LIPOPROTEIN METABOLISM IN THE NEPHROTIC SYNDROME IN MAN

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DECLARATION

I declare that the work presented in this thesis is entirely my own except where the assistance of others is acknowledged.

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ABBREVIATIONS

VLDL	very low density lipoprotein
LDL	low density lipoprotein
IDL	intermediate density lipoprotein
HDL	high density lipoprotein
NS	nephrotic syndrome
apo	apolipoprotein
apoLDL	total apolipoprotein content of LDL
Lp(a)	lipoprotein(a)
DGUC	density gradient ultracentrifugation
LCAT	lecithin cholesterol acyltransferase
ACAT	acyl-CoA cholesterol acyltransferase
CETP	cholesterol ester transfer protein
LPL	lipoprotein lipase
HTGL	hepatic triglyceride lipase
HMGCoA	3-hydroxy-3-methylglutaryl coenzyme A
FCR	fractional catabolic rate
AER	albumin excretion rate
d	density(g/mL)
TMU	tetramethylurea
CHD	1,2-cyclohexanedione
TCA	trichloroacetic acid
uCi	microcuries
BMI	body mass index
SD	standard deviation
SEM	standard error of mean

SUMMARY

The aim of this thesis was to characterise the abnormalities of plasma lipoprotein metabolism which occur in the nephrotic syndrome in man.

Both quantitative and qualitative changes in plasma lipoproteins were documented in a series of 45 consecutive patients with heavy proteinuria. Patients with proteinuria less than the nephrotic range(<3g/24 hours) did not have any significant elevations in cholesterol concentrations compared to controls but there was a relationship between urinary albumin loss and blood cholesterol. Primary hyperlipidaemia was not associated with increased urinary albumin losses.

The duration and magnitude of postprandial lipaemia following an oral fat load were not significantly greater in a group of nephrotic subjects compared to controls. However, both groups showed a high degree of inter-individual variation. The lipoproteins isolated in the d<1.006g/mL fraction following an oral fat load did not differ in composition between the two groups. Post-heparin lipase activities were not significantly different.

The transfer of apolipoprotein B along the delipidation cascade from very low to low density lipoproteins was

investigated using radioiodinated lipoproteins. Overall, apoB synthesis in nephrotic syndrome was not greater than in controls although the range in the nephrotic group was wide(up to four times normal). There was a significant increase in the production of apoB into the smaller, triglyceride-poor $VLDL_2$ density interval. Both $VLDL_1$ and $VLDL_2$ were cleared from the plasma at slower rates in the nephrotic syndrome. There was a modest increase in apoB transfer to LDL.

Studies of LDL metabolism revealed a consistent defect in fractional catabolism of LDL by the receptor-mediated pathway. LDL production was closely related to proteinuria and only rose when this exceeded 10g/day.

Inhibition of cholesterol synthesis with simvastatin produced dramatic falls in total and LDL-cholesterol in these subjects. Metabolic studies revealed a heterogeneous response with a majority of patients demonstrating increased catabolism of LDL but some showing reduced synthesis. Two serious and potentially worrying adverse events were witnessed during simvastatin therapy.

Particles corresponding to both HDL and LDL were isolated form urine collected from nephrotic subjects. The significance of this is uncertain but justified the corrections made for urinary LDL losses in the metabolic

studies and gives credence to the suggestion that filterd lipoproteins may be nephrotoxic.

The results of these studies are compared with the relatively few previous reports of lipoprotein metabolism in human nephrotic syndrome and the extensive literature on animal work. In man, catabolic defects appear to be at least as important as increases in lipoprotein production in the genesis of the hyperlipidaemia. However, some of the data is difficult to reconcile. Reasons for conflicting observations are discussed and areas requiring further research are highlighted.

CHAPTER 1 INTRODUCTION

1.1 Background

Hyperlipidaemia has been recognised as a complication of renal disease since the 19th century(1). Epstein, however, was the first to report the specific association between the nephrotic syndrome and hyperlipidaemia(2). Despite extensive clinical and animal research, the precise disturbances of lipid metabolism which initiate and maintain the hyperlipidaemia in human nephrotic syndrome have not been clearly elucidated(3).

An understanding of the changes in lipoprotein metabolism is important for several reasons. Firstly, investigation of dyslipidaemias may lead to important advances in our knowledge of both normal and abnormal lipoprotein metabolism. This is well illustrated by the example of familial hypercholesterolaemia. Secondly, there is now considerable epidemiological, experimental and clinical evidence that hyperlipidaemia plays an important role in the evolution of coronary artery disease in the general population. Although the role of hyperlipidaemia in the pathogenesis of coronary disease in nephrotic syndrome is debated, a substantial proportion of patients with renal disease die of cardiac disease. There is a general consensus that, in subjects with unremitting nephrotic

syndrome, hyperlipidaemia may accelerate atherosclerotic disease. Furthermore, recent experimental work suggests hyperlipidaemia may be implicated in the progression of renal failure. Finally, identification of the underlying metabolic defects permits therapy to be chosen on a rational basis thereby increasing efficacy and reducing the risk of adverse reactions.

The main aim of this thesis is to characterise the metabolism of apolipoprotein B-containing lipoproteins in human nephrotic syndrome. The fate of lipoproteins in the circulation has been investigated by labelling these particles and following their disappearance from the plasma. Application of multicompartmental modelling analysis generates a number of parameters describing the kinetic behaviour of lipoprotein subfractions. This permits comparisons with control subjects. In addition, a number of other related issues are considered including the relationships between proteinuria and lipid disturbances; treatment of nephrotic hyperlipidaemia; the effects of therapy on lipoprotein structure and metabolism; and urinary excretion of lipoproteins.

1.2 Lipoproteins - classification

Lipids are used by cells for a wide range of functions including membrane synthesis, hormone production and energy

requirements. The liver and intestine are the main sites of lipid synthesis and a directed and efficient transport mechanism has evolved in man and other species for their dispersion to other tissues. Neutral lipids(cholesterol esters, triglycerides) are hydrophobic and can only be transferred through the aqueous environment of plasma in macromolecular complexes containing protein (apolipoproteins) and more polar lipids(phospholipids). These "lipoproteins" form a spectrum of particles which can be divided into a number of classes according to differing physical and/or chemical properties.

The basic structure of a lipoprotein is shown in figure 1.1. It consists of a hydrophobic core of neutral lipids surrounded by a surface monolayer of protein and phospholipid. The polar regions of the latter are directed to the surface of the particle and facilitate its solubilisation in plasma. The more hydrophobic regions abut on the core lipid. Free cholesterol molecules occupy a position intermediate between these two parts of the micellar structure.

A number of classifications have been proposed for lipoproteins. The most common is that based on hydrated density and ultracentrifugal characteristics. This approach was developed during the 1950's by Gofman, Lindgren and co-workers(reviewed in 4) who identified

several discrete classes using a step-wise increase in background density to float lipoproteins in the ultracentrifuge. The procedure is still widely used today for preparative work although it is slow, labour-intensive and may result in apolipoprotein losses during centrifugation. Five main lipoprotein classes were recognised - chylomicrons, very low density(VLDL), intermediate density(IDL), low density(LDL) and high density(HDL) lipoproteins. The chemical composition and physical characteristics of particles within these subgroups are shown in tables 1.1 and 1.2. In recent years, the application of increasingly sophisticated techniques has led to the identification of structurally discrete subgroups within these classes.

Lipoprotein particles may also be defined by their flotation rates in the analytical centrifuge at a fixed background density. Lower density lipoproteins are separated by this method at a background density of 1.063g/mL; a density of 1.210g/mL is used for high density lipoproteins. The flotation rates are measured in Svedberg units(cm/sec/dyne/g x 10^{-13}) and the rates corresponding to the various lipoprotein classes are given in table 1.1.

From these initial methods, a number of developments have led to quicker and more accurate means of isolating intact lipoproteins, particularly for preparative work. The use

of swing-out bucket instead of fixed angle rotors reduces errors in separation due to lipoproteins impacting on the tube wall. The centrifugal force is generated along, rather than at an angle to, the long axis of the tube. Patsch(5) developed the technique of rate zonal ultracentrifugation where a linear gradient of increasing density(continuous or step-wise) is produced in a large capacity bowl rotor using a pump. This permits rapid and sensitive resolution of lipoproteins for both analytical and preparative purposes. Similarly, the use of pre-determined density gradients in swing-out rotor centrifuge tubes has been used to isolate rapidly lipoproteins from small quantities of plasma(density gradient ultracentrifugation - DGUC). Both of these techniques are used in this thesis and are described in more detail in methods.

Other chemical and physical properties have been used to separate and classify lipoprotein particles. Electrophoresis has been employed for more than 60 years to separate particles according to differences in size:charge ratio using a variety of matrix supports(paper, agarose, polyacrylamide). Three classes are identifiable by agarose gel electrophoresis(alpha, beta and pre-beta) which correspond approximately to HDL, LDL(including IDL) and VLDL. Chylomicrons are excluded from the gel by their large size and remain at the origin. This was the technique used

by Fredrickson et al.(6) to classify the common dyslipidaemias(table 1.3). This classification has many limitations which have been highlighted by advances in molecular biology. The lipoprotein patterns described do not represent homogeneous groups but rather the phenotypic expression of a number of genetically- and environmentally-determined defects which cannot be differentiated by electrophoretic methods. Fredrickson's classification is still widely used to describe lipoprotein patterns and, until further progress is made on the underlying metabolic defects of hyperlipoproteinaemias, it is unlikely to be superseded.

Particle size alone may be used to differentiate the plasma lipoproteins using molecular sieve gels of different pore size(7). Although this method is sensitive and has excellent resolution, it is not easily adapted for routine analysis of samples. Similarly, electron microscopy of negatively-stained lipoprotein samples allows direct visualisation of particles and measurement of sizes but is time-consuming, technically difficult and therefore not suitable for widespread use.

Gustafson and Alaupovic(8) were the first to propose a classification of the lipoproteins on the basis of their constituent apolipoproteins in the 1960's. The merit of this approach is that it emphasises the relationship

between lipoprotein structure and metabolism/function. The protein content of particles plays a crucial role in regulating lipoprotein metabolism in vivo.

1.3 Lipoproteins - physical and chemical characterisitics

Chylomicrons are the largest lipoprotein particles varying in diameter from 35 to >200nm. In normal individuals, chylomicrons are virtually absent in the fasting state. They are formed in the intestinal epithelial cells and their main lipid content, triglyceride, is synthesised from re-esterification of dietary monoglycerides and fatty acids. Triglycerides represent 90% by weight of chylomicrons. Protein accounts for 1-2% of the mass and the remainder is composed of phospholipid, cholesterol ester and free cholesterol in decreasing proportion. They contain apolipoproteins B48, A-I, A-IV and C initially. Other apoC's and apoE are acquired from HDL or VLDL particles in the lymphatic or systemic circulation.

Very low density lipoproteins are similar to chylomicrons with triglyceride as the main lipid constituent. These particles are secreted by hepatocytes and range in size from 35-75nm with a hydrated density <1.006g/mL. Apolipoproteins B-100, CII, CIII and E are the main protein constituents and have important regulatory roles in the catabolism of these particles.

Intermediate density lipoproteins(d=1.006-1.019g/mL) represent a transition from VLDL to LDL particles as a result of delipidation of the triglyceride moiety. They represent a small proportion of the circulating lipoprotein mass.

Low density lipoproteins are the main cholesterol-carrying lipoprotein in man and account for 70% of circulating cholesterol. They play a crucial role in cholesterol transport but are also associated with an increased risk of coronary artery disease when present in excess. Plasma levels of LDL are much higher in humans than comparable mammals and it has been postulated that this is the reason for our propensity to atherosclerosis. Apolipoprotein B100 is the main protein constituent accounting for 25-30% of particle mass. Recent evidence suggests that particles in the LDL density range(d=1.019-1.063g/mL) exist as several species with distinct structural and possibly metabolic characteristics(9).

High density lipoproteins are the most abundant lipoprotein particles(10) in human plasma although they only carry approximately 20% of circulating cholesterol. Epidemiological evidence suggests that HDL-cholesterol levels are inversely correlated with coronary heart disease. These particles are small and dense compared to other lipoproteins(d=1.063-1.210g/mL; particle size

5.5-12.0nm). A number of different species have been identified but in man HDL_2(d=1.063-1.125g/mL) and HDL_3(d=1.125-1.210g/mL) are the commonly recognised subclasses. Phospholipid and cholesterol ester are the main lipid constituents which are complexed with apolipoproteins AI and AII. Small amounts of other apolipoproteins(CI-III, D and E) may also be present.

1.4 The apolipoproteins

Because of their crucial role in maintaining the integrity and solubility of lipoproteins and regulating their metabolism, the apolipoproteins will be considered in some detail. The physicochemical properties and known physiological roles of these proteins are listed in table 1.4.

Apolipoprotein B in man exists in two forms designated B48 and B100. B100 is the predominant form. Its complete amino acid sequence has recently been reported(11). In plasma, it exists as a glycoprotein with a molecular weight of approximately 555000 daltons which is close to that calculated from its sequence - 512937 daltons. ApoB is highly insoluble in aqueous solution which has considerably hindered investigations into its biochemistry and function. B100 is secreted by the liver in VLDL particles on a one-to-one molar basis and transferred along the

delipidation cascade(see below) to LDL. In contrast to other apolipoproteins, it does not participate in exchanges between plasma lipoproteins. This unique property has been utilised to investigate the metabolism of apoB-containing lipoproteins in tracer experiments. ApoB100 acts as a ligand for the apoB/E receptor. Recent evidence suggests that variants of apoB100 which are poorly bound by receptors may be an important cause of hyperlipidaemia in the population(12).

ApoB48 in man is synthesised by intestinal cells and incorporated into chylomicrons. It is antigenically related to apoB100 although it does not bind to apoB/E receptors. It represents a truncated form of B100 which arises as a result of post-transcriptional editing of apoB mRNA(13).

ApoAI, the predominant protein of HDL, is produced by hepatic and intestinal cells and accounts for 70% of the protein content of HDL. Its main metabolic roles are as a co-factor for the enzyme, lecithin cholesterol acyl-transferase(LCAT), and in promoting cholesterol efflux from tissues. A number of defective apoAI variants have been described and some of these appear to be functionally less effective(e.g. as activators of LCAT). ApoAII is the second commonest protein in HDL. No physiological role for apoAII has been determined in man. ApoAIV was originally

identified in the rat but has been found in human plasma, mostly as free apolipoprotein, and in the intestinal mucosa.

The C class of apolipoproteins contains three forms - CI, CII and CIII. These are present in small quantities in VLDL and HDL particles. CII has an important role as a co-factor for lipoprotein lipase. No definite role for apoCI has yet been established. ApoCIII is a glycoprotein with a variable content of sialic acid residues which permits resolution into three subclasses by isoelectric focusing. Both in vitro and in vivo apoCIII inhibits lipoprotein and hepatic triglyceride lipase(14).

Much attention has been focused in recent years on apoE with the discovery of three common isoforms in the population. Isoelectric focusing has been used to identify E2, E3 and E4(15). Each is the product of a single gene which has three common alleles. The result is the appearance of six phenotypes which are, in decreasing order of frequency, E3/3, E4/3, E3/2, E4/2, E4/4 and E2/2. ApoE is an important ligand for both the B/E receptor and the putative chylomicron remnant receptor. It has a much higher affinity for the B/E receptor compared to apoB. This is attributed to interactions occurring at multiple binding sites between these molecules. The isoforms of apoE differ in their in vitro binding to apoB/E receptors

and this influences in vivo VLDL -> LDL transfer(see below). ApoE is present in VLDL and HDL. It is mainly synthesised in liver although many organs have been documented to produce small quantities.

Apolipoprotein(a) is a recently identified protein which is not strictly an apolipoprotein but is the characteristic component of a discrete group of lipoprotein particles known as Lp(a). These are apoB-containing lipoproteins which float at a density varying between 1.050-1.082g/mL and exhibit pre-beta mobility on electrophoresis. Recent studies have revealed that Lp(a) is an LDL-type particle with an extra glycoprotein molecule(apo(a)) linked to the apoB moiety by a disulphide bridge(16). Apo(a) shows considerable sequence homology with plasminogen(16). Plasminogen consists of a number of protein units known as kringles and an active protease site for cleaving fibrinogen. Apo(a) is now known to consist of an inactive protease region linked to a kringle 5 unit of plasminogen with a variable number of kringle 4 units attached. Recent studies have shown that apo(a) exists as a number of isoforms in man and that these appear to influence the plasma concentration(see below). High plasma apo(a) concentrations are associated with an increased incidence of cardiovascular disease(17). However, much remains to be discovered regarding the metabolism of Lp(a) and its importance and potential for modification in

atherosclerotic disease.

1.5 Lipoprotein metabolism

The control of lipoprotein metabolism in man is complex and not fully understood. The wide variation in lipid and lipoprotein levels within and between populations suggests that the homeostatic mechanisms regulating plasma lipoprotein levels are not under tight control. Many factors may disturb "normal" levels. Common genetic polymorphisms of proteins which modulate lipoprotein metabolism and diet are thought to be the most important factors. This variation is illustrated by the elevated cholesterol and triglyceride levels in the Western populations compared with Japan and third world countries. Countries such as Scotland and Finland with the highest mean levels have the highest incidence of cardiovascular mortality and morbidity(18). Until recently it has proved difficult to dissect out the precise pathophysiological disturbances responsible for the majority of primary and secondary hyperlipidaemias. With new developments in molecular biology and use of radioactive and stable isotope trace-labelled lipoproteins in kinetic studies, rapid advances are being made. This section attempts to summarise some of the current theories regarding the production and catabolism of the various lipoprotein subfractions. Where appropriate, examples of

dyslipidaemias due to specific genetic disorders or acquired conditions with known metabolic defects are used to illustrate the importance of these factors in maintaining normolipidaemia.

The two main pathways for lipid transport in man are termed the exogenous and endogenous pathways according to the source of the main lipid constituent(19). The liver acts as the central coordinator of these two pathways and is intimately involved in the secretion and removal of plasma lipoproteins. These two transport systems will be considered separately. In addition, a number of enzymes play important roles in plasma lipoprotein metabolism. These are listed in table 1.5.

1.5.1 Exogenous lipid transport

Dietary fat, glycerides and cholesterol, is transported in the exogenous pathway initially as chylomicrons which are synthesised in the intestinal villi. Synthesis of these lipoproteins is dependent both on the supply of exogenous fat from the intestinal lumen and on production of apoB48. In the rare condition of abetalipoproteinaemia, chylomicrons(and other apoB-containing lipoproteins) are absent from plasma and fat droplets accumulate in enterocytes(20). Chylomicrons are secreted both into the portal blood stream and the intestinal lymphatics. They

acquire apoE and apoC's in the interstitial fluid or circulation and these are important in their further metabolism. These particles bind to endothelial-bound lipoprotein lipase(LPL) in tissues, especially adipose tissue and skeletal muscle, and are rapidly hydrolysed with the release of free fatty acids. This reaction requires apoCII as a co-factor. Other apolipoprotein constituents of chylomicrons may inhibit this enzyme. The importance of this lipolytic enzyme is illustrated by familial LPL deficiency which is characterised by severe hyperchylomicronaemia with opalescent plasma even after a prolonged fast(21). Surface components(phospholipid, free cholesterol and some triglyceride) are transferred during lipolysis to HDL particles. The particles remaining are smaller, denser and contain a larger proportion of protein and cholesterol. Further hydrolysis occurs in the liver under the influence of hepatic triglyceride lipase(HTGL). The remnant particles formed are rich in apoE and are thought to be removed by receptor-mediated endocytosis in the liver. For some time the existence of a specific chylomicron receptor recognising apoE has been postulated and, recently, a candidate receptor protein(the LDL related protein) has been described(22).

The exogenous pathway is completed by the excretion of cholesterol as bile salts. This represents the only route by which significant quantities of cholesterol can be

ultimately excreted by the body. Small amounts may be lost as a result of desquamation of epithelial cells and as degraded sterol hormones in the urine. The production of bile acids from cholesterol is dependent on the activity of the rate-limiting enzyme, 7-alpha-hydroxylase. The activity of this varies inversely with the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase(HMG CoA reductase) which controls de novo cholesterol synthesis. In this way, cholesterol and bile acid synthesis are coordinated in an attempt to conserve intra-hepatic cholesterol pools although the precise relationship between these two pathways is not clear(19).

1.5.2 Endogenous lipid transport

These pathways are responsible for the redistribution of lipids to and from the liver. The metabolism of the various subfractions will be considered separately although it should be stressed that there is constant interchange between these particles which are in a dynamic equilibrium with each other and tissue lipids.

VLDL/IDL metabolism

Lipids arriving in the liver from the portal and systemic circulation or synthesised de novo must be packaged into lipoproteins to allow their transport through the plasma.

This is initiated by the assembly and secretion of very low density lipoproteins(VLDL). The secretion of these triglyceride-rich lipoproteins(like chylomicrons) is a function of the availability of fatty acids. Triglycerides are synthesised from either dietary fat delivered in chylomicrons or hepatic glucose and fatty acids stores. ApoB production is also essential for the secretion of these lipoproteins as shown by their absence in abetalipoproteinaemia(20). The relative importance of triglyceride and apolipoprotein synthesis in the control of VLDL secretion is unclear but it appears that increased triglyceride production is associated with a rise in both the number and size of VLDL particles(23). The latter reflects an increase in the triglyceride to protein ratio. Hormones, such as insulin, also influence the rate of secretion. Patients with poorly controlled non-insulin-dependent diabetes mellitus have an elevated level of triglyceride-rich lipoproteins due to increased hepatic secretion(24). This can be reversed by insulin therapy.

VLDL is secreted by the liver and carried to the periphery where, like chylomicrons, triglycerides and phospholipids are hydrolysed by lipoprotein lipase(LPL). This is facilitated by the acquisition of apoCII from HDL particles in the circulation. The larger VLDL particles(Sf 60-400) are preferentially hydrolysed by lipoprotein lipase while smaller VLDL and IDL remnant particles are substrates for

hepatic triglyceride lipase. This has been confirmed both by studies of hepatic clearance of these lipoproteins and by apoB kinetic investigations in rare cases of hepatic triglyceride lipase deficiency(25).

Considerable interchange of lipids also occurs between lipoproteins in the circulation. Triglyceride-rich particles acquire cholesterol ester from HDL_2 through the action of cholesterol ester transfer proteins. Lipolysis of the triglyceride component leads to a reduction in size and alteration in composition of these particles. ApoC and phopholipids are transferred to HDL in exchange for apoE with the formation of remnant particles(or IDL) in the density range 1.006-1.019g/mL. These are analagous to chylomicron remnants. Further remodelling results in the bulk of the particles passing to the LDL density range. Some of the remnant material is removed directly by hepatic B/E receptors. Recent evidence(25) suggests that there may be metabolic heterogeneity among subfractions of VLDL of differing densities. It is now clear that significant quantities of VLDL apoB are removed directly from this density range and are not transformed to LDL particles. ApoB kinetic studies have demonstrated that there is input and removal of these subfractions at a variety of points in the delipidation cascade. The precise metabolic fate may be determined by the initial particle size.

Another factor influencing the metabolism of VLDL particles is the common polymorphisms of apoE(15). The E2 polymorphism has a reduced affinity for the chylomicron and B/E receptor resulting in reduced clearance of remnant particles. Homozygosity for E2 gives rise to a specific dyslipidaemia which is accompanied by frank hyperlipidaemia in type III hyperlipidaemia(familial dysbetalipoproteinaemia). Affected individuals have fasting hypertriglyceridaemia and hypercholesterolaemia, high VLDL cholesterol: plasma triglyceride ratio, remnant accumulation and variable LDL levels. However, since 1% of the population are homozygous for the E2/E2 polymorphism and only 1/5000-10000 have type III hyperlipidaemia, another factor(e.g. obesity, alcohol excess) must co-exist with the E2/E2 phenotype for the full expression of this condition.

LDL metabolism

The majority of LDL particles are formed as the end-product of lipolysis of VLDL. A small part of the LDL pool may be secreted directly by the liver or as a very rapidly metabolised subfraction of VLDL which is difficult to detect by tracer experiments(27,28). The nature of this LDL production is still controversial. To date, there is no evidence of direct LDL-like particle secretion in

hepatic perfusion studies in a number of animals(29) although direct LDL synthesis may be quantitatively important in some types of hyperlipidaemia in man e.g. familial hypercholesterolaemia(27,28,30). In general, the production of LDL is linked to the catabolism of VLDL. The delipidation of the triglyceride content and the transfer of redundant surface material(phospholipid and free cholesterol) to HDL leads to the formation of smaller, denser particles within the LDL density range (d=1.019-1.063mg/L).

The mechanisms for removal of LDL from the plasma have been extensively investigated. A substantial proportion (approximately 50-70%) is cleared by the specific LDL receptor. This is a membrane-bound receptor found on most cells which recognises apoB and apoE, binds and internalises lipoprotein particles. These are then rapidly hydrolysed in lysozymes. The identification of the LDL(apo B/E) receptor and its role in cholesterol homeostasis(31) was recognised by the award of the Nobel prize to Goldstein and Brown in 1985 for work spanning more than a decade. Control of cellular cholesterol requirements is achieved by feedback regulation of the number of LDL receptors and of the production of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase which controls de novo cholesterol synthesis from acetate. The overriding requirement is to deliver adequate amounts of cholesterol to cells for membrane synthesis and

repair or sterol hormone production. Intracellular free cholesterol(or an oxidised derivative) controls the intracellular concentration by its effect on three crucial proteins - 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), acyl-CoA cholesterol acyltransferase (ACAT) and the LDL receptor. When intracellular supplies of free cholesterol are adequate, the number of LDL receptors expressed on the cell membrane is decreased as is HMG CoA reductase activity. Cholesterol is converted to cholesterol ester in the cell by ACAT for later use. If the intracellular cholesterol pool decreases, the opposite effects occur - LDL receptor numbers and HMG CoA reductase activity increases. These homeostatic mechanisms appear to be regulated by the effect of oxidised derivatives of free cholesterol on transcription and degradation of these proteins(32).

The alternative route(s) for catabolism of plasma LDL is poorly characterised but probably involves phagocytic cells. These "scavenger" pathways are thought to preferentially remove physiologically-altered LDL. This route for LDL removal appears to be unregulated and can lead to massive accumulation of cholesterol ester in smooth muscle and macrophage cells in atherosclerotic plaques. It has been postulated that modified LDL taken up by pathways independent of the LDL receptor leads to an increased risk of atherosclerosis(31). In vitro, acetylated LDL has been

shown to bind to a receptor on mouse macrophages(33). Another receptor identified in vitro on mouse macrophages binds and internalises beta-VLDL(cholesterol enriched) with the formation of foam cells. As yet, there is no clear evidence that similar pathways exist in vivo in mice or in man. The putative role of modified LDL, particularly oxidised LDL, in atherosclerosis has recently been reviewed(34).

The crucial role played by the LDL receptor in the control of plasma LDL is illustrated by the classical condition of familial hypercholesterolaemia(35). The clinical and genetic characteristics of this autosomal co-dominant condition have been well described. The diagnosis is based on a family history of premature coronary artery disease, tendon xanthomata and hypercholesterolaemia due to elevated plasma LDL-cholesterol and is associated with the onset of features of coronary insufficiency usually in the fourth or fifth decade. One in five hundred of the population have familial hypercholesterolaemia and there is a gene dose effect with homozygotes(frequency $1/10^6$) more severely affected. The latter often die from ischaemic heart disease before the age of twenty. Heterozygotes have only half of the normal number of functional LDL receptors resulting in a doubling of the plasma cholesterol; homozygotes have virtually no functional receptors and severe hypercholesterolaemia(3-4x normal). The rise in

plasma cholesterol levels permits sufficient cholesterol uptake by hormone-synthesising and actively dividing cells but at the expense of massive, unregulated uptake by alternative pathways which lack regulatory feedback. The changes in plasma cholesterol in this disease indicate the importance of LDL receptor activity in regulating plasma LDL levels. However, LDL synthesis can also affect the plasma level and kinetic studies of patients with familial hypercholesterolaemia indicate that many have an increased rate of LDL production. It is now recognised that the apoB/E receptor removes apoB-containing lipoproteins other than LDL(e.g. small VLDL and IDL) and the increased LDL production rate in familial hypercholesterolaemia may be due to increased availability of IDL for delipidation when its direct removal is impaired. In an unselected population, LDL-cholesterol levels have been correlated with both synthetic and catabolic rates suggesting that both play a role in determining the steady state level(36).

HDL metabolism

HDL metabolism is complex, poorly understood and inextricably linked to the metabolism of other lipoproteins. HDL is thought to play a critical role in preventing tissue accumulation of cholesterol. Nascent HDL is secreted by the liver(and probably other organs) as disc-like particles of phospholipid, free cholesterol and

protein(apoAI). These may also be produced from surface components of triglyceride-rich lipoproteins or by association of free phospholipid and apolipoprotein in plasma. These discs are not detected in plasma as they are rapidly converted to spherical particles as a result of the action of lecithin cholesterol acyltransferase(LCAT). Phospholipid and triglyceride are acquired from lower density lipoproteins and the HDL_3 particles formed (d=1.125-1.210g/mL) are thought to have a crucial role in "reverse cholesterol transfer"(10). Cholesterol from peripheral cells is transferred to these particles and esterified by LCAT. HDL particles enriched by cholesterol ester(HDL_2 d=1.063-1.125g/mL) have been shown to bind to hepatic membranes and other sterol-requiring cells by receptor-mediated mechanisms. Both apoAI and apoE may be ligands. As the liver is the only organ that can excrete significant amounts of cholesterol in the form of bile acids, this provides an important means of reducing tissue cholesterol levels. Cholesterol ester may also be transferred between HDL and lower density lipoproteins via cholesterol ester transfer proteins in the plasma.

HDL levels are linked to the metabolism of other lipoproteins. Measures of VLDL catabolism and apolipoprotein AI turnover show close correlations. Exchange of apolipoproteins between HDL and VLDL regulates the activity of lipoprotein lipase. The precise importance

of this and many other aspects of HDL metabolism have not been fully established.

1.6 Nephrotic syndrome - definition, causes and complications

The nephrotic syndrome is a clinical and biochemical syndrome rather than a single disease. A large number of pathological conditions may cause the syndrome which is arbitrarily defined as the presence of oedema, proteinuria in excess of 3.5g/day and hypoalbuminaemia(usually <30g/L). Although there is a broad correlation between these three features, some subjects maintain a normal serum albumin despite heavy proteinuria while others develop hypoalbuminaemia with smaller measured urinary losses. Treatment such as diuretics, albumin infusions and dietary intervention may modify the clinical picture. Since proteinuria is thought to be the primary manifestation leading to many other features of the syndrome, the nephrotic syndrome is often empirically defined on the basis of quantitative proteinuria alone. Although a useful practical approach, this value may vary considerably from day to day as a result of both intra-individual variation and the imprecision of urinary protein measurements.

In normal man, up to 150mg of protein is excreted in the urine per day. This represents the balance of protein

filtered in the glomeruli and reabsorbed in the tubules. A small amount of protein is also secreted by the tubules. In most cases, pathological elevation of urinary protein results from changes in the glomerular capillary wall (GCW). Rarely, proteinuria is secondary to excessive plasma proteins(e.g. light chain myeloma), decreased tubular absorption or lower urinary tract disease. It is unusual for any of these(with the possible exception of light chain myeloma where a number of these pathological mechanisms are operative) to give rise to proteinuria in the nephrotic range.

The GCW, consisting of the endothelial cell, basement membrane and the epithelial cell, restricts the passage of molecules into the urinary space on the basis of molecular size, configuration and electrical charge. Experimental studies, based on the differential clearance of dextran molecules over a range of sizes, have suggested that this barrier constitutes a series of cylindrical pores(37). The precise size and distribution of these theoretical pores remains controversial. The GCW also contains a fixed negative electrical charge which hinders the passage of anionic molecules such as proteins. Damage to the GCW may result in a change in the number and size of pores or a loss of negative charge leading to a marked increase in glomerular permeability. Opinions vary as to which of these defects is most important and this may differ between

diseases.

Many disorders can impair the integrity of the glomerular barrier and result in the clinical syndrome of proteinuria. Both primary glomerular diseases or systemic diseases with renal involvement may present in this way. The commonest causes in adults in the United Kingdom are listed in table 1.6. In many countries, infectious diseases(e.g. malaria, syphilis or hepatitis) account for the majority of cases. Light negative(or minimal change) nephropathy is the commonest cause of nephrosis in children in the UK. Although the diagnosis may on occasion be reached from the history and examination, the majority of cases will require renal biopsy for precise histological classification. This often has prognostic and, occasionally, therapeutic importance.

For many years, it has been widely held that the clinical syndrome and laboratory features of the nephrotic syndrome are initiated by the urinary loss of protein and modified by a number of compensatory mechanisms. However, there are still many unanswered questions regarding the metabolic changes which occur. Albumim homeostasis may be deranged by relatively small urinary losses. This has led some workers to invoke extensive tubular catabolism of filtered albumin. Increased albumin synthesis in response to proteinuria is not a universal finding. Studies have shown

that nephrotic patients on a low protein diet have albumin synthetic rates similar to controls(38). Although augmenting protein intake increases the rate of albumin synthesis, serum albumin levels do not rise and proteinuria increases. These findings have, in part, been confirmed by others(39). Since increasing proteinuria is associated with a poorer prognosis, clinicians are now reassessing the role of the traditional high protein diet in these patients. These examples illustrate our relative ignorance of many basic physiological and metabolic processes in this disease.

The nephrotic syndrome is accompanied by a wide range of disturbances of normal physiology(40). Hyperlipidaemia is a well-recognised association and will be considered in detail below. In addition, an increased incidence of thrombo-embolic events has been associated with changes in coagulation parameters(both activators and inhibitors of coagulation) and platelet function. A variety of disturbances of calcium homeostasis have been described including low ionised calcium and vitamin D deficiency. Osteodystrophy only develops in association with declining renal function. Infection has been a major cause of mortality and morbidity in nephrotic syndrome and this reflects an immunocompromised state. Immunoglobulin losses in the urine may be compounded by decreased synthesis and/or increased catabolism resulting in low IgG

and IgA levels. IgM levels are often high. Changes in cell-mediated immunity and in macrophage function have also been reported. Many other abnormalities including trace element deficiencies and altered thyroid status have been described. A comprehensive discussion of all these is outwith the scope of this thesis.

1.7 Hyperlipidaemia in nephrotic syndrome

1.7.1 Lipid and lipoprotein abnormalities

The association between renal disease and elevated blood lipids was first described by Blackall as long ago as 1813(1). Other workers subsequently confirmed his observations specifically in relation to glomerular disease(2,41). Despite the efforts of many investigators over the years, both the precise nature of the pathophysiological disturbances responsible for this phenomenon and the clinical importance remain uncertain.

The spectrum of lipid abnormalities found in nephrosis, involving both quantitative and qualitative changes in plasma lipoproteins, has been fairly well-characterised. However, the prevalence of abnormalities is not well documented and many studies have failed to control for other factors which may perturb lipid metabolism. Many early series included subjects with systemic diseases,

advanced renal failure or on treatment with corticosteroids. Diuretics and anti-hypertensive drugs are frequently needed in nephrotic subjects and the effects of these are difficult to avoid. All of these factors have independently been associated with alterations in plasma lipid levels.

Plasma cholesterol concentrations, both free and esterified, are consistently increased as are phospholipids. Triglyceride levels are much more variable. Baxter et al.(41) emphasised this wide variation with plasma triglyceride concentrations ranging from 76 to 3820mg/dL(0.9-43.3mmol/L). In this series, both triglyceride and cholesterol levels showed an inverse relationship with serum albumin although in more severe cases the ratio of cholesterol to triglyceride fell dramatically. At very low levels of serum albumin, lipid levels rose exponentially. The patients in this study were severely hypoalbuminaemic with 42 of 58 plasma lipid measurements associated with serum albumin <15g/L.

Changes in apoB-containing lipoproteins reflect the changes in neutral lipid levels with increases in LDL- and VLDL-cholesterol which in general show a positive relationship with the severity of the condition. Gherardi et al.(42) quantified the changes in plasma lipoproteins in a group of nephrotic children. They noted relationships between

serum albumin and three lipoprotein fractions. LDL_2 (d=1.019-1.063g/mL) was negatively correlated while HDL_2 (d=1.063-1.125g/mL) showed a positive correlation. Plasma VLDL-cholesterol(d<1.006g/mL) increased exponentially as serum albumin fell to 15g/L - a finding similar to Baxter's observations on triglycerides(41). Qualitative changes in the lipoprotein particles also occur with increased cholesterol ester and phospholipid content of both LDL and VLDL and a reduced protein content. A larger study of 59 children in relapse and remission of minimal change nephrotic syndrome confirmed the elevation of LDL-cholesterol concentrations(>95th centile for age) in 81% of patients but VLDL-cholesterol was only elevated in 60%(43). There was only a poor correlation with serum albumin and a significant number of patients had persistent lipid abnormalities in remission. However, another study, while confirming the elevated lipid levels in relapse, reported normalisation of all parameters including apolipoprotein concentrations within 7 weeks of remission(44).

In adults, there are few large studies of uncomplicated nephrotic syndrome which have clearly documented the prevalence and severity of hyperlipoproteinaemia using the current standard methods of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins for measuring cholesterol content of plasma lipoproteins. An early study(45) reported hyperlipidaemia in only 17 of 33

patients with nephrotic syndrome but 13 of these patients were in remission at the time of the study and all 7 who were actively nephrotic(serum albumin <30g/l; proteinuria >3g/day) had abnormal lipoprotein profiles. One third of the patients were on corticosteroids. These factors and the method of lipoprotein analysis make interpretation of this report difficult. Newmark et al.(46) have reported the largest series in a retrospective review of 96 cases of hyperlipidaemia in nephrosis caused by a variety of glomerular diseases including diabetes mellitus and systemic lupus erythematosus. Significant inverse associations were found between serum albumin and both total cholesterol and triglyceride. Although serum albumin was inversely related to urinary protein loss, no relationship was demonstrated between proteinuria and plasma lipid levels. Lipoprotein phenotypes were mainly classified as IIa, IIb or V. No clear differences between patients with primary or secondary glomerular disease were seen. However, as only patients with hyperlipidaemia were studied, this may not reflect the prevalence of hyperlipidaemia in an unselected nephrotic population. A study(47) of 20 consecutive patients with heavy proteinuria (>3g/day) demonstrated total cholesterol concentrations greater than the 95th centile for the population in 70% with figures of 55% and 30% for LDL- and VLDL-cholesterol, respectively. Total cholesterol concentration showed an inverse correlation with plasma albumin and oncotic

pressure. Joven et al. have also recently reported a series of 57 patients with nephrotic syndrome(48). Compared with published data, 33% had increased LDL-cholesterol with normal VLDL-cholesterol, 60% had increased levels of both and 7% had elevated VLDL-cholesterol alone. Plasma cholesterol, triglyceride, phospholipid and LDL-cholesterol were all inversely correlated with serum albumin but no association with proteinuia was observed.

HDL concentrations have been recorded as normal(44,48,49) or low(41,42,50) by most investigators although increased levels have also been found(51,52). HDL has been quantified in the urine in nephrotic patients but the urinary losses do not appear to be related to plasma levels although as with other proteins(e.g. albumin) the effect of tubular catabolism of filtered lipoprotein is uncertain. Changes in the composition of urinary HDL have been ascribed to this(53).

In general, changes in plasma apolipoprotein levels in man parallel the changes in lipoproteins. ApoB levels are invariably increased and apoAI reflect HDL-cholesterol levels. Levels of all three apoC's are increased although there is no change in the C-II/C-III ratio(44).

1.7.2 Altered lipoprotein metabolism

The metabolic changes resulting in the hyperlipidaemia in nephrotic syndrome are complex. Theoretically, hyperlipoproteinaemia may develop due to increased lipoprotein production, decreased catabolism or a combination of these mechanisms. Changes in both lipid and apolipoprotein metabolism occur. In both normal and abnormal lipoprotein metabolism, it is uncertain how the production of lipids and apolipoproteins is coordinated with the assembly and secretion of lipoprotein particles.

In nephrotic syndrome, the primary defect is generally considered to be increased hepatic secretion of lipoproteins. However, a number of catabolic defects have also been described. Much of the evidence for increased hepatic synthesis and secretion of lipoproteins is based on the work of Marsh and colleagues(54). Over a number of years, they have performed a series of experiments on animal models of nephrotic syndrome and consistently shown increased production of lipids, lipoproteins and apolipoproteins in intact animals and isolated perfused livers of nephrotic rats. These results have been confirmed by other workers by measuring the incorporation of labelled precursors into lipoproteins and apolipoproteins(55-58) and lipids(59-60). However, not all studies have reached this conclusion(61).

An alternative approach involving studies of the clearance of exogenously-labelled lipoprotein particles in nephrotic animals has demonstrated the presence of catabolic defects. Clearance of both HDL(62) and VLDL(63,64) particles appears to be reduced. A recent report on the fate of ^{125}I-labelled LDL particles in rats rendered nephrotic with puromycin aminonucleoside showed no difference in the fractional catabolic rate compare to controls(65). The elevated plasma LDL concentrations were ascribed to increased synthesis although this was only significant when compared on a mg/kg/day basis and was influenced by the failure of the nephrotic group to gain weight.

How far these results can be extrapolated to man is uncertain for a number of reasons. Firstly, the two main rat models of nephrotic syndrome which have been studied are induced by anti-kidney serum and puromycin aminonucleoside. Both experimental models have features which might perturb lipid and lipoprotein metabolism independent of proteinuria. Anti-kidney serum causes renal failure while puromycin is a general cytotoxin which is associated with hepatic toxicity. Secondly, lipoprotein metabolism shows marked inter-species differences. Man has an almost unique plasma lipoprotein profile with a greatly expanded LDL pool compared to other mammals. The main cholesterol carrying particle in plasma of most other species is HDL. Therefore, although the use of animal

models of nephrotic syndrome is convenient and has permitted some insights into abnormal lipoprotein metabolism, the results should be interpreted with caution. Confirmatory studies in man are required to validate the conclusions reached.

Similar techniques have been utilised in human nephrotic syndrome but on a relatively small scale compared to the extensive literature on animal work. Gitlin(66) performed pioneering studies on the use of radiolabelled lipoproteins to trace lipoprotein metabolism _in vivo_. Some of the earliest studies were performed on nephrotic children. The clearance of lipoproteins S_f 3-9, 10-200 and alpha-lipoproteins was investigated. It was concluded that S_f 3-9 lipoproteins were catabolised at a slightly faster rate in nephrosis but that there was a defect in conversion of less dense lipoproteins to this fraction. Alpha-lipoproteins were more rapidly removed from the circulation even when corrections were made for urinary losses. Similar studies of the metabolism of trace-labelled low density lipoproteins by Scott _et al_.(67) in the late 1960's showed a small but statistically insignificant decrease in the fractional catabolic rate of this lipoprotein fraction. They postulated that the increased LDL levels commonly found must be due to increased synthesis. A similar conclusion has recently been reached by other groups of investigators in studies of small numbers of nephrotic

subjects(48,68-70). The total number of nephrotic patients studied using these techniques is relatively small.

Other workers have looked at whole body lipid turnover by measuring the incorporation of radiolabelled precursors into triglyceride and cholesterol ester respectively. This is useful for assessing total lipid turnover but is unable to determine the metabolism of lipoprotein fractions because of lipid transfer between particles in the circulation. Using labelled glycerol and mevalonic acid, McKenzie and Nestel(71) demonstrated a reduction in the fractional clearance of both triglyceride and esterified cholesterol in nephrotic syndrome. However, there was a disproportionate increase in the lipid pool size suggesting that changes in both synthetic and catabolic mechanisms contribute to the hyperlipidaemia. Similar results were found for triglycerides by another group(72).

The stimuli to increased lipid and lipoprotein synthesis is uncertain. Cholesterol production may be increased due to disturbances of the metabolism of mevalonate, an important precursor of cholesterol biosynthesis. Mevalonate is both excreted by filtration and oxidised by the kidney. Clearance by both routes is reduced in nephrotic rats and increased substrate availability could enhance hepatic cholesterol production(73). However, the same mechanism could also account for decreased catabolism of low

density lipoproteins by its effect on intrahepatic sterol pools and, secondarily, on apo B/E receptor activity. Increased substrate availability has also been postulated as a cause of increased triglyceride synthesis. Fatty acids released by hydrolysis of triglycerides are normally bound to albumin. Hypoalbuminaemia increases plasma levels of free fatty acids which may be taken up by the liver and stimulate triglyceride synthesis(72).

Precisely how the changes in lipoprotein and apolipoprotein metabolism are mediated is not clear. Many workers have emphasised the central role of hypoalbuminaemia. Evidence for the importance of hypoalbuminaemia derives from studies in both animal models and man where amelioration of nephrotic hyperlipidaemia was achieved by infusion of large quantities of albumin or other oncotic agents(74,75). Furthermore, hyperlipidaemia accompanies the rare syndrome of analbuminaemia in man(76) and there is a broad inverse relationship between the degree of hypoalbuminaemia and hyperlipidaemia. Appel(47) also correlated the lipid abnormalities with plasma oncotic pressure. Early theories suggested that albumin and lipoproteins shared a common biosynthetic pathway and that a general increase in hepatic protein synthesis resulted in hyperlipoproteinaemia. However, the evidence for this is unconvincing. Increased albumin synthesis has not been consistently found in nephrotic syndrome and, recently, hypercholesterolaemia has

been demonstrated in a group of nephrotic patients maintained on a low protein diet who had normal albumin synthetic rates(38). Other factors must be involved since some patients develop hyperlipidaemia despite a normal serum albumin and hyperlipidaemia precedes hypoalbuminaemia in some animal models.

1.7.3 Enzymes involved in lipid and lipoprotein metabolism

Lipoprotein metabolism is dependent on a number of key enzymes and regulatory proteins(e.g. receptors and apolipoproteins). Defects in the concentrations or functional activities of some of these enzymes have been found in nephrotic syndrome in experimental animal models and in man.

Decreased post-heparin lipase activity has been recorded by a number of groups(63,77-79) and this has been localised to the lipoprotein lipase fraction(79). However, this is not a universal finding(51,80,81). Decreased lipase activity would explain the decreased catabolism of lighter lipoproteins found by Gitlin(66) and the marginally reduced capacity of nephrotic subjects to clear an intravenous fat load(82). Since apolipoprotein CII is a co-factor for this enzyme, increased urinary losses might result in decreased enzyme activity. Low plasma levels of apoCII have not been

found(44,83) although it has been suggested that the VLDL-associated apoCII may be relatively reduced(83). Staprans (84) found another lipase cofactor in the urine of nephrotic subjects which was identified as a glycosaminoglycan, heparan or chondroitin sulphate.

Similarly, lecithin cholesterol acyltransferase activity has been found to be decreased in nephrosis(85). Animal studies have confirmed this and related it to the degree of hypoalbuminaema(86).

Hypoalbuminaemia has also been implicated in the decreased activities of these enzymes. Albumin is an important acceptor of the products of these reactions(lysolecithin and free fatty acids, respectively) and accumulation of unbound products may limit the activities of the enzymes.

In summary, the nephrotic syndrome is associated with a complex series of changes in metabolism involving increased synthesis of lipids and apolipoproteins, catabolic defects of VLDL and HDL and altered activities of regulatory enzymes. However, many of these observations have been made in experimental nephrotic syndrome in contrast to the paucity of data in man. The factors which induce these changes and their relative importance in man have not been clearly determined.

1.7.4 Lipid abnormalities in other renal diseases

Abnormalities of plasma lipids and lipoproteins are also found in other renal diseases and these are considered briefly.

Renal failure

The lipid and lipoprotein changes in renal failure are distinct from those seen in the nephrotic syndrome. However, where the nephrotic syndrome is accompanied by reduced glomerular filtration a complex picture may result. Abnormalities of triglycerides have been reported when glomerular filtration rates decrease by 50%(87) - before most of the other clinical and biochemical features of renal failure develop. Although most of the studies of hyperlipidaemia in renal failure have been on maintenance dialysis patients, it is now clear that these precede end-stage renal failure.

The advent of dialysis therapy may accentuate hyperlipidaemia. Maintenance haemodialysis is associated with hypertriglyceridaemia, low HDL-cholesterol and normal total cholesterol concentrations. Many theories have been advanced to explain this. There appears to be a significant reduction in lipoprotein lipase activity. Reduced catabolism of VLDL leads to a reduction of HDL.

During the lipolysis of VLDL, excess surface material is transferred to HDL_3 particles which under the influence of lecithin cholesterol acyltransferase are converted to HDL_2. Failure of this mechanism is often associated with a fall in HDL-cholesterol concentration. Decreased LPL activity has been attributed to the repeated adminstration of heparin as an anticoagulant during regular dialysis. Heparin releases endothelial-bound lipoprotein lipase into the circulation and may lead to chronic exhaustion of the enzyme. However, lipase activity has been shown to be decreased in patients with impaired renal function before dialysis is required(88). Uraemic serum inhibits lipase activity in vitro(89).

In the dialysis population, other mechanisms have been implicated in the hypertriglyceridaemia. These include disturbances of carbohydrate metabolism due to insulin resistance, excessive dietary carbohydrate in low protein diets and acetate loading from dialysate. Carnitine deficiency has also been postulated as a cause. The precise mechanisms underlying the lipid abnormalities of chronic renal failure remain uncertain.

Organ transplantation

Hyperlipidaemia following organ transplantation is thought to be related to immunosuppressive therapy.

Corticosteroids, particularly in the larger doses used in the pre-cyclosporin era, cause insulin resistance and stimulate VLDL secretion. More recently, the advent of cyclosporin has been associated with a rise in cholesterol levels(90).

1.8 Hyperlipidaemia and atherosclerosis in the nephrotic syndrome

Over the last 20 years, substantial evidence has accumulated to implicate a causal role for primary hyperlipidaemia in premature coronary artery disease. Whether these findings can be extrapolated to other forms of hyperlipidaemia is less certain. It is often stated that the risk of cardiovascular events is increased in the nephrotic syndrome. However, this is based almost entirely on anecdotal or uncontrolled reports and has been challenged by some authorities. This controversy remains unresolved.

There are two main questions:-

1 . Is the nephrotic syndrome associated with an increased risk of cardiovascular disease?

2. If so, what is the contribution of the hyperlipidaemia?

Reports of small series of nephrotic subjects have suggested an increased incidence of cardiovascular events,

usually myocardial infarction(91,92). Coronary artery disease has been documented at a very young age in corticosteroid-refractory nephrotic syndrome(93). The only autopsy study of the coronary artery status of patients with this condition found an increase in the degree of coronary atherosclerosis compared to a group of matched controls(94). However, this study only included 3 patients with idiopathic glomerular disease. Thirteen had lupus nephropathy and 4 had diabetes mellitus. Both of these conditions may accelerate atherosclerosis independently. The prevailing opinion that the nephrotic syndrome confers an increased risk was challenged by Wass and colleagues(95). In what remains the largest epidemiological study of vascular disease in nephrosis, they followed a group of 159 consecutive adult nephrotics for a mean period of 5 years. When compared to a cohort of 1005 Greater London Council employeees, they were unable to detect an excess of deaths, morbid vascular events or electrocardiographic changes. A number of criticisms of this study have been made. Twenty-nine of 49 deaths were ascribed to terminal renal failure only 5 of whom received renal replacement therapy. Many of these deaths may have been due to cardiovascular disease rather than uraemia. Similarly, nearly half of the survivors had only a single short episode of proteinuria which may be a more benign condition. Separate analysis of those with persistent proteinuria showed a slight excess of cardiovascular

findings although this did not reach statistical signifigance. In contrast, a recent preliminary report of a similar large series has documented an increased incidence of ischaemic heart disease(96).

This controversy was highlighted by the juxtaposition in one journal of editorials supporting opposite opinions in 1981(97,98). However, some unanimity was reached over patients with persistent nephrosis with an unfavourably high LDL:HDL-cholesterol ratio who were considered to be at increased risk. A recent retrospective study of membranous glomerulopathy in the West of Scotland(99) found a significant increase in both arterial and venous thrombosis compared to a matched group with IgA nephropathy. The membranous group were characterised by heavy proteinura which is uncommon in IgA nephropathy. In summary, current opinion holds that those patients with a persistent nephrotic state are more likely to develop features of cardiovascular disease, particularly coronary artery obstruction.

Assuming this is true, how important is the hyperlipidaemia in accelerating atherosclerosis? The nephrotic syndrome may complicate other systemic disease and is often accompanied by a number of features which may increase cardiovascular risk. Hypertension and a hypercoagulable state(often manifesting as venous thrombosis) are perhaps

the most important. The preponderance of males with nephrotic syndrome, progressive renal failure and haemoconcentration due to over-vigorous diuretic therapy may also be relevant. However, the quantitative and qualitative changes in plasma lipoproteins documented in nephrosis are of a type commonly associated with an increased risk of coronary events in the general population (i.e. increased LDL-cholesterol, low or normal HDL-cholesterol and increased total and LDL:HDL-cholesterol ratio). Convincing experimental(100) and epidemiological (101) evidence exists causally implicating these abnormalities in the development of premature coronary heart disease. Recent studies(102,103) have shown that reductions in total and LDL-cholesterol are associated with a decreased incidence of cardiovascular events. It would seem reasonable to extrapolate these results to nephrotic syndrome where the calculated overall cardiovascular risk may be greater. However, the benefits of lipid lowering in nephrotic syndrome specifically have not been proven by the appropriate controlled trials.

1.9 The role of lipids and lipoproteins in progressive renal disease

Renal diseases are characterised by their progressive nature. Following renal injury and loss of filtration capacity, there is often a slow decline in the remaining

renal function even where the original process is apparently no longer active. For an individual, this decline is predictable with a linear fall in glomerular filtration with time. Work on animal models of renal failure led to the hypothesis that loss of renal excretory capacity was accompanied by glomerular hypertrophy and hyperfiltration in surviving nephrons(104). This protective response leads to further nephron loss by unexplained mechanisms although increased intraglomerular pressure has been implicated. Both systemic hypertension and protein load have been suggested as mediators of these glomerular changes. There is fairly convincing evidence that control of hypertension can at least retard the progression of renal failure both in experimental animal models and in man(105). Despite intensive study, the role of dietary protein restriction has not been conclusively proven(106) and many nephrologists do not prescribe low protein diets. Accumulation of inorganic salts(e.g. phosphate, calcium and urate) in the renal interstitium may accelerate renal failure by interfering with tubular function.

Recently, evidence has emerged to suggest that hyperlipidaemia may play a role in the progressive loss of renal function. This was first hypothesised in a comprehensive fashion by Moorhead in 1982(107). The evidence for this is based on a number of clinical and

pathological observations in man and in experimental models of renal disease. Glomerulosclerosis with mesangial expansion, foam cell accumulation and deposition of amorphous hyaline material is the common histological finding in many progressive renal diseases. For many years histologists have noted accumulation of lipid droplets in glomerular cells giving the cytoplasm a "foamy" appearance. These mesangial foam cells bear many similarities in terms of morphometry, contractility and histochemistry to the foam cells derived from macrophages and smooth muscle cells of atherosclerotic streaks(108). An autopsy study has correlated the presence of glomerular changes of ageing with atherosclerosis(109) raising the possibility of a common pathogenesis. Progressive renal disease in the rare familial lecithin cholesterol acyltransferase deficiency where cholesterol esters accumulate in peripheral tissues (110) anecdotally supports the concept of lipid nephrotoxicity.

The effect of manipulation of plasma lipids on the course of glomerulosclerosis, proteinuria and renal failure has been investigated in a number of normal species and models of renal injury. In early reports, dietary-induced hypercholesterolaemia was shown to promote glomerulosclerosis in the guinea pig(111) and this was confirmed in rats(112). Hypercholesterolaemia(dietary-induced or endogenous) in rats has been shown to accelerate

proteinuria, renal failure and renal histological abnormalities in obese Zucker rats, a remnant-kidney model (113) and puromycin aminonucleoside-induced nephrosis(114). These effects can be ameliorated, at least partially, by reducing the hyperlipidaemia with clofibrate or cholesterol synthesis inhibitors and are independent of changes in glomerular haemodynamics(113-115). As yet, no comparable data on the effects of lipid-lowering therapy on the course of chronic renal disease in man is available although an anecdotal report suggests that long term lipid-lowering with simvastatin may reduce proteinuria significantly(117).

The precise mechanisms whereby hyperlipidaemia may be nephrotoxic have been reviewed by Moorhead(107,116). Low density lipoproteins have cytotoxic effects on both endothelial and mesangial cells at high concentrations. Mesangial cell growth and expansion may be stimulated at lower concentrations(118). Although LDL carries an overall negative charge(pI 5.7), it contains positively charged domains which are essential for binding to the apoB/E receptor. In vitro, these can combine with negatively charged glycosaminoglycans. A similar occurence in vivo could lead to the neutralisation of the anionic sites of the glomerular capillary barrier and increase flux of anionic molecules. The role of filtered lipoproteins may also be important. Lipoproteins of all densities have been isolated from nephrotic urine(119) although HDL_3(5.5-9.5nm)

has been most commonly reported. Extensive tubular catabolism of filtered proteins and lipoproteins is thought to occur and direct or indirect effects on tubular cell function and viability may result. Further work is required to determine the precise role of hyperlipidaemia in progressive renal disease.

1.10 Treatment of hyperlipidaemia in the nephrotic syndrome

The rationale for treatment of hyperlipidaemia of nephrotic syndrome is that by reducing plasma lipid levels morbidity and mortality from cardiovascular disease will fall and, more contentiously, that the rate of progression of renal failure may be retarded. However, both of these propositions remain unproven. Where specific therapy is available for the nephrotic syndrome, remission of proteinuria will in most cases normalise the lipid profile. Alternative approaches to reducing proteinuria involving non-steroidal inflammatory agents, angiotensin converting enzyme inhibitors or protein restriction are available but there is little information on the effect of these interventions on plasma lipids and lipoproteins. In chronic, unremitting nephrotic syndrome, many physicians will actively treat hyperlipidaemia. The following section aims to summarise the published work on the hypolipidaemic measures employed in refractory nephrotic syndrome.

Few studies have looked at the effect of dietary restriction of cholesterol, saturated fat or calories alone in nephrotic hyperlipidaemia. Most drug studies, however, have incorporated some form of lipid lowering diet into the regimen. The precise importance of this is uncertain although, considering the degree of hyperlipidaemia in nephrotic syndrome, it is unlikely that dietary manipulation alone would reduce plasma lipids to "normal"levels.

In the last 20 years, a number of reports of pharmacological treatment have appeared. Edwards(120) performed a series of animal experiments demonstrating the efficacy of a wide range of agents in the hyperlipidaemia of experimental nephrosis and was one of the first to advocate a more aggressive approach to the problem in man. Enthusiasm for this was dampened by the experience of Bridgeman and co-workers who reported symptoms of muscle toxicity in 5 of 6 patients treated with clofibrate(121). Pharmacokinetic studies indicated that there was a greater proportion of free drug in plasma of nephrotic patients due to reduced protein binding and that the ratio of conjugated to unconjugated drug in the urine was lower. These differences would have led to a relative overdose of active drug. Recent experience with a newer fibric acid derivative, gemfibrozil, has shown that this group of drugs

can produce significant improvements in lipid parameters, especially triglyceride, without adverse effects(122,123).

Bile acid binding resins, which are still the mainstay of treatment of hypercholesterolaemia in the general population, have been used relatively infrequently in published studies of nephrotic syndrome although anecdotal experience suggests that they are poorly tolerated. In a placebo-controlled trial, colestipol reduced plasma and LDL-cholesterol in 6 out of 7 patients with average reductions of 20% and 32% respectively(124). The drug was generally well tolerated although others have reported problems with this group of drugs(123,125). Concern has been expressed that reduced absorption of vitamin D, which is already impaired in nephrotic syndrome, may precipitate osteomalacia.

Conflicting evidence has been reported on the efficacy of probucol in nephrosis(124,126) and experience with nicotinic acid derivatives is limited.

The advent of a new group of drugs which lower plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate limiting enzyme in cholesterol biosynthesis, has stimulated new interest in the field of cholesterol-lowering therapy(127). Reductions of 30-40% in total and LDL-cholesterol have been achieved

and the drugs appear to be well tolerated although long term experience is limited. Early reports(69,125,128,129) of their use in the nephrotic syndrome have been encouraging with reductions of total cholesterol of 35% and of LDL-cholesterol of >40%. The magnitude of reduction and the lack of side effects to date suggest this group of drugs may be eminently suitable for reducing hypercholesterolaemia in nephrotic syndrome.

TABLE 1.1 - PHYSICAL CHARACTERISTICS OF LIPOPROTEINS

	density (g/ml)	S_f value[a]	particle size(nm)	molecular weight (daltons)	EP[b] mobility
chylomicrons	<0.93	>400	75-1200	400×10^6	origin
VLDL	0.93-1.006	20-400	30-80	$10\text{-}80 \times 10^6$	pre-beta
IDL	1.009-1.016	12-20	25-35	$5\text{-}10 \times 10^6$	slow pre-beta
LDL	1.019-1.063	0-12	18-25	2.3×10^6	beta
HDL_2	1.063-1.125	3.5-20	9-12	3.6×10^5	alpha
HDL_3	1.125-1.210	0-3.5	5-9	1.8×10^5	alpha

a - Flotation rate in Svedberg units. ApoB-containing lipoproteins measured at d=1.063g/mL; HDL measured at d=1.210g/mL

b - electrophoresis on agarose gel

TABLE 1.2 - MEAN PERCENTAGE COMPOSITION OF LIPOPROTEIN CLASSES

	free cholesterol	cholesterol ester	triglyceride	phospho-lipid	protein
chylomicrons	5	2	84	7	2
VLDL	12	7	55	18	8
IDL	23	8	32	21	16
LDL	38	10	9	22	21
HDL_2	16	6	4	30	44
HDL_3	12	3	4	26	55

TABLE 1.3 - FREDRICKSON'S CLASSIFICATION

type	plasma appearance	plasma cholesterol	LDL cholesterol	plasma triglyceride	lipoprotein abnormality	electrophoretic pattern	examples of causes of phenotypic appearance
I	milky	raised	low	raised	excess chylomicrons	increased band at origin	lipoprotein lipase deficiency apo CII deficiency
IIa	clear	raised	raised	normal	excess LDL	increased beta band	familial hypercholesterolaemia, hypothyroidism, nephrosis
IIb	clear	raised	raised	raised	excess LDL &VLDL	increased beta and pre-beta	familial combined hyperlipidaemia diabetes mellitus, nephrosis
III	clear	raised	low/normal	raised	excess "remnants"	broad beta band	familial dysbetalipoproteinaemia
IV	milky	raised/ normal	normal	raised	excess VLDL	increased pre-beta	familial hypertriglyceridaemia, alcohol, renal failure, nephrosis, diabetes
V	milky	raised	normal	raised	excess VLDL & chylomicrons	increased pre-beta	familial hypertriglyceridaemia, alcohol excess

TABLE 1.4 - THE APOLIPOPROTEINS

	approximate molecular weight	subclasses	site of synthesis	metabolic roles
AI	28000		liver & intestine	major apolipoprotein of HDL modulates activity of LCAT
AII	8500		liver & intestine	20% of protein in HDL
AIV	46000		liver & intestine	?
B-48	264000		intestine	structural protein of chylomicrons
B-100	550000		liver	structural protein of VLDL,IDL & LDL apoB/E receptor binding
CI	6500		liver(& intestine)	activates LCAT
CII	9000		liver(& intestine)	C-II activates lipoprotein lipase
CIII	9000		liver	?inhibits receptor binding and lipase activity
E	34145	E-2, E-3, E-4	liver	apo B/E receptor binding ?ligand for chylomicron receptor

TABLE 1.5 - ENZYMES INVOLVED IN LIPOPROTEIN METABOLISM

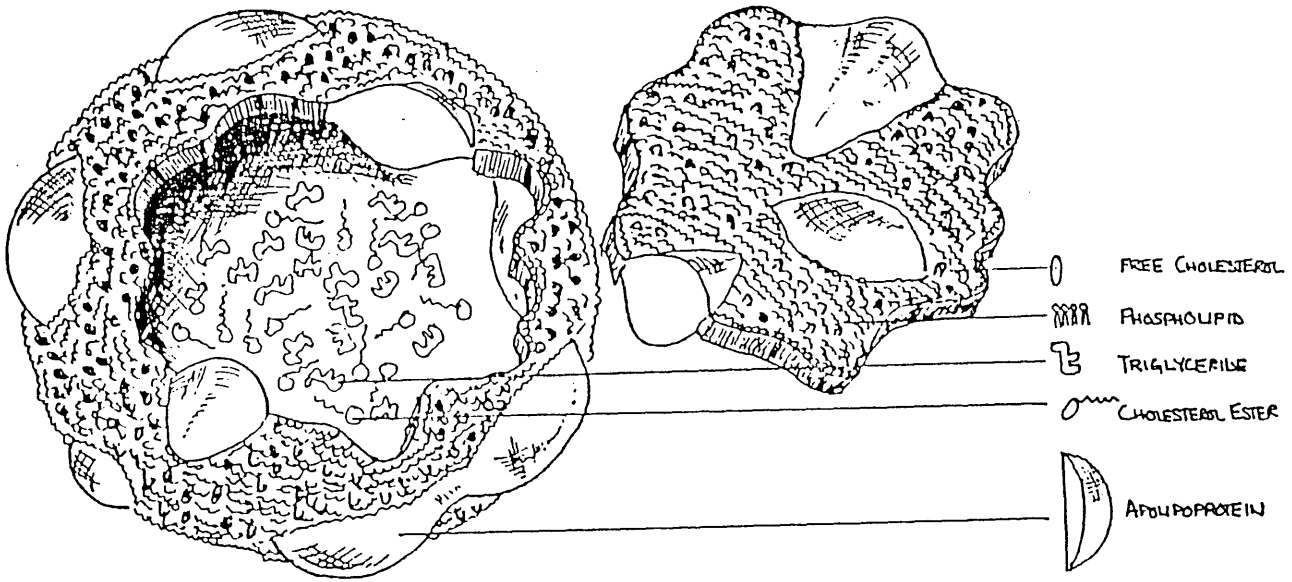
ENZYME	ESTIMATED MOLECULAR WEIGHT(daltons)	SITE OF ACTION	ACTION	CO-FACTORS/ INHIBITORS
lipoprotein lipase	34000-77000	endothelium	lipolysis of triglyceride in chylomicrons and large VLDL	activated by apoCII
hepatic triglyceride lipase	64000-69000	liver sinusoids	lipolysis of triglyceride and phospholipid in 'remnant' particles and in HDL_2	inhibited in vitro by purified apolipoproteins
lecithin cholesterol acyltransferase	60000-67000	plasma	esterifies cholesterol in HDL	activated by apoAI
plasma lipid transfer proteins(e.g. CETP)	41000-74000	plasma	transfer of neutral lipids and phospholipid between lipoproteins	inhibited by apoAI, apoE and albumin

TABLE 1.6 - CAUSES OF NEPHROTIC SYNDROME

PRIMARY GLOMERULAR DISEASES	SECONDARY GLOMERULAR DISEASES
minimal change	VASCULAR
focal glomerulosclerosis	renal vein thrombosis/arterial stenosis
membranous idiopathic	hypertension
secondary	INFLAMMATORY
mesangiocapillary type I & II	systemic vasculitides incl. SLE
mesangial IgA	anti-GBM disease
other mesangioproliferative GN	INFECTION
hereditary GN(Alport's, nail-patella)	post-streptococcal
congenital nephrosis	hepatitis B
	bacterial endocarditis/shunt nephritis
	NEOPLASTIC
	myeloma/light chain nephropathy
	MISCELLANEOUS
	diabetes mellitus
	amyloidosis
	pregnancy-associated
	transplant rejection
	severe right heart failure

Note: Primary glomerular diseases are diagnosed by histopathological features and the exclusion of systemic disorders which cause similar appearances.

FIGURE 1.1 - LIPOPROTEIN STRUCTURE



CHAPTER 2 GENERAL METHODS

2.1 Materials and methods

All biochemical parameters were measured in the routine laboratories of the Institute of Biochemistry, Glasgow Royal Infirmary. Measurements of cholesterol and triglyceride were performed by automated enzymatic assays (source: Boehringer Mannheim, East Lewes, Sussex, UK) on a Hitachi 704 analyser(source: Hitachi Ltd., Tokyo, Japan). Subfractionation of total plasma cholesterol was performed by "beta-quantification"(130). Very low density lipoproteins(d<1.006g/mL) were separated from 5mL of plasma by ultracentrifugation. Low density lipoproteins were precipitated from the d>1.006g/mL fraction with heparin/manganese. The precipitate was separated by centrifugation and HDL-cholesterol measured in the supernatant solution. LDL-cholesterol was calculated as the difference between total cholesterol and the sum of VLDL- and HDL-cholesterol.

The composition of lipoprotein subfractions was analysed using the previously described assays for total cholesterol and triglyceride. Free cholesterol was measured by a similar colorometric method omitting the cholesterol esterase step. Phospholipid was also assayed by an enzymatic method involving phospholipase(source: Boehringer

Mannheim, East Lewes, Sussex, UK). Protein content of isolated lipoproteins was measured by Lowry's method(131). Where samples were turbid, sodium dodecylsulphate(1mg/mL) was added to the copper alkali solution to disperse large, triglyceride-rich particles. Results were converted into mg/dL to calculate the total lipoprotein concentrations in plasma and the percentage composition by weight of each subfraction.

Apolipoprotein AI and B levels were measured by an automated immunoturbidometric method using commercially available kits(source: Orion Diagnostica, Espoo, Finland). Lipoprotein(a) was assayed using an enzyme linked immunosorbent assay(source: Immuno AG, Vienna, Austria). High density lipoprotein subfractions(HDL_2 and HDL_3) were measured by analytical ultracentrifugation(132). Apolipoprotein E phenotypes were defined by isoelectric focusing(133).

Serum electrolytes, urea, creatinine and proteins were assayed in the routine biochemistry laboratory using a multi-channel analyser. Urinary creatinine was measured by the Jaffe reaction on a Hitachi 717 autoanalyser(source: Hitachi Ltd, Tokyo, Japan). Total urinary protein was measured by the Ponceau-S dye-binding method(134) and urinary albumin concentration by a specific radioimmunoassay(135).

All chemicals and reagents used were obtained from Sigma (Poole, Dorset, UK) or BDH chemicals(Poole, Dorset, UK) except where otherwise indicated in the text. Ultra-centrifugation procedures were performed on L8-60 or L5 machines(source: Beckman, Palo Alto, California, USA) using Beckman rotors as detailed in individual methods.

Statistical tests are detailed in the text.

2.2 Patients

In an attempt to limit the effect of factors other than proteinuria/hypoalbuminaemia on plasma lipids and lipoproteins, these studies were restricted to patients(age 18-70years) who fulfilled the following criteria:-

- primary glomerular disease
- quantitative proteinuria >3g/day on two consecutive visits
- serum creatinine <400umol/L

Subjects with the following diagnoses were excluded:- diabetes mellitus, amyloid, systemic lupus erythematosus, malignant disease, immunosuppressive therapy and pregnancy. All of these conditions may independently perturb lipid metabolism.

Between 1st October,1988 - 1st October,1990, data was collected on 45 patients who fulfilled the above criteria. Over this period, a total of 242 patients with proteinuria in excess of 3g/day on at least one occasion were recorded on the renal unit computer base at Glasgow Royal Infirmary. One hundred and forty nine patients were excluded because of renal failure, interstitial renal disease or other systemic disease. The rest were excluded by other criteria, failed to attend when invited or had been lost to follow-up. These 45 subjects formed the population base for the subsequent studies. Fasting lipids and lipoprotein cholesterol concentrations were determined in all patients. Subfractionation of apoB-containing lipoproteins, apolipoprotein AI and B and lipoprotein(a) concentrations were performed in the majority of subjects.

Patients with hypercholesterolaemia(>7mmol/L) were invited to take part in the metabolic and drug studies to investigate the mechanisms responsible for hyperlipidaemia in the nephrotic syndrome and the response to treatment. Controls were recruited from laboratory and clinical personnel, from coronary screening projects in the Glasgow area and from patients attending the renal unit at Glasgow Royal Infirmary with minor renal disease. All subjects were studied as outpatients and asked to continue their normal diets except where indicated. For the metabolic studies, steady state conditions of lipoprotein metabolism

were confirmed by at least 3 measurements of plasma lipid and lipoprotein levels over the study period. The coefficient of variation for each patient's plasma cholesterol level was <10%. The figure for plasma triglyceride ranged up to 20%.

Participants in the metabolic investigations were given a personal explanation of the nature and aims of these studies and many were also given a pamphlet repeating this explanation. Written consent was obtained. Potassium iodate was administered orally for three days before each study and continued for four weeks to prevent thyroidal sequestration of radioiodide. All studies were approved by the Ethics Committee of Glasgow Royal Infirmary. Professor J Shepherd of the Institute of Biochemistry is licensed to administer radioactively labelled lipoproteins to human volunteers for the investigation of lipoprotein metabolism by the Adminstration of Radioactive Substances Advisory Committee of the Department of Health.

2.3 Lipoprotein kinetics - theory, modelling and computing

Over the last thirty years considerable information regarding normal and abnormal lipoprotein metabolism has accumulated from *in vivo* tracer studies using labelled lipoprotein particles. For a full consideration of the available techniques, readers are referred to Berman(136). The discussion here will be limited to the techniques used in this thesis including their theoretical basis, underlying assumptions and limitations and the analysis of experimental data.

The apoB-containing lipoproteins contain one molecule of apoB per particle. ApoB is integral to the structure of these particles, remains with them throughout their lifetime in the circulation and is removed with the particle from the plasma compartment. Therefore, by labelling apoB and following its metabolic fate in the plasma, the metabolism of these lipoprotein particles can be investigated. ApoB may be labelled endogenously by the intravenous administration of isotopically-labelled amino acids(e.g. [^{75}Se]selenomethionine or tritiated leucine) or exogenously with radioiodide. The former involves relatively large doses of radioactivity, labels all plasma proteins and is complicated by the re-cycling of tracer from rapidly catabolised proteins. However, it does

eliminate the possibility that artefacts introduced during ex vivo labelling alter the behaviour of the tracer and destroy tracer-tracee equivalence. In contrast, radio-iodination of lipoproteins involves relatively small doses of radioactivity and the free iodotyrosine released during catabolism is rapidly cleared via the renal tract with negligible re-cycling. The principal disadvantages are that only catabolism is examined directly and concern has been expressed that tracer damage may occur during the labelling process or that iodination may change the chemical properties of the apolipoprotein and interfere with its metabolism(137). However, studies in rabbits have shown that radiolabelled tracers, screened in vivo for 24 hours then re-isolated, decay at the same rate as newly labelled lipoproteins(138).

Radioiodinated lipoproteins were first used in tracer experiments in the 1950's(66) but their potential was not fully realised until Langer(139) reported a reduction in the fractional catabolic rate(FCR) of radioiodinated LDL in patients with familial hypercholesterolaemia. This observation laid the groundwork for the identification of the metabolic block in this condition. Subsequently, apoB metabolism has been extensively investigated in a range of dyslipidaemic states.

The protein moiety of lipoprotein particles is radioiodinated by the iodine monochloride method(140,141). The apolipoprotein content of LDL particles is 90-95% apoB and previous work(141) has shown that nearly all the radioactivity is associated with the protein moiety. Following intravenous injection, the radioactivity remains in the LDL fraction(figure 2.1) and the plasma radioactivity decay provides an estimate of the catabolic rate of LDL apolipoprotein(apoLDL). Free radioiodide released from LDL is rapidly cleared from the circulation.

When VLDL is labelled with radioiodide, significant quantities of label will be associated with other apolipoproteins(C and E) and lipids. In vivo, the apoB is transferred by progressive delipidation of VLDL particles to lipoproteins of higher density(i.e. IDL and LDL). To follow the metabolism of apoB, it is necessary to subfractionate(see chapter 3) the apoB-containing lipoproteins and measure the specific activity of apoB (counts per minute/mg apoB) in each fraction. This permits investigation of precursor-product relationships among these lipoprotein fractions(see chapter 6 methodology).

Studies with labelled lipoproteins generate data in the form of plasma total or specific radioactivity curves. Lipoprotein pool sizes are measured separately. These data can be analysed in a number of ways but the application of

multicompartmental modelling generates a number of kinetic parameters which quantitatively describe the metabolism of the lipoprotein fractions. These are useful for comparisons with normal or other disease states; for generating and testing quantitative hypotheses of lipoprotein metabolism in vivo; and for planning further experiments to help validate the model. In the modelling process, each apoB-containing lipoprotein fraction is considered to comprise a number of discrete metabolic compartments. In the steady state, the size of each compartment is stable i.e. transfer rates in and out are equal. The transfer between compartments is assumed to follow first order kinetics and can therefore be described by a linear function:-

$$R(i,j) = L(i,j) \times M(j)$$

R(i,j) is the flux from compartment j to i in mg apoB/day
L(i,j) is the rate constant for this flux in pools/day
M(j) is the mass of apoB in compartment j in mg
The shape of the plasma decay/appearance curves determines the number of exponentials which are required to explain the experimental data. The flux from a compartment is equal to the sum of the individual rate constants multiplied by the mass i.e.:-

$$R(x,j) = [L(i_1,j)+L(i_2,j)+L(i_n,j)+....] \times M(j)$$

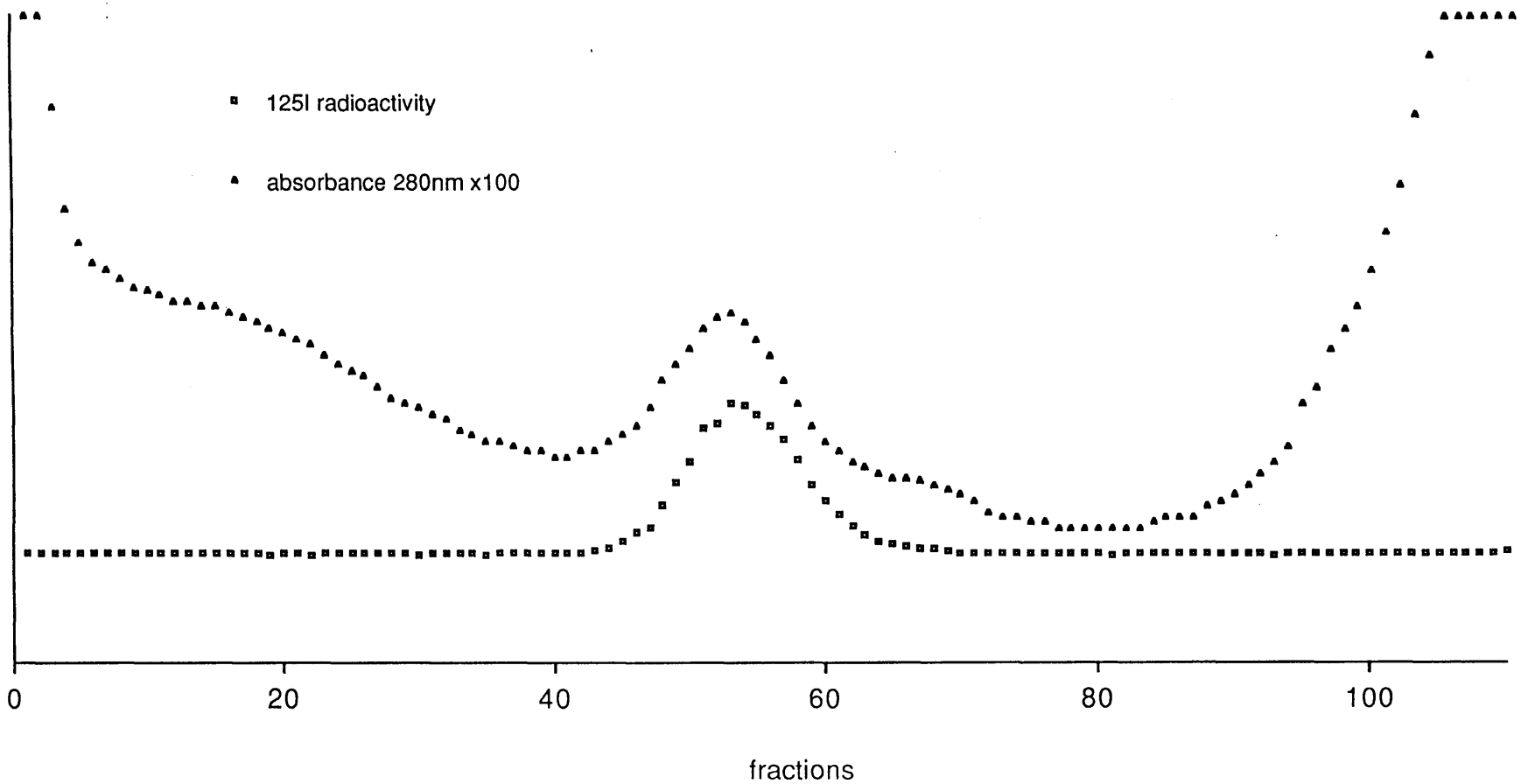
The series of differential equations thus generated can be solved simultaneously for the experimental data given initial estimates of the distribution of the tracer between the compartments in the pools. Fixed standard deviations of 5% were used for each data point. The modelling was done using the SAAM(simulated analysis and modelling) programme in its interactive mode(CONSAM) run on the VMS system of the Glasgow University computer network(142,143).

The SAAM programme generates a solution from the observed data by a least squares fit procedure and plots calculated plasma radioactivity curves for each compartment. These curves can then be "hand-fitted" i.e. the individual rate constants altered to approximate the observed and calculated data sets. When the sum of squares of the differences between these values is reduced below an arbitrary level, an automated iterative procedure is employed in which a series of changes in parameters is tested with the aim of reducing the overall sum of squares to a minimum value. Weighting of data points allows the operator to minimise the effect of outlying data points on the solution. Both radioactivity and mass data are used to provide a single solution containing rate constants, calculated pool sizes and synthetic inputs.

The compartmental models used in the kinetic studies of VLDL and LDL are described under methods for these sections

(chapters 5 and 6, respectively) but the general principles are applicable to both.

FIGURE 2.1 - DISTRIBUTION OF RADIOACTIVITY IN FRACTIONS ISOLATED FROM PLASMA BY ZONAL ULTRACENTRIFUGATION TO SHOW THE CONCORDANCE OF THE RADIOACTIVITY AND LDL PROTEIN PEAKS



CHAPTER 3 THE RELATIONSHIP BETWEEN HYPERLIPIDAEMIA AND PROTEINURIA

3.1 PREVALENCE OF LIPID AND LIPOPROTEIN ABNORMALITIES IN NEPHROTIC SYNDROME/PROTEINURIA

3.1.1 Introduction

Few studies have documented the prevalence of lipid and lipoprotein abnormalities in an unselected population of subjects with idiopathic nephrotic syndrome. The patients identified by the methods in section 2.2 formed the population base for subsequent studies. In this section, the abnormalities of lipid and lipoprotein are characterised and related to severity of disease.

3.1.2 Patients and methods

Subjects were contacted either at routine clinic visits or by post and invited to attend with a view to participating in these studies. Fasting blood was drawn for plasma lipids, beta-quantification of lipoprotein cholesterol, apolipoprotein E phenotype and lipoprotein(a) measurements. Twenty four hour urine collections and serum for creatinine and albumin were collected at the same time. A group of 192 patients selected from the data base of a coronary screening project(described below) were used as controls

for comparisons of plasma lipid and lipoprotein cholesterol concentrations. The assays were performed in the same laboratory. However, there was an interval of approximately 2-4 years between the periods of data collection and, in the control group, LDL-cholesterol levels were calculated by the Friedewald formula(144) rather than measured directly. The use of this formula has been validated up to a triglyceride concentration of 5mmol/L and all the control group had plasma concentrations below this value.

In a sub-group of patients, the apo B-containing lipoproteins were separated into $VLDL_1$(S_f 60-400), $VLDL_2$ (S_f 20-60), IDL(S_f 12-20) and LDL(S_f 0-12) by cumulatative flotation ultracentrifugation on a density gradient(see chapter 5 methods). Seven millilitres of fresh fasting plasma was adjusted to a density of 1.118g/mL by the addition of 1.194g of solid NaCl. Three x 2mL aliquots were then layered onto 0.5mL of a solution of d=1.182g/mL in a Beckman SW40 tube using a peristaltic pump. The density gradient was then layered on top as shown in figure 3.1. The four lipoprotein fractions were recovered by sequential ultracentrifugation using the protocol shown in the same figure. All runs were performed at 23^{o}C and decelerated with the brake off to minimise disruption of the gradient. The time of each run was corrected for acceleration and deceleration. $VLDL_1$ was recovered by

careful aspiration of the top 1mL of each tube which was then replaced by 1mL of d=1.0588g/mL. $VLDL_2$ and IDL were recovered in 0.5mL and LDL in 1mL fractions. These were analysed for lipid and protein by the methods described above and the plasma concentrations and compositions calculated.

These data were used to estimate the prevalence and nature of lipid and lipoprotein abnormalities and the relationship with serum albumin and proteinuria.

Preliminary frequency plots were used to judge whether the data were normally distributed. Plasma triglyceride and lipoprotein(a) both showed a highly skewed distribution but became Gaussian on log transformation of the data. Two sample t tests were used to compare data between groups except for the subfraction concentrations which demonstrated a wide variance and were analysed by distribution-free methods(Mann Whitney test). Least squares linear regression was used to explore the relationships between lipid values and proteinuria/hypoalbuminaemia. These are summarised as coefficients of determination(r^2 values) and the signifigance assessed by analysis of variance.

3.1.3 Results

Over a two year period, data on plasma lipids and lipoproteins were collected on 45 patients who fulfilled the criteria listed in chapter 2. The mean age was 45 years(range 17-68) and there was a preponderance of males(M:F=32:13). The period of follow-up(taken from time of renal biopsy) varied greatly from a minimum of 1 month to over 25 years in the case of one patient with membranous nephropathy, persistent proteinuria and very slowly declining renal function. The median time from diagnosis was 33 months.

All patients had an established histological diagnosis of primary glomerular disease. The main categories were idiopathic membranous nephropathy(27), minimal change(5), mesangiocapillary glomerulonephritis(4) and IgA nephropathy (3). There were two subjects with poorly defined mesangioproliferative lesions and single cases of focal glomerulosclerosis, glomerulosclerosis related to hypertension, nail patella syndrome and probable hereditary nephropathy. The average serum creatinine was 159umol/L(sd 83) and proteinuria ranged from 3.0-24.2g/day(mean 9.0). Mean serum albumin was 31g/L(sd 6).

The plasma lipid and lipoprotein cholesterol values and physical characteristics of the nephrotic and control

groups are compared in table 3.1. As expected, there were considerable increases in plasma cholesterol, triglyceride and LDL-cholesterol in the nephrotic group. HDL-cholesterol were decreased compared to controls. 89% of subjects had total cholesterol >6.5mmol/L - a level at which a number of authorities strongly recommend positive lipid-lowering measures in the general population(145). 34/45 patients had severe hypercholesterolaemia(i.e. >7.8mmol/L). A smaller number(84%) had LDL-cholesterol values greater than 4mmol/L. Elevated fasting triglyceride concentrations(>2mmol/L) were present in 66% with 5 patients exhibiting severe hypertriglyceridaemia (>6.5mmol/L). No patients had hypertriglyceridaemia and plasma cholesterol <6.5mmol/L.

Relationships between the severity of nephrotic syndrome and hyperlipidaemia were sought using quantitative proteinuria and serum albumin as explanatory variables. The coefficients of determination and the signifigance of the slopes of the regression lines determined by analysis of variance are listed in table 3.2. The strongest association was an inverse relationship between LDL-cholesterol and serum albumin. Serum albumin correlated only weakly with total cholesterol and not at all with triglyceride. In contrast, proteinuria showed some association with all three lipid measurements. However, there were significant inter-individual

variations. The subject with the highest LDL-cholesterol (16.3mmol/L) had only modest hypoalbuminaemia(29g/L). No relationships could be demonstrated between HDL-cholesterol and these parameters. Body mass index, age or length of follow up did not show any significant associations with lipid values.

Lipoprotein(a) concentrations were measured in 30 subjects. Values ranged from undetectable in one patient to 320mg/dL. The median value was 32mg/dL i.e. 50% of subjects had a plasma concentration greater than the level(30mg/dL) that has been postulated as indicating an increased risk of atherosclerosis(17). There was a significant correlation between the log transformed Lp(a) concentrations and serum albumin(r^2=35.9%, p=0.001) although there was no such association with proteinuria(figure 3.2).

Table 3.3 lists the plasma concentrations and composition of the four apoB-containing lipoprotein subfractions separated by density gradient ultracentrifugation in 33 nephrotic subjects and a group of 21 normolipidaemic controls. There were marked increases in the plasma concentrations(calculated from the sum of the masses of lipids and protein) in all four subfractions. The free cholesterol content of $VLDL_1$, $VLDL_2$ and IDL was increased in the nephrotic group but LDL isolated by this method had a normal composition.

3.1.4 Discussion

Plasma lipids and lipoprotein-cholesterol

These results broadly reflect the changes reported in the main published series on hyperlipidaemia in the nephrotic syndrome(41,46,48). In these studies, however, it is unclear whether all patients with nephrotic syndrome were screened for lipoprotein abnormalities. Thus, the occurrence of hyperlipidaemia in 100% of subjects may be a result of patient selection. In a smaller study of 20 consecutive patients with nephrotic syndrome, Appel et al.(47) recorded elevated plasma cholesterol in "most" patients with 70% lying above the 95th centile for age and sex. The paper does not give the individual lipid values but some patients were not considered hypercholesterolaemic. The data presented here and other studies confirm that hyperlipidaemia is a very common but not invariable complication of heavy proteinuria.

Differences between study populations may influence the nature and prevalence of lipid abnormalities even when other factors(e.g. diabetes mellitus, systemic lupus erythematosus, renal failure, corticosteroid therapy) are excluded. In both this study and Appel's(47), proteinuria alone was used to define the nephrotic syndrome while others have only included those with reduced serum albumin

- usually <30g/L. In Baxter's study(41), the subjects were severely hypoalbuminaemic with all but 15/58 lipid measurements taken when serum albumin was <20g/L. Only one subject in the current study had serum albumin <20g/L. This may reflect poorer nutrition thirty years ago, improvements in accuracy of albumin assays or simply that the study populations are truly different. In this study, only one patient with plasma cholesterol <6.5mmol/L had serum albumin <30g/L. A few months after diagnosis this patient died and, at autopsy, was found to have bronchial carcinoma. This may have been the underlying cause of his glomerular disease and may have modified the changes in lipoproteim metabolism produced by nephrosis.

Hyperlipidaemia and severity of nephrosis

In the current study, the association between severity of disease and hyperlipidaemia is best illustrated by the negative correlation between LDL-cholesterol and serum albumin. Proteinuria also correlated with total and LDL-cholesterol concentrations. More severe disease with heavier proteinuria and falling serum albumin was associated with an exponential rise in plasma triglycerides.

Lipoprotein(a)

Significant elevations of lipoprotein(a) were found in the group of thirty patients tested. A similar finding has recently been reported by Karadi et al.(146). The concentrations of Lp(a) in some patients were very high - up to 320mg/dL. The mechanism of the rise in Lp(a) in this condition is not known. The concentration of Lp(a) is markedly influenced by phenotypic variation in Lp(a) isoforms(147) and the interaction of these and nephrosis may be important in determining the final plasma concentration. Studies of Lp(a) metabolism in both normal and nephrotic subjects are required to elucidate the pathophysiology of these changes.

Apolipoprotein B-containing subfractions

The increases in plasma lipids in nephrotic syndrome are reflected in the substantial rises in all apoB-containing lipoprotein subfractions although the magnitude of these changes were very variable. $VLDL_1$, $VLDL_2$ and IDL were all enriched in free cholesterol. This conclusion is only tentative for $VLDL_1$ due to the very low content of free cholesterol in the controls which in some cases was below the limit of detection. However, the increased free cholesterol content in both $VLDL_2$ and IDL supports this finding. This could be explained by reduced LCAT activity

(86,87). The composition of LDL isolated by DGUC was not significantly different from controls. There are relatively few previous studies of lipoprotein composition in human nephrotic syndrome but in one report Gherardi et al.(42) found an increase in cholesterol ester and phospholipid content of VLDL and IDL at the expense of free cholesterol. Again, interpretation of this discrepancy is complicated by differences in the populations since Gherardi's study was on children.

In conclusion, these results emphasise that both quantitative and qualitative changes occur in the plasma lipoproteins in the nephrotic syndrome. The previously documented relationships between severity of nephrotic syndrome and hyperlipidaemia were confirmed. Lipoprotein(a) concentrations were elevated and significant changes in the concentration and composition of apoB-containing lipoprotein subfractions were found.

3.2 THE EFFECT OF NON-NEPHROTIC(<3.5g/day) PROTEINURIA ON BLOOD CHOLESTEROL

3.2.1 Introduction

Although the relationship between heavy proteinuria and hyperlipidaemia has been extensively reported, there is surprisingly little information on the effects of lower

levels of proteinuria on plasma lipids. This issue was addressed in a study looking at the relationship between urinary albumin excretion and blood cholesterol concentrations in an outpatient population attending a glomerulonephritis clinic.

3.2.2 Method and subjects

Aliquots of routine 24 hour urine collections and non-fasting serum were obtained from patients attending a renal outpatient clinic. Only patients with primary glomerular disease and well-maintained renal function(serum creatinine <200umol/L) were included. Those with other diseases known to effect lipoprotein levels were excluded(see chapter 2). Over a period of 9 months, samples were collected from 100 patients and urinary albumin excretion rates(AER) and serum cholesterol and albumin were measured.

Cholesterol measurements were compared with age, sex and weight matched controls. For each subject two controls were selected from a database of 10,000 subjects who participated in a coronary screening project between 1983 and 1985(148). Controls were selected in groups of ten taken from blocks of 500 participants and were matched for sex, age within 5 years and weight within 10%. The average values of the two controls were used for comparisons with

the groups defined by albuminuria(see below) and for investigating the relationship between cholesterol and both AER and serum albumin. Controls were not located for five patients <18 or >65years and these subjects were excluded.

Paired t tests were used to compare cholesterol values in the albuminuric and control groups. Both cholesterol and albumin excretion rate were negatively skewed in the patient group but became Gaussian on log transformation of the data. Linear regression of the log transformed values was used to examine the relationship between AER and serum cholesterol.

3.2.3 Results

The patient group contained patients with a range of primary glomerular disease(minimal change 19, mesangio-proliferative glomerulonephritis with IgA or IgM deposits 38, membranous nephropathy 27, mesangiocapillary glomerulo-nephritis 6, focal glomerulosclerosis 2, other 8). No account was taken of those whose disease was judged to be in remission since the end-point was the relationship between cholesterol and albumin excretion. The group encompassed a very wide range of AER from 1.6 to 15420mg/day(median 257). Serum cholesterol was also distributed over a wide range from 3.6 to 14.0mmol/l(median 6.6). The group were split into 4 subgroups according to

the rate of albumin excretion:-

1. <30mg/day i.e. normal(n=29)
2. 30-300mg/day - equivalent to a rate used to predict overt nephropathy in diabetes mellitus(n=21)
3. 300-3000mg/day - clinically significant proteinuria but less than nephrotic range(n=31)
4. >3000mg/day - nephrotic-range proteinuria(n=14)

Plasma cholesterol in each of these groups was compared with the values in the appropriate control subjects(table 3.4). The only group where there was a significantly increased cholesterol was the nephrotic group. However, when all 100 subjects were considered there was an increase in the plasma cholesterol concentrations in the proteinuric group and a relationship between serum cholesterol and albuminuria after log transformation(figure 3.3). This relationship persisted even when those subjects with albuminuria in excess of 3000mg/day were excluded although the association was predictably weaker(r^2=7.1%, $p<0.02$). When albumin losses increased to >5g/day, cholesterol rose exponentially.

3.2.4 Conclusion

There is little data available on the relationship of blood cholesterol with lower levels(i.e. non-nephrotic) of

urinary protein loss. By limiting this study to otherwise healthy patients with well-documented primary glomerular pathology, the effect of glomerular permeability alone on serum cholesterol was sought.

As expected those subjects with heavy proteinuria had raised cholesterol concentrations although in two subjects plasma cholesterol concentrations were <4mmol/L despite an AER >3000mg/day. In the other groups, there were no significant differences between the subjects and matched controls but overall there was a relationship between log albumin excretion and cholesterol suggesting that even at lower(i.e. non-nephrotic) rates of protein loss, serum cholesterol may be adversely effected. This may be of importance in the light of recent reports that microalbuminuria is a predictor of vascular disease in non-diabetic subjects(149,150) and of increased lipids in diabetics with microalbuminuria compared to well-matched diabetics without renal involvement(151). However, other factors such as hypertension may also be important in linking increased glomerular permeability to an increased risk of vascular disease.

3.3 URINARY ALBUMIN EXCRETION IN PATIENTS WITH PRIMARY HYPERLIPIDAEMIA

3.3.1 Introduction

Despite the evidence that hyperlipoproteinaemia may be nephrotoxic(116), there are no published studies of renal function in patients with primary hyperlipidaemia. In this section, urinary albumin excretion was used as a sensitive measure of renal dysfunction in patients with primary hyperlipidaemias.

3.3.2 Methods and subjects

Fasting plasma and random urine samples were collected from patients routinely attending the lipoprotein clinic at Glasgow Royal Infirmary. Patients with the following conditions were excluded:- diabetes mellitus, known renal disease or serum creatinine >120umol/L, hypertension or BP >170/95, malignancy, thyroid disease, connective tissue diseases, treatment with non-steroidal anti-inflammatory drugs. Over a period of 3 months, 141 samples were collected. Cholesterol and triglycerides were measured on plasma samples and albumin and creatinine on the urine samples. The results were compared with published data on albumin excretion rates. The relationship between albuminuria and hyperlipidaemia was tested by linear

regression after log transformation of the albumin and triglyceride data and by comparing AER's between the upper and lower quartiles of the cholesterol distribution.

3.3.3 Results

The plasma cholesterol in this group of 141 patients with primary hyperlipidaemia averaged 7.73mmol/L; range 4.15 to 13.3mmol/L. Urinary albumin concentrations and albumin: creatinine ratios demonstrated a negatively skewed distribution as shown in figure 3.4. These values are similar to those reported by Watts(152) for random urine samples in normal ambulant subjects. In this study, the upper 95% confidence limit was 29.6mg/L for albumin concentration and 2.3mg/mmol for albumin:creatinine ratio. Only 1.4% and 4.4% respectively of the results recorded in this study lie above these cut-off points.

There was no relationship between plasma cholesterol and either urinary albumin concentration(figure 3.5a) or albumin:creatinine ratio. A weak association was seen within the group between the log transformed plasma triglyceride concentration and urinary albumin concentration(figure 3.5b; r^2=3.1%, $p<0.05$). There was no difference between the mean values for albumin concentration between the upper and lower quartiles of the cholesterol distribution(0.735 sd 0.374 v 0.802 sd 0.286;

p=0.4).

3.3.4 Conclusion

There was no evidence from this study that primary hyperlipidaemia per se can cause renal dysfunction as assessed by urinary albumin excretion. Although there is accumulating evidence for a role of hyperlipidaemia in the progression of renal disease(mainly based on experimental animal models), this may only be a secondary phenomenon following a primary renal insult. This contention is supported by the absence of reports of renal disease in homozygous familial hypercholesterolaemia or in the animal model of this disease, the Watanabe rabbit. However, glomerulosclerosis can be induced by cholesterol feeding alone in some animals(111) and is a recognised feature of the rare familial lecithin cholesterol acyltransferase deficiency in man(110).

TABLE 3.1 - PHYSICAL CHARACTERISTICS AND LIPID PARAMETERS FOR CONTROL AND NEPHROTIC GROUPS

	CONTROL	NEPHROTIC	signifigance (two sample t test)
n	192	45	
sex(M:F)	120:72	32:13	ns[a]
age(years)	44(11)	45(14)	ns
BMI(kg/m^2)	26.1(4.1)	25.6(3.6)	ns
plasma cholesterol	5.9(1.2)	10.5(4.6)	<0.0001
plasma triglyceride	1.7(1.4)	3.9(3.8)	<0.0001[b]
LDL-cholesterol	3.75(1.03)	6.79(3.25)	<0.0001
HDL-cholesterol	1.43(0.38)	1.18(0.35)	0.0001

Figures in parenthesis are standard deviations. Lipid values are in mmol/L

a - chi-square test b - comparing log transformed values

TABLE 3.2 - CORRELATIONS BETWEEN LIPID PARAMETERS AND INDICES OF DISEASE SEVERITY

n	explanatory variable	response variable	r^2(%)	signifigance
42	albumin	LDL-chol	29.4	<0.001
44	albumin	plasma chol	9.2	0.05
43	albumin	plasma trig(log)	0	0.89
43	proteinuria	LDL-chol	17.9	0.005
45	proteinuria	plasma chol	14.4	0.01
44	proteinuria	plasma trig(log)	10.1	0.04

TABLE 3.3 - PLASMA CONCENTRATIONS AND COMPOSITION OF APO B-CONTAINING LIPOPROTEINS

	PLASMA CONCENTRATION (mg/dL)		PERCENTAGE COMPOSITION									
			free cholesterol		cholesterol ester		triglyceride		phospholipid		protein	
	C	NS	C	NS	C	NS	C	NS	C	NS	C	NS
VLDL1	38	150	1.4	4.0	14.1	14.8	58.3	56.1	15.6	15.5	11.3	9.6
	14-242	19-1213*	(2.1)	(3.3)**	(10.1)	(4.1)	(10.1)	(6.0)	(4.2)	(2.1)	(5.8)	(3.3)
VLDL2	46	188	5.6	9.3	23.9	22.7	35.7	34.9	19.9	19.9	14.9	13.3
	12-92	65-569*	(2.8)	(2.6)*	(6.0)	(5.8)	(5.6)	(7.7)	(2.8)	(1.7)	(3.0)	(2.7)
IDL	50	176	8.4	10.5	36.6	34.1	13.7	16.6	20.8	21.1	20.5	17.8***
	17-140	75-562*	(3.0)	(2.0)***	(4.4)	(4.6)	(4.6)	(6.5)	(2.4)	(1.5)	(3.3)	(2.6)
LDL	261	494	11.0	10.9	37.3	36.2	6.6	8.4	21.4	20.1***	23.7	24.6
	101-341	151-981*	(2.5)	(3.5)	(3.4)	(5.6)	(2.1)	(6.2)	(1.7)	(2.5)	(1.4)	(4.8)

Plasma concentrations are shown as median values and ranges and are compared by Mann Whitney test(non-parametric).

Compositional data are shown as mean values and standard deviations and compared by two sample t test

* $p < 0.0001$ ** $p < 0.001$ *** $p < 0.05$

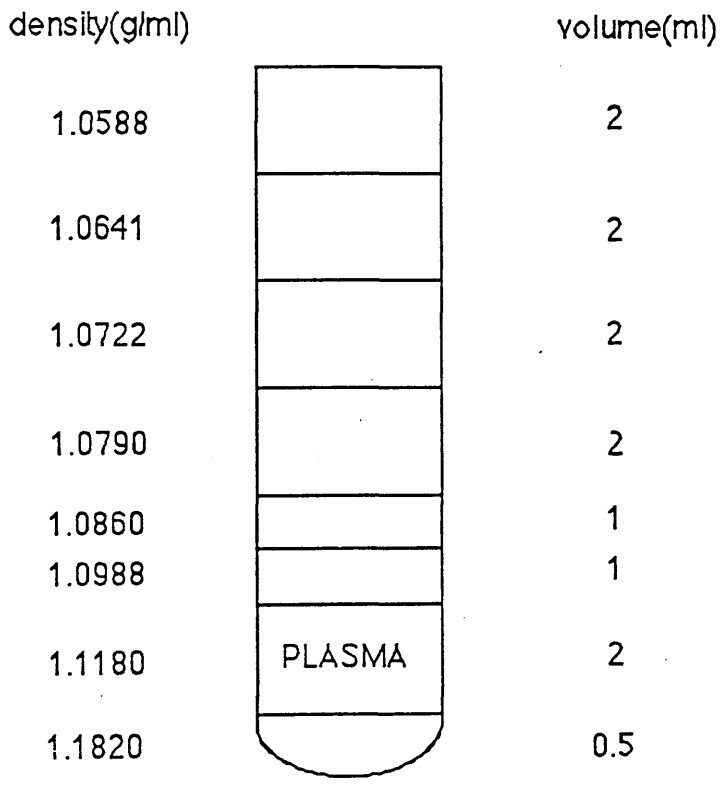
C - control group, NS - nephrotic group

TABLE 3.4 - SERUM CHOLESTEROL CONCENTRATIONS IN RELATION TO ALBUMIN EXCRETION RATE

ALBUMIN EXCRETION RATE (mg/day)	n	CHOLESTEROL(mmol/L) albuminuric	control	paired t test p
<30	29	5.9(1.3)	5.7(0.9)	ns
30-300	21	6.2(1.7)	5.7(1.0)	ns
300-3000	31	6.5(1.3)	6.0(0.9)	ns
>3000	14	8.5(2.4)	6.2(0.6)	<0.02

Cholesterol values are shown as means and standard deviations

FIGURE 3.1 DENSITY GRADIENT FOR SEPARATION OF apoB-CONTAINING LIPOPROTEINS



FRACTION (S_f)	SPIN SPEED (rpm)	SPIN TIME (hours:min)	INCREMENT $W^2t(x\ 10^{11})$	TOTAL $W^2t(x\ 10^{11})$
$VLDL_1$(60-400)	39000	1:38	1.03	1.03
$VLDL_2$(20-60)	18500	15:41	2.12	3.15
IDL(12-20)	39000	2:35	0.63	4.78
LDL(0-12)	30000	21:10	7.52	12.30

W - radians/sec(rpm/60 x 2π); t - time(sec)

FIGURE 3.2a
RELATIONSHIP BETWEEN QUANTITATIVE PROTEINURIA AND Lp(a)

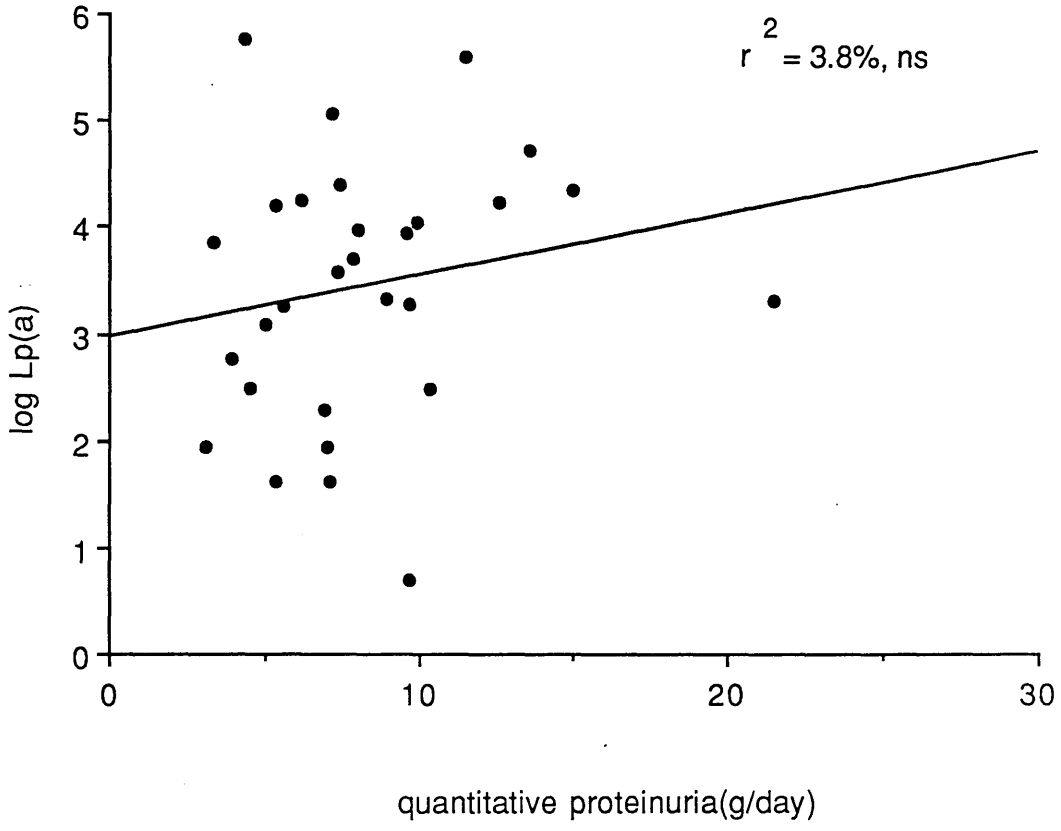


FIGURE 3.2b
RELATIONSHIP BETWEEN SERUM ALBUMIN AND Lp(a)

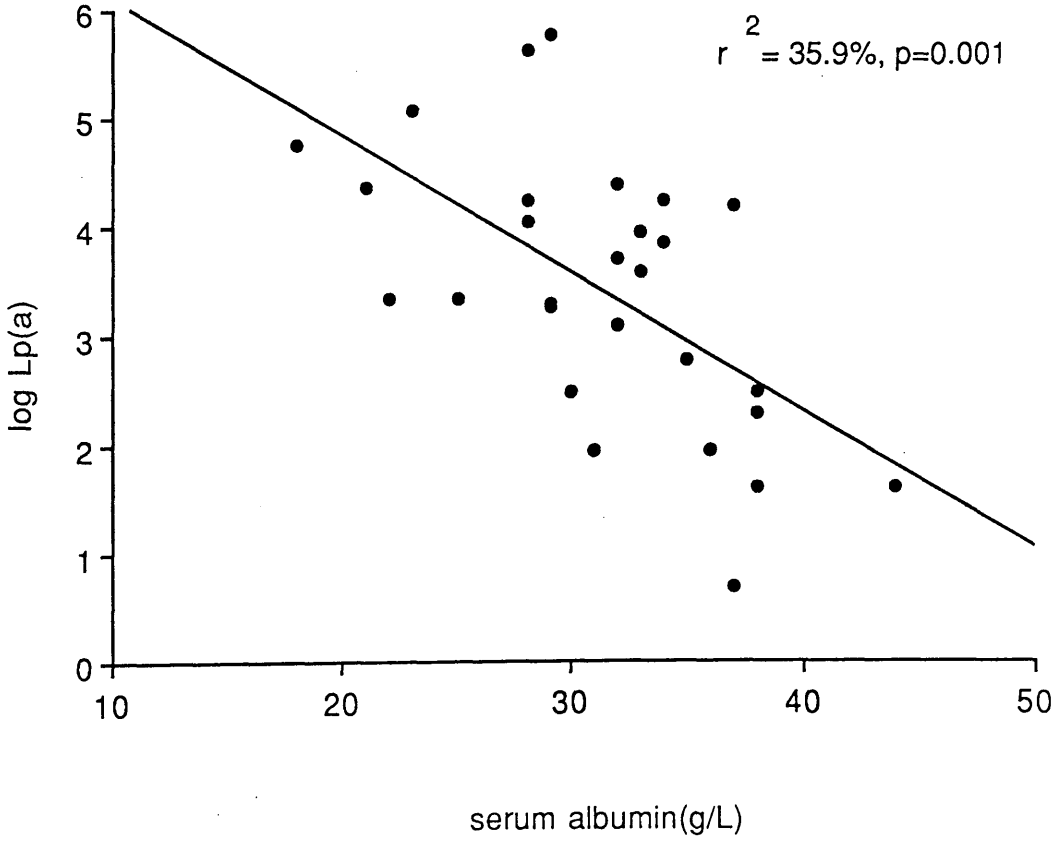


FIGURE 3.3
RELATIONSHIP BETWEEN ALBUMIN EXCRETION RATE AND SERUM CHOLESTEROL

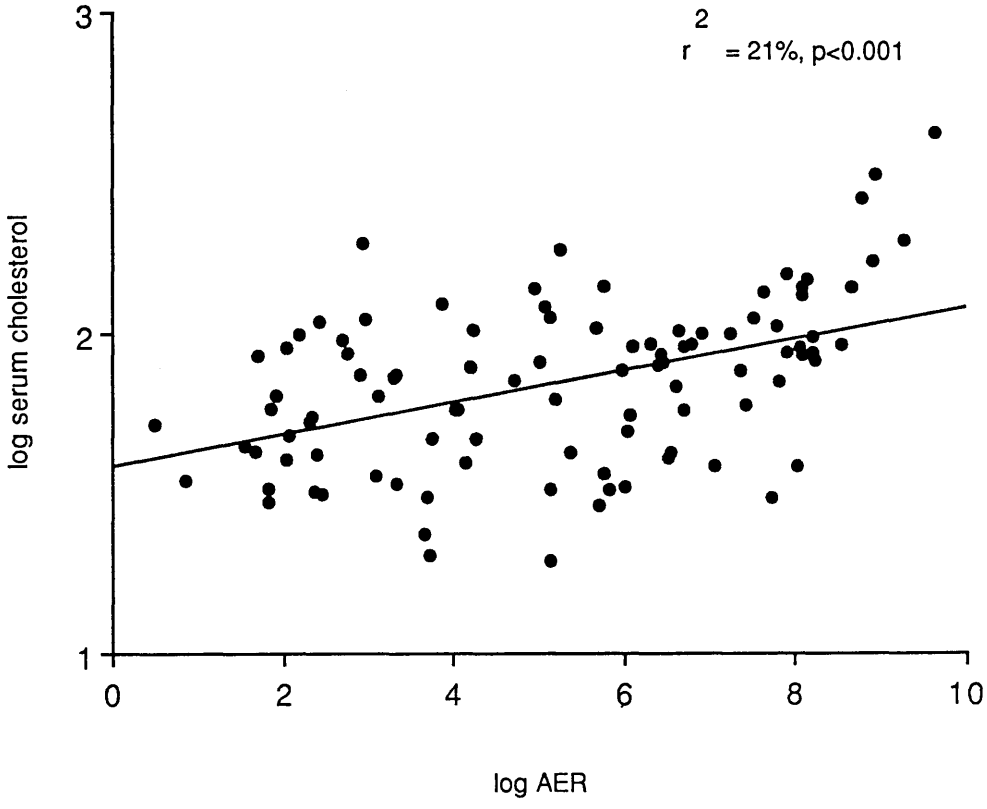


FIGURE 3.4
URINARY ALBUMIN CONCENTRATIONS IN SUBJECTS WITH PRIMARY HYPERLIPIDAEMIA

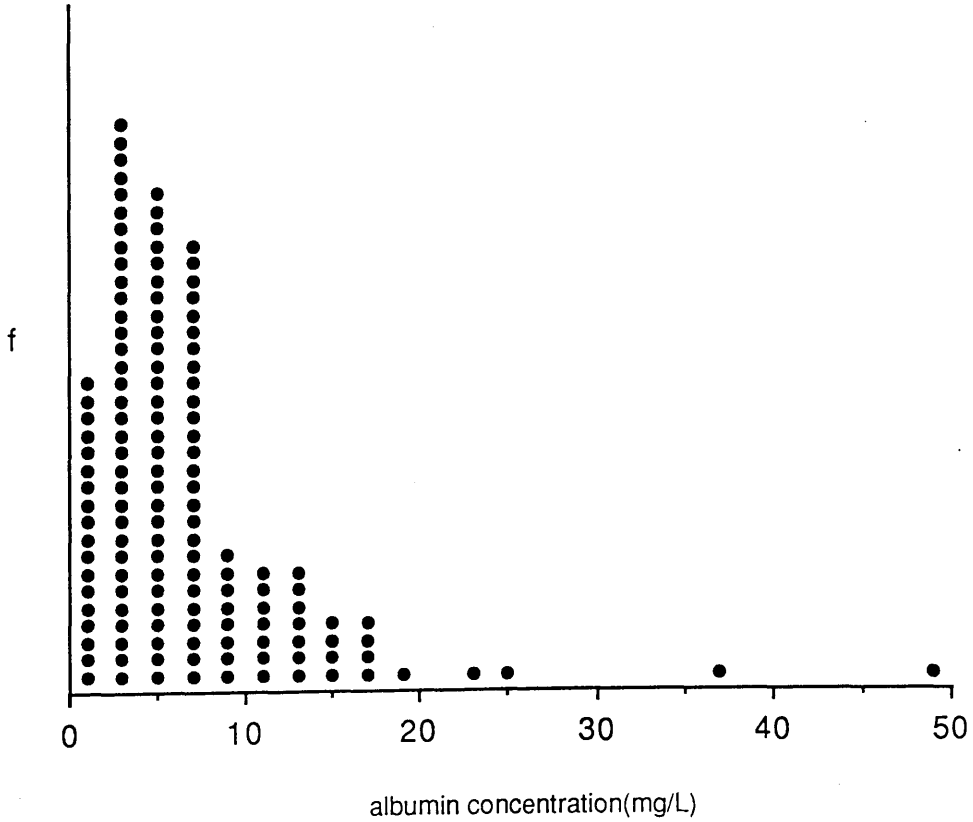


FIGURE 3.5a - RELATIONSHIP BETWEEN PLASMA CHOLESTEROL AND URINARY ALBUMIN CONCENTRATION

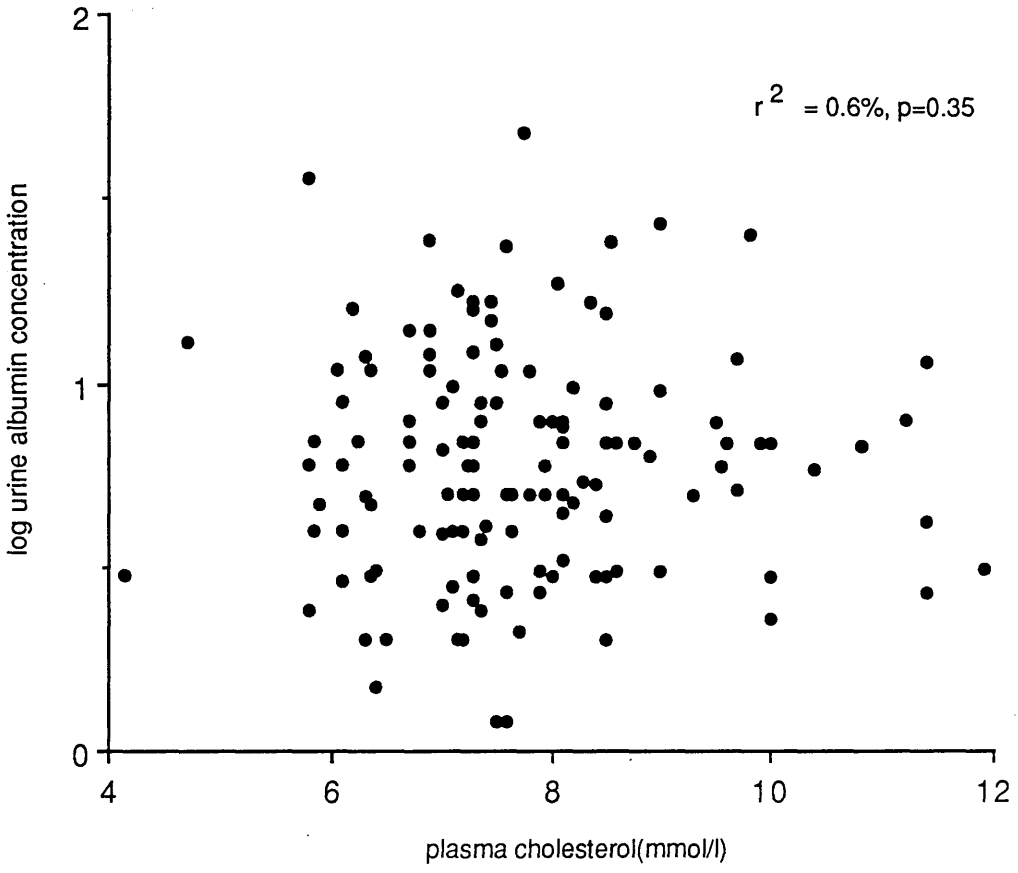
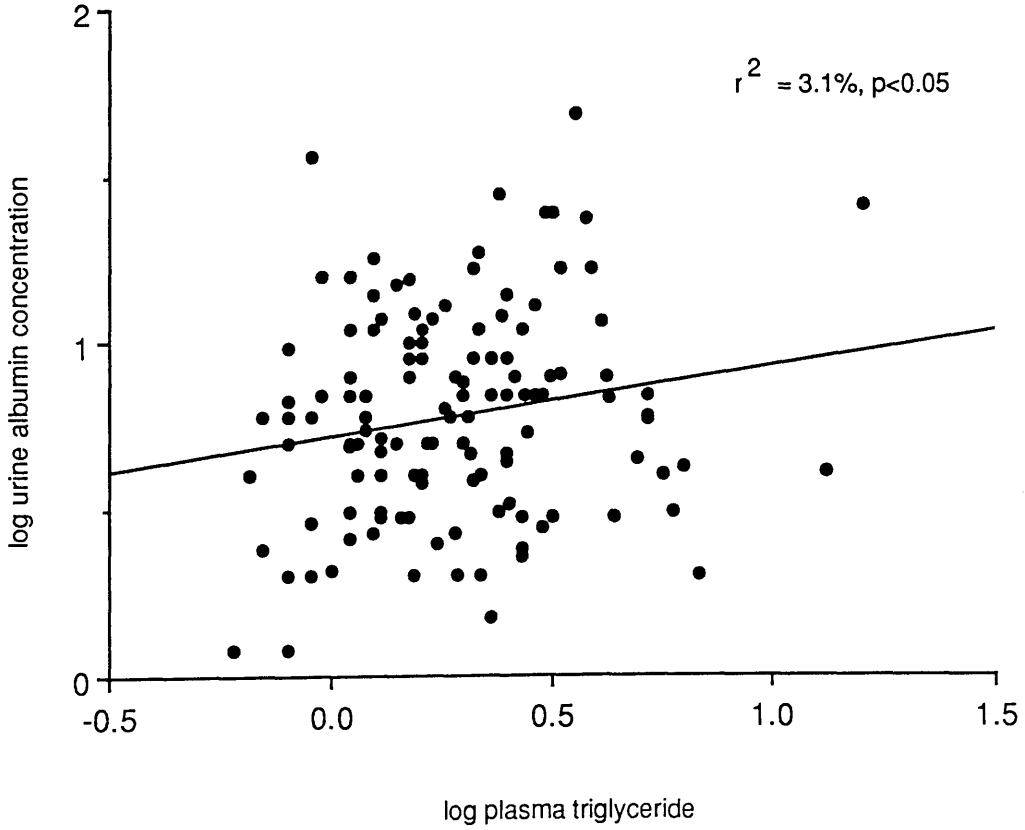


FIGURE 3.5b - RELATIONSHIP BETWEEN PLASMA TRIGLYCERIDE AND URINARY ALBUMIN CONCENTRATION



CHAPTER 4 POST-PRANDIAL LIPOPROTEIN METABOLISM IN NEPHROTIC SYNDROME

4.1 Introduction

The epidemiological, experimental and intervention studies which have examined the relationship between lipoproteins and ischaemic heart disease have focused on the association between atherosclerosis and hyperlipidaemia in the fasting state. However, the major part of our lives is spent in the post-prandial state. Abnormal metabolism of post-prandial lipoproteins(i.e. chylomicrons) may predispose to atherosclerosis(153). The metabolism of these triglyceride-rich lipoproteins is inextricably linked to that of other lipoproteins more commonly associated with atherosclerosis. Lipolysis of chylomicrons leads to a reduction in size and an increase in density of these particles with excess surface material(phospholipid and apolipoproteins AI and C) transferred to nascent HDL particles. LDL metabolism may be influenced by the delivery of intestinally-derived cholesterol to the liver. Although chylomicrons contain only a small percentage by weight of cholesterol ester(derived from the diet or incorporated by exchange with other lipoproteins in the plasma), they may contribute significantly to cholesterol flux to the liver due to their rapid turnover. Increasing intrahepatic sterol pools will reduce LDL receptor numbers.

The metabolism of post-prandial lipoproteins may be investigated by following the concentration of triglyceride and retinyl palmitate in the d<1.006g/mL fraction of plasma after a standardised oral fat load containing vitamin A (154). This fat-soluble vitamin is esterified, mainly as retinyl palmitate, and incorporated into chylomicron cores in the intestinal epithelium. The retinyl ester remains in the core of the chylomicron during progressive delipidation (mediated by endothelial-bound lipases) and is removed from this fraction when chylomicron remnant particles are taken up in the liver. Subsequently, retinol is re-secreted from the liver bound to retinol-binding protein which is not present in the d<1.006g/mL fraction of plasma. The concentration of retinyl palmitate in this fraction has been used as an indirect measure of circulating chylomicron remnants(154,155).

This method has been used to investigate chylomicron clearance in a number of disorders over the last 15 years. Delayed clearance of post-prandial lipoproteins has been reported in type III and type IV hyperlipidaemias (156,157), patients heterozygous for the E2 allele(158), chronic renal failure(159) and in older subjects(160). Recently, the potential role of these particles in atherogenesis has been supported by the observation that subjects with coronary artery disease have slower clearance rates(161). There are no published studies of post-

prandial lipoprotein metabolism in human nephrotic syndrome although a number of workers have addressed this question in experimental models. The aim of this study was to assess and quantify post-prandial lipaemia in human nephrotic syndrome.

4.2 Patients and methods

Subjects attended after a 12 hour fast and blood was drawn for fasting lipids and routine biochemistry. A standardised fat meal was then administered(161). 280mL of fresh double cream, 20g sucrose, 20g dried milk powder, 20mL flavoured syrup and 300,000units(equivalent to 150mg of retinol) of aqueous retinyl palmitate(RO-A-Vit - source: Roche Products, Welwyn Garden City, UK) was made up to a volume of 500mL with water. This emulsion contained approximately 137g fat, 11.4g protein, 50.3g carbohydrate and 400mg cholesterol(total calorie content 1480kcal). A volume equivalent to 65g fat/m^2 was consumed over ten minutes. Body surface area was calculated from a nomogram based on Du Bois' formula(162). The majority of subjects tolerated this well although a few reported some gastric fullness and mild nausea. One nephrotic patient, with probable irritable bowel syndrome, had moderately severe diarrhoea and has been excluded from the analysis. No other bowel symptom were reported. In previous studies, fat absorption with this test meal has been shown to be

almost complete(155) although this was not specifically determined in this study.

Subjects fasted for the first ten hours after the fat load with free access to water and a maximum of two cups of black tea or coffee. After ten hours, they were allowed a low fat, low vitamin A meal and then fasted until the final blood sample. Blood samples were taken at 2 hour intervals over the first ten hours and then at 14 and 24 hours. Plasma was separated at low speed centrifugation and stored at 4^{o}C protected from light. When all eight samples were collected, the d<1.006g/mL fraction was recovered from 4mL of plasma by overlaying with 2mL sodium chloride solution (d=1.006g/mL). Ultracentrifugation was performed for 16hours at 39000rpm(4^{o}C) in a Ti50.3 or Ti40.3 rotor. The d<1.006g/mL fraction containing chylomicrons, chylomicron remnants and hepatic VLDL was recovered in the top 2-3mL. Cholesterol and triglyceride were measured in plasma and this fraction. HDL_2 and HDL_3 were measured at 0, 6 and 24 hours and plasma from each of these time points was subjected to sequential ultracentrifugation at increasing densities of 1.019, 1.063, 1.125 and 1.210g/mL to obtain fractions corresponding to IDL, LDL, HDL_2 and HDL_3 respectively. After the final blood sample, subjects were given an intravenous bolus of heparin(70IU/kg) to assess heparin-releasable lipase activity. Plasma was obtained after 10 minutes, immediately separated at 4^{o}C and then

frozen at -20^{o}C till assayed.

Retinyl palmitate was measured in the d<1.006g/mL fraction from each time point by high pressure liquid chromotography (HPLC) - (161). 0.5mL of this fraction was diluted 1:1 with sodium chloride solution(d=1.006g/mL) and 50uL of 10% glacial acetic acid added. Fifty uL of retinyl acetate (10ug/mL) was added as an internal standard. Neutral lipids were extracted twice with diethyl ether. The lipid extract was blown down under nitrogen and kept protected from light at -20^{o}c till analysed(within 7-14 days). In some subjects, retinyl palmitate was also measured in the IDL and LDL fractions from the 6 and 24 hour samples to assess lipid transfer between lipoprotein fractions in the plasma. No retinyl palmitate was found in HDL subfractions.

Reverse phase HPLC was performed on a Brownlee aquapore RP300 column(source: Brownlee Laboratories, Santa Clara, California, USA) using acetonitrile and water as the mobile phase. Samples were dissolved in 250-500uL of isopropanol and loaded onto the column via an automatic injection loop(source: Rheodyne, Cotacti, California, USA). Peaks were detected at 345nm using a Holochrome monitor(source: Gilson, Middleton, Wisconsin, USA) and integrated electronically by a Hewlett-Packard integrator(source: Hewlett-Packard, Avondale, Philadelphia, USA). The area

under the curve was used to calculate the concentration of retinyl palmitate by comparison with standards. This was corrected for recovery(70-100%) of the retinyl acetate internal standard. At a plasma concentration of 1.5mg/dL, the intra-assay coefficient of variation(CV) was 5.6%(n=8) and the inter-assay CV was 14.5%(n=6). Previous studies have shown that a linear relationship exists between retinyl palmitate concentrations in a serially diluted plasma sample and the value obtained by this method(164).

Plasma triglyceride and d<1.006g/mL retinyl palmitate concentration were plotted against time for both nephrotic and control subjects. The area under the curve was calculated by the "trapezium" method(164) for both using the values at 0 hours as the baseline.

Post-heparin lipase activity was measured <u>in vitro</u> by estimating the rate of lipolysis of a triglyceride emulsion. Briefly, plasma was incubated with a ^{14}C-labelled triglyceride/gum arabic emulsion. Released free fatty acids were bound to albumin in the reaction mixture and extracted with solvents(165). Pooled normal plasma was added as a souce of apoCII activator. Selective measurement of lipoprotein lipase(LPL) was performed by inhibiting hepatic triglyceride lipase(HTGL) with sodium dodecylsulphate. HTGL was assayed in 1M NaCl which inactivates LPL.

Patients were selected according to the criteria in chapter 2 with the exception that two patients with more advanced renal failure were included in the nephrotic group. Control subjects with only minor renal disease(i.e. minor proteinuria and serum creatinine <250umol/L) were recruited from the renal outpatient clinic to assess the effect of heavy proteinuria alone on the lipaemic response. The characterisics of the two groups of subjects studied are compared in table 4.1. The groups were well-matched for age, sex, body mass index and smoking habits. There was no statistical difference between the mean serum creatinine concentrations. The nephrotic group had the anticipated elevations of fasting plasma cholesterol and triglyceride.

Statistical analysis

The purpose of this study was to compare the lipaemic response to a fat load measured by changes in triglyceride and retinyl palmitate concentrations in the d<1.006g/mL fraction of plasma in nephrotic subjects and controls. The main outcome measures were the peak and increments in triglyceride and retinyl palmitate concentrations; the areas under the concentration versus time curves for these two parameters; and post-heparin lipase activities. These were compared by two sample t tests. The relationships between the lipaemic response, fasting triglyceride

concentration, lipase activities and HDL subfraction levels for all 20 subjects were explored by linear regression although no firm conclusions were drawn from any observed associations as these were not the primary end-points. Stepwise multiple regression was used to determine if the lipaemic response(i.e. area under curve for triglyceride or retinyl palmitate) could be predicted from a combination of fasting triglyceride, HDL subfraction concentrations or lipase actvities.

4.3 Results

The most striking feature was the marked inter-individual variability within both nephrotic and control as demonstrated by the time course of plots of plasma concentration of both triglyceride and retinyl palmitate in the d<1.006g/mL fraction(figures 4.1 and 4.2). This heterogeneity of response is well-recognised although previous studies have shown that the response is generally reproducible within an individual(155,163). The mean triglyceride and retinyl palmitate concentrations in the d<1.006g/mL fraction are plotted in figures 4.3 for both groups. These parameters rose to a peak at 6 hrs and then declined to near basal values by ten hours in both nephrotics and controls. The shape of both curves is similar. Although the nephrotic group start with a higher basal triglyceride value(d<1.006g/mL concentration -

control 0.89mmol/L sd 0.45 v nephrotic 1.58 sd 0.81; $p<0.05$), the average increments(2.17mmol/L sd 1.07 v 2.73 sd 1.28) were the same as were the time to peak and return to baseline. One patient in the nephrotic group had a markedly decreased clearance rate of retinyl palmitate with persistently high levels at 14 hours and significant quantities present at 24 hours. This subject had the highest fasting plasma triglyceride level(4.45mmol/L) and the largest area under the curve for both triglyceride and retinyl palmitate in the d<1.006g/mL fraction. Two further patients(one in each group), who possessed one E2 allele, had an unusually high level of d<1.006g/mL retinyl palmitate at 24 hours. There were no differences between the areas under the curve from 0-24 hours for triglyceride (nephrotic 15.56mmol.hr/L sd 13.24 v control 12.59 sd 6.63) or retinyl palmitate in the d<1.006g/mL fraction (7.14mg.hr/dL sd 6.44 v 6.11 sd 4.05).

When data from all twenty subjects were considered, there was an association between fasting triglyceride levels and both the rise in triglyceride($r^2=0.56$, $p=0.016$) and the area described by the triglyceride curve($r^2=0.37$, $p=0.004$). A similar but weaker association was seen between triglyceride concentrations and the area under the retinyl palmitate curves($r^2=0.22$, $p=0.035$) and peak retinyl palmitate concentrations($r^2=0.20$, $p=0.046$).

Measurement of retinyl palmitate in IDL and LDL fractions revealed a small and variable transfer of retinyl ester into these higher density lipoproteins in both groups. In IDL, this amounted to an average of 5.1% of the d<1.006g/mL concentration at 6 hours and only 1.3% in LDL. Small quantities were also found at 24 hours in LDL. There were no differences between the controls and nephrotic subjects.

There were small but significant differences between the concentrations of HDL subfractions between the two groups with reductions in both HDL_2 (controls 84mg/dL sd 47 v nephrotic 45 sd 28; $p<0.05$) and HDL_3 (253mg/dL sd 61 v 201 sd 45; $p<0.05$) in nephrotic sydrome. However, this difference was no longer significant when the two patients with advanced renal failure who had the lowest HDL concentrations were excluded. There was a modest fall in HDL_3 concentrations at 6 hours (mean 21mg/dL sd 40; paired t test $p=0.035$). This had returned to normal by 24 hours and was seen in both groups. HDL_2 levels did not alter significantly.

The composition of particles in the d<1.006g/mL fraction at 0 and 6 hours were compared. At both time-points, there were small increases in the free and esterified cholesterol with a slightly reduced triglyceride content of these particles in the nephrotic group. These relatively small changes may reflect the higher circulating cholesterol in

the nephrotic group creating a concentration gradient for net movement of cholesterol to triglyceride-rich lipoproteins. The ratio of cholesterol:phospholipid (mmol/mg) was increased in the baseline sample(0.026 sd 0.004 v 0.021 sd 0.004, $p<0.01$) but was not significantly different at the 6 hour sample.

There were no differences in lipoprotein lipase, hepatic triglyceride lipase or total heparin-releasable lipase activity between the two groups(table 4.2). There was a weak relationship between LPL activity and d<1.006-triglyceride area under the curve following a fat load ($r^2=0.21$, $p<0.05$). HTGL activity may be important in the clearance of "remnant" particles formed by delipidation of triglyceride-rich lipoproteins(156) but no relationship was demonstrated between retinyl palmitate clearance(area under the curve) and HTGL activity.

When all these factors(fasting triglyceridaemia, lipase activities, HDL subfraction concentrations) were considered, fasting triglyceride remained the most powerful predictor of post-prandial lipaemia although still accounting for only 36.2% of the variability in this response. The predictive value could not be improved using any combination of HDL subfractions or lipase activities.

4.4 Discussion

Altough defective catabolism of triglyceride-rich lipoproteins is well-described in nephrotic syndrome, chylomicron metabolism has not been extensively investigated in human nephrotic syndrome. Studies in experimental nephrotic syndrome(166-168) have demonstrated an increased half-life of labelled chylomicrons. This could be reversed by heparin/albumin infusions(166) or by intravenous heparan sulphate isolated from nephrotic rat urine(167). Levy(168) attributed this defect partly to a 35% reduction in lipase activity. However, there was also a reduced clearance of homologous nephrotic chylomicrons in normal rats. Similar findings were recently reported from Japan for VLDL particles(64). Chylomicron synthesis and composition may be altered in experimental nephrotic syndrome with the production of large lipid-rich particles with reduced phospholipid, apo E and apo AI content (168,169). These changes may influence the catabolic potential of the particles by impairing binding to lipoprotein lipase and other proteins involved in the catabolism of triglyceride-rich lipoproteins. In human nephrotic syndrome, Newmark and colleagues(46) found a high incidence of fasting chylomicronaemia although other studies have not confirmed this(48).

In this study, there was no consistent defect in chylomicron metabolism assessed by the lipaemic response to a standard fat meal in patients with heavy proteinuria compared to controls. Although two nephrotic patients had significant renal impairment, this would have been predicted to increase any differences between the groups since renal failure is known to reduce lipolytic capacity and increase plasma concentrations of remnant particles (88,159). The wide inter-individual variation in both groups confirms previous studies. However, the shapes of the mean curves for triglyceride and retinyl palmitate and the mean areas under the curves are very similar in groups who were comparable in age, body weight, sex distribution and smoking habits. Inspection of the individual curves suggest that at least three subjects(two patients with nephrotic syndrome and one control) had a defect in the clearance of retinyl esters consistent with impaired removal of remnant particles. In two subjects, this may be attributed to the presence of an E2 isoform(158) but one of the nephrotic subjects demonstrating this pattern possessed an E4/E3 phenotype. The delayed clearance in this subject was associated with the highest peak triglyceride and the largest area under the curve although the precise reason for this is not clear. Lipoprotein lipase activity in this patient was 2.60umol FFA/mL/hour compared to a mean value for the nephrotic group of 3.71. The explanation for this response is not known.

The areas under the curves of triglyceride and retinyl palmitate in the d<1.006g/mL fraction are influenced by a number of factors other than removal rates. Absorption of dietary fat, synthesis and secretion of chylomicrons in the intestine and rate of lymphatic flow will effect the appearance of lipids in the blood after a fat load. None of these were measured directly in this study. However, the similarity in the rate of rise of both triglyceride and retinyl palmitate curves suggests that these factors are not significantly altered in human nephrotic syndrome.

The appearance of retinyl palmitate in IDL and LDL fractions has recently been reviewed in a re-assessment of this type of study for investigating the clearance of chylomicron remnants(160). However, the isolation of subfractions of lipoproteins prior to assay avoids the limitations of plasma retinyl ester measurements for this purpose. The quantity of retinyl ester found in lipoproteins of d>1.006g/mL is small although at 24 hours plasma retinyl palmitate concentration may overestimate the concentration in the d<1.006g/mL fraction. This probably represents transfer of retinyl esters between triglyceride-rich and low density lipoproteins mediated by circulating lipid transfer proteins. However, other possibilities are degradation of intestinally-derived lipoproteins to this density interval, contamination of the IDL/LDL fractions by remnant particles or re-secretion of retinyl esters in VLDL

by the liver.

The conflicting conclusions of previous studies on the activities of lipases in experimental and human nephrotic syndrome have been considered in chapter 1. Using specific assays for LPL and HTGL, no difference in the activities of these enzymes was seen between these two groups.

Clearance of triglyceride-rich very low density lipoproteins is decreased in nephrotic syndrome. This has been demonstrated by studies in experimental nephrotic syndrome, reports of reduced lipase activity and of impaired removal of VLDL particles in human nephrotic syndrome. Therefore, impaired tolerance of an oral fat load with a late and prolonged lipaemia might be anticipated. These studies do not confirm that such a defect is a consistent feature of the nephrotic syndrome although individual patients had delayed clearance of retinyl esters from the d<1.006g/mL fraction. The results of the dynamic studies are consistent with the normal post-heparin lipolytic activities and the absence of major compositional changes in triglyceride-rich lipoproteins at 6 hours which might retard their catabolism. The variability among all 20 subjects of the lipaemic response to a fat meal probably reflects the complexity of the mechanisms regulating an everyday metabolic process.

TABLE 4.1 - POST- PRANDIAL LIPAEMIA - CHARACTERISTICS OF SUBJECTS

	CONTROLS	NEPHROTIC
number	11	9
age(years)	49(11)	52(11)
sex M:F	8:3	6:3
body mass index(kg/m^2)	26.1(2.1)	24.1(2.1)
smokers	2	2
serum creatinine(umol/L)	130(52)	253(192)
quantitative proteinuria(g/day)	0.15(0.29)	6.3(4.7)
serum albumin(g/L)	42(1.6)	31(6.3)
plasma cholesterol(mmol/L)	5.9(0.8)	9.6(2.6)
plasma triglyceride(mmol/L)	1.6(0.8)	2.7(1.2)

Values quoted as mean(SD). Note nephrotic group included two subjects with more advanced renal failure(serum creatinine 640 and 520umol/L).

TABLE 4.2 - POST-HEPARIN LIPASE ACTIVITIES

	CONTROLS	NEPHROTIC
lipoprotein lipase	5.08(2.75)	3.71(1.22)
hepatic triglyceride lipase	19.9(7.2)	20.5(5.0)
total lipase	25.0(7.3)	24.2(5.0)

Values are mean(SD) expressed as umol free fatty acids/mL/hour

FIGURE 4.1a - TRIGLYCERIDE IN d<1.006g/mL FRACTION FOLLOWING FAT LOAD CONTROL GROUP

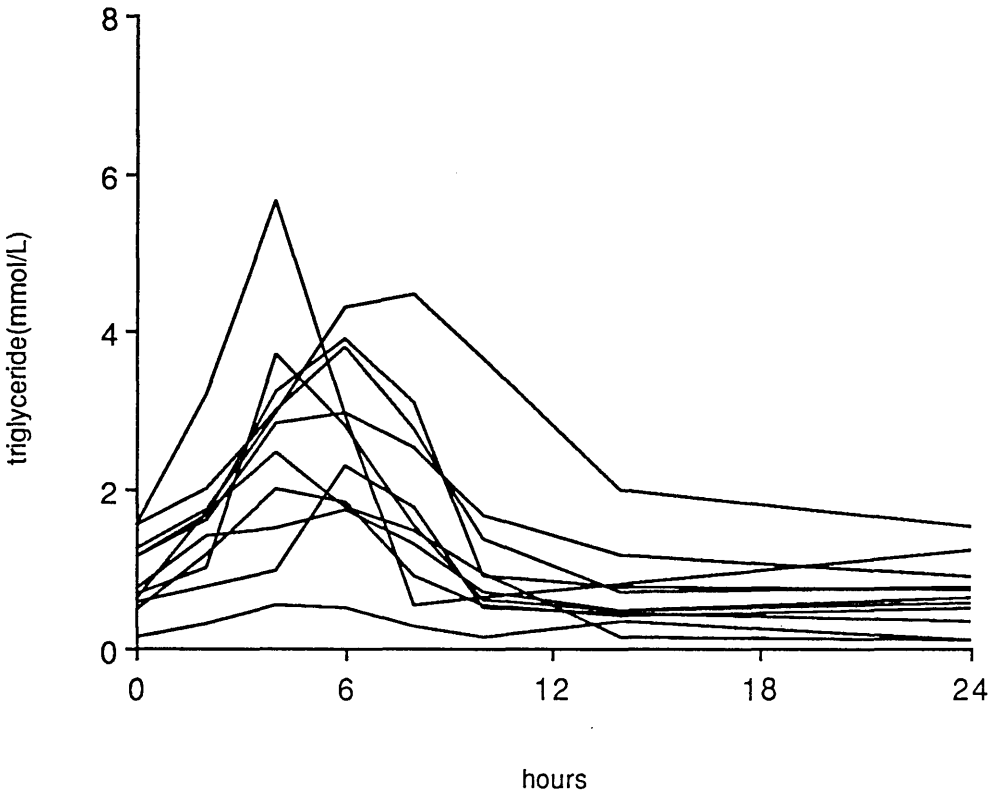


FIGURE 4.1b - TRIGLYCERIDE IN d<1.006g/mL FRACTION FOLLOWING FAT LOAD NEPHROTIC GROUP

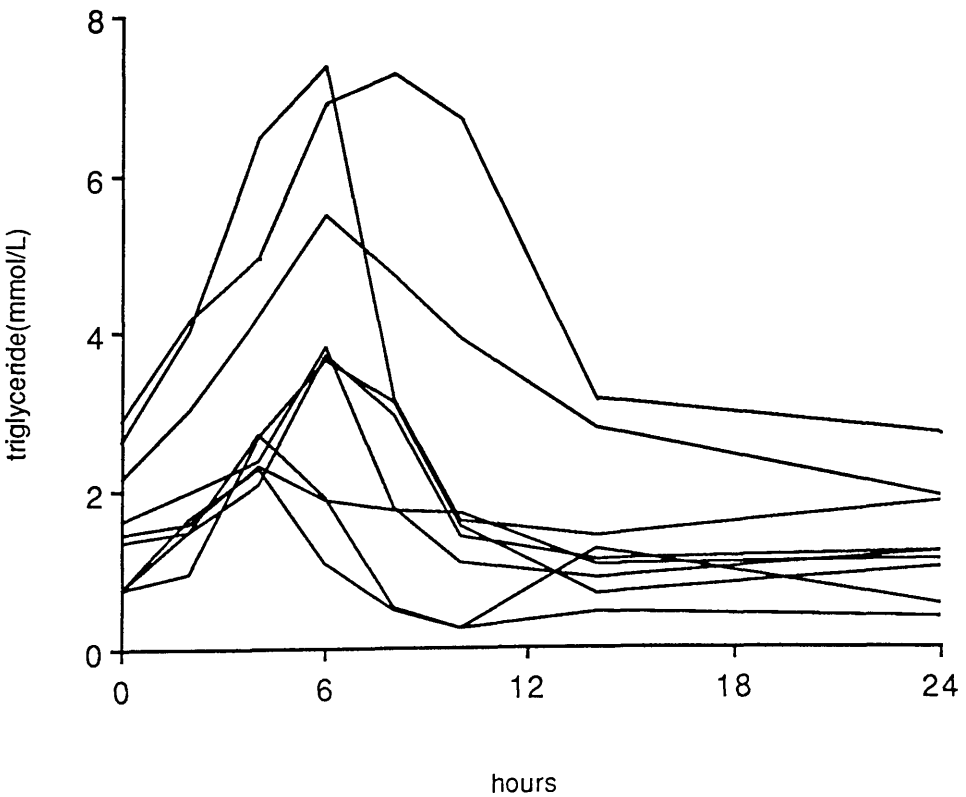


FIGURE 4.2a - RETINYL PALMITATE IN d<1.006g/mL FOLLOWING FAT LOAD
CONTROL GROUP

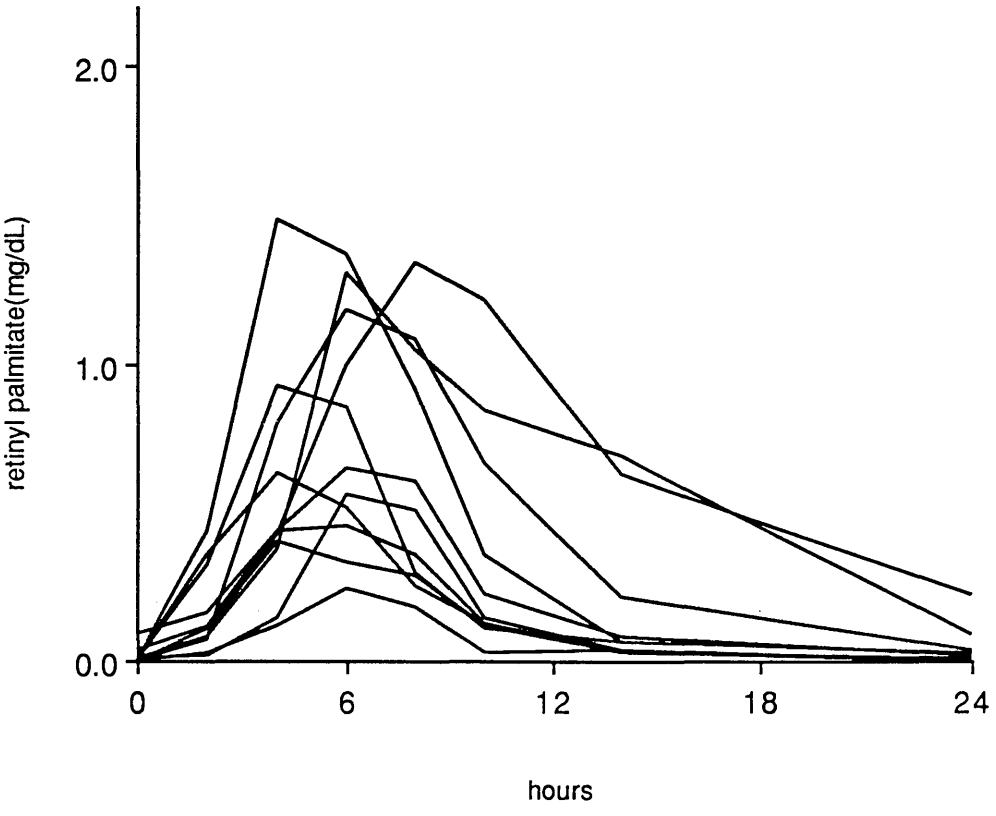


FIGURE 4.2b - RETINYL PALMITATE IN d<1.006g/mL FOLLOWING FAT LOAD
NEPHROTIC GROUP

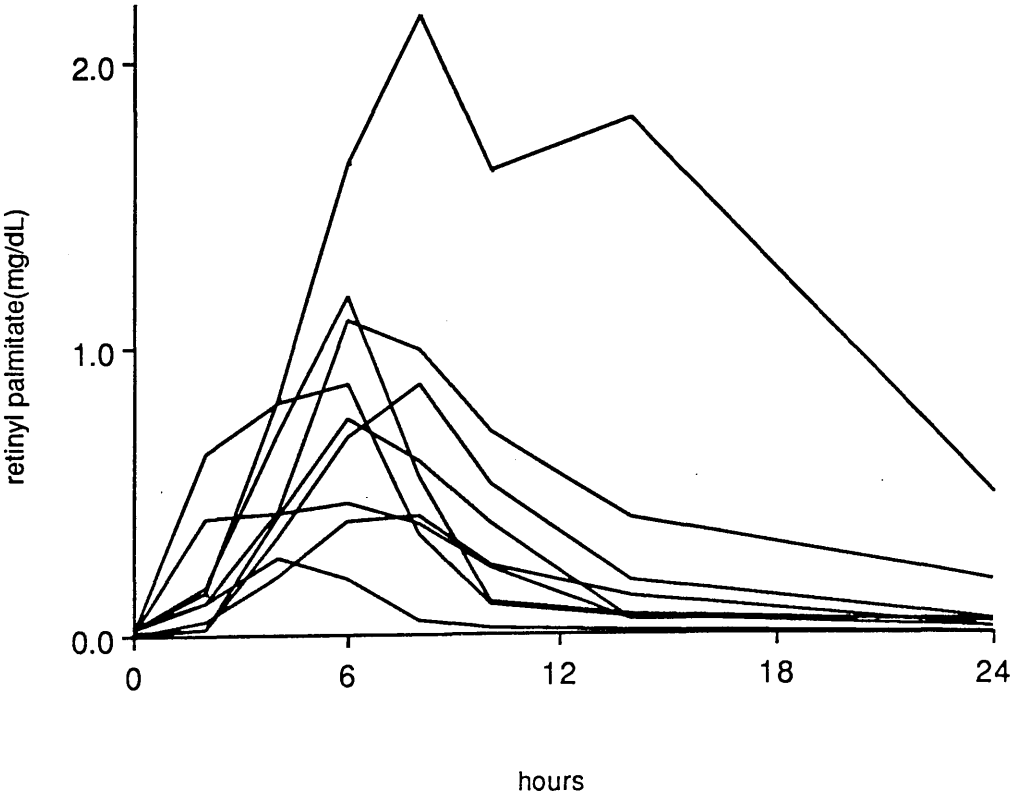


FIGURE 4.3a
MEAN TRIGLYCERIDE CONCENTRATION IN d<1.006g/ml FOLLOWING FAT LOAD

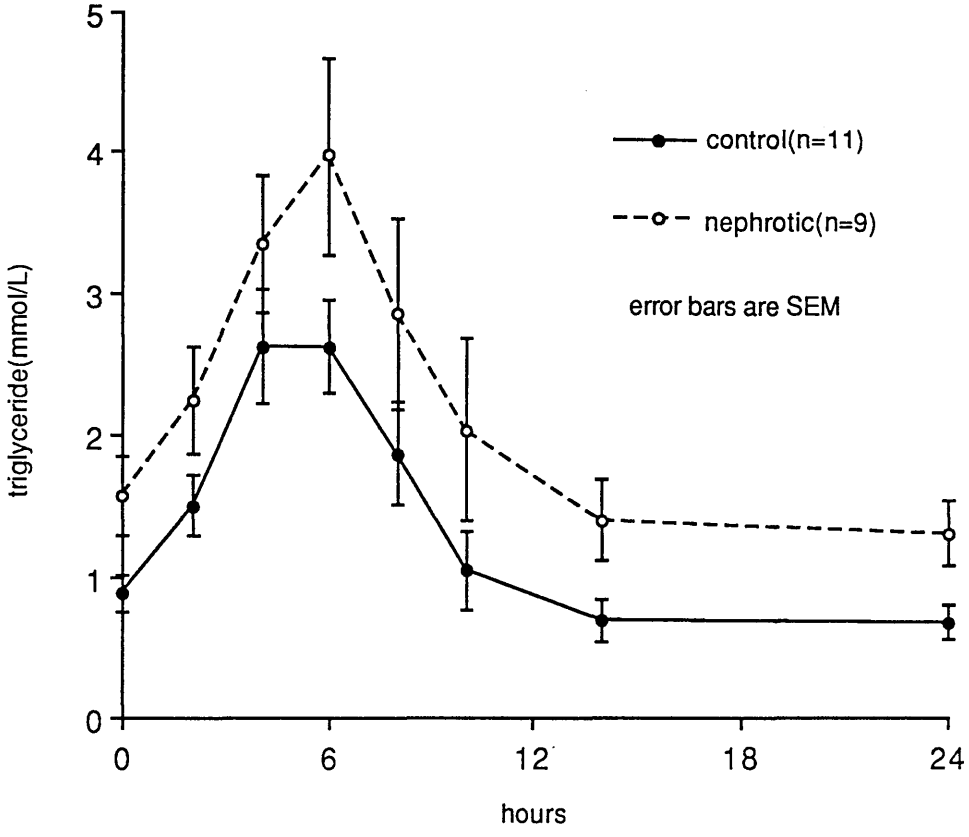
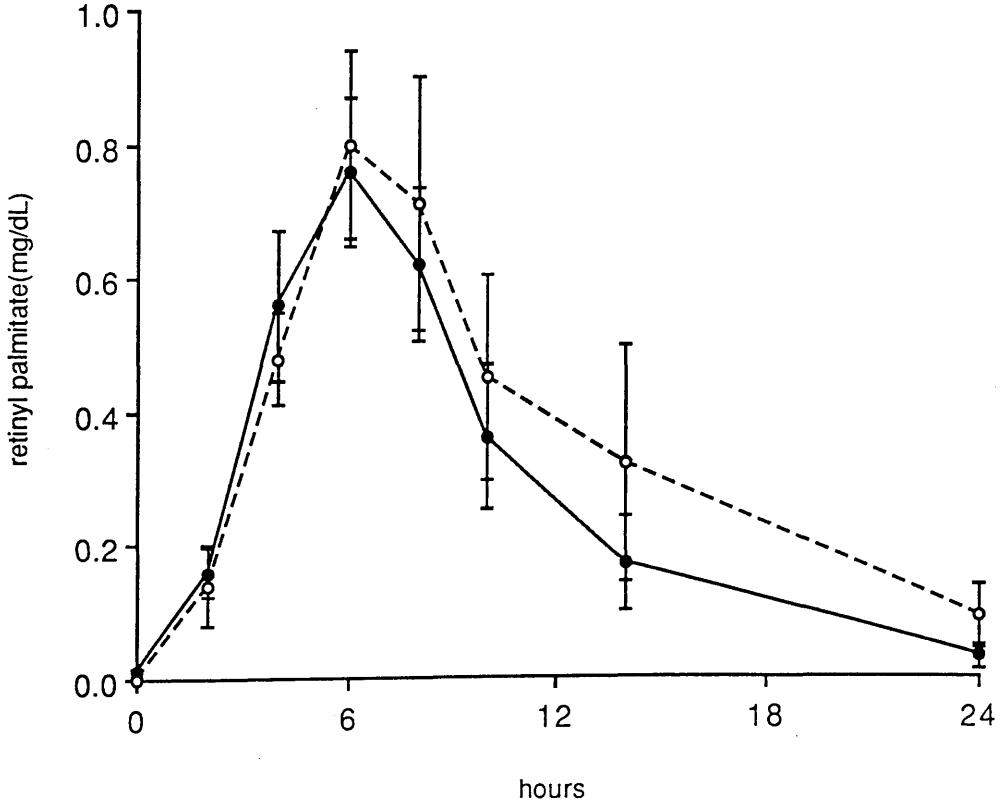


FIGURE 4.3b
MEAN RETINYL PALMITATE CONCENTRATION IN d<1.006g/mL FOLLOWING FAT LOAD



CHAPTER 5 VERY LOW DENSITY LIPOPROTEIN METABOLISM IN NEPHROTIC SYNDROME

5.1 Introduction

The metabolism of very low density lipoproteins bears many similarities to that of chylomicrons. Both are large triglyceride-rich particles originating in the liver and intestine, respectively. Endothelial-bound lipases are responsible for the hydrolysis of the triglyceride core of these particles. Chylomicrons are converted to "remnant" particles with subsequent removal by the liver, while VLDL is the precursor of IDL and LDL. It is widely held that the main cause of the hyperlipidaemia of the nephrotic syndrome is increased hepatic apolipoprotein, lipid and lipoprotein synthesis and secretion(54). This should be reflected in increased hepatic VLDL production. However, this hypothesis is based on experimental models of the nephrotic syndrome and few studies have investigated this directly (i.e. by the incorporation of labelled precursors into lipoproteins) in human nephrotic syndrome.

Catabolic processes can be examined by following the clearance of labelled tracers from the plasma. The theory of using labelled apoB as a tracer for the metabolic fate of apoB-containing lipoproteins was considered in chapter 2. In steady-state systems, information can be inferred

about synthetic rates from a knowledge of the plasma decay of the tracer and the pool size of the tracee. Despite the widespread use of apoB kinetic studies to investigate various dyslipidaemias, there have been few applications of this technique to the study of the mechanisms underlying nephrotic hyperlipidaemia. Decreased removal of VLDL particles has been described in both experimental models of nephrotic syndrome(63) and in man(66,69). These workers used tracers isolated in a wide density range and it is now recognised that VLDL (d<1.006g/mL) contains a heterogeneous mix of particles of differing metabolic origins and fates(26). The aim of this study was to compare the metabolism of discrete subfractions of VLDL and to follow the transfer of apoB from VLDL->IDL->LDL in nephrotic subjects and controls.

5.2 Patients and methods

Subjects attended after an overnight(>12 hours) fast and approximately 200-250mL of plasma was obtained by plasmapheresis. This was done by venesecting 400-500mL of blood into a citrated container, immediately separating the plasma by centrifugation and re-infusing the red blood cell concentrate(diluted in isotonic sterile saline) via the indwelling cannula. Total VLDL(d<1.006g/mL) was obtained by ultracentrifugation of 150mL(6 x 25mL) of plasma in a Beckman Ti60(or Ti50.2) at 39,000rpm at 10^{o}C

for 18 hours. The floating VLDL was aspirated in the top 2-4mL and then, if necessary, diluted with isotonic saline to achieve an initial plasma triglyceride concentration of 1.5mmol/L. This minimises carry over of $VLDL_1$ to $VLDL_2$ during preparative ultracentrifugation. The solution density was then raised to 1.118g/mL by the addition of solid sodium chloride(0.171g/mL). Six 2mL aliquots were subjected to cumulative flotation ultracentrifugation on a discontinuous salt gradient(figure 2.1) in a Beckman SW40 swinging bucket rotor as adapted from Lindgren(170).

$VLDL_1$(S_f 60-400) and $VLDL_2$(S_f 20-60) were isolated by this method and aliquots(2mL) labelled with ^{131}I and ^{125}I respectively by a modification of the iodine monochloride method(140,141,171). Free radioiodide and salt were removed by gel filtration on a PD10 column(source: Pharmacia, Uppsala, Sweden) followed by extensive dialysis against 0.15M NaCl, pH 7.4. Three days after the initial plasmapheresis subjects returned, having fasted for >12 hours, and approximately 50uCi of each tracer was administered intravenously. The tracers were sterilised by passage through a 0.45micron filter(source: Gelman Science, Northampton, UK) immediately prior to injection. Blood samples were collected at 10 minutes and then frequently over the first 10 hours after which a low fat meal was allowed. Further samples were taken at 14 and 24 hours and then daily fasting samples were obtained for 12 days. Each

plasma sample was subjected to ultracentrifugation as above to isolate four fractions - $VLDL_1$, $VLDL_2$, IDL(S_f 12-20) and LDL(S_f 0-12).

ApoB was precipitated(172) from each fraction by the addition of an equal volume of freshly-distilled 1,1,3,3-tetramethylurea(TMU). The precipitate was then delipidated and the specific activity of apoB in the precipitate measured after redissolving in 0.5M NaOH. Protein content was estimated by a modified Lowry assay(131) where standards were dissolved in 0.5M NaOH and radioactivity was measured in a twin channel gamma counter(source: Canberra Packard, Pangbourne, UK).

The plasma concentrations(in mg/dL) of $VLDL_1$, $VLDL_2$, IDL and LDL were estimated from compositional analysis of each fraction isolated from pooled plasma. The total cholesterol recovered in the four fractions by cumulative flotation ultracentrifugation was compared with the cholesterol content of the non-HDL cholesterol measured by routine beta-quantification of plasma. Recovery varied from 82-92%. The calculated lipoprotein masses were each corrected by this factor. This assumes that each fraction was lost in similar amounts and that the different components of the lipoproteins were also lost in equal proportion.

ApoB concentration was measured in aliquots of each fraction by precipitating apoB as above and measuring the protein content in the supernatant fluid. ApoB concentration was calculated as the difference between total protein and TMU-soluble protein. Intravascular pool sizes of apoB were obtained from the product of apoB concentration and plasma volume derived from the dilution of the $VLDL_2$ tracer at ten minutes.

Eight patients with nephrotic syndrome were recruited to this study. The characteristics of these subjects are listed in tables 5.1 and 5.2. The results of the kinetic studies were compared with a group of 5 normolipidaemic controls studied in a parallel investigation.

Kinetic Analysis

The radioactivity in apoB in each lipoprotein fraction was obtained by multiplying the specific activity(cpm/mg) at each time point by the pool size(mg apoB) of the appropriate fraction. All radioactivities were then expressed as a percentage of the total apoB activity present in the 10 minute sample calculated from the sum of radioactivity in $VLDL_1$, $VLDL_2$, IDL and LDL. Plasma decay curves(examples shown in figure 5.1) and pool sizes were then analysed by a multicompartmental model using the SAAM/CONSAM 30 programme(142,143). The apoB radioactivity

curves and estimated apoB pool sizes were used to derive fractional transfer and catabolic rates, flux rates between compartments and production rates for apoB in $VLDL_1$, $VLDL_2$ and, where necessary, LDL. Production and flux rates are expressed as mg apoB/kg body weight/day. Fractional transfer and catabolic rates are expressed as pools/day.

The model used to analyse apoB metabolism in very low density lipoproteins has been described previously (173,25,26,30). In summary, it consists of two parallel pathways from large and small VLDL through IDL to LDL. The transfer of apoB along this chain is thought to be dependent on step-wise delipidation mediated by lipoprotein and hepatic triglyceride lipase activities. Direct input of apoB occurs both at $VLDL_1$ and $VLDL_2$. Where the calculated mass in LDL falls significantly short(>10%) of the measured mass, direct synthesis of LDL is invoked. Removal of apoB occurs at each level of the pathway. However, this model did not allow an adequate fit of the data from the proteinuric group. The calculated pool mass for $VLDL_1$ was underestimated in a number of these subjects who had an expanded $VLDL_1$ pool. Several subjects also demonstrated a biexponential decay of $VLDL_1$ suggesting the presence of an additional component within this fraction which was being metabolised at a different rate. A revised model was therefore developed(figure 5.2). In this, $VLDL_1$ is composed of three compartments with input to and direct

removal from two parallel pathways. These extra compartments(compartments 29 and 26) were not required for the analysis of the control subjects.

Statistical analysis

Non-parametric methods were used because of the small numbers and the wide spread of results in the nephrotic group. Results are shown as medians and ranges. Differences between nephrotic and control groups were tested by the Mann-Whitney U test and correlations by Spearman's rank test. The plots comparing the specific activities of the two tracers between the groups are shown as mean and standard deviations. The compositional data for both groups were of comparable variance and were analysed by a two-sample t test.

5.3 Results

Baseline data

This group of patients with moderate to heavy proteinuria demonstrated varying increments in plasma cholesterol and triglyceride(table 5.3). There were no clear relationships between plasma lipids and measured urinary protein loss or serum albumin using non-parametric analysis. Serum albumin was remarkably well maintained even when quantitative

proteinuria approached 10g/day. LDL-cholesterol was moderately elevated in 7 patients with one pateint(JG) having a very high level of 10.0mmol/L. VLDL-cholesterol was high in 3 patients all of whom demonstrated a high VLDL cholesterol/triglyceride ratio suggesting the accumulation of VLDL remnant particles. One of these patients(WS) possessed an E2 allele of the apoE polymorphism. These changes were reflected in the increased plasma concentrations of three lipoprotein fractions($VLDL_2$, IDL, LDL) separated by cumulative flotation ultracentrifugation as calculated from the sum of compositional analysis(figure 5.3). In this small group of subjects and in contrast to the findings in chapter 3, there were no significant differences in the relative composition of all four lipoprotein fractions between controls and proteinuric subjects(table 5.4).

Kinetic studies

Figures 5.4a-g compare the metabolism of each tracer in the four subfractions by plotting the mean fraction of the total initial apoB radioactivity at each time point for both proteinuric and control groups. The results of the kinetic analysis are presented in tables 5.5a-d.

$VLDL_1$

The ^{131}I $VLDL_1$ apoB tracer was cleared more slowly in the nephrotic group than in controls primarily due to a consistent reduction in the fractional transfer of $VLDL_1$ apoB to $VLDL_2$ (median 0.93pools/day;range 0.22-3.33 v 3.66;2.48-5.16, $p<0.02$ - table 5.5a). Both nephrotic and control groups showed wide variation in the fraction of the $VLDL_1$ apoB pool which was catabolised directly from the plasma compartment. However, in order to fit the data for apoB specific activities and pool sizes, it was necessary to postulate that some nephrotic subjects produced a species of $VLDL_1$ which did not enter the apoB cascade (compartment 26 - figure 5.2). In the proteinuric group, the $VLDL_1$ apoB pool size varied from normal to eight times normal and this was related to plasma triglyceride level. The calculated rate of apoB flux from the large triglyceride-rich $VLDL_1$ to smaller $VLDL_2$ was reduced (1.9mg/kg/day;1.1-2.9 v 2.7;1.8-5.1, $p<0.05$) even in those patients with normal pool sizes(CF,MD,RG,JG). There was wide variation in the secretion of apoB into $VLDL_1$ (2.6-64.8mg/kg/day) in the proteinuric group. Although four patients had very high production, there was no statistically significant differences between the groups.

$\underline{VLDL_2}$

The $VLDL_2$ apoB tracer was also cleared from the plasma of the nephrotic group at a slower rate than normal. This was seen with both tracers i.e. directly labelled $VLDL_2$ and the material transferred from $VLDL_1$(figures 5.4b and e). Again, this was attributable to a reduced delipidation rate to IDL(or LDL by pathways 12->21 and 4->20) - table 5.5b. Direct removal of $VLDL_2$ apoB from plasma was highly variable and not significantly different between the two groups. The $VLDL_2$ apoB pool was expanded in the proteinuric group(2-3x normal) mainly due to a marked increase in *de novo* synthesis of apoB in $VLDL_2$ (14.6mg/kg/day;5.1-32.2 v 4.2;2.9-8.8, $p<0.02$). Reduced catabolism made a relatively smaller contribution to the expansion of the pool size. The larger $VLDL_2$ pool led to a modest increase in the amount of IDL apoB formation although this was not statistically significant (10.4mg/kg/day;4.4-17.2 v 5.8;2.7-8.7, $p=0.12$). This occurred despite the reduced fractional transfer rate from $VLDL_2$ to IDL.

$\underline{IDL}$

The mean plasma radioactivity curves for ^{125}I and ^{131}I reach comparable peak values in IDL at similar times for both groups(figures 5.4c and 5.4f). Thereafter, the curves

diverge with the normals showing an initial more rapid decay that was due to direct catabolism of the lipoprotein (table 5.5c). There was no obvious difference in the rates at which both groups of subjects accumulated ^{125}I and ^{131}I in LDL apoB indicating a similar degree of IDL to LDL conversion(table 5.5c). The expansion of the IDL apoB pool in nephrotics was due to a combination of increased synthesis from $VLDL_2$ and diminished direct removal. This had the net effect of channelling an increased amount of apoB from IDL to LDL(table 5.5d).

LDL

The appearance curves for ^{125}I and ^{131}I apoB radioactivity in LDL are similar for control and nephrotic groups(figures 5.4d and 5.4g). Peak values are achieved at a later time in the latter group but the extent of transfer from $VLDL_1$ and $VLDL_2$ is approximately the same. Normal subjects have a biphasic LDL decay curve consisting of a relatively rapid catabolism up to 120 hours and a slower terminal phase. Visual inspection of the averaged data from the patients with nephrotic syndrome indicated a lack of the early rapid phase of LDL degradation.

The observed fractional catabolic rate for LDL should be corrected for urinary loss of whole lipoprotein. This is not possible to determine from urine collections during a

VLDL turnover because a high proportion of injected radioactivity is present in components other than apoB(i.e. apolipoproteins C and E and lipids). In other studies(see chapters 6 and 7) using trace-labelled LDL in nephrotic syndrome, the average urinary excretion of undegraded lipoprotein was estimated as 7% of total urine radioactivity and the values in table 5.5d overestimate the true FCR by this proportion. The LDL FCR was subnormal in most of the disease group but three subjects(CM, WS and CF) had normal or even high values and, overall, there was no significant difference from controls even when corrected for estimated urinary losses. Likewise, there was a variable expansion of the LDL apoB pool. Its size ranged from normal to twice normal. The production of LDL from $VLDL_2$ and IDL was significantly increased in the nephrotic group compared to normal.

5.4 Discussion

Reduced lipoprotein catabolism has been described in nephrotic syndrome and several lines of evidence suggest that catabolic defects are present along the VLDL->IDL->LDL delipidation cascade. Early work by Gitlin *et al.*(66) demonstrated a delay in the catabolism of radioiodinated S_f 100-400 lipoproteins. More recently, Vega and Grundy (69) have shown reduced clearance of a combined VLDL and IDL tracer(d<1.019g/mL) in four nephrotic subjects compared

to controls. The delipidation of VLDL is catalysed by endothelium-bound lipases in hepatic and extra-hepatic tissues(skeletal muscle and adipose tissue). Decreased activities of these enzymes have been reported in this disorder(76-79) although this finding has not been confirmed in all studies(51,80,81). The studies presented in chapter 4 failed to show a difference in lipase activities between nephrotic and controls and both groups had, on average, similar lipaemic responses. These contradictory findings led to the present detailed investigation of VLDL metabolism in the nephrotic syndrome.

The eight subjects studied had persistent heavy proteinuria and had been followed-up for a period varying from 10 months to 35 years(table 5.1). They had moderate hyperlipidaemia except for JG who had a markedly elevated LDL-cholesterol level. Plasma triglyceride levels varied from low normal(1.2mmol/L) to high(4.5mmol/L) emphasising the heterogeneous nature of the study group. In this small group, there was no obvious relationship between disease severity(proteinuria or hypoalbuminaemia) and plasma lipid levels. When apoB-containing lipoproteins were fractionated by cumulative flotation ultracentrifugation significant increases were seen in plasma levels of $VLDL_2$, IDL and LDL(figure 5.3). The composition of all four lipoprotein fractions was not significantly different between the two groups. However, there was a trend towards

an increased free cholesterol content in VLDL subfractions as seen in chapter 3. There was no substantial accumulation of cholesterol ester-rich VLDL remnants in the $VLDL_2$ and IDL density ranges despite high plamsa triglyceride/VLDL cholesterol ratios in some patients.

Three significant perturbations of apolipoprotein B metabolism were noted in the nephrotic subjects. First, the rates of transfer of $VLDL_1$ apoB to $VLDL_2$ and of $VLDL_2$ apoB to IDL were reduced. This was true even in normotriglyceridaemic nephrotic subjects(MD,RG,CF in table 5.5a) who had normal $VLDL_1$ apoB pool sizes. Thus, the delayed clearance cannot be ascribed to saturation of the delipidation mechanism. These metabolic steps are believed to involve the action of lipoprotein lipase and, to some extent, hepatic triglyceride lipase(25). As noted above, a number of studies in both human and experimental nephrotic syndrome have examined post-heparin plasma lipase activities with contradictory results. Chan and co-workers (79), using a substrate-specific method, documented a decrease in lipoprotein lipase with normal hepatic triglyceride lipase activity. Oetliker(51) could not find any significant difference compared to controls for either enzyme using a more standard immuno-inhibition method although there was an inverse relationship between lipoprotein lipase activity and triglyceridaemia. *In vitro*, nephrotic serum may inhibit lipase activity(79) - a

similar finding has been reported in uraemia(89). However, the degree of inhibition *in vitro* did not correlate with reductions in lipase activity in the patients from whom the sera were obtained.

Several hypotheses have been advanced to explain the possible reduced lipase activity. Apolipoprotein CII, an important activator of lipoprotein lipase, has been isolated from nephrotic urine. However, total plasma concentrations are normal in nephrotic syndrome although Kashyap(83) has reported that the absolute amount associated with VLDL is reduced. Alterations of the lipid and/or apolipoprotein composition of VLDL may alter its catabolic potential and account for a recent report that VLDL from nephrotic rats has a reduced clearance rate in normal animals and is more resistant to lipolysis *in vitro*(64). The modest increases in free cholesterol content of VLDL species seen in the nephrotic subjects may affect their metabolic potential.

Two other explanations have been proposed. Staprans and co-workers(84) isolated a separate lipase co-factor from the urine of nephrotic patients which they identified as a glycosaminoglycan and Bernard(40) has reviewed the possibility that, in hypoalbuminaemic states, decreased removal of free fatty acids which are normally protein-bound may impair lipolysis.

Second, the synthesis of apoB in the density interval of $VLDL_2$ was increased several-fold on average. This effect was more consistent than the highly variable values seen for $VLDL_1$ apoB synthesis in the nephrotic subjects. The increased direct synthesis(table 5.5b) more than compensated for the reduced flux from $VLDL_1$. This in turn led to overproduction of IDL and LDL(tables 5.5c and 5.5d). Thus, hepatic oversynthesis of apoB was a feature of this group and contributed to the expansion of the $VLDL_2$, IDL and LDL pools. As discussed above, hepatic oversynthesis of apoB-containing lipoproteins is common in animal models of nephrotic syndrome(54-58) and has also been reported in man(70). In the latter study, markedly increased rates of apoB production were found in a group of severely hyperlipidaemic nephrotic patients although the full details of these subjects have not been reported. The stimulus to overproduction is unclear. In the current study, the finding that triglyceride-poor $VLDL_2$ rather than $VLDL_1$ is overproduced suggests that increased hepatic apoB rather than triglyceride synthesis was responsible for the elevation in plasma VLDL. This contrasts with the kinetic changes that underly the hypertriglyceridemia of non -insulin dependent diabetes mellitus(24). In this condition larger, lipid-rich $VLDL_1$ particles are secreted in excessive amounts but insulin therapy results in a decrease in $VLDL_1$ production and an increase in $VLDL_2$. This is thought to reflect limitation of carbohydrate and

free fatty acid flux to the liver which in the insulin-deficient state stimulates hepatic triglyceride synthesis and production of $VLDL_1$.

The third abnormality observed in nephrotic subjects was a modest increase in the production rate of LDL apoB. However, unlike Vega and Grundy(69), we did not require to invoke a significantly increased level of direct LDL apoB synthesis. In most subjects, all of the apoB in LDL of nephrotics could be accounted for by delipidation of VLDL and IDL. The fractional catabolic rates of LDL were on average slightly decreased although the nephrotic group covered a wide range and the difference was not statistically significant(see chapter 6).

The signifigance of a separate VLDL species in the nephrotic group(compartment 26 - figure 5.2) is uncertain. Alterations of hepatic lipid and protein metabolism may lead to the production of VLDL sub-species which, because of compositional changes, cannot feed into the delipidation chain and are removed directly from the circulation. The only alternative explanation is that denaturation occurred during the preparation of the $VLDL_1$ tracer in some of the nephrotic group leading to its rapid clearance from the plasma by phagocytic mechanisms. However, there does not appear to be any reason for this to be limited to nephrotic subjects and this has not been seen in normal and

other hyperlipidaemic groups which have been studied in similar investigations.

The results of this study suggest that increased hepatic synthesis of lipoproteins at the level of $VLDL_2$ is a feature of the nephrotic syndrome and this, together with a diminished capacity in some subjects to catabolise IDL and LDL, leads to hypercholesterolaemia. Elevation of plasma triglyceride is associated with high concentrations of the large triglyceride-rich $VLDL_1$ which derives again from a combination of overproduction and a reduced delipidation rate. It should be noted that this group of patients was atypical in some respects with well-maintained serum albumin despite heavy proteinuria. Hypoalbuminaemia has been closely correlated with hyperlipidaemia in this condition. The absence of severe hypoalbuminaemia in our patients is reflected in the relatively modest elevations of plasma and LDL-cholesterol and of LDL apoB pools.

CHAPTER 6 LOW DENSITY LIPOPROTEIN METABOLISM IN NEPHROTIC SYNDROME

6.1 Introduction

The potential role of apoB kinetic studies in the investigation of lipoprotein metabolism was first fully appreciated following the work of Langer et al.(139). These workers identified a decreased fractional catabolic rate of LDL as a consistent feature of familial hypercholesterolaemia(type IIa hyperlipoproteinaemia). Subsequent work established that this was due to genetic defects of the apoB/E receptor and provided a rational explanation for the delayed catabolism of LDL. These initial studies used LDL prepared by sequential ultracentrifugation as the tracer. This approach allows a more direct and accurate determination of LDL kinetics compared to studies which trace apoB through a series of lipoprotein fractions(chapter 5).

A further advantage of labelling LDL directly is the ability to differentiate between the two pathways for LDL catabolism - receptor and non-receptor(36). Modification of the apoB moiety of LDL by 1,2-cyclohexanedione(CHD) alters the arginyl residues of the receptor-binding site of apoB and abolishes ligand-receptor binding. The use of two isotopes(^{125}I and ^{131}I), to label native and chemically-

TABLE 5.1 - VLDL METABOLISM - CLINICAL CHARACTERISTICS OF NEPHROTIC[a] GROUP

subject	sex	age (years)	weight (kg)	body mass index (kg/m^2)	histology	duration of disease (months)	treatment
CM	M	60	77	26.3	FGS[b]	310	nifedipine, metoprolol, ranitidine
AP	M	55	98	30.9	membranous	42	frusemide, atenolol, nifedipine
WS	M	58	75	27.9	membranous	55	atenolol, enalapril, warfarin
JH	M	53	90	29.4	IgA	10	digoxin, bumetanide, warfarin
JG	M	48	57	24.1	membranous	420	nifedipine, atenolol, frusemide
MD	F	28	49	19.4	MCGN[c] II	159	phenobarbitone
RG	M	66	80	25.0	membranous	31	nifedipine
CF	F	34	61	22.6	MCGN II	66	frusemide

a - control group n=5, M:F = 3:2, age 36-46years, weight 71-89kg

b - focal glomerulosclerosis c - mesangiocapillary glomerulonephritis

TABLE 5.2 - VLDL METABOLISM - RENAL FUNCTION TESTS

subject	serum creatinine (umol/lL)	serum albumin (g/L)	creatinine clearance (mL/min/1.73m$^{2)}$	quantitative proteinuria (g/day)
CM	310	33	19	6.6
AP	190	36	35	10.8
WS	160	33	43	6.1
JH	100	38	58	3.3
JG	420[d]	32	18	9.6
MD	90	33	90	6.2
RG	250	27	40	8.3
CF	70	31	89	4.5

Values represent mean of repeated measures over the study period

d - serum creatinine rose from 370 to 450umol/L over the study period

TABLE 5.3 - VLDL METABOLISM - PLASMA LIPID AND LIPOPROTEIN CONCENTRATIONS

subject	E/E phenotype	chol (mmol/L)	trig (mmol/L)	VLDL-chol (mmol/L)	LDL-chol (mmol/L)	HDL-chol (mmol/L)	VLDL-chol:plasma trig ratio	apoB pool[e] size (mg)
nephrotic								
CM	4/3	9.4	4.5	3.0	5.4	1.1	0.67	4306
AP	4/3	8.6	3.5	1.6	6.0	1.0	0.46	5718
WS	3/2	8.6	3.4	3.0	4.8	0.8	0.88	5061
JG	3/3	12.8	3.1	2.1	10.0	0.8	0.68	4848
JH	3/3	8.3	2.8	1.1	6.0	0.9	0.38	3181
MD	3/3	7.3	1.9	0.9	4.9	1.5	0.47	2038
RG	3/3	7.3	1.6	0.6	5.4	1.3	0.38	4796
CF	4/3	7.6	1.2	0.6	5.4	1.7	0.46	2640
controls								
NC	3/3	5.0	1.7	0.8	3.1	1.0	0.46	1970
CD	3/3	6.2	2.6	1.0	4.1	1.4	0.40	2860
MF	3/3	5.8	1.1	0.6	4.0	1.2	0.56	3000
EK	3/3	5.0	1.0	0.4	2.8	1.9	0.37	1450
MM	3/3	5.6	2.2	1.0	3.7	1.2	0.45	2270

e - sum of apo B pools in the four lipoprotein fractions

TABLE 5.4 - COMPOSITION OF APO-B CONTAINING LIPOPROTEIN SUBFRACTIONS

NEHPROTIC SYNDROME v CONTROL

	$VLDL_1$ (S_f 60 - 400)		$VLDL_2$ (S_f 20 - 60)		IDL (S_f 12 - 20)		LDL (S_f 0 - 12)	
	NS	C	NS	C	NS	C	NS	C
free chol	3.5(2.4)	1.5(1.8)	9.4(2.9)	6.4(1.9)	9.5(2.2)	8.9(3.0)	10.5(1.7)	11.6(1.6)
chol ester	16.0(3.2)	16.2(3.2)	24.5(3.4)	22.4(4.3)	35.1(4.0)	35.6(4.7)	37.0(1.4)	37.0(2.0)
triglyceride	56.6(3.3)	57.4(4.1)	32.9(4.5)	36.9(2.9)	16.1(3.7)	14.3(1.7)	6.8(1.5)	6.0(0.9)
phospholipid	15.7(0.7)	15.4(2.6)	20.5(0.5)	20.4(2.3)	21.2(0.7)	22.1(1.7)	20.4(1.3)	22.0(0.5)
protein	8.2(0.6)	9.4(2.0)	12.8(0.8)	14.1(1.4)	18.1(1.2)	19.0(1.7)	25.4(2.7)	23.4(1.4)

Values are mean(sd) % composition by weight. Summed values may not equal 100% due to rounding.

NS - nephrotic n=8; C - control n=5

TABLE 5.5a - KINETIC PARAMETERS OF APO B METABOLISM IN $VLDL_1$

subject	production	pool size	fractional catabolism	
			direct	to $VLDL_2$
nephrotic	(mg/kg/day	(mg)	(pools/day)	
CM	25.3	413	4.54	0.22
AP	11.9	297	3.11	0.85
WS	7.8	210	1.74	1.02
JG	64.8	117	30.6	1.05
JH	2.8	195	0.58	0.74
MD	34.5	83	19.5	0.70
RG	23.0	87	20.1	1.01
CF	2.6	46	0.04	3.33
median	17.5	156	3.83	0.93
controls				
NC	8.8	67	4.17	5.16
CD	6.1	52	5.75	4.49
MF	14.5	97	10.8	2.48
EK	11.5	47	14.7	2.71
MM	14.7	106	6.91	3.66
median	11.5	67	6.91	3.66
p	ns	ns	ns	$p<0.02$

TABLE 5.5b - KINETIC PARAMETERS OF APO B METABOLISM IN $VLDL_2$

subject	production		pool size	fractional catabolism	
	direct	from $VLDL_1$		direct	to IDL
nephrotic	(mg/kg/day)		(mg)	(pools/day)	
CM	14.6	1.2	642	0.44	1.43
AP	15.7	2.6	714	1.40	1.11
WS	14.5	2.9	570	0.03	2.26
JG	11.2	2.2	488	0.0	1.54
JH	5.1	1.6	336	0.61	1.18
MD	32.2	1.2	162	8.44	1.66
RG	17.3	1.1	505	1.55	1.36
CF	9.8	2.5	233	0.12	3.12
median	14.6	1.9	497	0.53	1.49
controls					
NC	4.2	4.9	160	0.18	3.88
CD	8.8	2.7	231	2.14	2.14
MF	5.6	2.7	199	0.92	2.81
EK	2.9	1.8	69	2.14	2.74
MM	3.7	5.1	218	1.15	1.93
median	4.2	2.7	199	1.15	2.74
p	$p<0.02$	$p<0.05$	$p<0.02$	ns	$p<0.05$

TABLE 5.5c - KINETIC PARAMETERS OF APO B METABOLISM IN IDL

subject	production from $VLDL_2$	pool size	fractional catabolism direct	fractional catabolism to IDL
nephrotic	(mg/kg/day)	(mg)	(pools/day)	
CM	11.9	730	0.02	1.2
AP	8.1	1131	0.01	0.69
WS	17.2	1122	0.58	0.56
JG	12.0	595	0.09	1.15
JH	4.4	554	0.0	0.71
MD	5.5	322	0.08	0.75
RG	8.5	754	0.02	0.88
CF	11.7	527	0.21	1.13
median	10.4	663	0.05	0.82
controls				
NC	8.7	241	0.76	1.80
CD	5.8	408	0.18	1.02
MF	5.8	358	0.26	1.17
EK	2.7	174	0.37	0.68
MM	5.1	351	0.33	0.76
median	5.8	351	0.33	1.02
p	ns	$p<0.05$	$p<0.05$	ns

TABLE 5.5d - KINETIC PARAMETERS OF APO B METABOLISM IN LDL

subject	production		pool size	fractional catabolism	total apo B production in
	direct	from $VLDL_2$ and IDL			$VLDL_1$ + $VLDL_2$ + LDL
nephrotic	(mg/kg/day)		(mg)	(pools/day)	(mg/kg/day)
CM	4.8	11.9	2623	0.49	44.8
AP	0.0	8.0	3617	0.22	27.6
WS	4.7	8.4	3187	0.32	27.2
JG	0.0	12.4	3426	0.21	76.1
JH	0.0	4.6	2247	0.18	8.0
MD	0.0	4.9	1315	0.18	66.7
RG	0.0	8.4	3129	0.22	40.3
CF	0.0	10.0	1842	0.33	12.3
median	0.0	8.4	2876	0.22	33.9
controls					
NC	0.0	6.1	1433	0.30	11.4
CD	2.7	4.9	2505	0.26	13.7
MF	0.1	5.2	2044	0.23	13.4
EK	2.6	1.7	1160	0.26	14.3
MM	2.0	4.0	1620	0.28	14.9
median	2.0	4.7	1620	0.26	13.7
p	ns	ns	ns	ns	ns

TABLE 5.6a - RATE CONSTANTS k(i,j) IN POOLS/DAY FOR CATABOLISM AND TRANSFER

subject	(0,1)	(1,29)	(2,1)	(0,26)	(4,2)	(6,2)	(0,6)	(0,4)	(13,4)	(20,4)
CM	0	24.0	4.73	4.80	1.21	0.06	0.28	14.7	24.0	0
AP	3.6	29.1	0.98	1.22	1.63	0.43	0.71	1.15	1.07	0
WS	2.66	34.5	2.50	1.23	0.94	0.08	0.60	0	1.04	0
JG	0	24.0	5.59	39.7	0.58	0	0	0	24.0	8.72
JH	2.82	1.65	3.59	13.34	2.59	0.49	0.53	0.78	1.39	0.09
MD	2.37	36.0	4.13	24.0	2.31	0.41	0.95	8.06	4.78	0
RG	0	29.0	8.03	24.0	2.07	0.22	0.39	7.75	3.62	1.36
CF	0.31	4.06	21.8	36.0	2.53	0.05	0.60	1.67	7.85	0.98
median	1.34	26.5	4.43	18.7	1.85	0.15	0.57	1.41	4.2	0.05
controls										
NC	4.17		5.16	0	6.22	0.03	0.56	0	5.76	0
CD	5.75		4.49	0	7.16	0.02	0.41	1.92	2.22	0
MF	10.8		2.48	0	6.96	0.13	0.76	0	3.14	0.68
EK	14.7		2.71	0	7.03	0.10	0.90	0.33	10.4	0
MM	6.91		3.66	0	8.92	0.09	0.63	1.63	2.19	0.08
median	6.91		3.66	0	7.03	0.09	0.63	0.33	3.14	0

See figure 5.2 for compartment numbers and routes of metabolism between compartments.

TABLE 5.6b - RATE CONSTANTS k(i,j) IN POOLS/DAY FOR CATABOLISM AND TRANSFER(continued)

subject	(17.4)	(0,12)	(15,12)	(21,12)	(0,15)	(21,15)	(0,13)	(20,13)	(0,17)	(0,21)	(0,20)
CM	0	0.45	1.57	0	0	1.31	0.24	0.61	0.17	0.50	0.33
AP	0	1.97	1.63	0	0	0.72	0	0.54	9.93	0.21	0.32
WS	0	0	7.72	0	0.62	0.61	0.44	0.39	0./20	0.31	0.35
JG	24.0	0	2.33	0	0	1.40	0.01	0.41	2.66	0.21	0.21
JH	0	0.72	1.66	0.07	0	0.75	0	0.57	0.39	0.17	0.29
MD	0	10.4	1.97	0	0.09	0.80	0.06	0.40	0	0.18	0.29
RG	0	1.65	1.49	0	0	0.94	0.30	0.10	0	0.22	0.16
CF	0	0	3.89	0	0.23	1.98	0.19	0.25	0.70	0.37	0.19
median	0	0.59	1.82	0	0.01	0.87	0.13	0.40	0.30	0.21	0.29
controls											
NC	0.90	0.56	5.45	0	0	4.09	1.65	2.04	0.52	0.42	0.21
CD	0.60	2.72	2.42	0	0	1.39	0.80	1.10	0.32	0.30	0.15
MF	0	1.87	3.23	0	0	1.58	0.61	0.70	0.37	0.28	0.16
EK	0	3.65	1.56	0	0	0.80	1.21	1.39	0.24	0.28	0.25
MM	1.26	1.24	2.37	0	0	1.44	0.49	0.44	0.38	0.32	0.23
median	0.60	1.87	2.42	0	0	1.44	0.80	1.10	0.37	0.30	0.21

TABLE 5.7 - CALCULATED COMPARTMENTAL MASSES AND INPUTS

subject	M(1)	M(26)	M(29)	M(2)	M(4)	M(6)	M(12)	M(13)	M(15)	M(17)	M(20)	M(21)	U(26)	U(29/1)	U(12)
CM	19	391	3	70	2	16	555	62	668	0	115	1743	1877	72	1121
AP	256	1	40	122	90	74	428	176	955	0	305	3312	1	1166	1541
WS	86	111	13	210	190	29	141	238	883	0	263	1756	134	446	1090
JG	22	90	5	212	2	0	274	121	455	19	325	3101	3577	120	638
JH	40	0	155	46	53	43	194	128	426	0	265	1982	0	256	462
MD	14	66	3	22	4	9	127	41	281	0	57	1258	1584	108	1576
RG	11	73	3	37	6	21	441	54	700	0	85	3044	1752	87	1385
CF	7	0	39	60	15	5	153	258	269	0	405	1437	0	158	595
median	21	70	9	65	11	19	234	125	562	0	264	2716	859	139	1106
control															
NC	67	0	0	56	52	3	50	81	66	94	793	640	0	625	301
CD	51	0	0	32	48	3	148	56	256	95	417	1206	0	5232	761
MF	97	0	0	34	62	6	98	148	199	11	920	1124	0	1288	500
EK	47	0	0	18	11	6	39	42	76	56	231	219	0	1120	203
MM	106	0	0	43	99	2	70	231	114	5	485	606	0	819	280
median	67	0	0	34	52	3	70	81	76	56	485	640	0	819	301

a. Minor discrepancies in flux rates in and out of compartments are due to the effect of rounding up or down the rate constants and compartment masses.

b. U29 and U1 are equivalent inputs into the system. The control subjects did not require an extra delipidation step and apoB production was considered to occur directly into compartment 1 rather than 29.

FIGURE 5.1a
EXAMPLE OF APOB RADIOACTIVITY CURVES DERIVED FROM ^{131}I VLDL$_1$ TRACER

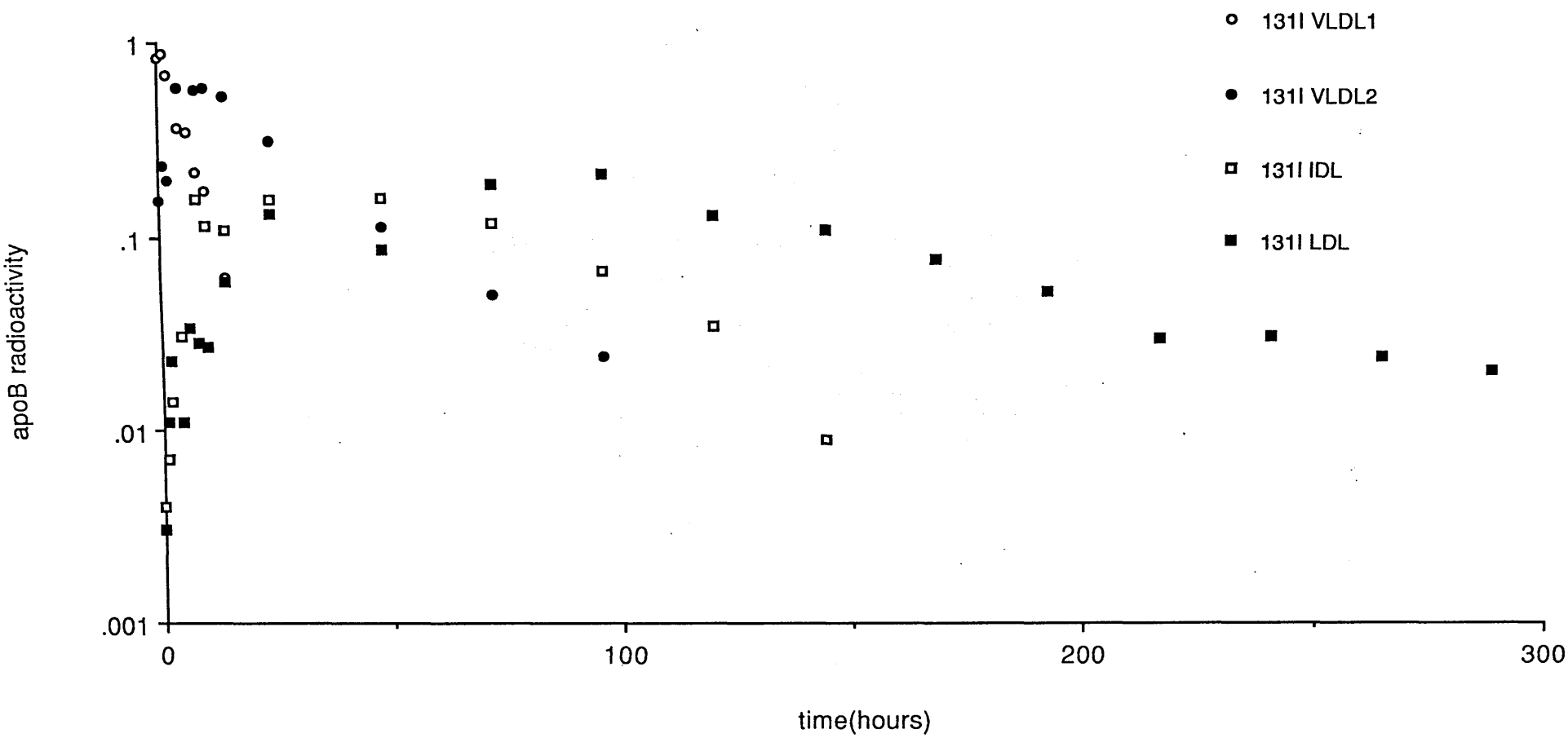
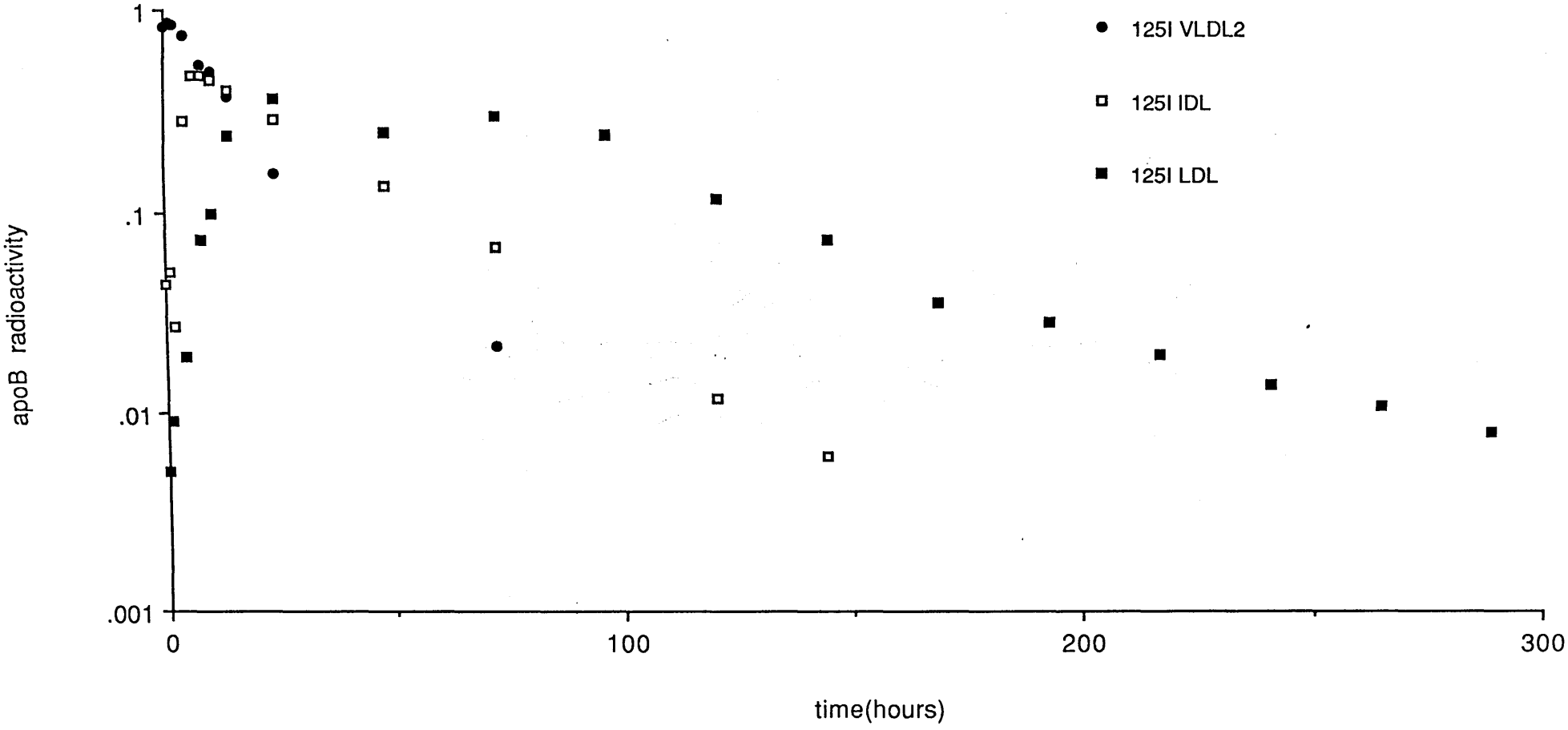


FIGURE 5.1b
EXAMPLE OF APOB RADIOACTIVITY CURVES DERIVED FROM ^{125}I VLDL$_2$ TRACER



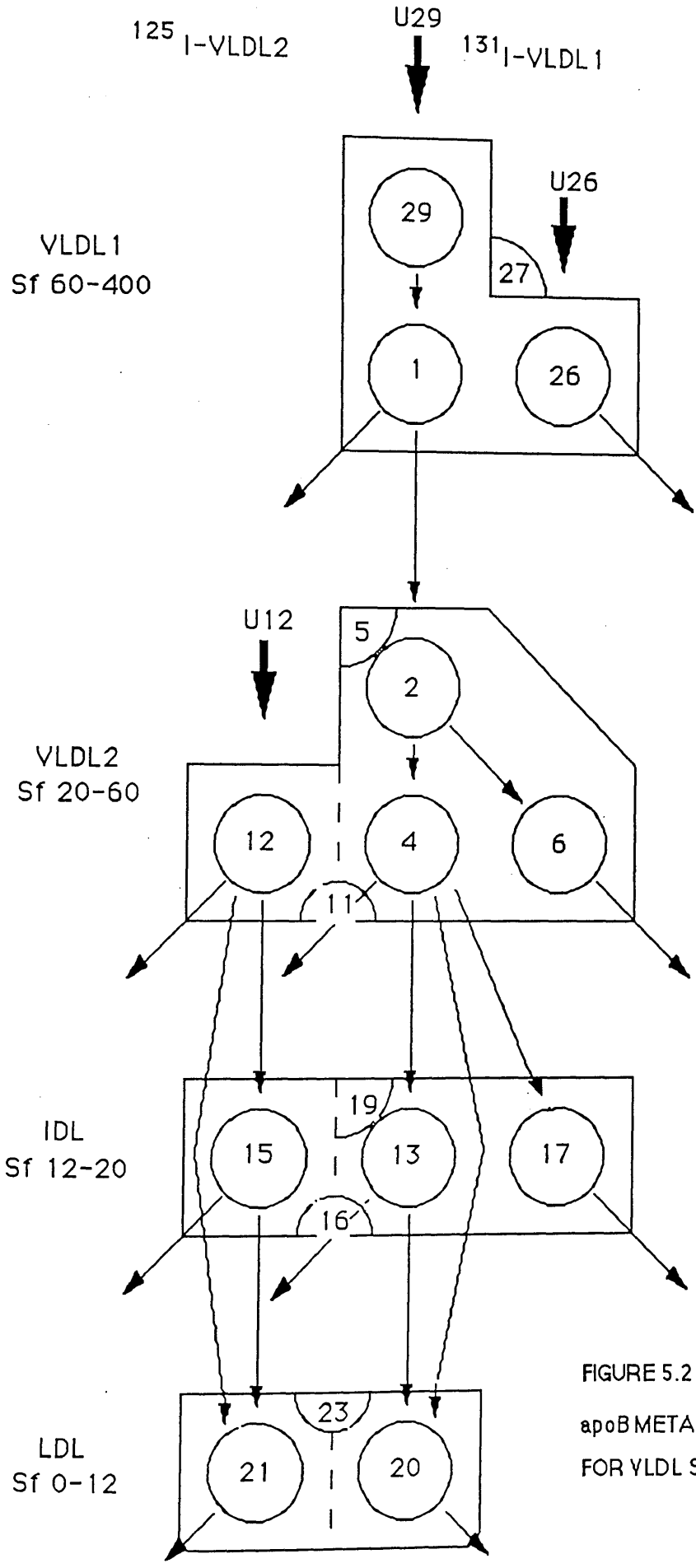


FIGURE 5.2-MODEL OF apoB METABOLISM USED FOR VLDL STUDIES

FIGURE 5.3 - PLASMA CONCENTRATIONS OF LIPOPROTEIN SUBFRACTIONS

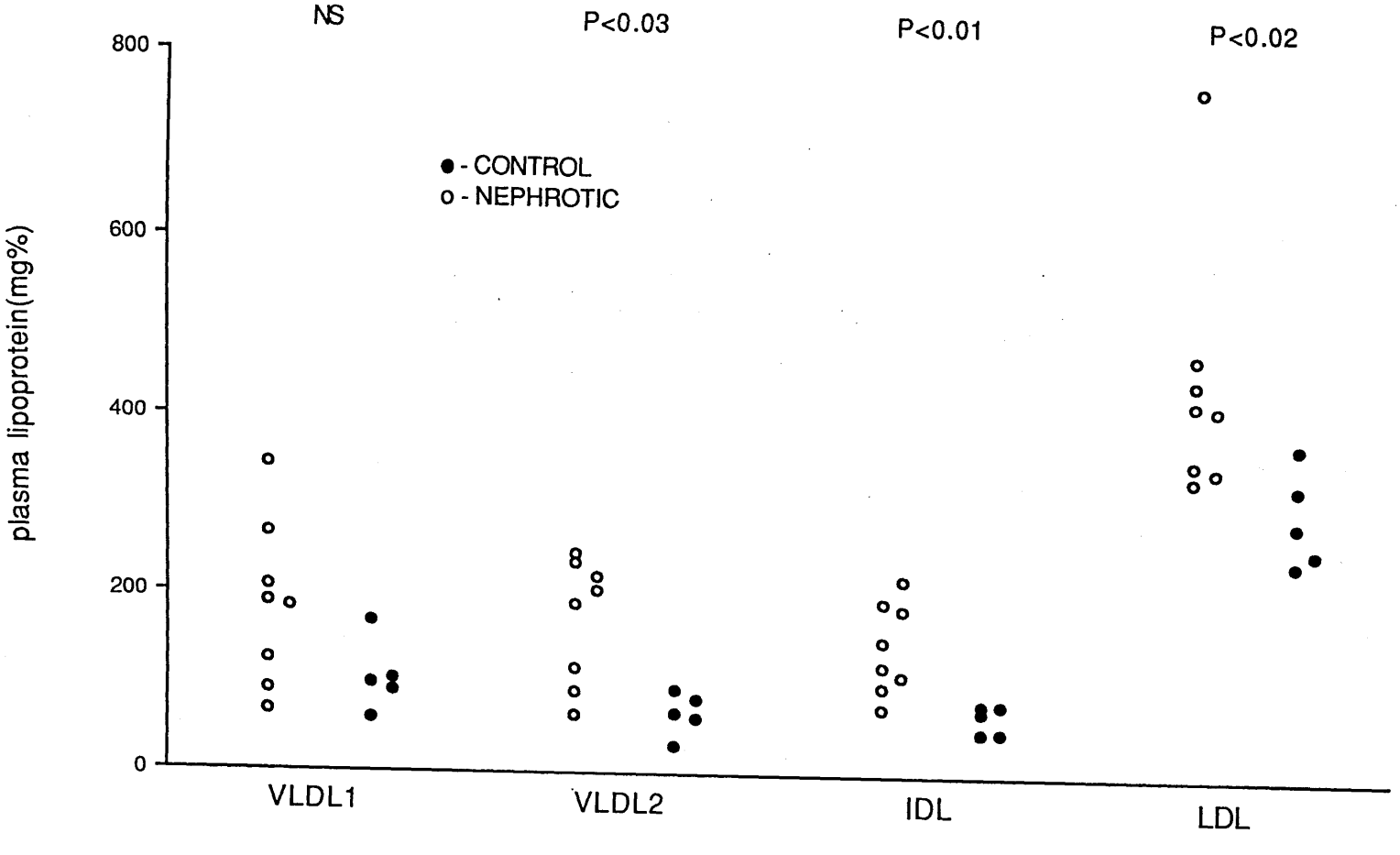


FIGURE 5.4 - MEAN apoB SPECIFIC ACTIVITY CURVES

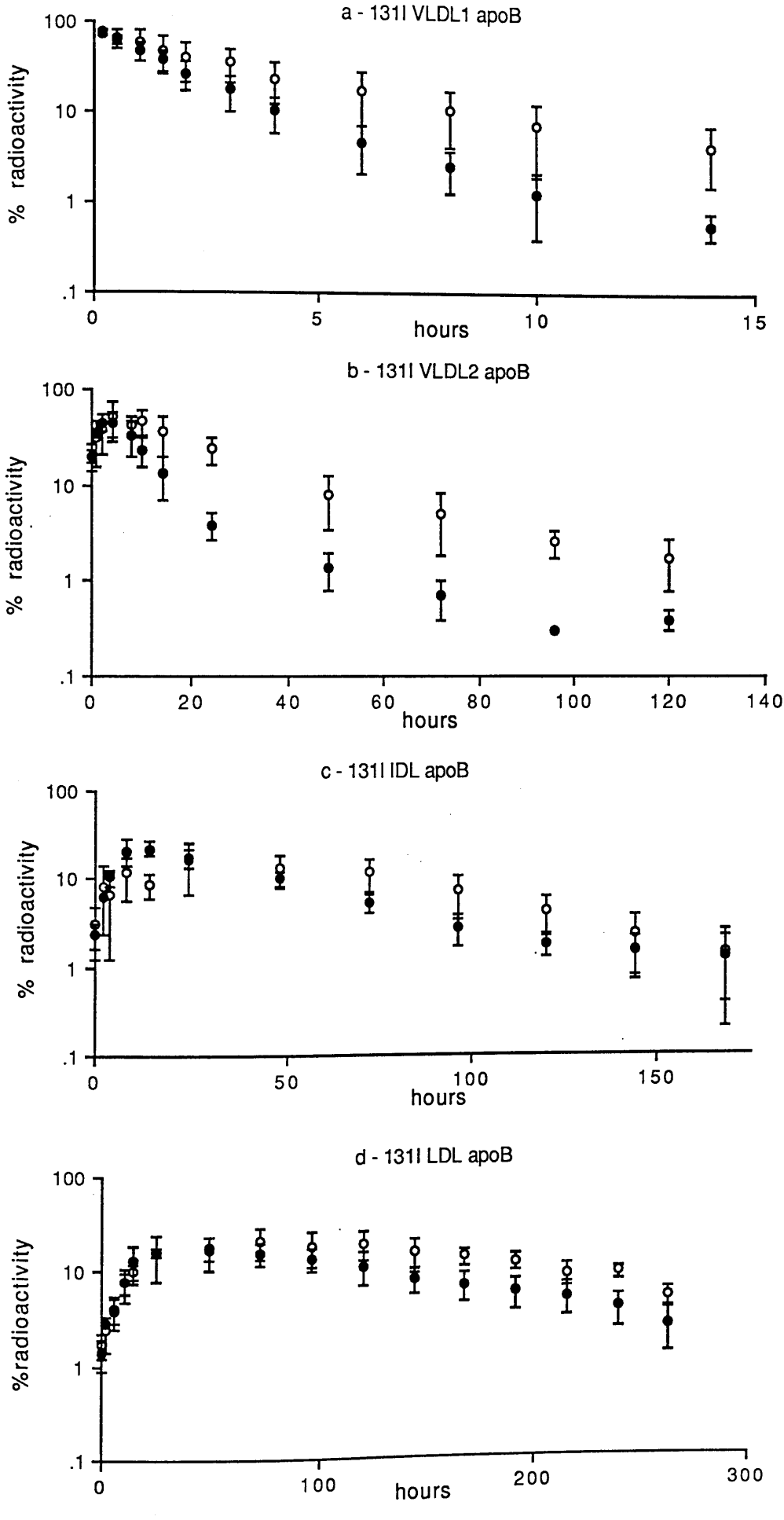
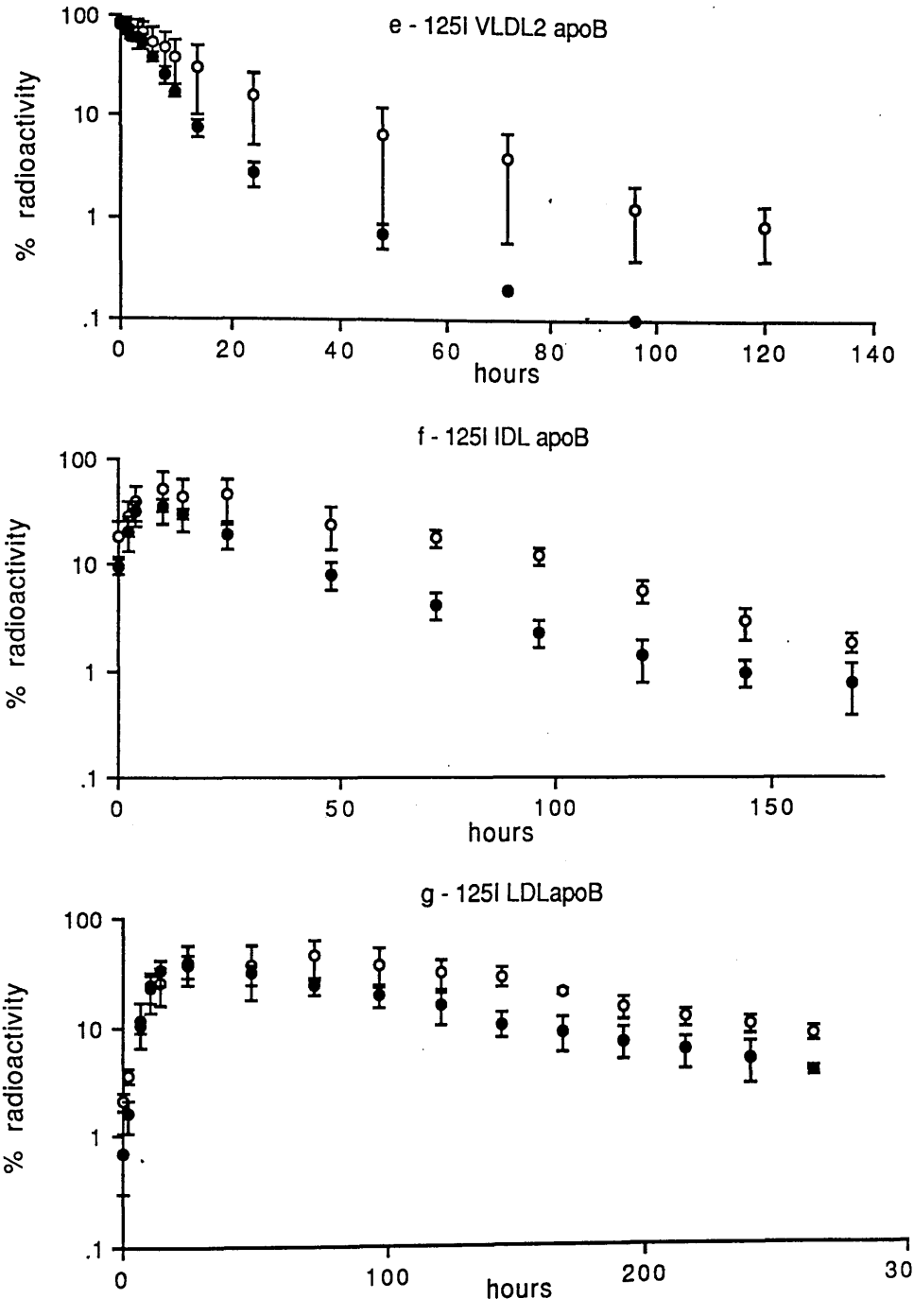


FIGURE 5.4(cont) - MEAN apoB SPECIFIC ACTIVITY CURVES



Nephrotic subjects are shown by open circles(o); controls by closed circles(●)

modified LDL respectively, permits the estimation of total and receptor-independent catabolism, the difference being a measure of receptor-mediated catabolism. This method has been validated in animal studies and in man(138,174).

The aim of this study was to investigate in more detail the metabolism of LDL in nephrotic syndrome and, specifically, to determine the relative contributions of these two catabolic pathways.

6.2 Subjects and methods

Low density lipoproteins were isolated from approximately 40mL of fasting plasma by rate zonal ultracentrifugation(5) in a Beckman Ti14 rotor using a linear gradient of density range 1.0-1.3g/mL. The LDL peak was collected in 2-3 x 15mL fractions and concentrated and desalted in a pressurised Amicon ultrafiltration system(source: Amicon, Stonehouse, Gloucester, UK) using an XM300 membrane(nominal MW cut-off 300,000d) and 0.15M NaCl/0.01% w/v Na_2EDTA/pH 8.1 as buffer. The final concentration of LDL protein was 1-3mg/mL(estimated by absorption of UV light at 280nm assuming an extinction coefficient of 1.0). Aliquots were then labelled with ^{125}I and ^{131}I(140,171) and unbound iodide and glycine buffer removed by gel filtration on a PD10 column(source: Pharmacia, Uppsala, Sweden). The ^{131}I tracer was modified with 1,2-cyclohexanedione(CHD). A

freshly prepared solution of CHD(0.15M in 0.2M borate buffer, pH 8.1) was incubated with an equal volume of ^{131}I-LDL for 2 hours at 35^{o}C. Free CHD was then removed by gel filtration on a PD10 column as above.

The radiolabelled tracers were administered intravenously to their respective donors approximately 8 hours after the initial blood sample. Immediately before injection, the labelled LDL was sterilised by membrane filtration through a 0.22micrometre filter(source: Gelman Science, Northampton, UK) and the activity in a 10uL sample was counted. Each subject received approximately 25uCi ^{125}I LDL and 25-50uCi of ^{131}I CHD-LDL as an intravenous bolus. The precise dose was calculated from the difference in the weight of the syringes before and after each injection. A blood sample was drawn at ten minutes from the opposite arm and then daily fasting samples were obtained between 08:00 and 09:00 for 14 days. Twenty four hour urine samples were collected on a daily basis. Patients were assumed to be in a steady state with respect to low density lipoprotein metabolism throughout the study and this was confirmed by repeated measurements of plasma lipids and lipoprotein cholesterol.

LDL was isolated by sequential flotation between 1.019-1.063g/mL on 3-4 occasions and the protein content used to calculate the plasma apoLDL concentration(>95% apoB). The

pool size of apoLDL was calculated as the product of apoLDL concentration and plasma volume. The latter was derived from the dilution of the ^{125}I-LDL tracer at 10 minutes.

At the end of the study, the radioactivity in 2mL of plasma from each time point was measured in a twin-channel gamma counter. Previous studies have shown that >95% of the plasma radioactivity is in the LDL density range and this was confirmed by re-isolating LDL from plasma by rate zonal ultracentrifugation(figure 2.1). The distribution of radioactivity and protein(measured by absorption at 280nm) in the LDL density region are very similar. Correction was made for spill over from the ^{131}I to the ^{125}I channel. Two mL samples of urine were also counted and total urinary radioiodide losses calculated from the product of cpm/mL and daily urine volume. In proteinuric subjects, correction was made for urinary losses of protein-bound radioactivity which was assumed to be LDL. Two mL of urine were added to 1.33mL of cold 50% trichloroacetic acid(TCA) and stood at 4^{0}C for 1 hour. The precipitate was separated at low-speed centrifugation, washed once with 20% TCA and the radioactivity in the precipitate measured. No significant protein-bound radioactivity was found in normal subjects.

Kinetic analysis

Decay curves of plasma radioactivity versus time were drawn for both tracers. The fraction of total radioactivity administered which was excreted per day in the urine was also plotted against time and the daily urine:plasma radioactivity ratio calculated. The data(plasma decay curves, urine radioactivities and apoLDL pool sizes) were analysed by multicompartmental modelling to obtain fractional catabolic rates for both tracers(142,143). The model utilised for this analysis is shown in figure 6.1 with parallel pathways for native and modified LDL. The shape of the plasma decay curves(figure 6.2a) suggests a biexponential decay. This is interpreted as a function of two LDL pools. One represents the intravascular apoLDL pool and tissue spaces in rapid equilibration with this pool. The other comprises slowly exchanging material in extravascular spaces. LDL is removed only from the plasma compartment and the free iodide generated by catabolism of LDL is excreted into the urine at a fixed rate of 2.5pools/day. The fractional catabolic rates(pools/day) for the native and modified LDL are given by rate constants L(3,1) and L(7,5) respectively. The difference between the fractional catabolic rates represents receptor-mediated catabolism. FCR's were adjusted for the urinary losses of TCA-precipitable radioactivity. Fractional catabolic rates can also be calculated by the daily plasma:urine ratio as

shown in figure 6.2b. Absolute turnover(or production rates in a steady-state system) were calculated as the product of apoLDL pool size and FCR and are expressed per kg body weight(mg apoLDL/kg/day).

There are a number of underlying assumptions in this model:-

(a) Catabolism is assumed to occur from, or close to, the intravascular compartment only. This is an over-simplification although the contribution of extravascular routes is probably small.

(b) LDL is considered to be homogeneous and this ignores variation due to the presence of specific LDL subfractions with differing metabolic fates(175).

(c) Although previous studies have shown that the CHD-modified LDL appears to be stable in vivo(138) some investigators have questioned this(176).

Nine patients with nephrotic syndrome were studied using this technique. The clinical characteristics of this group are shown in tables 6.1 and 6.2. Kinetic data were compared with a group of eight normal volunteers selected from laboratory and clinical staff.

Statistical analysis

Results of compositional analysis and kinetic parameters

(fractional catabolic and production rates) were compared by two sample t tests. Relationships between plasma lipids, production rates of apoLDL and proteinuria and hypoalbuminaemia were examined by linear regression.

6.3 Results

Plasma lipid and lipoprotein cholesterol levels

This group of patients illustrated the typical abnormalities of lipids and lipoproteins in the nephrotic syndrome(table 6.2). Total plasma cholesterol was elevated between 7.5 and 16.7mmol/L. This was mainly due to an elevation of LDL-cholesterol levels(4.6 to 12.9mmol/L) although several patients also had marked increases in VLDL-cholesterol(DE,MJ,JB,EA). This reflected moderate hypertriglyceridaemia in these patients with EA also demonstrating a very high VLDL-cholesterol:plasma triglyceride ratio suggesting accumulation of cholesterol ester-rich remnant particles. HDL-cholesterol concentrations and were slightly reduced compared to the control group. There were significant associations between daily protein loss and the three plasma lipid measures - total cholesterol(r^2=81%, $p<0.0005$), LDL=cholesterol (r^2=59%, $p<0.002$) and triglyceride(r^2=52%, $p<0.01$). No such relationships were seen with serum albumin.

Compositional analysis of LDL(d=1.019-1.063g/mL) isolated by sequential ultracentrifugation demonstrated a modest decrease in free cholesterol content(nephrotic 6.9% sd 2.1% v control 12.1% sd 1.2%, $p<0.001$) and a rise in triglyceride content(nephrotic 11.4% sd 3.1% v control 7.8% sd 1.2%, $p<0.01$). The plasma apoLDL concentration was markedly increased in the nephrotic group with a mean value of 251mg/dL(range 118-427) compared to a mean value of 93mg/dL in the control group.

Kinetic analysis

The results for the kinetic parameters are listed in table 6.3. In the nephrotic group, there was a significant reduction in the fractional catabolic rate(FCR) of the native ^{125}I-LDL compared to the control group(0.219 sd 0.061 v 0.384 sd 0.057 pools/day, $p<0.001$). There was only a slight reduction in the clearance of the modified LDL (0.169 sd 0.043 v 0.219 sd 0.039, $p<0.05$). The main difference in the clearance rate is therefore due to decreased catabolism by the receptor-mediated pathway. The mean value was reduced by an average of 70% in the nephrotic group. The values for the fractional catabolic rates were corrected for the proportion of urinary iodide present as protein-bound radioactivity(i.e. TCA-precipitable). This was assumed to be intact LDL as there is negligible exchange of radioiodide in plasma and this

was thought to be unlikely to occur in the urine. This figure averaged 6.9% and varied from 1.2-15.7%(calculated from the mean daily value for both tracers). There was considerable day-to-day variation within individuals. There was no difference between urinary losses of native or chemically-modified LDL.

The absolute turnover rate(or production rate) was elevated in the nephrotic group(20.7mg/kg/day sd 8.3 v 14.2 sd 2.3, $p<0.02$) but the range was wide. There was a striking correlation between quantitative proteinuria and apoLDL turnover(figure 6.3) but only where proteinuria exceeded 10g/day did turnover exceed normal values.

6.4 Discussion

These results indicate that plasma LDL concentrations are elevated in nephrotic syndrome due to a combination of decreased catabolism and increased synthesis. There is a consistent defect in the removal of apoLDL(and therefore LDL particles) from the intravascular compartment in the nephrotic syndrome. This was almost entirely due to a reduction in the fraction of the apoLDL pool catabolised by the receptor-mediated pathway. In contrast, although apoLDL turnover rates were slightly raised overall, inspection of the individual results and the relationship between production rates and quantitative proteinuria

suggest that synthesis was only increased above normal when proteinuria exceeded 10g/day. Thus, the main cause of the expanded apoLDL pool is a reduction in catabolism although in some subjects increased synthesis also contributes.

The results of this study and those described in the previous chapter can be compared. Although there was a slight reduction in the FCR for IDL and LDL in the VLDL apoB kinetic studies, this was not statistically significant. There are three possible explanations. Firstly, the fractional catabolic rate for LDL apoB derived from VLDL apoB isolated by cumulative flotation ultracentrifugation is consistently lower than for apoLDL prepared by rate zonal or sequential ultracentrifugation and labelled directly(25,26). This reflects an accumulation of errors in the calculation of LDL kinetics in the former studies and the inclusion of IDL particles in tracers used in the latter. Nephrotic LDL prepared by sequential flotation ultracentrifugation(d=1.019-1.063g/mL) had a higher triglyceride content(11.4%) compared to that isolated by DGUC(6.8%) - table 5.4. This is due to the higher plasma triglyceride concentrations in the study group in this chapter and the methodological differences in the ultracentrifugation procedures. Secondly, the FCR's in chapter 5 were not corrected for urinary lipoprotein loss. Finally, and most importantly, the group of patients studied in chapter 5 were less severely nephrotic than the

current group who have lower serum albumin concentrations and higher LDL-cholesterol levels. An increase in severity of the disease may lead to a greater fall in LDL FCR with a further rise in plasma cholesterol.

The relationship between LDL pool size and fractional catabolic rate is controversial. The evidence supports the contention that apoLDL pool size is a function of the FCR and that reduction of FCR in hypercholesterolaemia does not simply represent saturation of catabolic pathways. Langer et al.(139) first argued this on the basis that familial hypercholesterolaemia could not be ascribed to increased LDL synthesis since this was normal in their studies. The identification of the LDL receptor defect further confirmed that the primary metabolic problem was in LDL catabolism. The effect of acute reduction in pool size on catabolism of apoLDL was studied by Thomson(177) who performed plasmapheresis on two subjects(one normal and one homozygous FH) during LDL turnover studies. The slope of the plasma decay curve and the urine:plasma ratio remained constant suggesting the fractional catabolic rate was independent of acute changes in pool size. For these reasons, the LDL FCR is generally held to be an important determinant of the apoLDL pool size which is not influenced by acute changes in pool size. However, measures of LDL catabolism, even where supplemented by modified LDL tracers to quantify receptor-independent catabolism, can only

indirectly assess LDL receptor activity. More precise information regarding receptor numbers and degree of saturation can only be obtained by documenting changes in FCR following alterations in pool size where receptors numbers are kept constant(178). Since most perturbations of LDL pool size will automatically alter receptor numbers(e.g. treatment with bile acid-binding resins), these conditions are difficult to achieve in vivo.

Until recently there have been few published studies on the kinetic characteristics of LDL particles in the nephrotic syndrome. Early work by Gitlin and colleagues(66) showed delayed clearance of a "low density lipoprotein". However, the tracer used covered a wide density range(S_f 10-100 with a preponderance of particles of S_f 20-60) which would now be classified as VLDL or IDL particles. In addition, the preparation of the tracer, involving dextran precipitation of the apoB-containing lipoproteins and their subsequent dissociation by centrifugation, may have led to changes which could influence tracer-tracee relationships. In 1970, Scott and colleagues(67) reported LDL FCR's for nephrotic subjects which were marginally but not significantly lower than controls(mean values - nephrotic 0.227 pools/day v controls 0.308). These values are similar to those reported in this study with the exception that the FCR for the control group is lower in Scott's series. The reason for this discrepancy is not clear

although again methodological differences make direct comparisons difficult. Scott's tracer, partly prepared by precipitation steps, was isolated over a wider density cut than in the current investigation and contained a relatively high proportion of free iodide(up to 9%). In the current study, the young age of the control group may have exaggerated the differences between the FCR's of the two groups(179) but the mean value of 0.384pools/day is similar to the value of 0.371pools/day derived from a review of LDL turnover studies in normal subjects(180).

Two recent studies(48,69) have also examined LDL metabolism in the nephrotic syndrome in man. In a group of 4 subjects (69), the mean FCR of LDL apoB was similar to that of a group of historical controls(0.28 v 0.30pools/day) but there was a significant increase in the production rate (mean values 18.6 v 13.5mg/kg/day - similar to values in table 6.3). Joven *et al*.(48) also found no significant reduction in LDL FCR in 6 nephrotic subjects although the mean value was lower than control(0.294 v 0.330pools/day). However, LDL production rates were more than twice the control values and repeat turnovers in 3 patients following remission of proteinuria showed normal LDL apoB pool sizes, no change in FCR's and normal production rates. The same workers have reported similar results in a rat model of nephrotic syndrome induced by puromycin aminonucleoside (65). In contrast, calculation of apoLDL production rate

by a different technique involving the rate of recovery of steady state plasma LDL concentrations after LDL apheresis did not show any evidence of increased LDL synthesis(181).

The differences between these studies and the current investigation are difficult to explain. The characteristics of the patients studied and the methodologies used are similar. None of these studies corrected for urinary losses of intact lipoprotein particles and, although this may have led to an overestimate of the true LDL FCR, this would not be of sufficient magnitude to explain the differences. Inter-individual variation, perhaps due to dietary or genetic influences, may account for these differences since even in the results reported here there was a wide range of FCR's (0.140-0.325pools/day) in the nephrotic group. However, the present investigation is the only study to examine the relative contribution of the two LDL catabolic pathways in nephrotic syndrome and the consistent reduction in receptor-mediated FCR suggests this is a significant factor in the genesis of the hypercholesterolaemia in this condition.

There are several plausible explanations for a reduced LDL receptor clearance of LDL in the nephrotic syndrome. Studies of mevalonate metabolism in nephrotic rats have demonstrated reduced clearance of mevalonate through the

renal shunt mechanism(73). Increased availability of mevalonate may stimulate intracellular cholesterol synthesis with subsequent down-regulation of LDL receptors. A number of studies of experimental nephrotic syndrome have documented increased hepatic cholesterol synthesis(55,59). Another possibility is an alteration in the characteristics of the ligand. In vitro studies of LDL from subjects with renal failure have shown decreased binding and degradation by normal cultured fibroblasts(182). Although no similar studies have been reported for nephrotic subjects, LDL isolated from hypertriglyceridaemic patients has been shown in vitro to bind less well to LDL receptors(183).

TABLE 6.1 LDL METABOLISM - PATIENT CHARACTERISTICS 1 - CLINICAL DETAILS

subject	sex	age (yrs)	weight (kg)	BMI (kg/m^2)	renal histology	follow-up(yrs)	treatment
RG	M	54	77	24.0	membranous	1	bendrofluazide
JJ	M	51	71	23.2	FSGS	6	frusemide, enalapril
MM	F	55	70	28.8	MCGN type II	7	frusemide, enalapril
BT	M	52	59	19.7	membranous	1	frusemide
RM	M	30	107	31.8	membranous	0.5	none
DE	M	53	70-	25.1	membranous	1	frusemide, nifedipine, spironolactone
MJ	F	65	49	22.7	membranous	1	frusemide
EA	M	66	96	39.4	membranous	1	frusemide, isosorbide
JB	M	30	63	20.9	membranous	1	frusemide
mean(SD)		51(13)	74(18)				
controls	all M n=8	26(6)	71(12)				

Abbreviations: BMI - body mass index; FSGS - focal segmental glomerulosclerosis; MCGN - mesangiocapillary glomerulonephritis

TABLE 6.2 LDL METABOLISM - PATIENT CHARACTERISTICS 2 - BIOCHEMICAL PARAMETERS

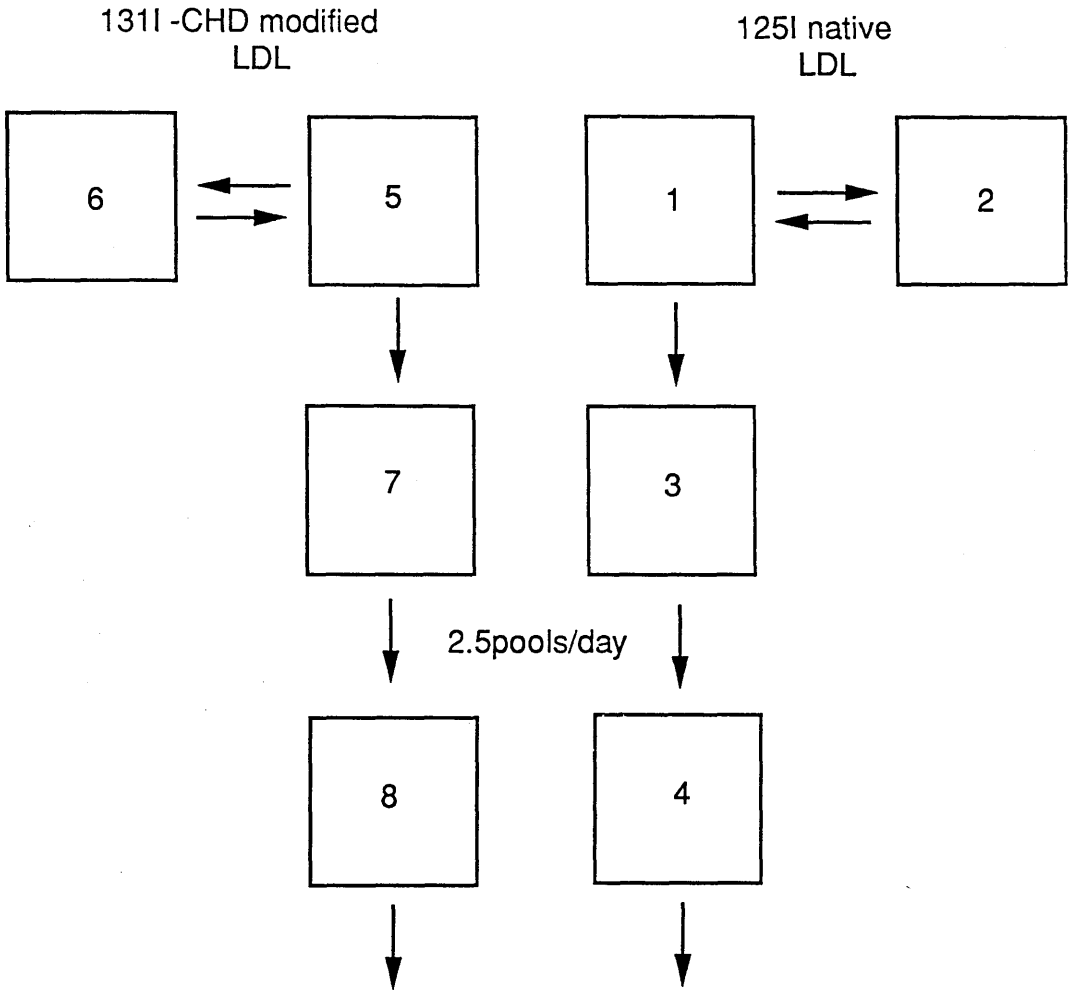
subject	serum creatinine (umol/L)	creatinine clearance (mL/min/1.73m2)	serum albumin (g/L)	quant. proteinuria (g/day)	plasma chol (mmol/L)	plasma trig (mmol/L)	VLDL chol (mmol/L)	LDL chol (mmol/L)	HDL chol (mmol/L)
RG	122(9.5)	86(5.7)	23	6.1(1.0)	8.5(0.2)	2.1(0.1)	1.1(0.1)	6.3(0.2)	1.2(0.1)
JJ	224(8.4)	39(3.9)	25	5.2(1.3)	7.5(1.00	3.1(0.7)	1.8(0.6)	4.8(1.3)	0.9(0.1)
MM	123(10.0)	45(6.0)	30	6.6(1.3)	8.4(0.4)	2.2(0.1)	1.1(0.1)	6.1(0.4)	1.3(0.2)
BT	106(8.5)	63(11.0)	25	5.4(1.5)	10.3(0.2)	2.9(0.5)	1.4(0.3)	7.5(0.4)	1.5(0.1)
RM	107(4.2)	115(18.50	34	9.7(3.4)	9.4(0.1)	2.2(0.1)	0.9(0.2)	7.0(0.3)	1.4(0.1)
DE	95(9.3)	84(14.0)	20	15.3(4.30	14.1(0.7)	4.3(0.5)	2.5(0.9)	10.2(0.6)	0.9(0.1)
MJ	239(14.0)	17.6(2.1)	18	11.9(2.1)	12.1(0.4)	5.5(0.8)	2.8(0.4)	8.5(0.7)	0.8(0.1)
EA	127(7.2)	75(8.0)	28	12.0(2.3)	9.6(0.4)	5.6(0.2)	4.2(0.2)	4.6(0.30	0.7(0.1)
JB	102(4.6)	85(5.6)	21	18.0(6.0)	16.7(1.8)	4.9(0.6)	2.9(0.9)	12.9(1.1)	0.9(0.20
mean(SD)	138(54)	68(29.7)	25(5)	10.0(4.6)	10.7(3.0)	3.6(1.4)	2.1(1.1)	7.5(2.7)	1.1(0.3)
controls	100(5.7)	102(7.1)			5.2(1.0)	1.2(0.5)	0.6(0.2)	3.1(0.6)	1.6(0.3)

Values are mean(sd) calculated from repeated measures(>3) over the study period except albumin which was only measured at the start.

TABLE 6.3 KINETIC PARAMETERS OF APOLDL TURNOVER

	plasma apoLDL (mg/dL)	% urinary loss	fractional catabolic rate total (pools/day)	fractional catabolic rate receptor independent (pools/day)	fractional catabolic rate receptor mediated (pools/day)	total production rate (mg/kg/day)	absolute catabolic rate receptor independent (mg/kg/day)	absolute catabolic rate receptor mediated (mg/kg/day)
subject								
RG	224	2.0	0.188	0.145	0.043	18.1	14.0	4.1
JJ	118	1.2	0.292	0.210	0.082	12.8	9.2	3.6
MM	215	15.7	0.163	0.137	0.026	12.0	10.5	2.2
BT	244	6.9	0.140	0.120	0.020	14.0	12.0	2.0
RM	238	7.5	0.237	0.219	0.018	19.5	18.0	1.5
DE	347	4.5	0.181	0.127	0.054	29.6	20.8	8.8
MJ	279	4.4	0.242	0.177	0.065	27.2	19.9	7.3
EA	171	8.0	0.325	0.236	0.089	22.2	16.1	6.1
JB	427	12.6	0.200	0.148	0.052	35.3	26.2	9.1
mean	251	6.9	0.219	0.169	0.050	20.7	16.32	4.9
(SD)	(92)	(4.7)	(0.061)	(0.043)	(0.026)	(8.3)	(5.5)	(3.0)
controls	93		0.384	0.219	0.165	14.2	8.1	6.2
(SD)	(12)		(0.057)	(0.039)	(0.051)	(2.3)	(1.1)	(2.1)
p	<0.001		<0.001	<0.05	<0.001	<0.02	<0.05	NS

FIGURE 6.1 - MULTICOMPARTMENTAL MODEL USED FOR ANALYSIS OF apoLDL METABOLISM



COMPARTMENT No.	POOL
1	intravascular LDL
2	extravascular LDL
3	total body iodide
4	urine iodide

compartments 5-8 are equivalents for modified LDL tracer

FIGURE 6.2a - PLASMA RADIOACTIVITY CURVE DURING LDL TURNOVER

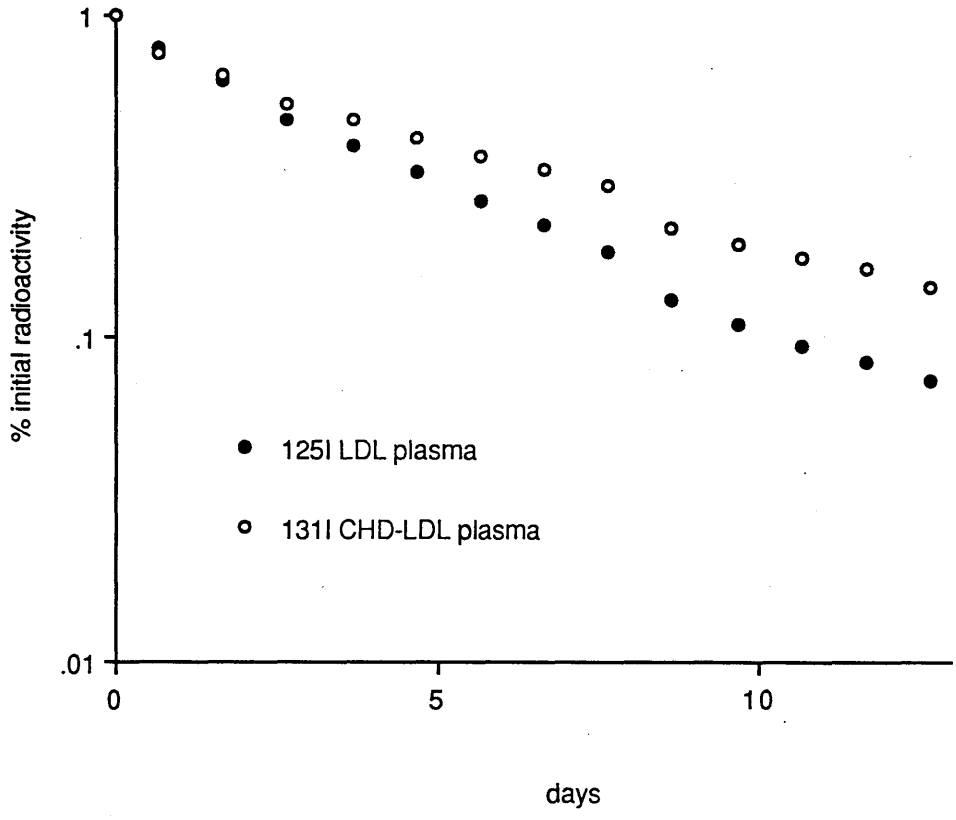


FIGURE 6.2b - URINE:PLASMA RADIOACTIVITY RATIOS DURING LDL TURNOVER

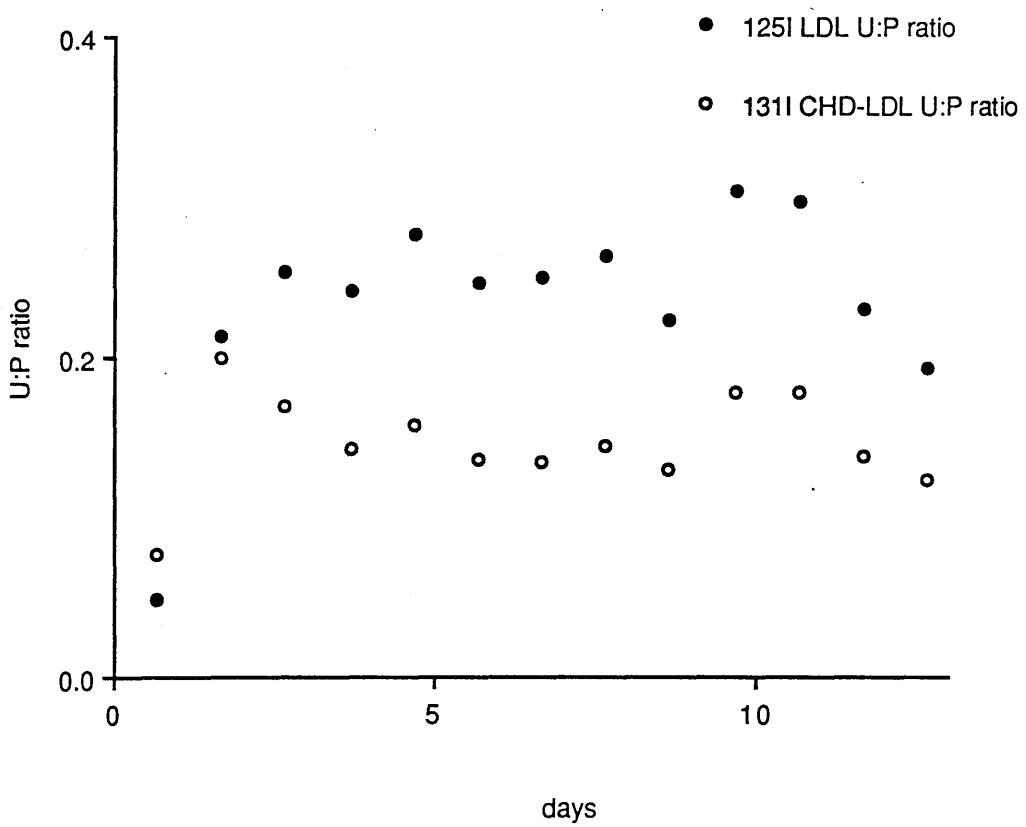
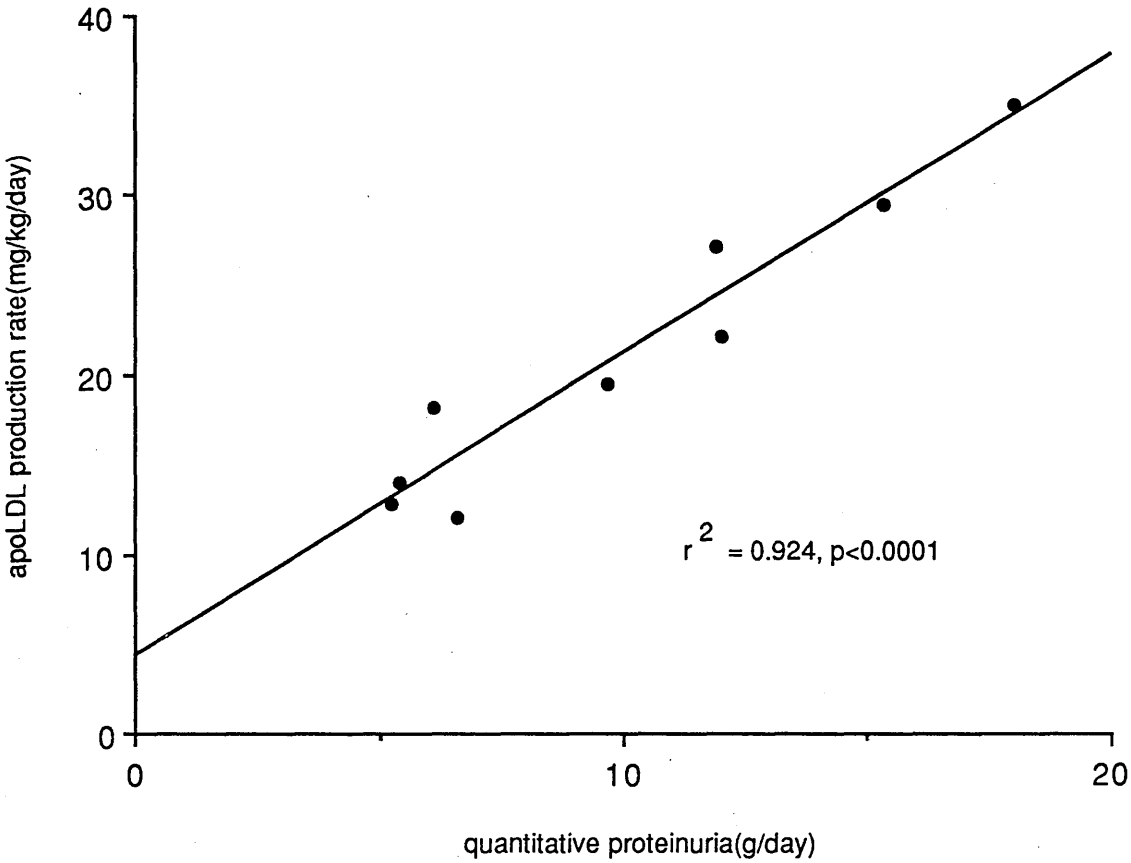


FIGURE 6.3 - RELATIONSHIP BETWEEN PROTEINURIA AND apoLDL PRODUCTION RATE



CHAPTER 7 THE EFFECT OF SIMVASTATIN(AN HMG CoA REDUCTASE INHIBITOR) ON PLASMA LIPIDS, LIPOPROTEINS AND LDL METABOLISM IN NEPHROTIC SYNDROME

7.1 Introduction

Treatment of hyperlipidaemia in nephrotic syndrome remains controversial. In contrast to primary hyperlipidaemia (102,103), the risk:benefit ratio of long-term hypolipidaemic therapy has not been quantified. No studies have compared the incidence and severity of adverse events related to drug therapy with the reductions in morbidity and mortality from vascular diseases. The relatively poor efficacy and tolerability of drugs such as bile acid-binding resins and fibric acid derivatives in nephrotic syndrome has not favoured widespread use of lipid-lowering agents in this condition. However, a number of recent short-term studies(125,128,129) have suggested that a new group of drugs, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors(184), are more effective and better tolerated.

These drugs competitively inhibit the rate-limiting enzyme in the cholesterol biosynthetic pathway(185) - figure 7.1. Whole body cholesterol synthesis is reduced by approximately 30%(186,187). This leads to a reduction in intrahepatic sterol pools and initiates a number of

mechanisms to conserve these. The mass of HMG CoA reductase increases and hepatocytes express more LDL receptors(188,189) thereby increasing uptake from plasma and increasing the fractional catabolic rate of LDL(190). Since the study described in chapter 6 found a consistent reduction in receptor-mediated LDL catabolism in nephrotic syndrome, this group of drugs appears to be ideally suited to lower plasma cholesterol in this condition.

The aims of this study were to assess:-
(1) short-term efficacy and tolerability of one of these drugs(simvastatin) in patients with nephrotic syndrome
(2) changes in LDL metabolism by receptor and non-receptor pathways in an attempt to link the putative action of the drug with changes in LDL turnover.

7.2 Subjects and methods

Sixteen subjects with nephrotic syndrome were recruited to an open study of the effect of simvastatin on plasma lipids and lipoprotein turnover according to the previously defined criteria. The characteristics of these subjects are listed in table 7.1.

The protocol for this study is illustrated in figure 7.2. Patients were seen by a dietitian and instructed on a cholesterol-lowering diet. Those of normal body weight

were given an isocaloric diet containing 100mg cholesterol per 1000 calories/day and a total fat content <35% of diet with a polyunsaturated:saturated ratio of >0.5. Subjects adjudged to be overweight were also prescribed calorie restriction. The diet was continued for at least eight weeks including a run-in period of four weeks on placebo preparation and plasma lipid values rechecked. Subjects whose total cholesterol was still >6.5mmol/L entered the active phase of the study. Treatment was started at 10mg of simvastatin per day taken with the evening meal. Patients were seen four weekly when standard biochemical and haematological indices were monitored and plasma lipids, lipoprotein cholesterol, HDL subfractions and apolipoproteins AI and B were measured in fasting plasma. The dose of simvastatin was titrated upwards to 20mg then 40mg according to total plasma cholesterol values. The aim was to maintain total plasma cholesterol between 3.5 and 5.2mmol/L. Patients were asked to keep a three day food diary on two occasions during the study to monitor adherence to diet. Compliance with drug therapy was assessed by tablet counts at each visit. Physical examination, electrocardiographs and full ophthalmological examination including slit lamp microscopy were performed before and at the end of the study.

A subgroup of eight patients(see table 7.1) underwent studies of apoLDL turnover as described in chapter 6. The

first turnover was performed during the placebo phase and was repeated in the last two weeks of the study on optimum drug therapy. The data from these studies were analysed according to the model described in chapter 6. The turnover data was supplemented by measures of plasma lathosterol by capillary gas chromotography as an index of cholesterol biosynthesis(191,192).

Statistical analysis

Paired t tests were used to assess changes in lipid and lipoprotein parameters over the active phase(0-12weeks) of the study. Kinetic parameters generated from the modelling procedure were also compared by paired t tests.

7.3 Results

Plasma lipid and lipoprotein concentrations

Fifteen subjects completed the study. Twelve of these required the full dose of 40mg/day and some were still significantly hyperlipidaemic. In three subjects, total plasma cholesterol fell to <5.3mmol/L on 20 mg/day. Overall, there were substantial reductions in total and LDL-cholesterol with mean falls of 32.9% and 39.5% respectively(table 7.2). Plasma triglyceride levels showed a modest fall(mean 14.4%) while HDL-cholesterol

concentrations rose significantly due to an increase in the HDL_2 subfraction. Apolipoprotein B concentrations in plasma fell by an average of 29.7% while there were no significant changes in apo AI.

The changes in plasma cholesterol and LDL-cholesterol have been plotted for the individual patients in figure 7.3. There was a rapid initial fall on 10mg of simvastatin but further decreases were observed on both 20 and 40mg doses. Total cholesterol concentrations were reduced below 6.5mmol/L in all but 3 patients. Of these three, one had the highest concentration(15mmol/L) at the start of active therapy and in another plasma cholesterol level, after initially falling on lower doses, rose to the starting concentration on 40mg/day simvastatin. The reason for this is unclear - on careful questioning the patient denied non-compliance and the tablet count was consistent with uninterrupted therapy. The results of the third "poor responder" are difficult to interpret. This patient had been shown to have minimal change histology on two occasions. Over the last few years, the nephrotic syndrome had been unresponsive to steroids and immunosuppressive therapy and proteinuria and severe hypercholesterolaemia had been persistently documented. During the study, there was a transient spontaneous remission of nephrotic syndrome with proteinuria falling to 0.6g/day and serum albumin rising to 42g/L. However, a further relapse occurred

almost immediately and, at the end of the study, proteinuria and hypoalbuminaemia were present to a similar degree as at the start. Plasma cholesterol at the start of the study was 19.1mmol/L but fell to 7.85mmol/L on 40mg/day simvastatin.

Renal function, serum albumin and proteinuria

There were no significant changes in renal function as judged by serum creatinine and creatinine clearance, proteinuria or serum albumin concentrations over the study period(table 7.2).

Tolerability

Most subjects tolerated drug therapy well in keeping with the reported low incidence of side-effects in other dyslipidaemias(127). However, two significant adverse events occurred during treatment although their precise relationship to the drug is uncertain. One subject developed a rapid decline in his visual acuity(from 6/6 to 6/24) in one eye within 14 days of starting the active drug. This was due to the rapid evolution of a pre-existing minor posterior subcapsular opacity noted at baseline ophthalmological examination. This was attributed to a course of steroids completed some 4 months earlier. This developed into a pronounced opacity involving the

visual axis. The drug was stopped immediately and, over the following year, there was no significant improvement or decline in visual acuity or the appearance of the lens. It is difficult to say with certainty that this deterioration was directly due to the drug therapy. However, a number of observations support this contention i.e. the close temporal association; the stability over a further year after drug withdrawal; the previous reports of cataract formation with older inhibitors of cholesterol synthesis (e.g. triparanol) and with HMG CoA reductase inhibitors at very high doses in dogs. The details of this case have been reported to the Committee on the Safety of Medicines.

A second patient had a marked rise in serum creatine kinase(CK) activity associated with generalised muscle symptoms described as "cramps". A peak value of 9400IU/L (upper limit of normal 150IU/L) was recorded three days after the acute symptoms and the time course of the serum concentration is plotted in figure 7.4. Drug therapy was not interrupted as it was initially thought that her symptoms were due to salt and water depletion related to a partial remission of proteinuria, hypokalaemia and over-vigorous diuretic therapy. When the serum CK level was checked a few days after the peak, it was falling rapidly and the drug was continued. Serum CK rose again to 1000IU/L by the end of the study but was not accompanied by any symptoms.

Low density lipoprotein metabolism

In the eight subjects investigated by apoLDL turnover studies, there was a consistent fall in the apoLDL pool size from a mean value of 5158mg to 3376mg - an average decrease of 33.1%(sd 11.6). There was also a relative depletion of cholesterol in the LDL particles isolated in the density range d=1.019-1.063g/mL. Overall, there was a small but significant decrease in the total cholesterol: protein(w/w) ratio from 1.72(sd 0.20) to 1.53(0.26),$p<0.05$.

The plasma decay curves and urinary radioactivities of the native(^{125}I-labelled) and cyclohexanedione-modified(^{131}I-labelled) LDL were used to estimate fractional catabolic rates(as described in chapter 6) before and after drug therapy. In one patient, before drug therapy, there was a very rapid early decay of the ^{131}I tracer which was probably due to denaturing of the protein during the modification procedure. For this patient, only the decay of the unmodified tracer was analysed giving a value for the total LDL fractional catabolic rate.

The changes in the apoLDL pool sizes, production rates and fractional catabolic rates for total, receptor-mediated and receptor-independent pathways are illustrated for each subject in figure 7.5. The mean values before and after treatment are shown in table 7.3. Five patients exhibited

increases in the total FCR while the rest were unchanged or fell slightly. Overall, the mean changes were not significant. Similarly, the FCR for the receptor-mediated pathway did not alter consistently in all 7 subjects although again a majority(5/7) demonstrated an increase in this parameter. The effect of drug therapy on the contribution of the receptor-independent pathway showed great variation among subjects(figure 7.5f) although the mean values before and after treatment were similar. Absolute apoB production rates were decreased in four subjects, unchanged in three and rose in one.

The amount of TCA-precipitable radioactivity in urine was used to estimate urinary losses of intact LDL. This varied widely from <1% to 14% of the daily turnover of LDL(taken as the average of 14 days) both among and within individuals. The mean values were lower than in the previous study(chapter 6). This was attributed to a more rigorous washing of the precipitate to remove any free radioiodide. The values quoted for the FCR's are corrected for the % of protein-bound urinary radioactivity. The average value for both ^{125}I and ^{131}I was used since there did not seem to be any consistent difference between the two tracers.

Plasma lathosterol concentrations fell in all eight subjects from a mean value of 127(sd 59) to 61(sd 23)ug/mg

cholesterol. The average decrease was 48%. There was no association between the decrease in lathosterol and changes in plasma cholesterol, LDL cholesterol, apoB pool size or kinetic parameters.

7.4 Discussion

Efficacy and tolerability

A number of recent studies have reported on the use of HMG CoA reductase inhibitors in nephrotic hyperlipidaemia(table 7.4). Reductions of 27-36% have been achieved in total cholesterol and of 27-45% in LDL-cholesterol. These reductions were compared favourably to the effect of cholestyramine by Rabelink(125). Plasma triglyceride and VLDL-cholesterol concentrations were also generally reduced and there was a trend towards an increased HDL-cholesterol concentration. All these studies reported minimal side-effects in short term use and, in one report(117), longer term therapy(48 weeks) satisfactorily controlled plasma cholesterol and was associated with reductions in proteinuria. The results of the current study are broadly in keeping with these previous reports with substantial falls in total and LDL-cholesterol and plasma apoB concentrations. Triglyceride and HDL-cholesterol both showed modest improvements and there was a significant increase in the cardioprotective HDL_2 subfraction. These

results confirm the utility of this group of drugs in reducing nephrotic hyperlipidaemia.

However, two serious adverse events were witnessed during this study, one of which resulted in a permanent disability. Although it is not certain that the drug therapy was causal in these adverse events, both of these side-effects have been previously reported for this group of drugs. An early inhibitor of cholesterol synthesis (triparanol) was associated with alopecia and lens opacities when used in clinical trials in the 1960's (193,194). This was attributed to the accumulation of toxic sterol metabolites late in the cholesterol biosynthetic pathway. For this reason, newer inhibitors of cholesterol synthesis have been extensiviely tested in animals with particular reference to possible lens toxicity. Large doses of both simvastatin and lovastatin (20-50x the equivalent dose in man) have been shown to cause an increased incidence of subcapsular lens opacities in dogs(195). The mechanism is thought to relate to the avascular nature of the lens combined with relatively high requirements for cholesterol for the differentiation and growth of lens epithelial and fibre cells. The lens has a high rate of cholesterol synthesis to compensate for its inability to utilise circulating cholesterol. Although little active drug will also reach the lens, even small decreases in cholesterol synthesis may be critical.

However, double-blind controlled studies in man have failed to show any increased incidence of opacity formation when compared to placebo or other lipid-lowering agents and the doses used in dogs to induce minor lens changes are much greater than would be used in clinical practice.

Muscle problems are also a recognised complication of this group of drugs. Asymptomatic rises in creatine kinase and vague muscle tenderness or weakness have been reported with a frequency of approximately 1%(196). More severe cases of myopathy and rhabdomyolysis have also been recorded(197). There appears to be an increased risk of muscle problems when used in conjunction with fibrates, cyclosporin A and nicotinic acid. Recently, it has been proposed(198) that the muscle toxicity results from depletion of mitochondrial ubiquinone stores. Ubiquinone is a product of mevalonate (see figure 7.1) and, although serum levels are not reduced by HMG CoA reductase inhibitors, mitochondrial supplies may be impaired(199).

These adverse events sound a note of caution amidst growing enthusiasism for the use of these drugs in renal disease. Careful monitoring of biochemical indices and ophthalmological review is advisable until more experience of HMG CoA reductase inhibitors in nephrotic syndrome is available.

Mechanism of plasma cholesterol lowering by HMG CoA reductase inhibition

Only one previous study has investigated the kinetic mechanisms underlying the reductions of plasma lipoproteins by this group of drugs in nephrotic syndrome(69). In 3 patients, radioiodinated VLDL/IDL(d<1.019g/mL) and LDL(d=1.019-1.063g/mL) tracers were used to follow apoB metabolism. Both increases in fractional catabolism and decreases in apoB production were observed in response to lovastatin therapy. A similar heterogeneity of response was found in the current study. Baseline results confirmed the low fractional catabolic rate of LDL(see chapter 6) in the nephrotic syndrome, mainly attributable to a decrease in receptor-mediated clearance. Despite consistent and substantial falls in apoLDL pools in all patients, the changes in apoLDL kinetics underlying these reductions were variable. A majority of subjects had increased catabolism by the receptor-mediated pathway in keeping with increased expression of LDL receptors. However, in four patients there were falls in apoLDL production of 23-51% and this was reduced by 13% on average. There were no apparent distinguishing factors between those who responded with an increase in receptor-mediated catabolism or those with decreased apoLDL production in terms of underlying renal disease, severity of nephrotic syndrome, apoE phenotype or fall in plasma lathosterol.

The relative importance of synthetic and catabolic processes in determining the steady state levels of plasma lipoproteins was considered in chapters 1 and 6. There is considerable evidence(31) that the major determinant of steady state plasma LDL concentrations is the rate of LDL receptor-mediated catabolism. This is controlled by the intrahepatic sterol pools which when depleted(e.g. by inhibiting cholesterol synthesis) results in increased synthesis of LDL receptors, increased LDL catabolism and reduction in plasma concentration. It is generally held that the plasma cholesterol lowering effect of HMG CoA reductase inhibitors is due to increased removal of LDL from plasma by the liver. Studies by Shepherd et al.(200) previously demonstrated this for cholestyramine where interruption of the enterohepatic bile salt circulation depleted hepatic sterol pools and increased the fractional catabolic rate of LDL by the receptor-mediated route. Early work in dogs confirmed that a similar mechanism existed in vivo for lovastatin(188). Although this paper is often quoted in support of this theory, this interpretation may be unjustified. The dosages of lovastatin used(10 and 25mg/kg/day) are much larger than would be used in clinical practice in man and at the lower dose the main cause of the fall in plasma cholesterol was a reduced apoLDL production rate. Although there was an increase in the number of receptors expressed on hepatic membrane preparations, there was no increase in the

fractional catabolic rate of radioiodinated human LDL. At the higher dose, these workers did demonstrate an increased fractional catabolic rate of LDL and increased high affinity binding of LDL to hepatic membrane preparations. Studies in humans have demonstrated the ability of these drugs to increase fractional catabolism of LDL in both heterozygous familial hypercholesterolaemia(190) and, more recently, in normal volunteers(201). Further support for this hypothesis is also available from *in vitro* studies of LDL receptors expressed on monocyte-derived macrophages (202) and circulating lymphocytes(203) from subjects treated with these drugs and by a recent elegant study by Reihner *et al*.(189). In this latter study, treatment of patients with lovastatin prior to elective cholecystectomy led to an increase in the amount of LDL receptor mRNA and high affinity LDL binding sites expressed on liver biopsy samples compared to controls.

However, apoB kinetic studies in other dyslipidaemias treated with lovastatin or pravastatin have not confirmed that increased catabolism of LDL is the principal cause of the lipid lowering effect. In two studies of patients with primary moderate hypercholesterolaemia(i.e. total cholesterol >6.5mmol/L), Vega and Grundy(204,205) documented increases in fractional catabolic rates of LDL in only 9/21 patients despite similar reductions in LDL-cholesterol and protein. In a majority of patients, the

kinetic explanation for the falls in apoLDL pool size was a reduced production rate. Studies in small numbers of patients with type III dysbetalipoproteinaemia(206) and in cholesterol ester storage disease(207) have produced similar findings. A recent study of lovastatin in familial combined hyperlipidaemia also concluded that the main metabolic change was a decreased LDL apoB production rate, specifically a decrease in the production of LDL which was not derived from VLDL(208).

Vega and Grundy(204,205) have attempted to explain the dual effects of HMG CoA reductase inhibitors on LDL metabolism by postulating the presence of a nascent pool of rapidly metabolised VLDL particles. Following drug therapy, these lipoproteins were removed by receptor-mediated clearance thus decreasing the amount of apoB available for transfer to LDL. Why "up-regulation" of LDL receptors leads to increased removal of precursors in some patients and enhanced LDL removal in others has not been satisfactorily explained. An alternative explanation is that decreased cholesterol availability due to inhibition of synthesis may reduce secretion of apoB-containing lipoproteins from the liver. The consistent reductions in plasma lathosterol concentrations(34-71%) suggest that hepatic cholesterol synthesis was significantly reduced in all these patients but the effect of this on LDL turnover is not clear. There is surprisingly little experimental work which has

investigated the effects of alterations of hepatic sterol metabolism on the assembly and secretion of lipoproteins. This has not been studied directly in man by methods examining synthesis directly(i.e. incorporation of radioactive or stable isotopes into VLDL apoB).

Indirect measures derived from studies with radioiodinated VLDL and LDL in rabbits(209) and miniature pigs(210,211) suggest that the cholesterol-lowering effect of lovastatin is achieved by inhibiting direct secretion of LDL by the liver. There is great debate about the existence of a pathway for direct hepatic LDL production in man(see ref 208 for a full discussion). The discrepancy between LDL apoB production and flux from VLDL identified by some workers in animals and in man may be due to one of two mechanisms:-

(i)direct secretion of LDL particles by the liver

(ii)a rapidly metabolised pool of nascent particles which are converted to the LDL density range in the hepatic sinusoids or in the circulation possibly by binding to lipoprotein lipase binding sites during "first pass"

The present study does not differentiate between reduced hepatic VLDL production, changes in flux of apoB from VLDL to LDL or variation in direct LDL secretion by the liver in these patients. However, the results presented in chapter 5 do not suggest that direct hepatic secretion of LDL is a

major metabolic pathway in nephrotic hyperlipidaemia. A recent study of VLDL-triglyceride metabolism in nephrotic rats treated with pravastatin showed that limitation of hepatic sterols may normalise VLDL synthetic rates(212). However, the results of this experiment must be interpreted with caution as the failure of the drug to reduce plasma cholesterol and apoB concentrations in control or nephrotic animals illustrates the fundamental differences in lipoprotein metabolism between rats and man.

Finally, at least part of the cholesterol-lowering effect of simvastatin is achieved by depleting low density lipoproteins of cholesterol with a decrease in the cholesterol:protein ratio. This has been shown previously by other investigators(204).

Further studies are required in man to fully elucidate the kinetic mechanisms underlying the reductions in cholesterol in these subjects. One approach would be to study the effects on VLDL apoB metabolism using the methods in chapter 5. However, a more useful and direct assessment of the relationship between secretion of apoB-containing lipoproteins and alterations of cholesterol metabolism could be gained by utilising stable isotopes to look at synthesis of apolipoproteins and lipids directly.

TABLE 7.1 - CHARACTERISTICS OF SUBJECTS TREATED WITH SIMVASTATIN

	age	sex	BMI (kg/m^2)	histology	serum creatinine (umol/L)	serum albumin (g/L)	quant. protein-uria(g/day)	plasma chol (mmol/L)	plasma trig (mmol/L)	relevant drug therapy
CM	22	F	25.2	membranous	70	28	6.2	9.15	2.35	frusemide
JS*	60	M	26.9	membranous	170	26	14.5	7.5	1.9	frusemide
LP	22	F	19.3	MCN	65	29	4.3	19.1	3.0	frusemide
CF	34	F	23.3	MCGN	75	29	4.4	7.15	1.1	frusemide,amiloride
JH*	45	M	20.9	membranous	130	28	12.6	8.85	3.05	bisoprolol
JB	32	M	19.9	membranous	210	26	8.0	12.65	2.2	frusemide
MS	52	F	32.4	MES-PROL	150	34	6.2	6.85	1.55	thyroxine, oestrogen
RC*	58	M	30.5	membranous	80	34	6.4	6.9	1.6	atenolol, frusemide
GB	57	M	24.0	MES-PROL	95	30	8.4	8.95	1.75	
JP*	67	M	28.8	membranous	150	28	9.6	7.85	3.1	frusemide, isosorbide
MY	29	M	21.1	MCGN	210	24	21.5	7.85	1.1	
RM*	31	M	33.8	membranous	110	35	4.3	6.75	1.95	atenolol
WT*	29	M	24.5	membranous	140	22	20.5	12.95	1.35	frusemide, amiloride
MM*	57	F	25.6	MCGN	210	36	8.8	8.4	1.6	enalapril, thyroxine
RB*	48	F	25.6	membranous	70	21	6.2	9.85	3.9	frusemide
JA	57	F	24.7	membranous	180	23	23.0	10.1	6.95	frusemide, captopril, nifedipine, atenolol

MES-PROL - mesangioproliferative ; MCN - minimal change; MCGN - mesangiocapillary
*indicates turnover subjects. Values shown are taken from first visit(week -4)

TABLE 7.2 - BIOCHEMICAL PARAMETERS DURING SIMVASTATIN THERAPY

	weeks of study					% change from baseline	p value
	-4	0	4	8	12		
total cholesterol(mmol/L)	9.43(3.19)	9.47(2.12)	6.96(1.68)	6.59(1.59)	6.14(1.66)	-32.9(14.5)	<0.0001
LDL-cholesterol(mmol/L)	6.94(2.97)	7.00(1.79)	4.96(1.38)	4.42(1.33)	3.99(1.34)	-39.5(20.7)	<0.0001
triglyceride(mmol/L)	2.40(1.45)	2.44(1.35)	1.83(0.90)	2.14(1.35)	2.00(1.02)	-14.4(28.6)	0.02
HDL-cholesterol(mmol/L)	1.15(0.25)	1.18(0.19)	1.28(0.26)	1.23(0.23)	1.32(0.29)	+12.0(22.8)	0.05
apo AI(g/L)	1.32(0.20)	1.25(0.21)	1.25(0.16)	1.33(0.30)	1.36(0.26)	+10.0(26.9)	ns
apo B(g/L)	1.86(0.53)	1.80(0.59)	1.28(0.49)	1.23(0.49)	1.22(0.37)	-29.7(13.9)	<0.0001
HDL_2(mg/dL)	43(18)	45(18)	56(31)	55(22)	64(25)	+52.0(71.9)	0.02
HDL_3(mg/dL)	226(48)	249(54)	257(38)	254(56)	238(47)	-2.5(18.3)	ns
serum albumin(g/L)	28(5)	30(5)	30(5)	30(5)	29(5)	2.3(10.7)	ns
serum creatinine(umol/L)	132(53)	138(60)	136(64)	139(65)	141(65)	+8.7(19.5)	ns
creatinine clearance ($mL/min/1.73m^2$)	65(32)	64(32)	68(37)	61(28)	56(27)	-10.3(23.1)	ns
proteinuria(g/day)	9.8(6.40)	10.2(4.6)	8.0(5.3)	7.9(6.2)	9.2(5.8)	-7.6(38.4)	ns

Values are means and standard deviations(n=16)

TABLE 7.3 - CHANGES IN apoLDL KINETIC PARAMETERS FOLLOWING SIMVASTATIN THERAPY

	PRE	POST	SIGNIFIGANCE (paired t test)
apoLDL pool size (mg)	5158(947)	3376(510)	p<0.001
LDL cholesterol: protein ratio(w/w)	1.72(0.25)	1.48(0.24)	p<0.05
plasma lathosterol (ug/mg chol)	127(59)	61(23)	p<0.005
total FCR (pools/day)	0.196(0.053)	0.252(0.076)	ns
receptor-mediated FCR	0.077(0.040)	0.111(0.051)	ns
receptor-independent FCR	0.126(0.023)	0.140(0.063)	ns
absolute turnover rate(mg/kg/day)	984(218)	871(343)	ns

values are means and standard deviations

TABLE 7.4 - PREVIOUS STUDIES OF HMG CoA REDUCTASE INHIBITORS IN NEPHROTIC SYNDROME

author/ year	reference number	no. of subjects	drug	dosage	% decrease			side-effects
					plasma cholesterol	LDL-cholesterol	plasma triglyceride	
Rabelink 1988	125	10	simvastatin	40mg	36	39	58	none
Golper 1989	128	10	lovastatin	80mg	33	45	25	transient abnormalities of liver function tests
Vega 1988	69	3	lovastatin	40mg	30	33	38	none
Kasiske 1989	129	14	lovastatin	40mg	27	27	30	diarrhoea

FIGURE 7.1 - CHOLESTEROL BIOSYNTHETIC PATHWAY

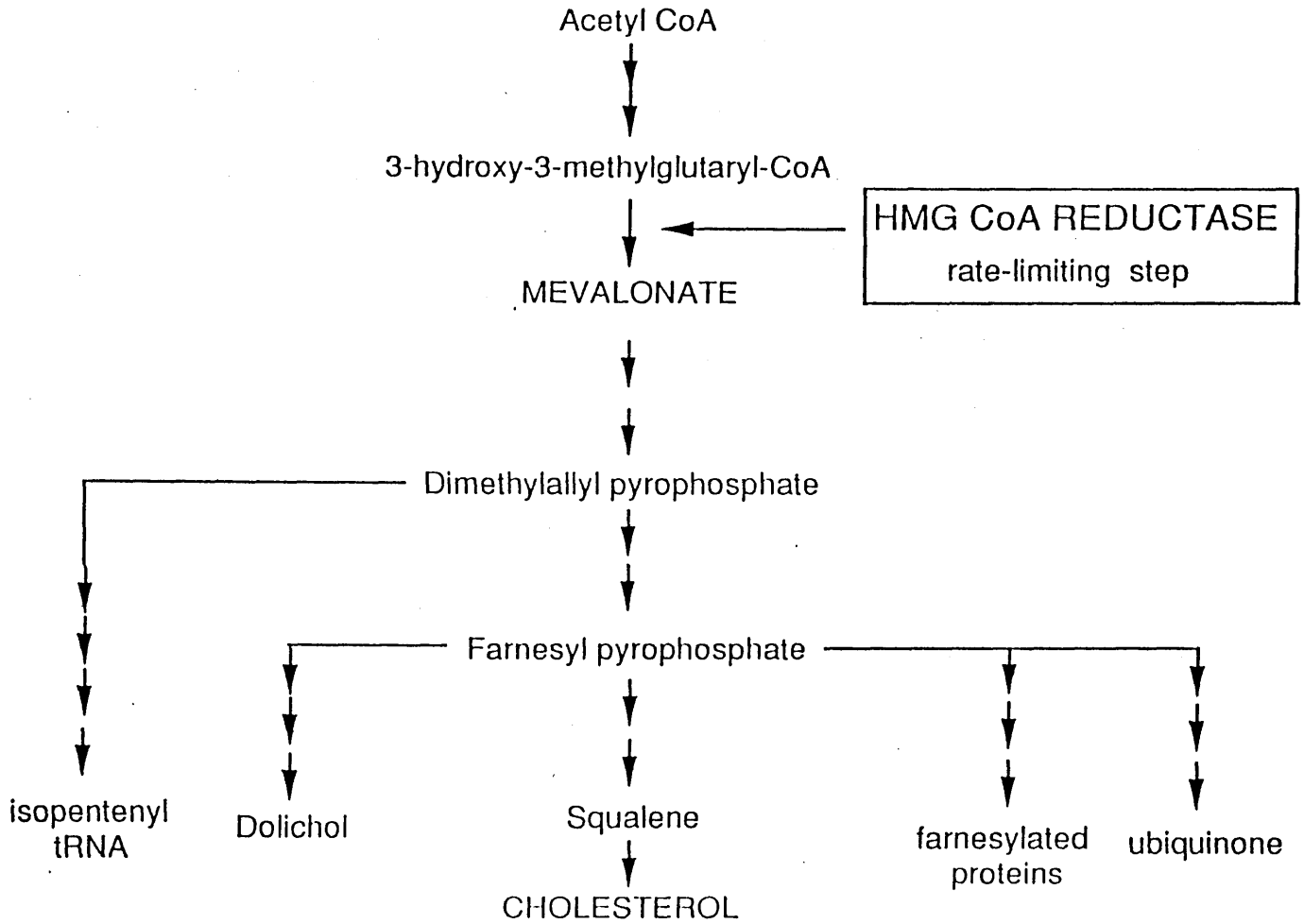
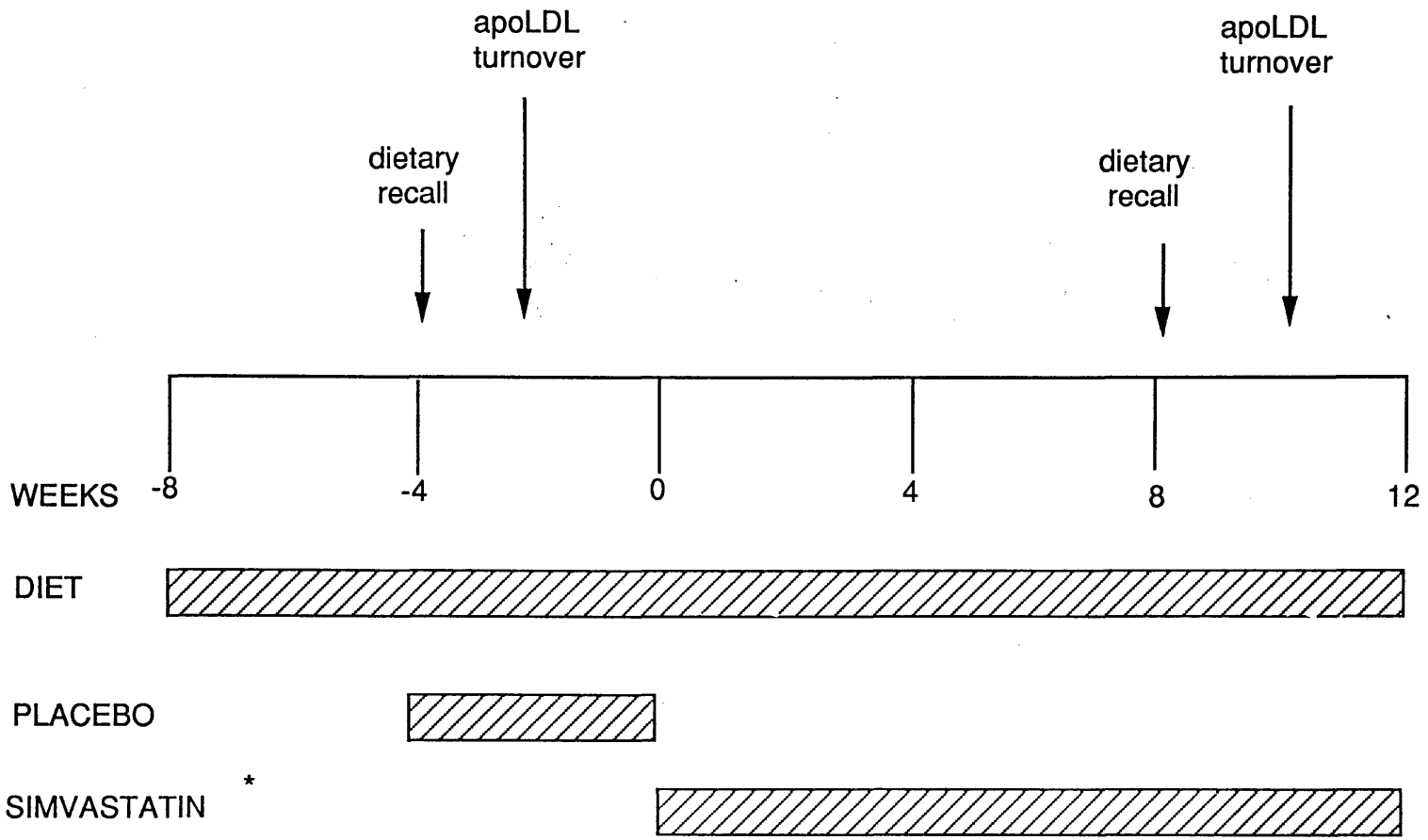


FIGURE 7.2 - PROTOCOL FOR SIMVASTATIN STUDY



* dose adjusted from 10 - 40mg to reduce total cholesterol to 3.5 - 5.3mmol/l

FIGURE 7.3a - PLASMA CHOLESTEROL CHANGES ON SIMVASTATIN

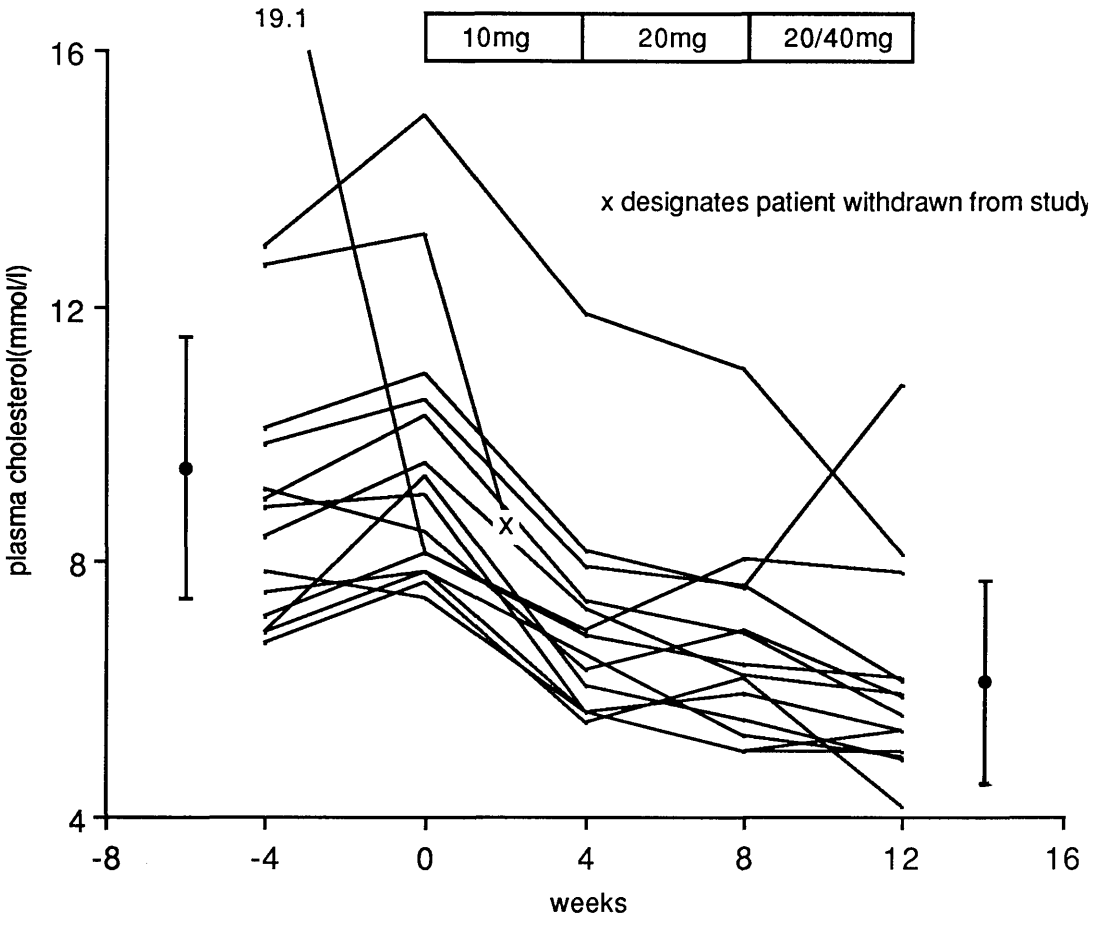


FIGURE 7.3b - PLASMA LDL-CHOLESTEROL CHANGES ON SIMVASTATIN

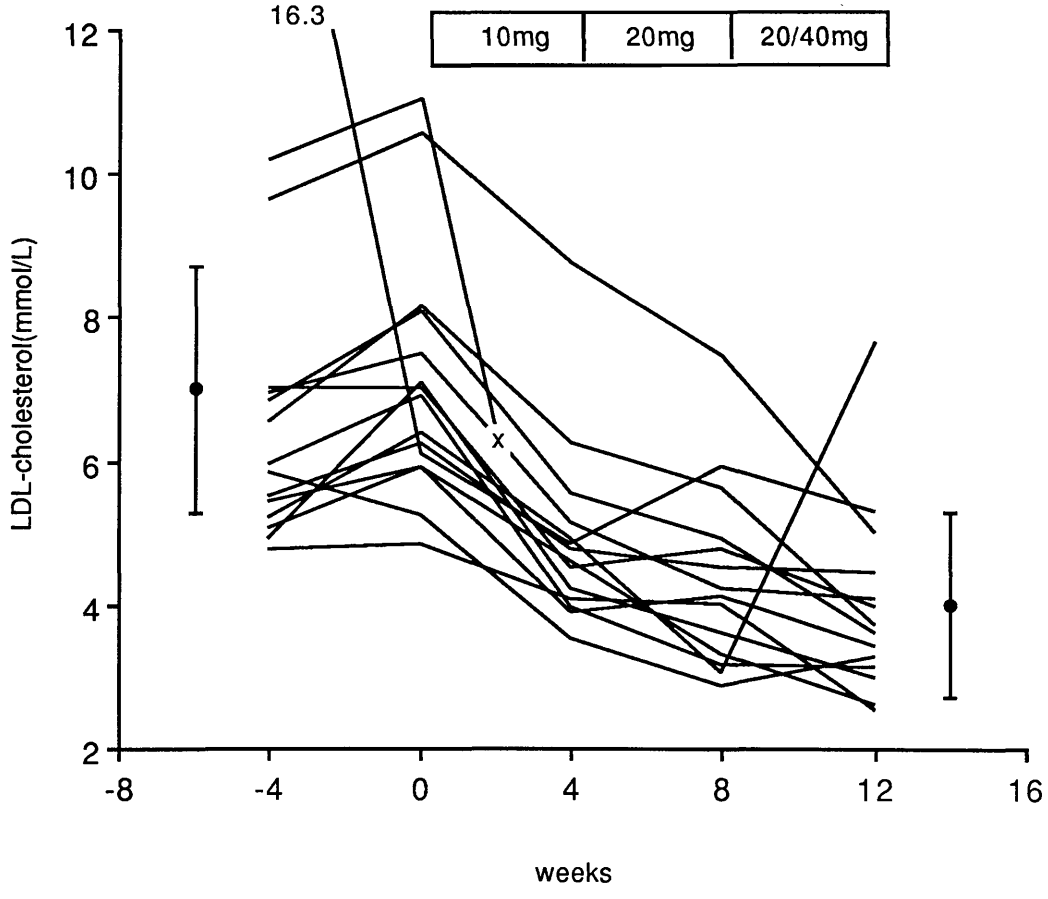


FIGURE 7.4 - SERUM CREATINE KINASE IN RELATION TO SIMVASTATIN THERAPY IN SUBJECT LP

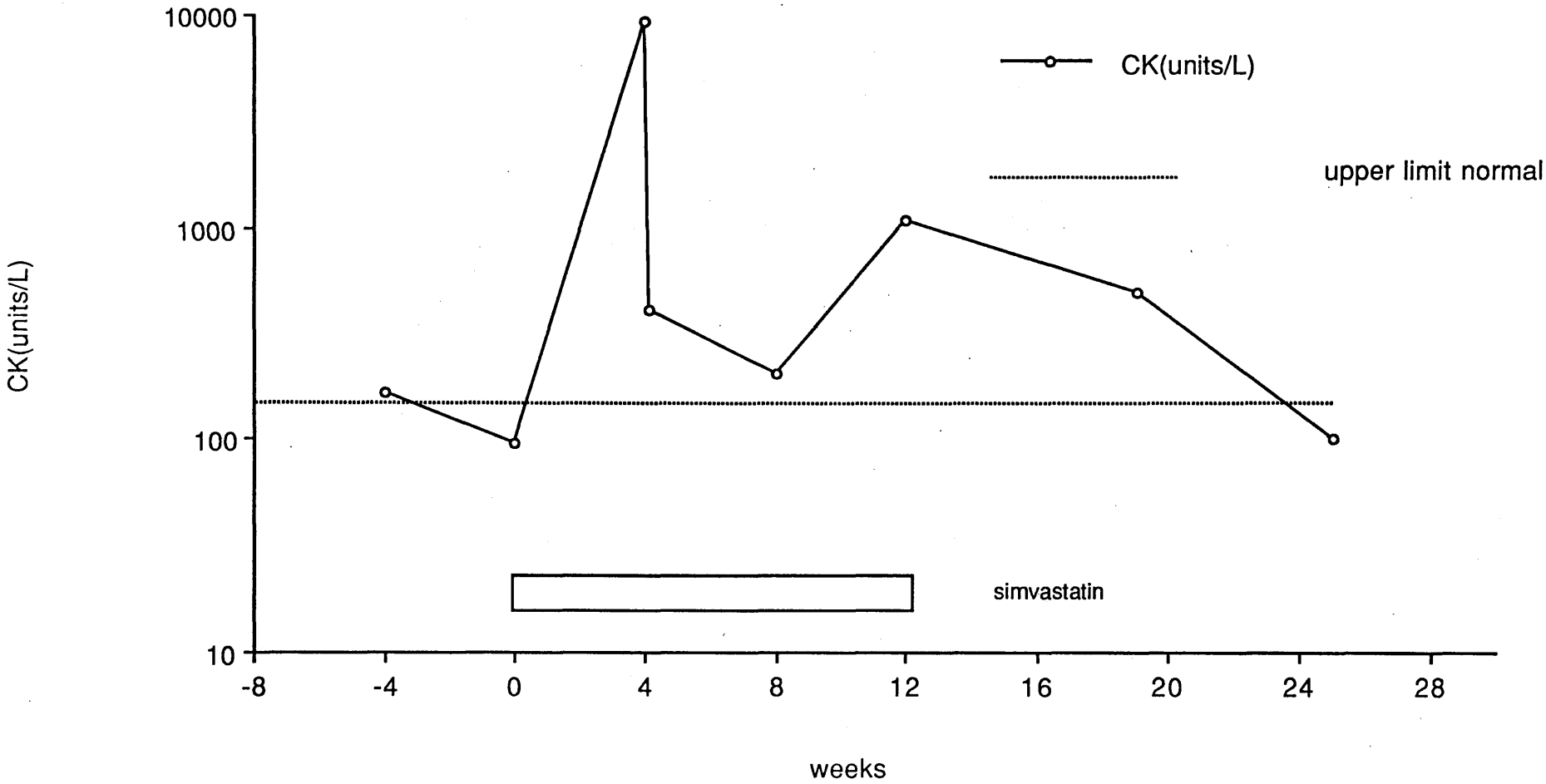
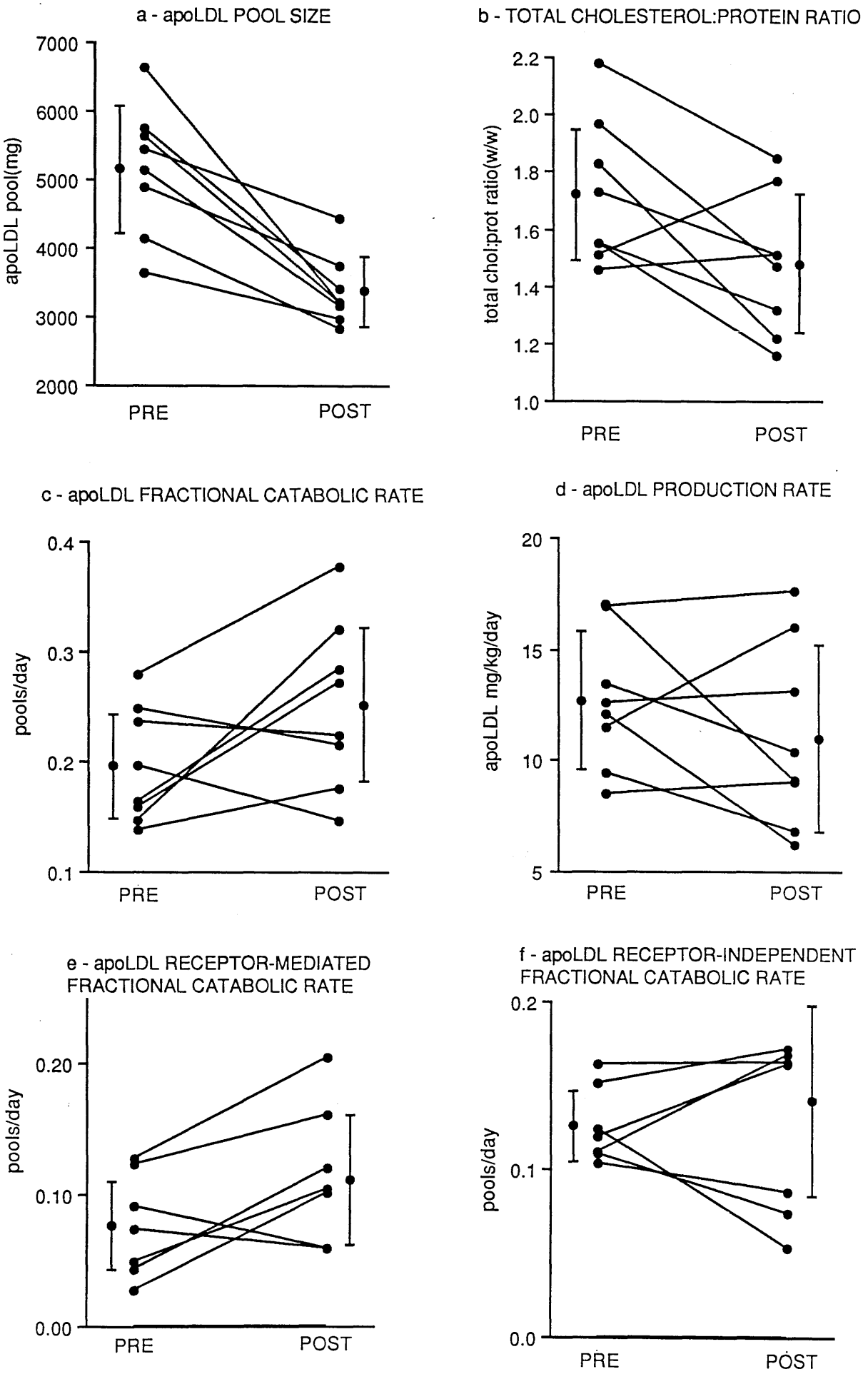


FIGURE 7.5 - EFFECT OF SIMVASTATIN ON apoLDL METABOLISM



CHAPTER 8 LIPOPROTEINURIA IN NEPHROTIC SYNDROME

8.1 Introduction

Increased urinary excretion of lipids is well-recognised in the nephrotic syndrome and has been included by some in the definition of the clinical syndrome. However, the nature and quantity of urinary lipids has been infrequently investigated. Urinary lipids may exist in four discrete physico-chemical forms(213):-

1.Cell membrane phospholipids
2.Large lipid droplets composed of cholesterol ester which are visible as oval fat bodies under the light microscope and as "Maltese crosses" under polarised light.
3.Small quantities of cholesterol monohydrate in solution
4.Lipoprotein particles

The aim of this study was to characterise and quantify urinary excretion of lipoproteins in relation to proteinuria. No attempt was made to investigate losses of the first three categories of lipid. A few previous studies have demonstrated excretion of lipoprotein particles, particularly HDL, in the nephrotic syndrome (53,119). HDL apolipoproteins have also been demonstrated in the urine of both normal and nephrotic subjects. This is probably due to filtration of apolipoproteins

circulating free of lipoprotein complexes(214). The finding of small but significant quantities of protein-bound radioactivity in the urine of patients with nephrotic syndrome undergoing radioiodinated LDL turnovers(chapter 6) stimulated further investigations designed to confirm that this was due to urinary losses of LDL particles. The molecular weight(approximately 2.3 x 10^6d), size(18-25nm) and net negative charge of LDL particles should retard glomerular filtration.

8.2 Subjects and methods

Lipoproteins were isolated from urine by concentrating large volumes of fresh, timed urine collections using a combination of ultrafiltration and ultracentrifugation. Fasting blood and 24 hour urine collections were collected from eleven patients with nephrotic syndrome. The patients studied were selected at random and included patients with several distinct glomerular diseases, proteinuria from 3.3 to 24.2g/day and a range of plasma lipid concentrations. Fasting lipids and lipoprotein cholesterol, serum creatinine and albumin and urine total protein and creatinine were measured as described in chapter 2.

Urine samples were collected with thymol or sodium azide as a preservative. The urine was concentrated 20-50 fold

using an ultrafiltration system within hours of the end of the collection period. An Amicon HIP 100-20 hollow fibre cartridge with a nominal MW cut-off of 100,000d(source: Amicon, Stonehouse, Gloucester, UK) was employed. Using this system, it was possible to concentrate urine samples (1-3litres) to a final volume of approximately 20-50mL over a period of several hours. The concentration factor was calculated from careful measurement of the volumes of the initial urine collection and the final concentrate. A membrane with a high molecular weight cut-off was deliberately chosen to remove most of the albumin and other small proteins from the concentrate while retaining lipoproteins. This helped to maintain the ultrafiltration rate during concentration and reduced the risk of albumin contamination of lipoprotein fractions. Studies with radioiodinated LDL and HDL showed that 60% of the radioactivity was recovered in the retentate and approximately 10% in the ultrafiltrate. The rest was assumed to have bound to connecting tubing or the filtration membrane. No correction was made for recovery in these experiments and the lipoprotein masses recovered represent a minimum estimate of urinary losses.

The retentate was further concentrated(x2) and fractions corresponding to IDL/LDL(1.006-1.063g/mL) and HDL (1.063-1.21g/mL) obtained by sequential flotation ultracentrifugation at the appropriate density. To

ascertain the background density of the solution, a preliminary ultracentrifugation step was performed where 6mL aliquots of the retentate were subjected to ultracentrifugation at 39,000rpm in a Ti 40.3 rotor for 16 hours at $4^{o}C$. The top 1mL was aspirated and its density measured in a densitometer(source: Anton Parr KG, Graz, Austria). The remaining 5mL aliquot was then adjusted to a density of 1.063g/mL by the addition of solid sodium bromide according to the formula:-

$$M = \frac{v(d_2 - d_1)}{1 - V_s d_2}$$

where v = volume of initial solution

M = mass of sodium bromide in grams

V_s= partial specific volume of salt at relevant temperature and concentration

d_2= 1.063g/mL

d_1= initial density of background solution

The solution was then made up to a final volume of 6mL by overlaying with d=1.063g/mL solution. A fraction corresponding to LDL was recovered in a volume of 2mL by careful aspiration after further ultracentrifugation. Solid NaBr was then added to bring the density to 1.210g/mL for isolation of HDL.

The lipoprotein fractions obtained were analysed for cholesterol, triglyceride and phospholipid content as outlined in chapter 2. Protein content was measured by Bradford's method(215). The minimum concentration of protein detectable was equivalent to excretion of approximately 5mg/day of lipoprotein. Values below this were considered too small to quantify accurately although small amounts of lipid were often measurable. Total lipoprotein concentration(mg/dL) was calculated from the sum of these concentrations and the daily excretion calculated from the urine volume and the concentration factor. To confirm the presence of intact lipoprotein particles, selected samples were subjected to non-denaturing gradient gel electrophoresis(216) and electron microscopy of preparations(217) negatively stained with phosphotungstate.

8.3 Results

Lipoproteins were detected in the urine of all eleven patients studied although in some this was too small to quantify accurately. In six primary hypercholesterolaemic patients with normal renal function, a small quantity of HDL was recovered from the urine in only one patient. The presence of "intact" lipoprotein particles was confirmed by direct visualisation of particles by electron microscopy where preparations of both HDL and LDL revealed circular

particles consistent with lipoproteins(figure 8.1). The estimated size of these(up to 50nm) was larger than predicted but this may be an artefact resulting from the extensive processing required to isolate the lipoproteins and the tendency for these particles to flatten and spread-out on EM grids. Further confirmation was obtained from gradient gel electrophoresis where both HDL particles over a range of sizes and LDL particles(occasionally in discrete subfraction bands) were identified(figures 8.2 & 8.3).

HDL was found in all eleven patients and could be accurately quantified in nine with values ranging from 29.3-107.0mg/day. Significant quantities of LDL were found in 6 patients varying from 11.1-57.8mg/day. Of those with no measurable LDL in the urine, four had the lowest levels of total protein excretion(3.3-6.2g/day). The fifth had the highest quantitative proteinuria at 24.2g/day. This discrepancy may be explained by a continuing degree of size selectivity even at this larger particle size since this patient had steroid-responsive, minimal change disease and would be predicted to have "selective" proteinuria.

Overall, there was a significant correlation between urinary losses of total protein and HDL(r^2=55.2%, p=0.005) - figue 8.4d. The numbers with LDL in urine are too small to draw any firm conclusions but there was a trend to

increased LDL in those with higher levels of proteinuria. There was no relationship between plasma LDL or HDL cholesterol concentrations and the urinary excretion of these lipoproteins(figures 8.4a and b).

8.4 Discussion

These results confirm that measurable quantities of both HDL and LDL are present in the urine of many nephrotic subjects. These particles have similar physical characteristics to their plasma counterparts. Glomerular filtration is the most likely route of entry into the urine as the quantity of lipoprotein generally mirrors total protein losses. A degree of selectivity occurs as demonstrated by the absence of urinary LDL in the patient with minimal change disease despite the highest total protein losses and the highest plasma LDL-cholesterol concentration. This raises fundamental questions about the nature and size of the defects in the glomerular capillary wall in glomerular disease. Current theories favour a model consisting of a membrane bridged by water-filled pores(218). Estimates of the size of these pores in normal subjects ranges from 4.5-5.5nm. In nephrotic syndrome, it is postulated that there is heterogeneity in the size of these pores. The area of the glomerular capillary wall covered by a subset of larger pores is increased at the expense of a decrease in number of smaller pores favouring

the filtration of larger molecules(e.g. albumin) to water and salt. The size of these larger pores is uncertain but these results suggest a few at least must approach the diameter of LDL particles. It is possible that these particles are deformable and may be able to migrate through smaller pores. Alternative theories consider the glomerular basement membrane to be similar to a thixotropic gel(219) which when subjected to physical stress(e.g. haemodynamic forces) becomes less viscous and allows the migration of larger particles to the urinary space.

Lipoproteins may reach the urinary space by processes other than filtration. There may be breaks or defects along the glomerular basement membrane allowing direct leakage of plasma constituents into the urinary space. A similar mechanism may explain the appearance of red blood cells of glomerular origin in the urine. There is evidence that filtered lipoproteins are catabolised and altered by tubular cells(53,119) and it is unlikely that tubular cells could assemble and secrete lipoproteins.

Although the presence of lipiduria in nephrotic syndrome has been known for many years, surprisingly few studies have attempted to characterise and quantify these losses. The oval fat body is perhaps the best recognised form of urinary lipid. This consists of an accumulation of cholester ester in cellular debris or casts. It has a

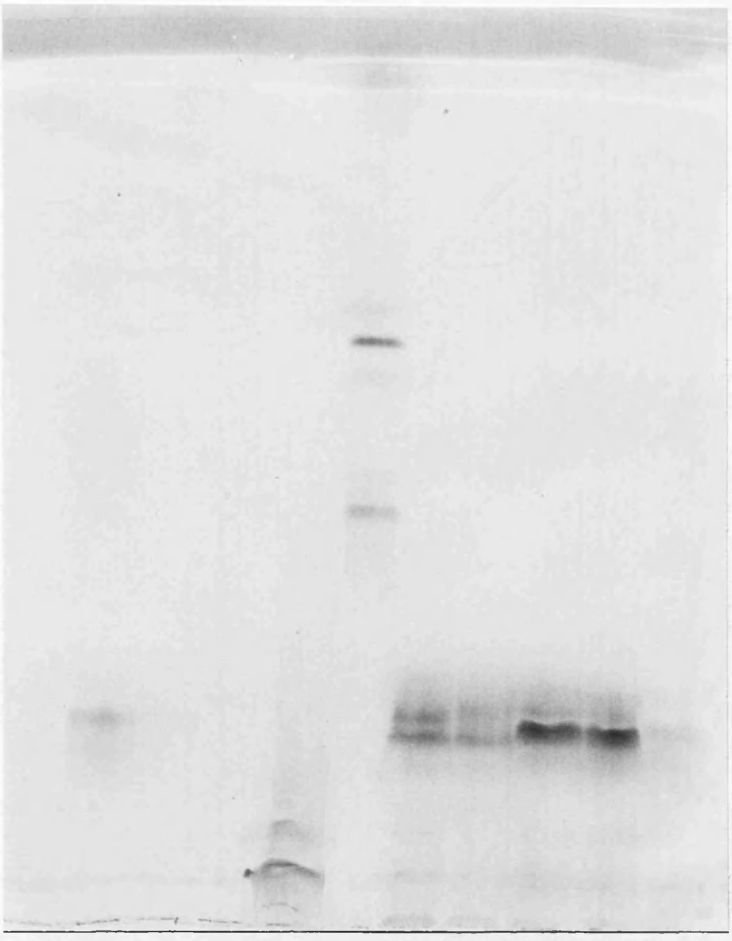
typical appearance under the microscope. However, the precise mechanism of the formation of these collections of fat is not known. Only a few reports have confirmed the presence of lipoprotein particles by a combination of chemical and physical methods(119,220,221,53) although there presence was predicted by earlier workers(222) and by the identification of apolipoproteins in urine. Jungst et al.(221) also identified a macromolecular protein/lipid complex in both normal and nephrotic urine. This accounted for most of the lipid in normal urine and a substantial fraction in nephrotic urine. Although thought to be a membrane derived complex, it contained significant quantities of albumin, possibly as a contaminant. Interestingly, it floated at a density between 1.1-1.3g/mL raising the possibility that it could be mistaken for HDL.

There are several important consequences of these findings. Firstly, as discussed above it raises important questions about the nature of the defect in the glomerular capillary wall in nephrotic syndrome. Second, it gives credence to the theory(107) that filtered lipoproteins may be capable of damaging tubular cells. Finally, it is important in the context of metabolic studies of radiolabelled lipoproteins in nephrotic subjects since losses of the magnitude measured here would represent up to 5% of HDL and up to 3% of LDL protein turnover each day. Since almost certainly these are underestimates due to losses during processing of

samples, the total urinary excretion may account for a significant fraction of daily apolipoprotein flux justifying attempts to correct for this in these studies.

FIGURE 8.1 - ELECTRON MICROGRAPH OF NEGATIVELY-STAINED LDL PARTICLES ISOLATED FROM NEPHROTIC URINE
(final magnification x100000)

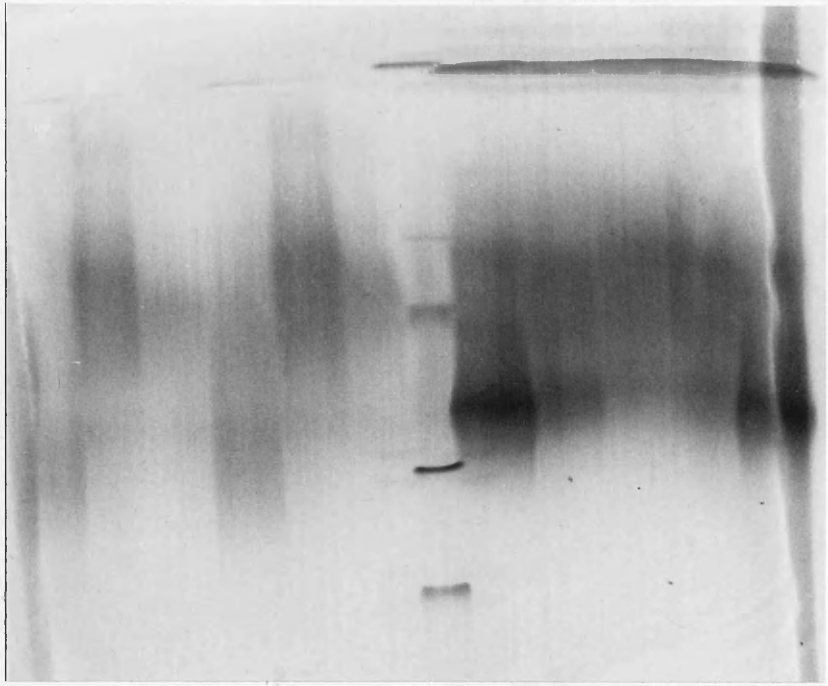
FIGURE 8.2 - GRADIENT GEL ELECTROPHORESIS OF LDL ISOLATED FROM NEPHROTIC URINE



1 2 3 4 5 6 7 8 9 10

1 - 3 urinary LDL samples; 4 - 5 molecular size markers; 6 - 10 plasma LDL samples

FIGURE 8.3 - GRADIENT GEL ELECTROPHOREISIS OF HDL ISOLATED FROM NEPHROTIC URINE



1 2 3 4 5 6 7 8 9 10 11 12

1 - 6 urinary HDL samples; 7 molecular size markers; 8 -12 plasma HDL samples

FIGURE 8.4a - PLASMA LDL-CHOLESTEROL AND LDL EXCRETION

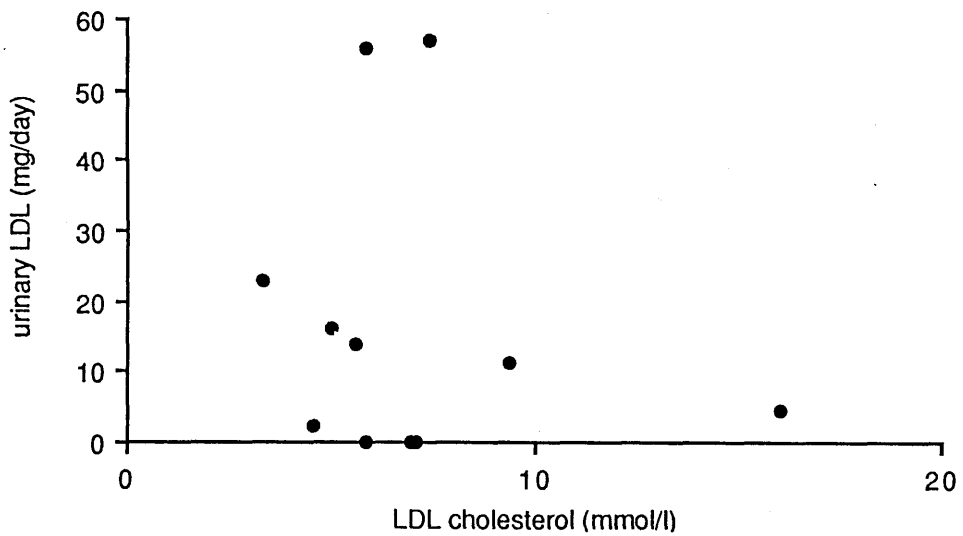


FIGURE 8.4b - PLASMA HDL-CHOLESTEROL AND HDL EXCRETION

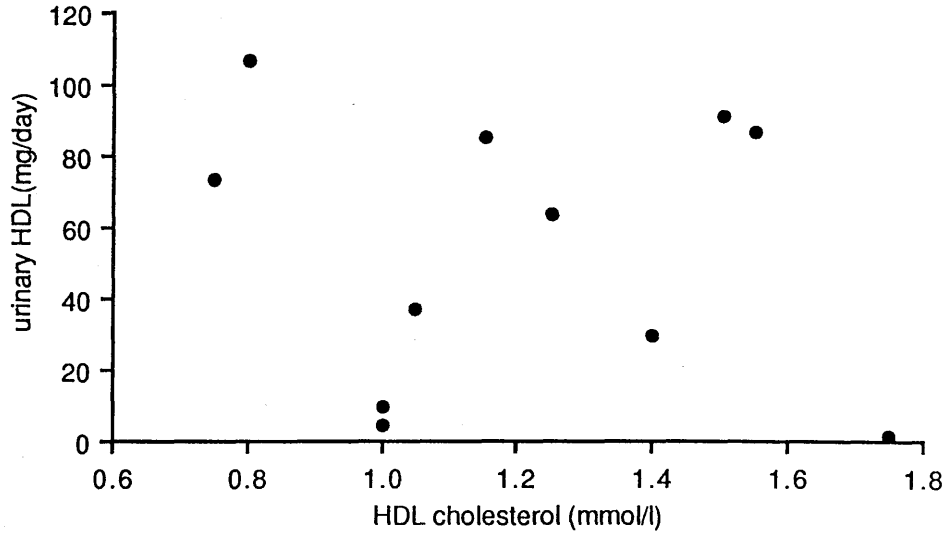


FIGURE 8.4c - URINARY TOTAL PROTEIN AND LDL EXCRETION

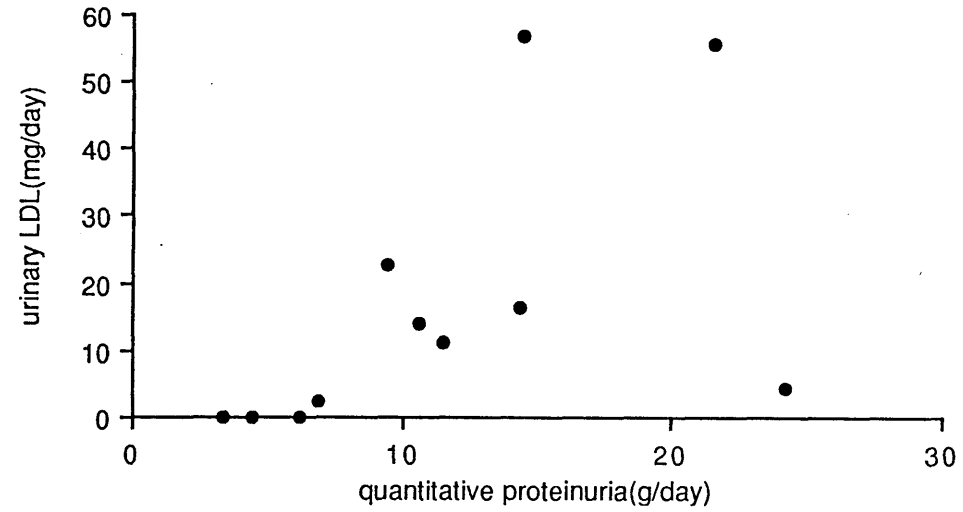
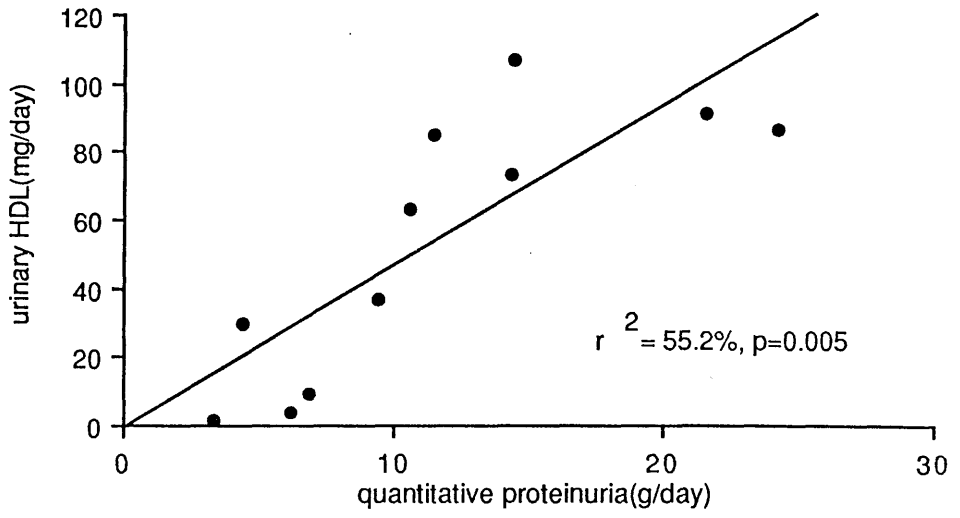


FIGURE 8.4d - URINARY TOTAL PROTEIN AND HDL EXCRETION



CHAPTER 9 GENERAL DISCUSSION

This final section considers the contribution of the preceding studies to current understanding of lipoprotein metabolism in nephrotic syndrome and highlights possible directions for further study. Three main areas are discussed:-

9.1 The relationship between hyperlipidaemia and nephrotic syndrome. This explores the prevalence and nature of hyperlipidaemia in the nephrotic syndrome and the effect of increasing severity of renal disease(chapters 3 & 8).

9.2 Lipoprotein metabolism in nephrotic syndrome. This is the main topic of this thesis and addresses the underlying abnormalities of lipoprotein metabolism and their possible causes(chapters 4-6).

9.3 Treatment of nephrotic hyperlipidaemia. The continuing uncertainty surrounding if, when and how to treat this complication of proteinuric disease is discussed(chapter 7).

9.1 THE RELATIONSHIP BETWEEN HYPERLIPIDAEMIA AND NEPHROTIC SYNDROME

Although hyperlipidaemia is a very common complication of nephrotic syndrome(41-48,53,223), its precise incidence has been poorly documented. Few studies have adequately defined their population base and have reported on a selected series of patients in whom hyperlipidaemia, often severe, has been identified. With two exceptions(47,53), it is not clear in these surveys whether all nephrotic subjects seen in a given time period were included. Other factors which hamper interpretation and comparisons of studies include the absence of well-defined "normal" plasma lipid and lipoprotein levels, differences in the analysis and classification of hyperlipidaemia, variations in the severity of nephrosis and the influence of co-existent factors on plasma lipids(e.g. systemic disease, drugs, renal failure).

The current investigation confirms previous surveys documenting the main lipid and lipoprotein abnormalities. Established nephrotic syndrome with heavy proteinuria, hypoalbuminaemia(<30g/L) and oedema is complicated by hyperlipidaemia in nearly all cases. Plasma cholesterol and LDL-cholesterol are invariably increased and, in more severe disease, plasma triglycerides and VLDL-cholesterol also rise and may become the dominant abnormality. In

these cases, plasma cholesterol may reach as high as 30mmol/L. However, in less severe cases with proteinuria >3g/day but well-maintained serum albumin, plasma cholesterol may be only modestly elevated or even "normal"(i.e. <6.5mmol/L). The studies presented here also demonstrate that, although lower levels of proteinuria are not associated with significant rises in cholesterol compared to controls, there is a weak but continuous relationship between these two variables. Further evidence(146) is presented confirming the significant elevations of plasma lipoprotein(a) concentrations and a strong relationship was demonstrated with the inverse of serum albumin.

Abnormalities of lipoprotein composition have been described in nephrotic subjects(42,49) but the consistent increases in free cholesterol in the VLDL and IDL subfractions demonstrated here are at variance with these previous reports. Gherardi et al.(42) reported an increase in the esterified cholesterol and phospholipid content of VLDL but the subjects in this study were all children which may go some way to explaining the discrepancy. Muls(49) identified two peaks in the LDL density range in some nephrotic subjects with the presence of higher density, cholesterol- and protein-rich particles. These findings were not confirmed by compositional changes over the whole LDL range. However, preliminary results of LDL

subfractionation by density gradient ultracentrifugation and gradient gel electrophoresis(224) in the Glasgow Royal Infirmary lipid research laboratory have consistently shown the presence of a denser species of LDL(LDL-III) in these subjects. A similar finding has been reported in patients with proven coronary artery disease(224). These particles are thought to be formed in the presence of hypertriglyceridaemia where slowly removed VLDL species are remodelled by lipases activity to produce small dense LDL particles.

Most studies of nephrotic hyperlipidaemia have emphasisied the relationship between the degree of hyperlipidaemia and the severity of renal disease as measured by hypoalbuminaemia or quantitative proteinuria. The three largest series(41,46,48) have found the closest association to be between plasma cholesterol and the inverse of serum albumin. No significant relationship was demonstrated with quantitative proteinuria in the two more recent studies (46,48). The data presented here shows serum albumin to be inversely related to LDL-cholesterol, only very weakly to total cholesterol and not at all to triglycerides. Relationships with all three parameters were found for proteinuria. Extending the study to subjects with lower levels of proteinuria(quantified accurately by urinary albumin assay) revealed that this relationship with cholesterol concentrations existed even at lower levels of

protein excretion. This may be important in view of reports suggesting microalbuminuria may predict a higher risk of future cardiovascular disease(149,150).

It has been postulated that the relationships between hyperlipidaemia and both hypoalbuminaemia and proteinuria are causal. Increased hepatic synthesis of lipoproteins has been shown in a variety of animal models. In man, lowered serum albumin levels may stimulate a general increase in hepatic protein synthesis resulting in elevated plasma concentrations of lipoproteins and other larger proteins(e.g. IgM, alpha-2-macroglobulin, fibrinogen). This may be mediated directly by albumin or by plasma oncotic pressure(47). The studies of Baxter and others(73,74) which demonstrated the ability of infusions of albumin, dextrans and other oncotic agents to ameliorate the lipid abnormalities provide strong support for this theory. However, recent work in man and animal models (38,225) has demonstrated a divergence between albumin synthetic rates and hyperlipidaemia. Others have proposed that the loss of a "liporegulatory" substance in the urine is responsible for the reduced catabolism of very low density lipoproteins and that proteinuria is the best predictor of hyperlipidaemia. ApoCII was an obvious candidate but although detectable in nephrotic urine it does not appear to be depleted in plasma. Staprans et al.(84) isolated a glycosaminoglycan co-factor from

nephrotic urine and this may yet prove to be very important. However, it seems certain that more than one mechanism accounts for the hyperlipidaemia and this is considered in more detail below.

Further studies

There have been relatively few studies of the apolipoproteins in patients with nephrotic syndrome(44). Further work is required to document the quantity and distribution of apolipoproteins among lipoprotein subfractions. Disturbances of apolipoproteins may explain some of the defects of lipoprotein metabolism in this condition. It is increasingly realised that the protein moieties of lipoproteins play a crucial role in determining their metabolic fate(226).

9.2 LIPOPROTEIN METABOLISM IN NEPHROTIC SYNDROME

The kinetic studies presented in this thesis were designed to elucidate the metabolic abnormalities underlying nephrotic hyperlipidaemia. The conclusions will be considered separately for triglyceride-rich(chylomicrons/ VLDL) and low density lipoproteins.

9.2.1 Triglyceride-rich lipoproteins

Despite a number of animal studies demonstrating a defect in chylomicron metabolism in nephrosis(166-168), there is a paucity of data on humans. Few studies have carefully evaluated the presence of fasting hyperchylomicronaemia in nephrotic syndrome. The available evidence is conflicting (46,48). Difficulties in separating chylomicrons from large, triglyceride-rich VLDL species may be resolved by newer specific assays of apoB48.

Using the technique of a vitamin A-loaded, standardised fat meal followed by serial measurements of triglyceride and retinyl palmitate in the d<1.006g/mL fraction, no overall difference in post-prandial lipaemia was demonstrated between a group of patients with nephrotic-range proteinuria and a control group. However, this summary statistic masks the fact that in both groups there was substantial inter-individual variation in the duration and magnitude of post-prandial lipaemia. Subjects with an E2 allele were shown to have a persistence of retinyl palmitate at 24 hours consistent with impaired removal of chylomicron remnants(158). However, the most abnormal lipaemic response was seen in a nephrotic patient with an E4/E3 phenotype and the reason for the delayed clearance in this case is unclear. Post-heparin LPL and HTGL activities were not significantly different between the two groups.

This contrasts with the significant decrease in the removal of two species of VLDL in nephrotic subjects shown in chapter 5. This is consistent with studies in rats(63,64) and in man(66,69). In Vega's study(69) using a similar technique, the average transit time for VLDL apoB was increased 3x above normal in four nephrotic subjects. In contrast, a preliminary report by Moorhead(70) suggested that the half life of VLDL apoB was no different from controls although there was a very wide spread of results in the nephrotic group. The discrepancy between the catabolism of chylomicrons and VLDL in the current studies may be due to compositional changes in VLDL which are not seen in chylomicrons in nephrotic syndrome in man. This is supported by the normal lipase activities. Compositional analysis of the apoB-containing lipoproteins fractionated by DGUC showed an increase in the free cholesterol content of these lipoproteins. This compositional changes has been shown to decrease the rate of lipolysis of triglyceride-rich lipoproteins(227). Chylomicrons were not specifically separated from VLDL species in the fat load tests. Therefore, no clear conclusions can be drawn regarding the composition of these particles. However, the small increases in free and esterified cholesterol resulting in a higher cholesterol:phospholipid ratio at baseline disappeared six hours after a fat meal suggesting that, in contrast to those of hepatic origin, the composition of lipoproteins of intestinal origin is not altered in

nephrosis. In neither study was the apolipoprotein content of the triglyceride-rich lipoproteins determined and it is possible that changes in the absolute or relative amounts of apoC's and apoE may influence the catabolic potential of these particles. This is certainly worthy of further study although simple, accurate and precise assays of individual apolipoproteins are not readily available.

Although catabolic defects of triglyceride-rich lipoproteins have been recognised in nephrotic syndrome, most workers have emphasised the importance of increased hepatic lipid, protein and lipoprotein synthesis as the main cause of the hyperlipoproteinaemia. The data in chapter 5 show that in nephrotic patients the rates of apoB synthesis vary widely from normal to 4x normal. Overall, there was no significant difference between the two groups although there was a consistent increase in the production of $VLDL_2$ apoB. This suggests apoB production is increased relative to triglyceride with the elaboration of smaller, triglyceride-poor VLDL species. These results are in accord with those of Vega(69) who found an average apoB production rate in VLDL of 10.7mg/kg/day(range 6.0-18.5). However, Moorhead's study(70) had much higher apoB production rates(mean 75.0mg/kg/day). These differences may reflect the heterogeneity of the nephrotic population since Moorhead's patients may have been more severely nephrotic as judged by their higher plasma lipid

concentrations than the patients in the current study. In this relatively small group of subjects, there was no association between apoB production rates and serum albumin or proteinuria and the reason for the wide variation in apoB production rates is not clear.

9.2.2 LDL Metabolism

Elevations of LDL-cholesterol are the earliest and most consistent lipid abnormality found in nephrotic syndrome. In chapter 6, evidence is presented that both catabolic and synthetic defects of apoLDL turnover are responsible. There was a highly significant reduction in the fractional catabolic rate of LDL, mainly due to decreased activity of the receptor-mediated pathway. Possible mechanisms for this were discussed. It was hypothesised that disturbances of mevalonate metabolism may stimulate hepatic cholesterol synthesis and thereby reduce apo B/E receptor activity (73). However, further work on mevalonate metabolism in human nephrotic syndrome is required to confirm the animal studies.

Three previous studies of LDL metabolism in nephrotic syndrome have been published over the last twenty years although none of these differentiated between the two pathways for LDL catabolism. Early work by Scott *et al.* (67) showed a modest decrease in apoLDL FCR compared to

controls but this was not statistically significant. Two more recent studies(48,69) have found virtually normal FCR's although in both the FCR's of the control groups were relatively low(0.30 and 0.33pools/day respectively) compared to the controls reported here(0.38pools/day) and other studies(180). None of these studies took account of urinary loss of LDL which would have led to a small overestimate of the true FCR but would not alter the results significantly. Other possible reasons for these discrepancies were discussed in chapter 6. In the further studies described in chapter 7, the low apoLDL FCR's were confirmed and this does appear to be a consistent finding in these subjects using the methods presented here.

All three previous studies of LDL metabolism in nephrotic syndrome have concluded that increased LDL production is the main cause of the elevated plasma level. In the current work, there was a very strict relation between apoLDL production and quantitative proteinuria and only those with >10g/day clearly had increased synthesis.

These studies demonstrate that both decreased catabolism and increased production of LDL are responsible for the hyperlipidaemia and that reduced receptor-mediated catabolism is the earliest detectable and, perhaps, most important metabolic defect. The cause of this has not been determined.

These results offer new insights into lipoprotein metabolism in the nephrotic syndrome but need to be qualified by the limitations of the techniques employed. Firstly, these subjects are investigated in a new steady state, a prerequisite for these studies. The changes in lipoprotein turnover which initiate the hyperlipoproteinaemia could conceivably be different from those which maintain it. The metabolic changes which occur at the onset of nephrosis in man are not known and would be difficult to study even in patients with frequently relapsing nephrotic syndrome. Secondly, the limitations of radiolabelled autologous tracers need to be considered. These were discussed earlier but it should be stressed that the fractional catabolic rate is the only kinetic parameter measured directly(from the slope of the plasma decay curve). Production rates are derived from the product of pool size and FCR on the assumption of a steady state. Furthermore, the rationale of these investigations is that the plasma lipid and lipoprotein concentrations are governed by changes in metabolic parameters and not vice versa. The evidence for this stems from the classical studies of Langer et al.(139) who demonstrated a reduced LDL FCR in familial hypercholesterolaemia which was subsequently shown to be due to a well-defined defect of the LDL receptor. Other work by Thomson et al.(177) has shown that acute reductions in the LDL pool size by plasmapheresis during a LDL turnover did not change the FCR

measured by the urine:plasma ratio. If lipoprotein levels rise above the maximum capacity of a catabolic route, this will inevitably lead to a decline in the FCR of that particle. This has been considered in some detail in animals by infusing lipoproteins to alter steady state levels and repeating kinetic studies(178). In the studies presented above, the maximum capacity of the various metabolic pathways has not been determined. However, the reductions in fractional catabolism of $VLDL_1$ apoB were seen even in subjects with only slightly expanded $VLDL_1$ apoB mass and normal production rates indicating that reduced catabolism was the primary defect.

Further studies

Considerably more work needs to be done before the sequence and importance of the various metabolic disturbances in nephrotic syndrome can be identified. The use of stable isotopes detected by mass spectroscopy offers new opportunities for research into lipoprotein metabolism in the next decade. These isotopes will permit safe and repeated measures of synthesis of apolipoproteins and lipids. The relationship with synthesis of other proteins, particularly albumin, could be assessed relatively easily and subjects, including children, could be studied in relapse and in remission. These studies may be seen as complementary to the use of radiolabelled tracers.

The effect of changes in composition of VLDL and LDL species on their metabolic fate might be investigated by crossover studies in man(initially performed in 1958 by Gitlin) where lipoprotein tracers prepared from both normal and nephrotic subjects labelled with different isotopes are administered to both to compare their plasma decay. However, there are considerable practical and ethical difficulties in ensuring that the donors are not harbouring infective agents and, at present, these have not been considered feasible.

9.3 TREATMENT OF HYPERLIPIDAEMIA IN NEPHROTIC SYNDROME

The arguments concerning the risk:benefit ratio of lipid lowering therapy in nephrotic subjects were considered in chapter 1. In the absence of well-conducted, controlled, prospective studies, a number of workers have advocated an individualised approach to lipid-lowering therapy(228,229). Initially, all other modifiable cardiovascular risk factors should be corrected with special attention to smoking and blood pressure control. Where the nephrotic syndrome is persistent, renal function is well-preserved and hyperlipidaemia is present, specific measures to reduce plasma and LDL-cholesterol are probably worthwhile. A low cholesterol diet(<300mg/day) with a polyunsaturated: saturated fat ratio of approximately 1.0 is advisable although by itself is unlikely to lower plasma cholesterol

to the "normal" range. The decision to add drug therapy is entirely empirical. Total cholesterol levels above 7.8mmol/L may well justify treatment and some would advocate a threshold of 6.5mmol/L or even lower for drug therapy. In all cases, the patients overall coronary risk profile may influence a decision to start therapy - where other factors are present a lower threshold may be adopted.

The study presented in chapter 7 and other published work(125,128,129) indicate that inhibitors of HMG CoA reductase are very effective in reducing plasma and LDL-cholesterol although not always to "normal" levels. In these and longer term(117) studies of small numbers of nephrotic patients, there have been few significant side-effects. However, in the series of patients presented here, the rapid progression of a posterior capsular lens opacity in one patient shortly after starting therapy warrants further study. Careful follow-up with ophthalmological assessment is advisable until more data is available. Serum creatine kinase levels also need careful monitoring.

The mechanism whereby HMG CoA reductase inhibitors reduce plasma and LDL-cholesterol appears to differ among individual patients(chapter 7). This is in accord with studies of some other hyperlipidaemias, particularly primary moderate hypercholesterolaemia(204,205). These

studies challenge the contention that inhibition of cholesterol biosynthesis reduces intra-hepatic cholesterol pools, increases apoB/E receptor expression and accelerates catabolism of LDL. This is an oversimplification of the action of these drugs on the complex metabolic processes involved in the assembly, secretion, interconversion and removal of circulating lipoproteins.

Experience with other lipid-lowering agents was considered in chapter 1. There are relatively few studies of there use although gemfibrozil(123) seems to be a safe fibric acid derivative and may have the advantage of raising HDL-cholesterol and lowering triglyceride(103). There is no data however on the potential atherogenicity of hypertriglyceridaemia in the nephrotic syndrome nor do these subjects appear to be at risk from pancreatitis.

The role of lipid-lowering therapy in the progression in renal disease has recently received considerable attention (230). Several authors(107,108) have hypothesised on the possible mechanisms of lipid nephrotoxicity. There are a number of studies in animal models of renal disease which have demonstrated the ability of lipid lowering drugs to ameliorate some measures of renal damage(230,115). As yet, there is no evidence to prove that such treatment is effective in man.

Further study

The indications for lipid-lowering therapy in nephrotic syndrome will only be clearly defined by prospective, double-blind controlled studies. There are two possible end-points of such studies - cardiovascular morbidity/ mortality and progression of renal failure. At a conservative estimate, these studies would require 250-500 subjects to be recruited and followed-up for 3-5years. Nephrotic syndrome in adults has an annual incidence of 1/100000. If only newly-presenting patients were eligible, this would entail recruiting all new patients in the UK to finish recruitment in one year! The logistical problems are therefore formidable and may preclude such studies. At present, some nephrologists have adopted an individualised approach to therapy as described above while others do not actively attempt to correct the lipid abnormalities.

9.4 Conclusion

Investigations of hyperlipidaemia in human nephrosis may lead to advances in our understanding of both normal and abnormal lipoprotein metabolism and the complex pathophysiological changes that accompany proteinuria. The results of the studies presented here detail some of the changes found in the kinetics of circulating lipoproteins. There is considerable heterogeneity among nephrotic

subjects and the precise basis of these kinetic alterations remains to be established.

Drugs are now available which can substantially reduce the hypercholesterolaemia of this condition. However, their place in routine practice will only be proven by appropriate, controlled trials and longer term experience.

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Metabolism of apolipoprotein B-containing lipoproteins in subjects with nephrotic-range proteinuria

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Metabolism of apolipoprotein B-containing lipoproteins in subjects with nephrotic-range proteinuria. Although hyperlipidemia is a well recognized complication of the nephrotic syndrome, the precise disturbances of lipoprotein metabolism which cause the elevated plasma lipid and lipoprotein concentrations have not been clearly defined in humans. This study examines the metabolism of apolipoprotein B-containing lipoproteins in patients with nephrotic-range proteinuria and in healthy controls. Two radioiodinated tracers of very low density lipoproteins ($VLDL_1$, Sf 60 to 400, and $VLDL_2$, Sf 20 to 60), were used to trace the metabolism of apolipoprotein B through the delipidation cascade from very low density lipoproteins (VLDL) to low density lipoproteins (LDL). The data from the apoB specific radioactivity curves and the pool sizes of apoB in four subfractions were analyzed by a multicompartmental modeling procedure using the SAAM 30 program. The main findings in the nephrotic group were: 1.) a consistent decrease in the fractional rate of apoB transfer from $VLDL_1 \rightarrow VLDL_2$ (median values—nephrotic 0.92 pools/day vs. controls 3.66, $P < 0.02$) and from $VLDL_2 \rightarrow$IDL (1.49 vs. 2.74, $P < 0.05$); 2.) increased secretion of apoB into VLDL2 (14.5 mg/kg/day vs. 4.2, $P < 0.02$); 3.) a trend towards decreased removal of IDL and LDL attributable to a defect in LDL receptor-mediated removal as previously shown (*Metabolism* 39:187–192, 1990). These findings suggest that catabolic defects of the apo B-containing lipoproteins are as important as increased hepatic synthesis in the pathogenesis of nephrotic hyperlipidemia in humans.

Hyperlipidemia has long been recognized as a complication of human nephrotic syndrome. However, the metabolic changes which result in the elevations of plasma lipids and lipoproteins have not been satisfactorily established [1, 2]. Evidence from experimental animal models of nephrotic syndrome has demonstrated increased hepatic lipoprotein secretion as the major cause of the hyperlipoproteinemia with some contribution from delayed lipoprotein catabolism [3]. It is not certain, however, that these findings can be extrapolated to humans. In recent years a considerable body of information regarding normal and abnormal lipoprotein metabolism has been obtained from techniques which employ radiolabeled tracers to follow the fate of lipoprotein particles in vivo [4]. Despite a number of early studies in nephrotic syndrome [5–8], these methods have not been widely employed to investigate this form of secondary hyperlipidemia.

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Hyperlipidemia in nephrotic syndrome may take various forms [1]. The most common finding is an elevation of low density lipoprotein (LDL) cholesterol levels. In more severe cases with heavy proteinuria and severe hypoalbuminemia, hypertriglyceridemia and elevated very low density lipoprotein (VLDL) cholesterol levels may become the dominant abnormalities [9, 10]. High density lipoprotein (HDL) cholesterol levels have been variously recorded as high, low or normal [11, 12]. The main abnormalities therefore involve the apolipoprotein B-containing lipoproteins. In the general population this pattern is associated with an increased risk of coronary atherosclerosis. Patients with nephrotic syndrome and/or renal failure are considered to have an increased incidence of ischemic heart disease [13], although this has been challenged by some [14] and the benefits of lipid-lowering therapy have not been clearly shown. Recent interest has also focused on the possible role of hyperlipidemia in the progression of many renal diseases [15, 16]. A clearer understanding of the pathophysiological disturbances of lipoprotein metabolism may allow more precise targeting of future therapeutic efforts to reduce the hyperlipidemia.

The kinetic characteristics of very low and low density lipoproteins can be followed by trace-labeling of the apoB moiety. Each particle contains one molecule of apoB which is integral to its structure and remains with the lipoprotein during its lifetime in the circulation. Radiolabeled apoB can therefore be used to follow the metabolic fate of lipoprotein particles. Recent reports have examined LDL apoB kinetic studies in patients with nephrotic syndrome and hypercholesterolemia [17, 18]. In our own study of the condition we concluded, on the basis of experiments using dual tracer methods, that the main reason for an increased LDL apoB pool in nephrotic syndrome was a reduced clearance (that is, fractional catabolic rate) of LDL by the receptor-mediated pathway [19]. Synthetic rates were increased only in those patients with proteinuria in excess of 10 g/day. There is little data on the metabolism of apoB in VLDL. In a recent report of four nephrotic subjects Vega and Grundy found delayed catabolism of VLDL with normal production rates [17]. However, these workers used a tracer of a wide density range which included both VLDL and IDL particles. Since there is considerable heterogeneity in the metabolism of VLDL particles, more precise information may be obtained by using tracers with well-defined flotation characteristics [20].

Table 1. Clinical characteristics of nephrotic group[a]

Subject	Sex	Age *years*	Weight *kg*	Body mass index *kg/m²*	Histology	Duration of disease *months*	Treatment
CM	M	60	77	26.3	FGS[b]	310	Nifedipine, metoprolol, ranitidine
AP	M	55	98	30.9	Membranous	42	Frusemide, atenolol, nifedipine
WS	M	58	75	27.9	Membranous	55	Atenolol, enalapril, warfarin
JH	M	53	90	29.4	IgA	10	Digoxin, bumetanide, warfarin
JG	M	48	57	24.1	Membranous	420	Nifedipine, atenolol, frusemide
MD	F	28	49	19.4	MCGN[c] II	159	Phenobarbitone
RG	M	66	80	25.0	Membranous	31	Nifedipine
CF	F	34	61	22.6	MCGN II	66	Frusemide

[a] Control group: $N = 5$, M:F = 3:2, age 36 to 46 years, weight 71 to 89 kg
[a] Focal glomerulosclerosis
[a] Mesangiocapillary glomerulonephritis

Table 2. Renal function tests

Subject	Serum creatinine *umol/liter*	Serum albumin *g/liter*	Creatinine clearance *ml/min/1.73 m²*	Quantitative proteinuria *g/day*
CM	310	33	19	6.6
AP	190	36	35	10.8
WS	160	33	43	6.1
JH	100	38	58	3.3
JG	420[a]	32	18	9.6
MD	90	33	90	6.2
RG	250	27	40	8.3
CF	70	31	89	4.5

Values represent mean of repeated measures over the study period.
[a] Serum creatinine rose from 370 to 450 μmol/liter over the study period.

The aim of this study was to investigate the metabolism of the apoB moiety of very low density lipoproteins in patients with nephrotic syndrome. Quantitative assessments of apoB production, catabolism and flux through the delipidation cascade from VLDL to LDL were derived from multicompartmental analysis of apoB pool sizes and plasma decay/appearance curves.

Methods

Patients

Patients were recruited from the outpatient clinic of the renal unit at Glasgow Royal Infirmary according to the following selection criteria: 1.) quantitative proteinuria > 3.0 g/day; 2.) serum creatinine < 400 μmol/liter; 3.) plasma cholesterol > 7 mmol/liter. All patients had a primary glomerular disease proven by renal biopsy. Diabetes mellitus, thyroid disorders, liver disease, pregnancy, amyloid and neoplasia were exclusion criteria. Treatment with corticosteroids was also considered an exclusion criterion although patients on diuretic and/or antihypertensive therapy were included. Clinical details of the patients are given in Tables 1 and 2. A group of five normolipidemic volunteers was also studied. They were recruited from a coronary screening program and were selected because of homozygosity for the E3 polymorphism of apolipoprotein E. This is the most common apo E phenotype, and since apo E polymorphisms may influence apo B metabolism [20] these subjects were selected as normal controls. Throughout the studies patients were asked to follow their normal diets. None of the proteinuric group had been prescribed a specific diet. The study was approved by the Ethics Committee of Glasgow Royal Infirmary.

The technique for performing apoB kinetic studies has been described previously [21, 22]. Briefly, subjects were asked to attend fasting and approximately 200 to 250 ml of plasma was removed by plasmapheresis. Oral potassium iodate (170 mg twice a day) was started and continued for four weeks to suppress thyroidal uptake of radioiodide. VLDL (daily < 1.006 g/ml) was recovered from the plasma by ultracentrifugation in a Beckman Ti60 rotor (18 hr, 39000 rpm, 10°C). Total VLDL was removed by aspiration of the top 2 to 4 ml from each 25 ml tube and diluted with the infranatant salt solution to achieve a lipoprotein concentration equivalent to a plasma triglyceride level of 1.5 mmol/liter (for example, triglyceride 3 mmol/liter – dilute 1:2). This minimises carryover of $VLDL_1$ to $VLDL_2$ during preparative ultracentrifugation. The solution density was then raised to 1.118 g/ml by the addition of solid sodium chloride (0.171 g/ml). Six 2 ml aliquots were subjected to cumulative flotation ultracentrifugation on a discontinuous salt gradient in a SW 40 rotor as adapted from Lindgren, Jensen and Hatch [23]. $VLDL_1$ (Sf 60 to 400) and $VLDL_2$ (Sf 20 to 60) were isolated by this method and aliquots (2 ml) were then labeled with ^{131}I and ^{125}I, respectively, as previously described [24]. Free radioiodide and salt were removed by gel filtration on a PD10 column (Pharmacia, Uppsala, Sweden) followed by extensive dialysis against 0.15 M NaCl pH 7.4. Three days after plasmapheresis the subjects returned, having fasted for more than 12 hours, and approximately 50 μCi of each tracer was administered intravenously. The tracers were sterilized by passage through a 0.45 micron filter (Acrodisc; Gelman Science, Northampton, UK) immediately prior to injection. Blood samples were collected at 10 minutes and then frequently over the first 10 hours, after which a low fat meal was allowed. Further samples were taken at 14 and 24 hours and then daily fasting samples were obtained for 12 days. Each plasma sample was subjected to ultracentrifugation as above to isolate four fractions: $VLDL_1$, $VLDL_2$, IDL (Sf 12 to 20) and LDL (Sf 0 to 12). ApoB was precipitated [25] from each fraction by the addition of an equal volume of freshly-distilled 1,1,3,3-tetramethylurea (TMU). The pellet was then delipidated and the apoB specific activity calculated from the protein content of the precipitate redissolved in 0.5 M NaOH (measured by a modified

Lowry assay [26]), and the radioactivity counted in a twin channel gamma counter (Canberra Packard, Pangbourne, UK).

The plasma concentration of each lipoprotein fraction was estimated from the sum of the concentrations of lipids and protein in each fraction isolated from pooled plasma. Total cholesterol, free cholesterol, triglyceride and total phospholipids were measured by enzymatic colorimetric assays (Boehringer Mannheim, Lewes, East Sussex, UK). Protein was measured by the Lowry method [26]. The mass of each fraction was corrected for ultracentrifugal losses by comparing the total cholesterol recovered in the four fractions obtained by cumulative flotation ultracentrifugation with the cholesterol content of the non-HDL cholesterol measured by routine beta-quantification [27]. Recovery varied from 82 to 92% and the calculated lipoprotein masses were each corrected by this factor. This assumes that each fraction was lost in similar amounts and that the different components of the lipoproteins were also lost in equal proportion.

Apo B concentration was measured in aliquots of each fraction by precipitating apoB with TMU and measuring the protein content in the supernatant solution [25]. ApoB concentration was derived from the difference between total and TMU-soluble protein. Intravascular pool sizes of apoB were obtained from the product of apoB concentration and plasma volume estimated from the dilution of the $VLDL_2$ tracer at ten minutes. Steady state conditions of lipoprotein metabolism were confirmed by measurements of plasma lipid and lipoprotein levels at regular intervals over the study period. The coefficient of variation for each patient's plasma cholesterol level was < 10%. The figure for plasma triglyceride ranged up to 20%. Apolipoprotein E phenotyping was performed by isoelectric focusing [28, 29].

Kinetic analysis

The radioactivity in apoB in each lipoprotein fraction was obtained by multiplying the specific activity (cpm/mg) at each time point by the pool size (mg apoB) of the appropriate fraction. All radioactivities were then expressed as a percentage of the total apoB activity present in the 10 minute sample calculated from the sum of radioactivity in $VLDL_1$, $VLDL_2$, IDL and LDL. Plasma decay curves and pool sizes were then analyzed by a multicompartmental model using the SAAM/CONSAM 30 program [30, 31]. The rate constants and estimated compartmental masses were used to derive fractional transfer and catabolic rates, production rates for apoB in $VLDL_1$, $VLDL_2$ and, where necessary, LDL and flux rates between compartments. Production and flux rates are expressed as mg apoB/kg body weight/day. Fractional transfer and catabolic rates are presented as pools/day.

The model used to analyse apoB metabolism in very low lipoproteins has been described previously [22, 32]. In summary, it consists of two parallel pathways from large and small VLDL ($VLDL_1$ and $VLDL_2$, respectively) through IDL to LDL. The transfer of apoB along this chain is thought to be dependent on stepwise delipidation mediated by lipase activity. Direct input of apoB occurs both at $VLDL_1$ and $VLDL_2$. Where the calculated mass in LDL derived from these precursors falls significantly short (>10%) of the measured mass, direct synthesis of LDL is invoked. Removal of apoB occurs at each level of the pathway. This model, however, did not allow an adequate fit of the data from the proteinuric group in that the calculated pool mass for $VLDL_1$ was underestimated in a number of these subjects who had an expanded $VLDL_1$ pool. Several subjects also demonstrated a biexponential decay of $VLDL_1$, suggesting the presence of an additional slowly metabolized component within this fraction. A revised model was therefore developed for the nephrotic subjects (Fig. 1). The numbering of these compartments reflects the evolution of the mathematical model and is presented in the form used in these studies. In this version, $VLDL_1$ is composed of three compartments with input to and direct removal from two parallel pathways one of which does not feed into the delipidation chain and contribute to the mass of other lipoprotein fractions. These extra compartments (29 and 26) were not required for the analysis of the control subjects.

Statistical analysis

Non-parametric methods were used because of the small numbers and the wide spread of results in the nephrotic group. Results are shown as medians and ranges. Differences between nephrotic and control groups were tested by the Mann-Whitney U test and correlations by Spearman's rank test. The plots comparing the specific activities of the two tracers between the groups are shown as mean and standard deviations. The compositional data for both groups were of comparable variance and were analyzed by a two-sample *t*-test.

Results

This group of patients with moderate to heavy proteinuria demonstrated variable rises in plasma cholesterol and triglyceride (Table 3). Serum albumin was remarkably well maintained even when quantitative proteinuria approached 10 g/day, and the inverse relationship between serum albumin and plasma cholesterol often seen in nephrotic syndrome did not exist. LDL cholesterol was moderately elevated in seven patients with one patient (JG) having a very high level of 10.0 mmol/liter. VLDL cholesterol was high in three patients who all demonstrated a relatively high VLDL cholesterol/plasma triglyceride ratio. One of these patients (WS) possessed an E2 allele of the apoE polymorphism. The increased plasma lipid levels were reflected in increased concentrations of $VLDL_2$, IDL and LDL. $VLDL_1$ was not significantly increased (Fig. 2). There were no significant differences in the relative composition of all four lipoprotein fractions between controls and proteinuric subjects (data not shown).

Kinetic studies

Figure 3 compares the metabolism of apoB from each tracer in the four subfractions by plotting the mean radioactivity at each time point for both proteinuric and control groups. The results of the kinetic analysis are presented in Tables 4A to D.

$VLDL_1$

The ^{131}I $VLDL_1$ apoB tracer was cleared more slowly in the nephrotic group than in controls primarily due to a consistent reduction in the fractional transfer of $VLDL_1$ apoB to $VLDL_2$ (median 0.92; range 0.22 to 3.31 vs. 3.66; 2.48 to 5.16, $P < 0.02$; Table 4A). Both nephrotic and control groups showed wide variation in the fraction of the $VLDL_1$ apoB pool which was catabolized directly from the plasma compartment. In the

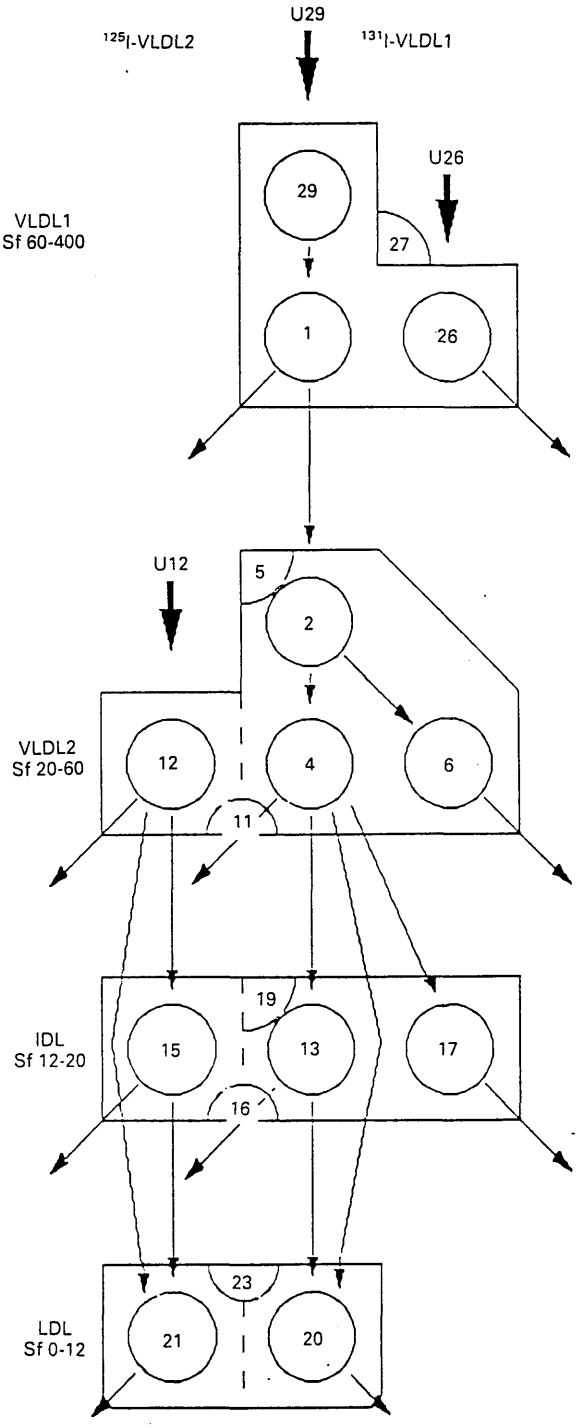

Fig. 1. *Model of lipoprotein metabolism used for multicompartmental analysis.* This model has been built up over several years from apoB kinetic studies in various dyslipidemic conditions. ApoB production occurs in both $VLDL_1$ and in $VLDL_2$, which is shown as U29/U26 and U12, respectively. Parallel pathways are shown for apoB derived from the two VLDL species. ApoB is transferred along a delipidation chain or removed from the system according to the arrows. The metabolic pathways are considered to be unidirectional and to follow first order kinetics.

proteinuric group, the $VLDL_1$ apoB pool size varied from normal to eight times normal and this was related to plasma triglyceride level (Table 3). The calculated rate of apoB flux from the large triglyceride rich $VLDL_1$ to smaller $VLDL_2$ was reduced significantly (1.9 mg/kg/day; 1.1 to 2.9 vs. 2.7; 1.8 to 5.1, $P < 0.05$) even in those patients with normal pool sizes (CF, MD, RG, JG). There was wide variation in the secretion of apoB into $VLDL_1$ (2.6 to 64.8 mg/kg/day) in the proteinuric group. Although four patients had very high production there were no statistically significant differences between the groups.

$VLDL_2$

The $VLDL_2$ apoB tracer was also cleared from the plasma of the nephrotic group at a slower rate than normal. This was seen with both tracers, that is, directly labeled $VLDL_2$ and the material transferred from $VLDL_1$ (Fig. 3B and 3E). Again this was attributable to a reduced delipidation rate to IDL (or LDL by pathways 12→21 and 4→20; Table 4B). Direct removal of $VLDL_2$ apoB from plasma was highly variable and not significantly different between the two groups. The $VLDL_2$ apoB pool was expanded in the proteinuric group (2 to 3 × normal) mainly due to a marked increase in de novo synthesis of apoB in $VLDL_2$ (14.5 mg/kg/day; 5.1 to 32.2 vs. 4.2; 2.9 to 8.8, $P < 0.02$). Reduced catabolism made a relatively smaller contribution to the expansion of the pool size. The larger $VLDL_2$ pool led to a modest increase in the amount of IDL apoB formation, although this was not statistically significant (10.4 mg/kg/day; 4.4 to 17.2 vs. 5.8; 2.7 to 8.7; $P = 0.12$). This occurred despite the reduced fractional transfer rate from IDL to LDL.

IDL

The mean plasma radioactivity curves for ^{125}I and ^{131}I reached comparable peak values in IDL at similar times for both groups (Fig. 3 C and F). Thereafter, the curves diverged with the normals, showing an initial more rapid decay that was due to direct catabolism of the lipoprotein (Table 4C). There was no obvious difference in the rate at which both groups of subjects accumulated ^{125}I and ^{131}I in LDL apoB, indicating a similar degree of IDL to LDL conversion (Table 4C). The expansion of the IDL apoB pool in nephrotics was due to a combination of increased synthesis from $VLDL_2$ and diminished direct removal. This had the net effect of channeling an increased amount of apoB from IDL to LDL (Table 4D).

LDL

The appearance curves for ^{125}I and ^{131}I apoB radioactivity in LDL were similar for control and nephrotic groups (Fig. 3 D and G). Peak values were achieved at a later time in the latter group but the extent of transfer from $VLDL_1$ and $VLDL_2$ was approximately the same. The most pronounced difference in the curves was delayed clearance of both tracers from the LDL of nephrotic subjects. Normal subjects had a biphasic LDL decay curve consisting of a relatively rapid catabolism up to 120 hours, and then a slower terminal phase. Visual inspection of the averaged data from the patients with nephrotic syndrome indicated a lack of the early rapid phase of LDL degradation. The observed fractional catabolic rate for LDL should be corrected for urinary loss of whole lipoprotein. This is not possible to determine from urine collections during a VLDL turnover because a high proportion of injected radioactivity is

Table 3. Plasma lipid and lipoprotein concentrations

Subject	E/E phenotype	Cholesterol	Triglyceride	VLDL-cholesterol *mmol/liter*	LDL-cholesterol	HDL-cholesterol	VLDL-cholesterol: plasma triglyceride ratio	apoB pool[a] size *mg*
Nephrotic								
CM	4/3	9.4	4.5	3.0	5.4	1.1	0.67	4306
AP	4/3	8.6	3.5	1.6	6.0	1.0	0.46	5718
WS	3/2	8.6	3.4	3.0	4.8	0.8	0.88	5061
JG	3/3	12.8	3.1	2.1	10.0	0.8	0.68	4848
JH	3/3	8.3	2.8	1.1	6.0	0.9	0.38	3181
MD	3/3	7.3	1.9	0.9	4.9	1.5	0.47	2038
RG	3/3	7.3	1.6	0.6	5.4	1.3	0.38	4796
CF	4/3	7.6	1.2	0.6	5.4	1.7	0.46	2640
Controls								
NC	3/3	5.0	1.7	0.8	3.1	1.0	0.46	1970
CD	3/3	6.2	2.6	1.0	4.1	1.4	0.40	2860
MF	3/3	5.8	1.1	0.6	4.0	1.2	0.56	3000
EK	3/3	5.0	1.0	0.4	2.8	1.9	0.37	1450
MM	3/3	5.6	2.2	1.0	3.7	1.2	0.45	2270

[a] Sum of apo B pools in the four lipoprotein fractions

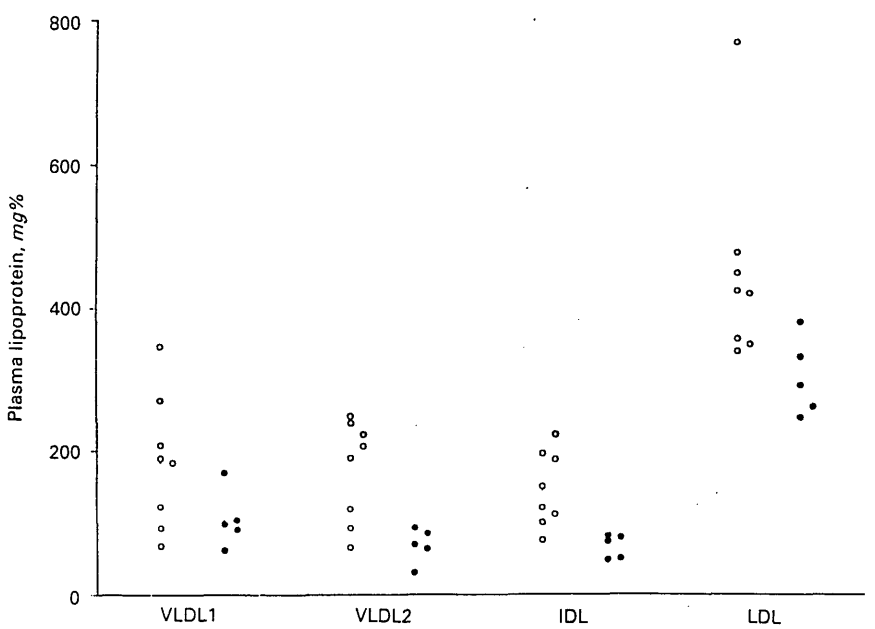

Fig. 2. *Plasma concentrations of lipoprotein subfractions prepared by cumulative flotation ultracentrifugation calculated from the sum of the compositional analysis.* Open circles (○) represent nephrotic subjects and closed circles controls (●). *P* values represent comparisons between control and nephrotic groups: $VLDL_1$ P = NS; $VLDL_2$ $P < 0.03$; IDL $P < 0.01$; LDL $P < 0.02$.

present in components other than apoB (that is, apolipoproteins C and E and lipids). In a previous study of trace-labeled LDL in nephrotic syndrome [19], we estimated the average urinary excretion of undegraded lipoprotein as 7% of total urine radioactivity; the values in Table 4D overestimate the true FCR by this proportion. The LDL FCR was subnormal in most of the disease group, but three (CM, WS and CF) showed normal or even high values, and overall there was no significant difference from controls even when corrected for estimated urinary losses. Likewise, there was a variable expansion of the LDL apoB pool. Its size ranged from normal to twice normal. The production of LDL from $VLDL_2$ and IDL was significantly increased in the nephrotic group compared to normal.

Discussion

Hyperlipidemia is common in nephrotic syndrome. Despite this its origins remain unexplained. Most investigators have considered that the major pathogenic mechanism is increased hepatic synthesis and secretion of lipoproteins [33]. The stimulus for this may be decreased serum albumin, either directly or indirectly via changes in plasma oncotic pressure [34] or viscosity. It has been suggested that there is a non-specific increase in hepatic protein synthesis in response to these factors. However, recently Kaysen et al [35] have examined the relationship between hyperlipidemia and albumin synthetic rates. By replacing a high protein with a low protein diet in patients with nephrotic syndrome, mean albumin synthetic rates were reduced from 17.60 to 12.61 g/1.73 m^2/day, which is in the low/normal range. Although lipoprotein synthetic rates were not measured directly, hyperlipidemia persisted in the face of normal albumin synthetic rates.

Reduced lipoprotein catabolism has also been described in nephrotic syndrome. Several lines of evidence suggest that catabolic defects are present along the VLDL→IDL→LDL

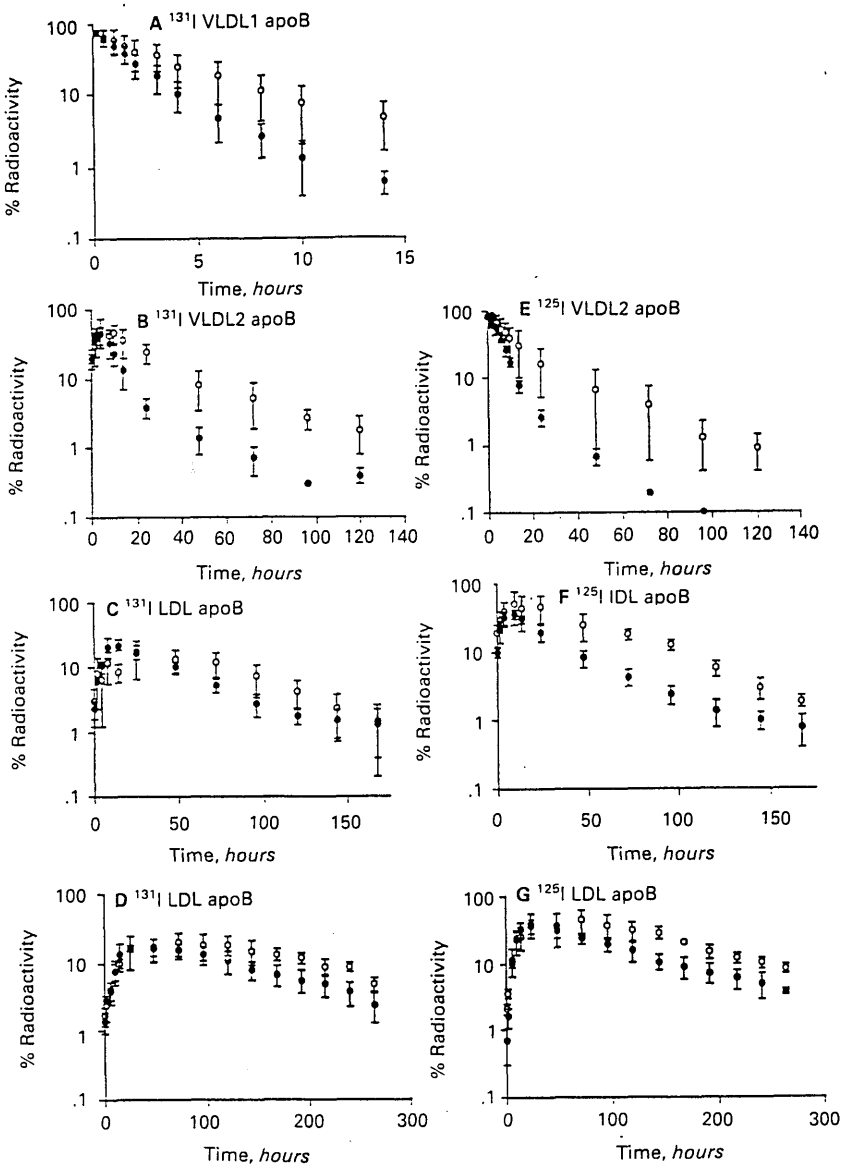

Fig. 3A-G. *Plots of mean radioactivity for ^{125}I and ^{131}I apoB tracers in each subfraction.* Open circles (○) represent nephrotic subjects and closed circles controls (●). Error bars are one standard deviation.

delipidation cascade. Early work by Gitlin et al [5] demonstrated a delay in the catabolism of radioiodinated Sf 100 to 400 lipoproteins. More recently Vega and Grundy [17] have shown reduced clearance of a combined VLDL and IDL tracer (daily < 1.019 g/ml) in four nephrotic subjects compared to controls. The delipidation of VLDL is catalyzed by endothelium-bound lipases in hepatic and extra-hepatic tissues (skeletal muscle and adipose tissue). Decreased activities of these enzymes have been reported in this disorder [36–39], although this finding has not always been confirmed [40–42]. Our own study of LDL metabolism in nephrotic syndrome [19] emphasized the decreased catabolic rate of the lipoprotein as the major contributor to the elevated plasma levels in the majority of subjects investigated. These observations imply that the metabolic disturbances in the lipoprotein transport system in this condition are complex. This led us to undertake the present detailed investigation of apoB kinetics in the nephrotic syndrome.

The eight subjects studied had persistent heavy proteinuria and had been followed up for a period varying from 10 months to 35 years (Table 1). They had moderate hyperlipidemia except for JG who had a markedly elevated LDL cholesterol level. Plasma triglyceride levels varied from low normal (1.2 mmol/liter) to high (4.5 mmol/liter), emphasizing the heterogeneous nature of the study group. Previous larger, cross sectional studies of the incidence of hyperlipidemia in nephrotic syndrome [9, 10] have generally reported relationships between hyperlipidemia and severity of nephrotic syndrome (hypoalbuminemia or proteinuria). These associations were not found in this study, but this probably reflects the small numbers which can be practically studied in lipoprotein kinetic investigations. When apoB-containing lipoproteins were fractionated by cumulative flotation ultracentrifugation significant increases were seen in plasma levels of $VLDL_2$, IDL and LDL (Fig. 3). The composition of all four lipoprotein fractions was normal, indicating that there was no substantial accumulation of cholesterol ester-rich VLDL remnants in the $VLDL_2$ and IDL density

Table 4A. Kinetic parameters of apo B metabolism in $VLDL_1$

Subject	Production *mg/kg/day*	Pool size *mg*	Fractional catabolism *pools/day* Direct	to $VLDL_2$
Nephrotic				
CM	25.3	413	4.54	0.22
AP	11.9	297	3.11	0.85
WS	7.8	210	1.74	1.02
JG	64.8	117	30.6	1.05
JH	2.8	195	0.58	0.74
MD	34.5	83	19.5	0.70
RG	23.0	87	20.1	1.01
CF	2.6	46	0.04	3.33
Median	17.5	156	3.83	0.93
Controls				
NC	8.8	67	4.17	5.16
CD	6.1	52	5.75	4.49
MF	14.5	97	10.8	2.48
EK	11.5	47	14.7	2.71
MM	14.7	106	6.91	3.66
Median	11.5	67	6.91	3.66
P	NS	NS	NS	<0.02

P value by Mann-Whitney comparing groups. Significance taken as $P < 0.05$.

Table 4B. Kinetic parameters of apo B metabolism in $VLDL_2$

Subject	Production *mg/kg/day* Direct	From $VLDL_1$	Pool size *mg*	Fractional catabolism *pools/day* Direct	To IDL
Nephrotic					
CM	14.6	1.2	642	0.44	1.43
AP	15.7	2.6	714	1.40	1.11
WS	14.5	2.9	570	0.03	2.26
JG	11.2	2.2	488	0.0	1.54
JH	5.1	1.6	336	0.61	1.18
MD	32.2	1.2	162	8.44	1.66
RG	17.3	1.1	505	1.55	1.36
CF	9.8	2.5	233	0.12	3.12
Median	14.6	1.9	497	0.53	1.49
Controls					
NC	4.2	4.9	160	0.18	3.88
CD	8.8	2.7	231	2.14	2.14
MF	5.6	2.7	199	0.92	2.81
EK	2.9	1.8	69	2.14	2.74
MM	3.7	5.1	218	1.15	1.93
Median	4.2	2.7	199	1.15	2.74
P	<0.02	<0.05	<0.02	NS	<0.05

P value by Mann-Whitney comparing groups. Significance taken as $P < 0.05$.

Table 4C. Kinetic parameters of apo B metabolism in IDL

Subject	Production from $VLDL_2$ *mg/kg/day*	Pool size *mg*	Fractional catabolism *pools/day* Direct	To IDL
Nephrotic				
CM	11.9	730	0.02	1.2
AP	8.1	1131	0.01	0.69
WS	17.2	1122	0.58	0.56
JG	12.0	595	0.09	1.15
JH	4.4	554	0.0	0.71
MD	5.5	322	0.08	0.75
RG	8.5	754	0.02	0.88
CF	11.7	527	0.21	1.13
Median	10.4	663	0.05	0.82
Controls				
NC	8.7	241	0.76	1.80
CD	5.8	408	0.18	1.02
MF	5.8	358	0.26	1.17
EK	2.7	174	0.37	0.68
MM	5.1	351	0.33	0.76
Median	5.8	351	0.33	1.02
P	NS	<0.05	<0.05	NS

P value by Mann-Whitney comparing groups. Significance taken as $P < 0.05$.

Table 4D. Kinetic parameters of apo B metabolism in LDL

Subject	Production *mg/kg/day* Direct	From $VLDL_2$ and IDL	Pool size *mg*	Fractional catabolism *pools/day*	Total apo B production *mg/kg/day*
Nephrotic					
CM	4.8	11.9	2623	0.49	44.8
AP	0.0	8.0	3617	0.22	27.6
WS	4.7	8.4	3187	0.32	27.2
JG	0.0	12.4	3426	0.21	76.1
JH	0.0	4.6	2247	0.18	8.0
MD	0.0	4.9	1315	0.18	66.7
RG	0.0	8.4	3129	0.22	40.3
CF	0.0	10.0	1842	0.33	12.3
Median	0.0	8.4	2876	0.22	33.9
Controls					
NC	0.0	6.1	1433	0.30	11.4
CD	2.7	4.9	2505	0.26	13.7
MF	0.1	5.2	2044	0.23	13.4
EK	2.6	1.7	1160	0.26	14.3
MM	2.0	4.0	1620	0.28	14.9
Median	2.0	4.7	1620	0.26	13.7
P	NS	NS	NS	NS	NS

ranges despite high plasma triglyceride/VLDL cholesterol ratios in some patients.

Three significant perturbations of apolipoprotein B metabolism were noted in the nephrotic subjects. First, the rates of transfer of $VLDL_1$ apoB to $VLDL_2$ and of $VLDL_2$ apoB to IDL were reduced. This was true even in normotriglyceridemic nephrotic subjects (MD, RG, CF in Table 4A) who had normal $VLDL_1$ apoB pool sizes. Thus the delayed clearance could not be due simply to saturation of the delipidation mechanism. These metabolic steps are believed to involve the action of lipoprotein lipase and, to some extent, hepatic triglyceride lipase [22]. As noted above, a number of studies in both human and experimental nephrotic syndrome have examined post-heparin plasma lipase activities with contradictory results. Chan and co-workers [39], using a substrate-specific method, documented a decrease in lipoprotein lipase with normal hepatic triglyceride lipase activity, while Oetliker et al [42] could not find any significant difference compared to controls for either enzyme using a more standard immunoinhibition method. In vitro, nephrotic serum appears to be able to inhibit lipase activity [39]; a similar finding has been reported in uremia. However, the degree of inhibition did not correlate with reduc-

tions in lipase activity in the patients. Several hypotheses have been advanced to explain the possible reduced lipase activity. Apolipoprotein CII, an important activator of lipoprotein lipase, has been isolated from nephrotic urine. However, estimates of total plasma levels have not confirmed a reduced concentration although recently Kashyap et al [43] have reported that the absolute amount associated with VLDL is reduced. Alterations of the apolipoprotein composition of VLDL may alter its catabolic potential, and account for a recent report that VLDL from nephrotic rats has a reduced clearance rate in normal animals and is more resistant to lipolysis in vitro [44]. Two other explanations have been proposed. Staprans and co-workers [45] isolated a separate lipase co-factor from the urine of nephrotic patients which they identified as a glycosaminoglycan, and Bernard [1] has reviewed the possibility that in hypoalbuminemic states decreased removal of free fatty acids which are normally protein-bound may impair lipolysis.

Second, the synthesis of apoB in the density interval of $VLDL_2$ was increased several-fold on average. This effect was more consistent than the highly variable values seen for $VLDL_1$ apoB synthesis in the nephrotic subjects. The increased direct synthesis (Table 4B) more than compensated for the reduced flux from $VLDL_1$. This in turn led to overproduction of IDL and LDL (Table 4 C and D). Thus, hepatic oversynthesis of apoB was a feature of this group and contributed to the expansion of the $VLDL_2$, IDL and LDL pools. As discussed above, hepatic oversynthesis of apoB-containing lipoproteins is a feature of animal models of nephrotic syndrome [33, 45–49] and has also been reported in humans [50]. In the latter study [50], markedly increased rates of apolipoprotein B production were found in a group of severely hyperlipidemic nephrotic patients, although the full details of these subjects have not been reported. The stimulus to overproduction is unclear. However, in the current study, the finding that triglyceride-poor $VLDL_2$ rather than $VLDL_1$ is overproduced suggests that increased hepatic apolipoprotein rather than triglyceride synthesis was responsible for the elevation in plasma VLDL. This contrasts with the kinetic changes that underly the hypertriglyceridemia of non-insulin-dependent diabetes mellitus [51]. In this condition larger, lipid-rich $VLDL_1$ particles are secreted in excessive amounts but insulin therapy results in a decrease in $VLDL_1$ production and an increase in $VLDL_2$. This is thought to reflect limitation of carbohydrate and free fatty acid flux to the liver, which in the insulin-deficient state stimulates hepatic triglyceride synthesis and production of $VLDL_1$.

The third abnormality observed in nephrotic subjects was a reduced clearance rate (FCR) for both IDL and LDL (Table 4 C and D). The reduction was consistent and significantly different from normal for IDL, whereas the LDL FCR varied from subnormal values in five patients (AP, JG, JH, MD and RG) through normal (WS and CF) to an elevated level in CM. Overall there was a trend towards lower LDL catabolic rates in the nephrotic group in keeping with previous studies [17, 19], although there was no statistically significant reduction. We believe that patient selection may have contributed to the lack of a significant reduction in the LDL FCR compared to the control group, since the present group did not exhibit as severe hypercholesterolemia as our previous study (mean LDL cholesterol 6.0 mmol/liter vs. 7.5 mmol/liter). In addition, there are methodological differences between the two studies. LDL studied using the cumulative flotation ultracentrifugation technique often displays a lower FCR compared to LDL prepared by rate zonal ultracentrifugation or fixed angle centrifugation. Indeed, the values obtained for the control group are lower than published data utilizing radiolabeled apoLDL as a tracer.

Synthesis of LDL apoB was found to be higher than normal, but unlike Vega and Grundy [17], we did not require a significantly increased level of direct LDL apoB synthesis. In most subjects all of the apoB in LDL of nephrotics could be accounted for by delipidation of VLDL and IDL.

The results of this study expand our current knowledge of the abnormalities in lipoprotein metabolism in the nephrotic syndrome. Increased hepatic synthesis of apoB-containing lipoproteins at the level of $VLDL_2$ was a feature of the syndrome, and this together with a diminished capacity to catabolize IDL and LDL led to the hypercholesterolemia observed in these subjects. Elevation of plasma triglyceride was associated with high concentrations of the large triglyceride-rich $VLDL_1$ which derived again from a combination of overproduction and a reduced delipidation rate. It should be noted that this group of patients studied was atypical in some respects with well-maintained serum albumin despite heavy proteinuria. The explanation for this is not clear; it is unusual but not unknown for patients with moderately heavy proteinuria to maintain serum albumin > 35 g/liter. Hypoalbuminemia has been most closely correlated with hyperlipidemia in this condition. The absence of severe hypoalbuminemia in our patients is reflected in the relatively modest elevations of plasma and LDL cholesterol and of LDL apoB pools. On the basis of our previous study we predict that an increase in LDL to a level more commonly found in nephrotic syndrome would be accompanied by further reduction in the fractional catabolic rate of the lipoprotein.

Renal failure is associated with a different spectrum of lipid and lipoprotein abnormalities than is found in the nephrotic syndrome. Elevations of plasma triglycerides, reduced HDL-cholesterol and normal or marginally elevated LDL-cholesterol concentrations are the typical findings, and some of these changes have been reported with mild renal impairment [52]. We attempted to minimize the effect of renal failure by restricting this study to patients with only modestly elevated serum creatinine concentrations. One patient studied did have serum creatinine > 400 μmol/liter, but had a lipoprotein profile typical of the nephrotic syndrome with markedly raised LDL. Although this patient had a high rate of apoB synthesis in $VLDL_1$, other kinetic parameters did not show any systematic differences from those with well-maintained renal function.

Further studies comparing well defined groups of nephrotic subjects on the basis of proteinuria, serum albumin, renal function and lipoprotein patterns together with comparisons of patients in relapse and remission of minimal change disease would be useful. The advent of methods involving stable isotopes [53] will permit more extensive and repeated applications of these techniques.

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Appendix 1. Rate constants K(i) pools/day

	(0,1)	(1,29)	(2,1)	(0,26)	(4,2)	(6,2)	(0,6)	(0,4)	(13,4)	(20,4)	(17,4)	(0,12)	(15,12)	(21,12)	(0,15)	(21,15)	(0,13)	(20,13)	(0,17)	(0,21)	(0,20)
Subject																					
CM	0	24.0	4.73	4.8	1.21	0.06	0.28	14.74	24.0	0	0	0.45	1.57	0	0	1.31	0.24	0.61	0.17	0.50	0.33
AP	3.6	29.14	0.98	1.22	1.63	0.43	0.71	1.15	1.07	0	0	1.97	1.63	0	0.01	0.72	0	0.54	9.93	0.21	0.32
WS	2.66	34.46	2.50	1.23	0.94	0.08	0.60	0	1.04	0	0	0	7.72	0	0.62	0.61	0.44	0.39	0.20	0.31	0.35
JG	0	24.0	5.59	39.74	0.58	0	0	0	24.0	8.72	24.0	0	2.33	0	0	1.40	0.01	0.41	2.66	0.21	0.21
JH	2.82	1.65	3.59	13.34	2.59	0.49	0.53	0.78	1.39	0.09	0	0.72	1.66	0.07	0	0.75	0	0.57	0.39	0.17	0.29
MD	2.37	36.0	4.13	24.0	2.31	0.41	0.95	8.06	4.78	0	0	10.44	1.97	0	0.09	0.80	0.06	0.40	0	0.18	0.29
RG	0	29.0	8.03	24.0	2.07	0.22	0.39	7.75	3.62	1.36	0	1.65	1.49	0	0	0.94	0.30	0.10	0	0.22	0.16
CF	0.31	4.06	21.76	36.0	2.53	0.05	0.60	1.67	7.85	0.98	0	0	3.89	0	0.23	1.98	0.19	0.25	0.70	0.37	0.19
Median	1.34	26.5	4.43	18.7	1.85	0.15	0.57	1.41	4.20	0.05	0	0.59	1.82	0	0.01	0.87	0.13	0.40	0.40	0.21	0.29
Controls																					
NC	4.17		5.16	0	6.22	0.03	0.56	0	5.76	0	0.90	0.56	5.45	0	0	4.09	1.65	2.04	0.52	0.42	0.21
CD	5.75		4.49	0	7.16	0.02	0.41	1.92	2.22	0	0.60	2.72	2.42	0	0	1.39	0.80	1.10	0.32	0.30	0.15
MF	10.79		2.48	0	6.96	0.13	0.76	0	3.14	0.68	0	1.87	3.23	0	0	1.58	0.61	0.70	0.37	0.28	0.16
EK	14.73		2.71	0	7.03	0.10	0.90	0.33	10.35	0	0	3.65	1.56	0	0	0.80	1.21	1.39	0.24	0.28	0.25
MM	6.91		3.66	0	8.92	0.09	0.63	1.63	2.19	0.08	1.26	1.24	2.37	0	0	1.44	0.49	0.44	0.38	0.32	0.23
Median	6.91		3.66	0	7.83	0	0.65	0.33	3.14	0	0.60	1.87	2.42	0	0	1.44	0.80	1.10	0.37	0.30	0.21

Notes to appendices:

1). Controls were analyzed with a model containing a rate constant (17,6). This accounted for a very small percentage of apoB flux to IDL and for comparisons with nephrotic subjects this material has been moved to (0,6).

2). Minor discrepancies in flux rates in and out of compartments are due to the effect of rounding up or down the compartment masses and rate constants.

3). U29 and U1 are equivalent inputs into the system. The control subjects did not require an extra delipidation step and apoB production was considered to occur directly into compartment 1.

Appendix 2. Calculated masses and inputs

	M(1)	M(26)	M(29)	M(2)	M(4)	M(6)	M(12)	M(13)	M(15)	M(17)	M(20)	M(21)	U(26)	U(29/1)	U(12)
Subject															
CM	19	391	3	70	2	16	555	62	668	0	115	1743	1877	72	1121
AP	256	1	40	122	90	74	428	176	955	0	305	3312	1	1166	1541
WS	86	111	13	210	190	29	141	239	883	0	263	1756	134	446	1090
JG	22	90	5	212	2	0	274	121	455	19	325	3101	3577	120	638
JH	40	0	155	46	53	43	194	128	426	0	265	1982	0	256	462
MD	14	66	3	22	4	9	127	41	281	0	57	1258	1584	108	1576
RG	11	73	3	37	6	21	441	54	700	0	85	3044	1752	87	1385
CF	7	0	39	60	15	5	153	258	269	0	405	1437	0	158	595
Median	21	70	9	65	11	19	234	125	562	0	264	2716	859	139	1106
Controls															
NC	67	0	0	56	52	3	50	81	66	94	793	640	0	625	301
CD	51	0	0	32	48	3	148	56	256	95	417	1206	0	522	761
MF	97	0	0	34	62	6	98	148	199	11	920	1124	0	1288	500
EK	47	0	0	18	11	6	39	42	76	56	231	219	0	1120	203
MM	106	0	0	43	99	2	70	231	114	5	485	606	0	819	280
Median	67	0	0	34	52	3	70	81	76	56	485	640	0	819	301

1990 and at the European Dialysis and Transplantation Association in Vienna, Austria on 5–8 September, 1990.

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Low-Density Lipoprotein Metabolism in the Nephrotic Syndrome

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Hyperlipidemia is a consistent feature of the nephrotic syndrome. In this study, low-density lipoprotein (LDL) metabolism has been investigated in nine patients with nephrotic syndrome and varying degrees of proteinuria. In subjects with moderate proteinuria (less than 10 g/d), total plasma cholesterol values were elevated to approximately 160% of normal due mainly to an increase in circulating LDL cholesterol. Metabolic studies showed that a defect in LDL clearance via the receptor pathway was responsible for its accumulation. The total amount of LDL apolipoprotein catabolized by this mechanism was only 55% of the value seen in controls; 60% more LDL was channelled into alternative, receptor-independent, catabolic pathways. Heavier proteinuria was associated with substantial increases in plasma triglyceride and very-low-density lipoprotein (VLDL) levels. The defect in LDL catabolism was aggravated by oversynthesis of the lipoprotein, which expanded the plasma LDL pool to 250% of normal. These observations indicate that the hyperlipidemia of the nephrotic syndrome is multifactorial in origin. The altered catabolism of LDL may be important in predisposing these subjects to premature atherosclerosis.

HYPERLIPIDEMIA is a consistent feature of the nephrotic syndrome. Individuals with persistent nephrosis have elevated plasma cholesterol levels that are considered to contribute to the accelerated atherosclerosis prevalent in this condition.[1-3] More recently it has been proposed that hyperlipidemia is an important factor in the progression of chronic renal failure[4] and this is currently the subject of much research. However, the mechanisms responsible for the hyperlipidemia in the nephrotic syndrome remain poorly understood.

Characteristically, total and low density lipoprotein (LDL) cholesterol are elevated in all subjects, while those with heavier proteinuria or marked hypoalbuminemia often demonstrate significant increases in triglyceride and very-low-density lipoprotein (VLDL) cholesterol.[5,6] The "cardioprotective" high density lipoprotein (HDL) fraction has variously been reported to be increased, decreased, or normal.[5-8]

The regulation of plasma LDL level is complex—both synthetic and catabolic mechanisms playing important modulatory roles. Much of our present understanding of the cellular metabolism of LDL stems from the work of Goldstein and Brown.[9] They identified the specific, high affinity, LDL receptor on cell membranes, which plays a central role in controlling the delivery and uptake of cholesterol by cells. In normal individuals, this receptor pathway accounts for about half of LDL catabolism while in familial hypercholesterolemia, deficient or absent receptor activity is associated with raised plasma LDL cholesterol levels and a substantially increased risk of premature atherosclerosis. In the presence of diminished receptor-mediated LDL removal, excessive amounts of the lipoprotein are channelled into alternative and, as yet, poorly defined, receptor-independent pathways that may involve the phagocytic system.[9]

The pathophysiological disturbances responsible for the hyperlipidemia of the nephrotic syndrome have not been clearly elucidated. Work on animal models[10-12] has indicated that increased hepatic lipoprotein synthesis may be the major metabolic abnormality but considerable differences in lipoprotein metabolism between species make direct comparison with human nephrotic syndrome difficult. However, some studies in humans have also suggested that oversynthesis is the most important disturbance in generating the hyperlipidemia.[7,13]

The aim of this study was to determine whether the accumulation of LDL in the nephrotic syndrome was a result of hepatic oversynthesis or decreased catabolism of the lipoprotein. In addition, the relative contributions of the two pathways responsible for LDL catabolism (receptor-mediated and receptor-independent) were examined as this may have important implications both for the pathogenesis of the hyperlipidemia and in assessing cardiovascular risk.

MATERIALS AND METHODS

Subjects

Nine patients (seven male, two female), aged between 30 and 66 years, gave informed consent to this study. They were recruited from the outpatient population attending the Renal Unit of Glasgow Royal Infirmary according to the following criteria: (1) serum creatinine < 300 μmol/L, (2) quantitative proteinuria consistently > 5 g/d, (3) total plasma cholesterol > 7 mmol/L.

Subjects suffering from other diseases or on treatment that might significantly alter plasma lipids were excluded. These included neoplastic and untreated endocrine disorders, diabetes mellitus, amyloidosis, systemic lupus erythematosis, or corticosteroid therapy. Women of child-bearing age were also excluded. Treatment with diuretics or antihypertensives was not considered an exclusion criteria. All subjects had an established histological diagnosis and were under regular renal review. No patient had a family history of premature vascular disease and there were no external stigmata of hyperlipidemia.

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Table 1. Clinical Details of Nephrotic Subjects

Patient	Sex	Age (yr)	Weight (kg)	Body Mass Index (kg/m^2)	Renal Histology	Follow-up (yr)	Treatment
RG	M	54	77	24.0	Membranous	1	Bendrofluazide
JJ	M	51	71	23.2	FSGS	6	Frusamide, enalapril
MM	F	55	70	28.8	MCGN type II	7	Frusemide, enalapril
BT	M	52	59	19.7	Membranous	1	Frusemide
RM	M	30	107	31.8	Membranous	0.5	None
DE	M	53	70	25.1	Membranous	1	Frusemide, nifedipine, spironolactone
MJ	F	65	49	22.7	Membranous	1	Frusemide
EA	M	66	96	39.4	Membranous	1	Frusemide, isosorbide
JB	M	30	63	20.9	Membranous	1	Frusemide
Mean(SD)		51(13)	74(18)				
Controls (all male, n = 8)		26(6)	71(12)				

Abbreviations; FSGS, focal segmental glomerulosclerosis; MCGN, mesangio-capillary glomerulonephritis.

The clinical and biochemical features of the study subjects are listed in Tables 1 and 2. Eight normal volunteers were also studied. The study conformed to the requirements of the Ethical Committee of Glasgow Royal Infirmary.

Protocol

LDL apolipoprotein metabolism was examined in the nephrotic subjects according to previously published procedures.[14,15] Briefly, blood was withdrawn after an overnight fast and used to produce LDL ($1.030 < d < 1.050$ g/mL) by rate zonal ultracentrifugation.

Table 2. Biochemical Features of Nephrotic Subjects

Patient	Serum Creatinine (μmol/L)	Creatinine Clearance (mL/min)	Serum Albumin (g/L)	Quantitative Proteinuria (g/d)
RG	122 (9.5)	86.0 (5.7)	23	6.1 (1.0)
JJ	224 (8.4)	39.3 (3.9)	25	5.2 (1.3)
MM	123 (10.0)	45.0 (6.0)	30	6.6 (1.3)
BT	106 (8.5)	63.0 (11.0)	25	5.4 (1.5)
RM	107 (4.2)	115.0 (18.5)	34	9.7 (3.4)
DE	95 (9.3)	84.0 (14.0)	20	15.3 (4.3)
MJ	239 (14.0)	17.6 (2.1)	18	11.9 (2.1)
EA	127 (7.2)	75.0 (8.0)	28	12.0 (2.3)
JB	102 (4.6)	84.8 (5.6)	21	18.0 (6.0)
Mean(SD)	138 (54)	67.7 (29.7)	25 (5)	10.0 (4.6)
Controls (n = 8)	100 (5.7)	102.4 (7.1)		

NOTE. Serum albumin was determined at start of study. Other values represent mean (SD) of daily measures throughout the study period.

This was then dialyzed against 0.15 mol/L NaCl/0.01% Na_2EDTA/0.01 mol/L Tris, pH 7.4, and divided into two aliquots that were labeled[16] with ^{131}I and ^{125}I. Unbound iodide and glycine buffer were removed by gel filtration.[15] After this maneuver, more than 98% of the radioactivity was covalently bound to apolipoprotein LDL. The ^{131}I-LDL tracer fraction was then treated with 1,2-cyclohexanedione (CHD), which blocks the arginyl residues of apolipoprotein B of LDL and prevents receptor binding.[17] The modified LDL is otherwise indistinguishable from native LDL and is handled as such by other metabolic pathways. Twenty-five μCi of each isotope were administered intravenously (IV) to their respective donors after sterilization through a 0.22 micron filter (Millipore UK, London). Exact weighing of syringes before and after injection permitted calculation of the dose administered. A blood sample was drawn at 10 minutes and then daily for 14 days. Subjects collected all their urine and the completeness of each day's collection was checked by measuring creatinine excretion. Two-milliliter aliquots of plasma and urine samples were stored for counting in a scintillation counter (Packard Instruments, Oberlin, OH) at the end of the study period. Urine samples were treated with trichloroacetic acid (1 vol urine:3 vol 20% TCA) to precipitate undegraded LDL lost directly into the urine and the radioactivity in the precipitate measured.

Studies were performed on outpatients eating a normal West of Scotland diet. None had been prescribed a specific diet as a result of their renal disease. Plasma lipid and lipoprotein levels were checked serially and did not alter significantly (ie, within 10% of mean value) over the study period indicating apolipoprotein B metabolism was in a steady state. Plasma cholesterol, triglyceride, and lipoprotein

Table 3. Plasma Lipid and Lipoprotein Concentrations in Control and Nephrotic Subjects

Patient	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	VLDL Cholesterol (mmol/L)	LDL Cholesterol (mmol/L)	HDL Cholesterol (mmol/L)
RG	8.5 (0.2)	2.1 (0.1)	1.1 (0.1)	6.3 (0.2)	1.2 (0.1)
JJ	7.5 (1.0)	3.1 (0.7)	1.8 (0.6)	4.8 (1.3)	0.9 (0.1)
MM	8.4 (0.4)	2.2 (0.1)	1.1 (0.1)	6.1 (0.4)	1.3 (0.2)
BT	10.3 (0.2)	2.9 (0.5)	1.4 (0.3)	7.5 (0.4)	1.5 (0.1)
RM	9.4 (0.1)	2.2 (0.1)	0.9 (0.2)	7.0 (0.3)	1.4 (0.1)
DE	14.1 (0.7)	4.3 (0.5)	2.5 (0.9)	10.2 (0.6)	0.9 (0.1)
MJ	12.1 (0.4)	5.5 (0.8)	2.8 (0.4)	8.5 (0.7)	0.8 (0.1)
EA	9.6 (0.4)	5.6 (0.2)	4.2 (0.2)	4.6 (0.3)	0.7 (0.1)
JB	16.7 (1.8)	4.9 (0.6)	2.9 (0.9)	12.9 (1.1)	0.9 (0.2)
Mean(SD)	10.7 (3.0)	3.6 (1.4)	2.1 (1.1)	7.5 (2.7)	1.1 (0.3)
Controls	5.2 (1.0)	1.2 (0.5)	0.6 (0.2)	3.1 (0.6)	1.6 (0.3)

NOTE. Values are mean(SD) of three separate determinations over the study period.

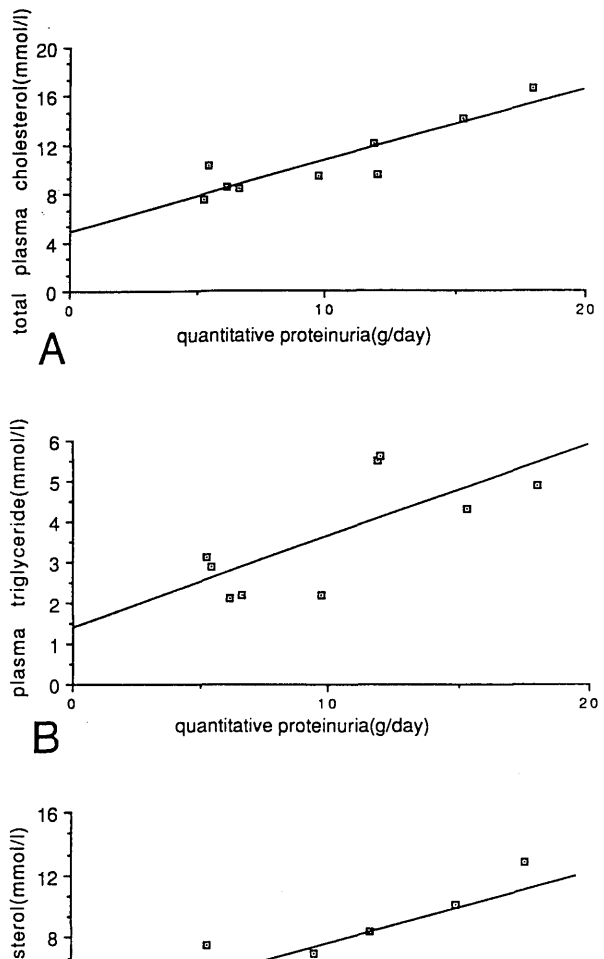

Fig 1. Relationships between plasma cholesterol, LDL cholesterol, plasma triglyceride, and proteinuria in the nephrotic syndrome. All three lipid parameters showed positive correlations with daily protein loss. Linear regression analysis gave the following relationships: (A) plasma cholesterol versus proteinuria, y = 4.84 + 0.59x, r = .90, P < .0005; (B) plasma triglyceride versus proteinuria, y = 1.39 + 0.22x, r = .72, P < .01; (C) LDL cholesterol versus proteinuria, y = 3.07 + 0.45x, r= .77, P < .002.

cholesterol were measured according to the Lipid Research Clinic's standard procedures.[18] Plasma LDL apolipoprotein levels were determined from the compositional analysis of the 1.019 to 1.063 g/mL LDL fraction.[15]

Kinetic Analysis

The plasma and urine radioactivity were used to calculate the plasma fractional catabolic rate (FCR)—ie, the fraction of the intravascular LDL pool catabolized per day—using the SAAM compartmental modeling program.[19] A two-compartmental model containing intra- and extravascular pools was used, together with two urinary excretion pathways—one for loss of intact or partially degraded LDL (ie, TCA-precipitable) and the other for degraded radioactivity. Both the native and the CHD-modified LDL fractional catabolic rates were corrected in this manner.

Native LDL acts as a tracer for total LDL catabolism while the modified LDL is cleared by receptor-independent mechanisms. The difference between the two clearance rates is, therefore, a measure of receptor-mediated clearance. From a knowledge of the intravascular apolipoprotein B pool size (calculated from apoLDL plasma concentration and plasma volume derived from the dilution of the injected LDL at 10 minutes) the absolute catabolic rate was obtained. In a steady state, this is equal to the production rate and this is conventionally expressed as milligram of apoLDL per kilogram of body weight per day.

Statistical analyses were performed using Student's t test to compare differences between groups. Correlations were performed by linear regression analysis.

RESULTS

The patients in this study had marked elevations of total cholesterol and triglyceride (Table 3) in accord with previous reports in the nephrotic syndrome.[5,20-23] Plasma LDL cholesterol was elevated in all nine subjects. Triglyceride levels and VLDL cholesterol were only marginally increased compared with controls in those subjects with moderate proteinuria (5 to 10 g/d), whereas in the group with heavier proteinuria (DE, MJ, EA, JB) much higher values were found. HDL levels tended to be lower in the latter group and overall were significantly lower compared with normals. Urinary protein loss was positively correlated with total cholesterol, LDL cholesterol, and triglyceride (Fig 1).

There were differences in the composition of LDL between controls and subjects with nephrotic syndrome. Protein, phospholipid, and esterified cholesterol content were unchanged but LDL triglyceride was increased (11.4% SD 3.1% *v* 7.8% SD 1.2%, P < .01) while free cholesterol fell (6.9% SD 2.1% *v* 12.1% SD 1.2%, P < .001).

LDL Apolipoprotein Kinetics

There was a significant reduction in the FCR of LDL in nephrotic subjects compared with controls (0.219 SD 0.061 *v* 0.384 SD 0.057 pools/d, P < .001; Table 4). This was almost entirely due to a reduction in the activity of the receptor-mediated clearance pathway. Clearance by this route was reduced by 70% in the nephrotic subjects (0.050 SD 0.026 pools/day *v* 0.165 SD 0.051, P < .001). There was a smaller reduction in the receptor-independent FCR (0.169 SD 0.043 *v* 0.219 SD 0.039 pools/day, P < .05; Table 4). The FCRs were corrected for measured urinary losses of "intact" LDL, ie, TCA-precipitable. This accounted for a small proportion (1% to 5%) in four subjects but was a potentially significant source of error (5% to 16%) in the remainder. The percentage urinary loss of TCA-precipitable radioactivity was similar for both ^{125}I-native LDL and ^{131}I-CHD modified LDL. The mean value is given in Table 4. This adjustment, therefore, did not alter the balance between receptor-mediated and receptor-independent clearance.

Overall, the mean absolute catabolic rate (ACR) was increased but this was not a uniform finding in the study group. Those patients with moderate proteinuria (patients

Table 4. Kinetic Parameters Describing apoLDL Turnover in Control and Nephrotic Subjects

	Plasma apoLDL (mg/dL)	% Urinary* Loss	Fractional Catabolic Rate† (pools/d)			Absolute Clearance Rate‡ (mg/kg/d)		
			Total	Receptor Independent	Receptor Mediated	Total	Receptor Independent	Receptor-Mediated
Patient								
RG	224	2.0	0.188	0.145	0.043	18.1	14.0	4.1
JJ	118	1.2	0.292	0.210	0.082	12.8	9 2	3.6
MM	215	15.7	0.163	0.137	0.026	12.0	10.5	2.2
BT	244	6.9	0.140	0.120	0.020	14.0	12.0	2.0
RM	238	7.5	0.237	0.219	0.018	19.5	18.0	1.5
DE	347	4.5	0.181	0.127	0.054	29.6	20.8	8.8
MJ	279	4.4	0.242	0.177	0.065	27.2	19.9	7.3
EA	171	8.0	0.325	0.236	0.089	22.2	16.1	6.1
JB	427	12.6	0.200	0.148	0.052	35.3	26.2	9.1
Mean	251	6.9	0.219	0.169	0.050	20.7	16.3	4.9
(SD)	(92)	(4.7)	(0.061)	(0.043)	(0.026)	(8.3)	(5.5)	(3.0)
Controls								
Mean	93		0.384	0.219	0.165	14.2	8.1	6.2
(SD)	(12)		(0.057)	(0.039)	(0.051)	(2.3)	(1.1)	(2.1)
P§	<.001		<.001	<.05	<.001	<.02	<.05	NS

*Percentage of urinary radioactivity precipitated with trichloroacetic acid.

†Data is corrected for measured urinary losses of protein-bound radioactivity.

‡Absolute clearance is the product of fractional clearance rate and plasma pool size expressed per kilogram of body weight.

§*P* values represent differences between nephrotic and control groups.

RG, JJ, MM, and BT) cleared the same amount of LDL each day as controls while in those with urinary protein losses of greater than 10 g/d, this amount was substantially increased. There was a strong positive correlation between the level of proteinuria and the overall ACR (Fig 2, r = .929, $P < .00005$). Since in steady state conditions catabolism and synthesis are equal, this implies that apoLDL synthesis is directly related to proteinuria.

DISCUSSION

High levels of plasma lipids are found in individuals with the nephrotic syndrome. The development of this secondary hyperlipidemia appears to be independent of the underlying renal disease but directly related to the degree of proteinuria and/or hypoalbuminemia. Previous investigations of the lipid and lipoprotein profiles of nephrotic patients have revealed a pattern of abnormalities[5,20,21] that was also present in our subjects. Mild to moderate proteinuria (generally with serum albumin greater than 20 g/L) is primarily associated with hypercholesterolemia due to raised LDL levels and its degree is proportional to measured urinary protein loss. Heavier proteinuria (in excess of 10 g/d) is associated with an increase in VLDL cholesterol and triglyceride levels, further rises in LDL, and a decrease in plasma HDL cholesterol. Several reports[20-23] recorded falls in a specific HDL sub-fraction—HDL_2 (d = 1.063 to 1.125 g/mL)—but this is not a universal finding.[24] Clearly, the alterations in lipoprotein transport caused by the nephrotic syndrome are complex and multiple mechanisms are involved in their etiology.

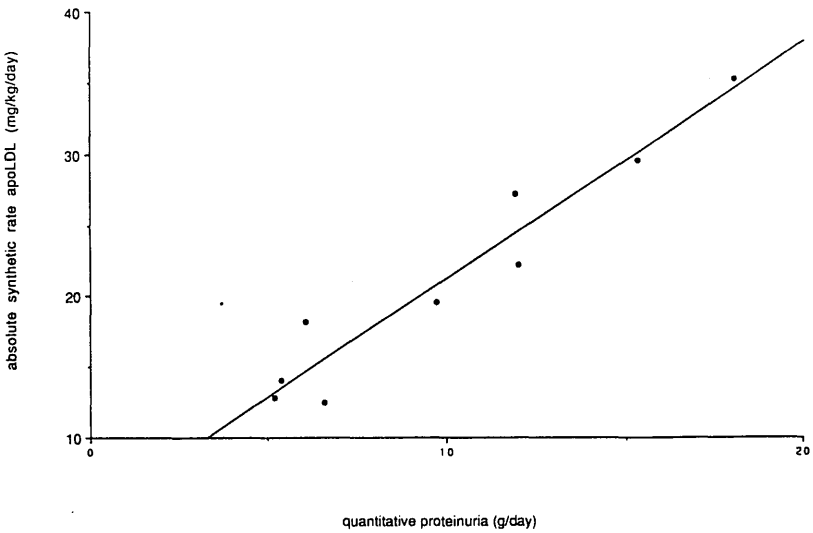

Fig 2. Correlation between apoLDL synthetic rate and daily urinary protein loss. Linear regression analysis provided the following relationship: y = 4.44 + 1.68x, r = .93. $P < .00005$.

The precise nature of the pathophysiological disturbances responsible for the hyperlipidemia of the nephrotic syndrome remains uncertain. Work on a rat model, using isolated liver perfusion and rat liver slices,[10,11] has demonstrated a rise in lipoprotein synthesis. However, differences in lipoprotein metabolism between species make extrapolation to the human condition unreliable.

Early studies of triglyceride turnover[6,25] and cholesterol synthesis[6] in humans have suggested that hepatic overproduction of these lipids is a feature of the disease. Few metabolic investigations have followed the turnover of apolipoprotein B, the major protein component of VLDL and LDL, and the only constituent that is integral to both particles throughout their lifetime in the plasma. Pioneering work by Scott et al[26] and Gitlin et al[27] suggested that human LDL catabolism was decreased in nephrotic syndrome. Scott et al reported values of 0.224 pools/d compared with 0.308 pools/d in normal subjects. However, this difference was not significant due to the wide variation in the control population (0.21 to 0.35 pools/d) and these investigators were led to invoke increased synthesis as the main contributor to the high LDL levels in nephrotics. No corrections were made in their measurements for loss of intact or partially degraded LDL in the urine. Small amounts of LDL were found in the urine of these patients using an immunoassay method. In addition, the injected tracer contained 2% to 9% nonprotein bound iodide. Both these factors may have falsely increased the FCR of the lipoprotein. Two more recent studies of small groups of adult nephrotics[13,28] reported virtually normal FCRs for radiolabeled LDL and concluded that the most important change was increased synthesis of this lipoprotein. However, the investigators again did not correct for urinary loss of protein-bound radioactivity which, if as extensive as we have found (up to 16%), would cause a substantial overestimate of the FCR from the plasma compartment. The level of proteinuria in their subjects was comparable with that of our group. The most consistent finding in the present study was an overall reduction in the mean FCR for apoLDL (0.219 SD 0.061 *v* control values of 0.384 SD 0.057 pools/day) although the range is wide and encompasses most of the results seen in the previous studies.[13,26,27] The reduced clearance rate was primarily due to a fall in catabolism via the LDL receptor pathway (0.050 *v* 0.165 pools/day in normals, Table 4). Indeed, the values seen in these nephrotics are similar to those observed in heterozygous familial hypercholesterolemia.[14,29,30]

Several mechanisms might result in suppression of LDL receptor activity in the nephrotic syndrome. Golper et al[31] have shown that, in experimental nephrosis in the rat, reduced renal clearance of plasma mevalonate led to increased hepatic cholesterol synthesis from this precursor. Expansion of the intrahepatic sterol pool (as suggested by animal studies[11]) would reduce LDL receptor activity. An alternative explanation is that nephrosis modifies the binding capability of LDL by altering the conformation of apoB on the particle's surface as has been reported in patients with chronic renal failure maintained on dialysis.[32] Certainly, in other situations, LDL with an increased triglyceride content binds less well to fibroblast receptors in vitro.[33]

In the four subjects with moderate proteinuria (RG, JJ, MM and BT) reduced LDL clearance was the main cause of the elevated plasma level (Table 4). The synthetic rate of the lipoprotein (mean value 14.4 mg/kg/d) was not significantly different from normals. Subjects with heavier proteinuria (DE, MJ, EA, and JB) exhibited a higher than normal apoLDL synthetic rate in addition to a lower FCR. As a group they also showed higher triglyceride and VLDL cholesterol levels suggesting that this additional apoB came via the VLDL-LDL delipidation cascade. The remaining subject (RM) showed an intermediate pattern.

The present study together with earlier work on lipoprotein patterns at different stages of the nephrotic syndrome[5] suggests that the hyperlipidemia is caused by at least two independent mechanisms. At moderate levels of proteinuria (less than 10 g/d), LDL catabolism is impaired either due to reduced receptor activity or defective ligand-receptor interaction. Above a proteinuria threshold of about 10 g/d, increased LDL (and possibly VLDL) synthesis aggravates the hyperlipidemia and triglyceride levels rise, often to become the dominant abnormality. The factors that precipitate the increase in synthesis are uncertain, although there is some evidence to suggest that a decrease in circulating albumin or plasma oncotic pressure may be responsible by stimulating a general increase in hepatic protein synthesis.[34] Finally, the effect of altered rates of lipoprotein synthesis and catabolism in nephrotic syndrome may be modified by postsecretory changes in lipoprotein particles.[35] Reduced activity of lipolytic and other enzymes has been recorded in nephrosis.[36,37]

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Primary hyperlipidaemia is not associated with increased urinary albumin excretion

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SUMMARY

Urinary albumin excretion was measured in a group of 141 otherwise healthy subjects attending a lipoprotein clinic to determine whether primary hyperlipidaemia was associated with evidence of early renal dysfunction. There was no evidence of increased urinary albumin concentrations or albumin:creatinine ratios when compared with data for normal controls. There were no differences in these parameters when the values for the upper and lower quartiles of the cholesterol distribution were compared and no relationship existed between plasma cholesterol and albuminuria. A weak association was shown between plasma triglyceride and urinary albumin concentration after log transformation of the data. We conclude that hyperlipidaemia per se is not associated with renal disease as measured by sensitive assays of albuminuria.

key words - hyperlipidaemia, lipoproteins, microalbuminuria

running title - hyperlipidaemia and albuminuria

INTRODUCTION

The factors responsible for the progressive nature of chronic renal disease have not been clearly elucidated. Recently it has been postulated that the hyperlipidaemia which accompanies proteinuric renal disease may play a role in the progressive decline in glomerular filtration rate (1). Studies in a number of experimental animal models of nephrotic syndrome and renal failure have shown that cholesterol feeding increases proteinuria and glomerulosclerosis and accelerates loss of GFR (2,3). Treatment of the hyperlipidaemia ameliorates these measures of renal damage. In man, an anecdotal report has suggested plasma cholesterol lowering may reduce proteinuria(4). Furthermore, renal disease has been documented in the rare familial lecithin cholesterol acyl transferase deficiency(5) and in association with morbid obesity(6). Other primary hyperlipidaemias do not appear to impair renal function although this has not been studied in a comprehensive fashion. To assess the potential nephrotoxicity of hyperlipidaemia we have looked at urinary albumin excretion in a group of patients with primary hyperlipidaemia attending a lipoprotein clinic.

METHODS

The patients studied were attending routine appointments at the lipoprotein clinic at Glasgow Royal Infirmary between October 1989 and January 1990. Patients were excluded if there was evidence of other diseases associated with increased albuminuria. These were:- hypertension or recorded blood pressure above 170/95 mmHg , diabetes mellitus, thyroid disease or abnormal thyroid function tests, renal disease or raised serum creatinine, history of malignancy, pregnancy, rheumatoid arthritis or connective tissue disease and treatment with non-steroidal anti-inflammatory drugs. Random urine samples were collected into empty sterile containers and stored at 4° C. Specimens were assayed between 4 and 10 weeks following collection. Fasting blood samples were obtained for plasma lipid and lipoprotein levels and renal function was checked if this had not been done previously.

Cholesterol and triglyceride were assayed using enzymatic colorometric methods on a Hitachi 717 discrete autoanalyser. Urinary albumin was assayed by a modification of the radioimmunoassay method described by Hutchison (7). The imprecision of this assay at a concentration of 12mg/L has been reported as <5%(within batch) and 6.7%(between batch). For these studies the working lower limit of detection was taken as 1mg/L. Urinary creatinine estimation was carried out on a Hitachi 704 discrete autoanalyser using the Jaffé reaction.

Statistical analysis was performed using the Minitab microcomputer package. Urinanry albumin concentrations, urinary albumin:creatinine ratios and plasma triglyceride concentrations all demonstrated a markedly skewed distribution which became Gaussian on log transformation of the data.The relationships between plasma lipids and urinary albumin concentrations were tested by linear regression. A two-sample t test was used to compare albumin excretion in the upper and lower quartiles for plasma cholesterol concentrations.

RESULTS

Urine and plasma specimens were collected from 141 patients. Fasting plasma cholesterol levels for this group ranged from 4.15 to 13.3mmol/l with a mean value of 7.73mmol/l. The distribution of urine albumin concentrations is shown in figure 1 and is highly skewed. The median urinary albumin concentration was 5.95mg/l (range <1.0- 48.5) and median albumin/creatinine ratio was 0.79mg/mmol (range <0.5 - 4.1). The normal range of urinary albumin excretion is difficult to define because of differences in methods of urine collection, subject preparation and inter-assay variation. However, Watts et al(8) have quoted 29.6mg/L as the upper 95% confidence limit for urinary albumin concentrations in untimed collections in ambulant subjects and only 2(ie 1.4%) of our results lie outwith this range. Similarly only 4/90(4.4%) had an albumin:creatinine ratio greater than the 95% confidence limits quoted in the same paper(0.1-2.3mg/mmol).

There was no relationship between plasma cholesterol and log transformed values of urinary albumin(figure 2) concentration or albumin/creatinine ratio (r^2=0.9, p=0.9). Fasting triglyceride values also

showed a markedly skewed distribution and after log transformation a weak relationship was demonstrated between triglyceride and urinary albumin concentration (figure 3). There was only a very weak correlation between albumin concentrations in urine and the urinary albumin:creatinine ratio (r^2=5.7%; p=0.022) in contrast to Hutchison's group of diabetic patients(7). There was however a clear relationship between these variables when we considered a group with high albumin concentrations in patients who were excluded on the basis of hypertension(data not shown).

Finally there was no difference in the urinary albumin concentrations (after log transformation) between the upper and lower quartiles of the plasma cholesterol distribution (0.735 SD 0.374 v 0.802 SD 0.286; p=0.4).

DISCUSSION

Increased urinary albumin excretion('microalbuminura') has been shown to predict the development of renal failure in diabetes mellitus(9). The precise mechanisms responsible for the rise in urinary albumin levels are unclear. Increased glomerular filtration, decreased tubular reabsorption or both may occur. Elevated urinary albumin excretion rates have been found in a number of other conditions including hypertension(10), postoperatively(11) and psoriasis(12) and are thought to be an early indicator of renal dysfunction.

There has been recent interest in the role of lipids in the development of glomerulosclerosis or tubular injury(1-3). Hyperlipidaemia secondary to proteinuria or renal failure following an initial renal insult may be capable of promoting progressive renal injury. The precise mechanism is unknown although an increase in glomerular permeability due to neutralisation of anionic sites, mesangial cell accumulation of lipoproteins and tubular cell toxicity from filtered lipoproteins have all been proposed(1).

Most workers have used models where an initial renal injury is induced by toxic, immunological or surgical measures and investigated the role of hyperlipidaemia on the subsequent course of the disease. Little data exists on renal function in primary hyperlipidaemias. There are no reports of renal disease in familial hypercholesterolaemia or in the animal model of this disease, the Watanabe rabbit. However there are a number of primary

disorders of lipid metabolism which have been associated with renal disease. Glomerulosclerosis develops in the spontaneously hyperlipidaemic obese Zucker rat(2)and has been reported in morbid obesity in man(6). We hypothesised that if hyperlipidaemia per se was capable of inducing renal damage in man then this might first be manifested as an increase in urinary albumin excretion.

We were unable to show any correlation between serum cholesterol concentrations and albumin excretion nor did we find a higher mean albumin excretion in the hypercholesterolaemic group. Mean values for albumin excretion compare closely to those obtained by other authors in normal patients using random or timed samples(8,13). Data for random urine samples are limited. They may produce elevated results in patients with orthostatic proteinuria or following exercise particularly in the diabetic patient and are therefore not ideal for prognostic use in this group(13). There is no evidence that they may produce low results. The absence of an association between albumin concentration and albumin:creatinine ratio contrasts with previous studies and our own observations on subjects excluded from the current study. This can most probably be explained by the consistently low levels of albuminuria. The albumin:creatinine ratio is consequently dependent to a greater degree on the urinary creatinine concentration than in those with higher albumin concentrations.

The signifigance of the weak relationship between the log transformed values of plasma triglyceride and urinary albumin concentration is uncertain. This may reflect variability in other factors(eg obesity, blood pressure) which may influence albumin excretion.

We conclude that primary hyperlipidaemia is not associated with increased urinary albumin excretion. If hyperlipidaemia has a role in the progression of renal disease this must be as a secondary permissive effect following renal injury rather than an initiating factor.

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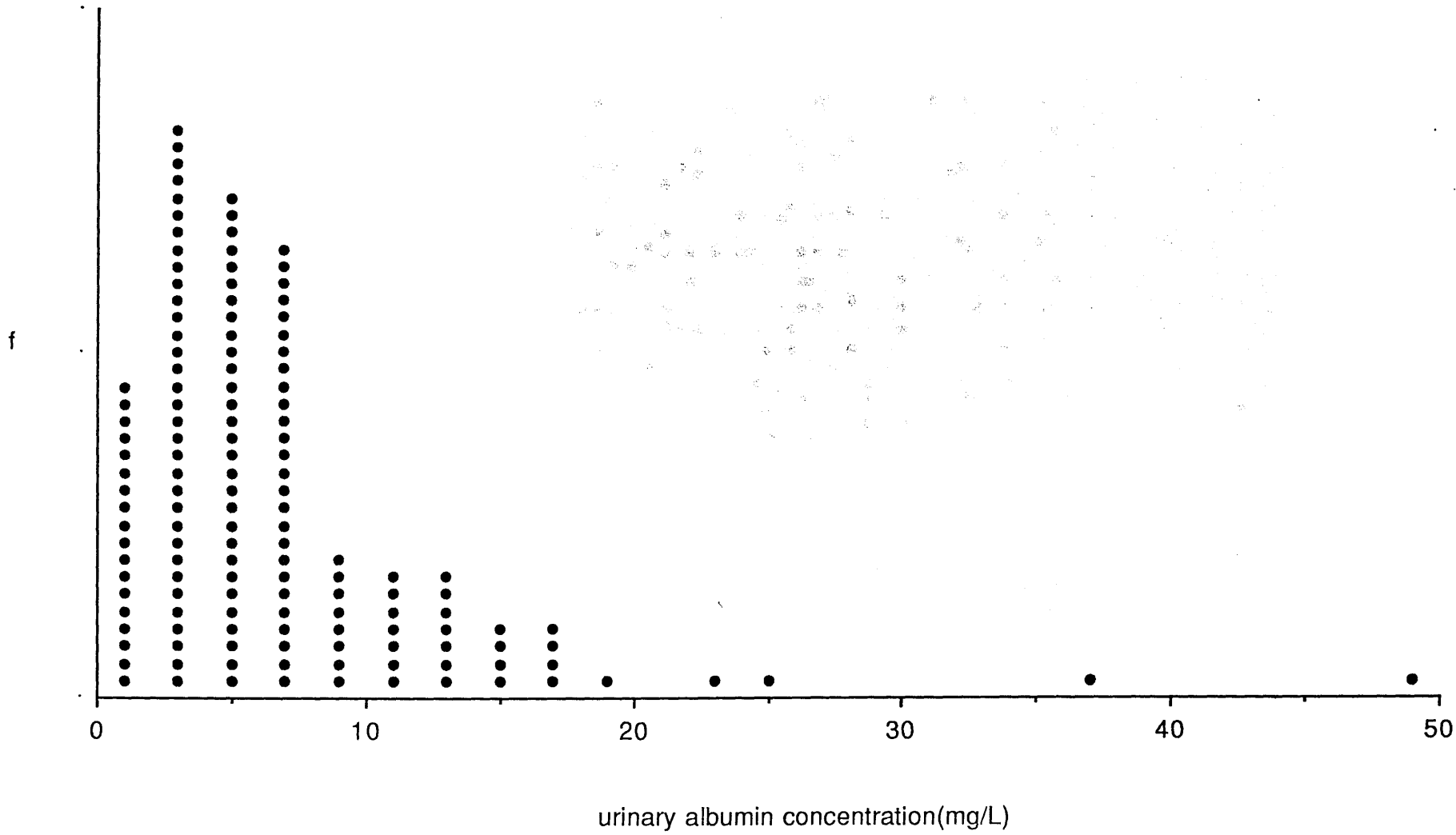
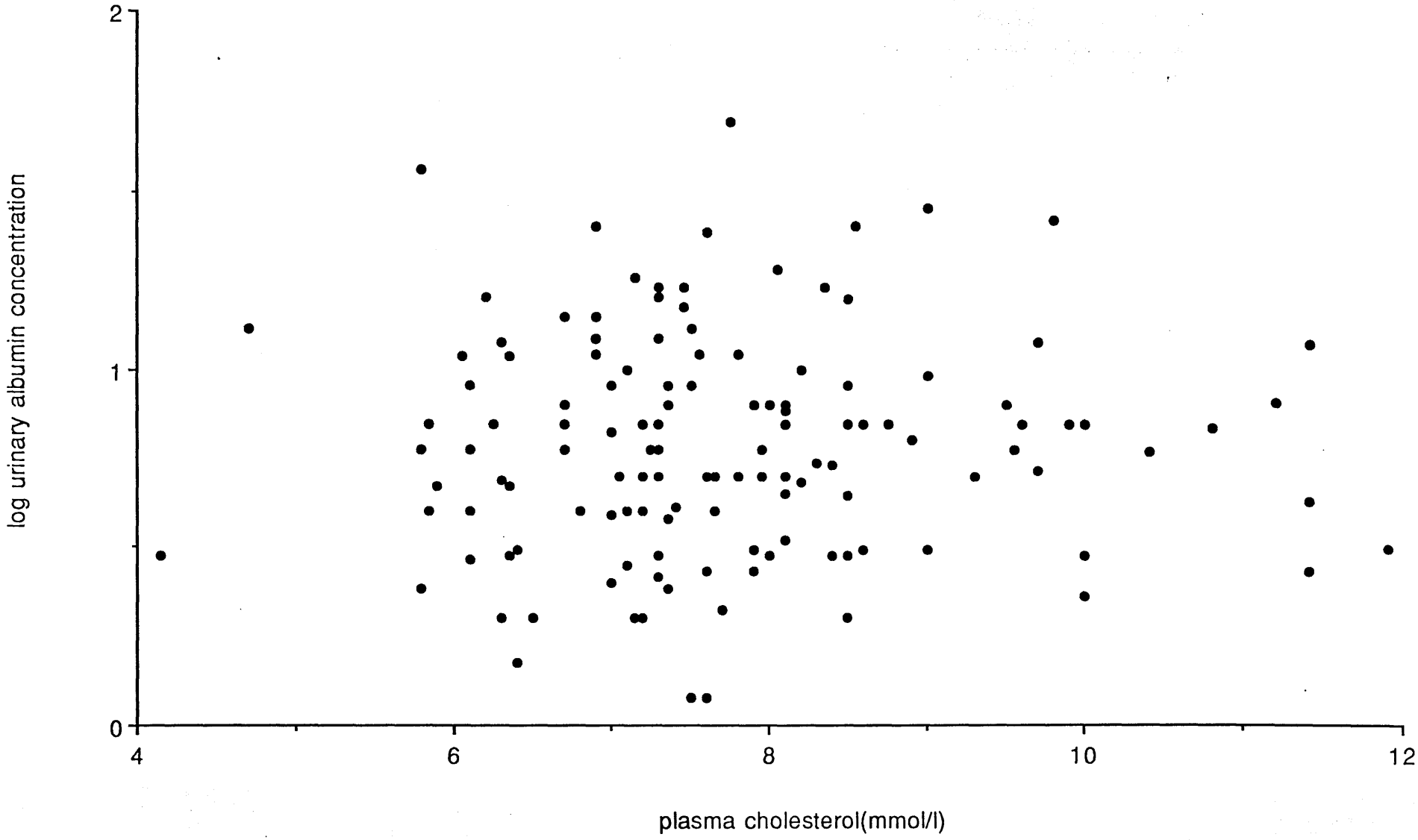
LEGENDS

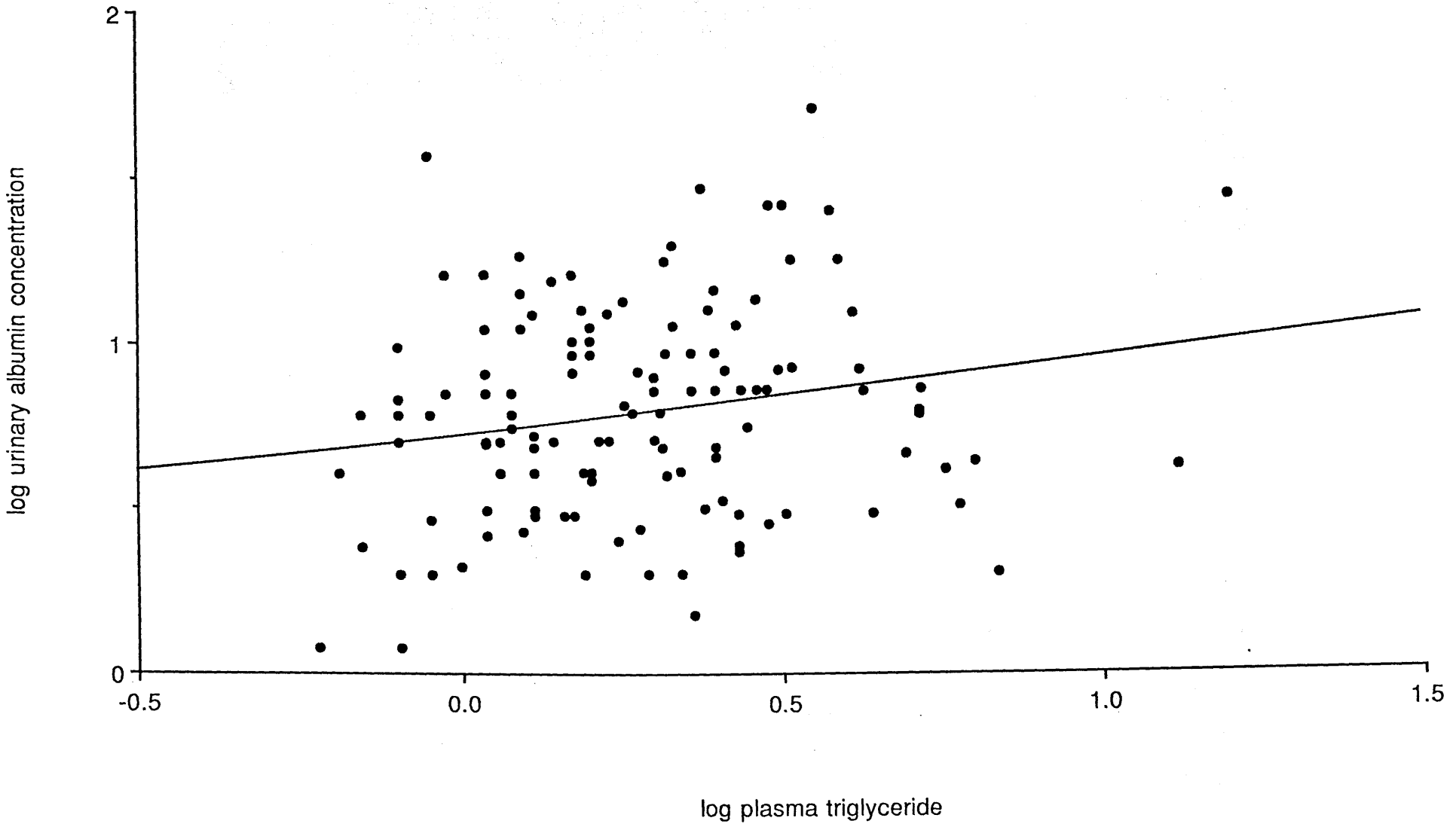
Figure 1: The frequency distribution of urinary albumin concentration in study population(n=141).

Figure 2: The relationship between plasma cholesterol and log urinary albumin concentration (r^2=0.9%, p=0.91).

Figure 3: The relationship between the log transformed values of plasma triglyceride and urinary albumin concentration(r^2=3.1%, p<0.05)


f
0
10
20
30
40
50
urinary albumin concentration(mg/L)


log urinary albumin concentration
2
1
0
4
6
8
10
12
plasma cholesterol(mmol/l)


log urinary albumin concentration
2
1
0
-0.5
0.0
0.5
1.0
1.5
log plasma triglyceride