 1.21). (*A*) Normal rat HDL; (*B*) nephrotic rat HDL; (*C*) HDL from nephrotic rats injected with heparin; (*D*) HDL from nephrotic rats after treatment of whole plasma in vitro with lipoprotein lipase; (*E*) HDL from an aliquot of the same nephrotic rat plasma treated with heat-inactivated lipase. Approximately 20 μg of protein was present in each gel except for the standard in *A* which contained 40 μg.

disclosed no differences; no measurements of hepatic lipase have been reported. A recent report by Calandra et al. (27) did not show significant differences in heparin-releasable plasma

*Table VII. Effect of Lipoprotein Lipase on the Apoprotein Composition of HDL when Added to Whole Serum from Nephrotic Rats**

	Percentage of total apolipoprotein			
	Apo A-IV	Apo E	Apo A-I	Apo C
Experiment 1				
(a) Heated enzyme				
1st spin	0.7	0.6	95.1	3.5
2nd spin	0.3	1.1	86.6	11.9
(b) Active enzyme				
1st spin	0.8	2.9	86.0	10.3
2nd spin	—	4.7	84.7	10.6
Experiment 2				
(a) Heated enzyme				
1st spin	0.3	0.4	91.2	8.0
2nd spin	—	0.9	90.8	8.3
(b) Active enzyme				
1st spin	0.4	2.6	83.0	13.9
2nd spin	—	4.0	79.7	16.3

* 0.5 of whole serum was added to 3 ml of enzyme solution (see Methods) and 3 ml of 3.0% bovine serum albumin (fatty acid-free) in 0.2 M Tris, pH 8.2, and incubated (with or without prior heating of the enzyme solution for 10 min at 80°C) for 2 h at 37°C. Sequential density ultracentrifugation at d 1.006, 1.020, 1.071, and 1.21 was then carried out. A portion of the HDL fraction was recentrifuged for 24 h at d 1.21 at 50,000 rpm in the 50 Ti rotor at 5°C. SDS-PAGE was carried out as described in Methods. The apoprotein composition was determined as described in the footnote to Table VI.

*Table VIII. Heparin-releasable Lipase Activity in Plasma from Normal and Nephrotic Rats**

	Hepatic lipase	Lipoprotein lipase
	U/ml	*U/ml*
Experiment 1		
Pair-fed normal rats (4)	80.1±2.09	54.8±4.47
Nephrotic rats (4)	65.7±6.92	22.1±8.16
Experiment 2		
Pair-fed normal rats (4)	73.6±0.99	80.2±5.15
Nephrotic rats (4)	26.8±2.21	18.5±2.30

The numbers in parentheses represent the number of rats.
* Lipase activity was measured as described in Methods. A unit of enzyme activity is defined as the liberation of 1 μmol of fatty acid from triolein per hour under the conditions of the assay. The mean±SEM for the groups of rats are indicated. The values for the nephrotics were significantly different from those of the controls in exp. 1 ($P < 0.05$ or 0.01) and 2 ($P < 0.001$).

lipase activity between control and nephrotic rats. In our experiments, the lipoprotein lipase and hepatic lipase activity of plasma, as measured 10 min after intravenous injection of heparin, was decreased by >50%, even under pair-fed conditions, compared with normal animals. Similar results were obtained by measurements of heparin-releasable lipase by perfused rat livers. Further evidence for decreased lipolysis was obtained by following the disappearance of labeled VLDL from plasma after intravenous injection. Three parameters that were related to lipolysis were measured—the apo B_L/apo B_H labeling ratio, the distribution of labeled apoproteins other than apo B, and the distribution of labeled lipid (mainly phospholipid) in the lipoprotein density classes that remained in plasma after 30 min. Whereas the apo B_L/apo B_H labeling ratio declined from 4.0 to 1.4 in the control rats, there was no significant change in the ratio in the nephrotic rats, even after the injection of heparin. Liver perfusion studies (10) have shown that VLDL lipolysis is necessary for the differential catabolism of apo B_L compared with apo B_H, although the extent of lipolysis that is required has not been precisely determined. In the normal rats, 30 min after injection of labeled VLDL, 17% of the total remaining labeled phospholipid in plasma appeared in HDL and 12% in the $d > 1.21$ fraction, while in the nephrotic rats only 3% of the phospholipid label was in HDL and 0.5% was in the $d > 1.21$ fraction.

Table IX. Hepatic Lipase Activity in Perfusates of Normal and Nephrotic Liver

	Hepatic lipase	Lipoprotein lipase*
	U/g liver	*U/g liver*
Experiment 1, rats pair-fed		
Normal rats (4)	12.4±0.81	5.4±0.78
Nephrotic rats (3)	6.2±1.03‡	2.4±0.68‡
Experiment 2, rats pair-fed		
Normal rats (5)	18.4±2.48	
Nephrotic rats (4)	9.0±1.51‡	
Experiment 3, rats fed ad lib		
Normal rats (5)	47.5±1.98	
Nephrotic rats (3)	24.8±0.87‡	

* Livers were perfused with medium that contained 40 U heparin/ml as described in Methods. Lipoprotein lipase was measured as the fraction of total lipase activity inhibited by 1 M NaCl. Heparin for experiment 3 was obtained from Sigma Chemical Co.; heparin for exps. 1 and 2 was obtained from Organon, Inc. Assay conditions are described in Methods. Lipoprotein lipase was estimated as the difference between total activity and that which was inhibited by 1 M NaCl (15).
‡ Significantly different from the mean of the normal rats ($P < .025$).

When heparin was injected into control and nephrotic rats, changes in the distribution of labeled apoproteins were observed at 30 min that complement the measurements of plasma lipase activity (measured at 10 min). In normal animals, heparin injection increased the amount of labeled apo C that was associated with HDL from 27 to 84%, due to its release from VLDL during the accelerated lipolysis. Much less occurred in the nephrotic rats; the percentage of total labeled apo C that was associated with HDL increased from 20 to 42%. After heparin, 31% of apo B_H and 46% of apo B_L were found in LDL in normal rats, while no apo B_H and only 4% of apo B_L were found in LDL in nephrotic rats, which again suggests decreased fractional lipolysis. At 30 min in the normal rats, considerable amounts of these two apolipoproteins were found in IDL (52 and 41%, respectively, for apo B_H and apo B_L), while there was very little (9 and 5%) in the IDL of the nephrotic plasma. We conclude that in spite of the decreased total triglyceride content of the plasma after the injection of heparin in the nephrotic rats, the resulting increase in average VLDL particle density was insufficient to cause a large shift into the $d < 1.02$ range. We did not separate chylomicrons from VLDL in these animals, so that a portion of the decline in total mass of plasma triglycerides may have been due to loss of chylomicron triglyceride. Finally, in these tracer experiments, the behavior of labeled apo E was qualitatively similar to that of apo C. In the nephrotic rats after heparin, labeled apo E shifted from VLDL to HDL, although less apo E appeared in nephrotic HDL than in controls. The most striking differences were seen in LDL, where the percentages of apo E were 14% (controls) compared with 3% (nephrotics). The changes in labeled apo E distribution in HDL were accompanied by changes in mass distribution (Table VI), and they were also observable by the direct addition of a purified lipoprotein lipase preparation to whole nephrotic plasma.

These observations provide strong evidence that apo E appears in the rat HDL density class as a consequence of

lipolysis of triglyceride-rich lipoproteins. The work of Weisgraber et al. (28) and of Blum (29) has clearly shown the ability of apo E to transfer from VLDL to HDL. In explaining the lack of apo E in nephrotic HDL as due to a failure of VLDL lipolysis, we must take into account the fact that perfused livers of nephrotic rats secrete appreciable amounts of apo E in the HDL density range (2). Our present hypothesis is that this apo E does not remain with nascent HDL after the latter reaches the plasma, but further experiments will be required to substantiate this point.

Rat HDL contains appreciable amounts of apo A-IV, which, in contrast to hepatically derived apo E, is largely an intestinal product (30). A decreased chylomicron lipolysis rate in nephrosis would therefore lead to an apo A-IV deficient HDL if apo A-IV behaved in a fashion similar to apo C and apo E. Our experiments with the in vivo injection of heparin in nephrotic rats suggest that this is so. However, much of plasma apo A-IV is in the $d > 1.21$ fraction (31), and this lipid-poor apo A-IV might also be a precursor of the apo A-IV of HDL. Because of its small size compared with that of the triglyceride-rich lipoproteins, it might be lost in the urine of the nephrotic rat. The fact that we were able to demonstrate an increase in the apo A-IV content of nephrotic HDL in vivo but not in vitro upon the addition of lipoprotein lipase may be explained by the low apo A-IV concentration in the total $d < 1.006$ fraction of nephrotic plasma. Presumably, in vivo the rapid metabolism of chylomicrons provides a continuous supply of apo A-IV. It is pertinent that in streptozotocin-diabetic rats that show decreased triglyceride and VLDL catabolism (32), the HDL is also deficient in apo A-IV and apo E (33, 34).

The conclusion that there is decreased fractional VLDL lipolysis in nephrosis is supported by the experiments presented here. The question then arises as to the mechanism. The lipase reactions that are responsible require not only the enzyme, but also, in the case of lipoprotein lipase, a cofactor, apo C-II, and an acceptor for the fatty acids that are formed, which is normally albumin. Furthermore, if catabolism is to proceed beyond the remnant stage, there must be an adequate hepatic capacity for remnant removal, since the liver is the main site of chylomicron and VLDL remnant uptake (36). With regard to the enzymes involved, lipoprotein lipase and hepatic lipase, our experiments are compatible with the idea that the amount of these enzymes may be decreased, but they do not rule out the possible presence of endogenous plasma inhibitors of lipase activity, nor the possible absence of lipase activators such as those described by Kashyap et al. (36) and Staprans et al. (37). The C apoproteins are present in large amounts in nephrotic plasma (8), and the lack of apo C-II would not seem to be a likely explanation for the greatly decreased VLDL fractional catabolic rate. Although puromycin aminonucleoside itself may have contributed to the decreased lipase activity, we consider that unlikely. The rats were studied 7–8 d after its administration, by which time any direct effects of puromycin aminonucleoside on lipase activity would be minimal.

The role of fatty acids as inhibitors of lipase reaction rates, and the role of albumin as a free fatty acid acceptor, can be addressed on the basis of our measurements of fatty acid levels in the various plasma density classes and in whole serum. In nephrotic rats that were fed ad lib., and killed at the same time of day as the controls, we did not find significant differences in the total free fatty acid levels, which is in agreement with earlier studies in humans (38). From the levels of albumin that were found, the free fatty acid to albumin molar ratio can be calculated. Assuming that all of the free fatty acids were bound to albumin, the molar ratio would be 0.5 in normals and 1.0 in nephrotics. This molar ratio is not high enough to produce a significant slowing in the rate of lipolysis (39). After the injection of heparin, the free fatty acid content at 30 min was increased 2.6-fold in controls and 5.2-fold in nephrotics. However, because of the high plasma levels of lipoproteins and the low levels of albumin, most of these fatty acids were not bound to albumin but to the lipoproteins themselves. Based on the distribution data shown in Table III, which are similar to the results in human nephrotic syndrome (38), the calculated free fatty acid to albumin molar ratio was 0.97 for controls and 0.76 for nephrotics, which suggests that even after heparin, saturation of binding sites for free fatty acids on the albumin molecule is not a likely cause for inhibition of lipolysis. The question of whether the increased amounts of free fatty acids in the VLDL fraction could contribute to a slowing of the rate of lipolysis may be examined by calculating the free fatty acid to triglyceride ratio based on the data in Tables II and III. Without heparin injection, the total free fatty acid levels are low enough that even if 25% were in VLDL, they would only amount to 0.3% of the triglyceride in nephrotic rats and 0.1% in normal rats. After heparin, the free fatty acids amounted to 2.7% of the triglyceride in nephrotics and 1.0% in normals, which may have contributed to an eventual slowing of the lipolytic rate.

Perhaps the most difficult question to answer on the basis of the present work is the effect of increased plasma VLDL concentration per se, as a result of increased hepatic synthesis (2), on the catabolic rate. At least two questions must be answered. First, are the endothelial-bound lipases saturated at a steady state concentration of VLDL which is sevenfold increased over normal? If they are, then the fractional catabolic rate would be decreased and we might not be able to observe a significant change in the apo B_L/apo B_H labeling ratio at 30 min because too small a percentage of the total pool was turned over. We do not believe this was the case. Although the apo B_L/apo B_H ratio was greatly reduced at 30 min in the controls, we have not observed significant changes in this ratio in nephrotics even at 60 min (data not shown), which is at least seven times the normal half-life of rat VLDL apo B (9). Second, are the hepatic remnant receptors saturated at the levels of VLDL or are its remnants present in nephrotic plasma? Further work is needed to answer this question.

At present, the most likely explanation for our results is a decrease in the absolute amount of lipoprotein lipase and

hepatic lipase in nephrotic rats. Preliminary experiments indicate that ^{125}I-VLDL from nephrotic rat plasma is readily catabolized in normal rats. Shafrir and Biale (40) have made the interesting suggestion that high circulating levels of VLDL may "leach" lipoprotein lipase from adipose tissue. We feel that the decreased lipase levels may reflect the general increase in the catabolism of body proteins, especially muscle, which occurs due to the metabolic channeling of amino acids to the liver for the synthesis of secretory proteins such as albumin and lipoproteins (41). Ultimately, this is a futile process, since the albumin is lost in the urine. It now seems clear that for the plasma lipoproteins, increased production is not accompanied by comparably increased removal rates.

Acknowledgments

The authors wish to thank Mrs. Sarah Massey, Mrs. Arline Ritz, and Mr. William Shriver for excellent technical assistance. We also thank Mrs. Holly McCloskey and Dr. Jane Glick for the lipoprotein lipase preparations and for their help with the lipoprotein lipase assays.

This work was supported in part from National Institutes of Health grant HL-22633 and from the W. W. Smith Charitable Trust.

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