[Frontiers in Bioscience 7, e326-338, August 1, 2002]

LIPOPROTEIN METABOLISM IN THE NEPHROTIC SYNDROME

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1. ABSTRACT

This review covers lipids, apolipoproteins, and receptors involved in the dyslipidemia of the nephrotic syndrome in humans and in rat or mouse models of the syndrome. It emphasizes research published during the last decade, though earlier work is cited. The focus is on the biosynthesis and catabolism of the plasma lipoprotein density classes and the role of receptors and enzymes in regulating lipoprotein metabolism in nephrosis. Although the factors responsible for the initiation of the hepatic and peripheral cellular responses to proteinuria and hypoalbuminemia remain elusive, recent work highlights the increased risk of atherosclerosis and the progression of renal disease associated with nephrotic dyslipidemia. Understanding of the role of the kidney in the catabolism of apolipoproteins entering the glomerular filtrate has been enhanced by the discovery of the receptor-mediated uptake of apolipoprotein A-I, the main apoprotein of HDL. The following aspects of lipid and lipoprotein metabolism in relation to nephrosis are discussed, with attention paid to differences between experimental nephrosis and the human nephrotic syndrome:(1) Albumin metabolism (2) Lipoprotein metabolism (3) Receptors (4) LCAT and CETP (5) Hepatic and Lipoprotein Lipase (6) Lipid metabolism (7) Lipiduria (8) Hypotheses and Questions (9) Summary.

2. INTRODUCTION

The nephrotic syndrome in humans or in animal models is characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Increases in the plasma levels of all of the apoB100-containing lipoprotein density classes- VLDL, IDL, and LDL- have been documented in rats (1) and humans (2). HDL levels are not increased in the human nephrotic syndrome, but in nephrotic rats or mice, high plasma HDL and apoA-I levels are present (3).

Albumin comprises about three-fourths of the urinary protein. The edema and the extent of expansion of the extracellular fluid volume are related to the degree of hypoalbuminemia. However, the relationship of the

changes in plasma oncotic pressure to lipoprotein metabolism needs to be clarified. As an explanation for the hyperlipidemia, both increased synthesis and decreased catabolism of apoB100-containing lipoproteins occurs in humans and in rat models of nephrosis (4,5). However, rats and mice carry most of their plasma cholesterol in HDL and lack neutral lipid transfer protein (CETP) activity, making them imperfect models of lipoprotein metabolism in human nephrosis.

It is generally assumed that the metabolic events following glomerular injury and subsequent proteinuria are independent of the underlying cause of the renal damage, but this is probably not true either for experimental nephrosis or the human nephrotic syndrome. For example, injection of puromycin aminonucleoside in rats, a common method of producing nephrosis, subjects the animal to possible hepatic injury before the compound is eliminated, and hence could affect the early hepatic response to proteinuria. Injection of antiserum against the kidney glomerular antigen megalin subjects the animal to stress-related phenomena as well as to antigen-antibody reactions in organs such as the lung, which also contain the antigen (6). Optimally, more than one experimental animal model should be used. In humans, careful selection of subjects is necessary since a systemic disorder such as AIDS, which can cause renal damage, has widespread metabolic effects. Nevertheless, progress in understanding the basic biological response to proteinuria has justified assuming a common underlying metabolic response to proteinuria.

The importance of a better understanding of the dyslipidemia of nephrosis is underscored by evidence supporting the view that the elevated apoB100-containing plasma lipoproteins, especially the triglyceride-rich components, leads not only to accelerated atherosclerosis, but to progression of the renal damage and subsequent renal failure (7).

Other reviews on lipoprotein metabolism in the nephrotic syndrome in animals and humans include earlier literature and complement the present review (1,4,8-14).

3.1. Albumin metabolism

Proteinuria is the signal event leading to the nephrotic syndrome.The liver can compensate for moderate urinary losses of albumin, resulting in little change in the plasma albumin concentration. As the albuminuria increases, hypoalbuminemia and edema ensue. Increased hepatic synthesis of albumin has been consistently demonstrated in nephrotic rats (15) and in humans (16-19).

Is the extent of proteinuria in the human nephrotic syndrome comparable to that seen in rats? A person weighing 70 kg with a daily excretion of 25 g, about the maximum generally observed, excretes 0.36 g/kg/d. A 250 g rat can excrete 450 mg of protein per day, or 1.8 g/kg, which is 5-fold more than humans based on body weight. However, the metabolic rate (estimated from the heart rate) is about 5-fold higher in rats compared to humans. Therefore, we may conclude that the severity of the nephrotic syndrome is at least metabolically comparable to that seen in rats. In both species, proteinuria can exceed the liver's ability to maintain a normal plasma level of albumin.

Does the decreased oncotic pressure trigger the subsequent dyslipidemia, or is the kidney damage and proteinuria itself the main culprit? Analbuminemic rats with normal renal function and decreased oncotic pressure show hyperlipidemia. (20,21). Maugeais *et al* (22) have reported hyperlipidemia in two sisters with analbuminemia and no proteinuria. In a study by Kaysen *et al* (23) in nephrotic rats, the infusion of albumin or a plasma expander such as Ficoll decreased nephrotic hyperlipidemia, though differences between the effects of albumin and Ficoll were noted. The elevated apoB100 and apoAI levels were reduced by Ficoll but only apoA-I levels were reduced by albumin, leading the authors to suggest that factors other than albumin concentration or oncotic pressure play a role in developing the hyperlipidemia. One problem relating to the use of an oncotic agent such as Ficoll is that changes in capillary permeability may result which could allow greater immediate loss of lipoproteins from the blood into the expanded extracellular space. In short term experiments, the lymphatic circulation will delay the return of these molecules to the blood, so that the decline in plasma levels may be, at least initially, a hemodynamic and not a metabolic phenomenon. Long-term replacement would be needed to answer this question. However, it remains plausible that the initial triggering event is related to a decreased oncotic pressure since most investigations have been carried out after the nephrotic hyperlipidemia was already established. The notion of an osmoreceptor remains attractive, especially since changes in atrial natriuretic peptide levels have been reported (24).

Can hypoalbuminemia and proteinuria contribute separately to the pathogenesis of nephrotic hyperlipidemia? Davies *et al* (25) reported that on a dietary protein intake of 40%, addition of Enalapril (an ACE inhibitor) reduced the proteinuria as well as the plasma lipids but did not affect albumin synthesis. They concluded that neither hyperlipidemia nor decreased lipoprotein clearance was linked to albumin synthesis. Shearer *et al* (26) compared the TG levels of nephrotic to those of analbuminemic rats. The TG levels were higher in the nephrotic rats, which also showed defective binding of their VLDL to aortic endothelial cells. VLDL from analbuminemic rats did not show such a defect, possibly owing to a higher amount of apoE relative to the TG content.

Joven *et al* (27) studied 29 subjects with proteinuria averaging 6.4 g/d, with or without hypoalbuminemia. Total apoB100 levels were not increased in individuals with normal albumin levels, but VLDL and IDL levels, as well as Lp(a), were increased. These two studies indicate that proteinuria and hypoalbuminemia, both resulting in hyperlipidemia, can be metabolically different. However, neither study addresses the question of the underlying triggering event.

Hypoalbuminemia itself results in a shift of some albumin-bound free fatty acids and lysolecithin to the

plasma lipoproteins, which may affect lipoprotein metabolism (28,29). In HepG2 cells, it has been shown that the secretion of human albumin is depressed by the addition of bovine serum albumin (BSA) in a dose-dependent manner (30). Moreover, the secretion of apoB, but not of apoA-I, was also decreased. In the presence of oleate, apoB secretion was increased, but the response was blunted at high concentrations (3-4,5%) of BSA. The effects on apoB secretion were related to a decrease in intracellular degradation. This effect in the presence of oleate was observed when oleate was bound to 1.5% BSA but not to 4.5% BSA. The authors concluded that free fatty acids bound to low concentrations of albumin in plasma might play a role in regulating the hepatic secretion of the apoB-containing lipoproteins.

This study (30) has an aspect which warrants some discussion. The effects observed with albumin were also observed with high concentrations of dextran, which does not bind fatty acids. The effects on apoB secretion can be explained by the re-uptake of apoB by the LDL receptor because albumin or dextran in the medium affect the depth of the unstirred water layer around the HepG2 cell, as shown in experiments by Williams *et al* (31). However, this does not negate the possible significance of hypoalbuminemia in the regulation of apolipoprotein B secretion. The relationship between hypoalbuminemia, the hepatic synthesis of albumin, and that of the apolipoproteins is further considered in the following section.

3.2. Lipoprotein metabolism

3.2.1. VLDL Metabolism

Increased hepatic synthesis of VLDL apoB100, the critical structural apolipoprotein of VLDL and LDL, has been demonstrated in nephrotic rats (1). In addition, decreased catabolism of VLDL has also been consistently found (4). In the nephrotic syndrome, the metabolism of VLDL apoB100 has been examined in three recent studies using amino acid precursors labeled with stable isotopes. This approach avoids potential artifacts in earlier work employing re-injection of the subject's VLDL after *in vitro* radioiodination,

Aguilar-Salinas *et al* (32) studied four subjects with nephrotic range proteinuria (>3.5 g/d) and found a significant 59% decrease of the fractional catabolic rate (FCR) of VLDL apoB100. Though the average production rate (PR) increased by 62%, this increase was not statistically significant (P=0.08). Three of the four subjects had elevated TG levels. In a study of 7 subjects, Demant *et al* (18) found that the increased plasma concentration of VLDL, particularly the small $VLDL_2$ subfraction, was due to decreased catabolism. Their subjects had average TG levels twice that of the controls. Because there was no correlation with the rate of albumin synthesis, it was concluded that a general increase in hepatic protein synthesis is not the primary cause of nephrotic hyperlipidemia.

De Sain-van der Velden *et al* (19) in a similar study of 6 subjects with elevated TG levels, found a decreased FCR of VLDL apoB100 (3.3 vs 6.4 pools/d) with no significant increase in production rate. Again, there was no correlation with an increased rate of albumin synthesis.

There seems little doubt from these studies and from earlier work with radioiodinated VLDL (33) that in humans with established nephrotic syndrome, VLDL apoB100 levels, as well as plasma TG levels, are elevated because of decreased catabolism. An increased PR, though seen in some subjects, is not a consistent finding. Furthermore, control of the synthesis of VLDL-apoB100 is not linked to that of albumin.

Once the hyperlipidemia is established, the apolipoprotein composition of VLDL changes. Deighan *et al* (34) reported that large VLDL particles ($VLDL_1$) were deficient in apoCII, apoCIII, and apoE. They indicated that these changes were associated with an increased free cholesterol to phospholipid ratio and a smaller average particle size. The smaller VLDL particles were associated with a decreased apoE content. Since apoC-II is an essential activator of lipoprotein lipase, and apoC-III inhibits the enzyme, these changes can explain decreased lipolysis independent of lipolytic enzyme activity or mass, and decreased VLDL catabolism. Decreased apoE, which is a ligand for VLDL-remnant (IDL) uptake, could contribute to the elevation of IDL. No increase in the mRNA for apoE in established Heymann nephritis was found.(35). Altered apoprotein composition could also affect receptor-mediated uptake of VLDL. In nephrotic rats, a decreased expression of the VLDL receptor, which is responsible for uptake of intact VLDL by muscle and adipose tissue, has been shown (36). It is interesting that in this rat model of focal glomerulosclerosis in which nephrosis progresses to chronic renal insufficiency (the Imai rat), the VLDL receptor was decreased in both adipose tissue and skeletal muscle.

3.2.2. Chylomicron Metabolism

The structural apolipoprotein of chylomicrons is apoB48. In contrast to studies of apoB100, there are few studies of its metabolism in humans (37) and none to date in the nephrotic syndrome. In rats and mice, but not in humans, apoB48 is secreted along with apoB100 in VLDL, another species difference. Levy *et al* (38) using PAN-nephrotic rats, found no increased intestinal production of apoB, even though lymph flow rates were increased. The intestine also synhesizes apoA-I. Neither Levy *et al* (38) nor Panduro *et al* (39) found any increase in intestinal apoA-I production in nephrosis.

Chylomicron clearance, like that of VLDL, is decreased in nephrotic rats (40). Kaysen *et al* (41) reported reduced TG uptake in heart, muscle, adipose tissue, and liver. Chylomicron remnants remaining in the plasma after initial lipolysis were also elevated, and decreased hepatic clearance was noted. These observations were followed by studies with chylomicrons isolated from the thoracic duct (42). The apoE/apoB48 ratios were similar in the control and Heymann-nephritic rats. After incubation with HDL from these rats, in comparison with chylomicrons from control rats incubated with normal HDL, a 50% reduction

in binding to endothelium was found even though the apoE content increased by 50%. Though the mechanisms involved are not easy to discern, these experiments support the view that alterations in the interactions of chylomicrons with endothelial-bound lipoprotein lipase may contribute to the decreased clearance. In addition, elevated VLDL levels will compete with chylomicrons for binding to endothelium-bound lipoprotein lipase.

In sharp contrast to this work in rats, Warwick *et al* (43) did not find any abnormality in postprandial TG clearance after an oral fat load in 9 subjects with nephrotic syndrome. This different result may relate to the fact that nephrotic rats generally show much higher TG levels. Differences in lipoprotein and hepatic lipase activity between humans and rats could be at the root of this discrepancy, as noted in section 3.5 below.

3.2.3. LDL Metabolism

In humans, elevated LDL levels in the nephrotic syndrome are accompanied by a preponderance of the small, dense particles (44) which are associated with an incresaed atherosclerosis risk in the general population. The metabolism of LDL can be studied by labeling its apoB100, either by radiolabeling *in vitro* or by endogenous labeling with an amino acid. In normal rats and mice, LDL levels are low. Increased secretion of apoB in the LDL density range has been found in perfused liver from nephrotic rats, which secrete apoB in every density class from d<1.006 to d<1.21(1). Most of the apoB production in rats is in VLDL.

Increased production of LDL-apoB100 in the human nephrotic syndrome has been found in several studies, but there has been some inconsistency in this finding. Earlier studies by Joven *et al* (45) using radiolabeled LDL concluded that an increased PR, and not a decreased FCR, raised LDL levels. Warwick *et al* (46) reached the opposite conclusion. A study by Vega *et al* (47) pointed to the TG level as an important variable correlated with the production of LDL apoB100. They found that subjects with elevated TG as well as elevated cholesterol overproduced LDL, whereas subjects showing only hypercholesterolemia had a decreased rate of clearance (FCR).

In the more recent studies, mainly using stable isotopes, an increased PR of LDL apoB100 has also been observed. In 4 subjects, compared to 4 controls, the PR was increased, with no increase in the transfer of apoB100 from VLDl to LDL (48). In another study of 7 subjects compared to 8 controls, a marked increase in PR was found, from 6.5 to 28 pools/day, with no decrease in the FCR (19) In a study of 7 nephrotic subjects, Stenvinkel *et al* (49) reported a significant two-fold increase in LDL-apoB100 production and a normal FCR, compared with 41 controls. The elevated PR was inversely correlated with the plasma albumin level (r=-0.82). They noted, however, that two of their seven nephrotics had a very low FCR of LDL apoB100. Demant *et al* (50) reported, in 7 subjects, significantly reduced LDL catabolism with only a trend towards an increased PR (+ 20%, P=0.1). Again, no correlations with the rate of albumin production were observed.

It seems reasonable to conclude that hepatic overproduction of LDL-apoB100 is a critical event in establishing the hypercholesterolemia of the nephrotic syndrome. Does this imply an increased conversion of VLDL to LDL or is there an increase in the secretion of LDL-like particles directly by the liver? Multicompartmental modeling of kinetic data from endogenous labeling in non-nephrotic control subjects generally requires assuming some direct secretion of LDL by the liver in order to give an optimal fit of the solutions of the differential equations to the data. Since decreased catabolism of VLDL is a consistent feature, it does not seem likely to suppose an accelerated conversion to LDL, and indeed this was not found by Aguilar-Salinas *et al* (48). In some circumstances in which a decreased FCR of LDL-apoB100 has been observed, down-regulation of the hepatic LDL receptor could explain the continuing hypercholesterolemia (see section 3.3 below).

3.2.4. Lipoprotein (a)

High levels of Lp(a) are associated with an increased risk of atherosclerosis in the general population (51). In humans, a small fraction of LDL is covalently attached to the large **(a)**-protein, forming a different lipoprotein class, Lp(**a**). However, some of the (a) protein is found in a free form in plasma. Lp(a) is of particular interest in relation to the nephrotic syndrome because high plasma levels of both Lp(a) (52) and free (a) (53) are found. Moreover, the kidney may play a prominent role in (a) catabolism since fragments of this large molecule are normally excreted in the urine (54). In the study by Stenvinkel *et al* (49), the production rate (PR) of the (**a**) of Lp(a) was increased and highly correlated with the PR of apoB100 (r=0.9). In spite of the fact that the kidney is involved in (**a**) catabolism, the high plasma levels of Lp(a) were due primarily to increased hepatic synthesis. The same conclusion was reached by Doucet *et al* (55) who also found that while the Lp(a) and its large fragments are passively filtered at the glomerulus, the smaller fragments may be secreted into the urine. Demant *et al* (56) have presented kinetic evidence in humans with normal renal function and high plasma Lp(a) levels indicating that the apoB100 and (a) proteins are catabolized at the same rate and suggesting that the assembly process is extracellular.

3.2.5. HDL and apoA-I Metabolism

HDL and apoA-I levels are high in nephrotic rats and unchanged in humans. Overproduction of apoA-I, with increased hepatic mRNA, has been documented in several studies in rats (4,39). In rats expressing the human apoA-I gene, PAN-nephrosis resulted in plasma levels of human apoA-I reaching 10 mg/ml in spite of urinary losses (57). In nephrotic mice, a 256 base pair in the promoter region, which responds to the transcription factor Early Growth Response Factor 1, appears to be the underlying mechanism responding to the as yet unknown stimulus (58).

In experimental nephrosis, the expanded pool of HDL leads to a saturation of the processes involved in HDL catabolism, resulting in a decreased fractional catabolic rate (FCR) (59,60). HDL isolated from daunomycin-nephrotic rats had an increased apoprotein content and when

iodinated, its uptake and degradation by primary rat hepatocytes was increased (61). This finding does not really contradict a finding of a decreased FCR in the intact animal since increased production is responsible for the initially increased pool size. It does suggest, however, that a primary decrease in hepatic catabolism is not the critical event. Moreover, there is some doubt as to the quantitative importance of the liver, as contrasted with the kidney, in HDL metabolism (62).

The reason for the failure of HDL levels to rise in humans is not understood. It is possible that in humans the apo A-I gene is not turned on to the extent seen in the rat. In nephrotic mice, the mRNA level is elevated two-fold, far less than the 5-fold elevation seen in rats (58). If a lesser degree of enhancement of mRNA levels in humans were to be combined with an increase in renal catabolism, contrary to the situation in the rat, HDL levels would not be elevated. Kinetic studies need to be undertaken to resolve this question.

3.3. Receptors

It is important to know the status of receptors involved in the catabolism of the plasma lipoproteins since they play a critical role in regulating plasma concentrations. The information we have at present has been obtained mainly in experimental nephrosis since tissue sampling is required to measure mRNA and receptor protein content. Extrapolation to the nephrotic syndrome in humans is uncertain, though it can sometimes be inferred from kinetic data. The LDL receptor is an example of this. In those subjects in which a decreased FCR of LDL-apoB100 has been found, it is reasonable to infer that down-regulation occurred, because plasma LDL levels in humans are largely determined by the number of hepatic receptors. However, in individuals in which apoB100 overproduction is the main kinetic feature, the status of the LDL receptors would be uncertain. LDL receptor levels in plasma monocytes from nephrotic subjects have not been measured. Even if they were, the status of hepatic receptors would not necessarily be reflected in peripheral blood.

Al-Shurbaji *et al* (63) found no change in expression of the hepatic LDL receptor in established PAN-nephrosis (day 7). Vaziri and Liang (64) reported down-regulation of the LDL receptor protein level in livers of PAN-nephrotic rats which was not accompanied by changes in mRNA levels or transcription rates. The down-regulation was not observed until 20 days after the onset of proteinuria. Liver cholesterol was not significantly elevated. Presumably, saturation of the receptors due to continued high levels of LDL caused either a decrease in receptor recycling or an increase in receptor protein degradation. However, the delayed down-regulation in these experiments indicates that it is not a factor in the initial elevation of LDL in the rat. In humans, in relatively long-standing nephrosis, down-regulation may be a central feature in the subset of subjects with a decreased FCR of LDL.

The LDL-receptor-related protein (LRP) which recognizes apoE (65) has so far not been investigated in nephrosis. Changes in its hepatic level could account for some of the elevation of chylomicron or VLDL remnants. As mentioned earlier, the decreased catabolism of VLDL can be partly explained by the decreased level of the VLDL receptor (66) in heart and skeletal muscle.

The scavenger receptor SR-B1 plays an important role in peripheral cell reverse cholesterol transport and in the hepatic selective uptake of cholesterol esters (67,68). A decreased hepatic level of this receptor has been shown in PAN-nephrotic rats (69) This could contribute to the elevation of plasma cholesterol esters and to an impairment in reverse cholesterol transport.

3.3.1. Effects of Statins

The statins inhibit cholesterol synthesis by inhibiting a rate-limiting enzyme of cholesterol biosynthesis, HMG CoA Reductase (70). They lower plasma levels of the apoB-containing lipoproteins in hyperlipidemic subjects, including those with the nephrotic syndrome (71). Steady state levels of LDL-C, HDL-C, and triglycerides in 56 subjects with glomerulonephritis and proteinuria greater than 3 g/d treated with simvastatin were reported to be changed by -47%, +1%, and –30%, respectively (71), but apoB levels were not reported. Because atherogenic LDL carries most of the plasma cholesterol, understanding the mechanism by which the statins decrease LDL is important. The paradigm is that decreasing the intracellular cholesterol level by inhibiting the pathway from acetyl-CoA up-regulates hepatic LDL receptors, resulting in enhanced clearance of LDL (72).

Kinetic studies in humans have not yielded consistent results supporting this paradigm. In hyperlipidemic individuals without renal disease, lovastatin decreased LDL levels by inhibiting its production, not by increasing catabolism (73). In PAN-nephrotic rats, fluvastatin was reported to decrease the number of VLDL particles secreted (74). However, LDL clearance was not measured in these experiments; and as we have noted, rats carry most of the plasma cholesterol in HDL.

Warwick *et al* (75) studied the effects of simvastatin in 8 nephrotic subjects. As expected, simvastatin decreased apoB100 and decreased plasma lathosterol levels. However, only 5 subjects showed a decrease in receptor-mediated catabolism of apoB100 with no significant alteration of production rates. Three years later, Aguilar-Salinas *et al* studied the effect of lovastatin treatment in 4 men with focal glomerulosclerosis (76). In this study, the drug decreased levels of LDL-apoB by dencreasing production owing to a decrease in the conversion of VLDL to LDL. Toto, Grundy, and Vega (2) studied the effect of pravastatin in 13 nephrotic subjects. It decreased labeled autologous LDL clearance by 18% with no change in the production rate. There was no effect on VLDL or IDL levels of apoB100.

At present it is hard to reconcile the conflicting results of the two kinetic studies in nephrotic subjects. The differences may be due to the fact that in the study by Aguilar-Sainas *et al* (76) the endogenous labeling

technique was employed, in contrast to the study by Toto *et al* (2) which employed re-injection of radioiodinated LDL. The latter method studies the catabolism of the mixed-age population of LDL while the former may give better insight into newly synthesized apoB100. However, endogenous labeling may not be as useful in studying apoproteins with long residence times, owing to the problem of amino acid recycling (77). It is a good possibility that statins act by decreasing hepatic apoB100 production as well as by decreasing catabolism, and that the balance between these two effects may depend on the level of hypercholesterolemia.

3.4. Plasma LCAT and CETP

During their stay in plasma, the lipids of the lipoproteins are subject to two kinds of modification – covalent bond modification (esterification and lipolysis), and exchange reactions between lipoprotein classes. Two circulating plasma proteins catalyze these reactions-lecithin-cholesterol acyl transferase (LCAT), and the neutral lipid (cholesterol ester) transfer protein (CETP). These have the functions, respectively, of forming cholesterol esters from cholesterol, and of exchanging triglycerides for cholesterol esters between lipoprotein classes.

In nephrotic syndrome, high levels of LCAT have been reported in humans (78). In rats, normal levels of LCAT were found when the assay was carried out with exogenous substrate but 40% lower when endogenous substrate was used. (79). This might be explained by the fact that lysophosphatidyl choline, a product of the LCAT reaction, redistributes to the lipoproteins (79,80), especially LDL (81), when albumin levels are low and this could inhibit the LCAT reaction. In PAN-nephrotic rats. Vaziri *et al* (82) reported reduced levels of LCAT which they ascribed to urinary loss of the enzyme. The mRNA levels were unchanged (relative to that of glyceraldehyde-3-P dehydrogenase). It is hard to reconcile this finding of reduced LCAT with the fact that Marsh and Sparks found no reduction in the extent of cholesterol esterification in total plasma, LDL, or HDL (83). However, Vaziri et al (82) waited 30 days after the initial induction of nephrosis with PAN, while Marsh and Sparks (83) studied their rats on day 7. It is noteworthy that LCAT is secreted by the liver.

CETP levels are increased in human nephrotic syndrome (84-86). In addition to high mass levels of the protein, its specific activity is enhanced by the increased concentration of free fatty acids in nephrotic lipoproteins (86).The implications of high levels of CETP are twofold-first, the protein is synthesized by the liver, conforming to the observation that many of the plasma proteins secreted by the liver are increased in nephrotic plasma. Second, normal or increased activity of LCAT followed by increased transfer of CE from HDL to VLDL as well as LDL would exacerbate the pro-atherogenic dyslipidemia. In addition, this would initially increase the TG content of HDL, which favors increased catabolism (87).

There is a third transfer protein, the phospholipid transfer protein, which remodels HDL and which acts particularly on TG-rich HDL and can cause proteolysis of apoA-I (88). It dissociates apo A-I in a lipid-poor form from HDL (89), and thus could contribute to the catabolism of free apo A-I by the kidney. Its level has not yet been measured in nephrosis, but one would predict that it would be increased and that such an increase, along with the increase in CETP action, could account for the normal or lower levels of HDL in human nephrotic syndrome.

3.5. Hepatic and lipoprotein lipase

In nephrotic rats, both enzyme activities measured after injection of heparin were found to be decreased in many, but not all, reported studies (1,4). Measurements in isolated perfused organs appear to give the most consistent results. Garber *et al* (90) found 50% decreases in both enzymes in liver perfusates from PAN-nephrotic rats. Hepatic lipase activity and its mRNA levels were decreased in PAN-nephrotic rats (91). Kaysen *et al* (92) found a 9-fold reduction in lipoprotein lipase released by heparin from perfused hearts in rats with Heymann nephritis. More recently, Liang and Vaziri (93) found low levels of immunoreactive lipoprotein lipase protein in the heart, skeletal muscle, and adipose tissue of PAN-nephrotic rats. The mRNA level, however, was decreased only in the heart. This laboratory also found decreased lipoprotein lipase in skeletal muscle and adipose tissue from rats with focal glomerulosclerosis (94). Since, as we have seen, VLDL and chylomicorn catabolism are impaired, the elevated TG levels seen in experimental nephrosis can be directly correlated to low levels of lipoprotein lipase.

Shearer and Kaysen (95) have recently provided evidence that aortic endothelial cells from rats when incubated with serum from nephrotic rats bind less lipoprotein lipase, which would contribute to the decreased VLDL clearance. They found the same effect with serum from Nagase analbuminemic rats and suggested that this was a phenomenon of serum with reduced oncotic pressure. Whether or not this applies *in vivo* is an interesting question. Incubation *in vitro* with hypooncotic serum *vs* normal serum will change the extent of the unstirred water layer surrounding the cultured endothelial cell, resulting in less exposure of the glycocalyx to added lipoprotein lipase.

In the human nephrotic syndrome, heparin-releasable lipoprotein lipase activity was originally reported to be decreased by 38% in 5 children (96) and negatively correlated with plasma TG levels in 9 children (97). Chan *et al* (98) found reduced lipoprotein lipase but not hepatic lipase activity in 18 adult patients. Kashyap *et al* (99) reported that 5 of 8 nephrotic subjects had significantly low levels of hepatic lipase and two of these also had significantly less lipoprotein lipase. Breier *et al* studied 10 nephrotic subjects and they too found decreased hepatic and lipoprotein lipase (100). However, Deighan *et al* in 27 subjects (101) and Demant *et al* (18) found no decreased heparin-releasable activity. Other factors besides decreased lipolysis, discussed in section 3.2 above, may be important in determining the elevated VLDL levels in humans.

3.6. Lipid metabolism

In the nephrotic syndrome, attention has been focused on the regulation of apolipoprotein metabolism

which determines plasma lipoprotein levels. The plasma lipoproteins, however, exist for the sake of the lipids they carry. Changes in plasma lipid concentrations could reflect changes in lipid metabolism which underlie the nephrotic syndrome. Could proteinuria trigger a rise in hepatic cholesterol and triglyceride synthesis which would drive apoB100 secretion and result in hypercholesterolemia?

Early work on hepatic cholesterol synthesis from labeled acetate in rats with Heymann nephritis induced by a single injection of antiserum indicated increased synthesis of cholesterol but not fatty acids by the liver (102). However, this may have resulted from the stress associated with the immune reaction. More recently, increased acetate incorporation into cholesterol and an increased hepatic content of cholesterol ester and a decreased triglyceride content has been reported in daunomycin-nephrotic rats (103). Increased incorporation of ^3H_2O was reported by Shafrir and Brenner (105). But Thabet *et al* (105) found no increase in HMG-CoA reductase or in cholesterol 7? -hydroxylase activity in PAN-nephrosis. This question has recently been re-examined. In PAN-nephrosis and again no change in hepatic HMG-CoA reductase activity was found (106). There was also no change in the plasma level of mevalonate nor in the plasma lathosterol/cholesterol ratio, which is a marker of cholesterol synthesis. Dullaart *et al* (107) also found no change in the lathosterol/cholesterol ratio and there was no effect of ACE-inhibitor therapy on this ratio. These findings are compatible with the fact that no significant increases in liver cholesterol content in nephrotic rats have been found in this and other studies (64,108).

Hepatic triglyceride synthesis and phospholipid metabolism in experimental nephrosis have not received much recent attention. In cultured hepatocytes from daunomycin-nephrotic rats, no change in palmitate incorporation into triglycerides or phospholipids was found, but there was an increased incorporation of labeled acetate into esterified fatty acids.(104). In liver perfusions, Brenner and Shafrir (109) found a 3-4-fold increase in oleate incorporation into secreted VLDL and HDL total lipids with only a modest increases in the liver itself. Total plasma free fatty acid concentrations do not change, although as mentioned more are carried in the lipoproteins rather than albumin.(90).

With respect to enzymes involved in the triglyceride synthesis pathway, Agbedana *et al* (110) reported that two microsomal enzymes, phosphatidate phosphohydrolase and diacylglycerol acyltransferase, were increased. ACAT (acyl CoA acyl transferase) activity was unchanged. These observations indicate that increased de novo synthesis of triglyceride accompanies the increased VLDL production.

3.7. Lipiduria

An early hypothesis of the origin of nephrotic hyperlipidemia was that of Marsh and Drabkin (111). It was postulated that proteinuria led to overproduction of hepatic secretory proteins, especially albumin, and including lipoproteins. The lipoproteins, too large to enter the glomerular filtrate even in the presence of glomerular damage, would then continue to rise in the plasma. There is little doubt that size and charge selectivity still apply to glomerular filtration of macromolecules in the nephrotic syndrome (112). Although some urinary loss of LDL is occasionally observed, almost all of the urinary lipoprotein is HDL, modified during its passage through the kidney. These observations apply to both humans (113) and rats (114).

However, the urinary loss of HDL accounts for only a small fraction of the overall catabolism of HDL. In studies from the author's laboratory (unpublished data), an average of 1.7 ± 0.8 mg of HDL per g of urinary protein was excreted in 4 subjects having a daily excretion of protein ranging from 0.7 to 21 g. More than half of the HDL protein was in the $1.21<d<1.25$ fraction. In the subject with 21 g of urinary protein/day, 55 mg of HDL was excreted. This subject had an estimated apoA-I absolute catabolic rate of 1.5 g/day, so that urinary excretion accounted for only 3.7 % of HDL catabolism. Although we did not measure lipid-poor apoA-I excretion, this is not HDL excretion *per se.* Urinary catabolism of apoA-I includes that taken up by the proximal tubules as well as the amount escaping into the urine.

The recent discovery of the dual role of cubilin, the vitamin B-12 receptor, as a renal receptor for HDL and/or apoA-I, emphasizes the role of the kidney in HDL catabolism (115). It is striking that internalization of the bound apoA-I requires the participation of megalin, an LDL receptor family member (115). Anti-megalin antibodies are responsible for the induction of Heymann nephritis in the rat, a further link between proteinuria and lipoprotein metabolism.

3.8. Hypotheses and questions

Though much progress has been made during the past decade, the important humoral signaling molecules which begin the process of responding to proteinuria have not yet been discovered, either in humans or in animal models. It remains difficult to distinguish between early events establishing the hyperlipidemia and the subsequent maintenance of new steady-state levels of plasma lipoproteins

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3.8.1. Is the initiating event hepatic overproduction of lipoproteins?

Overproduction of apoB100-containing lipoproteins has been repeatedly demonstrated in the animal models of nephrosis. In humans, most studies have also found overproduction of apoB100. A meta-analysis of all of the published work in humans ought to prove worthwhile. One reason why this is such an attractive hypothesis is that hepatic overproduction of albumin as well as that of many other hepatic secretory proteins, such as fibrinogen and transferrin (116), has been demonstrated in nephrotic humans and rats. Moreover, no such overproduction of any apolipoprotein by the intestine has been found. If, therefore, the signal received by the liver is a general one, overproduction of apoB100 would be a logical consequence. This does not mean that independent

control of the biosynthetic and catabolic pathways for each protein would be lost. Consequently, the fact that the rate of albumin synthesis is not correlated with that of apoB100, as in the study reported by Demant *et al* (18) remains compatible with the original overproduction hypothesis of Marsh and Drabkin (111).

Hepatic overproduction of apoA-I and HDL has been firmly established in nephrotic rats. In humans, steady state levels of apoA-I and HDL are not increased. No studies of production rates have yet been reported, so that hepatic overproduction compensated by increased catabolism is possible.

3.8.2. Is the initiating event decreased lipolysis of triglyceride-rich lipoproteins?

Decreased catabolism of VLDL and chylomicrons has been repeatedly demonstrated in humans and animal models. A decrease in VLDL catabolism via lipoprotein lipase action would increase its plasma concentration but would not explain a rise in LDL, unless one assumes a longer residence time might allow a greater conversion rate, which has so far not been found. If decreased LDL catabolism was the primary cause of the elevated LDL and its associated hypercholesterolemia, this could occur only if the primary event was down-regulation of the LDL receptor. There is little evidence to support this idea. It is hard to see, therefore, why decreased catabolism would be the initial event. In the case of VLDL, it would be possible as a consequence of urinary loss of an essential co-factor for lipoprotein lipase and/or hepatic lipase. Although there were early reports of urinary loss of activators (117,118), no subsequent work has emerged. Furthermore, apoC-II levels are not low in nephrosis (1). It seems reasonable to conclude that the decreased catabolism of the apoB100-containing lipoproteins which accompanies the nephrotic syndrome is secondary to the initial overproduction.

3.8.3. Why does impaired clearance of triglyceride-rich lipoproteins accompany nephrosis?

The overproduction hypothesis suggests that increased VLDL secretion is followed by an increase in the plasma pool size which might saturate lipoprotein lipase, resulting in a decrease in the fractional catabolic rate. Partitioning of free fatty acids into VLDL because of the hypoalbuminemia would contribute to a decrease in the rate of lipolysis. VLDL would compete successfully with chylomicrons for the lipase, accounting for the decreased chylomicron clearance. Any decreased LPL activity in conjunction with increased VLDL production strengthens these arguments. Other possibilities, for which there is not much evidence, involve apoC-II and apoC-III. ApoC-II might be more readily dissociated from VLDL when VLDL is enriched with free fatty acids. ApoC-III overproduction by the liver would result in impaired lipolysis of VLDL.

3.8.4. Is there an explanation for a decrease in lipase activity?

In addition to the redistribution of free fatty acids, which can affect lipoprotein lipase activity, a decrease in the mass of this protein, as well as hepatic lipase may also occur- at least in experimental nephrosis. Nuclear hormone receptors controlling lipolytic enzyme metabolism may be involved in a way not understood at present. Although there is amino acid channeling to the liver to synthesize albumin (4), whether or not this results in a catabolic state and a decreased enzyme synthesis in those tissues secreting the enzyme has not been shown.

3.9. Summary

Research to date has provided good evidence for both increased production and decreased catabolism of the apoB100-containing plasma lipoproteins in humans and animals with the nephrotic syndrome. Overproduction of apoA-I and high HDL levels due to increased transcription of the gene occurs in experimental nephrosis. However, HDL levels are not increased in humans. Very few studies of the metabolism of apoA-II, apoE, and the C apolipoproteins have been reported. Cholesteryl ester (neutral lipid) transfer protein activity is increased in human nephrotic syndrome.The hypoalbuminemia resulting from leaky glomeruli results in increased hepatic albumin synthesis and a re-distribution of fatty acids into the plasma lipoproteins. This phenomenon, along with decreases in lipoprotein lipase and hepatic lipase, and down-regulation of the VLDL receptor, can account for the decreased catabolism of VLDL and chylomicrons.

We suggest that the following areas need further investigation: (1) identification of signaling molecules responsible for increased hepatic albumin and apolipoprotein synthesis (2) studies of the role of nuclear hormone receptors in regulating cholesterol and lipoprotein metabolism (3) measurements of phospholipid transfer protein activity (4) kinetic studies of HDL metabolism in humans.

The increased risk of atherosclerosis and the deleterious effects of elevated lipids on the progression of the nephrotic syndrome in adults has been firmly established (119,120). As we learn more about the underlying pathophysiology of the nephrotic syndrome, newer approaches to therapy in individuals refractory to present-day treatment should emerge.

4. ACKNOWLEDGMENT

I wish to thank Dr. Ernst J. Schaefer and the Jean Mayer-USDA Human Nutrition Research Center on Aging at Tufts University for providing me with the environment which made the writing of this review possible. I also wish to thank Dr. Mark Sarnak for a critical reading of the manuscript.

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Key Words: Lipoproteins, Apolipoproteins, Nephrosis, Nephrotic Syndrome, Lipoprotein Metabolism, Review

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