Lipoprotein metabolism in experimental nephrosis

Julian B. Marsh

Department of Physiology and Biochemistry, The Medical College of Pennsylvania,
3300 Henry Avenue, Philadelphia, PA 19129

Proteinuria, hypoalbuminemia, edema, and hyperlipemia are the main features of the nephrotic syndrome. The proteinuria, which leads to the other characteristics of the syndrome, is caused by increased permeability of the glomerular capillary to plasma proteins. The glomerular filtration apparatus is a complex barrier formed by the surface glycoproteins of endothelial and epithelial cells which enclose the glomerular basement membrane (1). Damage to the filtration apparatus occurs in several human diseases, such as disseminated lupus erythematosus, interstitial glomerulosclerosis (seen in diabetes), idiopathic lipoid nephrosis, and others (2). It can also be produced experimentally by the intravenous injection of heterologous and homologous antibodies to glomerular glycoprotein antigens (3) or by administration of puromycin aminonucleoside (PAN) (4). The glomerular filtration apparatus is ordinarily more permeable to uncharged macromolecules than it is to proteins of the same size carrying a negative charge at pH 7.4. The loss of this charge selectivity, as well as the change in the size-selective barrier, is responsible for the proteinuria in nephrosis (5). Because it is much smaller than most plasma globulins, and because it comprises more than half of the total plasma proteins, plasma albumin is the main urinary protein found in nephrosis.

The hyperlipemia in the rat is a reflection of a 4- to 8-fold increase in the plasma concentration of all of the lipoprotein density classes (6). With the exception of HDL_3, all of the other plasma lipoproteins are too large to escape the damaged glomerular capillary. Human HDL_3, with an average diameter of about 70 Å is found in the urine of patients with nephrotic syndrome in appreciable amounts, so that human subjects with nephrosis may have normal plasma levels of HDL as a result of increased synthesis (7). In rats, however, most of the HDL is HDL_2 with a diameter of about 110 Å and its urinary loss is only 1.8 mg of cholesterol, or 10% of the plasma HDL cholesterol pool, per day (8).

Gherardi and co-workers (9) reported that in a group of seven children averaging 10 yr of age, and total serum cholesterols of about 580 mg/dl, the plasma concentrations of VLDL, IDL (1.006 < d < 1.019

g/ml), and LDL were, respectively, 6.3, 5.3, and 3.0 times normal. In contrast, HDL_2 and HDL_3 were 0.6 and 0.8 of normal. There were changes in lipid composition in all density fractions; perhaps the most striking changes were in the d < 1.063 g/ml fractions where the phospholipid and esterified cholesterol were increased and the free cholesterol and protein were decreased. In VLDL, cholesteryl esters tended to replace triglyceride. In nephrotic rats, similar but less striking changes have been observed (6, 10). As we shall point out later on, these changes in lipid composition may be related to a decreased lipoprotein lipase and hepatic lipase activity.

At about the time the Journal of Lipid Research was born, David L. Drabkin and I put forth the hypothesis (11) that the hyperlipemia of the nephrotic syndrome was due to hepatic overproduction of plasma lipoproteins, which were too large to be lost readily in the urine. The evidence in support of hepatic overproduction of lipoproteins was obtained in the isolated perfused rat liver (11). Studies by Radding and Steinberg (12) with liver slices reached the same conclusion. The increased synthesis of the protein moiety drives the hepatocyte to increase de novo lipid synthesis. Increased hepatic synthesis of total fatty acids, cholesterol, and triglycerides (13, 14) has been reported, but not phospholipids (15).

In collaboration with Dr. Charles E. Sparks, we have confirmed the findings of increased hepatic synthesis of lipoproteins in nephrosis and extended them to include the individual apolipoproteins (16). **Table 1** summarizes these results and compares them to those obtained two decades earlier with a different method of induction of nephrosis (anti-kidney serum) and a different methodology. Perhaps the theory of cancelling errors applies, but it is nevertheless gratifying to note the agreement between the two studies. The studies shown in Table 1, and similar observations reported by Calandra et al. (17), point to a far greater percentage increase in apoA-I synthesis by nephrotic liver than for the other apoli-

---

Abbreviations: AKS, anti-kidney serum; PAN, puromycin aminonucleoside; VLDL, IDL, LDL, and HDL, very low, intermediate, low, and high density lipoproteins, respectively.

TABLE 1. Lipoprotein and apolipoprotein secretion by perfused liver of normal control (C) and nephrotic rats (N)

Ref.	Conditions of Study	Total Protein		ApoA-I		ApoB		ApoC		ApoE	
		C	N	C	N	C	N	C	N	C	N
11	AKS nephrosis, immunoprecipitin measurement (1960)	108	230								
16	PAN nephrosis, ultracentrifugal isolation, SDS-PAGE (1979)	92	172	3	25	18	41	37	38	35	69

The header "Lipoprotein Output, μg Protein per g Liver per hr" spans the ApoA-I, ApoB, ApoC, and ApoE columns.

poproteins. Ordinarily, the rat liver is responsible for less than half of the total synthesis of apoA-I per day, the remainder coming from the intestine (18). The question, however, is why is there an increase in synthesis of any apolipoproteins? No answer has been given to this question, though of course there has been speculation. The liver appears to be responding to a general signal to increase the synthesis of all of the plasma proteins, especially albumin (11). What is the putative sensor responding to—decreased plasma oncotic pressure resulting from the hypoalbuminemia, or some change in plasma viscosity as suggested by Yedgar et al. (19)? Is some plasma substance that normally inhibits hepatic protein synthesis lost in the urine? The across-the-board nature of the hepatic response naturally suggests a hormonal effect, possibly originating in the hypothalamus. In nephrosis produced by anti-kidney serum (AKS), there is a precisely timed hyperplasia of the hepatocytes which begins 18 hr after AKS treatment and results in about a 30% increase in liver weight (20). This hypertrophy is not seen after PAN[1] possibly because the active metabolites of the compound are inhibitors of RNA synthesis (21). Nevertheless, increased hepatic plasma protein synthesis occurs in nephrosis, regardless of how it is induced. In plasmapheresis, where plasma proteins but not red cells are simply removed after bleeding, hyperlipemia and increased lipoprotein synthesis also occurs (22). This observation strengthens the conclusion that it is the loss of some plasma constituent, rather than something related to glomerular damage per se, which is involved. The fact that apolipoprotein synthesis is not increased to the same extent for each apolipoprotein suggests that feedback regulatory mechanisms exist which are superimposed on the overall stimulation of hepatic synthesis of secretory proteins.

The demand for plasma protein synthesis by the liver results in amino acid mobilization from muscle and

[1] No significant differences in the liver weight as a percentage of body weight were found in a series of 32 rats rendered nephrotic with PAN, as compared with controls, in the author's laboratory.

possibly other tissues as well. Consequently, the free amino acid concentration in muscle and plasma is low (23) and the animal is in negative nitrogen balance. This is not surprising considering the fact that a nephrotic rat can lose as much albumin in the urine per day as its normal total plasma pool (24). When precise measurements were made, it turned out that the animal's dietary intake of protein could compensate for urinary losses were it not for the fact that urea production was not appreciably reduced (21).

Returning to the problem of nephrotic hyperlipemia, while our laboratory was busy polishing the hepatic overproduction model, turnover studies in human nephrosis were conducted by Gitlin and co-workers (25) which concluded that the conversion of S_f 10–200 lipoproteins (VLDL) to S_f 3–9 (LDL) was impaired. These pioneering studies were among the very first in which radioiodinated plasma proteins were used to measure metabolic rates in the steady state. Delayed triglyceride clearance from plasma has also been reported (26).

A few years ago, our laboratory began a series of investigations designed to explore further the question of why a decreased fractional catabolic rate should exist for any of the lipoprotein classes. We began with a study of HDL catabolism. As shown in **Table 2**, nephrotic rat HDL ($1.063 < d < 1.21$ g/ml) is abnormal, having very little apoE or apoA-IV and a reduced amount of apoC. We carried out a crossover study in which labeled

TABLE 2. Apolipoprotein composition of plasma HDL from control and nephrotic rats[a]

Apoprotein	Control	Nephrotic
	percent of total apoprotein	
A-I	45 ± 2.50	75 ± 1.12
A-IV	11 ± 0.80	1.0 ± 0.49
C	32 ± 1.03	21 ± 0.87
E	13 ± 1.02	1.0 ± 0.61

[a] From the data in ref. 6. The values shown are means ± SEM and there were 16 control and 10 nephrotic rats used in the study.

TABLE 3. Fractional catabolic rate of normal and nephrotic ^{125}I-HDL in normal rats[a]

Type of ^{125}I-HDL	Fractional Catabolic Rate of Apolipoprotein				
	apoHDL	apoA-I	apoA-IV	apoE	apoC
Normal	0.098	0.098	0.108	0.098	0.105
Nephrotic	0.098	0.099	0.106	0.074	0.105

[a] From the data in ref. 28. In nephrotic rats, the fractional catabolic rate of normal HDL was 0.053. The values shown are the means of four experiments.

HDL from either normal or nephrotic rats was injected into both normal and nephrotic rats (27). The main conclusion from this study was that the catabolism of labeled HDL in rat plasma was not related to its content of apoA-IV, apoE, or apoC since normal or nephrotic HDL had the same residence time in normal rats. A summary of these data is given in **Table 3.** From these results, it is likely that apoA-I determines the rate of catabolism of the other apoproteins of the particle. The observations also suggest that the apoE-rich HDL species, which can be separated from the HDL mixture by heparin-Sepharose chromatography (28), must behave in a fashion quite different from apoE-rich VLDL or chylomicron remnants, which are rapidly metabolized. Severely nephrotic rats were found to have plasma apoA-I concentrations 7-times normal but the absolute catabolic rate increased only 2.7-fold (29). There was a direct relationship between the plasma HDL concentration and the biological half-life: that is, the higher the plasma level, the longer the $t_{1/2}$. The implication is that, if there are receptors for HDL uptake and degradation, as work from several laboratories has indicated (30–32), they are saturated at plasma levels of HDL that are not very much higher than normal. Thus, increased hepatic synthesis of HDL begins to expand the pool size and there may be no compensatory increase in the number of catabolic receptors. The HDL lost in the urine probably increases as the plasma concentration rises. HDL is altered during passage through the tubules (33) and work from Steinberg's laboratory suggests that the kidney may be an important organ for apoA-I catabolism (34).

The catabolism of VLDL in nephrotic rats was also examined after injecting ^{125}I-labeled VLDL. All of the results pointed to a decrease in the rate of catabolism. There was very little decrease in the amount of labeled apoB remaining in the plasma, especially in the case of apoB$_L$ (B-48) which is present in large amounts in rat VLDL and which has a higher catabolic rate than apoB$_H$ (B-100) in normal rats (35, 36). In addition, Garber et al. (37) found decreased levels of heparin-releasable lipoprotein lipase and hepatic lipase. The decreased hepatic lipase activity, in particular, would be expected to result in an increased phospholipid content of the circulating lipoproteins, since this enzyme has a considerable phospholipase activity. Such increases in phospholipids have been reported in human and rat nephrotic plasma, though the degree of change was modest, averaging 10% for all lipid classes in humans (9) and 12–14% in rats (6, 10). An important piece of evidence pointing to decreased intravascular VLDL catabolism in nephrosis was the fact that the abnormal apoprotein composition of HDL could be explained on this basis, since much of the apoE in HDL arises from VLDL catabolism (38). Other possible explanations for decreased VLDL catabolism, apart from low lipase levels, include the urinary loss of a lipoprotein lipase activator such as that described by Kashyap et al. (39) or by Staprans et al. (40). At present, it is difficult to evaluate these observations. ApoC-II, the accepted co-factor for lipoprotein lipase, is abundantly present in nephrotic rat plasma (6, 10, 41). Albumin itself when injected intravenously does not correct the catabolic defect.[2] Further work needs to be done to explore the possible saturation of VLDL remnant receptors in nephrosis. Increased hepatic synthesis of VLDL rapidly raises the plasma concentration, and if there were no increase in remnant receptors, there would be a decreased fractional catabolic rate even if lipase levels were normal. The reason for the low lipase levels is not apparent. It is not due to decreased dietary caloric intake, since pair-feeding did not eliminate the difference between nephrotics and controls (37). It is possible that amino acid mobilization from peripheral tissues, and utilization of amino acids for albumin synthesis by the liver, accounts for low tissue enzyme levels simply because of low concentrations of free amino acids, but there is no evidence for this at present. It is more likely that changes in hormones controlling tissue synthesis and degradation of intracellular enzymes occur. For example, circulating insulin levels have been reported to be low in nephrotic rats (42). The nephrotic syndrome, then, is characterized by hepatic overproduction of plasma lipoproteins unaccompanied by comparably increased catabolism. In fact,

---

[2] Unpublished experiments of D. W. Garber in the author's laboratory.

catabolism of triglyceride-rich lipoproteins not lost in the urine is actually decreased. The result is extreme hyperlipemia. There is evidence that this is accompanied by an increased severity of atherosclerosis (43). Certainly the risk must be greatly increased in humans since LDL levels are high and HDL levels are normal or low. Fortunately, steroid therapy of the nephrotic syndrome in humans has reduced the exposure time of individuals to such high plasma lipoprotein levels.

The fundamental mechanisms by which the liver is encouraged to increase its synthesis of plasma lipoproteins remain unknown. It would seem that we have a long way to go before we understand the metabolic events in the nephrotic syndrome. For example, only a brief report of an increased level of mRNA for apoA-I in nephrotic liver has so far appeared (44). I am confident that further explorations will lead not only to progress in understanding nephrosis, but in understanding the regulation of plasma lipoprotein synthesis in the physiological setting.▉▉

REFERENCES

1. Farquhar, M. G. 1981. The glomerular basement membrane: a selective macromolecular filter. *In* Cell Biology of Extracellular Matrix. E. D. Hay, editor. Plenum Press, New York. 335–378.
2. Pearl, M. A., R. R. Burch, E. Carvajal, B. H. McCracken, H. B. Woody, and W. H. Sternberg. 1963. Nephrotic syndrome: a clinical and pathological study. *Arch. Int. Med.* **112:** 716–726.
3. Boss, J. H. 1967. Antikidney antibodies. *Isr. J. Med. Sci.* **3:** 167–179.
4. Frenk, S., I. Antonowicz, J. M. Craig, and J. Metcoff. 1955. Experimental nephrotic syndrome induced in rats by aminonucleoside: renal lesions and body electrolyte composition. *Proc. Soc. Exp. Biol. Med.* **89:** 424–427.
5. Olson, J. L., H. G. Rennke, and M. A. Venkatachalam. 1981. Alterations in the charge and size selectivity barrier of the glomerular filter in aminonucleoside nephrosis in rats. *Lab. Invest.* **44:** 271–279.
6. Marsh, J. B., and C. E. Sparks. 1979. Lipoproteins in experimental nephrosis: plasma levels and composition. *Metabolism.* **28:** 455–465.
7. Baxter, J. H., H. C. Goodman, and R. J. Havel. 1960. Serum lipid and lipoprotein alterations in nephrosis. *J. Clin. Invest.* **39:** 455–464.
8. DeMendoza, S. G., M. L. Kashyap, C. Y. Chen, and R. F. Lutmer. 1976. High density lipoproteinuria in nephrotic syndrome. *Metabolism.* **25:** 1143–1149.
9. Gherardi, E., E. Rota, S. Calandra, R. Genova, and A. Tamborino. 1977. Relationship among the concentrations of serum lipoproteins and changes in their chemical composition in patients with untreated nephrotic syndrome. *Eur. J. Clin. Invest.* **7:** 563–570.

10. Gherardi, E., L. Vecchia, and S. Calandra. 1980. Experimental nephrotic syndrome in the rat induced by puromycin aminonucleoside. Plasma and urinary lipoproteins. *Exp. Mol. Pathol.* **32:** 128–142.
11. Marsh, J. B., and D. L. Drabkin. 1960. Experimental reconstruction of metabolic pattern of lipid nephrosis: key role of hepatic protein synthesis in hyperlipemia. *Metabolism.* **9:** 946–955.
12. Radding, C. M., and D. Steinberg. 1960. Studies on the synthesis and secretion of serum lipoproteins by rat liver slices. *J. Clin. Invest.* **39:** 1560–1569.
13. Marsh, J. B., and D. L. Drabkin. 1958. Metabolic channeling in experimental nephrosis. V. Lipide metabolism in the early stages of the disease. *J. Biol. Chem.* **230:** 1083–1091.
14. Shafrir, E., and T. Brenner. 1979. Lipoprotein lipid and protein synthesis in experimental nephrosis and plasmapheresis. I. Studies in rat in vivo. *Lipids.* **14:** 695–702.
15. Gherardi, E., and S. Calandra. 1979. Experimental nephrotic syndrome induced in the rat by puromycin aminonucleoside: hepatic synthesis of neutral lipids and phospholipids from ^{3}H-water and ^{3}H-palmitate. *Lipids.* **15:** 108–112.
16. Marsh, J. B., and C. E. Sparks. 1979. Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. *J. Clin. Invest.* **64:** 1229–1237.
17. Calandra, S., E. Gherardi, M. Fainaru, A. Guaitani, and I. Bartosek. 1981. Secretion of lipoproteins, apolipoprotein A-I and apolipoprotein E by isolated and perfused liver of rat with experimental nephrotic syndrome. *Biochim. Biophys. Acta.* **665:** 331–338.
18. Wu, A-L., and H. G. Windmueller. 1979. Relative contribution by liver and intestine to individual plasma apolipoproteins in the rat. *J. Biol. Chem.* **254:** 7316–7322.
19. Yedgar, S., D. B. Weinstein, W. Patsch, G. Schonfeld, F. E. Casanada, and D. Steinberg. 1982. Viscosity of culture medium as a regulator of synthesis and secretion of very low density lipoproteins by cultured hepatocytes. *J. Biol. Chem.* **257:** 2188–2192.
20. Marsh, J. B., D. L. Drabkin, and L. Poppe. 1970. Time sequence of macromolecule synthesis in experimental nephrosis. *Metabolism.* **19:** 392–399.
21. Taylor, J. M., and C. P. Stanners. 1968. The synthesis of RNA by mammalian cells treated with the aminonucleoside of puromycin. *Biochim. Biophys. Acta.* **155:** 424–432.
22. Brenner, T., and E. Shafrir. 1980. Lipoprotein lipid and protein synthesis in experimental nephrosis and plasmapheresis. II. Perfused rat liver. *Lipids.* **15:** 637–643.
23. Drabkin, D. L., J. B. Marsh, and G. A. Braun. 1962. Amino acid mobilization in plasma protein biosynthesis in experimental nephrosis. *Metabolism.* **11:** 967–977.
24. Marsh, J. B., and D. L. Drabkin. 1958. Metabolic channeling in experimental nephrosis. III. Influence of diet and of adrenalectomy; liver hypertrophy. *J. Biol. Chem.* **230:** 1063–1071.
25. Gitlin, D., D. G. Cornwell, D. Nakasato, J. L. Oncley, W. L. Hughes, Jr., and C. A. Janeway. 1958. Studies on the metabolism of plasma proteins in the nephrotic syndrome. II. The lipoproteins. *J. Clin. Invest.* **37:** 172–184.
26. Chan, M. K., J. W. Persaud, L. Ramdial, Z. Varghese, P. Sweny, and J. F. Moorhead. 1981. Hyperlipidemia in untreated nephrotic syndrome, increased production or decreased removal? *Clin. Chim. Acta.* **117:** 317–323.
27. Sparks, C. E., S. D. Tennenberg, and J. B. Marsh. 1981.

Catabolism of the apolipoproteins of HDL in control and nephrotic rats. *Biochim. Biophys. Acta.* **665:** 8–12.

28. Quarfordt, S. H., R. S. Jain, L. Jakoi, S. Robinson, and F. Shelburne. 1978. The heterogeneity of rat high density lipoproteins. *Biochem. Biophys. Res. Commun.* **83:** 783–786.

29. Sparks, C. E., S. D. Tennenberg, and J. B. Marsh. 1981. Catabolism of apoprotein A-I of HDL in normal and nephrotic rats. *Metabolism.* **30:** 354–358.

30. Kovanen, P. I., W. J. Schneider, G. M. Hillman, J. L. Goldstein, and M. S. Brown. 1979. Separate mechanisms for the uptake of high and low density lipoproteins by mouse adrenal gland in vivo. *J. Biol. Chem.* **254:** 5498–5505.

31. Bachorik, P. S., F. A. Franklin, D. G. Virgil, and P. O. Kwiterovich. 1982. High affinity uptake and degradation of apolipoprotein E-free high density lipoprotein and low density lipoprotein in cultured porcine hepatocytes. *Biochemistry.* **21:** 5675–5684.

32. Tamai, T., W. Patsch, D. Lock, and G. Schonfeld. 1983. Receptors for homologous plasma lipoproteins on a rat hepatoma cell line. *J. Lipid Res.* **24:** 1568–1577.

33. Shore, V. G., T. Forte, H. Licht, and S. B. Lewis. 1982. Serum and urinary lipoproteins in the human nephrotic syndrome: evidence for renal catabolism of lipoproteins. *Metabolism.* **31:** 258–268.

34. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.

35. Sparks, C. E., and J. B. Marsh. 1981. Metabolic heterogeneity of apolipoprotein B in the rat. *J. Lipid Res.* **22:** 519–527.

36. Elovson, J., Y. O. Huang, N. Baker, and R. Kannan. 1981. Apolipoprotein B is structurally and metabolically heter-ogeneous in the rat. *Proc. Natl. Acad. Sci. USA.* **78:** 157–161.

37. Garber, D. W., B. A. Gottlieb, J. B. Marsh, and C. E. Sparks. 1984. Catabolism of very low density lipoproteins in experimental nephrosis. *J. Clin. Invest.* **74:** 1375–1383.

38. Blum, C. B. 1982. Dynamics of apolipoprotein E metabolism in humans. *J. Lipid Res.* **23:** 1308–1316.

39. Kashyap, M. L., S. G. DeMendoza, M. Campbell, C. Y. Chen, R. F. Lutmer, and C. J. Glueck. 1978. Lipoprotein lipase activator deficiency in very low density lipoproteins in rat nephrotic syndrome. *Experientia.* **34:** 1044–1045.

40. Staprans, I., S. J. Garon, J. Hopper, Jr., and J. M. Felts. 1981. Characterization of glycosaminoglycans in urine from patients with nephrotic syndrome and control subjects, and their effects on lipoprotein lipase. *Biochim. Biophys. Acta.* **678:** 414–422.

41. Ohta, T., and I. Matsuda. 1981. Lipid and apolipoprotein levels in patients with nephrotic syndrome. *Clin. Chim. Acta.* **117:** 133–143.

42. Shafrir, E., T. Brenner, A. Gutman, M. Drevi, S. Diamant, and M. Mayer. 1972. Hepatic metabolic pathways and hormonal status in experimental nephrotic syndrome. *Isr. J. Med. Sci.* **8:** 271–284.

43. Mallick, N. P., and C. D. Short. 1981. The nephrotic syndrome and ischaemic heart disease. *Nephron.* **27:** 54–57.

44. Calandra, S., P. Tarugi, and E. Gherardi. 1984. Hyperlipoproteinemia in experimental nephrosis: mechanism for the increased HDL and apoA-I in plasma of rat with aminonucleoside nephrosis. *In* Treatment of Hyperlipemia. L. A. Carlson and A. G. Olsson, editors. Raven Press, New York. 259–262.