MINIREVIEW

Lipoprotein Metabolism in Experimental Nephrosis (44048)

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Abstract. **In experimental nephrosis, a decrease in plasma albumin resulting from proteinuria causes a decreased in the plasma oncotic pressure. The existence of an osmoreceptor, which responds to the low oncotic pressure and produces a factor(s) that signals the liver to increase the secretion of plasma proteins, is postulated. The hyperlipidemia characteristic of the nephrotic syndrome results primarily from increased hepatic secretion of apolipoproteins and lipoproteins representing the entire density spectrum from VLDL, IDL, and LDL to HDL. Not all plasma proteins and apolipoproteins are affected to the same extent. Increased mRNA levels due to increased transcription have been shown for albumin and apolipoprotein A-I (apoA-I). The increased secretion of VLDL, the major vehicle for triglyceride transport from the liver, appears to be due mainly to posttranscriptional events possibly related to increased lipogenesis. Once proteinuria begins, the demand for amino acids for albumin and apolipoprotein synthesis by the liver is increased. To meet this demand, protein catabolism in the peripheral tissues is increased. One manifestation of this process is a decrease in lipoprotein lipase which reduces VLDL catabolism, contributing to the sustained elevation of plasma VLDL. The spectacular overproduction of apoA-I in nephrosis in the rat is accompanied by a decreased fractional catabolic rate (FCR), contributing to the maintenance of high levels of HDL. Urinary loss of HDL and its renal catabolism does not account for the decreased FCR. The reason for the decreased FCR is not known. Work with nephrotic rats overexpressing transgenic human apoA-I has shown that human A-I forms smaller HDL_3-sized particles, rather than the larger HDL_2 of the rat. This may contribute to the failure of HDL levels to increase in the human nephrotic syndrome. High plasma VLDL and LDL with normal or low HDL probably account for the increased incidence of coronary artery disease in the nephrotic syndrome.** [P.S.E.B.M. 1996, Vol 213]

The nephrotic syndrome is characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia. In humans, several different diseases are associated with proteinuria, although most instances of the nephrotic syndrome appear to be autoimmune disorders (1). The development of animal models by Masugi (2) and by Heymann (3) using heterologous antibodies (the antigen is megalin, a member of the low-density lipoprotein (LDL)–receptor family [4]), and by Metcoff *et al.* (5) using puromycin aminonucleoside (PAN), laid the foundation for subsequent laboratory investigations of the pathophysiology of this disorder. The importance of understanding these events is underscored by the accumulating evidence that elevated plasma lipid levels from any primary cause exacerbate renal damage (6). There is also compelling evidence that long-standing nephrotic syndrome is associated with an increased incidence of coronary heart disease (7). For these reasons, the rat

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Work in the author's laboratory was supported by Grant HL-22633 from the National Heart, Lung, and Blood Institute with additional support from the Howard Heinz Endowment.

0037-9727/96/2132-0178$10.50/0

model of experimental nephrosis continues to provide a sound basis for clinical investigation. The recent development of transgenic and gene knock-out animal models in rats and mice (8, 9) has added a new dimension to the field.

This review focuses on recent research relating to the hyperlipidemia of rats with experimental nephrosis, as well as on some of the unanswered questions and hypotheses which may warrant further investigation. Both antibody- and drug-induced nephrotic syndrome will be considered. Work prior to 1984 has been reviewed (10), as have aspects of the human nephrotic syndrome (11, 12).

Hypoalbuminemia

Hypoalbuminemia, which follows proteinuria, decreases the plasma colloid osmotic (oncotic) pressure. This in turn is related to the expansion of the extracellular fluid volume seen as edema. The regulation of the extracellular fluid volume in nephrosis, particularly with respect to sodium retention, is complex, and it appears that hypoalbuminemia alone does not account for nephrotic edema in human nephrosis (13). One factor that has been shown to be increased in nephrotic rat plasma is the level of the atrial natriuretic peptide (ANP) which is secreted in response to pressure changes in the atrium of the heart (14). In nephrotic rats infused with ANP (15), no increase in sodium excretion took place and there was an increase in proteinuria accompanied by a rise in plasma LDL and a fall in plasma high-density lipoprotein (HDL). These findings involving a known hormonal response make it reasonable to suggest that an osmoreceptor exists which responds to changes in oncotic pressure by secreting a factor resembling the ANP. This factor then acts on hepatocytes, resulting in increased transcription of most of the genes responsible for the synthesis of the plasma proteins, including the apolipoproteins, resulting in hyperlipidemia.

The osmoreceptor hypothesis is not new (16), but recent studies in genetically analbuminemic rats support this concept (17, 18). Analbuminemic rats have no proteinuria, but they are hypercholesterolemic and have elevated plasma levels of apolipoprotein A-I (apoA-I). Though they are able to compensate for the lack of plasma albumin, they have a low oncotic pressure. In nephrotic rats, infusion of an oncotically active polymer such as ficoll or with albumin itself for 4 days, which restored oncotic pressure, returned cholesterol and apoA-I levels to normal (19). There was no effect on the urinary excretion of rat albumin. In these experiments, it would have been interesting to have determined the time course of the decline in plasma lipids since there is a theoretical possibility that the infusions increased capillary permeability, allowing the escape of lipoproteins from the plasma. However, it can be concluded from these studies in analbuminemic or nephrotic rats that neither proteinuria nor hypoalbuminemia *per se* is required for the maintenance of the hyperlipoprotinemia in nephrosis. The inference is strong that a low plasma oncotic pressure leads to hyperlipidemia.

Though the factors that might be involved as a consequence of the stimulation of a putative osmoreceptor remain to be discovered, one can ask whether or not this stimulus is general for all plasma proteins secreted by the liver, as originally proposed (16), or does it affect only certain classes of proteins? The fact that liver hyperplasia and hypertrophy occurs in nephrosis (20, 21) suggests that all secretory proteins would be increased. However, in Heymann nephritis, Sun *et al.* (22) reported that hepatic albumin and fibrinogen mRNA levels, and their transcription rates, were doubled and synthesis rates were increased 4-fold, while the synthesis of α_1-acid glycoprotein, an acute-phase protein like fibrinogen, was only increased by 50%. No changes were found in the mRNA level or transcription rate for this protein. Results similar to these have also been found in PAN nephrosis (23). Therefore, nephrosis does not increase the hepatic synthesis of all secretory proteins to the same extent, and the acute phase response, if involved at all, is not operative after the establishment of proteinuria. Reduction of proteinuria, by decreasing dietary protein intake (20, 24) or by administration of certain drugs (25, 26), is accompanied by a reduction in the hyperlipidemia. With decreased albuminuria, a rise in plasma albumin and therefore a rise in oncotic pressure would be expected to lessen the primary hepatic stimulus.

Lipoprotein Overproduction: Very Low Density Lipoprotein

The lipoprotein overproduction hypothesis, first formulated in 1960 (16), has been amply supported since that time (27, 28). The secretion of lipoproteins, as studied in the perfused liver, encompasses a wide spectrum of density classes including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL, and HDL (29). The hepatic secretion of apoB-100, the basic structural apoprotein of VLDL, is increased in nephrosis (27). The intestine, which secretes chylomicrons containing a truncated version of apoB-100 (apoB-48) and apoA-I, does not respond by increasing apoprotein secretion (30) although an increase in the intestinal mRNA for apoA-I has been reported in one laboratory (31). The mRNA for rat apoB-100 is edited in the intestine in all species, producing apoB-48. It is also edited in rat and mouse liver, resulting in the production of VLDL containing both B-100 and B-48 (32). Increased hepatic production of both forms occurs in nephrosis and no difference in the

extent of apoB-100 mRNA editing has been found in the nephrotic rat liver (33).

The supply of free fatty acids to the liver is a major determinant of VLDL secretion. Plasma levels of free fatty acids have been measured and are not increased in nephrosis (34). The plasma free fatty acid/albumin molar ratio does not rise in spite of hypoalbuminemia because the fatty acids redistribute into the circulating lipoproteins (35). Since there is no increase in the plasma free fatty acid concentration, this cannot be the driving force for increased VLDL secretion (36). Increased endogenous triglyceride synthesis (37, 38) or hepatic triglyceride stores must either be the main source of the fatty acids in the secreted VLDL, or else spare free fatty acids derived from the plasma which would otherwise be oxidized or stored within the liver. The cholesterol contained in the nascent VLDL, at least in the early stages of nephrosis, may be newly synthesized, and it has been suggested by Golper *et al.* (39) that one mechanism might be an impaired metabolism of circulating mevalonate by the nephrotic kidney. This would result in increased mevalonate delivery to the liver and incorporation into cholesterol. Increased activity of hepatic HMGCoA reductase was also found in this study, which supports earlier work indicating increased cholesterogenesis by the nephrotic liver (38, 40). However, Thabet *et al.* (41) did not find increased hepatic reductase activity, although they reported that the diurnal cycle of its activity was not demonstrable. Marsh *et al.* (33) also did not find changes in HMGCoA reductase mRNA in transgenic-nephrotic rats. These differences may be related to the use of PAN to induce nephrosis, which requires conversion to active metabolites before it is active (accounting for species differences in response) and which can be toxic at doses not much higher than those required to produce proteinuria. Differences exist in the toxicity of batches of PAN used to produce nephrosis. Liver hypertrophy, noted in Heymann nephritis (20) and in PAN-nephrosis by Golper *et al.* (39), was not found in a group of 35 PAN-nephrotic rats in our laboratory. When appropriate, studies of nephrosis induced by antibodies as well as by PAN ought to be carried out to minimize the possibility that the effects observed could be related to the agents used rather than to nephrosis itself.

Increased VLDL, as well as IDL and LDL production, in nephrosis appears to be driven in the early stages of nephrosis by increased apoB synthesis and secretion. Under most circumstances, the apoB-100 gene in the liver and its edited product in the liver and intestine, apoB-48, is expressed constitutively so that one might expect any increased secretion in nephrosis to be derived from posttranscriptional secretory events. However, Marshall *et al.* (42) reported 2.5-fold increases in the hepatic levels of apoB mRNA in PAN-nephrotic rats, as well as for albumin, apoA-I, apoA-II, and apoE. These increases were found on day 4 after the injection of PAN, a time when there is no measurable increase in proteinuria and no increase in plasma cholesterol levels. This supports the view that proteinuria *per se* is not the triggering mechanism for the increased apoB mRNA and that the initial increase in mRNA is not immediately followed by increased VLDL secretion. On Day 9, when proteinuria was at its height, though the mRNA levels were still elevated, no significant further increases in hepatic mRNAs were noted for most of the apoproteins, with the exception of apoA-I mRNA, which increased 5-fold. We have no evidence yet concerning mRNA stability for any of the apolipoproteins in nephrosis. It would appear that, in rats, the development of proteinuria causes continued increased transcription of the apoA-I and albumin genes. Continued increased VLDL apoB secretion after the initial burst of increased synthesis may therefore be mainly related to posttranslational processes, as we will discuss. Decreased VLDL catabolism promotes sustained elevation of VLDL levels.

One mechanism for increased VLDL secretion entails a decrease in the intracellular degradation of apo B, a process which has been demonstrated in cultured hepatocytes and hepatoma cell lines (43). Hirano *et al.* (44) found that intracellular degradation of apoB was diminished by lowering the albumin concentration in the medium of cultured HepG2 cells. Addition of fatty acids (complexed to albumin) such as oleic acid decreases the extent of intracellular apoB degradation in HepG2 cells, thereby enhancing apoB secretion (43). Increased hepatic lipogenesis in nephrosis could have the same effect. A two-step model for VLDL synthesis and secretion has been proposed in which an initial apoB particle containing only a small amount of lipid is formed, producing a molecule in the HDL density range (for reviews, see Refs. 45 and 46). This is followed by the addition of triglyceride possibly by fusion with triglyceride droplets within the cell. The initial lipidation step requires the participation of the microsomal lipid transport protein (MTP), whose absence results in failure of VLDL or chylomicron synthesis, as seen in human abetalipoproteinemia (47). No studies of the details of this process, nor of the MTP levels in nephrotic liver, have yet been reported.

Another theoretical mechanism for increased hepatic secretion of VLDL in nephrosis exists which has not been adequately explored. Decreased local reuptake of newly secreted particles within the space of Disse in the liver is possible. In cultured hepatoma cells, the HepG2 line, Williams *et al.* (48) have shown that decreasing the number of LDL receptors or the thickness of the unstirred water layer around the cells increased net apoB secretion. Their concept is that

owing to the unstirred water layer, newly secreted molecules are transiently trapped and exposed to cell surface receptors before they can enter the circulation. This phenomenon may explain the observations of Yedgar *et al.* (49), who demonstrated that apoB secretion was inhibited by addition of polymers such as dextrans or albumin which increase the viscosity of the incubation medium. This increases the thickness of the unstirred water layer, thereby restricting outward diffusion and increasing the re-uptake of particles containing apoB. Re-uptake of newly secreted VLDL might also explain the observations of Cianflone *et al.* (50), who found that reduction of the extracellular albumin concentration increased the concentration of apoB, but not of apoA-I, in the medium of HepG2 cells. This effect was greater in the presence of chylomicron remnants. Though native VLDL is not well recognized by the LDL receptor, exposure to lipoprotein and hepatic lipase in the space of Disse probably occurs and the smaller particles formed would allow the apoE and/or the apoB-100 which is present in the secreted VLDL to be in a conformation which now has high affinity for the LDL receptor. A second receptor system, the LDL-receptor–related protein (LRP, or the LRP/α_2 macroglobulin receptor) also recognizes apoE (51) and could also participate in the re-uptake of newly secreted VLDL. There is evidence that lipoproteins interacting with lipoprotein lipase may form complexes with cell surface heparan sulfate proteoglycans which are subsequently taken up by hepatocytes (52). The elevation of the apoB-containing lipoproteins could result in downregulation of hepatic LDL-receptors which would further diminish lipoprotein reuptake. At present, however, neither decreased reuptake nor decreased hepatic LDL receptor numbers in nephrosis has been demonstrated.

Lipoprotein Overproduction: HDL and Apo A-I

The increase in apoA-I production in experimental nephrosis is particularly striking, resulting in very high plasma levels of HDL in the rat despite urinary losses of HDL (53). Earlier work had shown that apoA-I synthesis and secretion by the liver was increased (27), and it was later shown, as mentioned above, that hepatic A-I mRNA levels were increased (54). Increased transcriptional activity of the apoA-I gene has been shown (22).

The availability of transgenic rats overexpressing human apoA-I in the liver (8) allowed a study of the effects of PAN-induced nephrosis in these animals (55). The human A-I gene responded strongly to the induction of nephrosis with PAN resulting in plasma levels of human apoA-I in the transgenic rats which exceeded 10 mg/ml (normal rat HDL apoA-I levels are approximately 0.6 mg/ml). The DNA construct included the human A-I promoter region from -256 to -41 bp upstream from the $(+1)$ transcription site. This region contains the regulatory elements which are necessary and sufficient for hepatic expression and contains at least three nuclear protein binding sites (56). It should be possible in the near future, therefore, to identify the transcription factors involved in the gene induction.

The high plasma levels of apoA-I in the transgenic-nephrotic rats followed a greater than 10-fold increase in human mRNA in the liver along with a 5-fold increase in endogenous rat A-I mRNA. The 5-fold increase was only slightly less than the 6-fold increase seen in nontransgenic nephrotic rats. Most of the apoA-I, both human and endogenous rat A-I, was present in the $d > 1.21$ fraction and not in HDL, contrasting sharply with the situation in nontransgenic rats in which almost all of the rat plasma apoA-I is in HDL. In fact, in the transgenic-nephrotic rats, the plasma HDL levels were below those in non-nephrotic transgenic rats and the average particle size was larger (55). This surprising observation has now been explained (33) by the size of the HDL particles excreted in the urine. The transgenic-nephrotic rats excreted four times as much HDL. These particles contained almost exclusively human A-I and were much smaller than the normal rat HDL which is mainly HDL_2 (8.2 vs 11 nm in diameter). Thus, the lower HDL levels and their larger size in the plasma of the transgenic-nephrotic rats reflected the urinary loss of the population of smaller human HDL.

An important conclusion can be drawn from these studies and from the studies of non-nephrotic A-I transgenic mice (9, 57)—namely, that the primary structure of apoA-I, as reflected in species differences in amino acid sequence, is a major determinant of HDL particle size. When the livers of transgenic-nephrotic rats were perfused, most of the apoA-I (human plus rat) was secreted as free A-I, not HDL. This also is true in normal rat liver as well as in cultured hepatocytes (58). However, HDL secretion was also increased in the transgenic-nephrotic rats and it contained only human A-I (33), indicating that human apo A-I competed successfully with rat A-I in the formation of an HDL particle. It is probable that these nascent HDL particles were formed in the space of Disse after secretion from the hepatocytes. It was found that the perfusate nascent HDL containing human A-I were smaller than those from nontransgenic nephrotics rats, averaging 20 nm in diameter versus 30 nm in the nontransgenic rats. Nascent HDL emerging from the liver contains more triglyceride and less cholesteryl ester than mature plasma HDL, which becomes smaller following lipolysis in the circulation (59). Even after filtration through the glomerulus and passage through the renal tubules, HDL is readily isolated by ultracentrifugation, though some alterations

of the apoproteins, possibly due to proteolysis, have been reported (60–62). In the human A-I transgenic-nephrotic rats, as well as in nontransgenic nephrotic rats, an additional 40% of the amount of urinary HDL ($1.063 < d < 1.21$) was recovered by ultracentrifugation at a density of 1.25, indicating the presence of even smaller, very high density (VHDL) particles. This agrees with the observation of Gherardi *et al.* (63) that only half of the urinary lipids in PAN nephrosis could be recovered by ultracentrifugation at a density of 1.21. These observations in the transgenic-nephrotic rats support the view that free human apo A-I forms small HDL particles, of about 5 nm in diameter, as well as particles of the size of HDL_3 (7–9 nm). Size selectivity in relation to plasma levels determines the extent of urinary loss. Pre-β HDL, which appears to be due to small amounts of lipid bound to only apo A-I, has been implicated as the first acceptor of free cholesterol during efflux from cells (64). Nephrotic rat plasma, however, does not appear to contain large amounts of pre-β HDL.

Catabolic Defects in Lipoprotein Metabolism: VLDL

Strong evidence has been obtained in several laboratories for a clearance defect in VLDL, using [^{125}I]-labeled VLDL (34) or [^{3}H]-glycerol labeling (65). In the latter experiments, VLDL from the nephrotic rats had a half-life when injected into normal rats which was twice as long as that for VLDL from normal rats. Furthermore, the *in vitro* rate of lipolysis of nephrotic VLDL was half that for normal VLDL. Preincubation of nephrotic VLDL with HDL from normal, but not from nephrotic rats, significantly increased the *in vitro* lipolysis rate. Since nephrotic HDL differs considerably in apoprotein composition from that of normal rat HDL, having much less apoE, apoA-IV, and the C apoproteins, it is possible that the catabolic defect was related to an abnormal apoprotein composition of nephrotic VLDL, which was partially corrected by incubation with normal rat HDL. However, no VLDL apoprotein analysis before and after incubation with HDL was reported. Since nascent hepatic VLDL from nephrotic rats does not appear to be very different from that of normal animals (27), it is hard to escape the conclusion that the resistance to lipolysis of mature VLDL taken from nephrotic rat plasma, as well as its decreased clearance in both nephrotic and normal rats, is a consequence of decreased *in vitro* exposure to lipoprotein lipase and hepatic lipase. This would produce a series of intact and remnant VLDL particles in which the conformation of the apoE was such that there would be little clearance by the liver. Reduced lipolysis would also prevent apoE from moving to HDL, accounting for much of the decreased apoE in nephrotic HDL. Another factor favoring decreased lipolysis is the partitioning of free fatty acids into VLDL, related to the hypoalbuminemia, which enriches VLDL in fatty acids and could theoretically slow the rate of lipolysis (34). Also, there may be changes in the apoC-III/C-II ratio in the nephrotic VLDL remnants, which ought to be further explored, especially since transgenic mice overexpressing apoC-III have very high triglyceride levels and have delayed clearance of VDL (66).

Though there are some negative reports in the literature concerning reductions in heparin-releasable lipoprotein lipase and hepatic lipase, most investigators have found decreased levels. Furukawa *et al.* (65) did not find significant decreases in either lipoprotein lipase or hepatic lipase at one time point (4 min) after heparin injection, though Garber *et al.* (34) reported 50% decreases in lipoprotein and hepatic lipase activity both in plasma at 10 min after heparin and in heparin-containing liver perfusates from PAN-nephrotic rats. The control rats in this study were pair-fed, which may partially explain the difference in results between the two laboratories.

Why should the levels of lipoprotein lipase be decreased? One possible reason might be that protein synthesis by peripheral tissues is decreased, owing to the mobilization of amino acids to the liver as originally suggested by Marsh and Drabkin (16) and supported by the recent work of Kaysen *et al.* (67). Further evidence for decreased lipolysis *in vivo* in nephrosis has come from the work of Levy *et al.* (68), who found both decreased VLDL and chylomicron catabolism. Kaysen *et al.* (69) have reported that both peripheral chylomicron catabolism and hepatic uptake of chylomicron remnants was impaired in Heymann nephrotic rats. They also found decreased heparin-releasable lipoprotein and hepatic lipase activity. The recent discovery of the VLDL receptor (70), which is present in peripheral tissues such as adipose tissue and muscle, but not in liver, represents another possible pathway by which the triglycerides of chylomicrons and VLDL can be made available to cells without the necessity for extracellular lipolysis. If its expression should prove to be low in nephrosis, it might explain why Levy *et al.* (68) found decreased triglyceride uptake by the heart in nephrotic rats even though they could not demonstrate a decrease in lipoprotein lipase in this tissue.

Catabolism of HDL

In contrast to the wealth of information about the catabolism of apoB-containing lipoproteins in normal animals and humans, relatively little is known about the catabolism of the apoA-I–containing lipoproteins. The residence time of apoA-I in plasma is relatively long (expressed at a $t_{1/2}$, it is about 10 hr in the rat and 120 hr in humans). When labeled with [^{125}I]-tyramine-

cellobiose which stays in lysosomes, apoA-I is taken up by many tissues and the kidney accounts for about 25% of its total catabolism (71). Though many cell types show specific HDL binding, internalization and degradation of the ligand is at least 10 times less than that of the LDL family of receptors (72). A putative HDL receptor has recently been described (73), but it appears to function mainly in steroid-producing tissues.

Increased synthesis and secretion of apoA-I and HDL is the primary reason for the elevation of plasma HDL levels in experimental nephrosis. Nevertheless, the question can be asked whether or not HDL, or apoA-I, catabolism is affected. In earlier work, Sparks *et al.* reported a sharp decline in the fractional catabolic rate of HDL (74), or of apoA-I (75), in PAN nephrosis. It was postulated that apoA-I catabolism was near its maximum in normal animals and that even small increases in synthesis would expand the plasma pool of HDL. Kaysen *et al.* (71) have reexamined this question in both Heymann nephritic and adriamycin nephrotic rats and confirmed the decrease in FCR. Their nephrotic rats had plasma levels of apoA-I averaging 1.3 mg apoA-I/ml (vs 0.46 mg/ml in controls) and in these rats they studied apoA-I kinetics after the injection of radiolabeled apoA-I incorporated into HDL. They estimated a synthesis rate for A-I of 2.2 times normal and a 33% decline in the FCR in the nephrotic rats and calculated that about 75% of the increase in plasma apo A-I concentration was due to increased synthesis. The decreased FCR contributed the remainder. In this study, by using [^{125}I]-tyramine cellobiose-apo A-I, the marker for tissue catabolic sites, they estimated that the kidney and the urine accounted for no more than one-fourth of the label. The decreased renal catabolism of apoA-I in the nephrotic rats was balanced by the urinary loss (presumably in HDL), and the urinary loss of apoA-I accounted for only 10% of the total apoA-I disappearance from plasma.

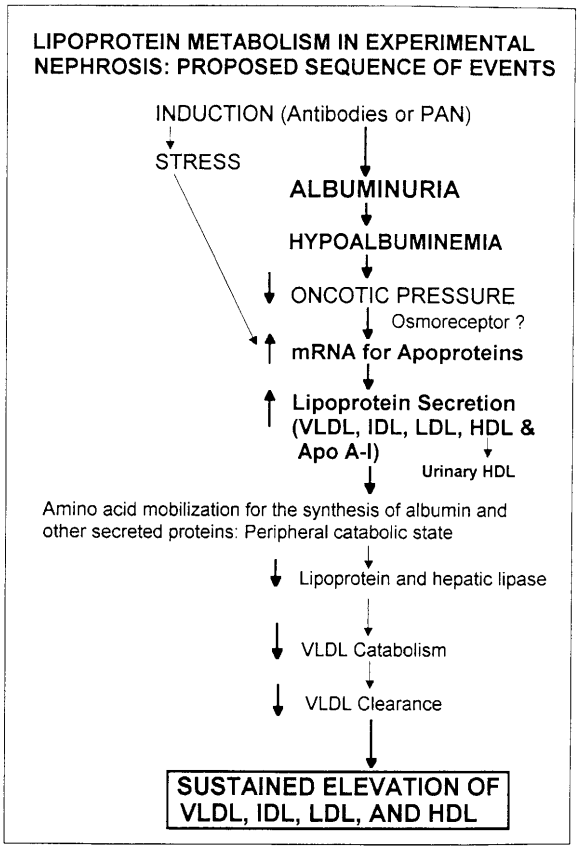

Figure 1. Sequence of events leading to the hyperlipidemia of experimental nephrosis.

The question is, what accounts for the decreased FCR? The experiments quoted above provide no clue. Indeed, if dissociation of apoA-I from HDL and subsequent filtration at the glomerulus accounts for the 25% of catabolism attributable to the kidney, the added urinary loss of HDL in nephrosis would predict an increase, not a decrease, in FCR and as we have just seen, no change in renal catabolism has been found. Our original view that catabolic sites may be close to saturation at normal levels of HDL may still hold. Perhaps HDL or apo A-I binding proteins are downregulated by HDL in nephrosis.

HDL in Experimental Nephrosis and the Nephrotic Syndrome in Humans

The most striking difference between the nephrotic syndrome in humans and experimental nephrosis in rats is that humans do not show high plasma levels of HDL. Rat HDL is mainly HDL_2 (9–12 nm in diameter) whereas human HDL is mainly HDL_3 (5–9 nm). It is possible that the difference in size allows more HDL_3 to be excreted in the urine in humans. But if renal losses in humans account for only a small fraction of HDL turnover as appears to be the case in rats, this simple explanation may be incorrect. If humans do not increase apoA-I synthesis to the same extent as rats, then urinary loss of HDL_3 could be critical even though most of the A-I catabolism may be extrarenal. Though our work with the human apoA-I transgenic rats shows that the human apoA-I gene has the elements necessary to respond to the stimulus of proteinuria, the magnitude of the stimulus might be much less. There has been one report (76) of an increased FCR for apoA-I in two cases of human nephrotic syndrome, so that humans may not show the decreased FCR seen in the rat. Humans have a plasma neutral lipid transfer protein (cholesteryl ester transfer protein, or CETP) which is absent in rats. CETP can transfer cholesteryl esters in HDL_2 to VLDL in exchange for triglyceride, thereby ensuring that the smaller HDL_3 molecules dominate, assuming that lipase rapidly hydrolyzes much of the triglyceride in the HDL. CETP levels are elevated in the nephrotic syn-

drome (77). If, then, HDL_3 particles are preferentially lost in the urine, and if the stimulus to apoA-I production is not as great in humans as it is in rats, the urinary loss of HDL_3 could explain the normal HDL levels in human nephrosis. If dissociation of A-I from HDL accounts for its catabolism, at least in the kidney, the smaller size of human HDL_3 would be important since dissociation from the smaller particles with a higher surface curvature is favored (78).

Figure 1 presents a concept of the sequence of events affecting lipoprotein metabolism in experimental nephrosis.

Helpful suggestions from Drs. M. Mahmood Hussain and Edward A. Fisher in the preparation of this manuscript are gratefully acknowledged.

1. Pesce AJ, First MR. Proteinuria. An Integrated Review. New York: M. Dekker, 1979.
2. Masugi M, Tomizuka Y. Uber die spezifischen zytotoxischen Veranderungen der Niere und der Leber durch das spezifische Antiserum (Nephrotoxin und Hepatotoxin). Zugleich ein Beitrag zur Pathogenense der Glomerulonephritis. Tr Jap Path Soc **21**:329–341, 1931.
3. Heymann W, Hackel DB, Harwood S, Wilson SGF, Hunter JLP. Production of the nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. Proc Soc Exp Biol Med **100**:660–664, 1959.
4. Farquhar MG, Saito A, Orlando RA, Kerjaschki D. The Heymann nephritis antigenic complex: Megalin (gp330) and RAP. J Am Soc Nephrol **6**:35–47, 1995.
5. Frenk S, Antonowicz I, Craig JM, Metcoff J. Experimental nephrotic syndrome induced in rats by aminonucleoside: Renal lesions and body electrolyte composition. Proc Soc Exp Biol Med **89**:424–427, 1955.
6. Diamond JR, Karnovsky MJ. A putative role of hypercholesterolemia in progressive glomerular injury. Annu Rev Med **43**:83–92, 1992.
7. Ordonez JD, Hiatt RA, Killebrew EJ, Fireman BH. The increased risk of coronary heart disease associated with nephrotic syndrome. Kidney Int **44**:638–642, 1993.
8. Swanson ME, Hughes TE, St. Denny I, France DS, Paterniti JR, Gfeller P, Burki K. High level expression of human apolipoprotein A-I in transgenic rats raises total serum high density lipoprotein cholesterol and lowers rat apolipoprotein A-I. Transgenic Res **1**:142–147, 1992.
9. Chajek-Shaul T, Hayek T, Walsh A, Breslow JL. Expression of the human apolipoprotein A-I gene in transgenic mice alters high density lipoprotein (HDL) particle size distribution and diminishes selective uptake of HDL cholesteryl esters. Proc Natl Acad Sci USA **88**:6731–6735, 1991.
10. Marsh JB. Lipoprotein metabolism in experimental nephrosis. J Lipid Res **25**:1619–1623, 1984.
11. Dorhout Mees EJ, Koomans HA. Understanding the nephrotic syndrome: What's new in a decade. Nephron **70**:1–10, 1995.
12. Appel GB. The hyperlipemia of the nephrotic syndrome. Am J Med **87**:5N–45N, 1990.
13. Koomans HA, Geers AB, Dorhout Mees EJ, Kortlandt W. Lowered tissue-fluid oncotic pressure protects the blood volume in the nephrotic syndrome. Nephron **42**:317–322, 1986.
14. Murlow PJ, Schrier R, eds. Atrial Hormones and Other Natriuretic Factors. Bethesda, MD, American Physiological Society, 1987.
15. Radin MJ, McCune SA. The effect of atrial natriuretic peptide infusion on renal haemodynamics and plasma lipoproteins in puromycin aminonucleoside nephrosis in rats. Clin Exp Pharmacol Physiol **20**:245–251, 1993.
16. Marsh JB, Drabkin DL. Experimental reconstruction of metabolic pattern of lipid nephrosis: Key role of hepatic protein synthesis in hyperlipemia. Metabolism **9**:946–955, 1960.
17. VanTol A, Jansen EHJM, Koomans HA, Joles JA. Hyperlipoproteinemia in Nagase analbuminemic rats: Effects of pravastatin on plasma apolipoproteins and lecithin:cholesterol acyltransferase activity. J Lipid Res **32**:1719–1728, 1991.
18. Yoshino G, Hirano T, Nagata K, Maeda E, Naka Y, Murata Y, Kazumi T, Kasuga M. Hypertriglyceridemia in nephrotic rats is due to a clearance defect of plasma triglyceride: Overproduction of triglyceride-rich lipoprotein is not an obligatory factor. J Lipid Res **34**:875–884, 1993.
19. Kaysen GA, Hoye E, Jones J Jr., VanTol A, Joles JA. Effect of oncotic pressure on apolipoprotein A-I metabolism in the rat. Am J Kidney Dis **26**:178–186, 1995.
20. Marsh JB, Drabkin DL. Metabolic channeling in experimental nephrosis. III. Influence of diet and of adrenalectomy; liver hypertrophy. J Biol Chem **230**:1063–1071, 1958.
21. Goldberg ACRK, Oliveira HCF, Quintao ECR, McNamara DJ. Increased cholesterol production due to liver hypertrophy in rat experimental nephrosis. Biochem Biophys Acta **710**:71–75, 1982.
22. Sun X, Martin V, Weiss RH, Kaysen GA. Selective transcriptional augmentation of hepatic gene expression in the rat with Heymann nephritis. Am J Physiol **33**:F441–F447, 1993.
23. Ibarra-Rubio ME, Pedraza-Chaverri J, Panduro A. Differential regulation in the expression of hepatic genes in nephrotic and pair-fed rats. Nephron **65**:119–124, 1993.
24. Kaysen GA, Jones H Jr., Martin V, Hutchison FN. A low protein diet restricts albumin synthesis in nephrotic rats. J Clin Invest **83**:1623–1629, 1989.
25. Hirano T, Mamo JCL, Ohnami K, Sugisaki T. Treatment of hyperlipidemia with probucol reduces proteinuria in puromycin aminonucleoside-induced nephrotic rats. Nephron **54**:358–359, 1990.
26. Thaiss F, Schoeppe W, Willaredt-Stoll JG, Batsford S, Mihatsch MJ. Cyclosporin A prevents proteinuria in an active model of membranous nephropathy in rats. Lab Invest **61**:661–669, 1989.
27. Marsh JB, Sparks CE. Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. J Clin Invest **64**:229–1237, 1979.
28. Calandra S, Gherardi E, Fainaru M, Guaitani A, Bartosek I. Secretion of lipoproteins, apolipoprotein A-I and apolipoprotein E by isolated and perfused liver of rats with experimental nephrotic syndrome. Biochim Biophys Acta **665**:331–338, 1981.
29. Marsh JB. Lipoproteins in a nonrecirculating perfusate of rat liver. J Lipid Res **15**:544–550, 1974.
30. Levy E, Ziv E, Bar-On H, Shafrir E. Chylomicron synthesis in experimental nephrotic syndrome. Biochim Biophys Acta **1005**:20–26, 1989.
31. Panduro AL, Castrillon L, Pedraza-Chaverri J, Vargas F, Ibarra-Rubio ME. Regulation of apolipoprotein A-I and E gene expression in liver and intestine of nephrotic and pair-fed rats. Nephron **65**:100–107, 1993.
32. Sparks CE, Marsh JB. Metabolic heterogeneity of apolipoprotein B in the rat. J Lipid Res **22**:519–527, 1981.
33. Marsh JB, Diffenderfer MR, Fisher EA, Sowden M, Dong M, Paterniti JR, Burkey BF. The effect of experimental nephrosis on hepatic lipoprotein secretion and urinary lipoprotein excre-

tion in rats expressing the human apolipoprotein A-I gene. J Lipid Res **37**:1113–1124, 1996.
34. Garber DW, Gottlieb BA, Marsh JB, Sparks CE. Catabolism of very low density lipoproteins in experimental nephrosis. J Clin Invest **74**:1375–1383, 1984.
35. Shafrir E. Partition of unesterified fatty acids in normal and nephrotic syndrome serum and its effect on serum electrophoretic pattern. J Clin Invest **37**:1775–1782, 1958.
36. Sniderman AD, Cianflone K. Substrate delivery as a determinant of hepatic apo B secretion. Arterioscler Thromb **13**:629–636, 1993.
37. Gherardi E, Calandra S. Experimental nephrotic syndrome induced in the rat by puromycin aminonuceloside: Hepatic synthesis of neutral lipids and phospholipids from ^{3}H-water and ^{3}H-palmitate. Lipids **15**:108–112, 1980.
38. Diamant S, Shafrir E. Lipogenesis in aminonucleoside-induced nephrotic syndrome. Biochim Biophys Acta **360**:241–251, 1974.
39. Golper TA, Feingold KR, Fulford MH, Siperstein MD. The role of circulating mevalonate in nephrotic hypercholesterolemia in the rat. J Lipid Res **27**:1044–1051, 1986.
40. Marsh JB, Drabkin DL. Metabolic channeling in experimental nephrosis. V. Lipid metabolism in the early stages of the disease. J Biol Chem **230**:1083–1091, 1958.
41. Thabet MA, Challa A, Chan JC, Pandak WM, Heuman DM, Vlahcevic ZR. Studies of alteration of hepatic cholesterol metabolism in puromycin-induced nephrotic syndrome in rats. Kidney Int **44**:789–794, 1993.
42. Marshall JF, Apostolopoulos JJ, Brack CM, Howlett GJ. Regulation of apolipoprotein gene expression and plasma high-density lipoprotein composition in experimental nephrosis. Biochim Biophys Acta **1042**:271–279, 1990.
43. Dixon JL, Furukawa S, Ginsberg HN. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from HepG2 cells by inhibiting intracellular degradation of apolipoprotein B. J Biol Chem **266**:5080–5086, 1991.
44. Hirano T, Furukawa M, Kurokawa M, Ebara T, Dixon JL, Nagano S. Intracellular apoprotein B degradation is suppressed by decreased albumin concentration in HepG2 cells. Kidney Int **47**:421–431, 1995.
45. Olofsson S-O. The molecular mechanism for the assembly and secretion of apoB-100–containing lipoproteins. Prog Lipid Res **30**:205–218, 1991.
46. Innerarity TL, Boren J, Yamanaka S, Olofsson S-O. Biosynthesis of apolipoprotein B-48–containing lipoproteins. J Biol Chem **271**:2353–2356, 1996.
47. Wetterau JR, Aggerbeck LP, Bouma M, Eisenberg C, Munck A, Hermier M, Schmitz J, Gay G, Rader DJ, Gregg RE. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science **258**:999–1001, 1992.
48. Williams KJ, Brocia RW, Fisher EA. The unstirred water layer as a site of control of apolipoprotein B secretion. J Biol Chem **265**:16741–16744, 1990.
49. Yedgar S, Weinstein DB, Patsch W, Schonfeld G, Casanada FE, Steinberg D. 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, 1982.
50. Cianflone K, Vu H, Sniderman AD. Effects of albumin on lipid synthesis, apo B-100 secretion, and LDL catabolism in HepG2 cells. Atherosclerosis **107**:125–135, 1994.
51. Hussain MM, Maxfield FR, Mas-Oliva J, Tabas I, Ji Z, Innerarity TL, Mahley RW. Clearance of chylomicron remnants by the LDL receptor-related protein/-α2-macroglobulin receptor. J Biol Chem **266**:13936–13940, 1991.
52. Mulder M, Lombardi P, Jansen H, Van Berkel TJC, Frants RR, Havekes LM. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. J Biol Chem **268**:9369–9375, 1993.
53. Marsh JB, Sparks CE. Lipoproteins in experimental nephrosis: Plasma levels and composition. Metabolism **28**:1040–1045, 1979.
54. Tarugi P, Calandra S, Chan L. Changes in apolipoprotein A-I mRNA level in the liver of rats with experimental nephrotic syndrome. Biochim Biophys Acta **868**:51–61, 1986.
55. Burkey BF, France D, Wang HX, Ma X, Brand B, Abuhani C, Diffenderfer MR, Marsh JB, Paterniti JR Jr., Fisher EA. Over expression of human apolipoprotein A-I in transgenic rats and the hyperlipoproteinemia associated with experimental nephrosis. J Lipid Res **36**:1463–1473, 1995.
56. Reischer SR, Fisher EA, Feinstein SI. Rabbit and rat liver nuclei both contain proteins which bind to the regions controlling apolipoprotein A-I gene expression. Eur J Biochem **216**:247–253, 1993.
57. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. Nature **353**:265–267, 1991.
58. Hussain MM, Zanni EE, Kelly M, Zannis VI. Synthesis, modification, and flotation properties of rat hepatocyte apolipoproteins. Biochim Biophys Acta **1001**:90–101, 1989.
59. Winkler KE, Marsh JB. Metabolism of triglyceride-rich nascent rat hepatic high density lipoproteins. J Lipid Res **30**:989–996, 1989.
60. Calandra S, Tarugi P, Ghisellini M, Gherardi E. Plasma and urine lipoproteins during the development of nephrotic syndrome induced in the rat by adriamycin. Exp Mol Pathol **39**:282–299, 1983.
61. Shore VG, Forte T, Licht H, Lewis SB. Serum and urinary lipoproteins in the human nephrotic syndrome: Evidence for renal catabolism of lipoproteins. Metabolism **31**:258–268, 1982.
62. Gomo ZAR, Henderson LO, Myrick JE. High-density lipoproteins in urine: I. Characterization in normal subjects and in patients with proteinuria. Clin Chem **33**:1775–1780, 1988.
63. Gherardi E, Vecchia L, Calandra S. Experimental nephrotic syndrome in the rat induced by puromycin aminonucleoside. Plasma and urinary lipoproteins. Exp Mol Pathol **32**:128–142, 1980.
64. Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res **36**:211–228, 1995.
65. Furukawa S, Hirano T, Mamo JCL, Nagano S, Takahashi T. Catabolic defect of triglyceride is associated with abnormal very-low-density lipoprotein in experimental nephrosis. Metabolism **39**:101–107, 1990.
66. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science **249**:790–793, 1990.
67. Kaysen GA, Carstensen A, Martin VI. Muscle protein synthesis is impaired in nephrotic rats. Miner Electrolyte Metab **18**:228–232, 1992.
68. Levy E, Ziv E, Bar-On H, Shafrir E. Experimental nephrotic syndrome: Removal and tissue distribution of chylomicrons and very-low-density lipoproteins of normal and nephrotic origin. Biochim Biophys Acta **1043**:259–266, 1990.
69. Kaysen GA, Mehendru L, Pan X-M, Staprans I. Both peripheral chylomicron metabolism and hepatic uptake of remnants are defective in nephrosis. Am J Physiol **263**:F335–F341, 1992.
70. Jingami H, Yamamoto T. The VLDL receptor: wayward brother of the LDL receptor. Curr Opin Lipidol **6**:104–108, 1995.
71. Kaysen GA, Hoye E, Jones H Jr. Apolipoprotein AI levels are increased in part as a consequence of reduced catabolism in nephrotic rats. Am J Physiol **268**:F532–F540, 1995.
72. Gottlieb BA, Marsh JB. High density lipoprotein binding by rat

Fu5AH hepatoma cells is not related to cholesterol content. Atherosclerosis **67**:251–259, 1987.
73. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science **271**:519–520, 1996.
74. Sparks CE, Tennenberg SD, Marsh JB. Catabolism of the apolipoproteins of HDL in control and nephrotic rats. Biochim Biophys Acta **665**:8–12, 1981.
75. Sparks CE, Tennenberg SD, Marsh JB. Catabolism of apoprotein A-I of HDL in normal and nephrotic rats. Metabolism **30**:354–358, 1981.
76. Saku K, Mendoza SG, Laver M, Hynd BA, Gartside PS, Kashyap ML. High-density lipoprotein apolipoprotein AI and AII turnover in moderate and severe proteinuria. Nephron **50**:112–115, 1988.
77. Dullaart RP, Gansevoort RT, Dikkeschei BD, deZeeuw D, de-Jong PE, VanTol A. Role of elevated lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein activities in abnormal lipoproteins from proteinuric patients. Kidney Int **44**:91–97, 1993.
78. Tall AR. Plasma high density lipoproteins: Metabolism and relationship to atherogenesis. J Clin Invest **86**:349–384, 1990.