GENETIC DETERMINANTS OF APOLIPOPROTEIN B METABOLISM

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To Juliane,

Angela and Martin

"That is what I find so wonderful,
that not a day goes by - to speak in
the old style - hardly a day, without
some addition to one's knowledge
however trifling, the addition I mean,
provided one takes the pains."

Samuel Beckett "Happy Days" 1961

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LIST OF ABBREVIATIONS

AVG	Average
BPB	Bromophenol blue
BSA	Bovine serum albumin
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHO	Carbohydrate
dCTP	Cytidine-tri-phosphate
DSS	Decyl sodium sulphate
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
FCR	Fractional catabolic rate
FCS	Fetal calf serum
FH	Familial hypercholesterolaemia
FSD	Fractional standard deviation
HAT	Hypoxanthine aminopterin thymidine solution
HL	Hepatic lipase
HLP	Hyperlipoproteinaemia
HDL	High density lipoprotein
HRP	Horseraddish peroxidase
HTG	Hypertriglyceridaemia
IDL	Intermediate density lipoprotein
IEF	Isoelectric Focussing
IgG	Immunoglobulin G
LCAT	Lecithin cholesteryl acyl transferase
LDL	Low density lipoprotein

LpL	Lipoprotein lipase
MAB	Monoclonal antibody
OD	Optical density
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
RES	Reticuloendothelial system
RFLP	Restriction fragment length polymorphism
RPMI 1640	Roswell Park Memorial Institute Medium 1640
SDS	Sodium dodecyl sulfate
S_f	Svedberg flotation coefficient at 1.063 kg/l
SSC	Salt sodium citrate solution
STD	Standard deviation
TAE	Tris-acetate-EDTA-buffer
TCA	Trichloroacetic acid
TE	Tris-EDTA-buffer
TEMED	Tetra methyl ethylene diamine
TMU	Tetra methyl urea
Tris	Tris hydroxy methyl amino methane - HCl
VLDL	Very low density lipoprotein
WHHL	Watanabe heritable hyperlipidaemic rabbit

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DECLARATION

The work presented in this thesis was performed solely by the author apart from areas of acknowledged collaboration.

Thomas Demant

SUMMARY

In this thesis the influence of genetic factors on the apolipoprotein B metabolism in humans was investigated. The phenotype of the apolipoprotein E polymorphism was determined for normolipidaemic subjects (n = 1600). The metabolism of apolipoprotein B in fifteen subjects, homozygous for apoE3, apoE4 or apoE2, was examined by VLDL-turnover studies, using trace-labelled $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60). Results were used for computer modelling of the apoB metabolism, which enabled quantitative comparisons between the three study groups. In apoE2/2 subjects, clearance of $VLDL_1$ and $VLDL_2$ as well as transfer from IDL into LDL was found to be delayed and in apoE4/4 subjects the LDL-FCR was reduced as compared to apoE3/3 normolipidaemics. These observations explain the correlation between apoE phenotypes and plasma cholesterol levels, which had been observed previously by others and were confirmed in the present study.

The XbaI restriction site polymorphism of the apoB gene was analysed in nineteen hypercholesterolaemic patients and correlated with fractional catabolic rates for LDL as defined by LDL-turnover studies. The X_2 allele was found to be linked with a decreased LDL-FCR, in line with previous reports of a correlation between X_2X_2 genotype and increased plasma cholesterol concentrations.

In addition to these studies of common genetic determinants of apoB metabolism, five patients with rare inherited disorders of lipoprotein metabolism were investigated. These conditions were homozygous familial hypercholesterolaemia, lipoprotein lipase deficiency and hepatic lipase deficiency. VLDL-turnovers in these subjects revealed the significance of the LDL-receptor and the two lipolytic enzymes for apolipoprotein B metabolism.

Finally, some conclusions were drawn about metabolic heterogeneity within the VLDL subfraction and about apoB synthesis.

1. INTRODUCTION

It has been known for more than 80 years that cholesterol is an important constituent of degenerative lesions of the arterial wall (1). As early as 1913 Russian and German investigators concluded from experiments, in which they fed egg yolk to rabbits, that dietary cholesterol was the cause of the development of atherosclerotic lesions in these animals (2). Thus the link between cholesterol intake and atherosclerotic disease has been established for some time, but more detailed studies of plasma lipid metabolism became possible only after the invention of high performance centrifuges, which are still the main tool for investigation in this field. Gofman and co-workers in 1950 were the first to describe in plasma the presence of distinct lipoprotein particles, which were characterised by their flotation properties in a centrifugal field (3).

1.1 Lipoproteins in Plasma

The principle structure of lipoproteins is depicted in Fig. 1. Hydrophobic lipids, like cholesteryl esters and triglycerides, form the core material of lipoproteins. This is surrounded by a closely packed 20-22 Å wide monolayer of polar lipids, comprising free cholesterol and phospholipids (4). Specific proteins, so-called

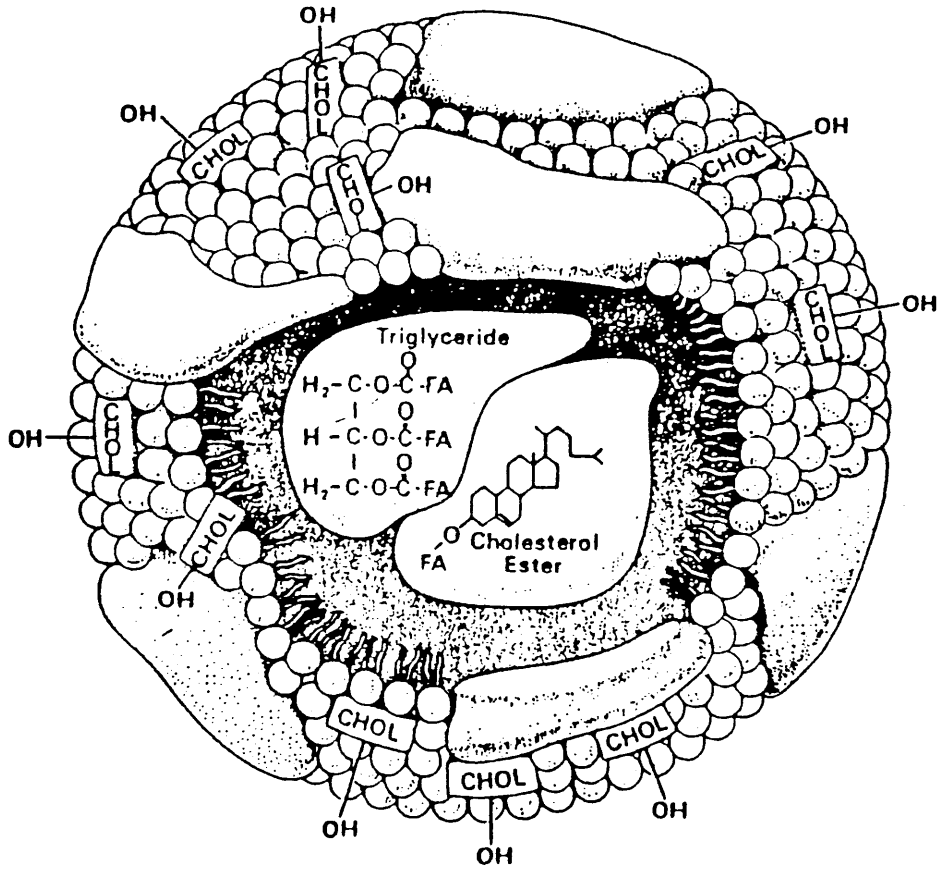


Fig. 1: Model of a human plasma lipoprotein. (Reproduced from: HB Brewer; Klin. Wochenschr. (1981), 59, 1027).

apolipoproteins, because of their orientation are able to both stabilise the lipoprotein and interact with key enzymes and receptors in plasma and on cells.

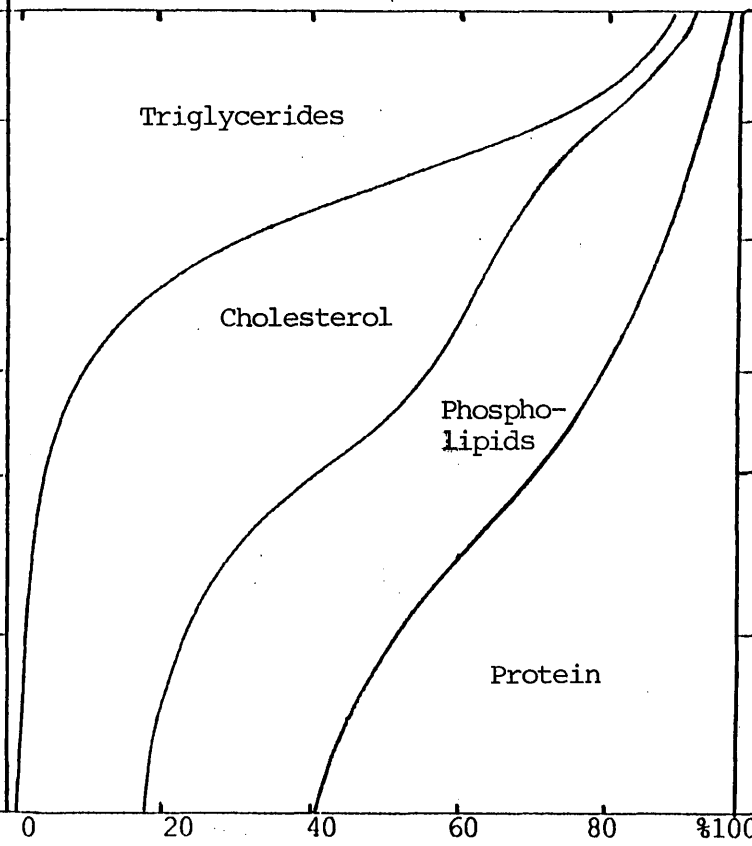
Table 1 illustrates the spectrum of human plasma lipoproteins: chylomicrons are triglyceride-rich particles with the lowest density, followed by very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) which together form the group of apolipoprotein B-100 containing lipoproteins. Chylomicrons are secreted by the intestine following absorption of dietary fat whereas VLDL is secreted by the liver. IDL and LDL are derived from VLDL through delipidation within or close to the plasma compartment (see paragraph 1.3.2).

In normolipidaemic plasma roughly two-thirds of total plasma cholesterol is associated with LDL, a lipoprotein whose concentration correlates strongly with the risk for coronary heart disease. A variant of LDL, called Lp(a), is detectable in plasma in variable amounts in the density interval between LDL and HDL_2 (5, 6). Lp(a) is an LDL, with another apoprotein, apo(a), coupled covalently to apoB probably via a disulphide bond.

HDL_2 and HDL_3 which differ by their size and apolipoprotein composition are distinct from the other lipoproteins in as much as they do not contain apolipoprotein B (7). In contrast to apoB containing

Table 1: Human Plasma Lipoproteins. - Physical Properties and Composition

Lipoproteins	Density Range (g/ml)	Sf (at 23°C & 1.063 g/ml)	Diameter Å	Apoprotein*
Chylomicrons	< 0.950	> 400	> 800	B-48, AI, AIV C, E (var. comp.)
$VLDL_{1/2}$	0.950 -1.006	60-400 20-60	300-800	B-100 (40%) C (50%) E (10%)
IDL	1.006 -1.019	12-20	250-300	B-100 (80%) C (10%) E (10%)
LDL	1.019 -1.063	0-12	200-250	B-100 (> 95%)
HDL_2	1.063 -1.125	-	100-200	AI (65%) AII (10%) C (15%) E (5%)
HDL_3	1.125 -1.221	-	75-100	AI (60%) AII (25%) C (5%) D (5%)



*Apoprotein values are mass% referring to the total protein content (= 100%) of respective lipoproteins. Data are derived from own measurements (apoB) and references (64, 244).

lipoproteins, the mass of cholesterol transported in HDL is negatively correlated to coronary heart disease.

Finally, two lipoproteins - ß-VLDL and HDL_C - are commonly referred to in the literature. They are usually not present in significant amounts in normolipidaemic plasma. Both lipoproteins were discovered in animals after cholesterol feeding (8). In man, ß-VLDL accumulates in familial dysbetalipoproteinaemia. Its potential significance for atherogenesis will be discussed later (paragraph 1.4.4). HDL_C or HDL_1, which is a subfraction of HDL-with-apoE as described by Weisgraber (9), may play a role in reverse cholesterol transport (see paragraph 1.3.3).

1.2. Apolipoproteins

The functions of apolipoproteins are (i) to stabilise the structure of lipoproteins, (ii) to act as specific activators or inhibitors of enzymes, which react with the lipid components and (iii) to bind to specific cell surface receptors which play a key role in lipoprotein metabolism.

Details characterising the structure and function of apolipoproteins are summarised in Table 2.

Table 2: Characteristics of Human Apolipoproteins

Apolipoprotein	Molecular Weight (kD)	Synthesis (see text)	Chromosomal Location	Lipoprotein Distribution	Special Functions (see text)
ApoAI	28.1	Intestine, Liver	11	HDL	Structural protein LCAT activator Ligand for HDL-receptor
ApoAII	17.4	Intestine, Liver	1	HDL	Structural protein hepatic lipase activator
ApoAIV	42.5	Intestine	11	Chylomicron	Function unknown
ApoB-100	514	Liver	2	VLDL, IDL, LDL	Structural protein Ligand for B/E-receptor
ApoB-48	246	Intestine	2	Chylomicron	Structural protein
ApoCI	6.6	Liver	19	VLDL, HDL	LCAT activator
ApoCII	8.8	Liver	19	VLDL (HDL)	Lipoprotein lipase activator
ApoCIII	8.8	Liver	11	VLDL (HDL)	Lipoprotein lipase inhibitor
ApoE	34.2	Liver	19	Chylomicron remnants VLDL, IDL, (HDL_1)	Ligand for B/E-receptor and for remnant-receptor
Apo(a)	300-700	Liver	6	Lp(a) chylomicron remnants	Function unknown

Molecular weights and synthetic origin from references (10), (19), (21), (31), (32), (50), (322); chromosomal location as given in (107).

1.2.1. Apolipoproteins AI, AII, AIV

Apolipoprotein AI is the main apolipoprotein of HDL. In adults it is mainly synthesised in the intestine (10) and secreted into the lymph either as a constituent of chylomicrons or as a component of intestinal HDL precursors. Another potential source of apoAI is the liver, where it is secreted possibly as part of nascent HDL (7, 322). LCAT, an essential enzyme for HDL metabolism, requires apoAI as a cofactor (11). The second major protein of HDL is apolipoprotein AII. It is more abundant in HDL_3 than in HDL_2, the former having a molar ratio of AII to AI of 1:3 versus 1:4 in the latter (7). In HDL_2 some apoAI-I is linked to apolipoprotein E by a disulphide bond (12). Both, apoAI and apoAII, have specific binding sites for phospholipids primarily in their amphipathic alpha-helical regions and are thereby important for the structural stability of HDL (14, 15). Recent findings by Oram (16, 17) and Schmitz (18) suggest that macrophages and fibroblasts express a protein which binds apoAI and apoAII but not apoE and possibly plays an important role in HDL-cell interactions as a cell-surface receptor, facilitating cholesterol mobilisation.

ApoAIV is an apolipoprotein synthesised in intestinal cells. It was first described as a constituent of chylomicrons in a chyluric patient (19). Its specific function is unknown.

1.2.2. Apolipoprotein B

Apolipoprotein B-100 is one of the largest monomeric proteins known. Its molecular weight is 514 kD. It is synthesised in the liver and incorporated into lipoproteins (VLDL) in the smooth endoplasmatic reticulum (20). Over more than two decades the primary structure of apoB-100 defied all efforts of elucidation due to the insolubility of this protein in aqueous buffers and its unusually large size. Only recently has the amino acid sequence been predicted by sequence analysis of corresponding cDNA (21, 22). The protein consists of 4536 amino acids which adopt a secondary structure with a high lipid binding potential mainly via amphipathic ß-sheet structures (20).

Apolipoprotein B-100 is a ligand for the LDL-receptor (B/E-receptor) which is expressed on cell surfaces throughout the body and provides the main catabolic route for apoB-100 containing lipoproteins (23). The other apolipoprotein which binds to the B/E-receptor is apolipoprotein E. The receptor binding domain of this latter protein has been identified as a basic amino acid sequence between residues 126 and 218 by testing apoE derived proteolytic peptides for their receptor-binding ability in-vitro (24). By analogy the basic sequences of apoB-100 between residues 3147-3157 and 3359-3367 have been suggested as receptor binding domains (21). This binding

region comprises three arginine and five lysine residues. By chemical modifications, such as covalent binding of cyclohexanedione to arginine residues or reductive methylation of lysine residues, the ability of apolipoprotein B to bind to the LDL-receptor can be abolished both in-vitro and in-vivo, confirming a crucial role of these basic amino acids for ligand-receptor interaction (25-27). The significance of apolipoprotein B mutations is discussed in paragraph 1.6.2.

A second apolipoprotein B isoform which is distinct from apoB-100 was isolated from chylomicrons (28). Chylomicrons are formed in the intestine following absorption of dietary fat. Apolipoprotein B associated with these particles differs from apolipoprotein B derived from VLDL when compared by SDS-electrophoresis: intestinal apoB having an apparent molecular weight of only 48% of hepatic apoB. The centile terminology for apoB isoforms, ie apoB-100 for apoB from the liver and apoB-48 for apoB from chylomicrons, was suggested by Kane (28). Recent work from Chen et al. and from Powell et al. has unravelled the unique molecular mechanism by which these two apoB lipoproteins are produced from the same gene (29, 30). In the intestine the apoB gene is transcribed into large mRNA as in hepatocytes. However immediately following transcription this mRNA is subjected to specific editing exchanging one cytosine into uracil in the codon CAA encoding for Gln 2153 which results in a UAA stop codon.

Thus the amino acid sequence of apoB-48 is terminated after amino acid 2152 and hence corresponds to the amino-terminal half of apoB-100. As noted above, the receptor-binding domain of apoB-100 is situated in the carboxy-terminal half of the molecule which explains why apoB-48 does not interact with the B/E-receptor.

1.2.3. Apolipoprotein (a)

Lp(a) was mentioned previously as an LDL-like lipoprotein, characterised by the presence of an additional apolipoprotein called apo(a). Apo(a) is synthesised by the liver (31). Its structure has been determined recently, again by use of cDNA analyses. It is very similar to human plasminogen and contains two types of plasminogen-like kringle domains, one of which is present in up to 37 repeats (32, 33). Despite this structural homology, apo(a) does not have any protease activity and its binding capacity for fibrin in-vitro is low. However, recent findings from two laboratories suggest that Lp(a) interacts with endothelial plasminogen receptors (307) and interferes with endothelial cell-mediated plasmin generation (308). This may result in impaired thrombolysis and thereby explain the role of Lp(a) as a risk factor for cardiovascular disease (35, 36, 314). On the other hand, apo(a) has been isolated not only from Lp(a) but also from chylomicron remnants which in-vitro caused lipid

accumulation in macrophages (280), providing additional scope for speculation about its involvement in atherogenesis.

Utermann et al. recently reported a size-polymorphism for apo(a), based on the number of kringle 4 repeats, which is controlled by several alleles for the apo(a) locus. Lp(a) phenotype and plasma Lp(a) concentration were found to be strongly correlated. The skewed curve for Lp(a) concentrations observed in population studies can be explained as a result of different gene frequencies for different Lp(a) isoforms (34). Published data on whether Lp(a) binds to the LDL-receptor are inconsistent, but most evidence points at Lp(a) being a less suitable ligand for the receptor than LDL (312,313).

1.2.4. Apolipoprotein CI, CII, CIII

Apolipoprotein C is the collective term for three smaller apolipoproteins (apoCI, apoCII and apoCIII) found mainly in chylomicrons, VLDL and HDL. ApoCII and ApoCIII appear to have opposing effects on the activity of lipoprotein lipase, the key enzyme in the metabolism of triglyceride-rich lipoproteins such as chylomicrons and VLDL. If apoCII is absent or defective a clinical syndrome similar to primary lipoprotein lipase deficiency (Type I HLP) results, with massive accumulation of chylomicrons and VLDL (37, 38). On the other hand an equally rare

condition with a genetic deficiency of apoCIII is marked by low triglyceride levels and accelerated VLDL degradation (39). In addition to these findings in inherited disorders an inverse correlation between the apoCII/CIII ratio and VLDL-triglyceride levels has been observed in hypertriglyceridaemic subjects, suggesting a strong influence of these apoproteins on lipoprotein lipase activity (40).

A further function of apoCIII may be the regulation of receptor mediated uptake of chylomicron remnants in the liver by steric interference with the apoE binding site (41).

1.2.5. Apolipoprotein E

Apolipoprotein E is a 34 kD glycoprotein first described as the "arginine-rich protein" due to its unusually high arginine content of about 10 mmol per 100 mmol protein (42). It is detectable in cell extracts from a number of different tissues including liver, adrenal gland, kidney, macrophages, skin and astrocytic glia (43-45). ApoE attached to lipoproteins seems to originate from the liver (31). In plasma it is mainly associated with VLDL, IDL, a subfraction of LDL and of HDL and with chylomicron remnants. The nucleotide sequence and structure of the apolipoprotein E gene has been analysed and the gene has been located on chromosome 11 (107, 328).

Phenotypic heterogeneity of apolipoprotein E was first discovered by Utermann (46), using isoelectric focussing electrophoresis to show the presence of three distinct protein bands. The molecular nature of this polymorphism was deduced from amino acid sequencing of the three apoE isoforms, which by agreement then were referred to as apoE2, apoE3 and apoE4 (47). ApoE3, by far the most common apoE isoform, has cysteine in position 112 and arginine in position 158 of the amino acid sequence. ApoE2 is derived from this by an arginine/cysteine interchange at position 158 (Arg 158 ⟶ Cys) and apoE4 is the result of a cysteine/arginine interchange at position 112 (Cys112 ⟶ Arg) (48-50).

ApoE is a ligand for the B/E-receptor as described previously. Its receptor-binding domain is thought to be in the region of the above mentioned mutations. In-vitro studies of the functionality of apoE isoforms have shown that apoE3 and apoE4 bind effectively to the B/E-receptor, while apoE2 binding affinity is reduced to about 2% of the normal value (51). Since arginine residues are essential for the receptor-ligand interaction the impaired apoE2 binding to the B/E-receptor can be explained by the mutation in position 158 which results in the loss of a positively charged arginine residue from the receptor-binding domain. Further evidence for the significance of a positive charge in this location was obtained from experiments showing that cysteamine treatment of apoE2

which converts cysteine into a positively charged lysine analogue, normalised receptor-binding completely (52). Functional apolipoprotein E, ie apoE3 and apoE4 binds not only to the B/E receptor but also to a second hepatic receptor, the apoE- or remnant-receptor. This putative receptor protein has been isolated recently from liver cell membranes (53).

Mutations other than the major isoforms apoE2, apoE3 and apoE4 have been described. They are rarer and show a variable degree of impaired receptor-binding. Examples are apoE2 (Arg 145 ⟶ Cys), apoE2 (Cys 146 ⟶ Gln), apoE2 (Arg 136 ⟶ Ser), apoE3 (Cys 112 ⟶ Arg, Arg 142 ⟶ Cys) apoE3 (Ala 99 ⟶ Thr, Ala 152 ⟶ Pro), apoE1 (Gly 127 ⟶ Asp, Arg 15 ⟶ Cys) (54-59). Other naturally occurring mutants have been identified but not sequenced yet (145-146). The significance of basic amino acids in positions 136, 140, 143 and 150 for receptor-binding was further demonstrated by site specific mutagenesis experiments, where these residues were replaced by neutral amino acids. The engineered variants all displayed defective binding pointing at the importance of ionic interactions in this process (329).

In addition to the genetically determined heterogeneity in apoE further variation in structure is caused by post translational modification, mainly covalent coupling of one or two sialic acid molecules to the protein (254).

For a review of apolipoprotein structure and function see reference (50).

1.3 Pathways of Plasma Lipoprotein Metabolism

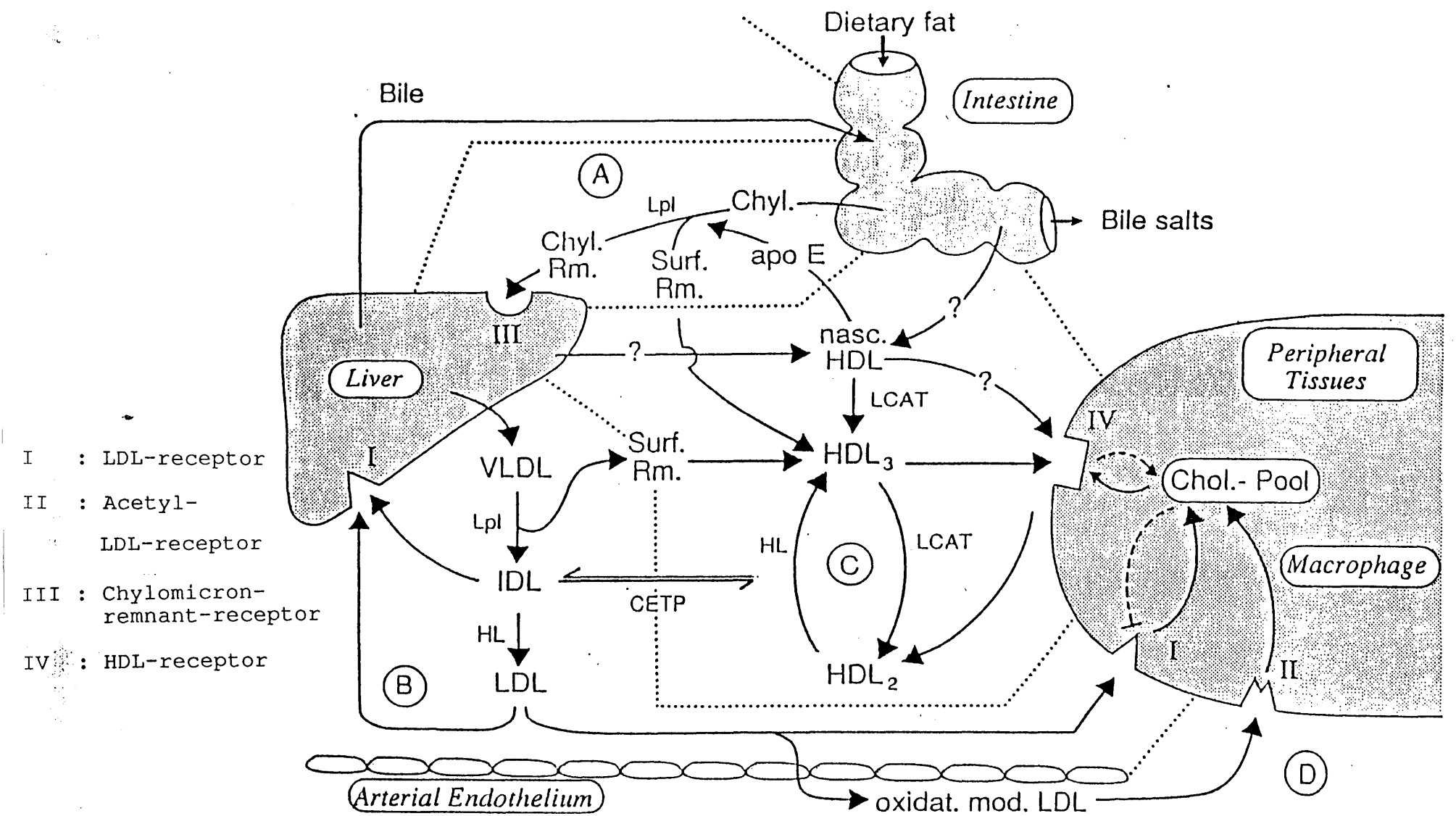
The major routes of lipoprotein metabolism are shown in Fig. 2. They interconnect in a complex fashion. However, three metabolic pathways can be distinguished:

- chylomicron metabolism
- metabolism of lipoproteins containing apoB-100
- HDL metabolism.

Each of these will be discussed in this section but the main emphasis will be on apolipoprotein B-100 metabolism. A fourth issue, illustrated in Fig. 2, has to do with the interaction between peripheral cells, in particular the cellular components of the arterial wall, and plasma lipoproteins. This is crucial for the development of arteriosclerotic disease and will be discussed in paragraph 1.5. Receptors and enzymes which play an important role in lipoprotein metabolism are listed in Table 3.

1.3.1 Chylomicron metabolism

Following intraintestinal hydrolysis, dietary fat is absorbed by enterocytes in the small intestine as ß-monoglycerides, fatty acids and free cholesterol.



I : LDL-receptor

II : Acetyl-LDL-receptor

III : Chylomicron-remnant-receptor

IV : HDL-receptor

Fig. 2: Pathways of lipoprotein metabolism. - A: chylomicron metabolism, B: apolipoprotein B metabolism, C: HDL-metabolism, D: lipoprotein metabolism in the arterial wall

Table 3: Receptors and Enzymes involved in Lipoprotein Metabolism

	Molecular Weight (kD)	Chromosomal Location	Function (see text)
RECEPTORS			
LDL-receptor	164	19	Binding of apoB-100 and apoE containing Lipoproteins
ApoE-receptor	500	?	Binding of apoE containing lipoproteins (chylomicron remnants)
HDL-receptor	110	?	Binding of HDL (apoAI and apoAII)
Acetyl-LDL-receptor	260	?	Binding of chemically or biologically modified LDL
ENZYMES			
Lipoprotein lipase	60.5	8	Lipolysis of triglyceride-rich lipoproteins
Hepatic lipase	65.2	15	Lipolysis to apoB containing lipoproteins, HDL_2 to HDL_3 conversion
LCAT	59	16	Formation of spherical HDL, HDL_3 to HDL_2 conversion
CETP	74	16	Cholesteryl ester-triglyceride exchange

Molecular weights from references (16), (53), (87), (281), (283), (315), (317); chromosomal location as given in reference (107).

Intracellularly, triglycerides and - to a lesser extent - cholesteryl esters are formed by re-esterification and these are packaged with apoB-48 to form chylomicrons, and secreted into lymph. From there chylomicrons enter the bloodstream, via the thoracic duct, where they undergo prompt changes. As a result of exchange with other lipoproteins, notably HDL, chylomicrons acquire apoC and apoE and lose most of their apoA (60); secondly, the triglyceride-rich core of chylomicrons is degraded rapidly by the action of lipoprotein lipase situated in the capillary bed of skeletal muscle and adipose tissue. As the core shrinks during hydrolysis, parts of the outer shell detach from the particle and form so-called surface remnants consisting of phospholipids, apoA and possibly some apoC. Surface remnants are considered by some investigators as a main source of HDL precursors (61).

The core unit of chylomicrons is transformed by lipoprotein lipase into particles, which are relatively cholesteryl ester-rich, triglyceride depleted and contain apoB-48 and apoE. These chylomicron remnants are catabolised in the liver by receptor mediated endocytosis, probably through a specific apoE binding remnant receptor. The amino acid sequence of a putative receptor protein has been published recently (53). Although, prior to this publication, the precise nature of this receptor was a matter of some controversy (62, 63), the existence of an apoE-specific pathway for chylomicron clearance,

independent from the LDL-receptor, was deduced from the observation that in homozygous familial hypercholesterolaemia, a condition with grossly impaired LDL-receptor function, chylomicron clearance is normal (64, 65). On the other hand, chylomicron clearance in subjects homozygous for apoE2 is markedly delayed (66, 67). Because of this evidence the concept of an independent apoE receptor is at present accepted by most investigators. However in-vitro experiments have demonstrated that chylomicron remnants can be internalised by human macrophages via the LDL-receptor indicating that the apoE-receptor is not an exclusive route of catabolism (68).

1.3.2. Metabolism of apoB-100 containing lipoprotein

The spectrum of apoB-100 containing lipoproteins in plasma is illustrated by Fig. 3. They cover a density interval from 0.950-1.063 kg/l with corresponding Svedberg flotation coefficients of S_f 0-400 at d = 1.063 kg/l. ApoB-100 containing lipoproteins are synthesised by the liver and secreted into the plasma compartment as VLDL particles (20, 69). Whether or not the liver can synthesise LDL directly is a matter of current debate (70-72). As indicated already in Table 1 the least dense particles - $VLDL_1$ - are triglyceride-rich with a low content of cholesterol and protein, the latter comprising apoC and apoE in addition to apoB-100. With increasing

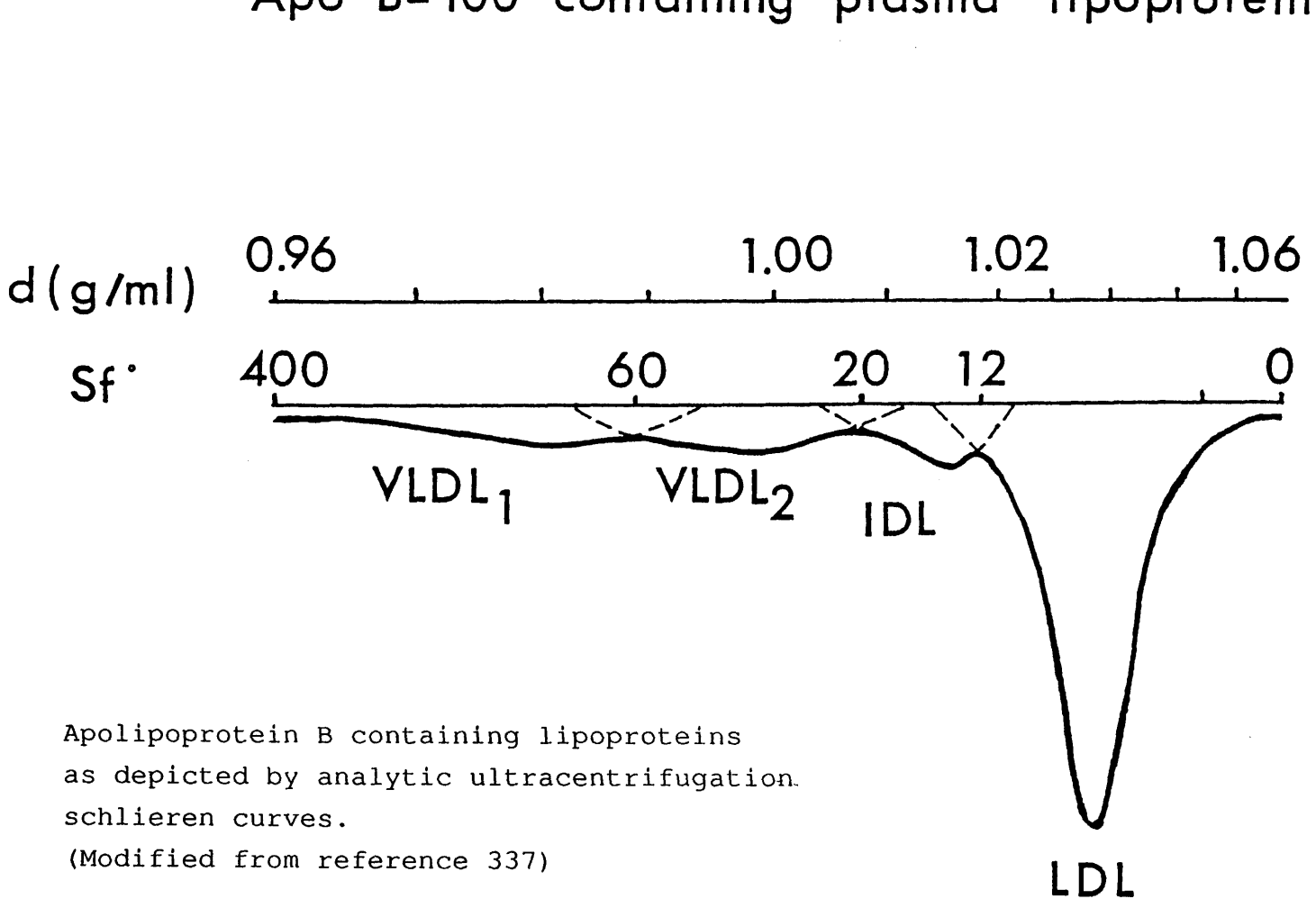


Fig. 3: Apolipoprotein B containing lipoproteins as depicted by analytic ultracentrifugation schlieren curves. (Modified from reference 337)

density the lipoproteins become triglyceride-depleted and enriched in cholesterol, cholesteryl ester and protein. By loss of apoC and apoE the composition of the protein component alters so that apoB becomes dominant, and accounts for almost all of the protein associated with LDL. This spectrum of lipoproteins represents a delipidation cascade in which the less dense S_f 60-400 VLDL is hydrolysed to form denser VLDL (S_f 20-60), then IDL (S_f 12-20) and finally LDL (S_f 0-12) as an end-product of the process.

The two lipolytic enzymes catalysing lipid hydrolysis are lipoprotein lipase and hepatic lipase. In-vitro studies suggest that their action is complementary inasmuch as lipoprotein lipase reacts preferentially with larger, triglyceride-rich particles while the ideal substrate for hepatic lipase seems to be denser lipoproteins of the IDL range (73). During VLDL delipidation surface remnants are formed in a fashion analogous to what has been described for chylomicron hydrolysis. These surface remnants are believed to be either HDL precursors or contribute to the HDL_3 to HDL_2 conversion (74). Concomitant with the loss of triglycerides, denser apoB-100 containing particles acquire cholesteryl esters which in LDL account for about 40% of the total mass. Cholesteryl esters are transferred from HDL to LDL precursors by cholesteryl ester transfer protein (CETP), partly in exchange for core triglyceride (75, 76).

A principal route of LDL catabolism is mediated by the LDL-receptor which was discovered by Goldstein and Brown in 1974 (77, 78). As mentioned earlier apoB and apoE are the specific binding proteins for this receptor with apoE showing an even higher binding affinity as compared to apoB (79, 80). Virtually all tissues express the LDL-receptor thus enabling cells to meet their cholesterol requirement at least in part by receptor mediated endocytosis. Cell culture experiments have shown that receptor-expression is regulated according to the LDL concentration in the medium, a mechanism by which cholesterol overloading via LDL-receptor mediated uptake is prevented (23, 81). From cholesterol feeding experiments in rabbits it became clear that the same principle applies to in-vivo conditions: as exogenous cholesterol supplies to the liver increased, the number of hepatic LDL-receptors were down regulated (82). Similarly in humans receptor dependent LDL catabolism was up-regulated when cholesterol was removed from hepatocytes by a drug interfering with the enterohepatic recirculation (83, 84).

Apart from LDL-receptor dependent degradation, LDL is catabolised by alternative routes. These are, on the one hand, other high affinity receptors of the reticuloendothelial system, such as the macrophage receptor for acetylated LDL, collectively referred to as the scavenger pathway (85-87). On the other hand non-specific, low affinity mechanisms like adsorptive endocytosis may

contribute significantly to LDL degradation. In normolipidaemic humans the relative contributions of LDL-receptor dependent and independent catabolism have been quantified by turnover studies using native and chemically modified LDL (27). About 50% of the LDL catabolism (0.19 pools/day out of a total FCR of 0.37/day) were attributable to LDL-receptor mediated degradation (27).

Studies investigating tissue-specific LDL degradation in various animals showed that approximately 50% of the total degradation takes place in the liver. This finding applies for receptor dependent and receptor independent LDL catabolism (88, 89). Following intracellular uptake, LDL is degraded in lysosomal vesicles. Hepatocytes are the only cells in the body with a capability of disposing of sterols. These are excreted via bile either as cholesterol or - after oxidative degradation - as bile salts.

The nature of most of the working principles in apoB-100 metabolism, like lipase-protein interactions, lipid exchange mechanisms or receptor mediated catabolism, could be worked out by in-vitro experiments. This approach, however, is not appropriate for developing a more detailed understanding of the quantitative aspects which govern the precursor-product relationship of the VLDL to LDL transformation. Only by employing in-vivo studies using radiolabelled lipoproteins as tracers did it become

possible to investigate the metabolic fate and inter-relations of distinct lipoprotein subfractions. The first experiments with trace-labelled VLDL in man demonstrated that radioactivity initially present in S_f 10-200 "VLDL" was rapidly transferred to the S_f 3-9 LDL density interval (90). Later with appreciation of the protein heterogeneity in VLDL, apoB was specifically examined and found to be the moiety that was conserved in this process in that all LDL apoB in the plasma could be attributed to the delipidation of VLDL (91). Sigurdsson et al. initially quantified this conversion and found that in normals not only did all LDL (d = 1.006-1.063 g/ml) come from VLDL but in addition all of the VLDL was catabolised to LDL (92). This rather strict precursor-product relationship was later shown to be not altogether correct in that while the majority of VLDL apoB did appear in the 1.006-1.019 kg/l density range (ie IDL), in normals a smaller proportion of this ultimately became LDL (93). The transient intermediate, IDL, is short lived and of low concentration in most subjects but can be substantially elevated in certain dyslipoproteinaemias. Further investigation of the VLDL-IDL-LDL metabolic cascade has revealed that there are multiple sites of entry and exit which can only be quantified using computer-based multicompartmental modelling techniques. These not only permit the calculation of apoprotein flux rates but also allow for the testing of quantitative hypothesis regarding

the physiology of the VLDL to LDL conversion (94, 95). An up-to-date concept of apoB metabolism in the plasma compartment will be discussed in section 4.5.

1.3.3 HDL metabolism

HDL metabolism is relatively poorly understood, partly because the synthesis and removal of these particles from plasma do not follow easily discernible metabolic routes. Three sources of HDL precursors have been suggested (7): as mentioned in previous paragraphs on chylomicron and apoB-100 metabolism, surface remnants generated during hydrolysis of triglyceride-rich particles are considered to be HDL precursors. Secondly, hepatic and intestinal cells may secrete directly discoidal "nascent" HDL and thirdly, phospholipid-apoprotein self-association may occur. Whatever the origin of HDL precursors, the discoidal shape of these complexes has to be converted into a spherical lipoprotein in order to form mature HDL_3. This process is catalysed by lecithin:cholesteryl acyl transferase (LCAT), an enzyme which esterifies cholesterol by acyl transfer from lecithin (96). Further surface remnant assimilation and LCAT – mediated cholesterol esterification converts HDL_3 into HDL_2 (74). The core diameter of HDL_2 is about 50% larger than that of HDL_3 (60 versus 40 Å) and its cholesteryl ester content is increased accordingly. Furthermore, on a cholesterol-rich diet an

even bigger HDL particle, HDL_1 or HDL_C, containing apoE but no apoAI, is generated, possibly by the same mechanism (97). On the other hand, in-vitro studies with cholesterol-loaded macrophages suggest that cholesterol, which is mobilised from these cells by interaction with HDL_3, plays a significant role in the formation of large, cholesterol-enriched HDL_1 (45,98). A similar mechanism has been shown to operate in-vivo in rabbits. After injection of a large dose of native or chemically modified LDL the HDL_2/HDL_3 ratio increased indicating that cholesterol deposited in peripheral tissues had been taken up by HDL_3 which in the process was converted into HDL_2 (99).

The HDL receptor, currently under investigation by several groups of researchers, seems to be involved in the mobilisation of intracellular cholesterol. At present, two competing concepts suggest that cholesterol is transferred into HDL either by receptor mediated translocation from intracellular pools (100, 101) or via a receptor mediated retroendocytosis pathway (18). At present it is not clear which mechanism is more important.

In light of the fact that almost half of the LDL degradation takes place in tissues which cannot break down or excrete sterols, a mechanism of cholesterol mobilisation from peripheral tissues, referred to as "reverse cholesterol transport", is of great importance. HDL_1, which usually is detectable only in low concentrations in human plasma, binds avidly to the B/E-receptor and is

probably catabolised by this route (102, 80). HDL_2 exchanges, via CETP, cholesteryl esters and triglycerides with triglyceride-rich apoB-containing lipoproteins (103). Subsequently, triglycerides are hydrolysed, primarily, by hepatic lipase and HDL_3 is finally regenerated (104-105, 340).

1.4. Genetic Disorders Affecting Apolipoprotein B Metabolism

Understanding of lipoprotein metabolism has been enhanced by the study of human diseases which are associated with disturbances of plasma lipoprotein concentrations or compositions. In this section the following genetically determined disorders of lipoprotein metabolism are discussed:

- Familial hyperchylomicronaemia (Type I HLP)
- Familial hepatic lipase deficiency
- Familial hypercholesterolaemia (Type II HLP)
- Familial dysbetalipoproteinaemia (Type III HLP)
- Familial hypertriglyceridaemia (Type IV HLP)
- Familial combined hyperlipidaemia.

1.4.1. Familial hyperchylomicronaemia

Familial hyperchylomicronaemia is a rare autosomal recessive disorder with a frequency estimated to be less

than one in a million. In the classical form of this disorder, patients have very low or absent lipoprotein lipase activity measurable in plasma after heparin injection, resulting in excessive hypertriglyceridaemia (106). While lacking enzyme activity, most patients have at least some enzyme mass, as detected by an immunosorbent test, consistent with structural mutations of the lipoprotein lipase gene, which is located on chromosome 8 (107, 108).

Typically, lipoprotein lipase deficiency is diagnosed in early childhood. Patients show failure to thrive, repeated attacks of abdominal pain, pancreatitis, splenomegaly and eruptive xanthomata. Their plasma is lactescent even after prolonged fasting due to delayed clearance of triglyceride-rich lipoproteins, both chylomicrons and large VLDL (109). Triglyceride levels in plasma often are as high as 50 mmol/l, caused by a marked increase of chylomicrons and large VLDL. In contrast, levels of VLDL (S_f 20-60) were described as normal to only moderately elevated (106). LDL as well as HDL levels are low, probably due to reduced availability of LDL and HDL precursors (108). Patients with lipoprotein lipase deficiency are not at risk for coronary heart disease, however pancreatitis which often occurs with triglyceride levels above 10 mmol/l is a serious complication. Therapy is a virtually fat-free diet, which can result in almost normal lipoprotein values.

As mentioned earlier, apoCII is an obligatory cofactor for lipoprotein lipase. In the absence of apoCII a phenotype indistinguishable from lipoprotein lipase deficiency develops (110-114). Several kindreds affected by this very rare condition have been analysed revealing a number of structural or regulatory mutations of the apoCII gene (115, 116). Another cause of functional lipoprotein lipase deficiency was observed in three related subjects in England (117). In this family lipoprotein lipase is blocked by an autologous plasma protein which was inherited as an autosomal dominant trait through four generations. Details about the VLDL metabolism in one of these patients are reported in section 3.4.

1.4.2. Familial hepatic lipase deficiency

Hepatic lipase deficiency is another extremely rare disease with only five patients described so far in the literature (118-120). The lipoprotein profile of these subjects is characterised by an increase of IDL and VLDL remnants (ß-VLDL), very low levels of LDL and an HDL fraction which consists of HDL_2 almost exclusively. VLDL levels vary widely between normal and markedly elevated. The triglyceride moieties of LDL and HDL are increased. Although the lipoprotein spectrum is clearly distorted, total cholesterol values are almost normal and at least two patients do not need lipid-lowering therapy. There is no

There is no evidence that hepatic lipase deficiency increases the risk of coronary heart disease. A study of the apolipoprotein B metabolism in one patient from Sweden (119) is reported in section 3.5.

1.4.3. Familial hypercholesterolaemia

Familial hypercholesterolaemia is a common inherited lipoprotein disorder with a gene frequency of about 1 in 500. It is caused by a defective gene encoding for the LDL-receptor (121, 122). The condition is inherited in an autosomal codominant fashion and therefore occurs in heterozygous and homozygous form (123). LDL-cholesterol levels in heterozygotes are usually two to three-fold increased as compared to normal subjects of the same age, with hypercholesterolaemia starting in early childhood. Clinically, patients show tendon xanthomas and arcus corneae and they are prone to premature cardiovascular disease. LDL levels in homozygous patients, who number about one in a million, are much higher, up to 20 mmol/l. Here the concentrations of LDL precursors, ie IDL and even $VLDL_2$, are often increased as well. The symptoms of severe coronary heart disease develop in most cases before the age of 20 years (122). The clinical course of familial hypercholesterolaemia both in its heterozygous and homozygous form, varies significantly, which is thought to result, in part, from the heterogeneity of mutations

affecting the structure of the receptor protein. Another factor, which may modify the course of disease is the concentration of Lp(a), which was found to be significantly higher in heterozygous FH patients with symptoms of coronary heart disease as compared to asymptomatic heterozygous subjects (318).

Mutations of the LDL-receptor may cause either no expression of the receptor at all (receptor-negative), or defects in the receptor function may occur, such as insufficient LDL-binding or impaired LDL internalisation into cells (122). At the genomic level more than twenty different mutations have been identified in different kindreds (124, 125). This is why most patients with the "homozygous" form of the disease are genetically compound heterozygotes. Because of this heterogeneity of the receptor defect it is impractical to diagnose familial hypercholesterolaemia by use of a single DNA gene probe or an individual restriction fragment length polymorphism (RFLP) (126). Only in genetically isolated small populations, like French Canadians, Afrikaner South Africans or Maronite Lebanese, familial FH is commonly linked with a single specific DNA mutation (127-129).

Metabolic studies have shown that increased levels of LDL in this condition primarily arise from delayed LDL clearance but in some patients oversynthesis of LDL also seems to contribute to the expanded plasma LDL pool (130, 131) with some LDL being derived through a VLDL independent

pathway (70, 132). Details of the apolipoprotein B metabolism in two patients with homozygous FH will be presented in section 3.6.

While lipid-lowering drug therapy is usually quite successful in heterozygous patients (133), homozygotes respond only poorly to diet and drug treatment and therefore require much harsher therapeutic interventions such as additional plasma exchange or LDL-apheresis (134-136).

1.4.4 Familial dysbetalipoproteinaemia

Patients with this disorder accumulate in their plasma remnant particles of both chylomicrons and VLDL, collectively termed ß-VLDL (137, 138). These particles are buoyant at a density smaller than 1.006 kg/l by ultracentrifugation but in contrast to VLDL they show ß-mobility, like LDL, on agarose gel electrophoresis (139). Compared to VLDL, ß-VLDL is cholesteryl ester-enriched at the expense of triglycerides (140). The apolipoprotein composition shows a decrease of apoC paralleled by an increase of apoE which accounts for their diminished electrophoretic mobility (141).

The delayed clearance of these remnant particles is probably due to the inheritance of apoE isoforms which do not bind normally to apoE specific cell-surface receptors (142, 143, 379). Most cases are homozygous for

apoE2 (Arg 158 ⟶ Cys) but rarely heterozygotes for the apoE2 allele are also affected (144). In addition a number of other rare apoE mutants with impaired receptor-binding affinity have been isolated from subjects with familial dysbetalipoproteinaemia (54-57, 146), some patients being only heterozygous for the defective allele. Familial apoE deficiency, a syndrome with only trace amounts of apoE in plasma, is also expressed phenotypically as dysbetalipoproteinaemia (147). Some degree of dysbetalipoproteinaemia without hyperlipidaemia is typical for normolipidaemic apoE2/2 homozygotes (142, 254).

Familial dysbetalipoproteinaemia occurs in about 1-2 in 10,000 subjects. Since apoE2 homozygosity is much more common than this, a second factor has to be postulated for the manifestation of the disease. This may be, for instance, another genetically determined hyperlipidaemia (148, 149) or a cause of secondary hyperlipidaemia such as diabetes or hypothyroidism (138). The fact that women are less often affected and that they are on average ten years older than men at the time of diagnosis suggests that oestrogen has an influence on the phenotypic expression of the disorder (150). Further evidence for genetic factors being involved in the manifestation of dysbetalipoproteinaemia come from two RFLPs which have been described in association with the condition: one for the apoB gene (XbaI) and one for the apoE-CI-CII gene cluster (HpaI),

the latter unassociated with normolipidaemic $apoE_2$ homozygosity (151, 152).

A comparative metabolic study with labelled apoE from patients and from normal controls confirmed that defective apoE metabolism is probably responsible for the lipoprotein abnormalities (153). Turnover studies with trace-labelled VLDL revealed a decreased fractional catabolic rate for VLDL and a reduced transfer from IDL to LDL, both explaining an accumulation of VLDL remnants (155, 274). Accordingly, labelled large triglyceride-rich lipoproteins of hepatic and intestinal origin (S_f > 60) containing apoB-100 or apoB-48 showed a delayed clearance following reinjection and no appreciable transfer into denser lipoproteins (67).

Typical clinical signs for dysbetalipoproteinaemia are xanthomata which may occur either as tuberous xanthoma of the skin or as linear yellow discolourations along palmar and interdigital creases (138). Cholesterol levels are usually in the range of 10-15 mmol/l and triglycerides 5-15 mmol/l. These lipid levels in combination with a VLDL-cholesterol/total triglyceride ratio of greater than 0.7 (mmol/mmol) and an apoE2/2 phenotype establish the diagnosis. Patients have a significantly increased risk for cardiovascular disease. This may be related to in-vitro observations showing that ß-VLDL is taken up avidly by macrophages which are eventually transformed into foam cells (156). The process which is mediated through

apolipoprotein E (156, 379) was thought to involve a specific ß-VLDL-receptor, but from recent studies it seems more likely now that ß-VLDL is internalised by the LDL-receptor (157). It is, however, not clear how the regulation of receptor-expression is circumvented which usually prevents cholesterol accumulation through the LDL-receptor pathway. Different rates of cellular uptake for LDL and ß-VLDL observed with macrophages from a particular homozygous patient with familial hypercholesterolaemia (158) may be explained by the specific mutation within the receptor gene in this case, affecting selectively the binding affinity for LDL but not for ß-VLDL (159, 160).

In dysbetalipoproteinemia therapeutic response to treatment usually is very good. Treatment starts with rectifying possible precipitating factors such as causes for secondary hyperlipidaemia. Lipid-lowering drugs may be considered in second line. Bezafibrate lowers plasma VLDL mainly by reducing VLDL synthesis without normalising the perturbed VLDL composition (161). In contrast, oestrogen (162) and lovastatin (163) both lower total cholesterol and triglyceride and normalise VLDL composition, probably by enhanced clearance of ß-VLDL. These drugs are known to up-regulate hepatic lipoprotein receptors. By this they help to overcome the inefficient interaction between apoE2-containing lipoproteins and receptors. The exception to this rule are seemingly patients with apoE deficiency who failed to respond

appropriately to oestrogen therapy (147). This observation indicates the need for some apoE, however defective, to mediate VLDL remnant removal.

1.4.5. Familial hypertriglyceridaemia

Familial hypertriglyceridaemia has to be diagnosed in subjects with hypertriglyceridaemia due to elevated VLDL levels and a family history of the same lipid disorder but no other hyperlipidaemias (for differentiation from familial combined hyperlipidaemia see following paragraph) (164). It is probably not a single nosologic entity and until now could not be linked to one or more clearly defined, genetic defects. Most patients with increased VLDL levels do not suffer from familial hypertriglyceridaemia but from secondary hyperlipidaemias, due to carbohydrate-enriched diet, alcohol intake or diabetes. Another more frequent disorder is familial combined hyperlipidaemia which may phenotypically present as hypertriglyceridaemia. Particle distribution within the VLDL range is skewed towards larger triglyceride-enriched particles in familial hypertriglyceridaemia as compared to normals (165).

Early metabolic studies in hypertriglyceridaemic subjects have shown that these individuals synthesise three times as much VLDL apoB as required for LDL synthesis (166). Therefore, the VLDL to LDL delipidation cascade

must allow for direct catabolism of LDL precursors at multiple sites along its length. The regulation of direct catabolism versus further delipidation is not well understood. One possible mechanism, suggested by in-vitro studies, is that if a VLDL particle has a prolonged residence in the plasma it may acquire too much cholesteryl ester in its core to permit it to shrink to the size of LDL (76). In support of this view, it has been shown that VLDL subfractions from normal subjects may be hydrolysed in the test-tube to LDL-like particles (167). Large VLDL from hypertriglyceridaemics on the other hand seems to be unable to be lipolysed sufficiently to form LDL in-vitro. In-vivo this would constitute a fraction of VLDL that fails to transit the delipidation cascade (168). Kinetic studies by Chait (169) and by Kissebah (170) investigated in parallel the metabolism of VLDL-apoB and VLDL-triglyceride in familial hypertriglyceridaemia as compared to normals. They found that the increase of triglyceride turnover was disproportionately greater than the increase of apoB suggesting that familial hypertriglyceridaemia may be due to hepatic oversynthesis of triglyceride which is then secreted as triglyceride-rich VLDL. In addition VLDL clearance was delayed, possibly because of saturation of lipoprotein lipase capacity (171). In a VLDL-turnover study by Packard et al. (154) large VLDL (S_f 100-400) and small VLDL (S_f 20-60) was used as metabolic tracers. It was shown that only the catabolism of the former was

delayed whereas the latter was converted to LDL at a normal rate. The reason for hepatic oversynthesis of large VLDL in this condition is unknown at present.

The risk for coronary heart disease does not seem to be significantly increased in familial hypertriglyceridaemia as long as other risk factors are absent (164, 321). On the other hand 5% of survivors of myocardial infarction were found to have familial hypertriglyceridaemia in contrast to a prevalence in the general population of less than 0.5% (173). Therapy should focus first of all on dietary changes with carbohydrates providing no more than 40 cal%. Only if high triglyceride levels persist lipid-lowering agents, such as nicotinic acid, may be given. Bezafibrate seems unsuitable, in particular in cases with border-line hypercholesterolaemia, as it lowers VLDL levels through enhanced delipidation and thereby may even increase LDL levels (172).

1.4.6. Familial combined hyperlipidaemia

Familial combined hyperlipidaemia was first described by several authors in 1973 following genetic analysis of lipid levels in families of myocardial infarction survivors (173, 174, 175). It is probably the most common inherited lipoprotein disorder. 10% to 30% of patients who had suffered a heart attack had raised levels of plasma cholesterol or triglyceride or a combination of

both and, in addition, a family history with the same spectrum of phenotypically different hyperlipidaemias. Lipoprotein levels are usually moderately elevated and pedigree studies have pointed at an autosomal dominant pattern of inheritance (176). In contrast to familial hypercholesterolaemia, hyperlipidaemia is not expressed in childhood and tendon xanthomata are rare. VLDL particles in familial combined hyperlipidaemia are smaller than normal but of normal composition (165). Metabolic studies have demonstrated an oversynthesis of VLDL-apoB (169, 93) and - in hypercholesterolaemic patients - also an oversynthesis of LDL-apoB, without appreciable direct LDL production (93). Therefore, the primary defect seems to be hepatic VLDL-apoB oversynthesis. Whether the condition phenotypically emerges more as hypertriglyceridaemia or as hypercholesterolaemia seems to depend on the efficiency of VLDL to LDL conversion. This view is supported by the inverse correlation between VLDL-apoB and LDL-apoB found in familial combined hyperlipidaemia (165). Obesity, showing a reduced VLDL to LDL transfer (177), may for example cause hypertriglyceridaemia in these circumstances. The reason for apoB oversynthesis is unknown.

Hyperapobetalipoproteinaemia as described by Sniderman et al. (179) is a condition positively correlated to cardiovascular disease and is marked by a relative increase of LDL-apoB without increased LDL-cholesterol levels. In this disorder too, LDL synthesis was found to be increased

secondary to increased VLDL synthesis (180). Hyperapobeta-lipoproteinaemia, however,does not follow a dominant pattern of inheritance. The relationship between this condition and familial combined hyperlipidaemia is not clear at present, but at least some overlap seems likely. Treatment for both follows the principles outlined for heterozygous familial hypercholesterolaemia and for familial hypertriglycerid-aemia.

1.5 Lipoproteins and Atherosclerosis

The link between plasma lipoproteins and in particular between apoB containing lipoproteins and cardiovascular disease has been mentioned repeatedly throughout the previous paragraphs. In the following more evidence for this correlation will be presented as derived from epidemiological studies during the last twenty years. In addition biochemical and cell-biological observations linking plasma lipoproteins with atherosclerotic changes of the vessel wall will be discussed.

1.5.1. Population studies and intervention trials

The positive correlation between plasma cholesterol and the prevalence of coronary heart disease was demonstrated by a number of population studies including

the Framingham Heart Study (181), the Pooling Project (182) and the Seven Countries Study (183). The best data come from the Multiple Risk Factor Intervention Trial (MRFIT) where 356,222 men, aged 35-57 years, were screened and followed up for six years (184). The number of deaths caused by coronary heart disease in relation to plasma cholesterol concentration is plotted in Fig. 4 (185). The correlation is continuous and dose-related in an exponential manner throughout the whole range of plasma cholesterol (186, 187). There is no threshold cholesterol level below which coronary heart disease is independent from the plasma cholesterol concentration. On the basis of quintile analysis it was estimated that nearly half of all observed deaths from coronary heart disease were attributable to serum cholesterol levels greater than 4.7 mmol/l (186). Detailed analysis of the Framingham data have shown that the same graded correlation between plasma cholesterol and risk of cardiovascular disease exists in the presence of other risk factors, notably cigarette smoking, diastolic hypertension or diabetes (188). Conversely, observations from Japan demonstrate that smoking combined with low plasma cholesterol does not result in increased incidence rates for coronary heart disease (183). Furthermore, data from Framingham provided supportive evidence for opposing correlations between LDL-cholesterol and HDL-cholesterol with regard to CHD risk (189). Based on twelve years follow-up data,

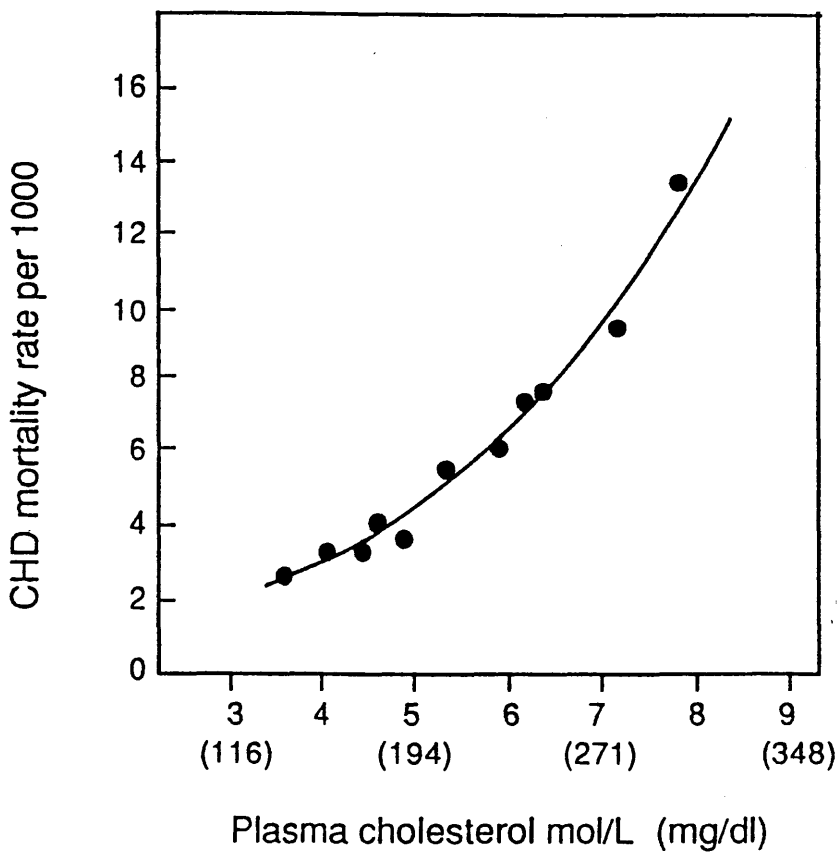


Fig. 4: Correlation between plasma cholesterol and mortality from coronary heart disease in participants of the Multiple Risk Factor Intervention Trial.
(Modified from reference 185)

individuals in the top quintile of HDL-cholesterol were found to have half the risk as compared with subjects in the lowest quintile (190). Autopsy studies have confirmed that serum cholesterol levels as well as high blood pressure are quantitatively related to the extent of atherosclerotic lesions (191).

Another line of evidence for a causal link between elevated plasma cholesterol levels and coronary heart disease comes from intervention trials where cholesterol levels were decreased with the aim of reducing coronary heart disease incidence. In the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) a 19% lower incidence of coronary heart disease was accomplished by a mean fall of 12% in LDL-cholesterol in men treated with cholestyramine as compared to placebo controls (192). In another placebo-controlled trial, the Helsinki Heart Study, a 10% decrease in LDL-cholesterol and a 10% increase in HDL-cholesterol were achieved by treatment with gemfibrozil (600 mg/day). This caused the cumulative rate of cardiac morbidity after five years to fall by 34% in the treatment group (193). However, due to an unexplained increase in non-cardiovascular mortality (ie death mainly by accidents or violence but not by cancer) both studies failed to produce a significant decrease of total mortality in the treatment group (193, 194). The Multiple Risk Factor Intervention Trial (MRFIT) with 12,866 high-risk men participating, also did not show a difference in death

rates between special intervention group and usual-care group, possibly because the beneficial effects of reduced cigarette smoking and dietetically lowered cholesterol were outweighed by an unfavourable response to antihypertensive drug therapy in a subgroup of hypertensive patients with arrhythmias. Statistical analysis was further complicated by the fact that risk factor levels declined not only in the intervention group but also, to a lesser extent, in the usual-care group (184). In the Oslo Study, an earlier, smaller intervention trial, the CHD incidence rate was lowered by 47%. Intervention included dietary advice and encouragement to stop smoking but no antihypertensive treatment. Plasma cholesterol levels were decreased in the intervention group by 13% as compared to controls (316).

A more rigorous regimen of treatment with lipid-lowering drugs (Colestipol 30 g/day and Niacin 3-12 g/day) was applied in a placebo-controlled angiographic trial, the Cholesterol Lowering Atherosclerosis Study (CLAS), resulting in a 43% reduction of LDL-cholesterol and a simultaneous 37% increase of HDL-cholesterol. Participants were 162 non-smoking men with previous coronary bypass surgery. Coronary angiograms from before and after two years of treatment showed that deterioration in overall coronary status was significantly less in drug-treated subjects and that regression of atherosclerotic lesions occurred in 16.2% of treated subjects versus 2.4% in controls (195).

In conclusion, despite the problems of interpretation with the largest study mentioned above, evidence for a causal link between high plasma cholesterol and increased risk for cardiovascular disease is very strong.

1.5.2. Lipoprotein interactions with the arterial wall

Recent progress in cell biological research has now unveiled some of the mechanisms which are important for the interaction between plasma lipoproteins and the cells of the arterial wall (196). Henricksen et al. observed in 1981 that LDL, after incubation with arterial endothelial cells, is taken up more rapidly by macrophages than native LDL (197). Similar to acetylated LDL or ß-VLDL but in contrast to native LDL, endothelial cell-modified LDL can transform marcophages into foam cells. Cells other than endothelial cells such as smooth muscle cells or macrophages can modify LDL in the same way (198, 199). The cell-induced modification is primarily a peroxidation of polyunsaturated fatty acids cleaved from phospholipids (200). Short-chain aldehydes, generated during the process, react covalently with lysine residues of apolipoprotein B, modifying the protein in a way similar to in-vitro acetylation (201, 202). Interestingly, the uptake of acetylated LDL by macrophages can be competitively inhibited to about 80% by biologically modified LDL,

meaning that both lipoproteins are binding to the same receptor. Thus, in-vivo oxidatively modified LDL seems to be a natural ligand for the acetylated LDL-receptor. As acetylated LDL is a far less effective competitor for the uptake of biologically modified LDL, the existence of a second scavenger receptor has been concluded (203). Oxidised LDL is antigenic, so antibodies could be raised against it, which were used to detect modified LDL by immunohistological methods in atherosclerotic lesions. Furthermore, autoantibodies against modified LDL can be demonstrated in human sera, providing evidence that biological LDL modification does occur in-vivo (204).

These observations form the basis for a model of atherogenesis, which was suggested first by Steinberg (205). It tries to explain the formation of fatty streaks, which are the earliest distinct lesions in the atherogenic process, starting from elevated LDL levels in plasma. LDL is transferred by endocytosis from plasma into the subendothelial space where it is oxidatively modified by appropriate cell contact and internalised by macrophages which by cholesteryl ester accumulation develop into foam cells. Biologically modified LDL is chemotactic for circulating monocytes but inhibits the mobility of resident macrophages, thus recruiting monocytes from the blood stream and accumulating macrophages in the subendothelial space (206). Morphologic studies have confirmed monocyte adhesion and foam cell accumulation as early events in the

process of fatty streak formation (207). Activated macrophages release growth factors which in turn recruit smooth muscle cells from the media and stimulate their growth (208). Moreover oxidatively modified LDL is cytotoxic and may damage, for instance, endothelial cells (198). Endothelial denudation, which again has been described by scanning electron microscopy in early atherosclerotic lesions, provides a focal point for platelet aggregation and facilitates further influx of plasma proteins (207). This model of fatty streak formation does not rule out other mechanisms to act simultaneously, for example, accumulation of chylomicron remanants (209) aggravated by cytotoxicity of lipolytic surface remnants (210). On the other hand, it brings together two often competing theories of atherogenesis - "lipid infiltration" versus "response to injury" - presenting them as complementary aspects of one process.

The progression of fatty streaks into fibrous plaques and eventually into necrotic lesions goes along with a continuation of the above outlined process (273). While foam cell formation continues at the luminal side of the vessel wall, foam cells at the bottom of the lesion start to die, possibly through intracellular precipitation of free cholesterol (211). In advanced lesions the content of free cholesterol, partly precipitated as crystals, is markedly higher than in fatty streaks (212). The

cholesteryl ester profile (cholesteryl oleate versus linoleate) of advanced lesions resembles more LDL than fatty streaks (213), suggesting that most of the cholesteryl esters stored in foam cells are hydrolysed when cells die, while LDL-proteoglycan interactions may account for at least some of the cholesteryl esters trapped in the lipid core of necrotic plaques (214, 215).

As evidenced by the results of CLAS (195), which were outlined earlier in this section, regression of atherosclerotic lesions can occur, if favourable conditions are provided. The role of HDL as a vehicle of reverse cholesterol transport has been addressed in paragraph 1.3.3. In-vitro studies (45, 98) have shown that in the presence of cholesterol acceptors, such as HDL_3, cholesteryl esters can be mobilised from foam cells. Animal experiments with rhesus monkeys suggest that regression of coronary lesions occurs uniformly if the total cholesterol/HDL-cholesterol ratio is smaller than 2.8 (216).

1.6. Factors Determining Plasma Cholesterol Levels

In contrast to most other plasma constituents, cholesterol levels are not regulated within narrow limits but vary greatly. This variation can be seen between individuals but also within one person. As with most biological variables influences can be either environmental

or genetic and probably most importantly a combination of both.

1.6.1. Influence of environmental factors on plasma cholesterol concentration

Since World War II cardiovascular disease has been the major cause of death in Western Societies. The LDL-cholesterol levels typical of a polulation with a high CHD rate are about five times higher than the minimum thought to be required to deliver cholesterol to body cells via the LDL-receptor (23). In contrast, societies with almost no CHD mortality have mean cholesterol levels nearer to this biochemically defined ideal level. Cross-cultural comparisons, such as the Seven Countries Study, demonstrate that the amount and the composition of dietary fat in the habitual diets on the one hand and the mean serum cholesterol levels and CHD rates on the other hand are strongly correlated (183). This points to diet as an important reason for high plasma cholesterol levels and for high CHD mortality in industrialised countries.

Anthropologists have developed some ideas of the composition of a "palaeolithic" diet which probably prevailed through almost all of man's two million years of evolutionary history (217). Interestingly, the total dietary energy in form of fat was only half of the current American diet (21% versus 42%) and the

polyunsaturated:saturated fat ratio was more than three times higher (1.41 versus 0.44), whereas cholesterol intake was very similar. In the light of these observations the current diet in "Western" countries seems unphysiological, resulting in lipoprotein levels which exceed by far natural requirements. Incidence rates for CHD are not static and in some countries, most notably in the USA, CHD rates are declining steadily after they had reached a peak in the early 1960s (218-220). These changes coincide with a trend towards lower mean cholesterol levels among US adults (221), a reduced per capita consumption of unskimmed dairy products, eggs and animal fats and with a marked increase in use of vegetable fats and oils (222). Since 1963 smoking has also declined significantly in North America. Major changes in lifestyle, some of which have had beneficial effects on plasma cholesterol, seem to have contributed to a significant decline of CHD mortality by more than 30% within three decades (218). A study from the Netherlands showed that as little as one fish meal per week (30 g per day) has a measurable effect in reducing the twenty year mortality from CHD (223). In this particular study lipoprotein levels were not measured. Not all dietary effects on CHD morbidity are caused by changes in lipoprotein metabolism (224) but from the observations outlined above it can be concluded that diet as an environmental factor has an important influence on plasma

lipoprotein levels and - mainly as a result of this - on the incidence rate of CHD.

1.6.2. Genetic determinants of plasma cholesterol concentrations

On the other hand, there is clear evidence for genetic influence on plasma cholesterol levels which has been estimated by family and by population studies to account for 50% to 60% of the total individual variance (225, 226). A comparative study of Japanese living in Japan, in Hawaii and in North America has shown that along with assimilation to "Western" diet and lifestyle both cholesterol levels and CHD rate increased (227). However, the CHD incidence of Japanese living in California was, in spite of similar environmental influences, only half the rate observed in Caucasian North Americans.

One of the most striking examples for genetically defined hyperlipoproteinaemia is familial hypercholesterolaemia, which is discussed in paragraph 1.4.3. However, while it is one of the most common inherited metabolic disorders it accounts for only 5% of myocardial infarctions under the age of 60 years (173). Although the effect of a defective receptor gene is very significant for an affected individual, the impact on cholesterol level variation in the population is only modest, because the defective gene

frequency of 1 in 500 is low with regard to the population as a whole. As the impact of a genetic trait on interindividual variation within a population is defined by the product of gene effect and gene frequency, common mutations exerting a relatively small effect on cholesterol concentrations may account for quite a large percentage of interindividual differences.

It has been proposed that there is a genetic contribution to LDL-receptor binding activity in normolipidaemic subjects. Receptor dependent LDL degradation by mononuclear cells or fibroblasts obtained from normolipidaemic monozygotic and dizygotic twins demonstrated a markedly bigger within-pair variation for the latter (228, 229). Plasma cholesterol levels did not vary accordingly, but the response to dietary cholesterol was found to be inversely linked to the LDL-receptor activity in-vitro (230). A number of RFLPs for the receptor gene have now been reported and it remains to be seen whether some of them correlate with hyperlipidaemia or CHD (107).

Variation of the apoB gene is another obvious possibility for genetic determination of apoB containing lipoprotein concentrations. Grundy et al. reported a turnover study in which the metabolism of autologous LDL and LDL from a normal donor were directly compared in moderately hypercholesterolaemic subjects (231). In five individuals, autologous LDL was cleared at a markedly lower

rate than control LDL. Fibroblast studies confirmed a reduced LDL-receptor binding affinity for LDL from one of these subjects (232) and subsequent sequencing of the receptor-binding domain revealed an Arg to Gln interchange in position 3500 of the amino acid sequence of apolipoprotein B (233). This mutant, now termed familial defective apolipoprotein B-100, has been found in several unrelated families where it also seems to be associated with moderate hypercholesterolaemia (234, 235).

Two other mutations of the apoB gene, originally defined as RFLPs for MspI and for EcoRI in the 3′ end of the gene, are now identified as Arg 3611 ⟶ Gln and Glu 4154 ⟶ Cys amino acid substitutions respectively (236, 237). Both mutations have been linked to significant variations in serum cholesterol levels and CHD risk (238-240). However, none of these correlations are sufficiently strong to be found consistently in all sample groups studied (240, 241).

In 62 subjects studied a Thr ⟶ Ile substitution at apoB amino acid residue 71 was associated with an apoB polymorphism defined by high or low binding affinity to the monoclonal antibody MB19 (242). In heterozygotes for this polymorphism (MB19+/M19-) this antibody was used to identify an allele specific difference in the expression of the apoB gene. This was found to be an autosomal dominantly inherited trait (243). The mechanisms of unequal apoB expression are unknown at present.

Finally, many workers have investigated an apoB gene polymorphism defined by an XbaI-RFLP. The two alleles differ due to a C to T interchange in the third position of Codon 2488 of the apoB gene which creates a cutting site for XbaI but is silent with regard to the amino acid sequence of apoB (245). Thus the mutation in itself does not change the apoB structure, but it seems to be in linkage disequilibrium with other, functionally significant mutations. Several studies show an association between serum cholesterol levels and the XbaI-RFLP (151, 246-248). Moreover, this polymorphism is closely linked to the x/y alleles of the antigenic group (Ag) system, an antigenic apoB polymorphism discovered by antisera from multiply transfused patients (237, 249). The Ag x/y antigen in turn has also been linked with different plasma cholesterol levels (250). Other Ag-antigens have been characterised by specific RFLPs and are listed in Table 4 (275-279). Not all studies have confirmed an association between the XbaI polymorphism and plasma cholesterol levels and unless a functionally relevant mutation in close linkage to the XbaI cutting site is found, the significance of this polymorphism is difficult to evaluate (241, 251, 252). This applies also to association studies of the XbaI polymorphism with regard to CHD, which have produced conflicting results (238, 241).

The best defined genetic factor determining variation of apoB lipoprotein concentrations within the

Table 4: Polymorphisms of the ApoB Gene as Defined by RFLP and ApoB Immunoreactivity

ApoB Protein	cDNA Mutation	RFLP*	Ag-System**	MAB	Correlation with Plasma Cholesterol or CHD (see text)	References
Thr 71 → Ile	ACC→ATC	ApaLI (n=62) Bsp 12861 (n=17)	c/g	MB19	MB19(+/-) correlated with allele-specific gene expression	(242,243) (275-276)
Val 591 → Ala	GCT → GTT	AluI (n=17)	a1/d	H11G3		(277)
Thr 2488 (no change)	ACC → ACT	XbaI	(x/y) (c/g)		Correlation with plasma cholesterol and CHD	(237) (246) (278,279)
Arg 3500 → Gln	CGG → CAG	(XbaI) (MspI)		MB47	Defective LDL receptor binding	(232-234)
Arg 3611 → Gln	CGG → CAG	MspI			Correlation with plasma cholesterol CHD	(236)
Glu 4154 → Lys	AAA → GAA	EcoRI (n=17)	t/z		Correlation with plasma cholesterol and CHD	(237) (279)

* The enzymes listed detect the respective apoB mutant by RFLP analysis, as the change occurs in the region of a specific cutting site. Enzymes in brackets refer to RFLPs which are in linkage disequilibrium with the respective mutation.

**Where correspondence between RFLPs and Ag-antigens was perfect, the number of tested individuals (n) is given. Lesser associations between RFLPs and Ag-antigens are indicated by brackets.

normal range is the apolipoprotein E polymorphism (253). The molecular basis of the apolipoprotein E polymorphism is explained in paragraph 1.2.4. The three apoE isoforms, apoE2, apoE3 and apoE4 commonly found in the population, are the gene products of three different alleles, e_2, e_3 and e_4, for the apoE locus (254). Inheritance is codominant resulting in three homozygous and three heterozygous phenotypes which can be distinguished by isoelectric focussing: apoE2/2, apoE3/3, apoE4/4 and apoE2/3, apoE3/4 and apoE2/4 respectively.

Gene frequencies observed in six Caucasian populations showed no statistically significant differences. The e_3 gene is by far the most common, accounting for an apoE3/3 phenotype in 60-65% of the individuals studied in each group (255). Comparisons between Caucasian and Asian populations demonstrated a higher e_3 frequency in Asians but no other consistent differences (256). Within the group of Caucasian populations Finns had a significantly higher e_4 frequency and a lower e_2 frequency as compared to all other groups (257).

Utermann was the first to observe that the majority of apoE2/2 homozygotes, not affected by familial dyslipoproteinaemia, still had an increased VLDL-cholesterol/triglyceride ratio but their LDL-cholesterol levels were lower than the population mean (142). Population studies which were carried out in various

countries have confirmed that apoE2/2 subjects have significantly lower LDL-cholesterol levels than apoE3/3 subjects. In contrast, apoE4/4 homozygotes have higher LDL-cholesterol levels and those of heterozygotes (apoE2/3, apoE3/4) fall between the values for the respective homozygotes. However, the impact of the e_2 and e_4 alleles on the LDL-cholesterol concentration is unequal. On average, the cholesterol reduction associated with e_2 is more than two times the cholesterol increase linked with e_4 (255). In addition, the cholesterol increase in apoE4/4 subjects is more variable with reports ranging from almost no difference to an increase of up to 12%. With regard to apolipoproteins the same correlations described for LDL-cholesterol were found for apolipoprotein B-100. Correlations for plasma apoE concentrations are inverse in that apoE2/2 subjects have the highest levels , apoE3/3 are intermediate and apoE4/4 levels are lowest (258, 259).

It has been mentioned previously that in-vitro apoE2 binding to the LDL-receptor is reduced to less than 2% of the binding observed with apoE3 and apoE4 which in cell binding assays are indistinguishable. In contrast, in-vivo studies in humans with trace-labelled apoE have demonstrated a more rapid catabolism of apoE4 as compared to apoE3 while the apoE2 clearance was delayed as expected (153, 260). These results are in line with the above mentioned correlations between apoE phenotype and apoE plasma concentrations. How different apoE isoforms

interfere with apoB metabolism is not yet entirely clear and will be discussed in section 4.2.

As apolipoprotein B levels are influenced by the apoE polymorphism, it is of obvious interest to see whether the apoE phenotype correlates with disorders of lipoprotein metabolism or with the risk for coronary heart disease. In hypertriglyceridaemia, notably in familial hypertriglyceridaemia, the e_2 allele is more frequent (261, 262) and in hypercholesterolaemia the frequency of the e_4 allele is increased in comparison with the population at large (261, 263). In severe hypertriglyceridaemia (Type V hyperlipidaemia with triglyceride >10 mmol/l and fasting chylomicronaemia) the e_4 allele frequency was found in two studies to be 2.5 to 3.5-fold increased (264, 265).

Only two (266, 267) out of several studies (266-271) have shown a higher frequency for the e_4 allele in CHD patients. In two studies the mean age at the time of the first infarction was lower in apoE4/3 than in apoE3/2 subjects (266, 269). Another hint for a potential atherosclerotic risk associated with apoE4 is given by the high frequency of the e_4 allele in the Finnish population, which coincides with high plasma cholesterol levels and a high incidence rate for CHD (257). Finally, a lower overall frequency for the apoE4 phenotype has been reported in healthy octogenarians and can be taken as an indication for a relatively increased mortality associated with the e_4 gene (272). A possible reason for the lack of a stronger

negative correlation between the e_2 allele and CHD is the prolonged plasma residence time for VLDL- and chylomicron remnants, which may counteract the benefit from low LDL concentrations (209, 343). Taken together this evidence supports the view that apoE isoforms influence the risk for CHD although this effect is rather small. About 7% of the inter-individual total variation in cholesterol concentrations is associated with the apoE polymorphism, whereas less than 3% of the variation in CHD risk may be ascribed to this gene locus (253, 255).

1.7. What Is The Purpose of This Study?

This thesis sets out to contribute to the understanding of how genetically determined factors interact with the metabolism of apolipoprotein B, the most important single risk factor for coronary heart disease. Two common genetic determinants of apoB metabolism, the apoE polymorphism and the XbaI apoB gene polymorphism were examined with regard to their impact on apoB metabolism in humans. The tools of investigation were turnover studies, either covering the whole of the apoB metabolism in plasma (ie VLDL-turnovers) or assessing the metabolism of LDL, the prevailing apoB containing lipoprotein in normolipidaemics as well as in most hyperlipidaemias. Apolipoprotein B metabolism was also studied in three genetically determined disorders of apolipoprotein B metabolism. These were

familial hyperchylomicronaemia, familial hepatic lipase deficiency and familial hypercholesterolaemia. All of them are biochemically well characterised and provide unique opportunities to learn more about the significance of specific defects for apolipoprotein B metabolism as a whole. All metabolic studies were analysed on the basis of a metabolic model for apoB metabolism. This allowed testing of the validity of the model and led to suggestions for further developments.

2. METHODS

2.1. Apolipoprotein E Preparation by Preparative Gel Electrophoresis

Apolipoprotein E was prepared by a modified version of the method of Meunier et al. (284).

2.1.1. Preparation of samples

(a) Preparation of VLDL

250 ml of plasma were obtained by plasmapheresis from a subject with plasma triglyceride concentration > 1.5 mmol/l. Total VLDL (d < 1.006 kg/l) was prepared by ultracentrifugation in a Beckman Ti60 rotor for 16 h at 36,000 rpm, 10°C. The supernatant VLDL (10-15 ml) was collected and dialysed against 0.01% EDTA, pH 7.4 at 4°C.

(b) Preparation of apo-VLDL

VLDL was delipidated at -20°C with ten volumes of ethanol:ether (3:1) twice and finally with ether once. The moist protein pellet was resolubilised in 6-12 ml of either:

(i) 0.1 M Tris, 3% SDS, 1% DTT, pH 6.7

(ii) 0.1 M Tris, 3% SDS, pH 8.2

Resolubilisation took place overnight at room temperature with gentle shaking.

Samples resolubilised in buffer (i) were used for apolipoprotein E preparation. The protein solution was heated in a boiling water bath for 3-5 min and subsequently glycerol was added to a final concentration of 20%. 20 ul BPB solution were added as a colour marker. This apolipoprotein preparation was aliquotted (2 ml) and could be stored at -20°C for several weeks.

(c) Dansylation of apo-VLDL

Samples resolubilised in buffer (ii) were used to produce dansylated apoproteins, which were used as markers on preparative electrophoretic gels (285). 2 ml of the protein solution were mixed thoroughly with 40 ul of 10% dansyl chloride in acetone. This was incubated in a boiling water bath for 2 min. Then 0.02 g of DTT were added and the sample boiled for another 1 min. Thereafter, it was dialysed against 200 ml 0.1 M Tris, 3% SDS, 1% DTT, pH 6.7. Finally glycerol was added up to a final concentration of 10%. Aliquots of 250 ul were stored at -20°C for up to several months.

2.1.2. Preparative gel electrophoresis

The separation of VLDL apolipoproteins was carried out on a vertical acrylamide slab gel (14 cm x 14 cm x 0.3 cm). The stacking gel was 1 cm high and had a concentration of 3.5% acrylamide in 0.06 M Tris, 0.1% SDS, pH 6.7. The separation gel concentration was 14%

(w/v) acrylamide in 0.5 M Tris, 0.1% SDS, pH 9.1. Both gels contained bisacrylamide in a concentration of 2.5% (w/w) of the acrylamide concentration. Polymerisation of gels was initiated by addition of 0.2% (w/v) ammonium persulfate and 0.03% (v/v) TEMED.

The running buffer was 49 mM Tris, 380 mM glycine, 0.1% SDS, pH 8.3. VLDL apolipoprotein preparation (2 ml) were applied on the stacking gel in a large central trough. As marker for VLDL apolipoproteins 100 µl of dansylated apo-VLDL were placed in each of the two lateral slots. For initial determination of molecular weight a 50 µl aliquot from a protein calibration kit was added to the apo-VLDL marker. Electrophoresis was performed at 100 V with a 40 mA current for the first 1.5 h and at 200 V, 80 mA for another 3 h.

Dansylated lipoproteins were visualised under UV light (λ = 254 nm). The fluorescent bands, which had been identified as apoE by molecular weight calibration, were located at both margins of the separation gel (Fig. 5). A horizontal strip of gel containing apoE bands at both ends was excised under UV light control and put on top of a vertical agarose slab gel held by a bottom layer of a 3.0% acrylamide plug gel. Concentration of agarose (low standard -mr; Biorad) was 0.8% in 0.24 M Tris, 0.1% SDS, pH 6.7. The same buffer was used for the plug gel.

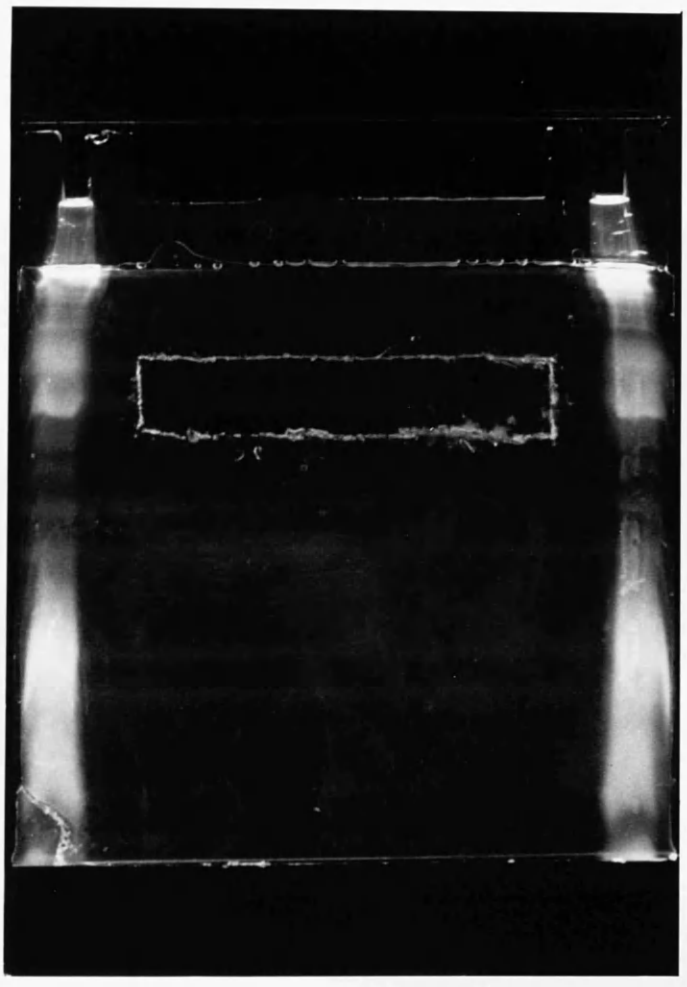


Fig. 5: Preparative SDS-gel electrophoresis after separation of VLDL apolipoproteins. ApoB, apoE and apoC are visualised in UV light by dansylation. A central gel section containing apoE has been removed for apoE preparation.

2.1.3. Preparation of apolipoprotein E

Protein was transferred from the acrylamide gel to the agarose gel by a 2 h electrophoresis at 70 mA. The protein was detectable in the agarose gel as a refringent band which was sliced out and placed in an ultracentrifuge tube. After centrifugation in a Beckman Ti60 rotor at 40,000 rpm for 20 min, apoE was recovered in 1-2 ml of supernatant.

The apoE solution was lyophilised and successively extracted twice with 20% TCA and once with acetone in order to remove SDS and salt (28). The precipitated protein was resolubilised in saline or in sample buffer for IEF.

2.1.4. Preparation of ^{125}I-labelled apolipoprotein E

(a) Preparation of TMU-soluble apo-VLDL

VLDL was prepared as described above. 2 ml VLDL were warmed up to 37°C and mixed with an equal volume of TMU. After another 30 min at 37°C the sample was centrifuged (3000 rpm, 20 min) and the fluid phase containing non-apoB lipoproteins recovered. The solid apoB pellicle was discarded (286). Protein was precipitated from the water/TMU phase by incubation with five volumes of chloroform:methanol (1:1) for 4 h at 4°C with gentle rocking. After centrifugation protein was washed with ether and resolubilised in 0.5 ml saline (0.9% NaCl in water).

Protein concentration was determined by the optical density at 280 nm.

(b) Radioiodination of TMU-soluble apo-VLDL

2 ml resolubilised protein were mixed with 0.5 ml 1 M glycine, pH 10, and 2 mCi ^{125}I. Finally ICl (25 nm/μl) was added at a molar ratio of 1:1 with regard to protein (molecular weight of protein assumed as 29 nM equivalent to 1 mg). During addition of ICl the reagent mixture was gently shaken to enhance an even distribution (287). Iodination was followed by an overnight dialysis against 0.1 M Tris, pH 6.7, for removal of free ^{125}Iodine. The solution of labelled proteins was then made 3% for SDS and 1% for DTT, boiled for 3 min in a water bath and finally made 10% for glycerol.

This sample was applied on a preparative SDS-acrylamide gel as described above. Protein bands could be detected with a Geiger-Muller tube, with maximal counts in regions where dansylated apo-VLDL bands could be visualised under UV light.

ApoE preparation from the acrylamide gel was identical with the procedure described for unlabelled protein.

2.2. Production of a Monoclonal Antibody Against Apolipoprotein E

2.2.1. Immunisation of mice

300 µg apoE solubilised in 600 µl saline were mixed thoroughly with 180 µl complete Freund's adjuvant until a creamy homogenous suspension was obtained. Ten mice were each injected intraperitoneally with 200 µl containing 20 µg apoE. After two weeks a booster injection, with incomplete Freund's adjuvant was applied. Dosage and route of injection were identical to the first injection.

Two weeks later the animals were anaesthetised with ether and 1-2 blood spots from the tail vein were collected on filter paper for antibody-binding tests. Three days before splenectomy for cell fusion (see 2.3.) a third apoE injection (20 µg) without adjuvant was administered into the tail vein.

2.2.2. Antibody-binding tests

Specific antibodies in murine plasma or cell culture medium were determined by a solid phase antibody-binding test (288).

(a) Solid phase anti-mouse γ-immunoglobulin

This was prepared using carbonyldiimidazole activated Sepharose-CL 4B as described by Chapman et al. (289, 290).

(b) Antibody-binding test from blood spot

A 6 mm disc was punched from the filter paper dried bloodspot and incubated overnight in 300 µl PBS, 10% sheep serum. Plasma was eluted at an estimated dilution of 1:150. Further dilutions of 1:450 and 1:1350 were prepared in the same buffer. Aliquots (100 µl) were taken in duplicate for each dilution and 200 µl PBS containing ^{125}I-apoE (about 20,000 cpm) were added. Samples were mixed thoroughly and incubated at room temperature overnight. Then 200 µl solid phase anti-mouse γ-immunoglobulin was added and incubated on an orbital shaker for another 1 h. After three washes with saline plus 0.2% Tween 20 radioactivity in the Sepharose pellet was determined and expressed as percentage of initial radioactivity. Non-specific binding of ^{125}I-apoE was determined in controls containing saline instead of plasma.

(c) Antibody-binding and displacement test from cell culture medium

Cell culture supernatant was diluted 1:3, 1:9 and 1:27 with PBS. Sheep serum was added to a final concentration of 6.6%. Aliquots (100 µl) were incubated with 50 µl ^{125}I-apoE (20,000 cpm) and either (a) 50 µl saline or (b) 50 µl unlabelled apoE at a concentration of

50 µg/ml. After overnight incubation the same procedure was followed as described in the previous paragraph. Samples (a) provided results for antibody-binding whereas samples (b) accounted for antibody displacement by excess of unlabelled apoE. The former is given as percentage of the initially added radioactivity. The latter is the difference between specific binding with and without excess apoE, expressed as percentage of the binding without unlabelled apoE.

2.2.3. Fusion protocol for myeloma-lymphocyte hybrid cells

Murine myeloma cells and spleen lymphocytes obtained after apoE immunisation were fused to form hybridoma cells following a modified protocol from Fazekas et al. (291). Two independent cell fusions with lymphocytes obtained from the same animal were performed in parallel.

(a) Myeloma cells

Myeloma cells (X63-Ag8-653) were grown up in 25 cm^2 flasks, then transferred into 75 cm^2 flasks until about 100 x 10^6 cells were obtained. Cells were collected, centrifuged (800 rpm, 10 min) and washed twice with RPMI-1640 and finally suspended in 10 ml RPMI-1640.

(b) Spleen lymphocytes

The spleen of an immunised mouse was dissected under aseptic conditions and placed in a Petri dish containing 10 ml RPMI-1640. By teasing the organ a cell suspension

was prepared which was allowed to settle a few minutes in a test tube. The cell suspension was centrifuged (800 rpm, 10 min) and cells were resuspended in 10 ml RPMI-1640.

(c) Preparation of murine peritoneal macrophages

Three mice were killed by exposure to ether vapour. The abdominal skin was dissected and 5 ml RPMI-1640 were injected intraperitoneally. After gentle massage of the abdomen RPMI-1640 was withdrawn slowly. Peritoneal washings from three mice were pooled and centrifuged (800 rpm, 10 min). Cells were diluted in 100 ml hybridisation medium (see below) or RPMI-1640 to approximately 30,000 cells/ml.

(d) Fusion protocol

(i) Hybridisation medium: 20% FCS, 2 mM L-glutamine 100 U/ml penicillin, 100 ug/ml streptomycin, 2.5 µg/ml fungizone, 2X HAT.

(ii) Polyethylene glycol solution: 5 ml RPMI-1640 were added to 10 g PEG-1500 and heated in a 50°C water bath until all PEG was dissolved. 0.1 M NaOH was added to adjust to pH 7.0. Total volume was made up to 20 ml with pre-warmed RPMI-1640 to give a final PEG concentration of 50%. Sterilisation was performed by membrane filtration or autoclav (15 min).

(e) Fusion procedure

5 ml aliquots of suspensions of myeloma cells and lymphocytes were mixed and centrifuged. The supernatant was removed quantitatively and the cell pellet was placed

in a water bath at 37°C. 0.8 ml PEG solution was added dropwise over 1 min (approximately 1 drop/3 sec) under constant shaking. 1 ml of pre-warmed RPMI-1640 was added in the same way followed by another 9 ml RPMI-1640 over 5 min. Cells were centrifuged and resuspended in 120 ml pre-warmed hybridisation medium containing macrophages (approximately 30,000 cells/ml). This cell suspension was distributed on five 96-well plates with 200 µl aliquots per well by means of a multiwell transfer device (Transplate). Plates were sealed with transparent adhesive tape and incubated at 37°C for 10-14 days.

2.2.4. Screening and cloning of hybridoma cell lines

(a) Screening of hybridoma cell lines for antibody secretion with antigen-coated plates

Hybridoma cell lines were screened for secretion of apoE-specific antibodies by a qualitative enzyme-linked immunosorbent assay (ELISA) using apoE-coated cell culture plates (292).

(i) Preparation of protein-coated plates: TMU-soluble apo-VLDL was prepared as described previously. Non-apoB protein derived from 2 ml VLDL was dissolved in 0.3 ml of 0.05 M sodium carbonate buffer, pH 9.6 and protein concentration was measured by the Lowry method (293). Protein concentration was adjusted to 2 µg/ml and aliquots of 150 µl per well were dispensed on

96-well cell culture plates. Plates were incubated for 4 h at room temperature, emptied and filled with 3% BSA, 0.05 M sodium carbonate buffer, pH 9.6. After another 3 h incubation plates were emptied again, covered with transparent foil and stored at -20°C until required.

(ii) Screening for anti-apoE antibodies: Plates coated with TMU-soluble apo-VLDL were rinsed with PBS, 0.05% Tween 20, pH 7.4 and 100 µl of cell culture supernatant were added. After 3 h of incubation wells were rinsed as above, then 100 µl of a 1:100 dilution of anti-mouse-IgG, linked to horseradish peroxidase were added. Following another 1 h incubation wells were washed three times and 100 µl of colour reactant was added. The colour reactant which released a soluble dye was made up as follows: 0.04% o-phenylenediamine-dihydrochloride, 0.012% hydrogen peroxide (30% v/v) in 0.05 M citric acid, 0.01 M disodium-phosphate buffer, pH 5.0 . Wells containing anti-apoE antibodies showed a colour development from yellow to dark orange within 5 min of addition of colour reactant.

(iii) Cloning anti-apoE producing hybridoma cell lines: Cell lines secreting anti-apoE antibodies had to be cloned to ensure that the antibody originated from a single hybridoma cell. Cell lines which were found to secrete anti-apoE antibodies were placed into 2 ml wells together with 2 ml RPMI-1640 containing peritoneal macrophages and grown up until approximately 50% confluence. 100 µl of this cell suspension were

transferred into each of the eight wells of the far left row of a 96-well plate which previously had been primed with 100 µl of RPMI-1640 per well. By subsequent transfer of 100 µl aliquots per well from one vertical row to the next, working from left to right, a serial dilution of cells ranging from 1:2 to $1:2^{12}$ was carried out. After addition of another 100 µl of RPMI-1640, containing peritoneal macrophages, the plate was sealed and incubated for 7-14 days at 37°C. Then six vertical rows with the lowest cell concentrations were screened for anti-apoE antibodies. Cells in the highest dilution still positive for anti-apoE antibodies were assumed to be monoclonal.

(b) Screening of hybridoma clones for anti-apoE antibody secretion

(i) Preparation of apoE-nitrocellulose test strips: Nitrocellulose strips with bound apoE were used for anti-apoE antibody screening. They were prepared in the same way as apoE Western blots (see 2.3.2.), except for the following modifications: 0.5 ml of plasma were delipidated in ethanol:ether twice. Delipidated proteins were resolubilised in 2-3 ml of sample buffer and 1 ml plus 20 µl BPB-marker was applied on top of an IEF gel which was prepared with an even surface without sample wells. Strips from the vertical margins and from the centre of Western blots were tested for apoE in order to locate the position of apoE on the blot. The apoE carrying area was excised and sliced into strips (approximately 2.0 cm x 0.1 cm)

which were used as test strips in the assay described below.

(ii) Anti-apoE antibody assay with apoE test strips: 50 µl of cell culture medium were transferred from each well into 3 ml test tubes containing 450 µl saline, 0.05% Tween 20, 10 mM Tris, pH 7.4. An apoE-nitrocellulose test strip was added to each test tube and was incubated for 2 h on an orbital shaker. The nitrocellulose strip was washed with buffer (same as above) and then incubated with 0.5 ml of a 1:100 dilution of anti-mouse-IgG-HRP conjugate. Finally, after another 2 h incubation, test strips were washed once more and apoE-binding antibodies were visualised by addition of 1 ml of colour reactant.

2.2.5 Production of murine ascites containing monoclonal anti-apoE antibodies

A hybridoma clone producing an anti-apoE antibody was transferred from a cloning plate into a 25 cm^2 flask and grown up in 5 ml RPMI-1640 in the presence of peritoneal macrophages. After 7-14 days cells were centrifuged (800 rpm, 10 min) and resuspended in six 0.5 ml aliquots RPMI-1640.

Six mice (female, approximately 20 g) which had been primed by an intraperitoneal injection of 0.5 ml pristane 1-3 weeks earlier were injected with 0.5 ml of the above cell suspension (5×10^5 to 8×10^6 hybridoma cells) into

the peritoneal cavity. Thereafter their weight was checked every second day and when weight had increased by 5-10 g ascites fluid was collected via a peritoneal cannula. This procedure was repeated a maximum of two times. The ascitic fluid was centrifuged (3000 rpm, 10 min), aliquoted (250 µl) and stored at -20°C. Serial dilutions of the fluid ranging from $1:10^3$ to $1:10^6$ were tested with apoE strips as outlined above.

2.2.6. Storage of Cells

Hybridoma cell lines not used for intraperitoneal reinjection directly after cloning were resuspended in FCS, 10% DMSO at a concentration of 10^4 cells per 0.5 ml and stored in liquid nitrogen.

Cells were reactivated by thawing in a 37°C water bath. 10 ml of ice-cold RPMI-1640 were added immediately and cells were then centrifuged and resuspended in RPMI-1640 for counting and viability testing (Trypan Blue). After another centrifugation cells were resuspended in RPMI-1640, containing macrophages, and grown up as described above.

2.2.7. Immunoglobulin Classification of Monoclonal Antibodies

Monoclonal antibodies from cell supernatants were characterised by a commercially available isotyping kit (Serotec) based on agglutination of antibody-labelled red cells.

2.3. Phenotyping of ApoE Isoforms

ApoE isoforms were differentiated by a combination of isoelectric focussing and Western blotting. The method applied was a combination of the procedures published by Menzel et al. (294) and Havekes et al. (295).

2.3.1. Separation of apolipoproteins by isoelectric focussing

(a) Delipidation of plasma samples

10 µl of plasma were injected into 2.5 ml of ethanol:ether (3:1) and kept at -20°C overnight. After centrifugation (3000 rpm, 10 min) the precipitated protein was washed with ether and finally dissolved in sample buffer (6 M urea, 0.1 M Tris, 5% beta-mercaptoethanol, 1% DSS, pH 10). After 30 min at 4°C the samples were ready for application on an IEF gel.

(b) Delipidation of VLDL

VLDL was prepared from 5 ml of plasma and delipidated (see 2.1.1.). The moist protein pellet was dissolved in 0.5-1.0 ml sample buffer as described above.

(c) Isoelectric focussing procedure

Vertical acrylamide slab gels, 8 M urea, pH 4-6, were used for IEF of delipidated plasma specimens. Slab size was 14 cm x 14 cm x 0.15 cm. Acrylamide concentration was 5.0% (w/v) and bisacrylamide accounted for 2.5% (w/w) of the acrylamide content. The ampholyte mixture added was composed of equal volumes of ampholyte pH 4-6 from LKB (Bromma, Sweden) and Serva (Heidelberg, FRG) and 20% ampholyte pH 3-10 (Serva). The final ampholyte concentration of IEF gels was 1.0%. TEMED at a concentration of 0.6% (v/v) and ammonium persulfate 0.17% (w/v) were added to initiate polymerisation. Acrylamide concentration of IEF gels was increased to 8.5% when delipidated VLDL was examined. Eighteen sample wells were formed per slab gel and 30 µl of sample were placed in each well and overlayered with layering solution (80% sucrose, 5% ampholyte). For isoelectric focussing a vertical electrophoresis slab gel unit from Hoefer Scientific Instruments was used. The top buffer tray was filled with 0.02 M NaOH, the bottom electrolyte solution was 0.1 M H_3PO_4. Electrical power applied for IEF was limited to 3 W per slab gel, ie voltage was set at maximal 250 V and current at maximal 24 mA for two slabs run in parallel.

After 15 h the voltage was increased to 500 V for another 1 h. The procedure was carried out at room temperature with water cooling.

(d) Protein staining of IEF gels

Proteins were stained on acrylamide gels after IEF by a modification of the method of Malik et al. (296). Staining solution was prepared as follows: 3 g of Coomassie Blue G-250 were dissolved in 150 ml water at 80°C. Then 75 ml of 3 N H_2SO_4 were added slowly under constant stirring and heated at 80°C for another 10 min. Subsequently the solution was passed through filter paper and adjusted to pH 5.5 with 10 M KOH. Finally 0.25 g DSS and 25% (w/v) TCA were added.

Gels were transferred after IEF into 10% TCA for 30 min and then incubated under gentle shaking in staining solution at 60-70°C for 1-2 h. For destaining gels were incubated under the same conditions in 20% ethanol for 4 h. Gels could be kept in 7.5% acetic acid for several weeks.

2.3.2. Western blotting of apolipoprotein E

(a) Electro-blotting of proteins

Following IEF, proteins were electrophoretically transferred from acrylamide slab gels to nitrocellulose membranes as originally described by Towbin et al. (297). IEF gels were recovered from the vertical slab gel apparatus and equilibrated for 30 min in transfer buffer

(198 mM glycine-25 mM Tris, 20% methanol, pH 7.9). For electro-blotting the Trans-Blot Cell from Biorad was used. Nitrocellulose sheets were cut to size and laid on IEF gels with intimate contact. Both gel and membrane were placed in the electrophoresis cell in a way that the gel was facing the cathode while the membrane was facing the anode. Electrotransfer was performed in transfer buffer (see above) at 100 V, 400 mA for 3 h at 4°C with water cooling.

(b) Immunostaining of apolipoprotein E

ApoE which was bound to the nitrocellulose membrane was detected by sequential incubation with an apoE-specific antibody, followed by an IgG-binding antibody linked to HRP, which in turn catalysed a colour reaction. Incubations were carried out on an orbital shaker at room temperature. The buffer for all incubations and washes was saline, 10 mM Tris, 0.05% (v/v) Tween 20.

Following electro-blotting nitrocellulose blots were transferred into a 3% (w/v) aqueous solution of skimmed milk powder for 30 min. Blots were washed (15 min) and then incubated for 2 h in 20 ml of a $1:10^3$ to $1:10^5$ dilution of an anti-apoE antibody preparation. This was either murine ascites fluid containing a MAB or polyclonal goat antiserum. A second wash was followed by another 2 h incubation with 20 ml of a 1:100 dilution of either an anti-mouse-IgG or an anti-goat-IgG antibody, both conjugated to HRP. After a final wash blots were incubated with colour reactant.

The colour reactant which produced an unsoluble dye was made up as follows: 60 mg 4-chloro-naphthol was dissolved in 10 ml of methanol and mixed with 60 ml saline containing 30 µl hydrogen peroxide (30% v/v) directly prior to use.

ApoE was clearly detectable on blots as dark blue bands after incubation for about 15 min. Blots were then washed, dried and stored in the dark.

2.4. RFLP Analysis of the Apolipoprotein B Gene

The methods in this section were applied as described by Maniatis et al. (304) unless stated otherwise.

2.4.1. Preparation of DNA

DNA was prepared from blood leucocytes by the Triton X-100 lysis method essentially as described by Kunkel et al. (298).

10 ml of blood were collected into EDTA tubes and stored at -20°C until DNA was prepared. After thawing the samples were kept on ice. Blood cells were mixed with 80 ml of ice-cold lysis buffer (0.32 M sucrose, 10 mM Tris, 5 mM $MgCl_2$, 1% Triton X-100, pH 7.5). After centrifugation (10,000 rpm, 10 min, 4°C) the pellet of white cells was resuspended in 4.5 ml 75 mM EDTA and mixed gently using a

disposable plastic Pasteur pipette. 250 µl of 10% SDS and 250 µl of proteinase K (2 mg/ml) were added and the mixture incubated overnight at 37°C.

The sample was extracted with liquid phenol (water-saturated), centrifuged (10,000 rpm, 5 min, 20°C) and the DNA-containing aqueous upper phase collected using a bent Pasteur pipette. This was followed by two extractions with 5 ml chloroform:isoamylalcohol (24:1). Addition of 0.5 ml of 3.0 M sodium acetate and 11 ml ethanol (100%) caused the DNA to precipitate. The precipitate was transferred into a 1.5 ml Eppendorf tube. After a short centrifugation the remaining ethanol was removed and DNA was dissolved in 0.5 ml of TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.6) at 4°C over 2-3 days. The DNA solution was then stored at -20°C for up to several months.

2.4.2. Endonuclease digest of DNA

DNA dissolved in TE-buffer was diluted 1:50 with water and the optical density (OD) determined at 260 nm. The OD_{260}-reading multiplied by 2500 gives the DNA concentration in ug/ml. Simultaneous reading of OD_{280} provides a measure for protein and phenol contamination of the DNA preparation. The OD_{260}/OD_{280} ratio for sufficiently pure DNA is greater than 1.6.

A 5 µg aliquot of DNA plus an aliquot of distilled water to a total volume of 40 µl was added to the following

reagent mixture: 2 µl 0.1 M spermidine, 5 µl enzyme buffer (10 x concentrate, as supplied by the enzyme manufacturer) and 20 units (usually 2 µl) of the appropriate endonuclease (XbaI, EcoRI or MspI). This was incubated at 37°C overnight. After addition of another ten units (1 µl) of enzyme, samples were incubated for a further 5 h at 37°C.

2.4.3. Separation of DNA fragments by agarose electrophoresis

DNA fragments resulting from an endonuclease digest were separated by agarose electrophoresis.

(a) Preparation of an agarose gel

0.8 g agarose (gelling temperature 36°C) were dissolved in 100 ml TAE-buffer (40 mM Tris-acetate, 1 mM EDTA) by cooking for about 2 min in a microwave oven. After cooling to about 50°C the warm agarose solution was poured into a mould formed by a perspex plate (113 cm x 11 cm) sealed with autoclave tape along the edges. Twelve wells for sample application were formed by an appropriate comb before the gel was set. For flat bed electrophoresis the gel was covered by TAE-buffer to a depth of about 1 mm.

(b) Agarose electrophoresis

50 µl of DNA sample were mixed with 10 µl of loading buffer (BPB and xylene cyanol in 5 x TAE-buffer, 50%

glycerol) and applied in one of the sample slots of an agarose gel. For calibration of DNA fragments λ-phage markers (2-23 kb, 0.5 µg) were applied in a separate well. Electrophoresis was carried out at 40 mA overnight. After electrophoresis the gel was stained by transfer into a plastic dish with 200 ml distilled water and 50 µl ethidium bromide (10 mg/ml). DNA was visualised under UV light as an orange band across the gel, representing numerous DNA fragments sorted according to their size. The λ-markers showed as distinct bands of known molecular weight.

2.4.4 Southern blotting

DNA fragments were transferred from agarose to nylon membranes by capillary blotting, a procedure first described by Southern et al. (299). After electrophoresis the agarose slabs were successively incubated with the following solutions: (a) denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 30 min, twice and (b) neutralising solution (1.5 M Tris, pH 5.5) for 30 min, twice.

Capillary blotting was performed as shown in Fig. 6. Transfer buffer was 10 x SSC (1.5 M NaCl, 0.15 M sodium citrate, pH 7.5). Capillary flow was limited to the agarose gel and the nylon membrane by plastic strips separating the edges of the gel from the membrane and paper towels on top of it. After overnight blotting the nylon filter was washed with 2 x SSC and air dried. The dry

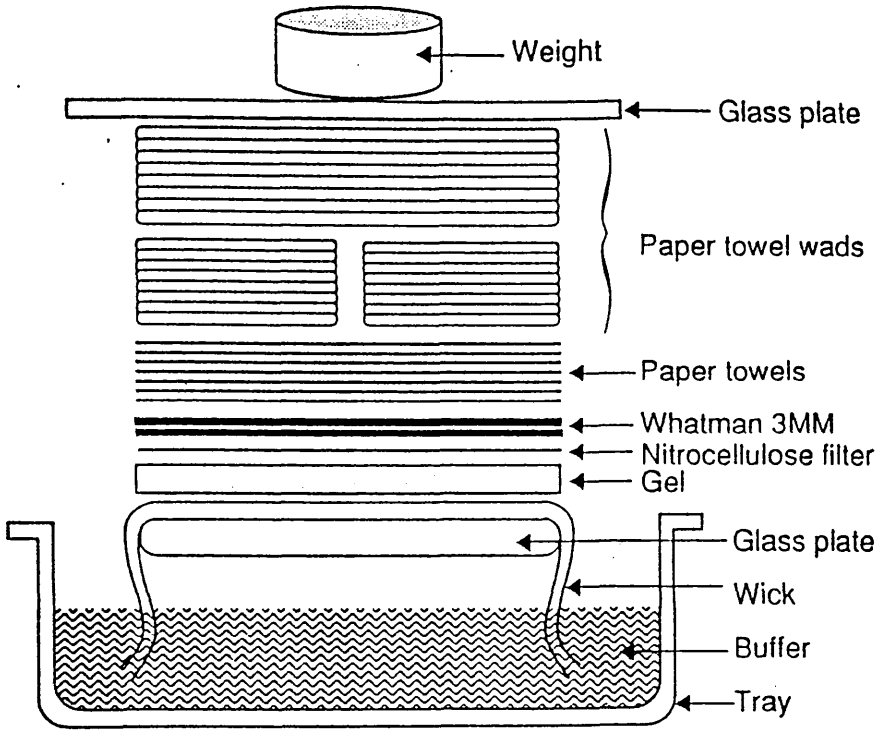


Fig. 6: Experimental arrangement for preparation of a Southern blot.
(Modified from: Davis LG, Dibner MD, Battey JF: "Basic methods in Molecular Biology". Elsevier, New York, 1989)

filter was irradiated for 3 min on an UV transluminator to covalently bind the DNA.

2.4.5. Preparation of a ^{32}P-labelled genetic probe

Genetic probes for the apoB gene were re-isolated from bacterial plasmids. Plasmid DNA was prepared by the alkaline lysis method using a 250 ml broth culture incubated overnight. 10 µg of plasmid DNA was digested using the appropriate enzyme to release the cloned human DNA from the plasmid backbone. The fragments were separated on a low melting point agarose gel and the probe region excised from the stained gel with a sterile scalpel. Single stranded DNA was obtained when agarose containing the probe was boiled for 3 min in a water bath.

For labelling the gene probe by random priming the following reagent mixture was incubated at 37°C for 5 h: 25 µl DNA (denatured genetic probe), 10 µl oligo-labelling buffer (see Reference 300), 1 µl BSA (20 mg/ml), 5 µl ^{32}P-dCTP, 0.3 µl Klenow (large fragment of DNA polymerase I), 8.5 µl water. ^{32}P-labelled DNA and free ^{32}P-dCTP were separated on a Sephadex G-50 column (NICK-column, Pharmacia) equilibrated with TE-buffer, 0.1% SDS. 100 µl fractions were collected and the peak fractions pooled. The equivalent of 3 x 10^6 cpm was used to prepare 5 ml of hybridisation mix.

2.4.6. Hybridisation of Southern filters

Prehybridisation solution was made up as follows: 1.50 ml 20 x SSC, 0.50 ml 50 x Denhardt's solution, 0.25 ml 10% SDS, 2.74 ml water, 10 µl salmon sperm DNA (10 mg/ml, denatured by 5 min boiling in water bath). Hybridisation solution had the same composition, except that 50 µl of denatured salmon sperm DNA and denatured ^{32}P-labelled gene probe (3 x 10^6 cpm) were added. Denaturing of the ^{32}P-probe was carried out just before use by boiling for 3 min and cooling rapidly on ice. Apolipoprotein B polymorphisms as defined by the restriction enzymes XbaI, EcoRI and MspI were detected: (a) by hybridising the two XbaI fragments designated X_1 (8.6 kb) and X_2 (3.5 kb) with the 3.5 kb probe pABC3.5; (b) in an EcoRI digest by hybridising the two fragments R_1 (10.5 kb) and R_2 (12.5 kb) with the cDNA probe pAB3 and (c) after digestion with MspI by probing with PH2, a 2 kb Hind III fragment subcloned from an apoB genomic recombinant. With the latter multiple hybridising fragments could be demonstrated (305). Those of about 2.6 kb were designated M_1 and those of about 2.2 kb were designated M_2 (section 3.7., Fig. 45).

A Southern blot (approximately 100 cm^2) was placed in a heat-sealable bag together with 5 ml of prehybridisation solution, the bag was sealed after careful elimination of air bubbles and incubated at 65°C for 4 h in a hybridisation oven. The prehybridisation solution was

replaced by hybridisation solution and incubation was continued for another 12 h. After hybridisation blots were successively washed in 200 ml of the following solutions:

- 2 x SSC, 0.5% SDS for 10 min, at room temperature
- 2 x SSC, 0.1% SDS for 15 min, at room temperature
- 2 x SSC, 0.5% SDS for 60 min, at 65°C
- 2 x SSC, 0.5% SDS for 30 min, at 65°C.

Blots were finally air-dried, wrapped in cling film and applied to an x-ray film (Kodak X-mat S). After 3-10 days storage at -70°C the film was developed. DNA fragments binding the labelled gene probe were detectable as black bands.

2.5. VLDL-Turnover Studies

The metabolism of large and small VLDL was investigated following protocols previously published by Shepherd, Packard et al. (154, 172). The multicompartmental kinetic model used to calculate kinetic rate constants and protein fluxes was basically the same as published previously (301).

2.5.1. Cumulative gradient ultracentrifugation

ApoB containing lipoproteins in plasma were subfractionated into large VLDL or $VLDL_1$ (S_f 60-400), small VLDL or $VLDL_2$ (S_f 20-60), IDL (S_f 12-20) and LDL (S_f 0-12)

by a modification of the cumulative gradient centrifugation technique described by Lindgren et al. (302). The density of 2 ml of plasma was increased to d = 1.118 kg/l by addition of 0.341 g NaCl. This was layered over a 0.5 ml cushion of d = 1.182 kg/l NaBr solution in a Beckman SW40 rotor tube and above it a discontinuous NaBr gradient ranging from d = 1.0988 kg/l to d = 1.0582 kg/l was placed as detailed in Fig. 7. Prior to gradient building centrifuge tubes were coated with polyvinylalcohol in order to obtain a wetable inner surface (282). Densities of salt solutions were checked with a digital densitometer.

For subfractionation of apoB-containing lipoproteins the rotor was centrifuged at 39,000 rpm for 1 h 38 min at 23°C and decelerated without braking. $VLDL_1$ was removed in the top 1.0 ml which was replaced with 1.0 ml of d = 1.0588 kg/l solution before continuing with the separation. $VLDL_2$ was then recovered with the top 0.5 ml of the gradient following centrifugation at 18,000 rpm for 15 h 41 min at 23°C. IDL was prepared from the top 0.5 ml after centrifuation at 39,000 rpm for 2 h 35 min and finally LDL was isolated from the top 1.0 ml after further centrifugation at 30,000 rpm for 21 h 10 min.

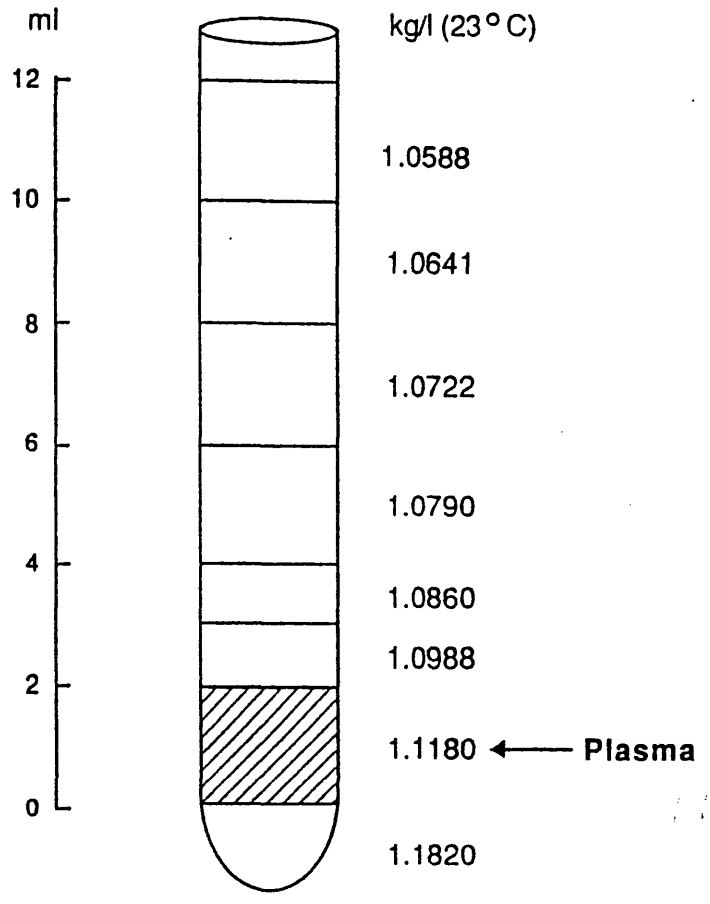


Fig. 7: Discontinuous NaBr-gradient as used for the subfractionation of apoB containing lipoprotein by ultracentrifugation.

2.5.2. Preparation of radiolabelled VLDL subfractions

(a) Subfraction of total VLDL

Total VLDL was prepared from 250 ml of plasma as detailed in paragraph 2.1.1. VLDL from subjects with plasma triglyceride concentration higher than 2 mmol/l was diluted with saline to a concentration corresponding to a triglyceride concentration of 1.5 mmol/l. This was necessary to avoid $VLDL_1$ carry-over into the $VLDL_2$ subfraction and to confine variation of VLDL concentrations so that a standardised protocol for VLDL radioiodination could be applied. 12 ml of this VLDL were then adjusted to d = 1.118 kg/l by addition of solid NaCl and $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) prepared as described above.

(b) Trace-labelling of VLDL subfractions

Radiolabelling was carried out by the ICl method as modified by Bilheimer et al. (287). 2 ml VLDL were mixed with 0.5 ml 1 M glycine, pH 10 and 2 mCi of radioactive carrier-free sodium iodide. Then 6 µl ICl (25 nM/µl) were added with gentle shaking. Usually $VLDL_1$ was labelled with ^{131}I and $VLDL_2$ with ^{125}I. Unbound radioiodine was removed by dialysis against three times 2 l saline.

Labelled VLDL were sterilised by filtration through plasma primed 0.45 µm disposable filters. Radioactivity concentration (µCi/ml) was calculated by counting radioactivity in 10 µl of labelled VLDL and comparing to ^{125}I and ^{131}I-simulated standards.

2.5.3. Analysis of apolipoprotein B in VLDL subfractions

ApoB lipoproteins in S_f 60-400 subfractions were analysed by SDS-acrylamide electrophoresis. The method was similar to the procedure described previously for preparative electrophoresis (2.1.2.).

Delipidated VLDL from 5 ml of plasma was resolved in 1-2 ml of 0.5 M Tris, 3% SDS, 1% DTT, pH 9.1 in a boiling water bath (3 min). 20 µl BPB and glycerol were added (final concentration 10%). Slab gels were 3% (w/v) acrylamide with 2.5% (w/w) bisacrylamide in 0.5 M Tris, 0.1% SDS, pH 9.1. Running buffer was 0.1% SDS, 49 mM Tris - 380 mM Gly, pH 8.3. Approximately 20 µg of resolubilised protein were applied per well and electrophoresis was carried out at 20 mA until the dye marker was at the bottom of the slab (approximately 3 h). Slabs were fixed in ethanol:water:acetic acid (9:9:2) for 2 h, stained in 0.0025% Coomassie Blue in 10% acetic acid (48 h) and destained in 10% acetic acid (28).

2.5.4. Protocol for VLDL-turnovers

250 ml of plasma were collected from a fasting subject by plasmapheresis. VLDL subfractions were prepared and labelled with radioactive iodine by the above procedure which took altogether two-and-a-half days. On the third day after sampling, autologous ^{131}I-VLDL$_1$ and ^{125}I-VLDL$_2$

were reinjected at 8.00 h into the fasting donor subject. 10 ml blood samples were collected 10 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h and 14 h after injection. The first meal was taken after the 10 h sample was drawn. During the following twelve days 10 ml fasting bloods were taken each morning.

ApoB-containing lipoproteins ($VLDL_1$, $VLDL_2$, IDL and LDL) were prepared from 2 ml plasma aliquots as described (see 2.5.1.).

All subjects participating in the study were prescribed KI (60 mg thrice daily) for three days prior to and one month after isotope administration in order to minimise thyroidal sequestration of radioiodide.

2.5.5. Determination of apoB-specific radioactivity

ApoB was isolated from lipoprotein preparations by precipitation with TMU as published by Kane et al. (286).

TMU was redistilled before apoB precipitation (boiling point 176°C). Lipoprotein solutions, obtained by cumulative gradient centrifugation, were adjusted to 37°C before an equal volume of prewarmed TMU was added. After vigorous mixing the samples were kept at 37°C for 30 min and centrifuged (3000 rpm, 20 min). The TMU/water phase was removed carefully and the remaining apoB pellicle was delipidated with ethanol:ether (3:1) at -20°C overnight followed by ether for 2 h at -20°C. ApoB was dried at 40°C

and finally hydrolysed in 1.0 ml 0.5 M NaOH at 40°C overnight. Radioactivity was counted in a γ-counter and the protein concentration of the specimen was determined by a modified Lowry procedure (Biuret reagent made up in water instead of 0.1 M NaOH). From these results specific activities for apoB were calculated as cpm/mg.

2.5.6. Determination of lipoprotein composition and of pool sizes for apoB-containing lipoproteins

$VLDL_1$, $VLDL_2$, IDL and LDL were prepared from 12 ml plasma pooled during the course of a VLDL-turnover. Total cholesterol, free cholesterol, triglycerides, phospholipids, total protein and TMU-soluble protein were measured for each lipoprotein fraction.

Total cholesterol and triglycerides were determined by enzymatic colorimetric assays on a Hitachi 704 autoautoanalyser. Free cholesterol was measured by the same cholesterol assay, omitting the cholesterol esterase step, and phospholipids by another enzymatic colorimetric test. Protein was measured by modified Lowry procedures: to clarify the reaction mixture 1 mg/ml DSS was added to the Biuret reagent for total protein; for TMU-soluble proteins 40 µl TMU were added to calibration standards and a fixed volume of 80 µl was used for protein determination. Standard curves were obtained with aqueous

solutions of human serum albumin.

All results were expressed as into mg/dl units and the composition of a lipoprotein species was calculated as g/100 g. ApoB concentrations were calculated as the difference between total protein and TMU-soluble protein and expressed in mg/dl or as percentage of total protein. Plasma volume was either calculated by isotope dilution or assumed to account for 4% of the body weight. Pool sizes for apoB in the four lipoprotein fractions were derived from plasma volume and apoB plasma concentrations.

2.5.7. Modification of the VLDL-turnover procedure for studies in patients with hyperchylomicronaemia

Prior to cumulative gradient centrifugation for preparation of apoB containing lipoprotein subfractions, chylomicrons had to be removed from plasma of lipoprotein lipase deficient patients. 5 ml aliquots of plasma were overlayered with 1.5 ml of saline and centrifuged in a Beckman Ti40.3 rotor for 30 min at 10,000 rpm, 10°C. After removal of the top 1.5 ml, containing chylomicrons, the remaining sample was mixed and the procedure described above was repeated once again.

When $VLDL_1$ and $VLDL_2$ were prepared for radio-labelling as metabolic tracers, the following protocol was adopted: chylomicrons were removed from plasma as explained and total VLDL was prepared as detailed in paragraph 2.1.1. The VLDL preparation (15-20 ml) was then

diluted 1:10 with plasma infranatant from the VLDL centrifugation. $VLDL_1$ and $VLDL_2$ were prepared and labelled as outlined in paragraph 2.5.2.

2.5.8 Kinetic analysis of VLDL-turnover data by the SAAM 29 program

On the basis of measurements of pool sizes for apoB-containing lipoproteins and apoB-specific radioactivities 10 min after tracer injection radioactivity of apoB recovered from each of the four lipoprotein fractions at various time points was calculated as percentage of the dose initially injected. These data defined apoB decay curves which together with the mass of apoB protein associated with each lipoprotein fraction were used to simulate apoB metabolism in a multicompartmental model computed by the SAAM 29 program (311). This model is depicted in Fig. 8. The main features are:

(a) Large VLDL-apoB (S_f 60-400) is represented as a single species which decays monoexponentially. It is either catabolised directly or transferred to the range of small VLDL (S_f 20-60).

(b) Within the S_f 20-60 lipoproteins there is an arrangement akin to that described by Berman et al (94). Some apoB enters a catabolic cascade and is converted to IDL (S_f 12-20) while other material is diverted into a pool of slowly metabolised remnant particles (compartment 6).

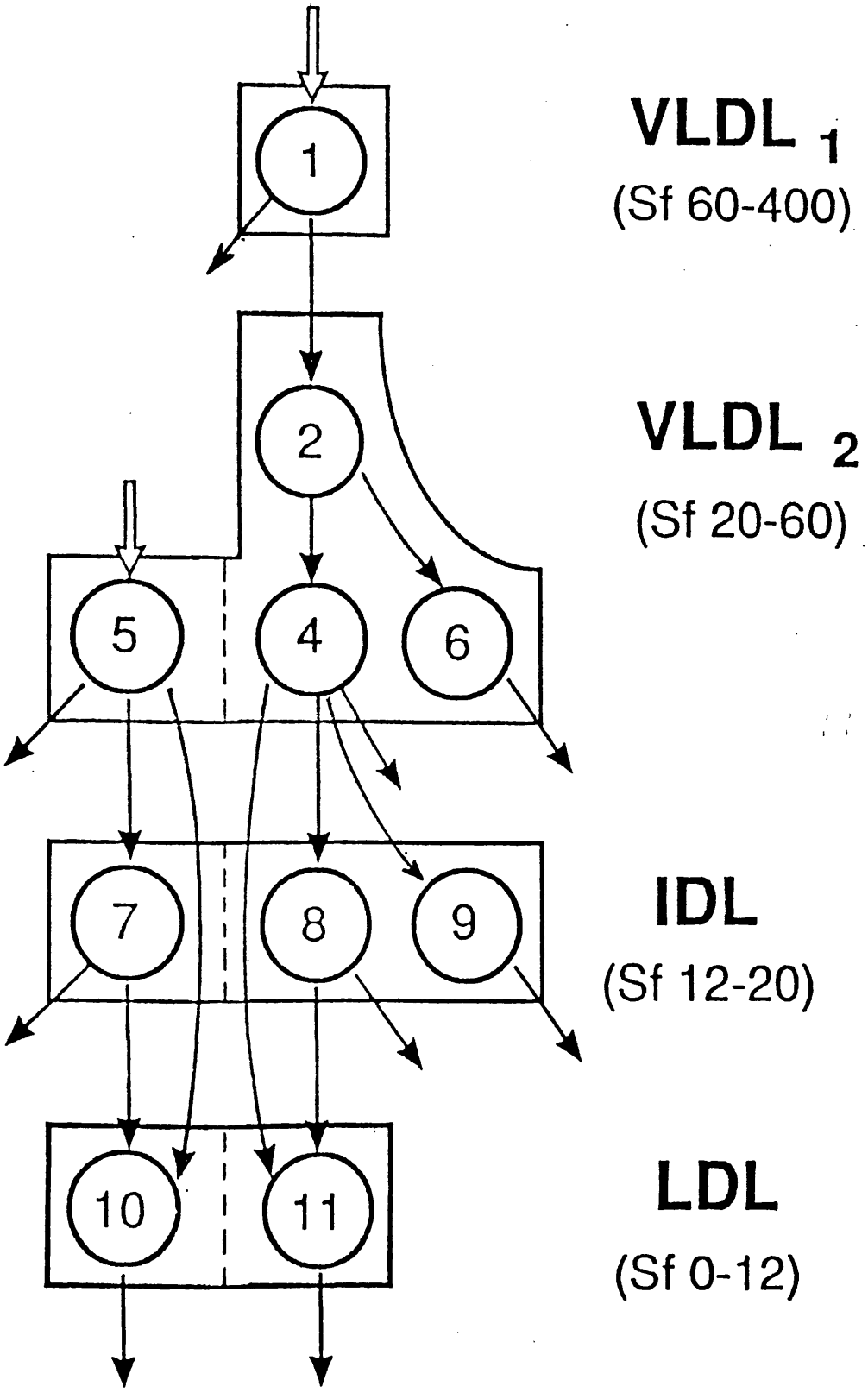


Fig. 8: Multicompartmental model describing the metabolism of apoB containing lipoproteins in plasma.

The lipolytic cascade comprised two or three sub-compartments. The apoB metabolism of patients with hepatic lipase deficiency (see 3.5.) and with familial hypercholesterolaemia (see 3.6.) was analysed with a model containing three serial subcompartments. As it was found that a third subcompartment had little impact on the curve fitting process, this was abolished and all other analyses were performed with a simplified model with only two serial $VLDL_2$ subcompartments.

(c) There is input of newly synthesised apoB into the S_f 20-60 density range (compartment 5). This is required because firstly not all of the S_f 20-60 apoB mass can be accounted for by transport from large VLDL and secondly when large and small VLDL are labelled separately the kinetics of appearance of these tracers in IDL and LDL apoB are different. Usually the radioactivity derived from labelled small VLDL appears more quickly in these denser fractions and accounts for a higher proportion of their mass. Provision is made for this phenomenon by incorporating in the model parallel pathways for $VLDL_1$ and $VLDL_2$ and their metabolic products appearing in IDL and LDL.

(d) In the IDL range it was necessary to postulate the existence of a slowly metabolised species (compartment 9).

(e) LDL was distributed between two plasma pools, accounting for different metabolic properties of LDL derived either from $VLDL_1$ (compartment 11) or from $VLDL_2$

(compartment 10). This model provided an acceptable fit to the observed data. The calculated kinetic rate constants were with few exceptions defined with a fractional standard deviation (FSD) of less than 0.1 (see Tables I-16, I-17, I-18). For $VLDL_2$ and for IDL the calculated masses derived from the kinetic analyses were within 10% of the measured values. With $VLDL_1$ and LDL deviations of calculated masses from measured pools were often greater. Reasons for these discrepancies will be discussed in paragraphs 3.4.3. and 4.4.

2.5.9. Nutritional Records

In order to estimate the impact dietary factors may have on lipoproteins during metabolic studies, participants were asked to record and weigh all food and fluid intake over seven days. These protocols were analysed by "Microdiet" (309), a computer program designed to calculate the percentage distribution of caloric intake from protein, carbohydrates and fat. In addition, total daily calories ingested are given and the ratio of polyunsaturated over saturated fatty acids (P:S ratio) is derived from analysis of the fat consumed.

Body weight was evaluated by an obesity index, ie the ratio of weight over height squared (310). An index smaller than 25 is considered to be normal, and an index of 25-30 represents slight obesity.

2.6. LDL-Turnover Studies

Trace-labelled native LDL and cyclohexanedione-modified LDL were used to study the LDL-receptor mediated and the LDL-receptor independent LDL catabolism in humans by turnover techniques published first by Shepherd et al. (27).

2.6.1. LDL preparation by rate-zonal centrifugation

LDL was isolated by rate-zonal centrifugation as described by Patsch et al. (303). 60 ml of plasma were adjusted to d = 1.300 kg/l by addition of 18 g NaBr. Separation of apoB-containing lipoproteins was carried out on a linear density gradient ranging from d = 1.000-1.200 kg/l in a Beckman Ti14 zonal rotor centrifuged at 45,000 rpm for 110 min at 10°C. The rotor was unloaded with heavy salt solution (d = 1.200 kg/l) and fractions from the LDL peak were pooled and concentrated by pressure filtration to a protein concentration of about 4-6 mg/ml.

2.6.2. Preparation of labelled native and cyclohexanedione-modified LDL

1 ml of concentrated LDL solution was mixed with 250 µl 1 M glycine, pH 10, and 1 mCi of either ^{131}I or ^{125}I. ICl (25 mM/µl) was added under gentle shaking at a

molar ratio of 25:1, the molecular weight of LDL being assumed as 640,000, ie equivalent to 1.56 nM. Non-reactant radioactive iodine was eliminated by passing samples over a PD-10 column (Pharmacia). ^{131}I-LDL was modified by binding to cyclohexanedione according to the method of Mahley et al. (25). 2 ml of LDL (protein concentration 2-5 mg/ml) were mixed with 4 ml of 0.15 M cyclohexanedione in 0.2 M sodium borate buffer, pH 8.1, and incubated for 2 h at room temperature under constant stirring. Non-reactant cyclohexanedione was eliminated by a second passage over a PD-10 column. Sterile filtration was carried out as described previously.

2.6.3. Protocol for LDL-turnover studies in humans

LDL used for tracer preparation (^{125}I-LDL and ^{131}I-CHD-LDL) was isolated from 120 ml of blood and reinjected into the donor subject. Ten minutes after injection and then daily over a two-week period 10 ml blood samples were collected. The plasma clearance of each tracer was followed by counting radioactivity at the end of the study in 2 ml aliquots of plasma and decay curves for native and CHD-modified LDL were constructed by plotting the percentage of initially injected dosage left in plasma against time. KI tablets were prescribed as explained before.

2.6.4. Kinetic analysis of LDL-turnover data

LDL metabolism was analysed on the basis of a two subcompartment model (319). One subcompartment representing the intravascular pool was in equilibrium with a second extravascular compartment. Mass input and fractional catabolic rate were linked to the intravascular compartment. This model assumes LDL-apoB is kinetically homogeneous and that degradation only occurs from the plasma compartment. It provided a good fit to the observed LDL decay curves.

2.7. Ethical Considerations

All subjects gave informed consent to the study which met the requirements of the Ethical Committee of each host institution.

2.8. Statistical Methods

Differences between metabolic parameters as presented in Tables 11 and 12 were analysed by a non-parametric correlation test (Whitney-Mann).

Compositional data (Table 9) and cholesterol means (Table 24) were compared by the unpaired t-test.

Differences in gene frequencies (Table 24) were evaluated by the X^2-test.

2.9. Materials and Equipment

Chemicals and reagents came from the following sources: 4-Chloro-1-naphthol, Dithiothreitol (DTT), Dansylchloride, o-Phenylenediamine-dihydrochloride, Spermidine, Salmon Sperm DNA, Agarose (gelling temp. 36°C), Pristane, Iodine-monochloride (ICl) Trishydroxymethylaminomethane (Tris) and Tetramethylurea (TMU) were obtained from Sigma (St Louis, MO, USA); N,N,N',N'-Tetramethylethylenediamine (TEMED) and Decyl-sodiumsulphate from Eastman Kodak (Rochester, NY, USA); Agarose (low standard -mr) and Coomassie Blue G-250 from Biorad (Richmond, CA, USA); Phenol (water saturated) from Rathburn Chemicals Ltd (Walkerburn, Scotland); 32-Phosphorus-labelled Cytidine-triphosphate (^{32}P-dCTP) from DuPont (Boston, MA, USA); ^{125}Iodide and ^{131}Iodide from Amersham (Amersham, England); Ampholyte pH 4-6 (Ampholine) from LKB (Bromma, Sweden); Ampholyte pH 4-6 and pH 3.10 (Servolyte) from Serva (Heidelberg, West Germany); RPMI 1640 medium and HAT solution from Flow Laboratories (Irvine, Scotland) Cyclohexanedione from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade and obtained from British Drug House (Poole, England).

A molecular weight calibration kit for proteins ("Rainbow Marker") was purchased from Amersham (Amersham, England); Sepharose-CL-4B, Nick-Columns and PD-10 Columns from Pharmacia (Uppsala, Sweden); Freund's Adjuvant from

Difco Laboratories (Detroit, MI, USA); an agarose gel electro-phoresis system from Corning Medical (Paolo Alto, CA, USA).

Biological and biochemical products were supplied by the following companies: Endonucleases (XbaI, EcoRI, MspI) by Anglia Biotech (Colchester, England); Proteinase K and enzymatic kits for measurements of cholesterol (CHOD-PAP) triglycerides (GPO-PAP) and phospholipids by Boehringer (Mannheim, West Germany); Klenow (large fragment of DNA polymerase I) by Gibco (Paisley, Scotland); sheep serum and HRP-anti-mouse IgG by SAPU (Carluke, Scotland); myeloma cells (X63-Ag8-653) by Flow Laboratories Ltd (Rickmansworth, England); an isotyping kit for mouse monoclonal antibodies from Serotec (Oxford, England); an immunochemical assay for apolipoprotein B from Orion Diagnostica (Espoo, Finland).

Cell culture plates and the multiwell transfer device (Transplate) came from Costar (Northumbria Biologicals Ltd, Cramlington, England); sterile filters (0.45 μM) from Millipore (Molsheim, France); filter paper (3 MM) from Whatman (Maidstone, England); nitrocellulose membrane (BA 85) from Schleicher and Schuell (Dassel, West Germany); nylon membrane from Amersham (Amersham, England).

Vertical slab gel electrophoresis was carried out with the electrophoresis unit SE 600 from Hoefer Scientific Instruments (San Fransisco, CA, USA). For Western blots the Trans-Blot-Cell and the power supply Model 200/2.0 from

Biorad (Richmond, CA, USA) were used. Density gradients were made with a six-channel roller pump from Technicon Ltd (Dublin, Ireland). Densities of aqueous solutions were measured with a digital densitometer (DMA 35) from Paar Scientific Ltd (London, UK). DNA hybridisations were performed in a hybridisation oven from Bachofer (Reutlingen, West Germany). Pressure filtration was performed with a concentration cell from the Amicon Corporation (Danvers, MA, USA) using XM 300 filters from the same manufacturer.

For centrifugations up to 3000 rpm standard table centrifuges (rotor r = 15 cm) were used. Centrifugations of 5000-15,000 rpm were performed in a J2-21 Beckman centrifuge (rotor r = 11 cm). Ultracentrifugations (>10,000 rpm) were carried out in Beckman ultracentrifuges using the specified rotors.

3. RESULTS

3.1. Production of a Monoclonal Antibody against Apolipoprotein E

3.1.1. Preparation of apolipoprotein E

Apolipoprotein E was isolated from delipidated VLDL by preparative electrophoresis. Approximately 20 mg of VLDL-apoprotein was applied per slab gel. The yield was estimated by the preparation of apoE from radiolabelled non-apoB VLDL-apoproteins (ie TMU-soluble proteins). 4% to 5% of the initial radioactivity was recovered with apoE. As apoB accounts for about 40% of VLDL-apoprotein and apoE for about 10%, the preparative yield can be estimated as approximately 30%. Most of the loss occurred at the TCA-precipitation step which followed electrophoresis, as indicated by radioactivity counts measured before and after this step. The purity of the apoE was demonstrated by isoelectric focussing and subsequent protein staining (Fig. 9).

3.1.2. Raising of a monoclonal antibody against apolipoprotein E

Ten mice were injected with apoE as outlined in paragraph 2.2.1. The antigenic response of each animal was

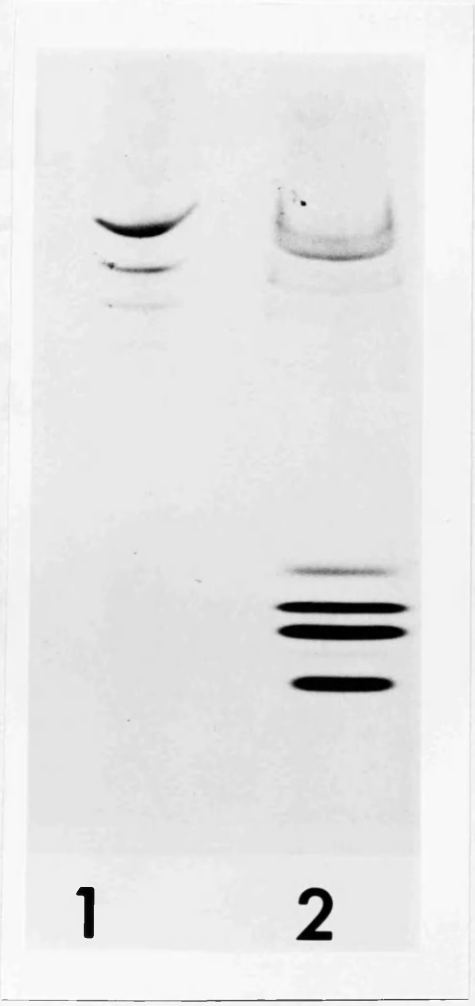


Fig. 9: Isoelectric focussing of apoE (1), prepared from VLDL, and VLDL apolipoprotein (2). Minor bands in the apoE range represent mono- and disialated apoE.

determined by a blood spot antibody-binding test (see 2.2.2). Specific antibody-binding ranged from 6-27% of the initial radioactivity. The spleen from the animal with the best antibody production was used for cell-fusion with myeloma cells. Two independent fusion procedures were carried out. When hybridoma cells were screened for anti-apoE antibody secretion, 94 wells out of a total of 960 were positive. These antibody producing cells were further characterised by an assay for specific antibody-binding and for antibody-binding displacement by excess of unlabelled apoE. The displacement assay measures antibody affinity, large displacement indicating high binding affinity. It thereby differentiates the cause of specific antigen-binding, which can be either high-affinity antibodies in relatively low concentrations or low-affinity antibodies in higher concentrations.

The culture medium of fifteen wells showed in at least one out of three dilutions specific binding of more than 10% (mean 24% ± 12%) and binding displacement of more than 50% (mean 61% ± 6.5%). Supernatants from eleven wells showed specific apoE bands, when used as first antibody source with an apoE-Western blot. The four wells failing to produce bands in this assay were those with the lowest specific apoE-binding. To ensure that the anti-apoE antibodies tested were monoclonal, ie originated from a single hybridoma cell, cells from five wells with the best binding characteristics were cloned twice. Finally, two

hybridoma cell lines were obtained producing two monoclonal antibodies against apoE. These were designated ME 21 and ME 59. Both were classified from cell supernatant as IgG_1 antibodies.

3.1.3. Production of antibody containing ascites

Pristane-primed mice were injected intraperitoneally with monoclonal hybridoma cells. Six to ten mice were used at one time for each cell line. Ascites yield varied considerably from 1 ml up to 23 ml per animal. Ascites dilutions ranging from $1:10^2$ to $1:10^6$ were tested with apoE Western blots. Antibody titers - as defined by the highest dilution still producing apoE bands on blots - varied from $1:10^2$ to $1:10^6$. Animals producing only modest amounts of ascites fluid (< 3 ml) tended to have higher antibody titers. Ascites volume and antibody titer were dependent on both the specific cell line and on the number of injected cells. With one cell line (ME 59) a small ascites volume (1.4 ± 0.3 ml/animal, n = 6) with a high antibody titer (> $1:10^4$) was recovered after injection of 5×10^6 cells/animal, in contrast to much larger ascites volumes (8.6 ± 6.0 ml/animal, n = 8) with a lower titer ($1:10^2$ to $1:10^3$) after injection of only 5×10^5 cells/animal.

With the other cell line (ME 21) 2.8 ± 1.0 ml ascites were produced per animal (n = 5) with a titer of $1:10^6$ after injection of 7×10^6 cells/animal.

3.2. Apolipoprotein E Polymorphism and its Correlation with Plasma levels of Cholesterol and Apolipoprotein B

Blood samples were collected as part of a cholesterol screening programme. Screening for elevated plasma cholesterol levels was opportunistic and Health Centre based. The apolipoprotein E polymorphism of 717 randomly selected subjects and of 858 subjects with cholesterol levels between 5.0 and 6.2 mmol/l was determined from plasma by isoelectric focussing and Western blotting (Fig. 10). Results for percentage distributions of apoE phenotypes and apoE allele frequencies are presented in Table 5 together with results obtained from population studies published by other investigators. The gene frequencies observed in the randomly selected group resemble closely the frequencies reported from other Caucasian populations, notably from Aberdeen (266) and from Münster, West Germany (268). In the group with plasma cholesterol levels ranging from 5.0-6.2 mmol/l the gene frequency for e_2 was lower (-25%) and the frequency for e_4 was higher (+34%) than in the random group, which can be explained by the association between apoE phenotypes and plasma cholesterol concentrations.

Mean values for plasma cholesterol were calculated for each apoE phenotype in the randomly selected study group. ApoE phenotypes could be ranked by their cholesterol

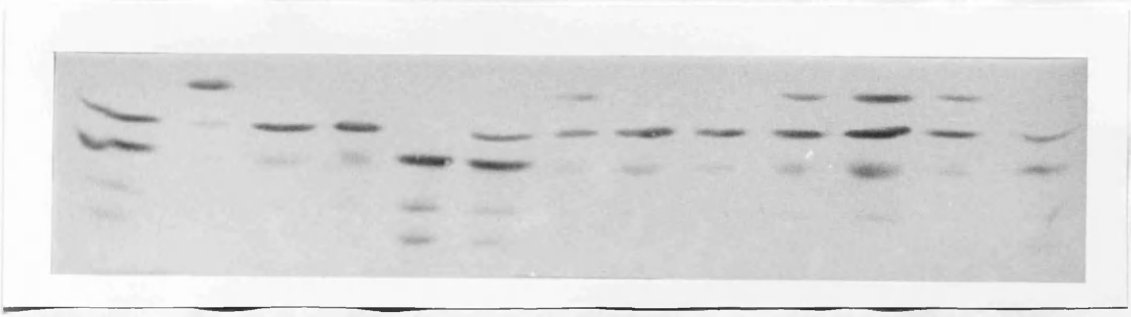


Fig. 10: Apo E phenotypes as visualised by Western blots.- From left to right: Apo E 3/2, E 4/4, E 3/3, E 3/3, E 2/2, E 3/2, E 4/3, E 3/3, E 3/3, E 4/3, E 4/3, E 4/3, E3/2.

Table 5: Percentage Distribution of ApoE Phenotypes and ApoE Allele Frequencies in a Population Sample from Glagow as Compared to Other Population Studies

n	E2/2	E3/2	E3/3	E4/3	E4/4	E4/2 (Percentage Distribution of Phenotypes)	e_2	e_3	e_4 (Gene Frequency)	Population Studied	Reference
750	1.7	11.8	65.5	16.1	2.6	2.2	0.089	0.793	0.118	Glasgow (Random Sample Group)	
858	0.2	9.8	60.5	24.2	2.1	3.1	0.067	0.775	0.158	Glasgow (Total Chol 5.0-6.2 mmol/l)	
400	0.5	12.8	58.3	24.8	1.0	2.8	0.083	0.770	0.145	East Scotland	(266)
2018	0.7	12.9	55.9	25.4	2.9	2.2	0.082	0.750	0.167	Netherlands	(258)
1000	0.8	11.0	62.7	20.3	2.3	3.0	0.078	0.783	0.139	West Germany	(268)
615	0.3	6.7	54.0	31.9	6.3	0.5	0.041	0.773	0.227	Finland	(257)
576	0.3	6.1	71.9	19.3	1.7	0.7	0.037	0.846	0.117	Japan	(256)

levels as follows: E3/2 < E2/2 < E3/3 < E4/3 < E4/4. Thus, the apoE2 allele was associated with lower plasma levels (-4%) and the apoE4 allele with higher plasma levels (+8%) as compared to apoE3 homozygosity (Table 6). In accordance with the reported gene frequencies the median cholesterol concentration for the whole of the population screened was determined as 5.6 mmol/l, which is between the means for apoE3/3 and apoE4/3 subjects. The low e_2 frequency observed in the group with cholesterol levels of 5.0-6.2 mmol/l is due to the fact that the cholesterol mean for apoE3/2 subjects is close to the lower limit of 5.0 mmol/l whereas the mean for apoE4/3 individuals is well within the range of sampling.

ApoE phenotypes were correlated with plasma apoB concentrations in the same sense as with plasma cholesterol. In apoE3/2 subjects they were 16% lower and in apoE4/3 subjects they were 10% higher than in apoE3/3 homozygotes. Numbers for apoE2/2 and apoE4/4 homozygotes were insufficient for proper evaluation.

Generally, these findings are well in line with the correlations found in a population study in the Netherlands (231). Total cholesterol concentrations there differed by -3.7% for apoE2/2, but only +3.7% for apoE4/4 subjects. ApoE3/3 individuals presented with a mean cholesterol level of 5.6 ± 1.02 mmol/l. Differences for total apoB in plasma were given as -32% for apoE2/2 and +9% for apoE4/4 as compared to apoE3/3 subjects.

Table 6: Mean Plasma Cholesterol and ApoB Levels for Different ApoE Phenotypes as Determined in a Population Sample from Glasgow

ApoE Phenotype	E2/2	E3/2	E3/3	E4/3	E4/4	E4/2	Median Cholesterol Concentration
Total Chol (mmol/l) (n = 750)	5.28 ± 0.71 (13)	5.23 ± 0.92 (87)	5.51 ± 0.72 (492)	5.75 ± 1.04 (121)	5.93 ± 1.18 (20)	5.59 ± 1.00 (17)	5.52
Total apoB (g/l)* (n = 264)	0.77 ± 0.11 (2)	0.85 ± 0.32 (33)	0.92 ± 0.27 (181)	1.01 ± 0.34 (43)	0.91 (1)	0.76 ± 0.28 (4)	0.92

*Total apoB in plasma was measured by an immunochemical assay (see 2.8.)

3.3. Apolipoprotein B Metabolism in Normolipidaemic Subjects Homozygous for ApoE2, ApoE3 or ApoE4

The metabolism of large (S_f 60-400) and small (S_f 20-60) VLDL was studied in normolipidaemic subjects homozygous for apoE2, apoE3 or apoE4. For each of the three groups five individuals were selected from participants in a cholesterol screening programme.

3.3.1. Characterisation of the study group

The median cholesterol level of the population from which participants for the study were selected was 5.6 mmol/l. For recruitment of volunteers a plasma cholesterol range of 5.0-6.2 mmol/l (median ± 10%) was observed. The mean lipid and lipoprotein concentrations of the five subjects in each group of apoE homozygotes are given in Table 7. Total cholesterol is only marginally lower in apoE2/2 and 10.5% higher in apoE4/4 individuals as compared to apoE3/3 individuals. This is similar to the correlation between total plasma cholesterol and apoE phenotypes observed in the population at large (Table 6).

LDL-cholesterol levels differed by -20% for apoE2/2 and +11.8% for apoE4/4 subjects in comparison to apoE3/3 subjects. Differences for apoB plasma concentrations were even more pronounced: total apoB levels were decreased by

Table 7: Normolipidaemics Homozygous for ApoE2, ApoE3 and ApoE4. - Comparision of Plasma Lipid and Lipoprotein Concentrations

	Total Trig (mmol/l)	Total Chol (mmol/l)	VLDL Chol (mmol/l)	LDL* Chol (mmol/l)	HDL Chol (mmol/l)	VLDL-Chol/ Trig. Ratio
E2/2 n = 4	1.72 ±0.10	5.46 ±0.60	1.19 ±0.15	2.84 ±0.40	1.43 ±0.06	0.69 ±0.07
E3/3 n = 5	1.69 ±0.56	5.51 ±0.49	0.75 ±0.25	3.54 ±0.51	1.32 ±0.29	0.45 ±0.06
E4/4 n = 5	1.47 ±0.49	6.09 ±0.53	0.72 ±0.30	3.96 ±0.51	1.40 ±0.23	0.49 ±0.11

* "LDL-Chol", as determined by the LRC-protocol (320) includes LDL (S_f 0-12) and most of IDL (S_f 12-20). Values are means + standard deviation.

-49% in apoE2/2 and increased by +11.5% in apoE4/4 subjects, as compared to apoE3/3 homozygotes (Table 8). The disproportionate changes observed in apoE2/2 homozygotes in total cholesterol and in LDL-cholesterol and apoB are evidence for major alterations within the spectrum of apoB containing lipoproteins in these subjects. This is further specified by the differences in percentage distribution of apoB among plasma lipoproteins as shown in Table 8. While percentage values for apoE3/3 and apoE4/4 are very similar and the LDL subfraction in both cases accounts for more than three-quarters of plasma apoB, concentrations of $VLDL_1$, $VLDL_2$ and IDL are relatively increased in apoE2/2 so that LDL represents less than half of the apoB in the plasma.

Compositional data for apolipoprotein B containing lipoproteins are given in Table 9. The figures for apoB quantify the relative contribution of apoB to the total protein component of the four lipoprotein subfractions. There is little difference between apoE2/2, apoE3/3 and apoE4/4. Data for lipid composition show that $VLDL_2$ in apoE2/2 is relatively cholesteryl ester-rich and triglyceride depleted, indicating the accumulation of ß-VLDL particles within the $VLDL_2$ density range. These findings, complemented by a VLDL-cholesterol/total triglyceride ratio of < 0.6 are the hallmarks of dyslipoproteinaemia, typical of the apoE2/2 phenotype. The

Table 8: Normolipidaemics Homozygous for ApoE2, ApoE3 or ApoE4. - Comparison of Apolipoprotein B Concentrations and Distribution Among Plasma Lipoproteins

	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma* Concentration (= 100%) (mg/ml)
	(Percentage Distribution)				
E2/2, n = 4	5.6 ±1.2	19.5 ± 3.1	28.5 ± 4.2	46.5 ± 3.3	0.45 ±0.07
E3/3, n = 5	3.7 ±1.1	6.6 ± 1.6	12.5 ± 0.9	77.2 ± 2.7	0.87 ±0.16
E4/4, n = 5	2.7 ±2.1	4.9 ± 1.6	10.8 ± 1.0	81.6 ± 4.1	0.97 ±0.18

* ApoB plasma concentrations were calculated as described in paragraph 2.5.6.

Table 9: Normolipidaemic Homozygotes for ApoE2, ApoE3 or ApoE4. - Composition of Apolipoprotein B Containing Lipoproteins.

		Free Cholest.	Cholesteryl Ester	Triglyceride	Phospholipids	Protein	ApoB
				(g/100 g)			(% of total protein)
$VLDL_1$	E2/2	4.7 ± 0.5**	19.9 ± 3.9	50.9 ± 4.2	17.3 ± 1.2	7.2 ± 1.2	34 ± 9
	E3/3	1.5 ± 1.8**	16.2 ± 3.2	57.4 ± 4.1	15.4 ± 2.6	9.4 ± 2.0	37 ± 2
	E4/4	3.6 ± 1.7	14.4 ± 5.8	57.0 ± 4.3	17.7 ± 2.7	7.5 ± 1.0	35 ± 5
$VLDL_2$	E2/2	8.6 ± 1.4	30.5 ± 3.7*	27.5 ± 5.2**	21.5 ± 1.4	12.0 ± 1.4	71 ± 5
	E3/3	6.4 ± 1.9	22.4 ± 4.3*	36.9 ± 2.9**	20.4 ± 2.3	14.1 ± 1.4	66 ± 4
	E4/4	7.0 ± 2.2	22.6 ± 2.1	34.8 ± 2.8	21.2 ± 0.8	14.5 ± 0.9	65 ± 7
IDL	E2/2	9.5 ± 1.6	38.3 ± 2.2	11.6 ± 2.8	23.7 ± 1.0	17.0 ± 0.8	86 ± 3*
	E3/3	8.9 ± 3.0	35.6 ± 4.7	14.3 ± 1.7*	22.1 ± 1.7	19.0 ± 1.7	91 ± 3*
	E4/4	9.4 ± 2.5	37.0 ± 1.7	11.4 ± 2.0*	23.2 ± 1.2	19.0 ± 1.4	87 ± 8
LDL	E2/2	8.4 ± 1.6*	38.2 ± 1.9	7.9 ± 1.2*	22.8 ± 0.7	22.8 ± 0.9	86 ± 10
	E3/3	11.6 ± 1.6*	37.0 ± 2.0	6.0 ± 0.9*,**	22.0 ± 0.5	23.4 ± 1.4	96 ± 2
	E4/4	11.2 ± 1.2	36.5 ± 0.7	4.4 ± 0.6**	21.9 ± 1.7	26.0 ± 2.4	95 ± 4

n = 5 for apoE3/3 and apoE4/4; n = 4 for apoE2/2; *: p 0.05, **: p 0.02, E2/2 and E4/4 were compared versus E3/3

triglyceride content of LDL is significantly higher in apoE2/2 and significantly lower in apoE4/4 subjects as compared to apoE3/3 controls.

Some physical characteristics of the three groups investigated are presented in Table 10. The female/male relation was different in the apoE2/2 group as compared to the two other groups. Body weight in the apoE3/3 group was about 20% higher than in the two other groups, which - in addition to the slightly higher body mass index - was due to a larger average body size (+6%).

Before starting metabolic studies, volunteers were advised to carry on with their usual diet in order to maintain steady-state conditions. Diets were recorded by seven days weighed intake protocols. Daily energy intake (kcal/day) varied considerably between the study groups, probably because of different occupations in addition to the differences in body weight. Food composition, in contrast, was quite similar, in particular with regard to the percentage of dietary fat. The polyunsaturated: saturated fatty acid ratio (P/S ratio) showed substantial variation within each group ranging from 0.1-0.8. It is assumed that variation of the P/S ratio within this range has no major effect on lipoprotein metabolism.

The above characterisation of subjects participating in this study is based on mean values for the three apoE phenotype groups. Individual data for each subject are presented in Table I-1 to I-9.

Table 10: Normolipidaemics Homozygous for ApoE2, ApoE3 or ApoE4. - Comparision of Physical Parameters and Diet.

	Sex (M/F)	Age (Years)	Body Weight (kg)	Body Weight Index	kcal/ day (= 100%)	Energy (%) from Protein	CHO	Alcohol	Fat	P/S Ratio
E2/2 n = 4	1 M 3 F	47 ± 6	62 ± 8	23.3 ± 1.7	1612 ± 653	15 ± 4	47 ± 8	4 ± 5	32 ± 1	0.36 ± 0.31
E3/3 n = 5	3 M 2 F	42 ± 3	79 ± 8	26.6 ± 3.3	2219 ± 309	14 ± 4	40 ± 6	8 ± 11	37 ± 9	0.26 ± 0.18
E4/4 n = 5	3 M 2 F	36 ± 6	65 ±15	23.7 ± 3.2	2093 ± 517	16 ± 3	42 ± 7	3 ± 4	39 ± 5	0.24 ± 0.10

3.3.2. VLDL-turnover studies in apoE2/2, apoE3/3 and apoE4/4 subjects

VLDL turnover studies were carried out as described in paragraph 2.5. Results are shown in Figs. 11-13 as percentages of initially injected radioactivity recovered as either $VLDL_1$ (S_f 60-400), $VLDL_2$ (S_f 20-60), IDL (S_f 12-20) or LDL (S_f 0-12) plotted against time. The curve patterns displayed in these figures were obtained after simultaneous injection of radiolabelled $VLDL_1$ (top panel) and differentially labelled $VLDL_2$ (bottom panel).

Mean percentage values as measured in the five subjects homozygous for apoE3 are plotted versus time in Fig. 11 and means for five apoE4/4 subjects are illustrated in Fig. 12. Means (AVG), standard deviations (STD) and fractional standard deviations (FSD) are given in Tables I-10 and I-11. FSD were usually well below 0.5. Four of the five apoE2/2 subjects showed a similar curve pattern which was distinctly different from the curves observed in apoE3/3 and apoE4/4 subjects. These curves are illustrated in Fig. 13 and the corresponding data are listed in Table I-12. The fifth subject (P.M.) homozygous for apoE2 displayed decay curves which were different from all other normolipidaemic subjects studied (Fig. I- 5). Therefore, her data were not included when apoE2/2 averages were calculated. Individual VLDL-turnover curves for each subject are shown in Figs. I-1 to I-15. In order to compare

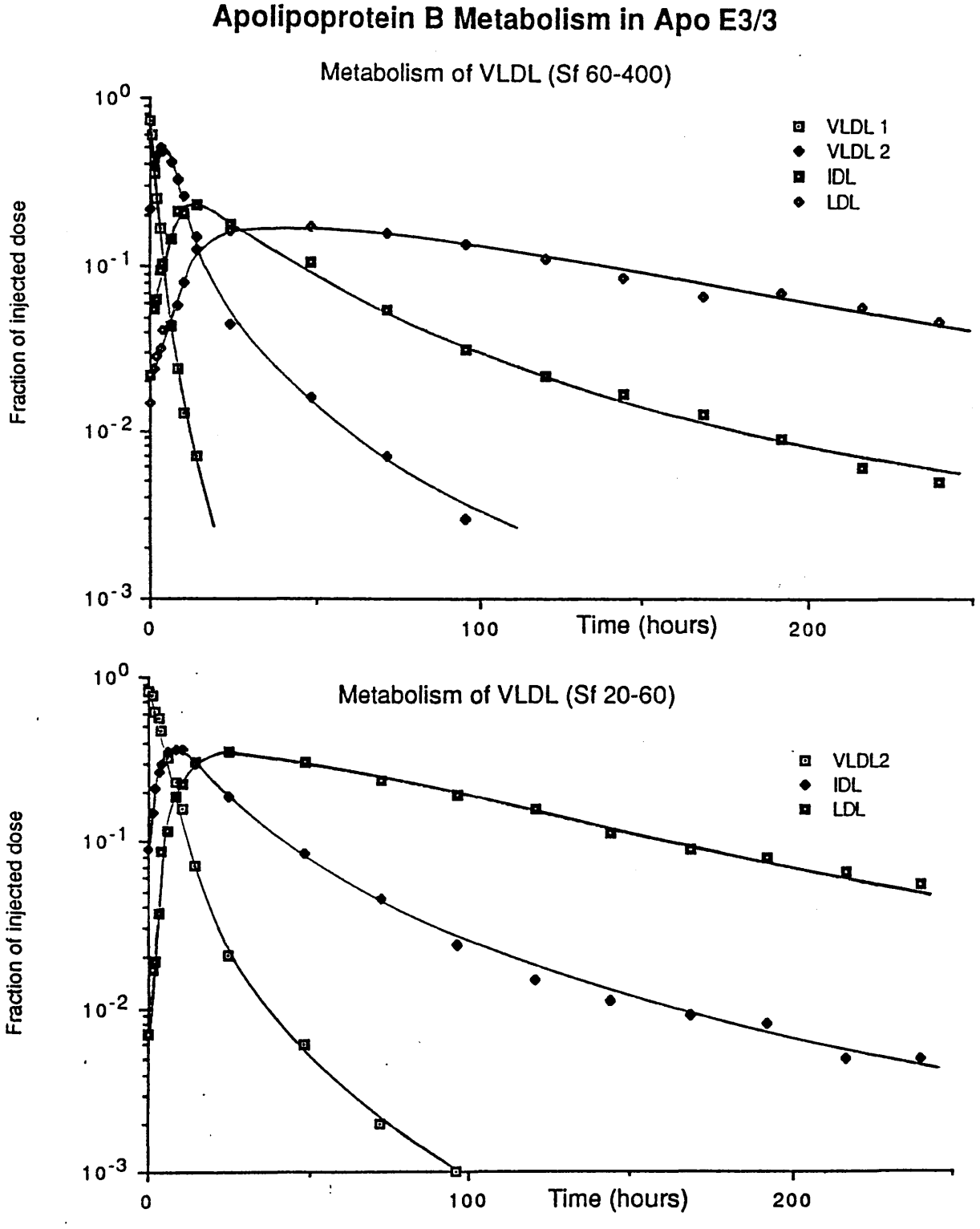


Fig. 11: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in apoE3/3 subjects (n = 5). - Averages from Table I-10 A/B of individual data as shown in Fig. I-1 to I-5.

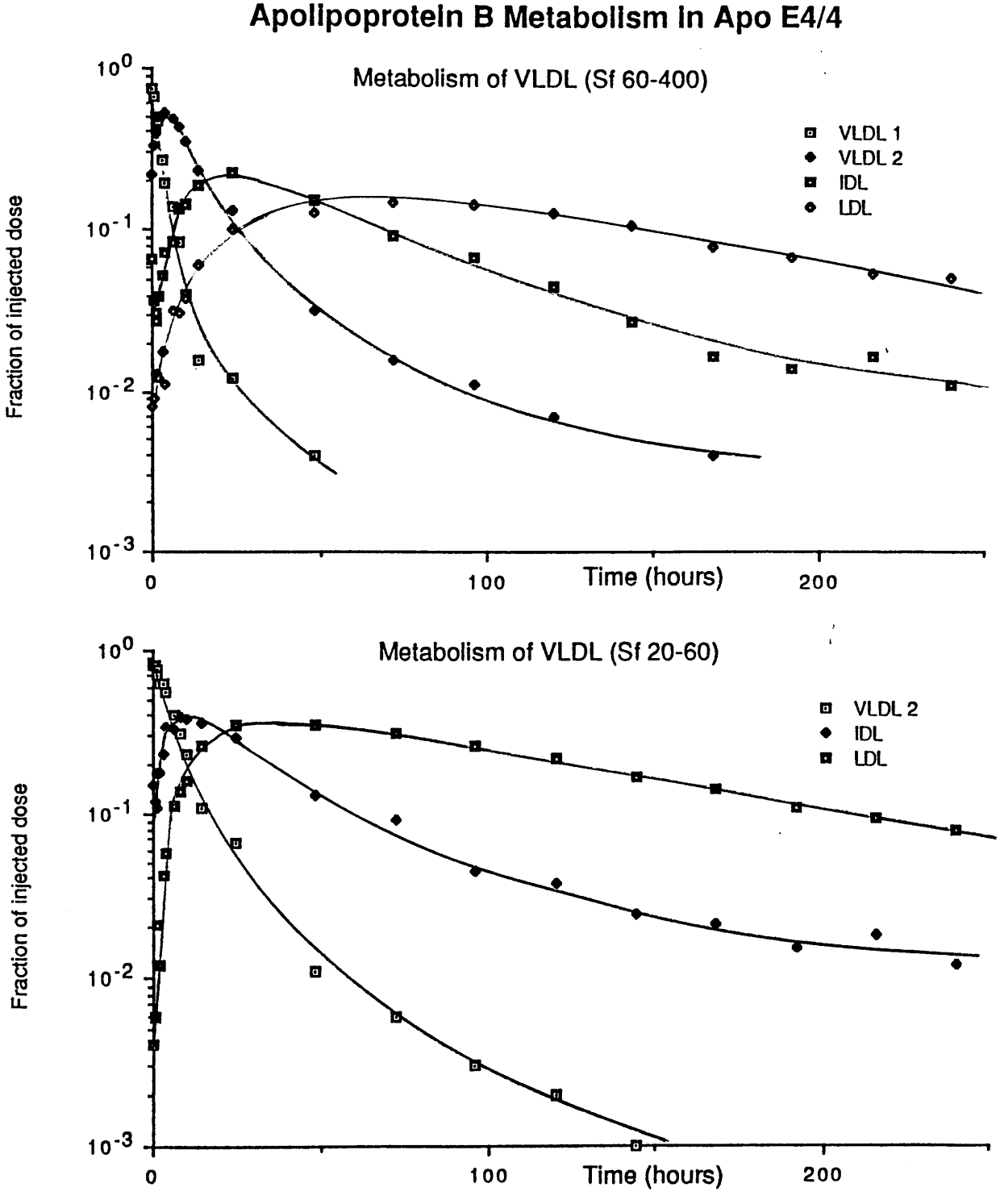


Fig. 12: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in apoE4/4 subjects (n = 5). - Averages from Table I-11A/B of individual data as shown in Fig I-6 to I-10

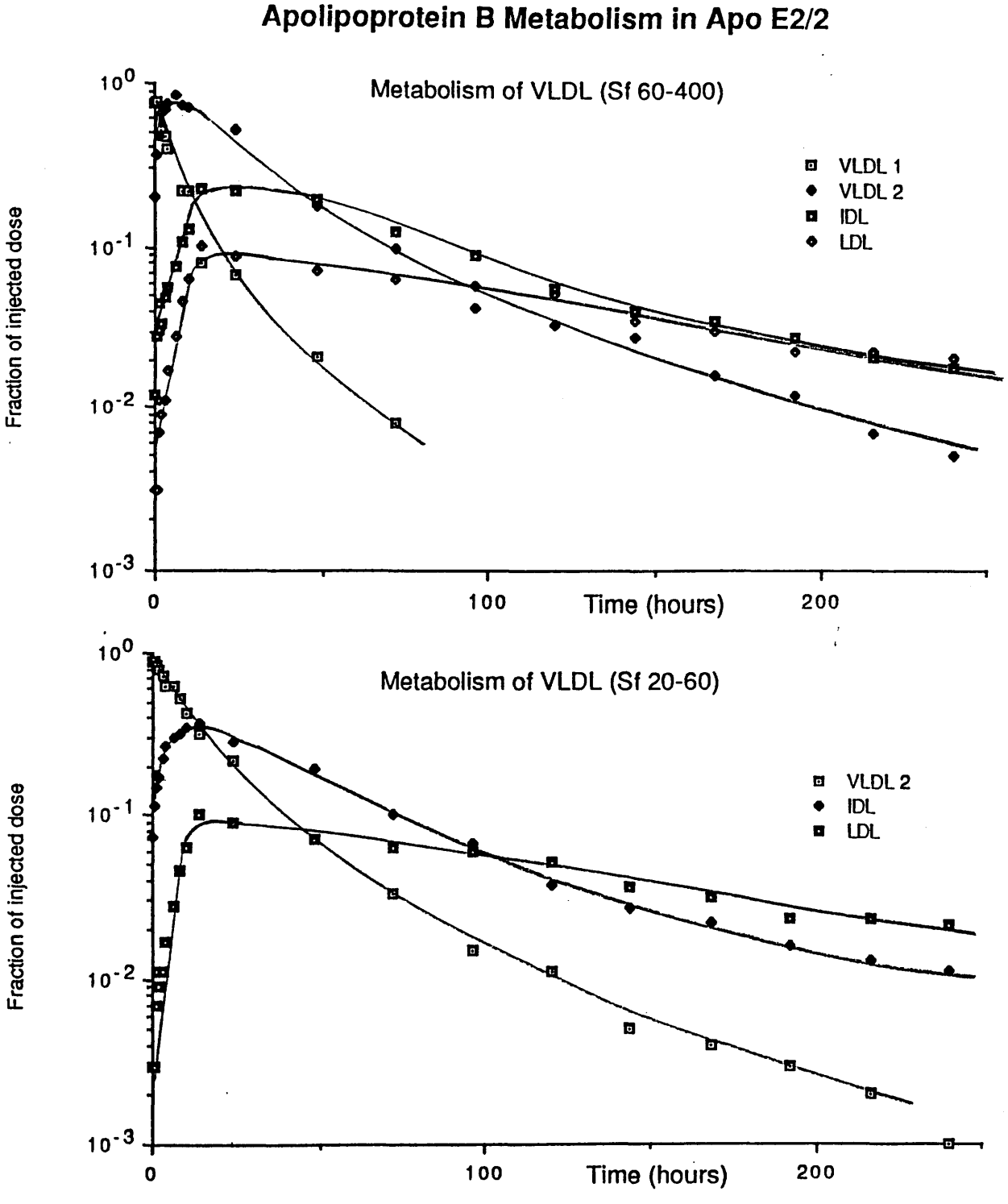


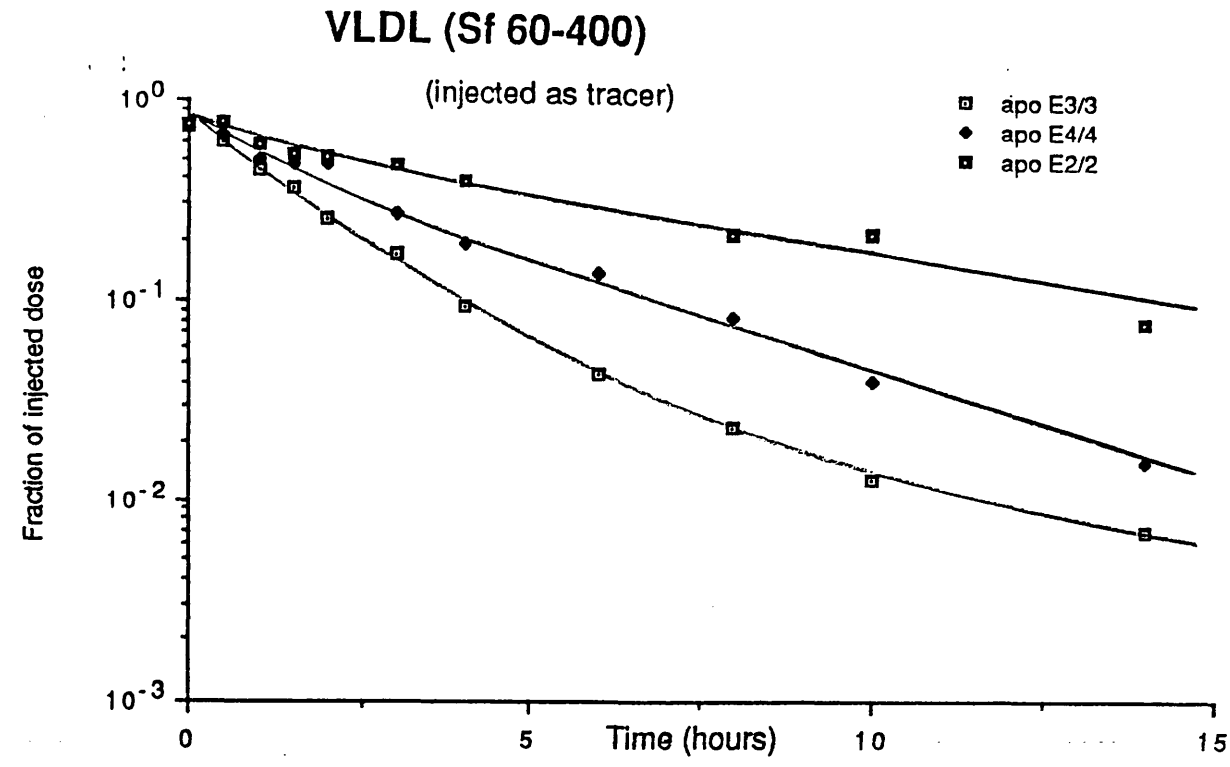
Fig. 13: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in apoE2/2 subjects (n = 4). - Averages from Table I-12 A/B of individual data as shown in Fig. I-11 to I-14.

the decay curves obtained for different apoE phenotypes, curves for the same lipoproteins were displayed together in one diagram (Figs. 14-20).

$VLDL_1$, injected as tracer, disappeared from the plasma compartment rapidly in apoE3/3 and apoE4/4 subjects with about 1% of the initially injected dose left after 14 h. In apoE2/2 individuals clearance of this lipoprotein subfraction was much slower with about 10% remaining in plasma after 14 h (Fig. 14). Basically, $VLDL_1$ catabolism could be described by a straight line on semilogarithmic paper, indicating a monoexponential decay process. In some subjects a second exponential occurred but this accounted only for less than 3% of the injected dose.

$VLDL_2$, derived from $VLDL_1$, was cleared with the highest rate in apoE3/3 subjects. Clearance in apoE4/4 individuals was slower. After 72 h 0.7% and 1.6% respectively of the initially injected dose were left in the plasma compartment. In apoE2/2 subjects proportionally more $VLDL_1$ was transferred into $VLDL_2$, which in turn was cleared at a much lower rate than seen in the other apoE phenotypes (about 10% left in plasma 72 h after injection) (Fig. 15).

Transfer of $VLDL_2$ (derived from $VLDL_1$) to IDL and IDL decay were fastest in apoE3/3 individuals. IDL clearance in apoE2/2 individuals was slower, although the difference towards apoE3/3 subjects was less than with the previous two lipoprotein subfractions (3.6% versus 1.3%



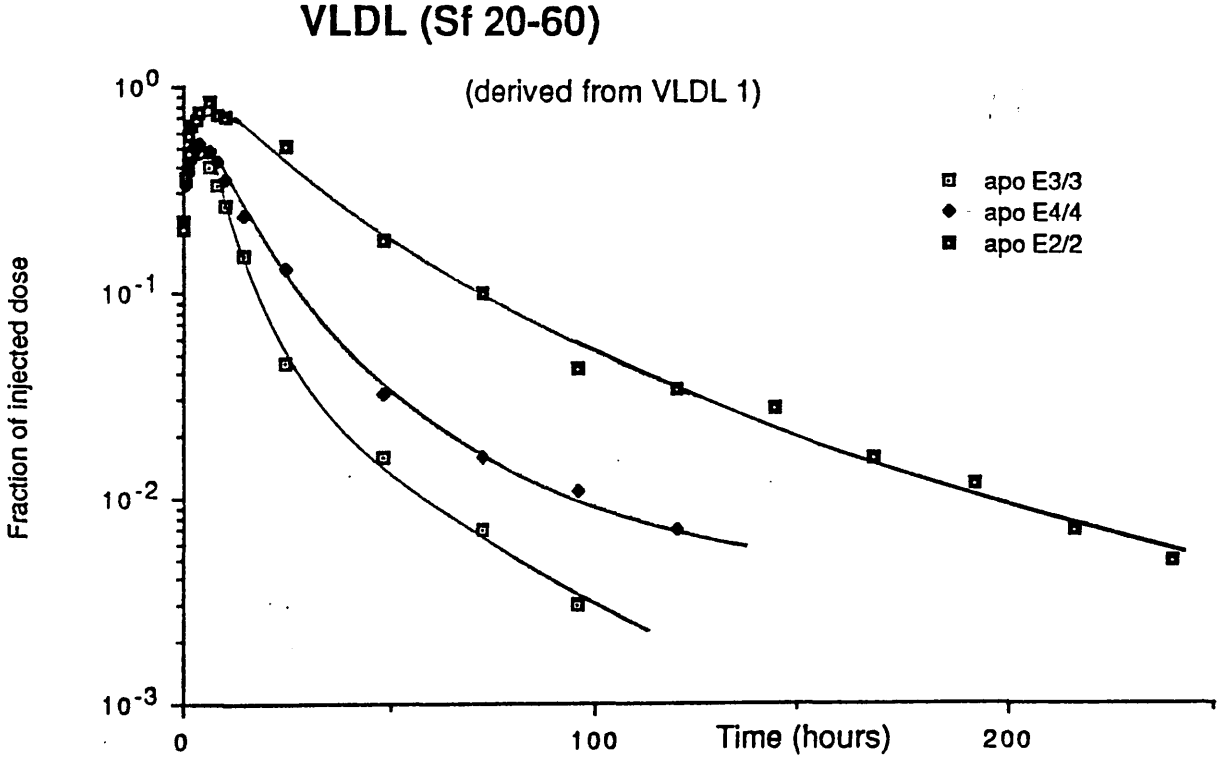
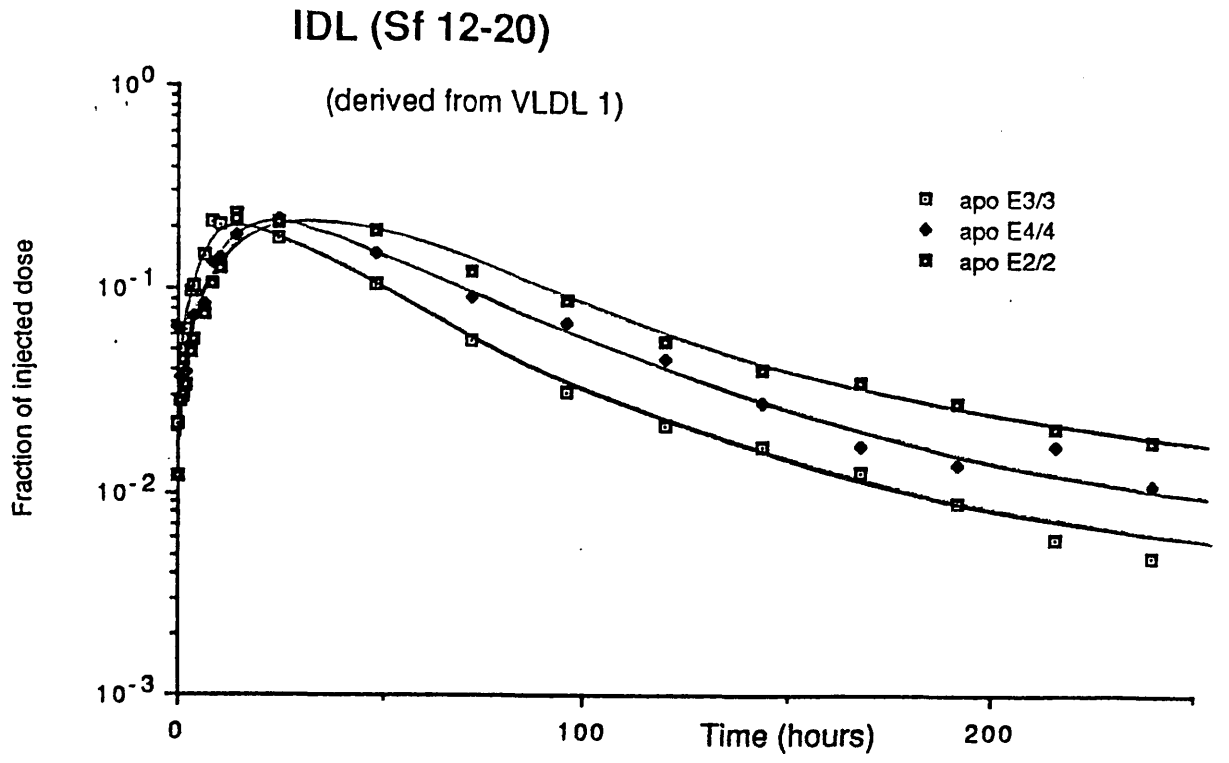


Fig. 14 and Fig. 15:

Metabolism of $VLDL_1$. - Decay curves for $VLDL_1$ and VLDL2 derived from $VLDL_1$ in apoE3/3, apoE4/4 and apoE2/2 normolipidaemic subjects.



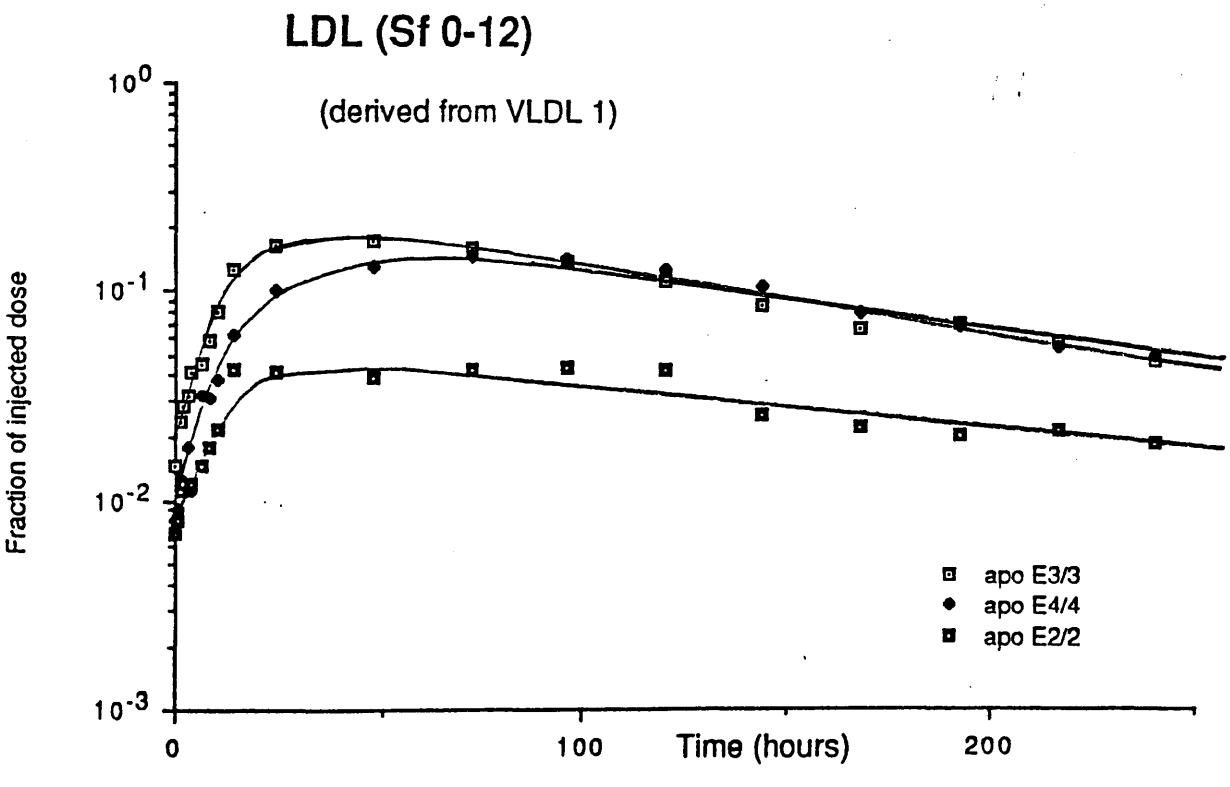
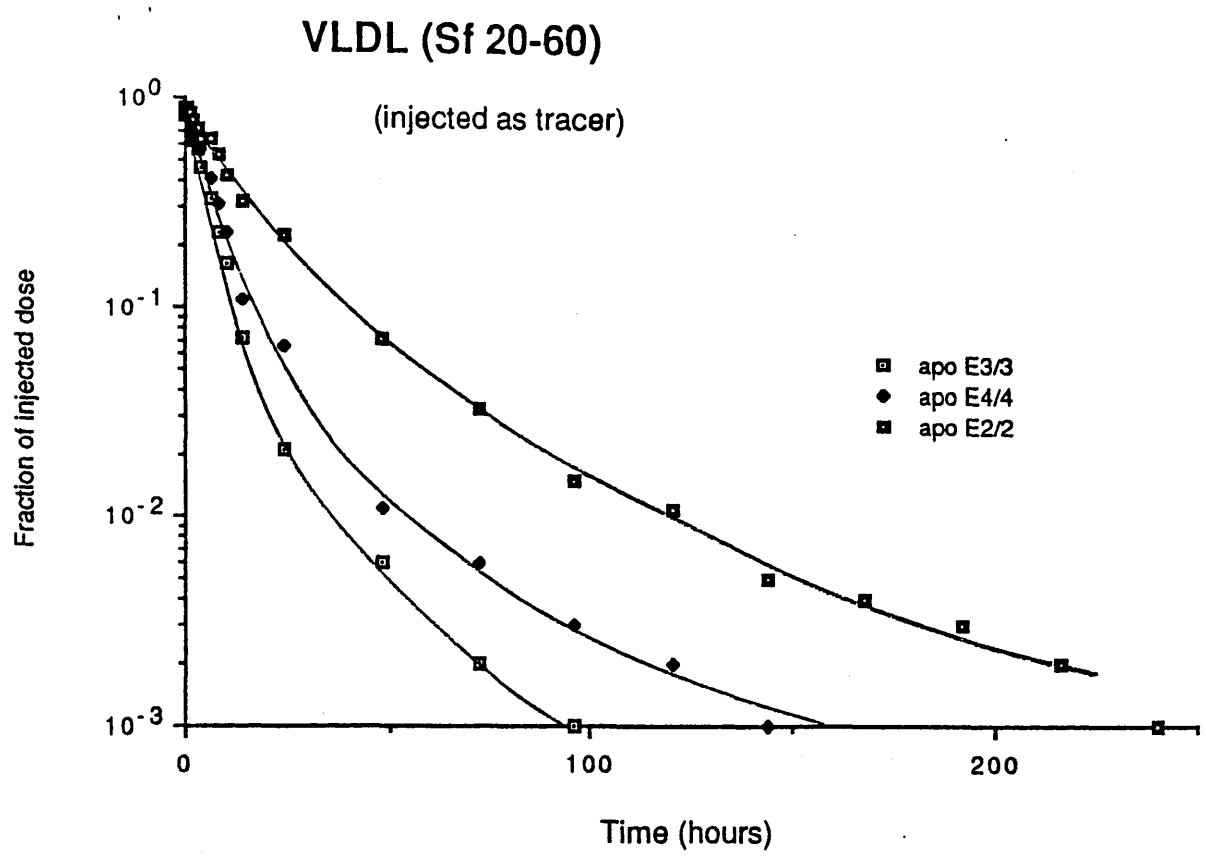


Fig. 16 and Fig. 17:

Metabolism of $VLDL_1$. - Decay curves for IDL and LDL derived from $VLDL_1$ in apoE3/3, apoE4/4 and apoE2/2 normolipidaemic subjects.



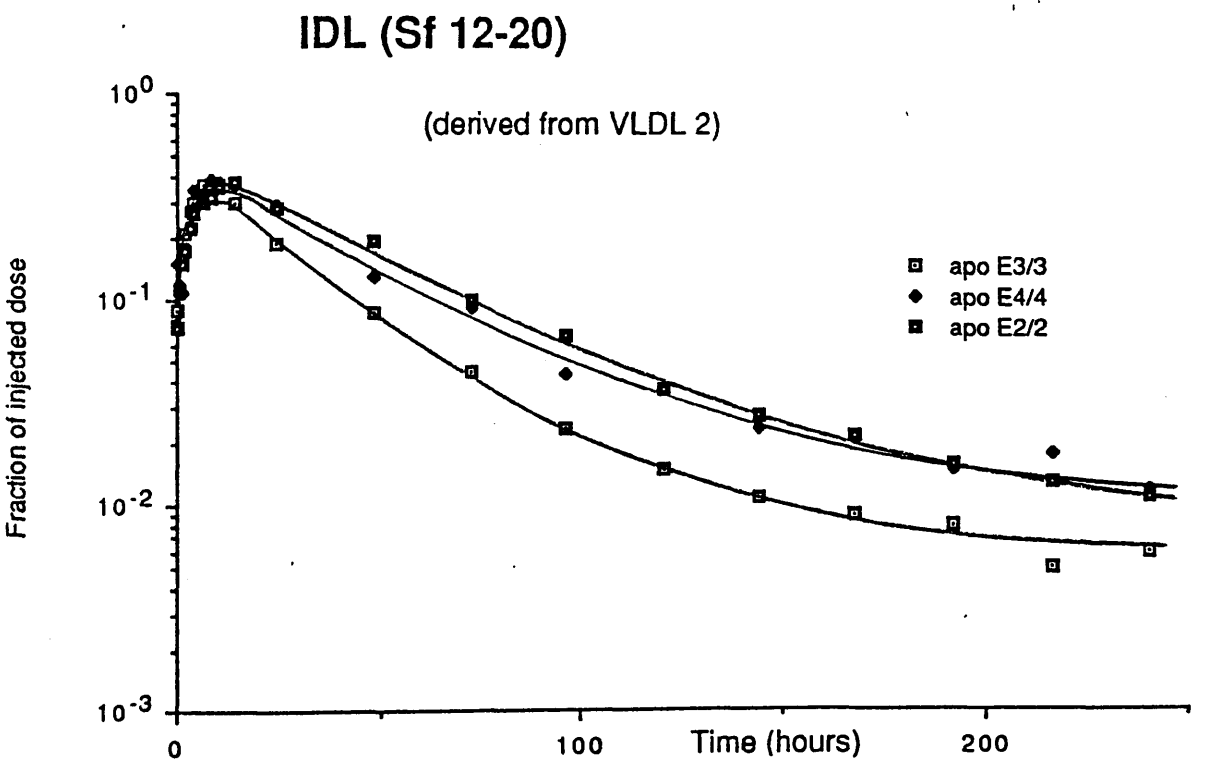


Fig. 18 and Fig. 19:

Metabolism of $VLDL_2$. - Decay curves for $VLDL_2$ and IDL derived from $VLDL_2$ in apoE3/3, apoE4/4 and apoE2/2 normolipidaemic subjects.

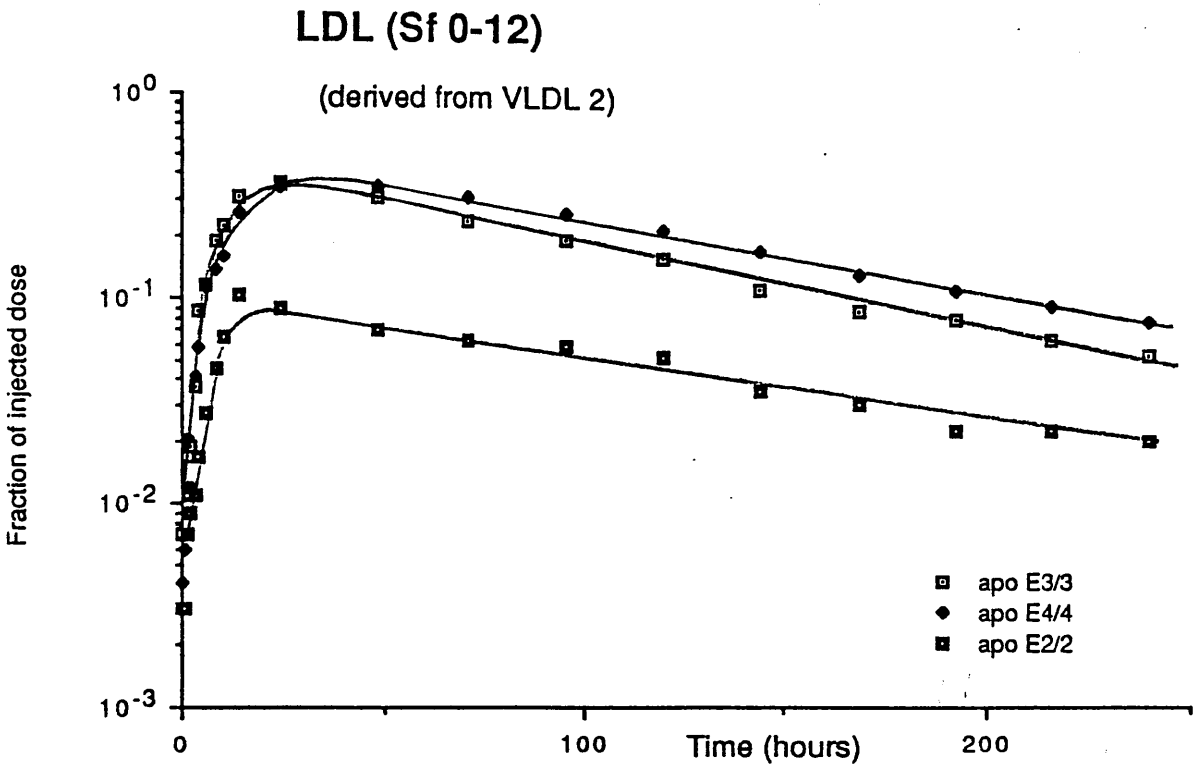


Fig. 20: Metabolism of $VLDL_2$. - Decay curve for LDL derived from $VLDL_2$ in apoE3/3, apoE4/4 and apoE2/2 normolipidaemic subjects.

after 168 h). IDL clearance in apoE4/4 individuals was intermediate between the rates seen for the two other groups (Fig. 16).

In apoE3/3 subjects $VLDL_1$-derived LDL reached a peak of nearly 20% within 48 h. The slope of the following decay curve was slightly steeper than observed in apoE2/2 and apoE4/4 subjects. The latter reached the peak of the LDL curve at nearly 15% after 72 h. In apoE2/2 subjects transfer from IDL to LDL was substantially lower. Less than 5% of the injected dosage reached the LDL density range (Fig. 17).

With $VLDL_2$ injected as tracer, the following observations were made: $VLDL_2$ clearance in apoE2/2 individuals was much slower than observed in apoE3/3 and apoE4/4 (1.5% versus 0.1% and 0.3%) respecively (Fig. 18). Throughput towards IDL occurred at the same rate, but IDL clearance in apoE2/2 and in apoE4/4 subjects was somewhat slower than in apoE3/3 subjects (Fig. 19).

The peak of the LDL curve (35% of the injected dose) was reached within 48 h by both apoE3/3 and apoE4/4 subjects. The slope of the LDL curve in apoE4/4 was again slightly less than observed with apoE3/3 homozygotes. In apoE2/2 individuals only 12% of the labelled precursor was transferred into LDL. The slope of the decay curve was less well defined. However, it clearly was not steeper than the slope of LDL decay curves observed in E3/3 and E4/4 subjects (Fig. 20).

In summary, apoE2/2 subjects differed from apoE3/3 and apoE4/4 by a markedly slower catabolism of large and small VLDL and a significantly reduced transfer of IDL into LDL. Differences between apoE3/3 and apoE4/4 subjects were much smaller with a tendency for decreased clearance of all apoB containing lipoprotein subfractions.

It has been mentioned already that one of the apoE2/2 subjects (P.M.) showed lipoprotein decay curves with little similarity to the curve pattern observed in four other apoE2/2 homozygotes (Fig. I- 5). Her metabolism of $VLDL_1$ and $VLDL_2$ was as rapid as observed in apoE3/3 subjects, but catabolism of IDL and notably of LDL was faster than seen in this group. As in the four other apoE2/2 subjects IDL to LDL interconversion was impaired although to a lesser extent with LDL curves peaking at 16% and 23% respectively.

Finally, a comparison of curves for LDL derived either from $VLDL_1$ or from $VLDL_2$ is given in Figs. 21-23. In the three study groups two features were consistently observed: Firstly, $VLDL_1$ to LDL transfer occurred at a lower rate than transfer from $VLDL_2$ to LDL and secondly, LDL derived from $VLDL_1$ was catabolised more slowly as compared to LDL derived from $VLDL_2$.

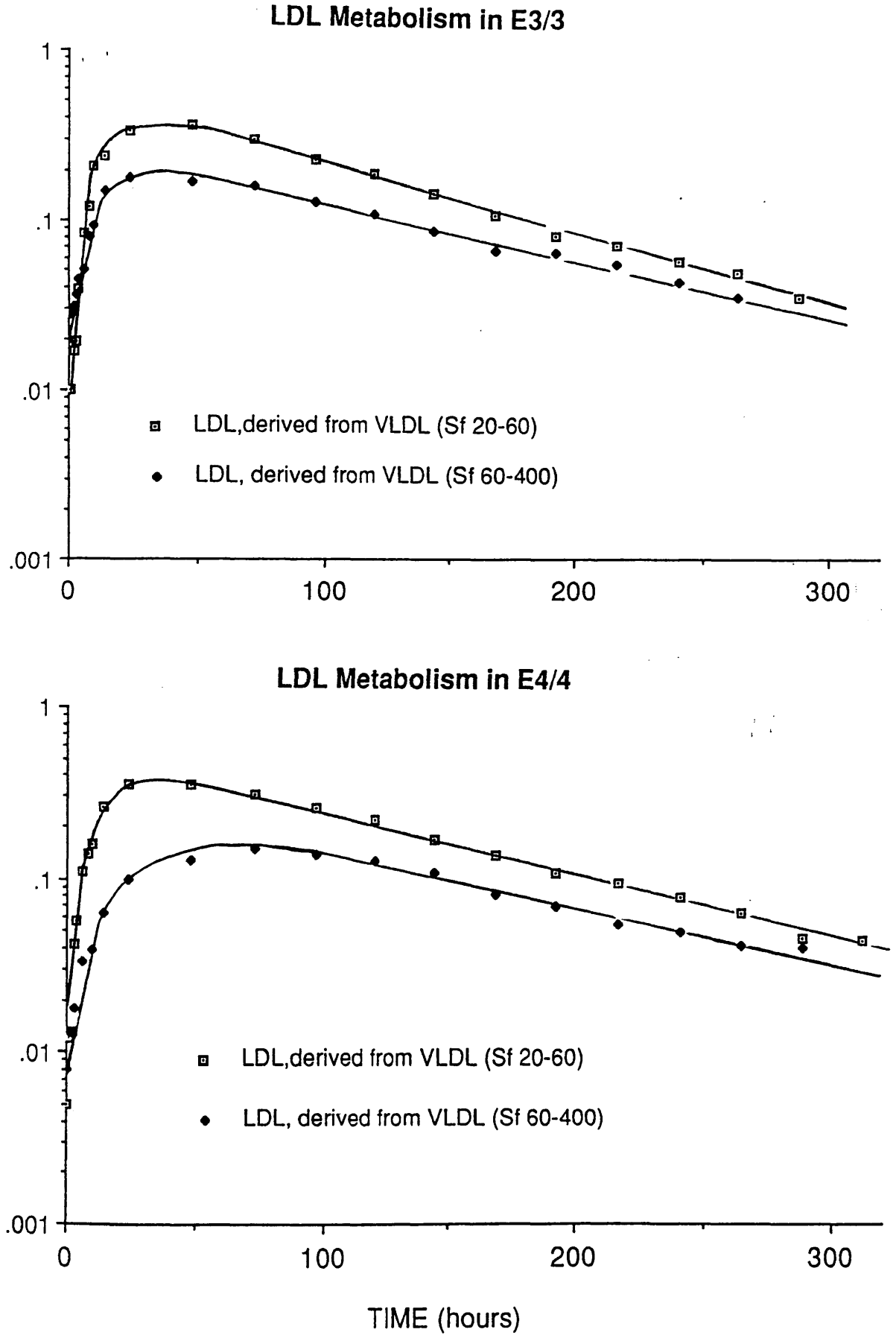


Fig. 21 and Fig. 22:

Metabolism of LDL derived from $VLDL_1$ or $VLDL_2$ in apoE3/3 and apoE4/4 homozygous normolipidaemic subjects.

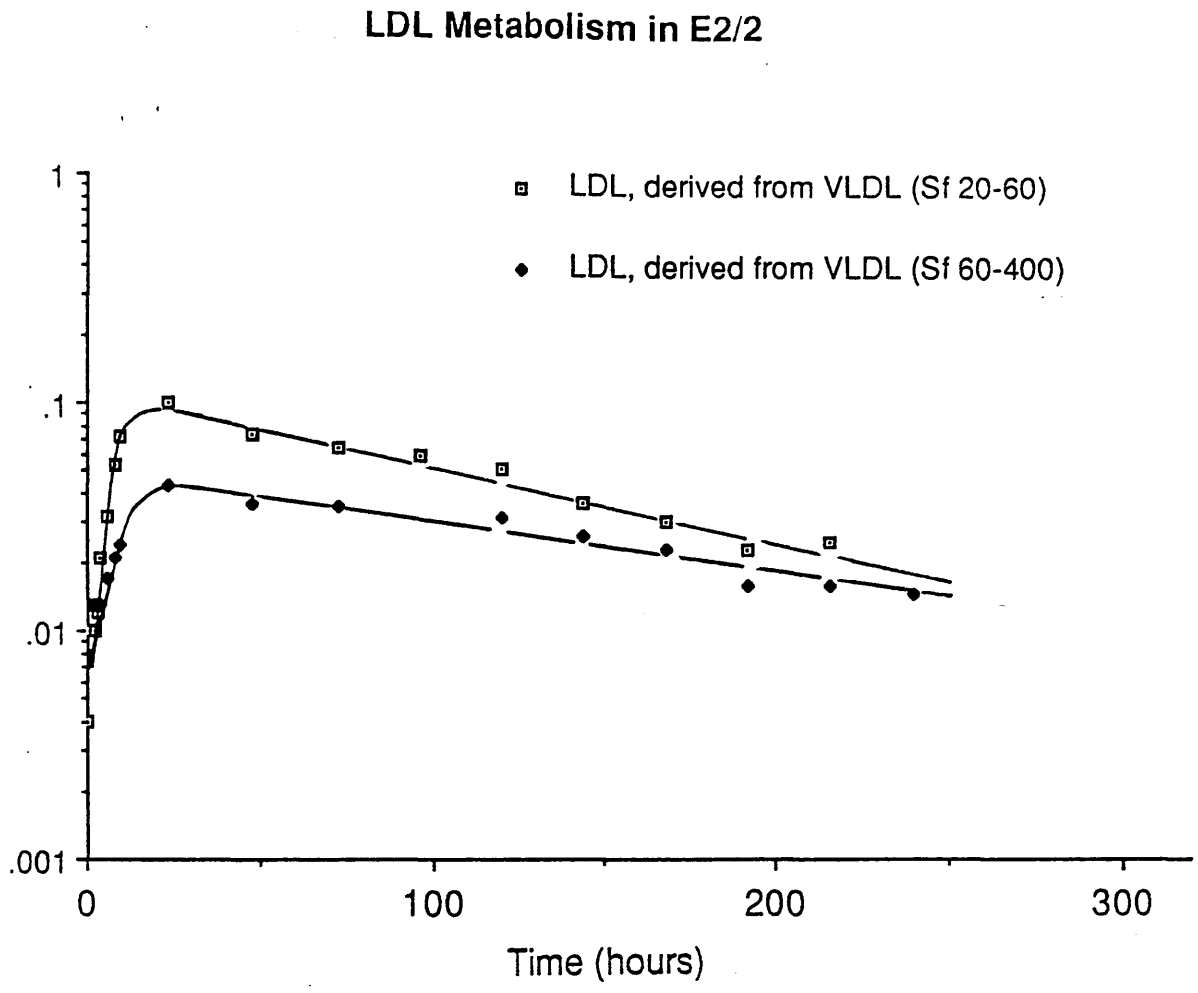


Fig. 23: Metabolism of LDL derived from $VLDL_1$ or $VLDL_2$ in apoE2/2 homozygous normolipidaemic subjects

3.3.3. Analysis of apolipoprotein B metabolism in apoE2/2, apoE3/3 and apoE4/4 subjects by computer modelling

The decay curves for the four apoB containing lipoprotein subfractions which were discussed in the previous section were analysed using the SAAM 29 computer program. The model of apolipoprotein B metabolism which provides the basis for computer modelling and curve fitting is depicted in Fig. 8 and has been described in paragraph 2.5.8. For comparison of the metabolic behaviour of apoB containing lipoproteins each set of individual turnover data was analysed using the same kinetic model. The computer calculations were aimed at an optimal fitting of observed data, ie decay curves as previously described, and calculated curves, thus minimising the sum of squared differences between the two. Calculated curves were derived from observed data on the basis of the kinetic model. This required the calculation of kinetic rate constants k(I,J) and masses M(I) for each subcompartment. Kinetic rate constants in the above notation describe the fractional transfer rate in terms of pools/day for material being transferred from compartment J to compartment I. Individual values for kinetic rate constants and masses for subcompartments are listed in Tables I-13 for apoE3/3, Table I-14 for apoE4/4 and Table I-15 for apoE2/2 subjects. The quality of the data as defined by fractional standard

deviations (FSD) can be evaluated from Tables I-16, I-17 and I-18. The FSD values were usually well below 0.1.

For comparison of the three apoE phenotypic groups, averages of the calculated metabolic parameters are presented in Table 11. In these comparisons, values obtained from apoE3/3 subjects serve as a normal reference, as the apoE3 allele is by far the most frequent. When kinetic rate constants from apoE3/3 and apoE2/2 subjects were compared, the following statistically significant differences were revealed: k(0,1), k(0,4), k(8,4), k(0,6), k(10,7) and k(11,8) were all smaller in the apoE2/2 homozygotes than in apoE3/3 homozygotes, whereas k(0,7) and k(0,8) were larger in the former as compared to the latter (Fig. 24). A comparison of rate constants between apoE4/4 and apoE3/3 individuals showed only one significant deviation. This was observed for k(0,10), which was smaller in apoE4/4 as compared to apoE3/3 subjects ($p < 0.01$). The only statistically significant difference between subcompartment masses was observed for M(11) with apoE2/2 values smaller than values obtained from apoE3/3 subjects.

From these calculated values the following parameters of apolipoprotein B metabolism were derived for each of the four apoB containing lipoproteins:

(a) Rate of direct synthesis, which is - steady-state conditions prevailing - equal to the sum of the fractional catabolic rates multiplied by the plasma pool.

TABLE 11:

Comparison of calculated metabolic parameters in apoE2/2, apoE3/3 and apoE4/4 homozygotes

	M(1)	k(0,1)	k(2,1)	M(2)	k(4,2)	k(6,2)
E2/2, n=4						
mean	51	0.3*	3.75	9.5	21.1	0.25
± s	±12	±0.32	±1.35	±4.03	±3.0	±0.24
E3/3, n=5						
mean	61	5.26*	6.3	18	21.9	0.16
± s	±20	±4.47	±1.18	±5	±2.7	±0.11
E4/4, n=5						
mean	53	5.87	4.58	18.8	15.1	0.12
± s	±35	±6.32	±2.08	±17.2	±7.8	±0.10

	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
E2/2, n=4						
mean	159	0.03*	0.83**	0.24	0.05	45.8
± s	±44	±0.04	±0.10	±0.14	±0.04	±39.2
E3/3, n=5						
mean	91	1.61*	2.48**	0.52	0.06	64
± s	±43	±0.76	±1.26	±0.17	±0.12	±41
E4/4, n=5						
mean	74	1.02	1.23	0.28	0.11	40.8
± s	±33	±0.86	±0.63	±0.12	±0.18	±37.0

	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	M(7)
E2/2, n=4						
mean	0	10.2	0.04	5.5	0.28**	165
± s		±4.9	±0.07	±1.2	±0.03	±64
E3/3, n=5						
mean	2.01	3.33	0.24	3.8	0.62**	94
± s	±1.13	±1.03	±0.48	±1.7	±0.19	±54
E4/4, n=5						
mean	0	9.68	0	2.6	0.41	141
± s		±4.42		±1.6	±0.09	±54

* : $p<0.05$, ** : $p<0.025$,

	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)	M(9)
E2/2, n=4						
mean	0.84**	0.72*	64	3.11**	0.16**	114
± s	±0.38	±0.28	±47	±1.58	±0.28	±50
E3/3, n=5						
mean	0**	2.41*	120	0.68**	1.26**	105
± s		±1.36	±75	±0.42	±0.51	±24
E4/4, n=5						
mean	0	2.02	83	0.39	0.72	74
± s		±0.56	±62	±0.21	±0.38	±28

	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
E2/2, n=4					
mean	0.31	383	0.29	77**	0.18
± s	±0.08	±109	±0.06	±40	±0.07
E3/3, n=5					
mean	0.34	723	0.28***	702**	0.2
± s	±0.08	±287	±0.01	±402	±0.03
E4/4, n=5					
mean	0.24	1165	0.22***	402	0.17
± s	±0.08	±254	±0.01	±224	±0.04

* : $p<0.05$, ** : $p<0.025$, *** : $p<0.01$

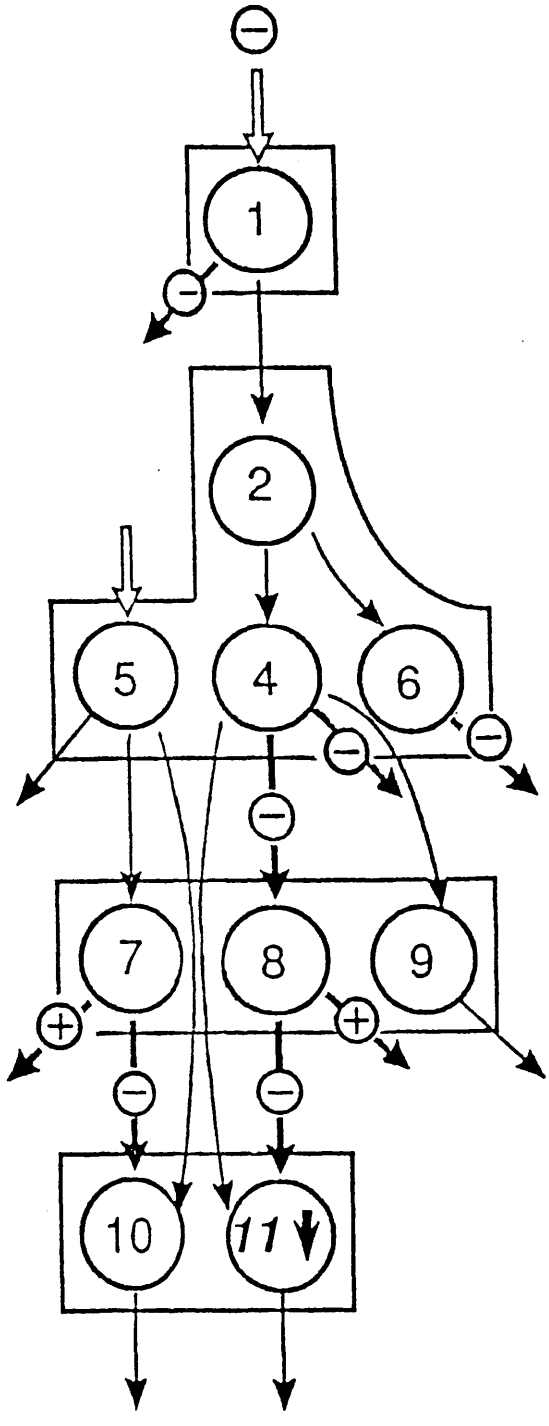


Fig. 24: Kinetic model of apoB metabolism in apoE2/2 subjects. - Significant differences in comparison to apoE3/3 homozygotes are indicated (⊕, ⊖, ↓).

(b) Plasma pool, ie the sum of masses from subcompartments accounting for each lipoprotein.

(c) Fractional rates for either direct catabolism or for transfer to denser lipoproteins.

Averages for the three study groups are presented in Table 12, calculated from individual data compiled in Tables I-19, I-20 and I-21.

In apoE2/2 subjects fractional rates for direct catabolism of $VLDL_1$ and $VLDL_2$ were smaller than observed in apoE3/3 subjects. IDL to LDL transfer was significantly reduced and direct catabolism of IDL was increased. The fractional catabolic rate for LDL was increased but this difference between apoE2/2 and apoE3/3 subjects was not statistically significant.

In apoE4/4 subjects, direct $VLDL_2$ catabolism was also significantly reduced but in contrast to apoE2/2 subjects this was partly compensated for by an increase of the transfer rate from $VLDL_2$ to IDL. This increase however did not reach statistical significance. The fractional catabolic rate (FCR) for LDL was significantly lower in apoE4/4 as compared to apoE3/3 subjects.

The plasma pool of LDL was smaller in apoE2/2 as compared to apoE3/3 reflecting the different apoB plasma concentrations in these subjects (see Table 8). Direct synthesis of $VLDL_1$ and total apoB synthesis were lower in apoE2/2 subjects. The same applied for total apoB synthesis in apoE4/4. In apoE2/2 subjects these

TABLE 12:

Comparison of apolipoprotein B metabolism in apoE2/2, apoE3/3 and apoE4/4 homozygotes

VLDL (Sf 60-400)

	direct synth. [mg/d]	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to VLDL2
E2/2, n=4				
mean	208**	51	0.3*	3.75
± s	±75	±12	±0.32	±1.35
E3/3, n=5				
mean	693**	61	5.26*	6.3
± s	±240	±20	±4.47	±1.18
E4/4, n=5				
mean	423	53	5.86	4.58
± s	±168	±35	±6.3	±2.08

VLDL (Sf 20-60)

	direct synth. [mg/d]	flux from VLDL1	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to IDL&LDL
E2/2, n=4					
mean	274	189	220	0.03**	2.08
± s	±142	±65	±12	±0.03	±0.58
E3/3, n=5					
mean	335	386	176	1.65**,*	2.58
± s	±179	±125	±55	±0.47	±0.49
E4/4, n=5					
mean	260	188	136	0.58*	3.38
± s	±64	±75	±69	±0.46	±1.29

* : $p<0.05$, ** : $p<0.025$

IDL (Sf 12-20)

	direct synth. [mg/d]	flux from VLDL2	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to LDL
E2/2, n=4					
mean	0	447	344	0.97**	0.41**
± s		±125	±95	±0.18	±0.15
E3/3, n=5					
mean	0	418	319	0.34**	1.02**
± s		±106	±81	±0.14	±0.32
E4/4, n=5					
mean	0	379	297	0.18	1.16
± s		±97	±103	±0.09	±0.32

LDL (Sf 0-12)

	direct synth. [mg/d]	flux from IDL&VLDL2	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
E2/2, n=4					
mean	0	142*	458**	0.28	482**
± s		±39	±86	±0.06	±150
E3/3, n=5					
mean	38	337*	1576**	0.24*	1102**
± s	±47	±102	±442	±0.02	±166
E4/4, n=5					
mean	19	327	1695	0.2*	721
± s	±37	±71	±375	±0.02	±168

* : $p<0.05$, ** : $p<0.025$

differences still persisted when body weight was taken into account. The body weight standardised total apoB synthetic rate in apoE4/4 subjects, however, was not significantly different from the rate of apoE3/3 subjects.

ApoB was synthesised in apoE2/2 subjects in about equal proportions either as $VLDL_1$ or as $VLDL_2$. In apoE3/3 and in apoE4/4 subjects about two-thirds of the apoB was synthesised as $VLDL_1$ and one-third as $VLDL_2$. Direct apoB synthesis in the LDL range occurred in some individuals of the apoE3/3 and the apoE4/4 phenotype but it hardly exceeded 10% of the total apoB synthesis.

In summary, in apoE2/2 individuals, the rates for direct catabolism of $VLDL_1$ and $VLDL_2$ are decreased and the transfer rate of IDL to LDL is reduced, while the rate of direct IDL metabolism is increased. In apoE4/4 subjects the rate of direct catabolism of $VLDL_2$ and the fractional catabolic rate of LDL are smaller than in apoE3/3 individuals.

3.4. Apolipoprotein B Metabolism in Familial Hyperchylomicronaemia

The VLDL metabolism of two patients with familial hyperchylomicronaemia was studied. One subject suffers from classical lipoprotein lipase deficiency while the other has an inherited plasma inhibitor of lipoprotein lipase activity.

3.4.1. Characterisation of patients

Study participants are characterised by the data compiled in Table 13. D.A., a 35 year old male, reported from his childhood and adolescence repeated bouts of abdominal pain and several attacks of acute pancreatitis. His plasma triglyceride levels were found to be well above 20 mmol/l with cholesterol ranging between 5-10 mmol/l. His apoE phenotype is apoE4/2. An assay for lipoprotein lipase revealed no enzyme activity in this patient.

The triglyceride and cholesterol concentrations given in Table 13 were measured during the administration of a low fat diet, which was maintained throughout the metabolic study. Lipoprotein levels were determined after removal of chylomicrons (see 2.5.7.).

D.S., a 58 year old woman, is a member of a family affected by familial hyperchylomicronaemia, which was described by Brunzell et al. (117) (see 1.4.2.). She gave a history of recurrent abdominal pain since the age of 16 years. Massive hypertriglyceridaemia of about 30 mmol/l was diagnosed when she presented with eruptive xanthomas on the outer aspects of both feet. On physical examination the spleen was palpable but the liver appeared to be of normal size. Her apoE phenotype is apoE4/3. Adipose tissue lipoprotein lipase activity in this subject and in her son who also has massive hypertriglyceridaemia was found to be increased and in-vitro incubation experiments demonstrated

Table 13: Lipoprotein Lipase Deficiency and Hepatic Lipase Deficiency. - Physical Parameters, Plasma Lipid and Lipoprotein Concentrations.

	Sex (M/F)	Age (Years)	Body Weight (kg)	Body Weight Index	Total Trig (mmol/l)	Total Chol	VLDL Chol	LDL** Chol (mmol/l)	HDL Chol
Lipoprotein lipase deficiency:									
D.A.*	M	35	66	24.2	19.5±2.3	7.4±1.0	4.5±1.0	0.9±0.1	0.4±0.1
D.S.*	F	58	62	25.8	20.0±5.7	7.6±1.0	4.0±0.2	0.7±0.1	0.4±0.1
Hepatic lipase deficiency:									
G.P.	M	63	70	23.9	2.8±0.5	5.1±1.1	0.8±0.4	2.2±0.3	2.0±0.3
Controls (n = 5)	3 M 2 F	39 ±11	73 ±12	24.2 ± 2.5	1.4±0.6	5.0±1.1	0.7±0.3	3.0±1.1	1.3±0.3

* VLDL, LDL and HDL were measured after removal of chylomicrons.
** "LDL-Chol" as determined by the LRC-protocol (320) includes LDL (S_f 0-12) and most of IDL (S_f 12-20).

an inhibitory effect of plasma from these patients on normal post-heparin plasma lipolytic activity (117). This was taken as evidence that in this family hyperchylomicronaemia is caused by a dominantly inherited plasma inhibitor of lipoprotein lipase activity.

Plasma lipid levels, as shown in Table 13, were determined under a low fat diet as described for patient D.A. Native plasma from D.S. after overnight incubation at +4°C, plasma after removal of chylomicrons and plasma infranatant after removal of VLDL is shown together with normal plasma in Fig. 25.

The relative distribution of apolipoprotein B in plasma was found to be very different from normolipidaemic controls, whereas apoB plasma concentrations were perfectly normal (Table 14). More than 50% of plasma apoB accumulated as $VLDL_1$-apoB. $VLDL_2$ pools were normal in D.A. but increasedin D.S. IDL pools were considerably lower than normal and LDL-apoB represented only one quarter to one-sixth of total plasma apoB.

Compositional studies of the four apoB containing lipoproteins revealed an increased triglyceride content for all four lipoprotein subclasses at the expense of cholesterol and cholesteryl ester. Phospholipid and protein compositions were little different from controls (Table 15).

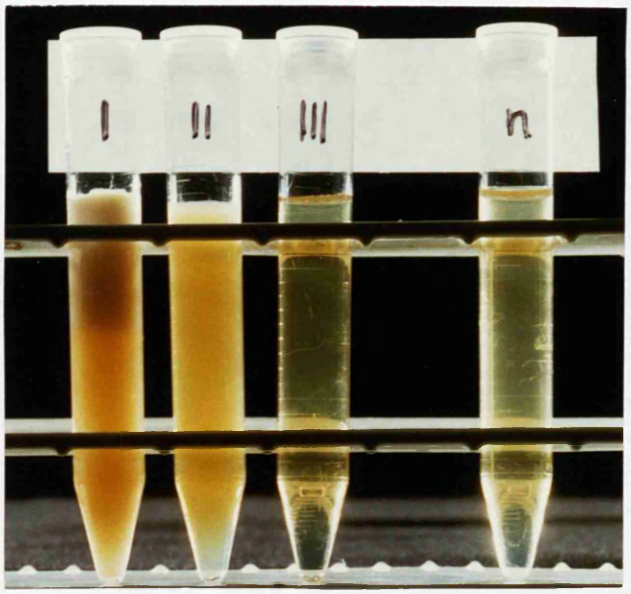


Fig. 25: Plasma from a patient with familial hyperchylomicronaemia. - I: Native plasma after 24 h at +4°C; II: plasma after removal of chylomicrons (see paragraph 2.5.7.); III: plasma infranatant after VLDL preparation; n = normal plasma.

Table 14: Lipoprotein Lipase Deficiency and Hepatic Lipase Deficiency. - Relative Distribution of Apolipoprotein B Among Plasm Lipoproteins.

	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma Pool (mg)	ApoB Plasma Concentration (mg/ml)
	(Percentage Distribution)				(= 100%)	
Lipoprotein Lipase Deficiency:						
D.A.	62.2	8.0	4.3	25.5	1568	0.63
D.S.	54.0	22.0	7.5	16.5	1808	0.73
Hepatic Lipase Deficiency:						
G.P.	0.6	19.3	64.4	15.6	1591	0.59
Controls (n = 5)	2.7 ±1.1	9.2 ±1.5	13.9 ± 4.4	74.3 ± 5.9	2105 ±1031	0.71 ±0.24

Table 15: Lipoprotein Lipase Deficiency and Hepatic Lipase deficiency. - Compositions of Apolipoprotein B Containing Lipoproteins.

		Free Cholesterol	Cholesteryl Ester	Triglyceride	Phospholipids	Protein	ApoB
				(g/100 g)			(% of total protein)
$VLDL_1$	Lpl(-)	4.6 ± 1.2	9.1 ± 1.2	66.4 ± 0.9	13.1 ± 0.7	7.0 ± 0.3	48 ± 1
	HL(-)	3.9 ± 1.8	9.3 ± 0.5	62.2 ± 3.1	14.6 ± 3.4	10.0 ± 0.2	57 ± 3
	Controls	1.7 ± 2.3	16.0 ± 4.3	56.2 ± 4.8	17.0 ± 1.4	9.1 ± 2.4	37 ± 2
$VLDL_2$	Lpl(-)	4.2 ± 0.7	12.6 ± 0.4	54.3 ± 2.0	17.1 ± 0.4	11.9 ± 2.0	47 ± 6
	HL(-)	13.3 ± 2.6	12.7 ± 2.2	34.6 ± 0.9	23.4 ± 1.2	16.0 ± 0.6	80 ± 5
	Controls	8.1 ± 1.4	21.1 ± 5.9	35.1 ± 4.0	21.4 ± 2.4	14.4 ± 1.6	66 ± 5
IDL	Lpl(-)	2.6 ± 2.6	26.4 ± 4.1	30.6 ± 2.0	21.5 ± 3.0	19.0 ± 0.5	56 ± 1
	HL(-)	12.7 ± 2.0	35.5 ± 3.7	27.9 ± 1.7	24.7 ± 1.2	21.9 ± 0.8	90 ± 6
	Controls	11.2 ± 2.3	33.4 ± 4.8	12.4 ± 2.0	23.9 ± 1.3	19.1 ± 2.3	91 ± 4
LDL	Lpl(-)	3.2 ± 3.2+	27.2 ± 1.3	20.2 ± 2.1	23.9 ± 2.3	25.8 ± 2.6	85 ± 0
	HL(-)	7.1 ± 0.2++	24.5 ± 4.9	23.6 ± 1.4	26.4 ± 1.2	18.6 ± 4.9	98 ± 3
	Controls	13.4 ± 1.5	34.8 ± 2.2	5.1 ± 0.2	23.0 ± 1.6	23.6 ± 1.6	96 ± 2

Lpl(-): Means ± s from two patients; HL(-): Means ± s from three measurements in one patient.
Controls: Means ± s from five subjects (Table I-23).

3.4.2. VLDL-turnover studies in familial hyperchylomicronaemia

The metabolism of large and small VLDL was studied using a protocol which included a step for chylomicron removal (see 2.5.7.) as this massively increased lipoprotein subfraction would have disturbed the subfractionation of the apoB-100 containing lipoproteins. $VLDL_1$ used for preparation of metabolic tracers was analysed by electro-phoresis on 3% SDS-acrylamide gels (see 2.5.3.). Only tracers of apoB-48, which was clearly present in total plasma and in chylomicron preparations, could be detected in preparations of $VLDL_1$ (Fig. 26). This is in line with results reported by Meng et al. (352), where even without centrifugation for chylomicron removal little apoB-48 was recovered from the VLDL fraction ($d < 1.006$ g/ml) of Type I patients after overnight fasting.

Lipoprotein decay curves, as obtained from VLDL-turnover studies, are depicted in Fig. 27 for D.A. and in Fig. 28 for D.S. The curve patterns from both subjects are very similar. Curves for each lipoprotein from patient D.A. are shown together with normal controls and with curves from a hepatic lipase deficient subject in Figs. 29-32.

The clearance of large VLDL (S_f 60-400) is markedly delayed but that of small $VLDL_2$ is only slightly slower than in normolipidaemic controls. In the IDL range less

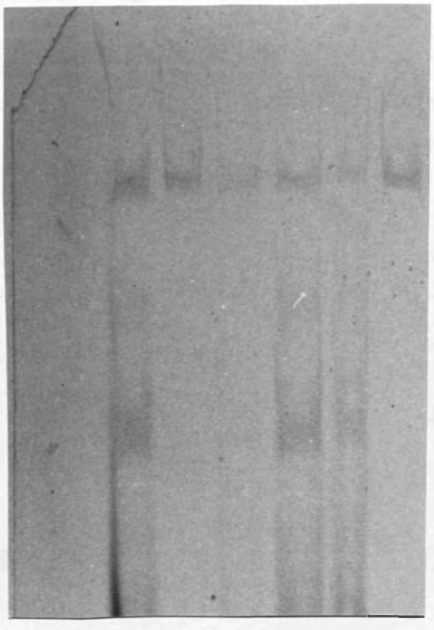


Fig. 26: SDS-gel electrophoresis of plasma (1), chylomicrons (2) and $VLDL_1$ (3) from a patient with familial hyperchylomicronaemia.- 50 ul and 100 ul were applied per lane.

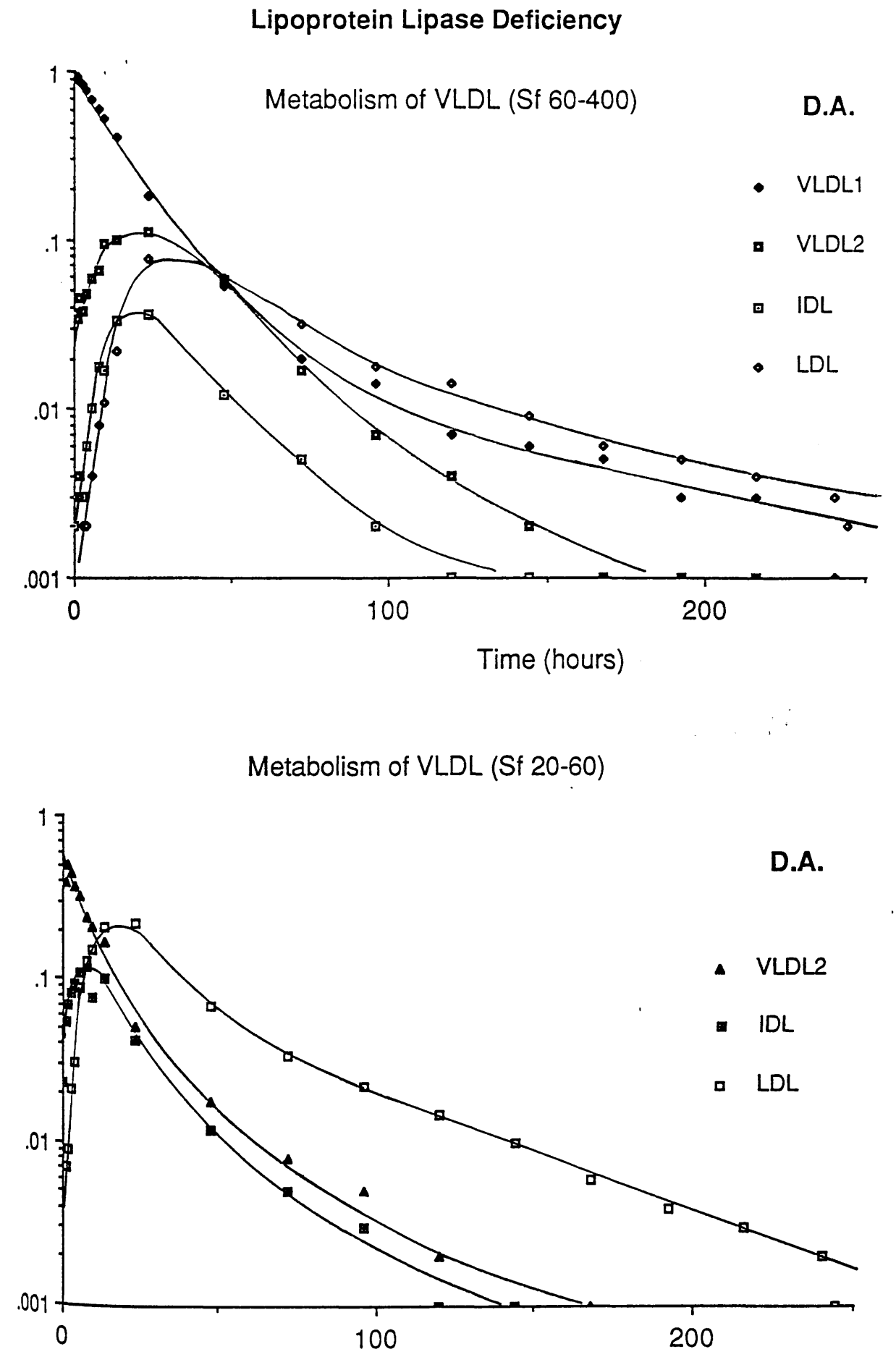
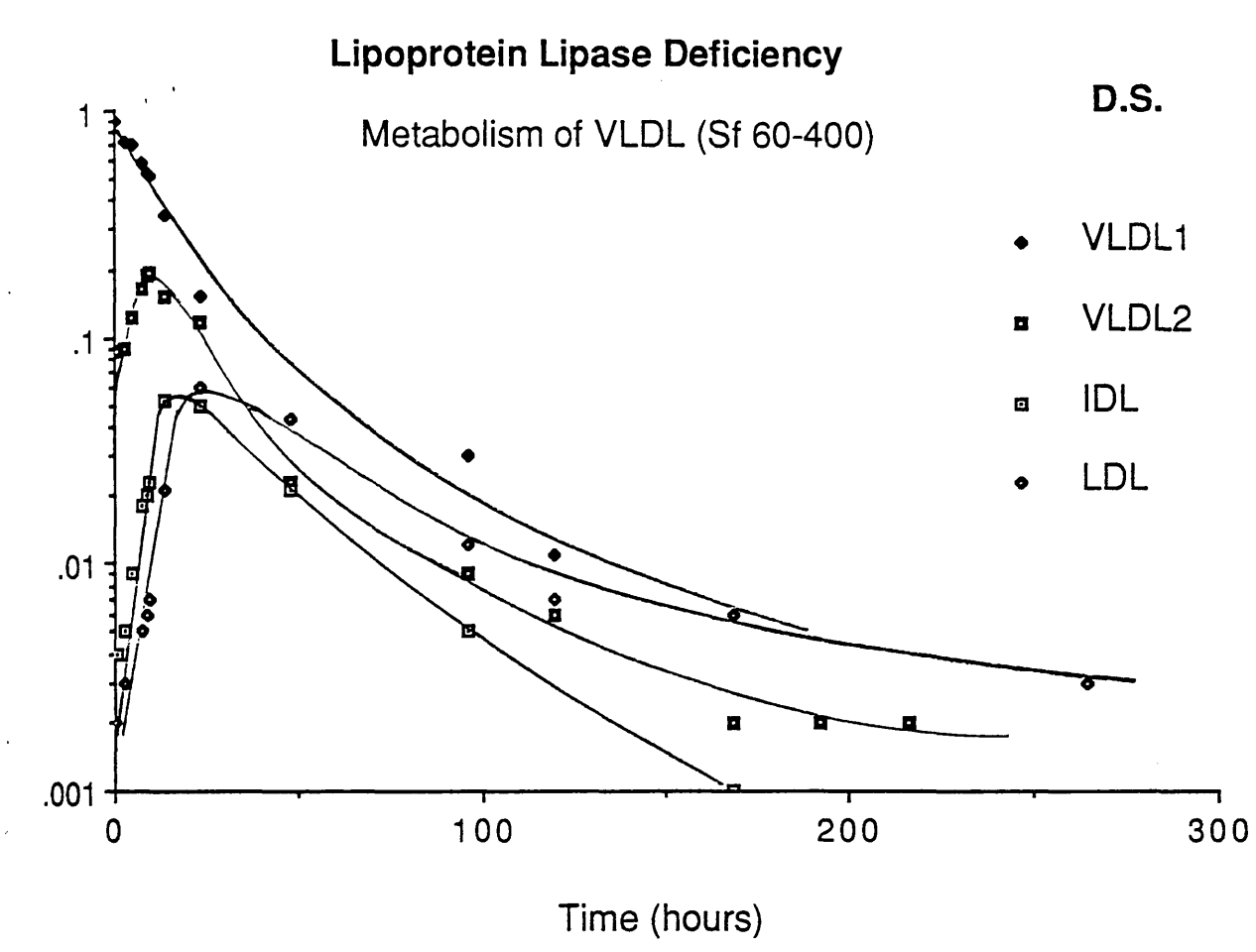


Fig. 27: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in a patient (D.A.) with lipoprotein lipase deficiency.



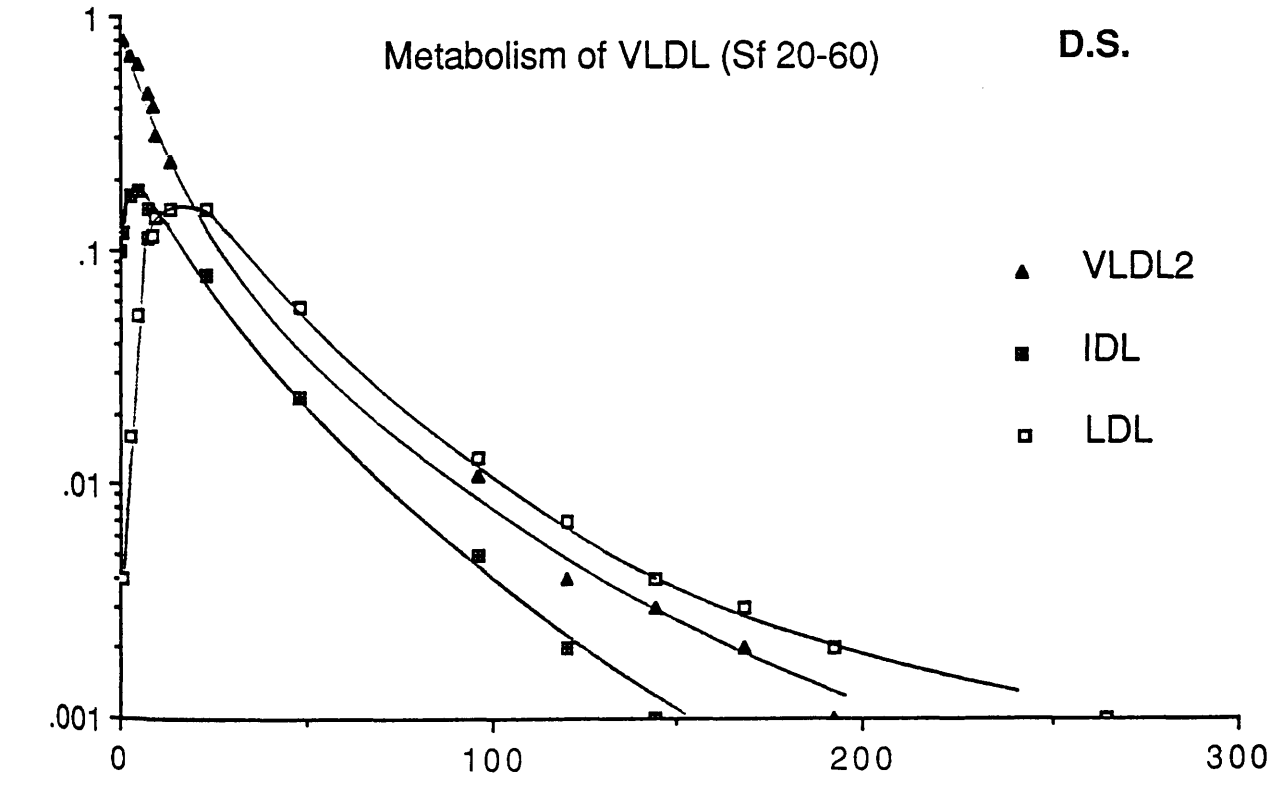
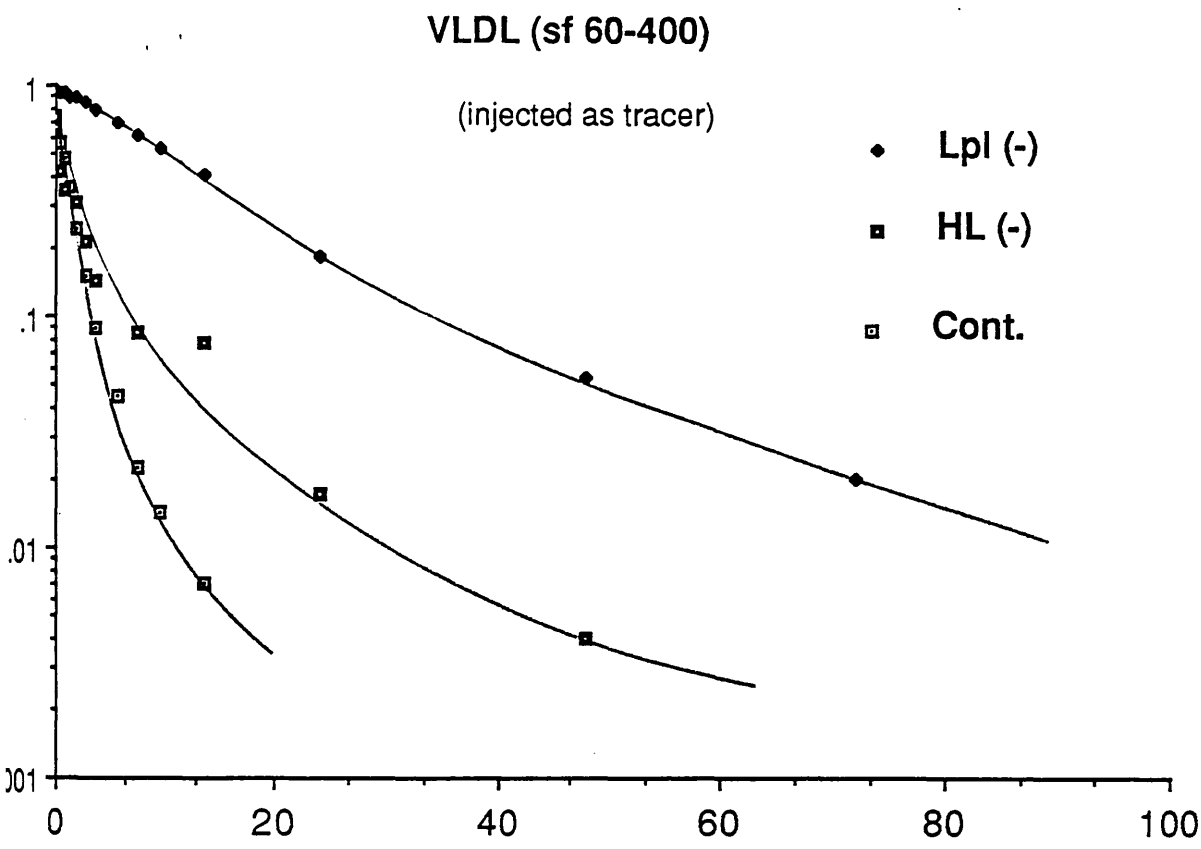


Fig. 28: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in a patient (D.S.) with familial hyperchylomicronaemia.



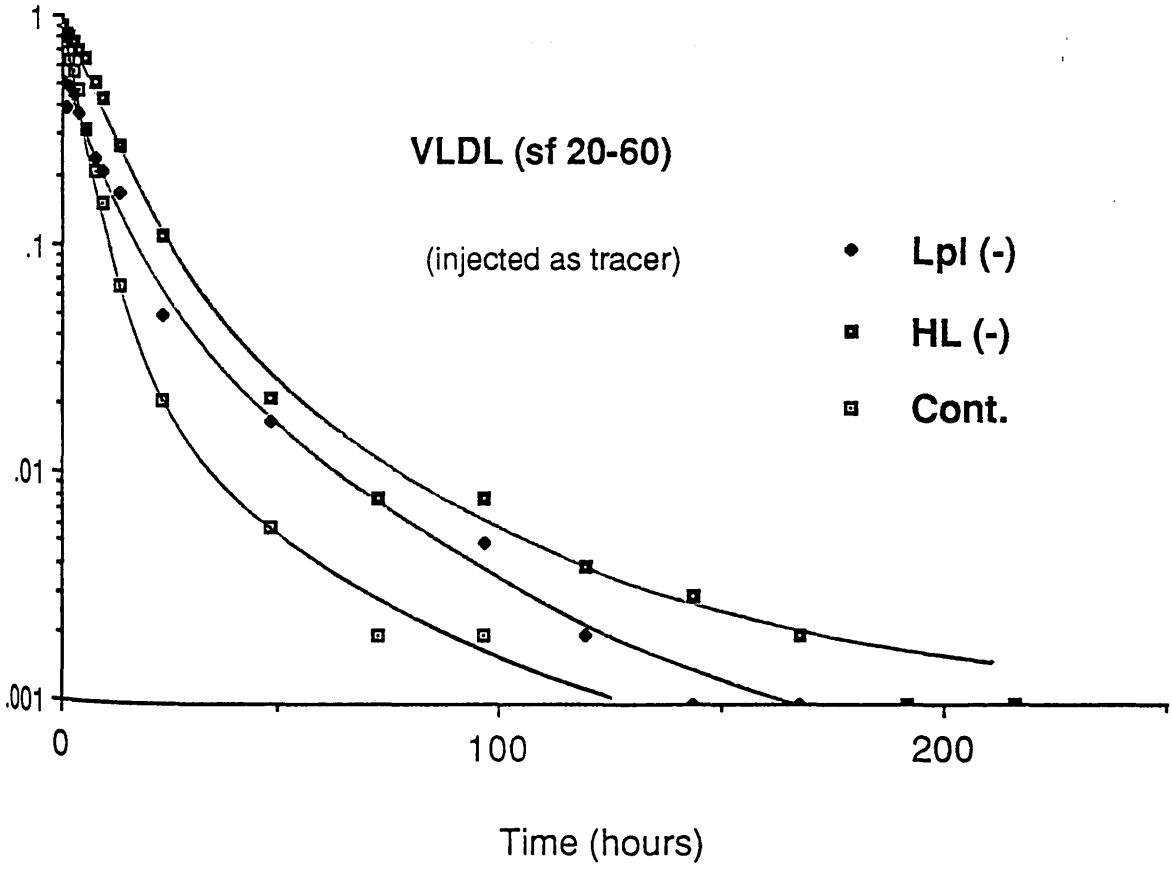


Fig. 29 and 30:

Metabolism of $VLDL_1$ and $VLDL_2$ in a patient with lipoprotein lipase deficiency (D.A.), a patient with hepatic lipase deficiency (G.P.) and in normolipidaemic controls (n = 5).

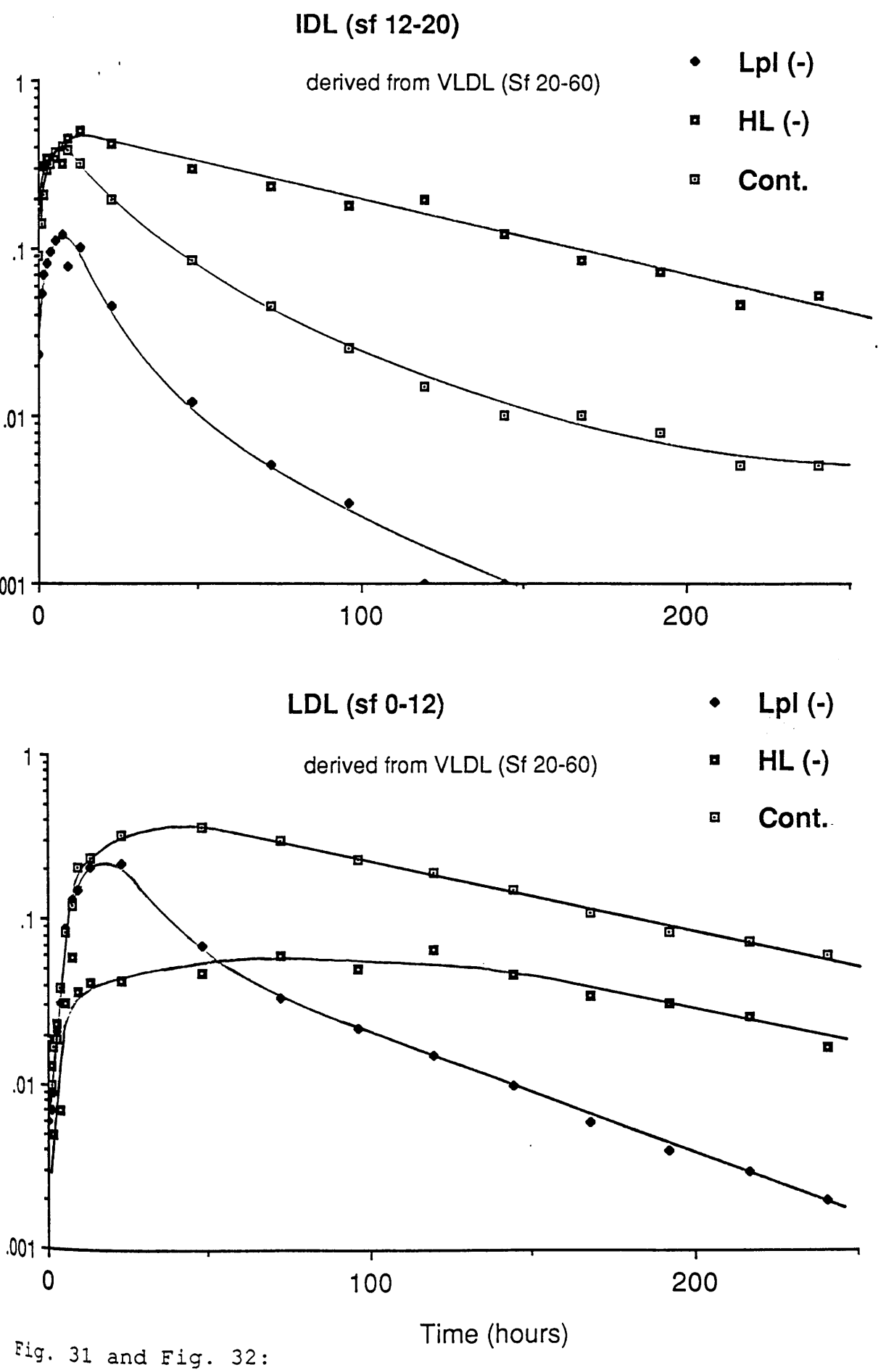


Fig. 31 and Fig. 32:

Metabolism of IDL and LDL derived from $VLDL_2$ in a patient with lipoprotein lipase deficiency (D.A.), a patient with hepatic lipase deficiency (G.P.) and in normolipidaemic controls (n = 5).

lipoprotein accumulates than in normal controls, and the slope of the decay curve is steeper, indicating an increased IDL catabolism. The curve for LDL (derived from $VLDL_2$) peaks at about 20% and falls off rapidly also showing an accelerated catabolism of this lipoprotein species.

3.4.3. Computer modelling of apolipoprotein B metabolism in familial hyperchylomicronaemia

When the data from the two metabolic studies of apolipoprotein B metabolism in familial hyperchylomicronaemia were analysed using the SAAM 29 program, it became apparent that the underlying model, as shown in Fig. 8, was not adequate for a situation with a massively increased $VLDL_1$ pool size. Less than half of the measured $VLDL_1$ pool could be accounted for by computer calculation. A tendency for underestimating the $VLDL_1$ pool size had been observed even with computer modelling of turnover data from normolipidaemics but this was now greatly exaggerated and therefore required a modification of the metabolic model. This, however, could not be accomplished easily within the capacity restraints of SAAM 29, as all available subcompartments were employed in the current metabolic model already. The solution was to create a model for metabolism of $VLDL_1$ and $VLDL_2$ and to link this at a second stage with the calculations based on the previous model,

which showed an acceptable fit for IDL and LDL decay curves and pool sizes. The model used for calculations of $VLDL_1$ and $VLDL_2$ metabolism is illustrated in Fig. 33. The new feature in this model are three subcompartments accounting for $VLDL_1$, two in serial alignment and one for "remnant" particles, which do not rejoin the delipidation cascade. With this model calculated masses derived from the kinetic analyses were within 20% of the measured values.

It has to be emphasised, that kinetic rate constants and pool sizes as determined by these calculations were not optimised on the basis of an integral metabolic model. In this sense calculated metabolic parameters for these turnover studies remain preliminary. Fractional standard deviations (FSD) for calculated rate constants were considerably higher than those in previously reported analyses. A final interpretation requires a more powerful computer program, capable of coping with more subcompartments at a time than SAAM 29. An advanced version of the current program, SAAM 30, will be available in the near future. The metabolic data as displayed in Figs. 27 and 28 will be re-analysed on the basis of an improved metabolic model with three $VLDL_1$ subcompartments.

The parameters of apolipoprotein B metabolism as defined by the currently available kinetic rate constants, are summarised in Table 16. The $VLDL_1$ pool size is more than ten-fold increased in both patients (D.A. and D.S.). The transfer rate of $VLDL_1$ to $VLDL_2$ is reduced to about 5%

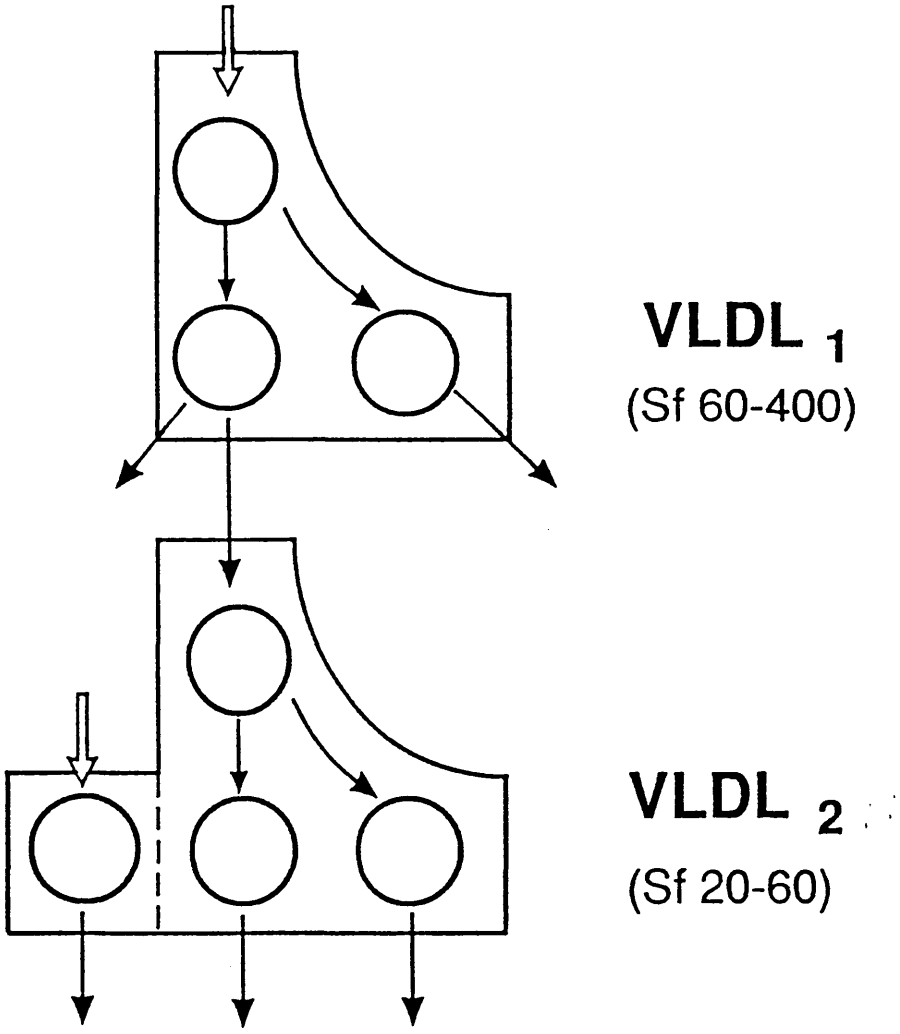


Fig. 33: Kinetic model for VLDL metabolism in familial hyperchylomicronaemia.

TABLE 16:

Apolipoprotein B metabolism in lipoprotein lipase deficiency Lpl(-) and in hepatic lipase deficiency HL(-)

VLDL (Sf 60-400)		direct synth. [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to VLDL2 [pools/d]
DA *	Lpl(-)	1005	787	1.05	0.23
DS *	Lpl(-)	960	784	0.74	0.48
Contr.,	mean	560	66	5.5	7.6
n=5	± s	±280	±44	±6.4	±4.7
GP	HL(-)	91	9	1.8	8.3

VLDL (Sf 20-60)		direct synth. [mg/d]	flux from VLDL1 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to IDL&LD [pools/d]
DA *	Lpl(-)	150	178	206	0.61	1.76
DS *	Lpl(-)	767	377	468	1.5	0.95
Contr.,	mean	278	331	191	0.49	2.8
n=5	± s	±91	±145	±91	±0.13	±1.2
GP	HL(-)	480	74	265	1.08	0.97

IDL (Sf 12-20)		direct synth. [mg/d]	flux from VLDL2 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to LDL [pools/d]
DA	Lpl(-)	0	207	78	0.78	1.87
DS	Lpl(-)	0	439	138	0.66	2.51
Contr.,	mean	0	441	277	0.52	1.3
n=5	± s		±204	±134	±0.34	±0.9
GP	HL(-)	0	262	886	0.25	0.05

* Preliminary data, see paragraph 3.4.3.

LDL (Sf 0-12)		direct synth. [mg/d]	flux from IDL&VLDL2 [mg/d]	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
DA	Lpl(-)	0	301	312	0.96	1155
DS	Lpl(-)	0	346	289	1.21	1727
Contr.,	mean	18	380	1571	0.31	857
n=5	± s	±36	±102	±788	±0.11	±349
GP	HL(-)	0	43	215	0.21	571

of the average value observed in normals. Rates for total $VLDL_2$ catabolism are not very different from normal controls, although the rates for direct catabolism tend to be higher and the fractional transfer rates for $VLDL_2$ to IDL transfer tend to be lower.

The fractional catabolic rates for IDL are higher than in the control group and equal or greater than the transfer rate from $VLDL_2$ to IDL. Thus, IDL does not accumulate in plasma which is reflected by the low peak of the IDL decay curve and the small IDL plasma pool observed in patients. The fractional catabolic rate for LDL is three to four-fold higher than typical for normolipidaemics. Accordingly, the LDL pool is much lower than in controls, in line with results reported before (see Table 14.).

3.5. Apolipoprotein B Metabolism in Hepatic Lipase Deficiency

Apolipoprotein B metabolism was assessed in a Swedish patient with hepatic lipase deficiency by means of a VLDL-turnover study (340).

3.5.1. Clinical data characterising the patient

G.P., a 63 year old male, is one of two brothers, first described in 1974 as having a new dyslipoproteinaemia

called hyper-alpha-triglyceridaemia because of a pronounced increase of triglycerides in the HDL fraction (118). Later it was discovered that this lipoprotein abnormality was due to the absence of hepatic lipase activity in the plasma (118). Besides their disorder of lipid metabolism both patients are healthy and show in particular no signs of cardiovascular disease. Their parents died at an advanced age of unknown causes. The apoE phenotype of G.P. was determined as apoE4/3.

Lipid and lipoprotein concentrations from G.P. are given in Table 13. Mild hypertriglyceridaemia and a relatively high HDL level were the only deviations from normal. However, agarose electrophoresis revealed the presence of ß-VLDL in the lipoprotein subfraction of $d < 1.006$ g/ml (Fig. 34).The plasma apoB concentration was also normal whereas apoB distribution among lipoproteins was distinctly different not only from normal controls but also from the two patients with no plasma lipoprotein lipase activity (Table 14). More than 60% of plasma apoB accumulated in the IDL density range. $VLDL_2$-apoB concentration was doubled and LDL-apoB concentration was only one-fifth of the usual value. Rate zonal ultracentrifugation of plasma from G.P. showed a continuous profile of the lipoproteins in the density range of 1.006-1.063 g/ml. The peak of the main apoB-lipoprotein was clearly shifted to the left towards lighter densities as

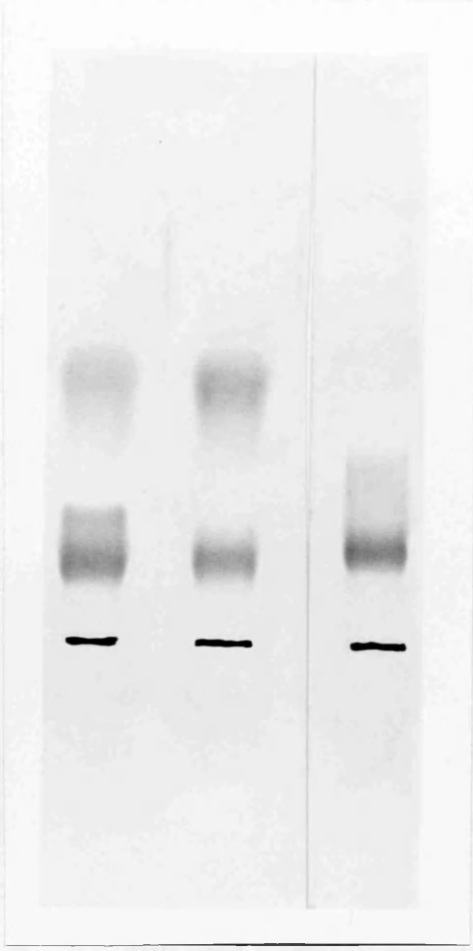


Fig. 34: Lipoprotein agarose gel electrophoresis with plasma (1) and VLDL (2) from a patient with hepatic lipase deficiency; n = normal plasma.

evidenced by comparison with a radioactively labelled LDL-marker prepared from normal plasma (Fig. 35).

Compositional data for apoB containing lipoproteins from three measurements are presented in Table 15. Large and small VLDL had normal triglyceride contents but were reduced in cholesteryl esters and enriched in free cholesterol. Triglycerides were markedly increased in IDL and LDL, at the expense of cholesteryl esters. Phospholipid and protein composition were normal.

3.5.2. VLDL-turnover study in a patient with hepatic lipase deficiency

The results of a VLDL-turnover study are illustrated in Fig. 36 and in Figs. 29-32, where decay curves for individual lipoproteins are compared with those from normal controls and from a subject with lipoprotein lipase deficiency.

In hepatic lipase deficiency $VLDL_1$ catabolism is slightly slower than in normal controls but far less affected than in lipoprotein lipase deficiency. Fourteen hours after injection of the $VLDL_1$ tracer, 0.8% of the initial dosage remained in the plasma as $VLDL_1$-apoB in normals compared to 4.0% in hepatic lipase deficiency and 40% in lipoprotein lipase deficiency.

$VLDL_2$ catabolism was delayed in hepatic lipase deficiency and in lipoprotein lipase deficiency to roughly

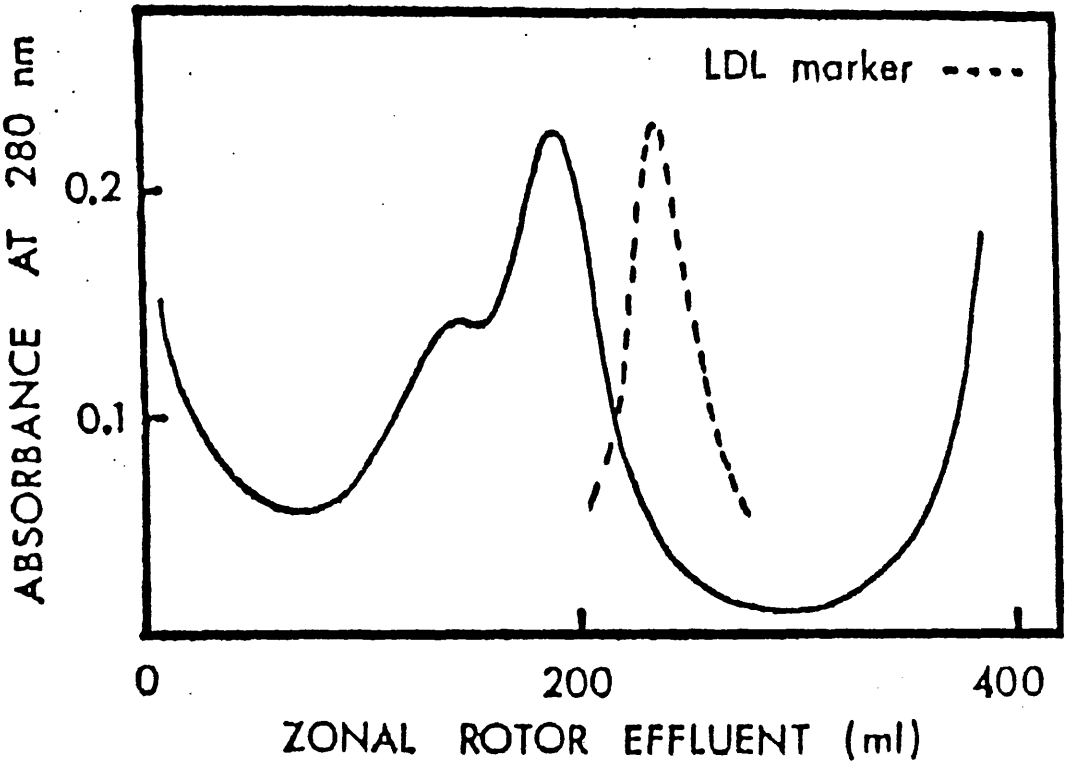


Fig. 35: Rate zonal ultracentrifugation profile of apoB containing lipoproteins in a patient with hepatic lipase deficiency. - The radioactive marker indicates the flotation characteristics of LDL prepared from a normal subject.

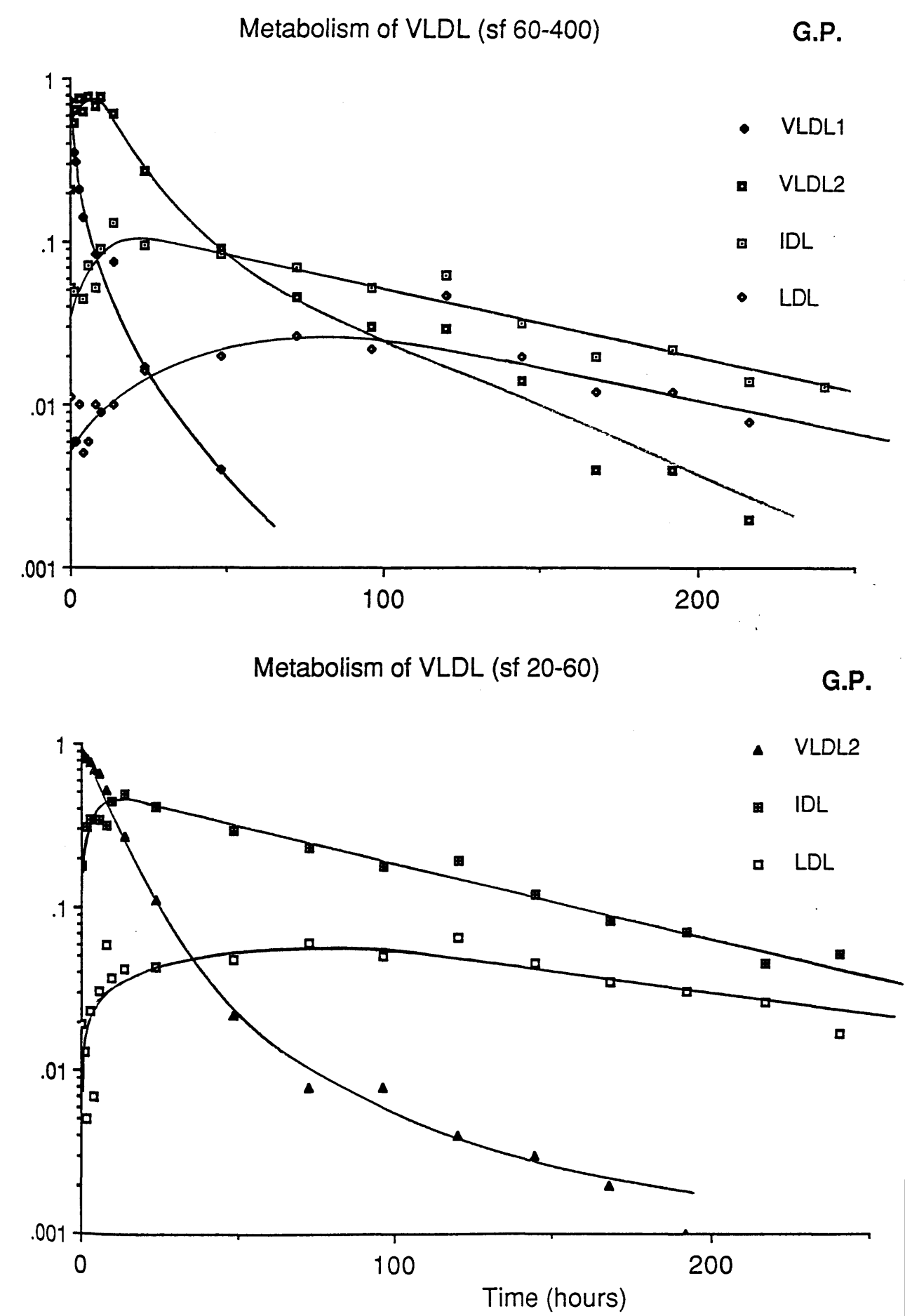


Fig. 36: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in a patient (G.P.) with hepatic lipase deficiency.

the same extent. After 48 h about 2% of the initial dosage was recovered as $VLDL_2$ in both lipase deficient conditions in contrast to an average of 0.6% in the control group.

IDL clearance was markedly reduced in hepatic lipase deficiency. In fact, the IDL curve had the shape of the curve for LDL usually seen in normolipidaemics. The LDL curve in hepatic lipase deficiency, in contrast, was peculiar in that it plateaued for about 3 days (60-130 h after injection) at a level of about 6% of the initially injected dosage. This indicated a very low throughput of IDL to LDL. The final slope of the LDL curve was about the same as observed with normal subjects.

3.5.3. Computer analysis of apolipoprotein B metabolism in hepatic lipase deficiency

The observed turnover data shown in Fig. 29 were analysed on the basis of the metabolic model as explained in paragraph 2.5.8. Calculated kinetic rate constants and masses for subcompartments are listed in Table 17. These are compared with average values from a group of normolipidaemics which are characterised by the data presented in Table I-22 and Table I-23. Kinetic rate constants observed in the hepatic lipase deficient subject, which were outside the range of normal values, are highlighted in Fig. 37. The figures from Table 17 are the

TABLE 17: Calculated masses and rate constants in hepatic lipase deficiency

Name	M(1)	k(0,1)	k(2,1)	M(2)	k(3,2)	k(6,2)
GP	9	1.8	8.3	8.5	3.4	5.3
Contr., n=5						
mean	66	5.5	7.6	33	10.1	0.7
± s	±44	±6.4	±4.7	±17	±4.0	±0.4

Name	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
GP	12	0.16	0.2	0.58	0	180
Contr., n=5						
mean	52	3.9	5.9	3.4	0.7	55
± s	±32	±6.0	±4.6	±5.3	±0.8	±24

Name	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	k(9,6)
GP	1.25	1.41	0	56	0.81	0
Contr., n=5						
mean	0.3	4.8	0.5	24	1.2	0
± s	±.7	±2.2	±1.0	±23	±0.4	

Name	M(7)	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)
GP	857	0.24	0.05	42	0.27	0.32
Contr., n=5						
mean	104	0.5	2.2	113	0.9	1.41
± s	±63	±0.7	±1.3	±90	±0.53	±1.09

Name	M(9)	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
GP	25	0.28	209	0.2	6.4	0.21
Contr., n=5						
mean	61	0.8	865	0.34	693	0.25
± s	±58	±0.4	±463	±0.14	±407	±0.11

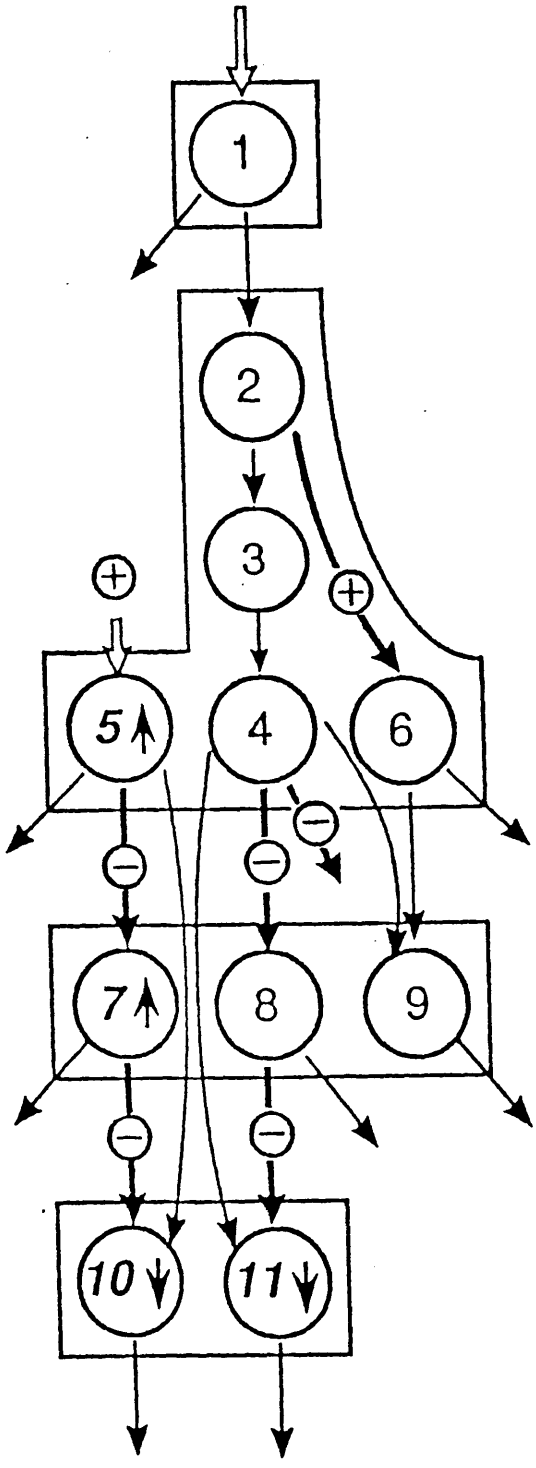


Fig. 37: Kinetic model of apoB metabolism in hepatic lipase deficiency. - Significant differences in comparison to normal controls are indicated (⊕, ⊖, ↑, ↓)

basis for a quantitative description of the apolipoprotein B metabolism in this patient, as given in Table 16. Fractional catabolic rates for $VLDL_1$ were within the normal range but the $VLDL_1$ pool was low due to a low rate of synthesis. The synthetic rate for $VLDL_2$, in contrast was increased as was, to a lesser extent, the $VLDL_2$ plasma pool. The transfer rate towards IDL was reduced by two-thirds but at the same time direct catabolism of $VLDL_2$ was doubled.

In spite of the reduced input from $VLDL_2$, the IDL plasma pool was markedly increased due to a substantial reduction in the fractional transfer rate of IDL to LDL, which was less than 5% of the normal value. Direct catabolism of IDL was not increased. As input from IDL was very low, the LDL pool was less than one-sixth of the average LDL pool observed in normolipidaemics. LDL was degraded at a low normal fractional catabolic rate.

3.6. Apolipoprotein B Metabolism in Homozygous Familial Hypercholesterolaemia

The metabolism of apolipoprotein B was investigated in two patients with homozygous familial hypercholesterolaemia. This was part of a larger study where the apoB metabolism of seven homozygous FH patients was analysed and compared to normolipidaemic controls (301).

3.6.1. Characterisation of patients

Data describing the two patients analysed here are presented in Table 18. J.C. is a 44 year old Englishman with widespread tendon xanthomas and severe left carotid artery stenosis. He is refractory to all lipid-lowering drug therapy including sequestrant resins. His three children are hypercholesterolaemic. Fibroblasts and lymphocyte assays failed to show any receptor activity (301). M.M., a 21 year old Ugandan of Indian extraction, was found to be severely hypercholesterolaemic in childhood. He exhibited widespread tendon xanthomas and had coronary artery bypass surgery for occlusive disease at the age of 15 years. His lymphocytes expressed less than 10% of normal receptor activity (301). The apoE phenotype is apoE4/3. Lipid and lipoprotein levels in these patients were typical for their disease with massive increases of total cholesterol and LDL-cholesterol. ApoB plasma concentrations were increased to the same extent (Table 19). The distribution of apoB among plasma lipoproteins, however, was similar to the distribution seen in normolipidaemics. In both cases LDL was by far the dominating apoB-lipoprotein species, accounting for about three-quarters of plasma apoB.

Compositional analyses of apoB containing lipoproteins are summarised in Table 20. The main differences occurred with $VLDL_2$ and IDL, which were both cholesteryl

Table 18: Homozygous Familial Hypercholesterolaemia. - Physical Parameters, Plasma Lipid and Lipoprotein Concentrations in Two Patients and in Controls.

Homozygous FH:	Sex	Age (years)	Body Weight (kg)	Body Weight Index	Total Trig (mmol/l)	Total Chol	VLDL-Chol	LDL-* Chol (mmol/l)	HDL-Chol
J.C.	M	44	70	24.2	1.9 ± 0.3	16.8 ± 1.1	0.9 ± 0.5	15.4 ± 1.6	0.8 ± 0.2
M.M.	M	21	50	20.8	2.9 ± 0.3	14.1 ± 1.3	1.5 ± 0.5	12.0 ± 1.3	0.7 ± 0.1
Controls (n = 5)	3 M 2 F	39 ±11	73 ±12	24.2 ± 2.5	1.4 ± 0.6	5.0 ± 1.1	0.7 ± 0.3	3.0 ± 1.1	1.3 ± 0.3

* "LDL-Chol" as determined by the LRC-protocol (320) includes LDL (S_f 0-20) and most of IDL (S_f 12-20)

Table 19: Homozygous Familial Hypercholesterolaemia. - Relative Distribution of Apolipoprotein B Among Plasma Lipoproteins.

	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma Pool (mg) (= 100%)	ApoB Plasma Concentration (mg/l)
	(Percentage Distribution)					
Homozygous FH:						
J.C.	0.6	2.4	13.6	83.4	10909	3.80
M.M.	1.7	9.2	12.9	76.2	6120	3.06
Controls (n = 5)	2.7 ±1.1	9.2 ±1.5	13.9 ± 4.4	74.3 ± 4.9	2105 ±1031	0.71 ±0.24

Table 20: Homozygous Familial Hypercholesterolaemia. - Compositions of Apolipoprotein B-Containing Lipoproteins in Patients and in Controls.

		Free Cholesterol	Cholesteryl Ester	Triglyceride	Phospholipids	Protein	ApoB
				(g/100 g)			(% of total protein)
$VLDL_1$	hm FH	4.3 ± 1.3	17.1 ± 1.4	53.3 ± 1.2	16.0 ± 0.1	8.8 ± 0.0	39 ± 8
	(Cont.)	1.7 ± 2.3	16.0 ± 4.3	56.2 ± 4.8	17.0 ± 1.4	9.2 ± 2.4	37 ± 2
$VLDL_2$	hm FH	11.1 ± 3.0	33.2 ± 0.4	20.5 ± 3.0	20.8 ± 0.2	14.6 ± 0.1	68 ± 4
	(Cont.)	8.1 ± 1.4	21.1 ± 5.9	35.1 ± 4.0	21.4 ± 2.4	14.4 ± 1.6	66 ± 5
IDL	hm FH	12.7 ± 1.1	38.4 ± 2.6	5.9 ± 0.9	22.4 ± 1.3	20.7 ± 0.8	78 ± 6
	(Cont.)	11.2 ± 2.3	33.4 ± 4.8	12.4 ± 2.0	23.9 ± 1.3	19.1 ± 2.3	91 ± 4
LDL	hm FH	7.7 ± 1.9	39.8 ± 2.4	4.3 ± 1.1	20.8 ± 0.9	27.5 ± 0.3	95 ± 1
	(Cont.)	13.5 ± 1.5	34.8 ± 2.2	5.2 ± 0.2	23.0 ± 1.6	23.6 ± 1.6	96 ± 2

hm FH: Homozygous Familial Hypercholesterolaemia, n = 2. - Cont: Normolipidaemic controls, n = 5 (Table I-23).

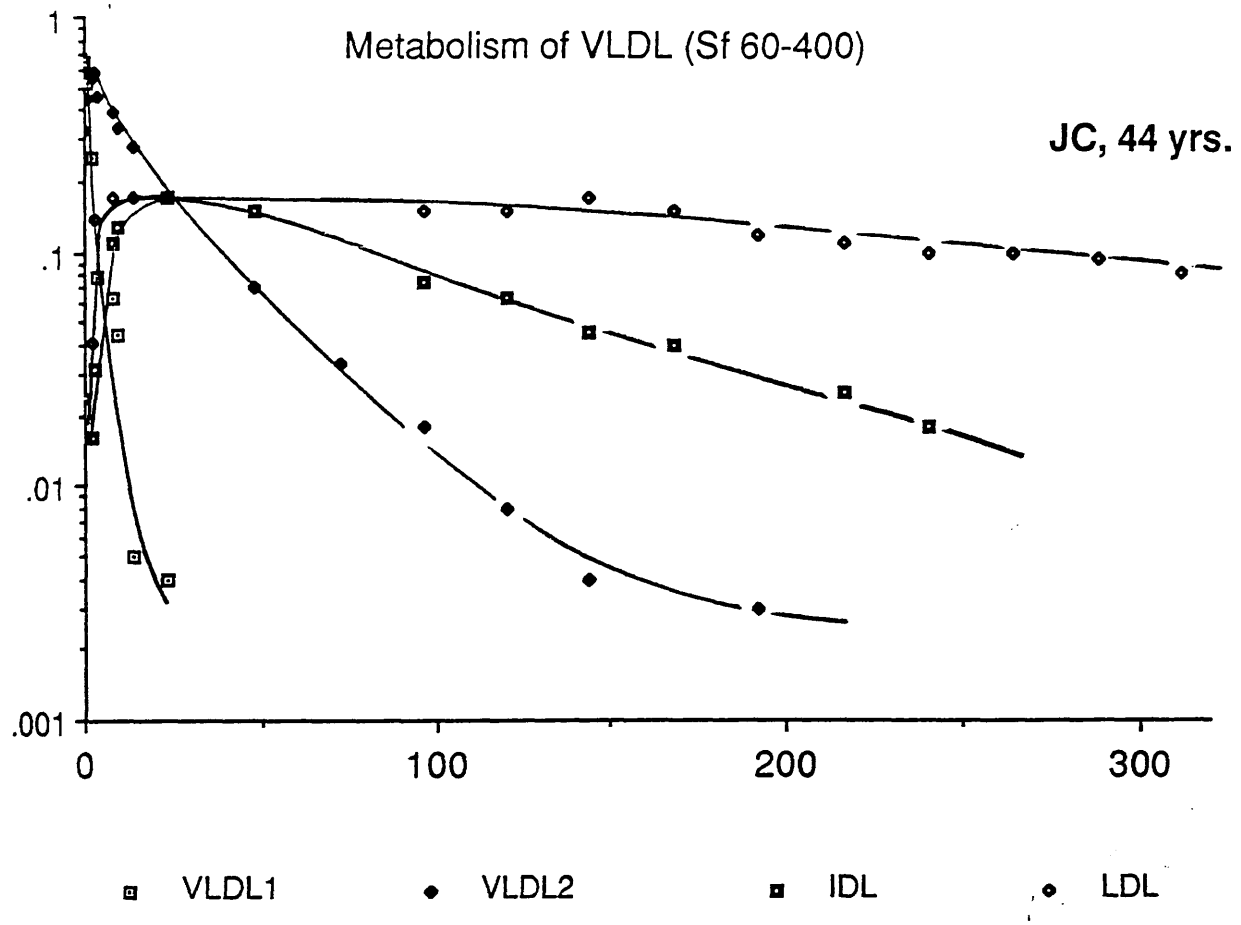
ester-enriched and triglyceride-depleted. The free cholesterol content of LDL was reduced, which was interpreted as an effect of the prolonged exposure to lecithin:cholesterol acyl transfer (LCAT) due to the long plasma residence time of these particles.

3.6.2. VLDL-turnover studies in homozygous familial hypercholesterolaemia

Lipoprotein decay curves produced by VLDL-turnover studies are shown in Figs. 38-39 and together with curves from normal controls in Figs. 40-43.

$VLDL_1$ catabolism was only slightly slower in FH patients compared to controls. In contrast, $VLDL_2$ clearance differed markedly from normals. In patients 1% and 4% respectively, of the initial dosage was isolated from $VLDL_2$ after 72 h as compared to 0.2% in controls.

The IDL curve reached a peak later in patients than in controls and the slope of the curve was less steep, indicating a delayed $VLDL_2$ to IDL transfer and a decreased IDL catabolism. Both patients showed a very shallow LDL decay curve in agreement with the slow clearance rate for this lipoprotein in FH. The rising section of the LDL curve and thereby the time required to reach the maximum of the LDL curve differed considerably between the two patients.



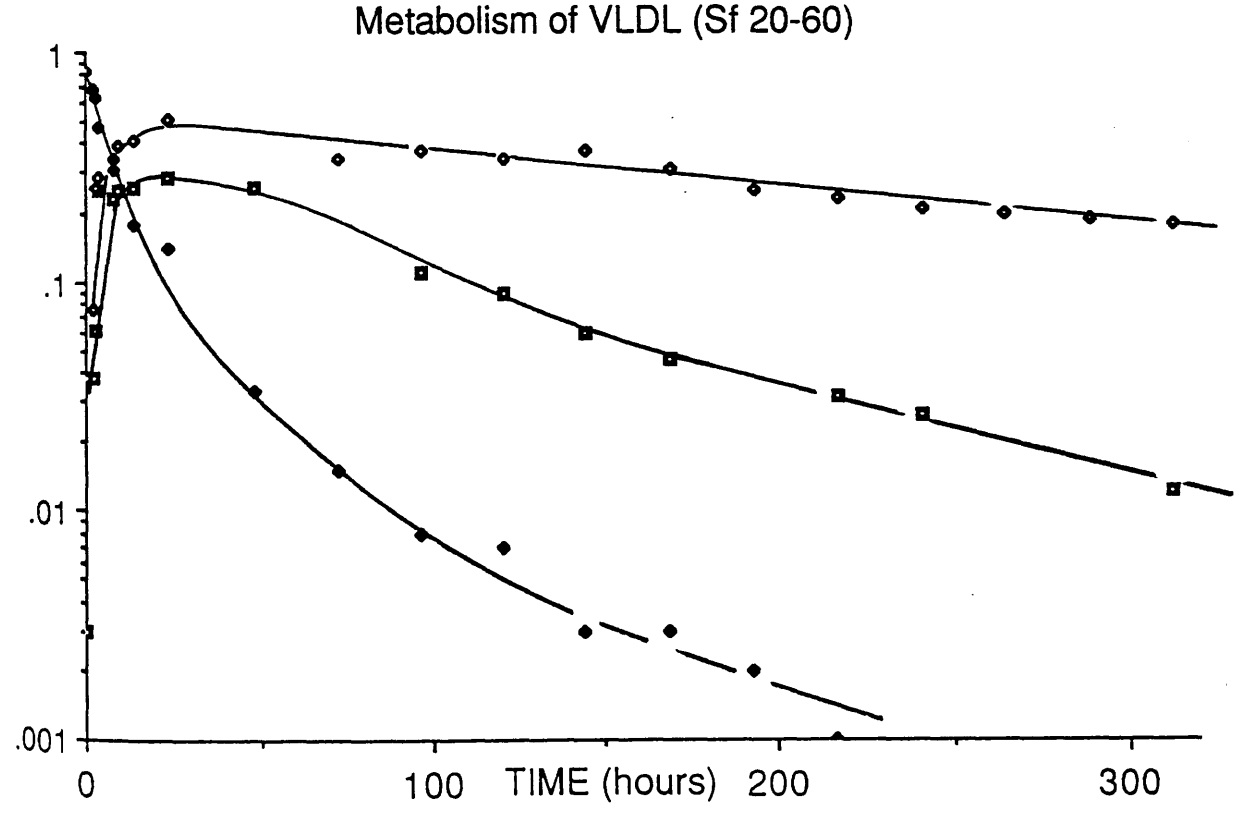


Fig. 38: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in a patient (J.C.) with homozygous familial hypercholesterolaemia.

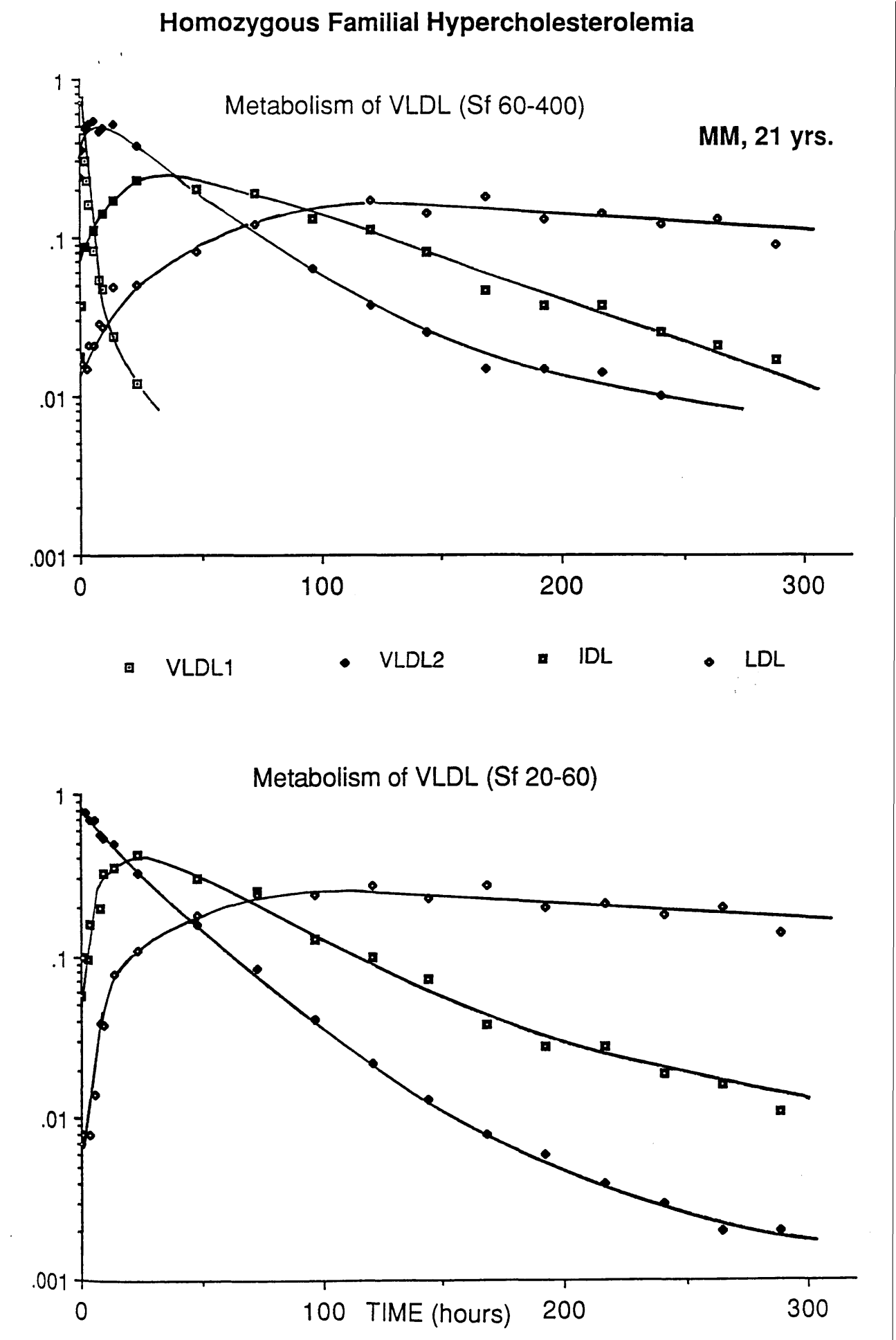
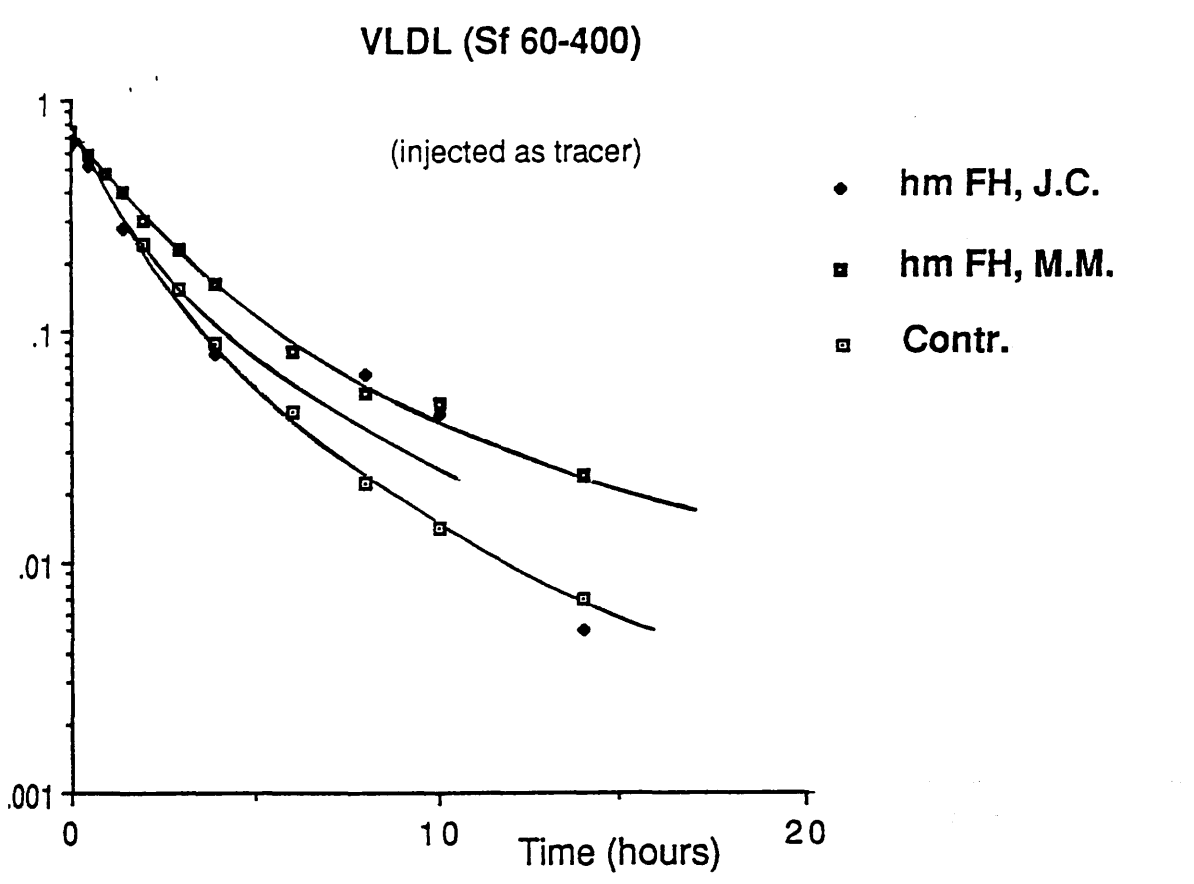


Fig. 39: Lipoprotein decay curves from $VLDL_1$ and $VLDL_2$ turnover studies in a patient (M.M.) with homozygous familial hypercholesterolaemia.



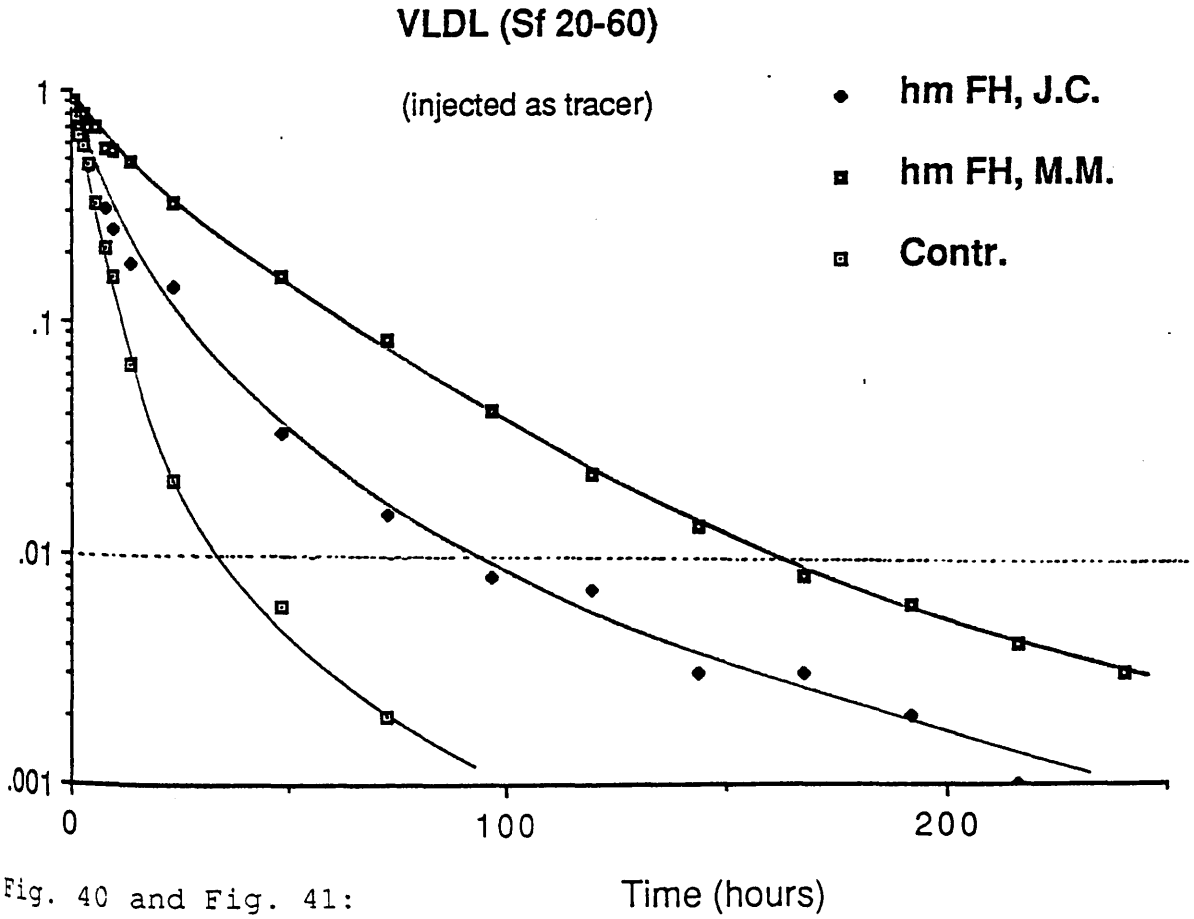
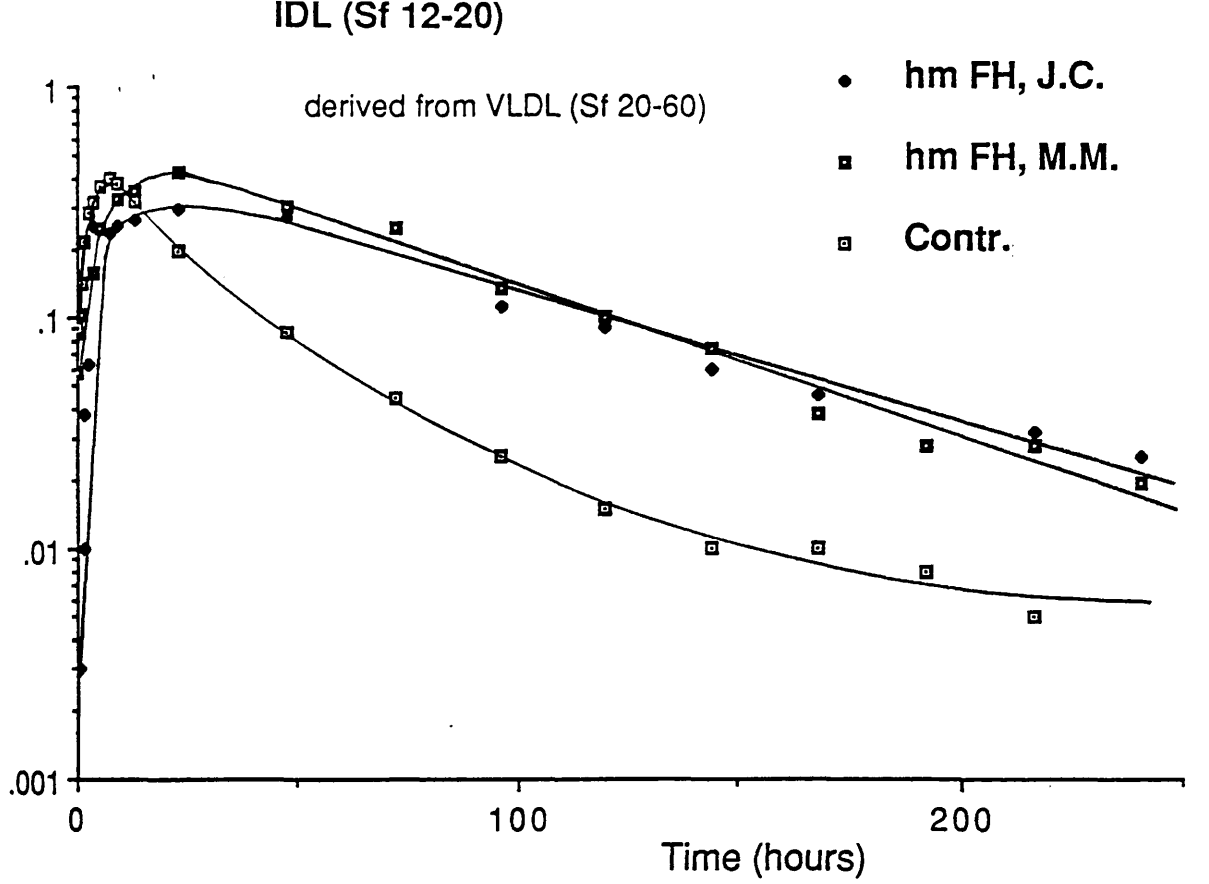


Fig. 40 and Fig. 41:

Metabolism of $VLDL_1$ and $VLDL_2$ in two patients (J.C.) and M.M.) with homozygous familial hypercholesterolaemia and in normolipidaemic controls (n = 5).



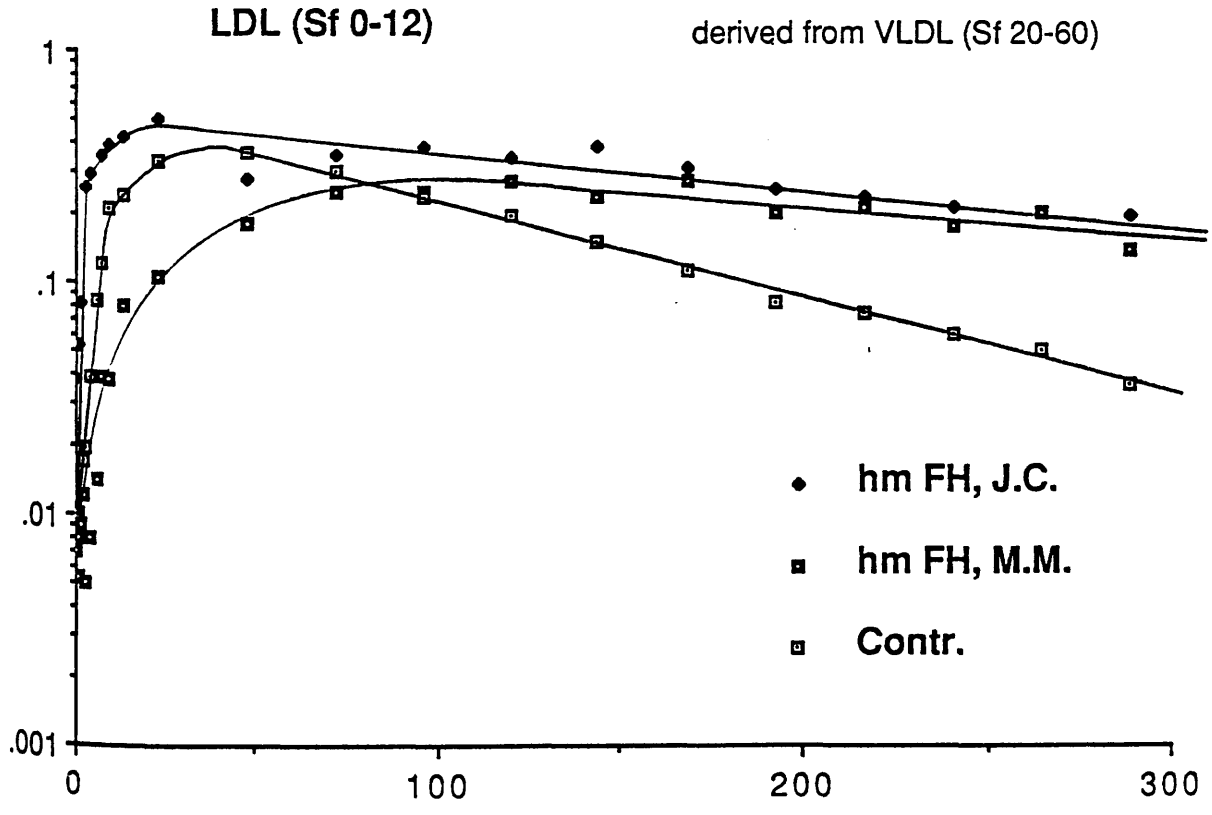


Fig. 42 and Fig. 43:

Metabolism of IDL and LDL in two patients (J.C. and M.M.) with homozygous familial hypercholesterolaemia and in normolipidaemic controls (n = 5).

3.6.3. Computer analysis of apolipoprotein B metabolism in homozygous familial hypercholesterolaemia

Kinetic rate constants and subcompartment masses were calculated as in paragraph 2.5.8. (Table 21). Values from the two patients are compared with normal averages calculated from individual data in Table I-23. Rate constants and masses outside the normal range are set in bold in Fig. 44. A quantitative description of apolipoprotein B metabolism in the two FH homozygotes is given in Table 22.

Pool size, direct synthesis and fractional catabolic rates for $VLDL_1$ were similar to values observed in the control group. The pool size for $VLDL_2$ was higher than normal because of a significant increase of the mass in subcompartment M(6) accounting for VLDL-remnant particles. The fractional transfer rate of $VLDL_2$ to IDL was reduced by 75%, whereas direct synthesis of $VLDL_2$, influx from $VLDL_1$ and direct $VLDL_2$ catabolism were normal.

The IDL pool was increased at least three-fold due to a significant reduction in both fractional rate of direct catabolism and transfer rate to LDL. ApoB flux from IDL to LDL, calculated as the product of increased IDL pool and decreased transfer rate, remained normal. The LDL pool size was three to six times above normal as the fractional catabolic rate for LDL was reduced to less than one-third of the normal value.

TABLE 21: Calculated masses and rate constants in homozygous familial hypercholesterolaemia

Name	M(1)	k(0,1)	k(2,1)	M(2)	k(3,2)	k(6,2)
JC	59	3.6	7.7	21	14.4	7.7
MM	72	4.4	4.1	45	2.4	4.2
Contr., n=5						
mean	66	5.5	7.6	33	10.1	0.7
± s	±44	±6.4	±4.7	±17	±4.0	±0.4

Name	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
JC	35	0	4.3	0	4.3	71
MM	81	0.1	1.2	0	0	59
Contr., n=5						
mean	52	3.9	5.9	3.4	0.7	55
± s	±32	±6.0	±4.6	±5.3	±0.8	±24

Name	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	k(9,6)
JC	0.1	1.3	1.7	166	0.96	0
MM	0	3.6	0	302	0.5	0.12
Contr., n=5						
mean	0.3	4.8	0.5	24	1.2	0
± s	±0.7	±2.2	±1.0	±23	±0.4	

Name	M(7)	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)
JC	770	0	0.24	618	0.24	0
MM	513	0.05	0.37	184	0.17	0.36
Contr., n=5						
mean	104	0.5	2.2	113	0.9	1.41
± s	±63	±0.7	±1.3	±90	±0.53	±1.09

Name	M(9)	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
JC	0	0	8586	0.11	1600	0.072
MM	144	0.25	3081	0.084	1563	0.084
Contr., n=5						
mean	61	0.8	865	0.34	693	0.25
± s	±58	±0.4	±463	±0.14	±407	±0.11

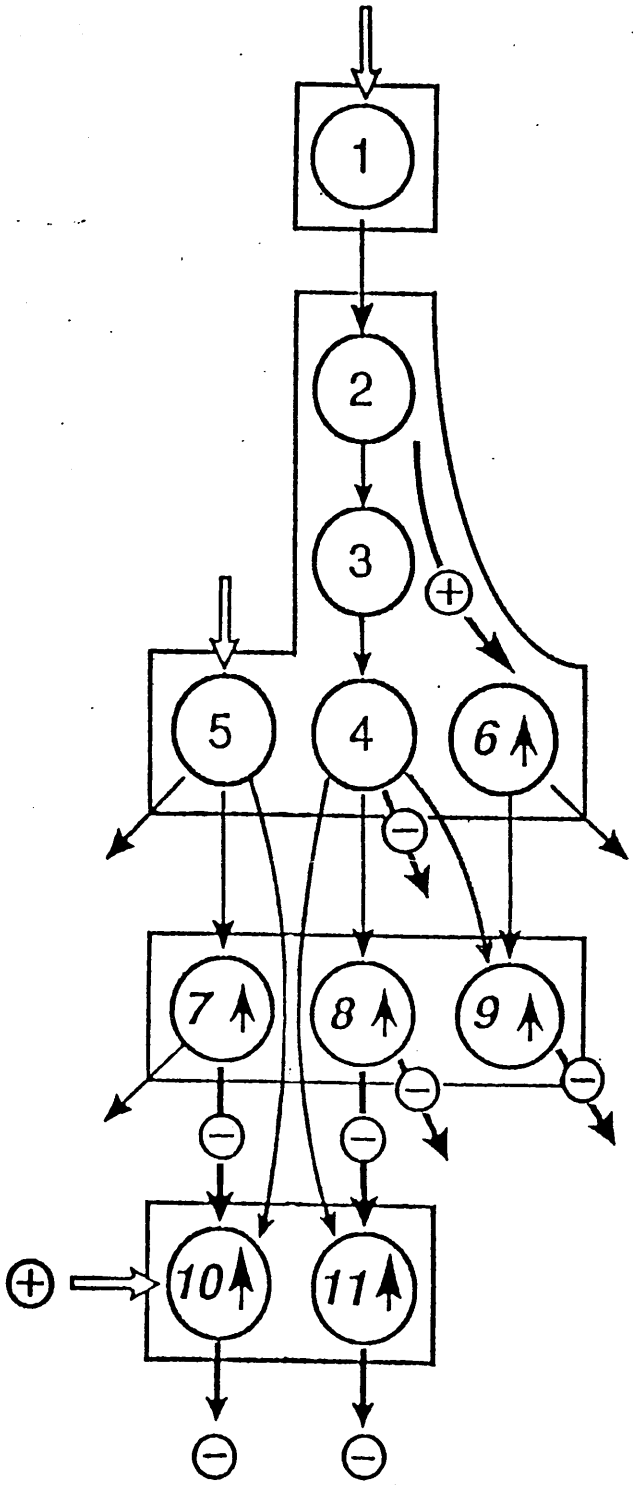


Fig. 44: Kinetic model of apoB metabolism in homozygous familial hypercholesterolaemia. Significant differences in comparison to normal controls are indicated (⊕, ⊖, ↑, ↓).

TABLE 22: Apolipoprotein B metabolism in homozygous familial hypercholesterolaemia

VLDL (Sf 60-400)		direct synth. [mg/d]	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to VLDL2
JC		674	59	3.6	7.7
MM		614	72	4.4	4.1
Contr., n=5	mean ± s	560 ±280	66 ±44	5.5 ±6.4	7.6 ±4.7

VLDL (Sf 20-60)		direct synth. [mg/d]	flux from VLDL1	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to IDL&LD
JC		217	462	310	0.53	0.78
MM		214	297	536	0.3	0.65
Contr., n=5	mean ± s	278 ±91	331 ±145	191 ±91	0.49 ±0.13	2.8 ±1.2

IDL (Sf 12-20)		direct synth. [mg/d]	flux from VLDL2	plasma pool [mg]	fract. rate of direct catab. [pools/d]	transfer to LDL
JC		94	241	1388	0.11	0.24
MM		0	349	842	0.11	0.3
Contr., n=5	mean ± s	0 0	441 ±204	277 ±134	0.52 ±0.34	1.3 ±0.9

LDL (Sf 0-12)		direct synth. [mg/d]	flux from IDL&VLDL2	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
JC		396	453	10186	0.083	962
MM		134	256	4644	0.084	1361
Contr., n=5	mean ± s	18 ±36	380 ±102	1571 ±788	0.31 ±0.11	857 ±349

In both patients total apoB synthesis was slightly higher than on average in controls. Total LDL-apoB synthesis and influx from IDL and VLDL was within the normal range in M.M., but markedly increased in J.C. Direct apoB synthesis in the range of LDL accounted in M.M. for 10% of the total apoB synthesis which was higher than the normal average. In J.C. direct apoB synthesis occurred not only in the range of LDL but also in IDL and in this subject 50% of the total apoB synthesis came from these two sources.

3.7. Genetic Apolipoprotein B Polymorphisms and Lipoprotein Metabolism

3.7.1. Apolipoprotein B gene polymorphisms and plasma cholesterol levels

ApoB genetic polymorphisms as defined by restriction enzymes XbaI, EcoRI and MspI were analysed in 19 moderately hypercholesterolaemic subjects (Fig. 45). Patients were 40-60 years of age. Hypercholesterolaemia was only diagnosed two months after dietary advice, designed to correct hyperlipidaemia had been given. Familial hypercholesterolaemia was ruled out by checking for tendon xanthomas, first-degree relatives with raised LDL-cholesterol or a strong family history of premature cardiovascular disease. Means for total cholesterol, LDL-cholesterol and apoB in

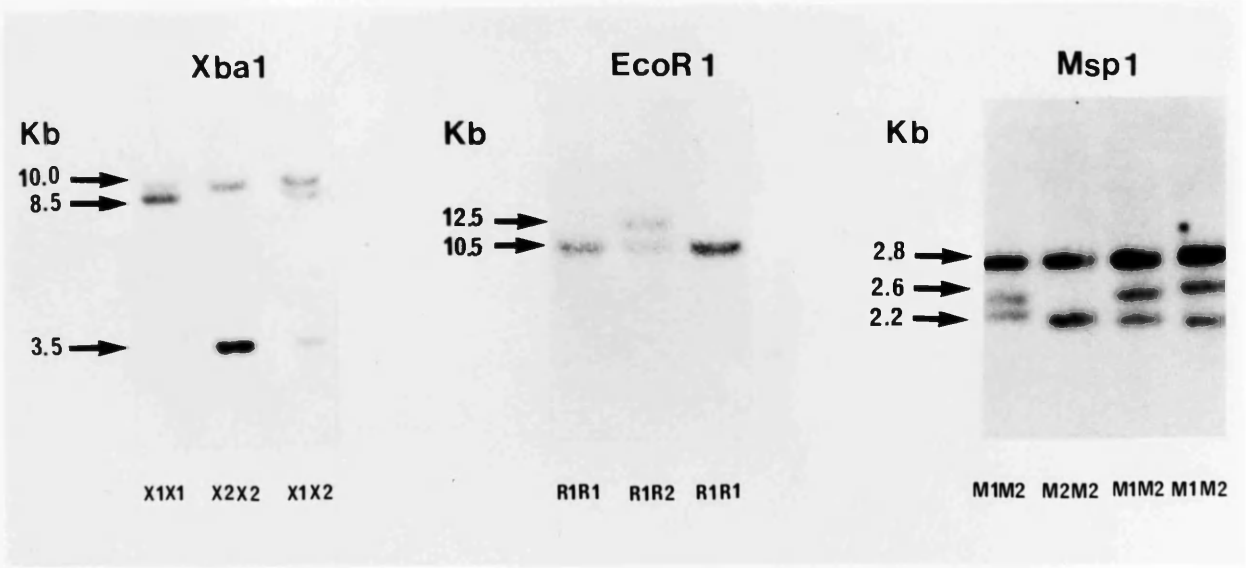


Fig. 45: Southern blot analysis for restriction site polymorphisms of the apoB gene.

plasma did not differ significantly for the three genotypes, X_1X_1, X_1X_2 and X_2X_2 as defined by XbaI (Table 23 and Table I-24). The same applied for the apoB polymorphisms detected by EcoRI and MspI. It should be noted that the MspI apoB polymorphism described here is due to a hypervariable satellite in the 3' flanking region of the gene (306). It was detected as described in paragraph 2.4.6 and is different from the MspI-RFLP in exon 26, mentioned earlier (236).

In a second study, the XbaI apoB polymorphism was determined for three groups of apoE3/3 homozygotes with low, intermediate and high total plasma cholesterol (Group I, II and III). ApoE3/3 homozygotes were selected in an attempt to eliminate the impact of another known genetic factor influencing plasma cholesterol levels. The allele frequency for X_1 (ie the allele without the XbaI cutting site) was higher for the group with a low cholesterol mean (Group I) as compared to the intermediate cholesterol group (Group II). Similarly, the allele frequency for X_2 (ie the allele with the cutting site) was higher for the high cholesterol group (Group III) (Table 24). If cholesterol values for each genotype from each of the three study groups were averaged, the mean for X_1X_1 was smaller than the mean for X_2X_2 with X_1X_2 falling in between. These findings fall short of statistical significance mainly because in group (I) there is no increase in X_1X_1 subjects corresponding to the higher number of X_2X_2

Table 23: Plasma Cholesterol and LDL Concentrations from Moderately Hypercholesterolaemic Patients with Different XbaI ApoB Genotypes

	X_1X_1	X_1X_2	X_2X_2
n	(5)	(8)	(6)
Total Cholesterol	7.76 ± 0.59	7.69 ± 0.72	7.73 ± 0.78
LDL Cholesterol	5.73 ± 0.47	5.41 ± 0.94	5.30 ± 0.78
ApoB	1.87 ± 0.19	1.68 ± 0.20	1.84 ± 0.34

Table 24: XbaI ApoB Polymorphism and Plasma Cholesterol Levels in ApoE3/3 Homozygotes

	I	II	III	Chol. mmol/l (mean ± SD)	n
Chol. mmol/l (mean + s)	4.2 ± 0.3	5.5 ± 0.2	6.9 ± 0.5		
n	27	25	26		
Gentoypes:					
X_1X_1	9	5	6	5.23 ± 1.10*	20
X_1X_2	10	12	7	5.44 ± 1.11	29
X_2X_2	8	8	13	5.83 ± 1.23*	29
Gene Frequencies:					
X_1	0.52$\mathcal{S}$	0.44	0.36$\mathcal{S}$		
X_2	0.48	0.56	0.63		

Groups I, II and III were age and sex matched (male/female ratio: 15/12, 14/11 and and 15/11; mean age: 35 ± 5, 38 ± 6 and 38 ± 6 years respectively). The three groups differed by their cholesterol mean as indicated.

Differences between cholesterol means (*) and gene frequencies ($\mathcal{S}$) were not significant as tested by unpaired t-test and X^2-test respectively.

subjects in group (III). However, the results indicate a trend which is in line with other studies (see paragraph 1.6.2.).

3.7.2. Apolipoprotein B gene polymorphisms and LDL catabolism

In the 19 moderately hypercholesterolaemic subjects, described in the previous paragraph, LDL catabolism was assessed by turnover studies. Native and chemically modified LDL tracers were injected and LDL-receptor dependent and LDL-receptor independent LDL catabolic rates were calculated. The total fractional catabolic rate for LDL differed between the genotype groups defined by the XbaI apoB RFLP as shown in Table 25. Individuals with the X_1X_1 genotype had on average 22% higher fractional catabolic rates for LDL compared with those with X_2X_2. Subjects with the genotype X_1X_2 had an intermediate mean FCR. When receptor dependent and receptor independent LDL-FCRs were calculated it became clear that the difference in overall catabolism was due to an increase in the fraction and amount of LDL degraded by the receptor route. Subjects of X_1X_1 genotype exhibited a 58% higher receptor mediated FCR than those with the genotype X_2X_2 and cleared 65% more LDL-apoB through this pathway. No such difference was observed in the fraction of LDL degraded by receptor

Table 25: LDL Kinetic Parameters and XbaI ApoB Genotype

	Fractional Catabolic Rate			Absolute Receptor Mediated Catabolic Rate	Synthetic Rate
	Total	Receptor Mediated	Receptor Indepentent		
	(pools/day)			(mg/kg per day)	
XbaI-RFLP					
X_1X_1 (n = 5)	0.271 ±0.020	0.082 ±0.013	0.189 ±0.028	6.06 ±1.25	19.99 ± 2.84
X_1X_2 (n = 8)	0.237 ±0.024	0.069 ±0.028	0.168 ±0.032	4.58 ±1.77	15.86 ± 1.89
X_2X_2 (n = 6)	0.222 ±0.031	0.052 ±0.019	0.170 ±0.031	3.67 ±1.15	16.38 ± 4.29
X_1X_1 v. X_2X_2	$p < 0.025$	$p < 0.025$	ns	$p < 0.010$	ns

independent mechanisms, nor was there a significant difference in the rate of LDL synthesis.

Kinetic parameters for the same individuals, grouped according to their apoB genotype as defined by the EcoRI and MspI endonucleases are given in Table 26. There is a tendency for higher receptor mediated LDL catabolism in R_1R_2 and M_1M_2 individuals but none of these correlations are statistically significant.

Details about individual values for plasma lipoprotein levels, kinetic parameters and apoB haplotypes are given in the original publication of this work (333) and in Table I-25.

Table 26: LDL Kinetic Parameters and EcoRI and MspI ApoB Genotypes

	Fractional Catabolic Rate			Absolute Receptor Mediated Catabolic Rate	Synthetic Rate
	Total	Receptor Mediated	Receptor Independent		
	(pools/day)			(mg/kg per day)	
ECoRI-RFLP					
R1R1 (n = 12)	0.234 ±0.031	0.063 ±0.019	0.170 ±0.028	4.62 ±1.50	17.33 ± 3.70
R2R2 (n = 7)	0.253 ±0.029	0.074 ±0.032	0.179 ±0.037	4.78 ±2.07	16.73 ± 3.01
MSpI-RFLP					
M1M2 (n = 9)	0.255 ±0.027	0.080 ±0.020	0.174 ±0.031	5.49 ±1.39	16.20 ± 6.87
M2M2 (n = 8)	0.227 ±0.032	0.064 ±0.014	0.163 +0.028	4.50 ±1.28	15.77 ± 3.00

4. DISCUSSION

As outlined before the metabolism of apolipoprotein B containing lipoproteins is influenced by environmental and genetic factors. Genetic determinants of apolipoprotein B metabolism are the focal point of this study.

Genetic factors can be mutations with relatively small effects on individuals or rare defects, causing major disturbances of lipoprotein metabolism. While the former, because of their frequency, contribute significantly to the interindividual variation in lipoprotein metabolism in the population at large, the latter are particularly informative in that they provide clues to the physiological role of the affected gene product. The XbaI polymorphism of the apoB gene and the apoE polymorphism are examples for common mutations with comparatively small effects on an individual basis. In contrast, lipoprotein lipase or hepatic lipase deficiency as well as homozygous familial hypercholesterolaemia, are conditions, where key elements of apolipoprotein B metabolism are defective resulting in massive accumulations of distinct lipoprotein species. By comparison with normal subjects, the importance of the different lipolytic enzymes or the LDL-receptor can be inferred.

4.1. The XbaI Restriction Site Polymorphism of the Apolipoprotein B Gene and LDL Metabolism

Two papers from Law et al. (247) and from Berg (246) reported in 1986 an association between an XbaI restriction site polymorphism of the apolipoprotein B gene and plasma concentrations for total cholesterol and apolipoprotein B in normolipidaemic healthy subjects (246, 247). In both studies, homozygotes for the apoB allele devoid of the XbaI cutting site (X_1X_1) exhibited cholesterol concentrations which were, respectively, 7% and 10% lower than those observed in heterozygotes (X_1X_2) or homozygotes for the allele possessing an additional cutting site (X_2X_2). Differences for apoB levels were 10% and 15%. Triglyceride levels were not significantly different in one study (246) but differed by 27% in the other study (247). Altogether 35 X_1X_1 subjects and 104 X_1X_2 and X_2X_2 subjects were examined. Gene frequencies in both study groups were 0.48 for X_1 and 0.52 for X_2. From these findings an autosomal dominant form of inheritance was deduced with the X_2 allele linked to an increase of plasma cholesterol concentrations. However, another study by Talmud et al. (151) showed intermediate cholesterol levels for X_1X_2 heterozygotes suggesting a codominant mode of inheritance, which is easier to reconcile with the fact that each LDL particle carries only one apoB molecule, encoded for either by an X_1 or an X_2 allele.

These findings formed the background for a study aimed at differentiation of possible reasons for the observed variation in cholesterol and apoB levels. Possible causes were either increased apoB synthesis or impaired apoB catabolism linked to the X_2 allele as compared to the X_1 allele. In order to distinguish between these options LDL-kinetic parameters defined by turnover studies were correlated with the genotype for the XbaI-RFLP of the apoB gene.

The subjects whose LDL metabolism was studied were all middle-aged (40-60 years) and had moderate, diet-refractory hypercholesterolaemia with an average of 7.7 ± 0.7 mmol/l. Subjects suffering from heterozygous FH were excluded from the study. Hypercholesterolaemia was caused in this group by a combination of oversynthesis and defective catabolism. The mean synthetic rate of 17.1 ± 3.3 mg/kg per day was in excess of normal values (11-13 mg/kg per day) and the mean total LDL-FCR was lower than observed in normal controls (0.24 ± 0.03 versus 0.35 ± 0.06 pools/day) (323). Receptor mediated LDL catabolism was on average only 28% of the total LDL-FCR, distinctly lower than the 50% observed in normolipidaemics (27).

When values for LDL-FCR were related to the XbaI apoB genotype, the group of five X_1X_1 homozygous subjects had a 22% higher total FCR than the six X_2X_2 homozygotes. Differences for receptor mediated LDL catabolism were even more marked while receptor independent catabolism was the

same in both groups. These findings suggest that apolipoprotein B as the product of the polymorphic gene has a structural defect in X_2X_2 individuals, which reduces its affinity for the LDL-receptor. In X_1X_2 heterozygotes, LDL particles carry either the defective apoB translated from the X_2 allele or the structurally intact apoB derived from the X_1 allele. This implies that LDL in these subjects is metabolically heterogeneous and as a consequence the FCR for LDL should be intermediate as observed in this study.

As mentioned before, the DNA sequence change which creates the XbaI restriction site does not result in a change of the amino acid sequence of the translated protein, which makes it unlikely that the XbaI polymorphism itself is functionally significant. Rather, this mutation is probably in linkage disequilibrium with a functionally important change elsewhere in the coding region of the apoB gene. The receptor-binding domain is an obvious candidate area (324) but so far efforts have failed to identify a common DNA mutation in this region specifying X_2X_2 in contrast to X_1X_1 subjects.

The association between the X_2 allele and lower LDL-FCRs provides an explanation for the higher plasma cholesterol levels observed in X_2X_2 homozygous normolipidaemics. In subjects with normal LDL levels LDL-FCR and LDL concentration in plasma are inversely correlated (323). Lower clearance rates for LDL, possibly

caused by impaired LDL-receptor binding in X_2X_2 individuals, may result in an accumulation of LDL in the plasma compartment. In hypercholesterolaemic patients, such as in the present study, oversynthesis of apoB partly overrides the correlation between LDL-FCR and LDL plasma concentration, thus explaining the lack of a correlation between the XbaI apoB genotype and LDL-cholesterol in this group of subjects (Table 23).

The correlation between LDL-FCR and the XbaI apoB genotype has been confirmed by two other papers: Houlston et al. (324) found in their study of 22 normolipidaemic males, aged 35-49 years, that X_2 homozygosity was associated with 13% higher cholesterol concentrations and a significantly lower LDL-FCR (-11%). X_1X_2 heterozygotes in this study had the lowest cholesterol levels and the highest LDL-FCR, a finding which could not be explained satisfactorily.

The second study by Series et al. (325) investigated in-vitro degradation of LDL isolated from X_1X_1 and X_2X_2 homozygotes. LDLs were labelled with different iodine isotopes and in-vitro degradation by fibroblasts was monitored. LDL derived from an X_1X_1 subject and LDL from an X_2X_2 subject were incubated in the same culture dish and paired rates of degradation were measured. In a group of nine normolipidaemics with a mean age of 48 years and in a group of fourteen hypercholesterolaemics (mean cholesterol 8.3 mmol/l; mean age 57 years) in-vitro degradation of LDL

from X_2X_2 subjects was significantly lower than observed with LDL derived from X_1X_1 subjects (-15% and -18% respectively, for the two study groups). No such difference was observed in a group of ten younger normolidaemics (mean age 31 years). These findings confirm the results of the in-vivo studies discussed before. In addition the possibility emerges of an age dependent manifestation of the effects a structural LDL-defect may have on LDL metabolism.

Another observation in support of the concept that the XbaI apoB gene polymorphism is responsible for structurally different apoB isoforms is the fact that this restriction site polymorphism is strongly associated with the Ag (c/g) antigenic polymorphism (237, 246, 278-279). The antigenic group (Ag) variation is due to a series of LDL specific antigens detected by antisera from multiple transfused patients (249). The close linkage between the Ag system and several apoB restriction site polymorphisms including the XbaI-RFLP (Table 4) suggests that DNA mutations are causing changes in the apoB structure which give rise to the development of homospecific antibodies. From a study in Finland it was reported that the combined genotype with both, X_2 and apoB(c), alleles present correlated even better with elevated plasma cholesterol levels than either allele alone (248).

The fact that the XbaI apoB polymorphism is not identical with the functionally important apoB mutation but

only linked to it may explain, in part, why some population studies failed to detect a significant association between plasma cholesterol and XbaI apoB genotype. An example is the study reported in paragraph 3.7.1., which showed trends for a higher X_2 allele frequency in hypercholesterolaemics but no corresponding increase of the X_1 allele frequency in subjects with low plasma cholesterol (Table 24). Other negative results are from Sweden (251), where 200 randomly selected subjects were analysed and from the United States (238), where 168 subjects with and without coronary heart disease were studied. In Japan no association between the XbaI RFLP and cholesterol levels was observed and an X_2 allele frequency of 0.04 was reported, a value significantly lower than 0.5, the frequency usually observed in Caucasian populations. This demonstrates that ethnic heterogeneity is another factor complicating population studies of this polymorphism.

So far only one study reported a significant correlation between the XbaI apoB gene polymorphism and coronary heart disease (238). Here the X_1 allele frequency was significantly higher in patients with myocardial infarction as compared to controls. As mentioned before, cholesterol levels did not correlate with the XbaI genotype. Similar results were reported by Myant et al. (241) with an increased allele frequency for X_1, though this difference was significant only in a subset of normolipidaemic patients. Again cholesterol levels did not

correlate with the XbaI polymorphism. In the study presented in paragraph 3.7.2 a trend towards higher LDL synthetic rates in X_1X_1 subjects is noticeable. Vega et al. have found overproduction of LDL to be associated with coronary heart disease in patients with hypertriglyceridaemia and in a subgroup of normolipidaemic patients (327). The same applies for familial combined hyperlipidaemia (93) and hyperapobetalipoproteinaemia (180) both conditions strongly associated with coronary heart disease. Further investigations will clarify the link between coronary heart disease, the X_1 allele of the apoB gene and possible oversynthesis of apolipoprotein B. While the X_2 allele is associated with increased plasma cholesterol levels, these future studies will show whether or not the X_1 allele is an independent risk factor for coronary heart disease.

4.2. Apolipoprotein B Metabolism in Normolipidaemics: The Influence of Apolipoprotein E Polymorphism

4.2.1. The current model

Many studies from a number of different countries including the one presented in paragraph 3.2. have demonstrated that plasma cholesterol levels are correlated with the apolipoprotein E phenotype (255). Homozygotes for apoE3, the common "wild type" variant, who account for 60-

70% in Caucasian populations, have plasma cholesterol levels higher than apoE3/2 and apoE2/2 subjects but lower than apoE4/3 and apoE4/4 individuals. It was this observation that initiated the present study of apolipoprotein B metabolism in individuals homozygous either for apoE3, apoE4 or apoE2. VLDL-turnovers in these subjects provide insights into the mechanisms by which different apoE isoforms might modulate the metabolism of apolipoprotein B. These can be compared with the current hypothesis of how apoE isoforms interact with apoB metabolism (258, 330).

The current concept of apoB metabolism is depicted in Fig. 46. The diagram for apoE3 homozygosity resembles Fig. 2, where the areas A and B illustrate the metabolism of chylomicrons and of apoB containing lipoproteins. As mentioned before (paragraph 1.6.2), the metabolism of apoE2 is delayed in-vivo as well as in-vitro when compared to apoE3 metabolism (51, 153). In contrast, apoE4 is catabolised at an increased rate when injected into normolipidaemic subjects (260) in spite of identical behaviour in-vitro (51). Further insights into the role of apolipoprotein E in apoB metabolism came from metabolic studies in patients with familial dyslipoproteinaemia or with an inherited deficiency of apoE. In both conditions the patients have decreased catabolic rates for VLDL and chylomicron remnants (137, 147). In patients with apoE deficiency transfer of VLDL into IDL and LDL is reduced

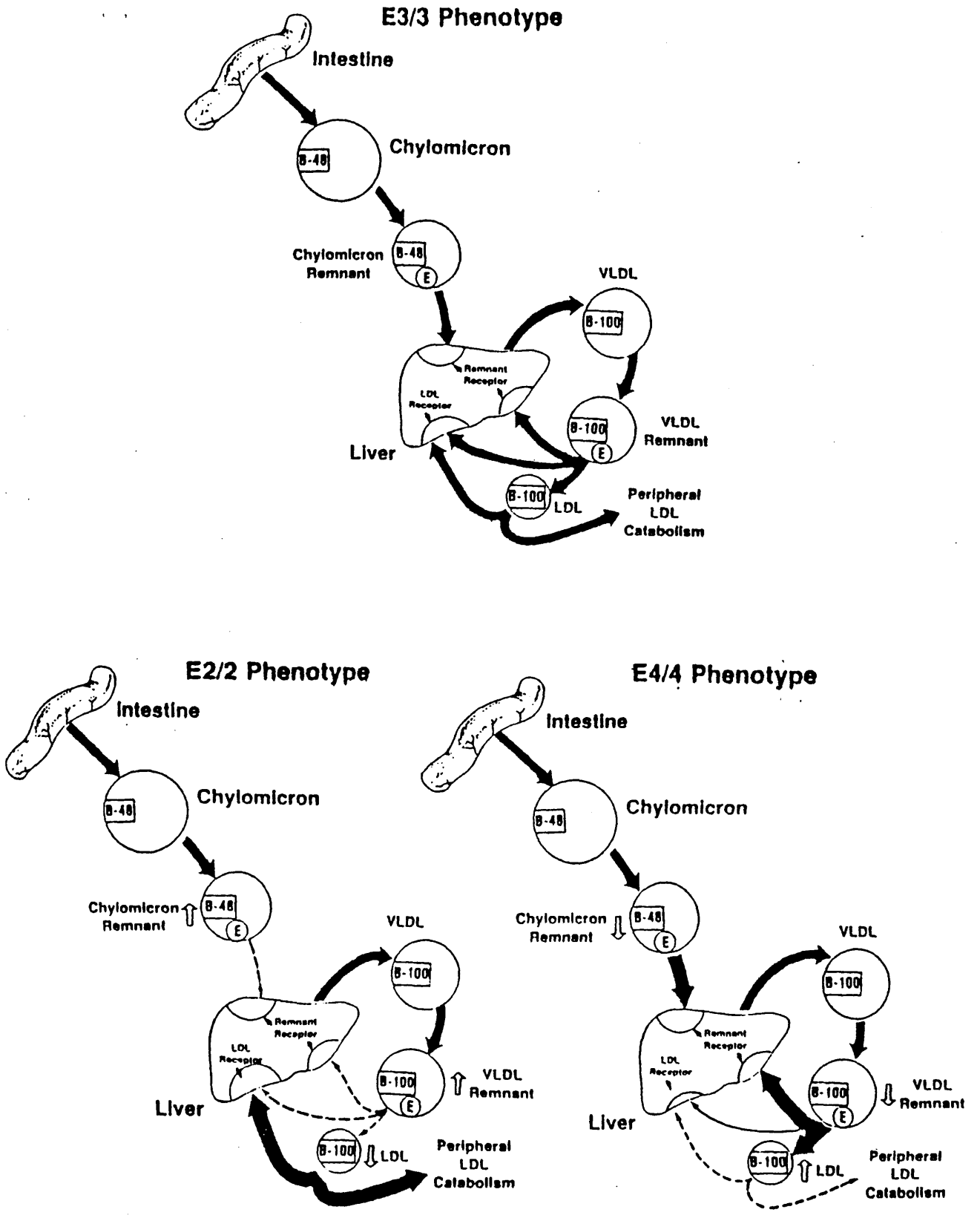


Fig. 46: Current model for the interaction between apoE isoforms and apoB metabolism. For details see text. (Reproduced from reference 255).

and the catabolism of normal LDL, produced by apoE3/3 homozygotes, is enhanced (147, 331). These observations led to the following model of apoB metabolism in apoE2/2 homozygotes: in the absence of functional apoE chylomicron uptake by the liver is impaired resulting in up-regulation of the LDL-receptor. This in turn increases the fractional catabolic rate of LDL and reduces LDL plasma levels. In addition the LDL synthetic rate is reduced as the conversion of LDL precursors into LDL seems to depend on functional apoE (Fig. 46). The inverse of these mechanisms may apply for apoE4/4 homozygotes whose more rapid clearance of chylomicrons may lead to down-regulation of the LDL-receptor and a consequent increase of LDL concentration (Fig. 46). LDL-receptor activity, according to this model, has a pivotal role in the apoE dependent modulation of apoB metabolism.

4.2.2. Apolipoprotein E2 and apoB metabolism

Results of VLDL-turnover studies in apoE2/2 homozygous normolipidaemics are reported in section 3.3.2. The main differences as compared to apoE3/3 homozygotes are repeated again: direct catabolism of large and small VLDL were reduced (0.3 versus 5.3 pools/day and 0.03 versus 1.7 pools/day, respectively) as was the transfer rate of IDL to LDL (0.4 versus 1.0 pools/day). In contrast, direct catabolism of IDL was faster (1.0 versus 0.3 pools/day) but

the fractional catabolic rate of LDL was not significantly increased. As pointed out previously, apoE2 does not bind efficiently to the LDL-receptor. However, this is not sufficient to explain reduced direct catabolism of $VLDL_1$ and $VLDL_2$ in apoE2/2 homozygotes, since in-vitro studies have shown that normolipidaemic VLDL is not degraded by the LDL-receptor pathway. This may be due to a conformational peculiarity of apoE in VLDL of normolipidaemics, which makes it inaccessible to receptor binding (334). An alternative explanation is that some apoE is lost from VLDL during preparation by ultracentrifugation before it is used for in-vitro studies. This view is supported by the observation that receptor mediated uptake and degradation of VLDL can be induced in-vitro by addition of purified apoE (335). Therefore, some VLDL clearance via the LDL-receptor seems possible in spite of the negative findings mentioned before.

The "apoE"-receptor provides another potential pathway for direct VLDL clearance. Evidence for the existence of a lipoprotein-receptor, distinct from the LDL-receptor, has been discussed in paragraph 1.3.1. The putative receptor protein was discovered by its close structural and biochemical similarities with the LDL-receptor (53). Thus, one would expect that apoE2 is also a poor ligand for this receptor. Reduced catabolic rates in-vivo for $VLDL_1$ and $VLDL_2$ in apoE2/2 homozygotes can then be

explained as the result of impaired receptor mediated degradation.

The second important finding in apoE2/2 homozygotes, a reduced transfer rate of IDL to LDL, parallels results of in-vitro studies with lipoproteins from patients with familial dysbetalipoproteinaemia (337, 338). In contrast to normal VLDL (167), apoB-100 containing ß-VLDL from these patients could be converted only into IDL but not into LDL by addition of lipoprotein lipase. The formation of "LDL" could be achieved by the further addition of apoE3 and lipoprotein deficient plasma (338). Another paper reports that VLDL from apoE2/2 normolipidaemics, containing varying amounts of ß-VLDL was a relatively poor substrate for lipoprotein lipase and hepatic lipase (362). This may be a consequence of a prolonged exposure to the action of CETP with the result of relative triglyceride depletion in exchange of cholesteryl esters derived from HDL. However, even if this is a contributing factor to the impairment of IDL to LDL conversion, the mechanism by which apoE3 can enhance this process remains obscure. ApoE3 does not seem to be a cofactor of lipolytic enzymes, since normal pre-ß-VLDL from patients with familial dysbetalipoproteinaemia can be converted to LDL by addition of lipoprotein lipase and apoCII (338).

VLDL-turnover studies in patients with familial dyslipoproteinaemia and with apolipoprotein E deficiency also confirm a reduced IDL to LDL conversion in the absence

of receptor-binding apoE (155, 274, 331). In summary, both in-vitro and in-vivo studies suggest an important role of apoE in the conversion of IDL into LDL but the underlying mechanism is not understood at present.

The significantly lower LDL plasma pool results mainly from the low influx of material from the IDL density range. The overall fractional catabolic rate for LDL is somewhat higher in apoE2/2 as compared to apoE3/3 subjects but this falls short of statistical significance, possibly because of greater interindividual variation among the four apoE2/2 subjects. LDL decay curves (Figs. 17 and 20) and the kinetic rate constants k(0,10) and k(0,11) show that FCRs for both LDL subspecies, whether derived from $VLDL_1$ or from $VLDL_2$, are very similar in apoE2/2 and in apoE3/3 subjects. However, the relative proportion of the LDL masses in subcompartment M(10) and M(11) is clearly different (ratios M(10) over M(11) are 4.97 and 1.02 in apoE2/2 and apoE3/3, respectively). LDL accounted for by M(10) is derived from $VLDL_2$ and is the dominant LDL subspecies in apoE2/2 subjects. In all apoE phenotypes this LDL subspecies is catabolised faster than $VLDL_1$-derived LDL, represented by M(11). The total LDL-FCR accounts for LDL catabolism as a whole, no matter what the relative contributions of metabolically distinct lipoprotein subspecies are. It is higher in apoE2/2 than in apoE3/3 subjects, in spite of almost identical LDL kinetic rate constants k(0,10) and k(0,11), because the

balance between LDL in M(10) and LDL in M(11) is shifted towards the former.

A crossover experiment by Gregg et al. has demonstrated that LDL from apoE2/2 homozygotes is catabolised at a slower rate than LDL from apoE3/3 subjects (339). Therefore, the LDL decay curve in apoE2/2 subjects does not necessarily reflect the level of LDL-receptor activity as compared to control subjects, as a decay curve with a normal descending slope may represent degradation of LDL with reduced binding affinity by an increased number of receptors. Direct catabolism of IDL, which is also mediated by LDL receptors (334), was significantly increased in apoE2/2 subjects. This probably reflects up-regulation of LDL-receptors in apoE2/2 subjects which because of the poor binding qualities of their LDL is masked when only LDL metabolism is taken into account.

Finally, the significantly lower rate of apolipoprotein synthesis in apoE2/2 homozygotes has to be addressed. At present the regulation of apoB synthesis is poorly understood at the molecular level. Evidence is emerging now from recent studies with macrophages that cholesterol derived from different lipoproteins, such as LDL or ß-VLDL, may enter different intracellular cholesterol pools (332). Whether or not this is of importance for the regulation of apoB synthesis is yet unknown.

One apoE2/2 subject (P.M.) provided VLDL-turnover data, which were markedly different from the other four subjects studied in this group (Fig. I- 5). Therefore these were excluded when average values were calculated. Repeated Western blot analyses showed that there is no doubt about the apoE phenotype of P.M. and this was confirmed by a method (347) using an oligonucleotide specific for the apoE2 (Arg 158 ⟶ Cys) allele (Dr. R. Houlston, unpublished observation). Dyslipoproteinaemia, reflected by the apoB distribution in plasma was further proof of apoE2 homozygosity. The VLDL-cholesterol/ triglyceride ratio in P.M., however, was lower than with the other apoE2/2 subjects, possibly because her mean total triglyceride value was increased by 65% (Table I-3). Also, the composition of $VLDL_1$ from P.M. was abnormal in that it was triglyceride-rich mainly at the expense of cholesteryl esters and phospholipids. Synthesis of $VLDL_1$-apoB was three-fold increased in comparison with the average rate for apoE2/2 homozygotes (Table I-21). These findings suggest that this patient presents a combination of apoE2/2 homozygosity and hypertriglyceridaemia due to an oversynthesis of large VLDL. The diet consumed by P.M. was not carbohydrate-rich, no alcohol intake was recorded (Table I-9) and there were no signs of conditions causing secondary hypertriglyceridaemia. Unfortunately, family members required for establishing the diagnosis of familial hypertriglyceridaemia were not available. It should be

noted that $VLDL_1$ oversynthesis only occurred in comparison to values from other apoE2/2 subjects but not if compared to apoE3/3 individuals. However, a comparison between P.M. and apoE3/3 subjects is invalid in this context, as apoE2 homozygosity per se seems to be associated with a reduced rate of apoB synthesis.

It is known that large VLDL from hypertriglyceridaemic subjects (HTG-VLDL) in contrast to normolipidaemic VLDL is degraded via LDL-receptor uptake (348). The receptor-binding domain of apoE associated with HTG-$VLDL_1$ can be blocked with a monoclonal antibody which still leaves appreciable binding affinity for the LDL-receptor (349). This is only abolished by complete proteolytic degradation of apoE (348). Thus, in HTG-VLDL apoB epitopes can replace apoE as a receptor ligand to some extent. This does not contradict the observation that apoE is necessary and sufficient for receptor mediated uptake of triglyceride-rich lipid particles as demonstrated by experiments with artificial liposomes with incorporated apoE (350).

The VLDL-turnover in P.M. can be interpreted as an in-vivo study, complementing the in-vitro observations mentioned above. $VLDL_1$ and $VLDL_2$ were cleared at normal rates, possibly because of the interaction of two independent abnormalities: firstly, oversynthesis of $VLDL_1$ produces particles which bind to the LDL-receptor, even in the absence of receptor-binding apoE; secondly, there is

some evidence for up-regulation of LDL-receptor activity in apoE2/2 homozygotes as mentioned in the previous paragraph. Both together result in an increased turnover where oversynthesis is balanced by increased catabolic rates. As hypertriglyceridaemia was only moderate, lipoprotein lipase activity was likely to be far from saturated, explaining the significant increase of $VLDL_1$ to $VLDL_2$ transfer (171). The transfer rate of IDL to LDL, however, was also distinctly higher than the typical rates for apoE2/2 subjects. This and the significantly increased FCR for LDL are difficult to explain, since the particle compositions were the same as measured for apoE2/2 and apoE3/3 normolipidaemics. Hypermetabolism as observed in severe hypertriglyceridaemia may be involved since it is conceivable that mild hypertriglyceridaemia combined with impaired lipoprotein-receptor binding activates alternative non-receptor dependent routes of metabolism which operate in severe hypertriglyceridaemia (see paragraph 4.3.1.). However, the precise mechanisms involved remain uncertain.

It is well possible that the combination of hypertriglyceridaemia and apoE2 homozygosity will develop into familial dysbetalipoproteinaemia in this subject. A follow-up measurement of her plasma lipoproteins after 18 months, however, has shown only insignificant changes.

4.2.3. Apolipoprotein E4 and apoB metabolism

The main finding with VLDL-turnovers in apoE4/4 homozygotes as compared to apoE3/3 subjects was a delayed direct catabolism for $VLDL_2$, IDL and LDL. These differences were significant for $VLDL_2$ and for LDL, but not for IDL. As discussed earlier, LDL decay curves for LDL derived from $VLDL_1$, and for LDL, derived from $VLDL_2$, were both less steep than the equivalent curves for apoE3/3 subjects. The kinetic rate constant k(0,10), ie the FCR for $VLDL_2$-derived LDL was significantly smaller in apoE4/4 than in apoE3/3 subjects ($p < 0.01$). The mass ratio of M(10) over M(11) was again in favour of M(10) although to a lesser extent than observed in apoE2/2 homozygotes (2.89 and 1.02 in apoE4/4 and apoE3/3, respectively). Nevertheless, the total FCR for LDL was significantly smaller in apoE4/4 subjects due to the marked reduction in k(0,10). Together these findings suggest that receptor mediated LDL degradation is reduced in apoE4/4 subjects.

The switch from apoE to apoB as the primary determinant of lipoprotein-receptor binding occurs within the range of $VLDL_2$ (Sf 20-60) (334). It is possible therefore that down-regulation of LDL-receptors results in a reduced up-take and degradation of $VLDL_2$, as some of these particles may be cleared physiologically by the LDL-receptor pathway. However, the decrease in direct catabolism is less marked than in apoE2/2 individuals,

because clearance by the "apoE"-receptor is unimpaired in apoE4/4 individuals.

From this study of apoB-100 metabolism it is not clear why LDL-receptor activity should be down-regulated in apoE4/4 homozygotes. Some hints may come from two recent papers which addressed the relationship between apolipoprotein E polymorphism and the metabolism of dietary fat. One study found the efficiency of intestinal cholesterol adsorption to be 35% higher in a group of apoE4/3 and apoE4/4 subjects than in apoE3/2 subjects with values for apoE3/3 subjects in between (341). In an earlier study with male Finns it had been demonstrated that the efficiency of cholesterol adsorption correlates well with plasma cholesterol levels (342).

In the second trial (343) the rate of dietary fat clearance was measured using the vitamin A fat loading test. Chylomicron remnants were cleared more rapidly in seven apoE4/3 subjects and more slowly in nine E3/2 subjects as compared to the clearance in ten apoE3/3 individuals. In another similar study a prolonged chylomicron remnant clearance time was observed only in apoE2/2 but not in apoE3/3 subjects, while apoE4/3 and apoE3/3 were not analysed separately (344).

As apoE3 and apoE4 show the same receptor-binding characteristics in-vitro in contrast to apoE2, it is not obvious why apoE3/3 and apoE4/4 subjects in-vivo should differ in the way described. A possible mechanism

explaining these observations has to do with the distribution of apoE among plasma lipoproteins (260, 345). Most apoE in apoE3/3 homozygotes is associated with HDL where at least some apoE forms a covalent complex with apoAII via a disulphide bond (12). In contrast, a markedly higher proportion of apoE4 is attached to VLDL, probably because apoE4, which lacks a cysteine residue in position 112, cannot form apoE-AII complexes in HDL (345). In addition the added positive charge in apoE4 seems to increase the affinity for lipoproteins of lower density (346). As a result apoE4 is catabolised faster than apoE3, which is to a greater extent associated with relatively slowly degraded HDL (260). Moreover, apoE4 is readily available for association with chylomicrons and chylomicron remnants which become targeted for rapid receptor mediated uptake by the liver. If in apoE4/4 homozygotes intestinal cholesterol adsorption is more efficient and exogenous fat clearance faster and probably more efficient, too, down-regulation of the LDL-receptor is a natural consequence of relatively high intracellular cholesterol supplies.

4.2.4. Conclusions: a modified model

In principle the model of apoE dependent modification of apolipoprotein B metabolism, which was introduced at the beginning of this chapter, is compatible

with the results of the metabolic studies discussed in the last two sections. By these studies the relative importance of specific mechanisms underlying the correlations between plasma apoB concentrations and apoE phenotypes has been clarified to some extent and it is now possible to draw a picture in some more detail.

In apoE2/2 homozygotes the most significant mechanism resulting in low LDL levels is reduced formation of LDL from IDL precursors. LDL decay curves on the other hand do not provide evidence for increased LDL catabolism as a result of up-regulated LDL-receptor activity. Nevertheless this cannot be ruled out and indirect evidence as increased catabolism of IDL suggests that LDL-receptor activity is indeed up-regulated to some extent. Receptor up-regulation might be a consequence of delayed metabolism of dietary fat.

In contrast, high LDL concentrations in apoE4/4 homozygotes can be explained by diminished LDL-receptor activity since the LDL-FCR is lower in apoE4/4 as compared to apoE3/3 subjects as reflected by a lesser gradient of the LDL decay curves. Higher efficiency of intestinal cholesterol adsorption and faster clearance of chylomicrons are appropriate causes for down-regulation of LDL-receptor activity in apoE4/4 homozygosity.

In summary, apoE2 lowers LDL levels mainly by reduced LDL-synthesis from precursor particles and apoE4

increases LDL concentrations indirectly as a consequence of a more efficient metabolism of dietary fat supplies.

These conclusions have interesting repercussions for the quantitative aspects of the correlation between plasma cholesterol and apoE phenotypes. In most population studies published so far, plasma cholesterol levels were markedly lower in apoE2/2 than in apoE3/3. In six studies, presented in a recent review by Davignon et al. (255), the mean difference was -16% or 0.8 ± 0.2 mmol/l. In contrast the increase of cholesterol levels observed in apoE4/4 was only +5% or 0.3 ± 0.2 mmol/l and appeared to be more variable, ranging from +12% in Helsinki to +2% in Framingham. The more prominent difference between apoE2/2 and apoE3/3 subjects may be caused by an inherited metabolic defect and some additional variation as a result of dietary habits. The increased cholesterol levels in apoE4/4 subjects, in contrast, probably depend largely on the diet ingested and are therefore quite pronounced in populations with high intake of saturated fatty acids and cholesterol and almost absent in communities on a low-fat diet. Thus, the correlation between plasma cholesterol and apoE phenotype is an example for a combined peristatic and genetic interaction (258).

In the study reported in paragraph 3.2. apoE2/2 subjects presented with a rather small reduction of cholesterol in plasma while the increase observed in apoE4/4 was above average. This may be an indication for

an "atherogenic" diet prevailing in the population studied, as suggested by the high incidence rate for coronary heart disease in the Scottish community.

4.3. Apolipoprotein B Metabolism in Genetically Defined Disorders of Lipid Metabolism

4.3.1 Familial hyperchylomicronaemia

Two etiologically distinct patients, one with classical lipoprotein lipase deficiency and one with lipoprotein lipase activity blocked by a plasma inhibitor (117) were studied. VLDL-turnovers produced results, which were remarkably similar: firstly, $VLDL_1$ conversion into $VLDL_2$ was markedly reduced, causing a massive increase of $VLDL_1$ plasma concentrations. Secondly, IDL and even more so LDL were metabolised at rates 1.5 to 3.5-fold higher than in normal controls.

The first apoB metabolic study of Type I hyperlipidaemia was published by Nicol et al. (73), who investigated two patients, one with lipoprotein lipase deficiency and one with apoCII deficiency. Total VLDL ($d < 1.006$ g/ml) was used as a tracer and conversion into IDL and LDL was monitored. Fractional catabolic rates for VLDL and the mean conversion time of VLDL to LDL were found to be within the normal range. As a result apoB-100 metabolism was described as unimpaired by lipoprotein

lipase deficiency (73). In another study Stalenhoef et al. (109) analysed the metabolism of $VLDL_1$ and chylomicrons in two lipoprotein lipase deficient subjects. They found clearance of both lipoproteins, which were distinguished by their content of apoB-100 and apoB-48 respectively, markedly delayed. No transfer of protein was observed from $VLDL_1$ or chylomicrons into the density range of LDL. A third metabolic experiment by Goldberg et al. was carried out in monkeys, whose lipoprotein lipase was blocked by an infusion of lipase-antibodies (351). Shortly afterwards trace-labelled human $VLDL_1$ and $VLDL_2$ were injected. The most significant findings were a marked decrease of $VLDL_1$ catabolism and a complete block in the transfer of $VLDL_1$ and $VLDL_2$ into LDL.

Results of the first publication are in obvious contradiction to the findings reported in the two other papers. Some of this inconsistency could be resolved by the double turnover approach used in the present study, where metabolism of autologous large and small VLDL was investigated simultaneously. The results, as presented in paragraph 3.4.2 and 3.4.3, were different from both, the previous study in humans (73) and from the animal experiment (351). In the following it is tried to explain how these discrepancies could occur.

Radioiodination of total VLDL (Sf 20-400) results in preferentially labelling $VLDL_2$ and only little $VLDL_1$ since the iodine reacts proportionally to the protein mass.

Therefore, the tracer used in the VLDL-turnover reported by Nicol (73) was in fact $VLDL_2$ which was cleared at low normal rates as in the present study (Table 17). The blocked transfer of $VLDL_1$ into $VLDL_2$ was obviously undetectable by a $VLDL_2$-tracer injection.

Marked reduction of in-vivo conversion of $VLDL_1$ into $VLDL_2$ as observed in the present study and by Stalenhoef et al. (109) is in line with in-vitro studies, where the enzyme activity was tested by incubation with various lipoprotein preparations. Native chylomicrons and large VLDL were the preferred substrates for lipoprotein lipase (353).

The complete block of LDL formation observed in the animal experiment could not be confirmed by the study in humans, in which apoB was found to be transferred from $VLDL_1$ and $VLDL_2$ into LDL at a percentage of 5% and 15% respectively. The difference may be explained by different pathways for preferential LDL formation. In cynomolgus monkeys a substantial proportion of LDL is not derived from circulating $VLDL_2$ but from a small pool of VLDL which is rapidly, within minutes, converted into LDL (72, 381). This process may be largely dependent on lipoprotein lipase activity, which was blocked in this experiment.

The second abnormality observed in the VLDL-turnover study of Type I patients was hypermetabolism of denser apoB containing lipoproteins. A similar observation was first made by Sigurdsson et al. as a result of LDL-turnovers in

patients with severe hypertriglyceridaemia (354). Six patients with a mean triglyceride concentration of 17.8 ± 9.2 mmol/l catabolised LDL at a rate almost double what had been determined for normal controls (0.7 ± 0.14 versus 0.41 ± 0.09 pools/day). The two patients in the present study had slightly higher triglyceride levels and LDL-FCRs were 3.5 times higher than normal. Further investigations by Shepherd et al., employing the double-turnover technique described in paragraph 2.6., provided insights into the underlying mechanisms (355). The five subjects with the highest triglyceride concentrations (mean 20.6 ± 9.4 mmol/l) in this study had a total LDL-FCR of 0.75 ± 0.34 pools/day. While 0.20 ± 0.10 pools/day were cleared via the LDL-receptor pathway, 0.55 ± 0.27 pools/day were degraded by non-receptor dependent mechanisms. Comparison with reference values shows that only the latter was significantly increased. This increase could be reversed by lowering triglycerides through drug treatment. The precise nature of LDL-receptor independent LDL degradation is still not known, but some evidence suggests an important role of the reticuloendothelial system in this process. Saturation of the RES by ethyloleate injections into rabbits produced a marked fall in receptor independent catabolism of human LDL (356) and in patients with myeloproliferative disorders LDL clearance by the non-receptor pathway was shown to be accelerated (357). Spleenomegaly, as present at least in

one of the patients (D.S.) reported in this study, is common in severe hypertriglyceridaemia and provides further evidence for involvement of the RES in this metabolic disorder (106).

4.3.2. Hepatic lipase deficiency

Hepatic lipase is the second lipolytic enzyme involved in apolipoprotein B metabolism. Its physiological role was analysed on the basis of observations, including a metabolic study, obtained from a patient with hepatic lipase deficiency (340).

A first hint on the role of hepatic lipase in apoB metabolism comes from the abnormal pattern of apoB distribution among plasma lipoproteins seen in this subject (G.P.) and reported from two other patients (118-120). The main apoB containing lipoprotein was recovered in the IDL fraction by cumulative gradient ultracentrifugation (Table 14), in accordance with the shift of the peak of apoB containing lipoproteins towards a smaller density observed in the rate zonal ultracentrifugation profile (Fig. 36). For another hepatic lipase deficient subject, the density of the major apoB-lipoprotein was determined as 1.023 g/ml, in contrast to 1.044 g/ml for normal LDL (120). A similar lipoprotein profile was found in a patient with acquired hepatic lipase deficiency as a consequence of vitamin A intoxication,

where a marked increase was noted for LDL of lighter density (359). None of these subjects had significant amounts of typical LDL. The lipoprotein subfraction recovered as LDL by cumulative gradient centrifugation, as listed in Table 14, represents the "tail" of the peak for the main apoB containing lipoprotein (Fig. 36) rather than a distinct lipoprotein species. As mentioned for lipoprotein lipase, hepatic lipase has been tested in vitro to determine its lipolytic activity for different lipoprotein substrates. The highest activities were found with IDL (S_f 12-20), VLDL ($d < 1.006$ g/ml) and LDL ($1.006 < d < 1.063$ g/ml) but lipolytic activity against chylomicrons was very low (73, 353). Prior to this study the only in-vivo experiments investigating the role of hepatic lipase for apoB metabolism had been animal experiments, where the enzyme was inactivated by infusion of appropriate polyclonal antibodies (360, 361). In an experiment with cynomolgus monkeys, within hours after antibody infusion the mass of VLDL, IDL and light LDL ($S_f > 9$) increased while the total mass of LDL decreased. Injection of radiolabelled VLDL revealed, that these changes were due to a decreased catabolic rate of VLDL, resulting in a delayed VLDL to LDL transformation.

In the present study of apoB metabolism in a hepatic lipase deficient subject the most significant finding was the almost complete block of IDL to LDL conversion, resulting in a markedly increased IDL pool. This was in

spite of a reduced transfer from $VLDL_2$ to IDL of less than half of the normal rate. In contrast, the rate of transfer from $VLDL_1$ to $VLDL_2$ was unaffected. The in-vitro findings mentioned above and the results of the in-vivo studies discussed here and in the previous paragraph suggest that both enzymes, lipoprotein lipase and hepatic lipase mediate the delipidation of $VLDL_2$ to form IDL. In contrast, for the transformation of IDL to LDL only hepatic lipase is essential. In the absence of hepatic lipase IDL or particles slightly denser than IDL become the end product of the apoB-lipoprotein delipidation cascade in plasma. Thus hepatic lipase is crucial for the formation of typical LDL, but also contributes to the delipidation of less dense apoB containing lipoproteins.

Total apoB synthesis in G.P. is below the normal average mainly because of very low $VLDL_1$-apoB synthesis, while $VLDL_2$-apoB synthesis is higher than normal. This constellation resembles the situation in apoE2/2 homozygosity, another condition associated with accumulation of ß-VLDL resulting from impaired IDL to LDL transformation (see paragraph 4.2.4.).

Another abnormality of lipoprotein metabolism in hepatic lipase deficiency is the marked increase of HDL_2 at the expense of HDL_3, as the HDL_2 to HDL_3 conversion dependents on hepatic lipase activity. This means that CETP can no longer effectively exchange triglycerides from apoB-lipoproteins with cholesteryl esters from HDL which

may explain a relative increase of the triglyceride content in these lipoprotein subfractions (Table 15).

4.3.3. Homozygous familial hypercholesterolaemia

In the previous discussion of apoB metabolism the role of LDL-receptors was repeatedly addressed. Patients with homozygous FH provide the unique opportunity to study human apoB metabolism in the absence of this metabolic route (301, 369). Langer et al. concluded in 1972 from their LDL-turnover studies in patients with heterozygous FH that hypercholesterolaemia in these subjects was due to a decreased FCR for LDL (130). In 1974 the discovery of the LDL-receptor and its defect in FH by Goldstein and Brown unveiled the molecular basis for this disorder of lipoprotein metabolism (77, 121). Only gradually it became apparent that the function of the LDL-receptor in apolipoprotein B metabolism is far more versatile than just to mediate the degradation of LDL via receptor-binding. Turnover studies in heterozygous and homozygous FH patients by Soutar et al. (363) suggested that the catabolism of IDL is also delayed, while VLDL metabolism was not significantly different from normal controls. However, a more detailed analysis of VLDL-turnover requires a comprehensive kinetic model of apoB metabolism as applied in the metabolic studies discussed in previous sections.

Results from two studies of apoB metabolism in homozygous FH patients were presented in paragraph 3.6.

While $VLDL_1$ metabolism was unaltered in both patients as compared to controls, $VLDL_2$ metabolism differed in several respects. The pool size of $VLDL_2$ was higher because of a significant increase of remnant particles, represented in the metabolic model by M(6) (Fig. 44, Table 21). This was reflected by a corresponding change in $VLDL_2$ composition with a cholesteryl ester/triglyceride ratio of 1.6 versus 0.6 in normal controls (Table 20). The accumulation of remnants is probably due to a prolonged $VLDL_2$ residence time in plasma allowing for more extensive cholesteryl ester transfer from HDL. A tendency for subnormal clearance rates k(0,6) also contributed to increased remnant concentrations. The main reason, however, for the increased plasma residence time of $VLDL_2$ (Fig. 41) and IDL (Fig. 42) was a delay in the delipidation process, mirrored by 75% reductions of the transfer rates of $VLDL_2$ to IDL and of IDL to LDL. These delipidation steps have been linked in the previous paragraph with the action of hepatic lipase. As there are no clues to a reduced hepatic lipase activity in homozygous FH, it can be inferred that the LDL-receptor is an additional important functional component for the conversion of VLDL to LDL by delipidation in the plasma compartment. A model of this process based on the results of the metabolic studies presented so far will be discussed in section 4.4.

Besides a reduced IDL to LDL transfer rate, the fractional rate for direct catabolism of IDL was significantly reduced. This confirms that IDL degradation is mediated in part by the LDL-receptor as previously shown by VLDL-turnover studies (363) and by direct investigation of IDL metabolism (358). As a result, the IDL pool size increased four-fold. Expectedly, LDL-FCRs in both patients were markedly reduced to less than one-third of the normal value. Studies with native and chemically modified LDL have shown that this residual LDL-FCR is almost entirely due to receptor independent LDL catabolism (364). Because of decreased catabolism LDL pool size was increased proportionally to the same extent as observed for IDL.

Two observations with the present study are related to apoB synthesis rather than to apoB catabolism. Firstly, both patients had relatively high rates of total apoB-synthesis and in the case of J.C. there was a marked increase of total LDL-apoB production. Five homozygous FH patients (including J.C. and M.M.) had on average a total apoB synthetic rate which was 65% higher than normal (301). This confirms findings from several other studies in heterozygous and homozygous FH patients, where on the basis of LDL-turnover data increased rates of LDL synthesis were calculated (70, 131). Secondly, direct synthesis of LDL and, in the case of J.C., also of IDL was far higher than

observed in the control group, again confirming previous reports (70, 132).

In this context, animal experiments, using the WHHL rabbit as a model for the human disease, are of particular interest (365). In one metabolic study a markedly increased conversion of VLDL to LDL was noted due to a reduction in direct catabolism of IDL which increased the fraction of IDL particles transformed through further delipidation into LDL (366). This mechanism was proposed as an explanation for LDL oversynthesis in humans (365). However, mass transfer from IDL to LDL in the two FH patients of the present study was not increased and was not significantly different from normal controls when three additional homozygous FH patients were considered (301). The effects of reduced IDL clearance were balanced in humans by an equally significant reduction in the transfer rate of IDL to LDL. While a diversion of LDL precursors from direct catabolism to conversion into LDL can, in principal, explain some of the observed increase of LDL-apoB synthesis, this mechanism does not contribute to an understanding, why total apoB synthesis is often increased in familial hypercholesterolaemia.

Another discrepancy between results obtained from experiments with WHHL rabbits and findings in patients with homozygous FH became apparent when the problem of direct LDL synthesis was addressed. In the animal experiments all apoB was found to be synthesised as VLDL accounting as a

precursor for the whole of LDL production (367). Accordingly, in the density range of LDL no apoB-100 was found in liver perfusates from WHHL rabbits (368).

The metabolic study of five homozygous FH patients, including the two subjects discussed in this paragraph, showed some variation of the degree of direct LDL-apo B synthesis ranging from no LDL-apoB synthesis in one subject to 29% of the total apoB synthesis as the other extreme. This correlates negatively with the total triglyceride level observed in these patients ($r = -0.84$) and with the percentage of apoB in plasma being associated with $VLDL_1$ ($r = -0.90$). The number of patients investigated is too small to draw further conclusions. Nevertheless, it is worth noticing that WHHL rabbits are usually mildly hypertriglyceridaemic. It can be speculated that the apoB metabolism in these animals represents the situation in human patients with homozygous FH combined with some hypertriglyceridaemia but little or no direct synthesis of LDL-apoB. Other patients with normal triglyceride levels showed significant input of LDL not derived from VLDL precursors into the LDL pool. Variability of triglyceride levels in homozygous FH has been noticed by several authors (122, 369) but observations regarding the clinical significance of this variation are scarce. In a group of 92 patients with heterozygous FH elevated triglyceride levels were of predictive value for the development of coronary heart disease (Dr M Seed, personal communication).

Recently quantitative and qualitative differences in the VLDL subfraction were reported from WHHL rabbits with high and low incidence rates for CHD (372, 373). It remains to be seen whether or not these observations are of significance for the understanding of the disease in humans.

4.4. The Current Concept of VLDL to LDL Interconversion

Throughout the previous sections the significance of various genetically determined factors for the metabolism of apolipoprotein B has been examined. In this final chapter the results will be presented as part of an integral concept based on the metabolic model which was used for the analyses of the metabolic studies. Moreover, some general features of apoB metabolism will be addressed.

The metabolic model illustrated in Fig. 8 provides for two parallel pathways of delipidation for particles which enter the plasma compartment either as $VLDL_1$ (S_f 60--400) or as $VLDL_2$ (S_f 20-60). Two independent delipidation routes are required because the two VLDL subfractions show quantitative and qualitative differences in their metabolic behaviour (154). In all turnover studies where the metabolism of both VLDL species was monitored simultaneously the following observations were made: $VLDL_2$ was transferred into LDL at a higher rate and more rapidly than $VLDL_1$ and

LDL derived from $VLDL_2$ was catabolised faster than LDL derived from $VLDL_1$. Consequently, the decay curves for LDL derived either from small or from large VLDL show different maxima and terminal decay rates (Fig. 21-23). This has led to the concept of metabolic channels in the VLDL to LDL conversion process. That is, the metabolic characteristics of an apoB containing lipoprotein are in part determined by its origin (374). It has been argued that, since it takes longer for large VLDL to get delipidated and reduced to the particle size of LDL, randomly occurring degradation and elimination from the plasma is more probable, thus reducing the chance of large VLDL becoming LDL. However, different metabolic properties of LDL subfractions derived from different precursor particles are strong evidence for genuine metabolic heterogeneity between VLDL subfractions and support the concept of metabolic channelling. Quantitatively, the percentage of $VLDL_2$ converted into LDL was about double that which had been observed for $VLDL_1$ to LDL conversion. This ratio was remarkably constant for normolipidaemic as well as hyperlipidaemic subjects, indicating that metabolic channelling is a universal feature of apolipoprotein B metabolism. The differences between FCRs for LDL, derived from $VLDL_2$, and LDL, derived from $VLDL_1$, was greatest in apoE2/2, intermediate in apoE3/3 and smallest in apoE4/4 subjects (see k(0,10) and k(0,11) in Table 11). It may be speculated that this has

to do with different levels of receptor-mediated LDL-clearance (see paragraph 4.2.4.) provided LDL derived from $VLDL_2$ binds better to the LDL-receptor than LDL derived from $VLDL_1$.

Examination of LDL metabolism in normolipidaemics with different apoE phenotypes (paragraph 4.2.2.) provides an example for the importance of metabolic heterogeneity within the VLDL density range. Due to different synthetic rates for $VLDL_1$-apoB and $VLDL_2$-apoB, the relative proportions of LDL subspecies were changed in apoE2/2 as compared to apoE3/3 subjects, resulting in a different overall catabolic rate for LDL despite very similar catabolic rates for LDL subspecies.

The mechanisms which predispose different VLDL subspecies for different metabolic fates are unknown yet. One possibility is that the particles differ in their apolipoprotein composition. Havel et al. studied, in rabbits, the metabolism of apoB containing lipoproteins with and without concomitant apoE (375, 376). They found that VLDL which contains both apoB and apoE, is removed from the plasma more rapidly than VLDL containing apoB only and that a smaller fraction of apoB/E particles was converted into LDL. There are obvious similarities between the results of these animal studies and VLDL-turnovers in humans. However, unless it is demonstrated that in humans particles derived from $VLDL_1$ differ in their apoE content from $VLDL_2$ derived lipoproteins, the significance of these

findings remains uncertain. Heterogeneity, other than defined by metabolic properties, has been demonstrated for all apoB containing lipoprotein subclasses (377). Equilibrium density-gradient ultracentrifugation and non-denaturing gradient-gel electrophoresis have revealed for instance the existence of three or even four LDL subclasses which are present in different proportions in males or females, normo- or hypertriglyceridaemia or subjects with or without CHD (378). For the production of IDL and LDL subspecies a hypothetical metabolic model with two pathways for large and small IDL has been proposed and it remains to be seen whether this coincides with the dual pathways of VLDL to LDL transfer outlined above.

A second more general aspect of VLDL to LDL conversion deals with the quantitative relationships between metabolic precursors, $VLDL_1$ and $VLDL_2$, and the end product, LDL. In normolipidaemic apoE3/3 subject about one-third of VLDL-apoB, synthesised as $VLDL_1$ or $VLDL_2$ was transferred into LDL while the remainder was directly catabolised from the VLDL and IDL subcompartments. In three out of five subjects this accounted for 80-100% of the measured LDL pool. In two subjects, however, VLDL precursors accounted for only 65% and 50% of the measured LDL pool, which made it necessary to accept direct LDL synthesis and input into the LDL pool. The rate of direct LDL-apoB synthesis was on average less than 10% of the total apoB synthesis. In apoE4/4 homozygotes approximately half of the VLDL-apoB was transferred into

LDL accounting, in four out of five subjects, for the measured LDL pool. The higher rates of apoB transfer from VLDL to LDL in apoE4/4 was the result of impaired clearance of potential LDL-precursors because of LDL-receptor down-regulation as previously discussed (see section 4.2.). Wherever direct LDL-apoB synthesis occurred, the assumption was that de novo input distributed evenly between the two LDL subcompartments. This, however, is not necessarily so, causing a degree of uncertainty for the calculation of the total LDL-FCR in this situation. In apoE2/2 subjects only one quarter of the apoB synthesised as $VLDL_1$ or $VLDL_2$ was converted into LDL due to the marked decrease of IDL to LDL transfer coupled with increased direct catabolism of IDL and despite the significantly reduced direct catabolism of VLDL lipoproteins. Direct LDL-apoB synthesis was not observed in these subject.

In summary, in normolipidaemic subjects 25-50% of VLDL-apoB are converted into LDL. In some individuals, notably of the apoE3 and apoE4 phenotype, direct LDL-apoB synthesis occurs, but this is less than 15% of total apoB synthesis. Higher percentages of direct LDL-apoB synthesis were observed in homozygous FH where it contributed up to 30% of the total apoB synthesis. These results are similar to findings reported in several studies reported by Grundy and his colleagues (95, 177, 380). The rate of VLDL

conversion into LDL was about 40% and LDL de novo synthesis occurred in a range between 0 and 50% of apoB synthesis.

Experiments with monkeys where the metabolism of deferentially labelled VLDL and LDL was studied simultaneously confirm that significant amounts of LDL are derived from sources other than circulating VLDL (72, 381). Several mechanisms for direct input of LDL are possible. Firstly, particles of the size of LDL may be secreted by the liver, but data are only available from cholesterol fed animals where the directly secreted lipoproteins were markedly different from circulating LDL (382). Secondly, direct input may occur in the density range of IDL with subsequent rapid transformation into LDL (178). Finally, a small VLDL pool may be converted rapidly, within minutes, into LDL. Such a pool would escape exogenous labelling and evidence for this mechanism, using ^{3}H-Leu labelled hepatic VLDL, has been established recently in African green monkeys (72).

A synopsis of the mechanisms involved in VLDL-LDL transformation in humans is given in Fig. 47. In normolipidaemics, apoB enters the plasma compartment as $VLDL_1$ or $VLDL_2$ at a ratio of about 2:1. Some $VLDL_1$ particles are rapidly removed from the plasma but most are delipidated by lipoprotein lipase to form particles of the same density as de novo synthesised $VLDL_2$. Further delipidation of the two lipoprotein subspecies within the density range of $VLDL_2$ and transfer into IDL is mediated by both lipolytic

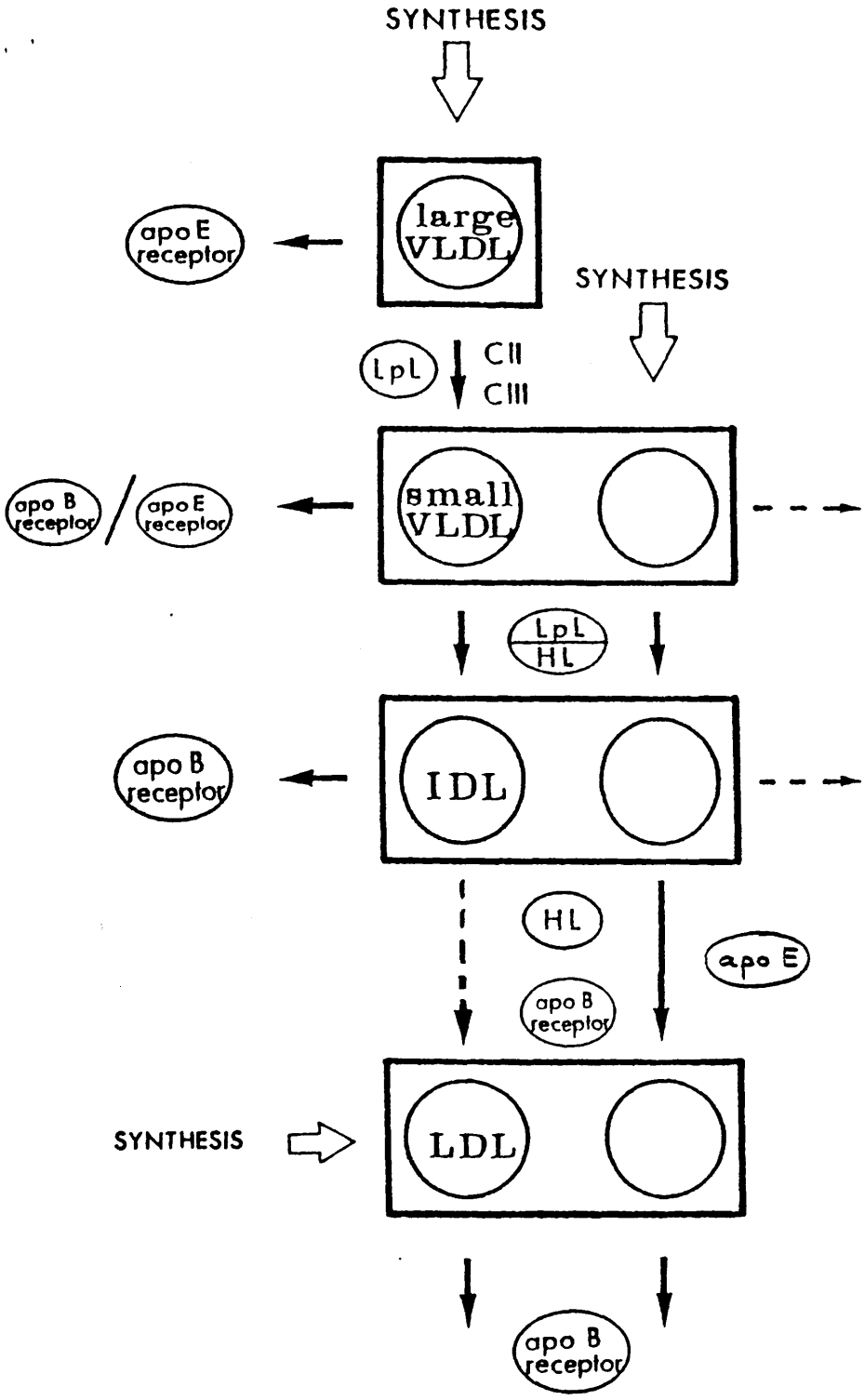


Fig. 47: Metabolic scheme for VLDL to LDL conversion in plasma. - LpL: Lipoprotein lipase; HL: Hepatic lipase; CII, CIII: Apolipoprotein CII and CIII

enzymes, lipoprotein lipase and hepatic lipase. Remnants from this density interval are cleared by receptor mediated uptake either through the "apoE"-receptor or through the LDL-receptor (334). The conversion of IDL into LDL depends on the interaction of three components: hepatic lipase, the LDL-receptor and functional apoE are all important. The anatomical site of IDL to LDL transformation is most likely the liver, as demonstrated by liver perfusion studies in humans, showing hepatic extraction of $VLDL_2$ and IDL (69). Hepatic lipase has been localised by immunofluorescence and immuno-electron microscopy in rat livers exclusively on the liver endothelial cells (383). Endothelial cells also express the LDL-receptor, however, when contact-inhibited they bind lipoproteins but do not internalise them (384). These observations, taken together suggest a mechanism whereby IDL in the hepatic sinusoids is first immobilised by binding to the LDL-receptor, then exposed to the lipolytic activity of hepatic lipase and subsequently released as mature LDL. In-vitro and in-vivo studies show that functional apolipoprotein E is also important for this step but the mechanism of action is not yet known.

The results presented in this thesis were derived from turnover studies, where the metabolic rate of one or two exogenously labelled lipoprotein species had been followed. Observed data from such studies described primarily the catabolism of lipoproteins and only by inference synthetic rates can be obtained. Some of the

most interesting results, such as reduced synthesis of apoB in apoE2 homozygosity or oversynthesis of apoB in homozygous FH, address the problem of how the synthesis of apolipoprotein B is regulated. Reliable measurements of synthetic rates for apolipoprotein B containing lipoproteins would probably enhance the understanding of the regulatory mechanisms involved in a way comparable to the contribution made by metabolic studies based on tracer kinetics. In a recent paper (385) a first approach in this direction was reported. Apolipoprotein B was labelled endogenously by infusions of a stable isotope incorporated in an amino acid (^{15} N-Gly). Fractional synthetic rates for VLDL-apoB were in good agreement with values obtained by tracer experiments. It is hoped that future investigations employing new techniques such as stable isotope kinetics for in-vivo studies in humans will produce useful information, enhancing our knowledge of apolipoprotein B metabolism and contributing to the prevention of atherosclerotic disease.

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APPENDIX

TABLES I-1 to I-25

Table I-1: ApoE3/3 Homozygous Normolipidaemics. - Plasma Lipids and Lipoproteins.

Subjects	Total Trig (mmol/l)	Total Chol	VLDL-Chol	LDL-Chol (mmol/l)	HDL-Chol	VLDL-Chol/ Trigly Ratio
N.C.	1.71 ± 0.38	4.96 ± 0.54	0.79 ± 0.21	3.14 ± 0.35	1.01 ± 0.13	0.46
C.D.	2.55 ± 0.71	6.23 ± 0.79	1.02 ± 0.25	4.09 ± 0.50	1.36 ± 0.19	0.40
M.F.	1.09 ± 0.13	5.76 ± 0.22	0.61 ± 0.14	3.96 ± 0.17	1.19 ± 0.08	0.56
E.K.	0.95 ± 0.06	4.97 ± 0.32	0.35 ± 0.08	2.76 ± 0.17	1.86 ± 0.12	0.37
M.M.	2.17 ± 0.29	5.63 ± 0.35	0.97 ± 0.21	3.74 ± 0.14	1.20 ± 0.05	0.45
Mean ± s	1.69 ± 0.56	5.51 ± 0.49	0.75 ± 0.25	3.54 ± 0.51	1.32 ± 0.29	0.45 ± 0.06

Table I-2: ApoE4/4 Homozygous Normolipidaemics. - Plasma Lipids and Lipoproteins.

Subjects	Total Trig	Total Chol	VLDL-Chol	LDL-Chol	HDL-Chol	VLDL-Chol/Trigly Ratio
	(mmol/l)			(mmol/l)		
T.S.	2.00 ± 0.75	6.55 ± 1.00	1.08 ± 0.54	4.37 ± 0.83	1.10 ± 0.22	0.54
C.R.	1.49 ± 0.28	6.74 ± 0.35	0.61 ± 0.23	4.66 ± 0.41	1.39 ± 0.19	0.41
W.B.	2.01 ± 0.59	6.19 ± 0.34	0.99 ± 0.50	3.96 ± 0.20	1.23 ± 0.14	0.49
M.W.	0.90 ± 0.27	5.66 ± 0.40	0.59 ± 0.28	3.35 ± 0.49	1.73 ± 0.09	0.66
M.D.	0.93 ± 0.18	5.33 ± 0.46	0.32 ± 0.14	3.45 ± 0.44	1.56 ± 0.11	0.34
Mean ± s	1.47 ± 0.49	6.09 ± 0.53	0.72 ± 0.30	3.96 ± 0.51	1.40 ± 0.23	0.49 ± 0.11

Table I-3: ApoE2/2 Homozygous Normolipidaemics. - Plasma Lipids and Lipoproteins.

Subjects	Total Trig (mmol/l)	Total Chol	VLDL-Chol	LDL-Chol (mmol/l)	HDL-Chol	VLDL-Chol/ Trigly Ratio
A.Y.	1.60 ± 0.79	4.88 ± 0.13	1.03 ± 0.64	2.43 ± 0.43	1.42 ± 0.19	0.64
M.B.	1.80 ± 0.27	4.85 ± 0.35	1.07 ± 0.10	2.45 ± 0.26	1.33 ± 0.08	0.59
G.S.	1.85 ± 0.27	6.14 ± 0.83	1.41 ± 0.47	3.25 ± 0.49	1.48 ± 0.23	0.76
E.W.	1.64 ± 0.08	5.96 ± 0.76	1.25 ± 0.24	3.24 ± 0.59	1.48 ± 0.21	0.76
Mean ± s	1.72 ± 0.10	5.46 ± 0.60	1.19 ± 0.15	2.84 ± 0.40	1.43 ± 0.06	0.69 ± 0.07
P.M.	2.85 ± 0.33	4.83 ± 0.38	1.48 ± 0.39	2.21 ± 0.13	1.14 ± 0.08	0.52

Table I-4: ApoE3/3 Homozygous Normolipidaemics. - Apolipoprotein B Concentration, Pool Size and Percentage Distribution Among Plasma Lipoproteins.

Subjects	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma Pool (mg) (= 100%)	ApoB Plasma Concentration (mg/ml)
		(Percentage Distribution)				
N.C.	4.4	8.3	11.4	76.0	1970	0.76
C.D.	2.2	6.6	12.5	78.7	2860	1.10
M.F.	3.8	6.0	11.7	78.5	3000	0.97
E.K.	2.8	4.1	12.9	80.1	1450	0.63
M.M.	5.4	8.2	13.9	72.5	2270	0.91
Mean	3.7	6.6	12.5	77.2	2310	0.87
± s	±1.1	±1.6	± 0.9	± 2.7	± 570	±0.16

Table I-5: ApoE4/4 Homozygous Normolipidaemics. - Apolipoprotein B Concentration, Pool Size and Percentage Distribution Among Plasma Lipoproteins.

Subjects	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma Pool (mg) (= 100%)	ApoB Plasma Concentration (mg/ml)
	(Percentage Distribution)					
T.S.	3.8	6.9	12.6	76.7	3250	1.08
C.B.	1.5	3.6	10.8	84.0	3090	1.23
W.B.	6.1	6.6	10.6	76.7	2270	0.76
M.W.	1.7	3.8	10.1	84.3	1510	0.76
M.D.	0.2	3.4	9.9	86.4	2050	1.02
Mean	2.7	4.9	10.8	81.6	2434	0.9
$\pm$ s	$\pm$2.1	$\pm$1.6	$\pm$ 1.0	$\pm$ 4.1	$\pm$ 652	$\pm$0.18

Table I-6: ApoE2/2 Homozygous Normolipidaemics. - Apolipoprotein B Concentration, Pool Size and Percentage Distribution Among Plasma Lipoproteins.

Subjects	$VLDL_1$	$VLDL_2$	IDL	LDL	ApoB Plasma Pool (mg) (= 100%)	ApoB Plasma Concentration (mg/ml)
		(Percentage Distribution)				
A.Y.	3.9	23.0	21.6	51.5	984	0.33
M.B.	5.9	20.2	28.5	45.4	995	0.47
G.S.	7.2	14.4	31.7	46.8	1390	0.53
E.W.	5.5	20.2	32.1	42.2	1090	0.47
Mean	5.6	19.5	28.5	46.5	1115	0.45
± s	±1.2	± 3.1	± 4.2	± 3.3	± 164	0.07
P.M.	7.7	20.4	24.0	47.9	1220	0.61

Table I-7: ApoE3/3 Homozygous Normolipidaemics. - Physical Parameters and Diet

Subjects	Sex (M/F)	Age (Years)	Body Weight (kg)	Height (cm)	kcal/ day	Energy (%) from: Protein	CHO	Alcohol	Fat	P/S Ratio
N.C.	M	36	71	179	2244	17	45	-	37	0.11
C.D.	M	43	86	172	2620	11	38	30	21	0.11
M.F.	M	46	89	173	2234	21	30	7	42	0.60
E.K.	F	40	71	175	2333	14	48	-	38	0.20
M.M.	F	44	76	161	1666	10	42	-	48	0.30
Mean ± s		42 ± 3	79 ± 8	172 ± 6	2219 ± 310	14.6 ± 4.0	40.6 ± 6.2	7.6 ±11.5	37.2 ± 8.9	0.26 ±0.18

Table I-8: ApoE4/4 Homozygous Normolipidaemics. - Physical Parameters and Diet

Subjects	Sex (M/F)	Age (Years)	Body Weight (kg)	Height (cm)	kcal/ day	Energy (%) from: Protein	CHO	Alcohol	Fat	P/S Ratio
T.S.	M	29	85	181	2229	18	38	6	38	0.11
C.B.	M	41	62	153	2954	17	35	-	48	0.31
W.B.	M	45	79	173	1990	18	37	9	36	0.19
M.W.	F	29	50	158	1934	11	55	-	34	0.20
M.D.	F	36	48	157	1358	14	46	-	40	0.40
Mean ± s		36 ± 6	65 ±15	164 ± 11	2093 ± 517	16 ± 3	42 ± 7	3 ±4	39 ± 5	0.24 ±0.10

Table I-9: ApoE2/2 Homozygous Normolipidaemics. - Physical Parameters and Diet

Subjects	Sex (M/F)	Age (Years)	Body Weight (kg)	Height (cm)	kcal/ day	Energy (%) from: Protein	CHO	Alcohol	Fat	P/S Ratio
A.Y.	M	47	74	170	-	-	-	-	-	-
M.B.	F	39	58	159	2325	13	53	-	34	0.80
G.S.	F	45	63	166	1044	20	36	12	32	0.19
E.W.	F	57	53	158	1466	12	53	4	31	0.10
Mean ± s		47 ± 6	62 ± 8	161 ± 6		15 ± 4	47 ± 8	4 ±5	32 ± 1	0.36 ±0.31
P.M.	F	35	58	154	1405	11	46	-	43	0.10

TABLE I-10 A:

Metabolism of large VLDL (Sf 60-400) in apoE3/3 homozygotes

n = 5

averages are % of initially injected radioactivity

E3/3: VLDL1 (injected as tracer)

TIME	AVG	STD	FSD
0	0.742	0.042	0.057
0.5	0.618	0.068	0.11
1	0.475	0.105	0.221
1.5	0.374	0.101	0.271
2	0.264	0.097	0.368
3	0.178	0.076	0.429
4	0.103	0.046	0.454
6	0.047	0.026	0.568
8	0.026	0.013	0.53
10	0.013	0.009	0.69
14	0.006	0.002	0.353
24	0.009	0.008	0.692

E3/3: VLDL2 (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.2	0.028	0.141
0.5	0.289	0.101	0.349
1	0.35	0.035	0.1
1.5	0.443	0.075	0.169
2	0.452	0.085	0.187
3	0.463	0.106	0.229
4	0.45	0.129	0.259
6	0.384	0.123	0.321
8	0.331	0.134	0.399
10	0.236	0.079	0.337
14	0.132	0.063	0.479
24	0.038	0.012	0.317
48	0.014	0.006	0.449
72	0.007	0.003	0.412
96	0.003	0	0.255
120	0.004	0.001	0.267

E3/3: IDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.023	0.007	0.31
1	0.057	0.02	0.36
2	0.063	0	0
3	0.1	0.034	0.331
4	0.106	0.023	0.22
6	0.147	0.057	0.392
8	0.21	0.071	0.376
10	0.194	0.05	0.259
14	0.224	0.043	0.194
24	0.174	0.042	0.245
48	0.1	0.022	0.224
72	0.053	0.012	0.238
96	0.028	0.011	0.249
120	0.018	0.005	0.305
144	0.015	0.007	0.483
168	0.013	0.009	0.672
192	0.008	0.005	0.608
216	0.005	0.002	0.396
240	0.003		

E3/3: LDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.014	0.005	0.35
1	0.02	0.005	0.285
2	0.029	ERR	ERR
3	0.028	0.004	0.159
4	0.041	0.01	0.286
6	0.041	0.013	0.322
8	0.059	0.028	0.469
10	0.077	0.03	0.397
14	0.132	0.057	0.434
24	0.163	0.083	0.508
48	0.166	0.063	0.382
72	0.154	0.039	0.259
96	0.132	0.037	0.284
120	0.109	0.04	0.37
144	0.078	0.023	0.3
168	0.068	0.023	0.345
192	0.057	0.021	0.381
216	0.051	0.019	0.376
240	0.038	0.015	0.408
264	0.025	0.012	0.481
288	0.024	0.012	0.501

TABLE I-10 B:

Metabolism of small VLDL (Sf 20-60) in apoE3/3 homozygotes

n = 5

averages are % of initially injected radioactivity

E3/3: VLDL2 (injected as tracer)

TIME	AVG	STD	FSD
0	0.82	0.026	0.031
1	0.737	0.035	0.048
2	0.587	0.028	0.048
3	0.54	0.05	0.093
4	0.444	0.089	0.177
6	0.329	0.053	0.176
8	0.206	0.047	0.231
10	0.148	0.026	0.241
14	0.061	0.016	0.263
24	0.019	0.008	0.437
48	0.005	0.002	0.413
72	0.002	<0.001	0.267
96	0.001	<0.001	0.222

E3/3: IDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.098	0.014	0.152
1	0.164	0.036	0.223
2	0.211	0.075	0.419
3	0.269	0.061	0.206
4	0.316	0.082	0.26
6	0.368	0.08	0.219
8	0.382	0.072	0.181
10	0.365	0.052	0.143
14	0.302	0.038	0.128
24	0.192	0.052	0.251
48	0.083	0.023	0.277
72	0.043	0.012	0.279
96	0.024	0.007	0.303
120	0.014	0.006	0.484
144	0.01	0.003	0.379
168	0.008	0.004	0.562
192	0.007	0.004	0.536
216	0.005	0.002	0.588
240	0.005	0.002	0.599
264	0.004	0.003	0.75

E3/3: LDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.007	0.004	0.571
1	0.017	0.008	0.514
2	0.016	0.005	0.374
3	0.036	0.017	0.475
4	0.088	0.047	0.531
6	0.114	0.05	0.438
8	0.191	0.091	0.482
10	0.225	0.093	0.414
14	0.327	0.089	0.289
24	0.37	0.091	0.249
48	0.306	0.063	0.208
72	0.235	0.043	0.171
96	0.196	0.048	0.247
120	0.156	0.053	0.34
144	0.105	0.027	0.262
168	0.089	0.031	0.346
192	0.073	0.024	0.329
216	0.06	0.02	0.337
240	0.05	0.021	0.418
264	0.039	0.004	0.122
288	0.041	0.013	0.393

TABLE I-11 A:

Metabolism of large VLDL (Sf 60-400) in apoE4/4 homozygotes

n = 5

averages are% of initially injected radioactivity

E4/4: VLDL1 (injected as tracer)

TIME	AVG	STD	FSD
0	0.742	0.104	0.141
0.5	0.673	0.116	0.172
1	0.497	0.139	0.279
1.5	0.463	0.209	0.452
2	0.462	0.176	0.381
3	0.269	0.183	0.679
4	0.193	0.151	0.776
6	0.142	0.109	0.771
8	0.085	0.067	0.794
10	0.041	0.041	1.012
14	0.016	0.014	0.866

E4/4: VLDL2 (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.222	0.096	0.435
0.5	0.334	0.155	0.462
1	0.394	0.179	0.456
1.5	0.422	0.175	0.415
2	0.502	0.135	0.269
3	0.496	0.128	0.258
4	0.518	0.076	0.148
6	0.476	0.101	0.213
8	0.426	0.133	0.314
10	0.346	0.073	0.212
14	0.226	0.069	0.311
24	0.129	0.037	0.289
48	0.032	0.012	0.387
72	0.016	0.007	0.449
96	0.011	0.003	0.374
120	0.007	0.003	0.484

E4/4: IDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.066	0.002	0.037
0.5	0.037	0.026	0.702
1	0.031	0.032	1.024
2	0.039	0.028	0.725
3	0.053	0.029	0.559
4	0.074	0.035	0.481
6	0.084	0.041	0.472
8	0.135	0.057	0.424
10	0.145	0.061	0.426
14	0.188	0.056	0.301
24	0.224	0.079	0.353
48	0.152	0.032	0.213
72	0.093	0.027	0.294
96	0.069	0.043	0.616
120	0.046	0.021	0.447
144	0.028	0.011	0.363
168	0.017	0.006	0.399
192	0.014	0.006	0.406
216	0.017	0.006	0.362
240	0.011	0.006	0.545
264	0.009	0.004	0.481
288	0.009	0.004	0.444

E4/4: LDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.008	0.005	0.614
0.5	0.009	0.004	0.453
1	0.012	0.008	0.657
1.5	0.013	0.007	0.555
2	0.012	0.004	0.322
3	0.018	0.005	0.302
4	0.011	0.008	0.809
6	0.032	0.021	0.646
8	0.031	0.022	0.727
10	0.038	0.024	0.634
14	0.062	0.031	0.503
24	0.102	0.043	0.427
48	0.129	0.054	0.417
72	0.148	0.058	0.394
96	0.144	0.043	0.302
120	0.127	0.027	0.212
144	0.106	0.021	0.195
168	0.081	0.025	0.314
192	0.069	0.021	0.305
216	0.055	0.017	0.322
240	0.051	0.015	0.308
264	0.041	0.014	0.341
288	0.039	0.005	0.133

TABLE I-11 B:

Metabolism of small VLDL2 (Sf 20-60) in apoE4/4 homozygotes

n = 5

averages are% of initially injected radioactivity

E4/4: VLDL2 (injected as tracer)

TIME	AVG	STD	FSD
0	0.811	0.059	0.073
0.5	0.809	0.079	0.098
1	0.781	0.095	0.122
1.5	0.712	0.102	0.144
2	0.616	0.094	0.152
3	0.629	0.154	0.244
4	0.555	0.149	0.269
6	0.406	0.122	0.301
8	0.309	0.097	0.316
10	0.225	0.058	0.258
14	0.108	0.034	0.315
24	0.066	0.026	0.391
48	0.011	0.003	0.328
72	0.006	0.002	0.413
96	0.003	0.001	0.415

E4/4: IDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.145	0.051	0.345
0.5	0.122	0.038	0.311
1	0.107	0.011	0.099
1.5	0.183	0.061	0.337
2	0.175	0.032	0.182
3	0.234	0.057	0.245
4	0.337	0.033	0.099
6	0.332	0.072	0.219
8	0.388	0.096	0.249
10	0.375	0.107	0.286
14	0.356	0.109	0.307
24	0.292	0.071	0.242
48	0.131	0.034	0.261
72	0.092	0.027	0.303
96	0.044	0.028	0.646
120	0.037	0.023	0.627
144	0.024	0.012	0.508
168	0.021	0.008	0.402
192	0.015	0.008	0.597
216	0.018	0.005	0.303
240	0.012	0.008	0.712
264	0.013	0.004	0.353
288	0.009	0.004	0.456

E4/4: LDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.004	0.002	0.431
0.5	0.006	0.001	0.272
1	0.012	0.007	0.642
1.5	0.021	0.011	0.583
2	0.012	0.004	0.339
3	0.042	0.021	0.492
4	0.057	0.014	0.247
6	0.113	0.063	0.553
8	0.139	0.075	0.541
10	0.159	0.044	0.275
14	0.258	0.071	0.275
24	0.348	0.088	0.254
48	0.351	0.088	0.251
72	0.309	0.071	0.231
96	0.264	0.051	0.195
120	0.217	0.039	0.182
144	0.174	0.026	0.152
168	0.135	0.019	0.144
192	0.113	0.021	0.191
216	0.095	0.018	0.197
240	0.079	0.014	0.185
264	0.065	0.017	0.271
288	0.047	0.029	0.633

TABLE I-12 A:

Metabolism of large VLDL (Sf 60-400) in apoE2/2 homozygotes

n = 4

averages are % of initially injected radioactivity

E2/2: VLDL1 (injected as tracer)

TIME	AVG	STD	FSD
0	0.741	0.051	0.068
0.5	0.756	0.071	0.094
1	0.585	0.015	0.025
1.5	0.532	0.025	0.047
2	0.512	0.116	0.227
3	0.466	0.145	0.311
4	0.394	0.115	0.293
6	0.197	0.045	0.231
8	0.217	0.106	0.491
10	0.214	0.101	0.473
14	0.081	0.042	0.529
24	0.067	0.042	0.629
48	0.021	0.006	0.331
72	0.008	0.005	0.677

E2/2: VLDL2 (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.198	0.022	0.111
0.5	0.356	0.081	0.225
1	0.465	0.118	0.254
1.5	0.581	0.118	0.204
2	0.638	0.102	0.159
3	0.691	0.116	0.169
4	0.744	0.132	0.177
6	0.833	0.033	0.041
8	0.732	0.067	0.092
10	0.695	0.062	0.089
24	0.508	0.042	0.083
48	0.175	0.009	0.059
72	0.104	0.041	0.401
96	0.043	0.003	0.087
120	0.034	0.021	0.627
144	0.028	0.015	0.556
168	0.016	0.006	0.389
192	0.012	0.005	0.461
216	0.007	0.003	0.505
240	0.005	0.003	0.596
264	0.005	0.001	0.399

E2/2: IDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.012	0.003	0.317
0.5	0.028	0.015	0.552
1	0.031	0.015	0.486
1.5	0.045	0.035	0.763
2	0.034	0.017	0.519
3	0.049	0.022	0.449
4	0.057	0.026	0.456
6	0.076	0.025	0.329
8	0.107	0.042	0.395
10	0.129	0.042	0.324
14	0.223	0.036	0.162
24	0.217	0.083	0.382
48	0.196	0.064	0.326
72	0.125	0.043	0.348
96	0.091	0.023	0.253
120	0.056	0.006	0.111
144	0.041	0.005	0.137
168	0.036	0.004	0.135
192	0.028	0.005	0.176
216	0.021	0.006	0.276
240	0.018	0.003	0.185
264	0.007	<0.001	0.066
288	0.006	<0.001	0.076

E2/2: LDL (derived from VLDL1)

TIME	AVG	STD	FSD
0	0.007	0.001	0.143
0.5	0.008	0.003	0.408
1	0.011	0.003	0.361
1.5	0.012	0.003	0.232
2	0.012	0.002	0.226
4	0.012	0.004	0.333
6	0.015	0.005	0.354
8	0.018	0.008	0.467
10	0.022	0.008	0.394
14	0.043	0.012	0.292
24	0.042	0.014	0.331
48	0.039	0.012	0.322
72	0.043	0.016	0.379
96	0.044	0.022	0.522
120	0.043	0.019	0.442
144	0.026	0.008	0.313
168	0.023	0.008	0.347
192	0.021	0.011	0.512
216	0.022	0.009	0.445
240	0.019	0.008	0.435

TABLE I-12 B:

Metabolism of small VLDL (Sf 20-60) in apoE2/2 homozygotes

n = 4

averages are % of initially injected radioactivity

E2/2: VLDL2 (injected as tracer)

TIME	AVG	STD	FSD
0	0.885	0.049	0.056
0.5	0.891	0.041	0.046
1	0.807	0.101	0.125
1.5	0.853	0.093	0.109
2	0.783	0.073	0.093
3	0.717	0.098	0.136
4	0.633	0.038	0.061
6	0.633	0.106	0.167
8	0.532	0.091	0.169
10	0.427	0.056	0.131
14	0.319	0.089	0.281
24	0.223	0.012	0.055
48	0.071	0.011	0.162
72	0.033	0.011	0.331
96	0.015	0.002	0.134
120	0.011	0.003	0.324
144	0.005	0.002	0.408
168	0.004	0.001	0.353
192	0.003	<0.001	0.128

E2/2: IDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.073	0.038	0.519
0.5	0.112	0.041	0.363
1	0.149	0.066	0.442
1.5	0.178	0.069	0.386
2	0.173	0.084	0.491
3	0.224	0.089	0.397
4	0.266	0.082	0.309
6	0.296	0.048	0.161
8	0.316	0.068	0.217
10	0.351	0.062	0.177
14	0.373	0.068	0.183
24	0.279	0.043	0.154
48	0.192	0.036	0.189
72	0.101	0.029	0.287
96	0.066	0.011	0.155
120	0.037	0.001	0.049
144	0.027	0.002	0.104
168	0.022	<0.001	0.037
192	0.016	0.002	0.159
216	0.013	0.003	0.252
240	0.011	0.003	0.264
264	0.005	0.001	0.201

E2/2: LDL (derived from VLDL2)

TIME	AVG	STD	FSD
0	0.003	0.002	0.622
0.5	0.003	0.002	0.509
1	0.007	0.004	0.536
1.5	0.011	0.002	0.143
2	0.009	0.001	0.129
3	0.011	0.001	0.129
4	0.017	0.007	0.411
6	0.028	0.007	0.253
8	0.046	0.019	0.427
10	0.064	0.018	0.286
14	0.102	0.032	0.321
24	0.089	0.021	0.229
48	0.071	0.012	0.172
72	0.063	0.017	0.269
96	0.059	0.007	0.131
120	0.052	0.005	0.109
144	0.036	0.011	0.322
168	0.031	0.008	0.284
192	0.023	0.008	0.378
216	0.023	0.005	0.248
240	0.021	0.005	0.279

TABLE I-13:

Calculated compartment masses and rate constants in apoE3/3 homozygotes

Name	M(1)	k(0,1)	k(2,1)	M(2)	k(4,2)	k(6,2)
NC	50	0.48	7.87	20	19.9	0.07
CD	47	2.13	6.94	19	17.4	0.04
MF	78	6.43	6.67	22	24	0.23
EK	41	13.28	4.43	8	24	0.34
MM	91	3.98	5.61	21	24	0.11
mean	61	5.26	6.3	18	21.9	0.16
± s	±20	±4.47	±1.18	±5	±2.7	±0.11

Name	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
NC	83	1.26	2.77	0.71	0	59
CD	66	2.91	1.44	0.62	0	143
MF	129	1.29	2.2	0.2	0.3	48
EK	29	0.69	4.76	0.68	0	36
MM	147	1.92	1.24	0.26	0.02	34
mean	91	1.61	2.48	0.49	0.06	64
± s	±43	±0.76	±1.26	±0.21	±0.12	±41

Name	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	M(7)
NC	2.61	4.05	0	3	0.51	49
CD	2.28	2.33	0	2	0.41	199
MF	0	3.66	0	7	0.72	66
EK	3.38	1.94	0	3	0.93	71
MM	1.74	4.67	1.21	4	0.52	85
mean	2.01	3.33	0.24	3.8	0.62	94
± s	±1.13	±1.03	±0.48	±1.7	±0.19	±54

Name	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)	M(9)
NC	0	4.92	71	1.1	2.15	125
CD	0	1.67	63	0.38	1.12	141
MF	0	2.63	241	0.31	0.87	83
EK	0	0.98	51	1.28	1.44	83
MM	0	1.86	176	0.34	0.7	93
mean	0	2.41	120	0.68	1.26	105
± s	0	±1.36	±75	±0.42	±0.51	±24

TABLE I-13 (contd.)

Name	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
NC	0.47	797	0.3	725	0.21
CD	0.29	1148	0.29	470	0.15
MF	0.31	644	0.27	1459	0.17
EK	0.24	257	0.27	308	0.24
MM	0.41	769	0.26	548	0.23
mean	0.34	723	0.28	702	0.2
± s	±0.08	±287	±0.01	±402	±0.03

TABLE I-14:

Calculated compartment masses and rate constants in apoE4/4 homozygotes

Name	M(1)	k(0,1)	k(2,1)	M(2)	k(4,2)	k(6,2)
TS	85	4.15	3.58	13	24	0.23
CB	39	4.57	5.29	9	24	0.24
WB	105	2.53	1.98	53	3.89	0.01
MW	21	18.1	3.91	7	12.43	0.08
MD	17	0	8.16	12	11.21	0.03
mean	53.4	5.87	4.58	18.8	15.1	0.12
± s	±35.3	±6.32	±2.08	±17.2	±7.8	±0.10

Name	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
TS	118	0.6	1.88	0.07	0	114
CB	87	1.56	0.46	0.28	0.04	25
WB	91	0	1.84	0.44	0	24
MW	34	0.5	1.5	0.36	0.06	13
MD	38	2.42	0.49	0.27	0.47	28
mean	73.6	1.02	1.23	0.28	0.11	40.8
± s	±32.5	±0.86	±0.63	±0.12	±0.18	±37.0

Name	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	M(7)
TS	0	2.84	0	5	0.53	212
CB	0	9.78	0	4	0.48	193
WB	0	7.51	0	2	0.28	89
MW	0	15.96	0	1	0.38	76
MD	0	12.29	0	1	0.4	135
mean	0	9.68	0	2.6	0.41	141
± s	0	±4.42	0	±1.62	±0.09	±54

Name	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)	M(9)
TS	0	1.53	202	0.52	0.58	52
CB	0	1.27	52	0	0.77	110
WB	0	2.03	84	0.58	1.41	103
MW	0	2.71	49	0.48	0.57	63
MD	0	2.54	28	0.39	0.28	41
mean	0	2.02	83	0.39	0.72	73.8
± s	0	±0.56	±62	±0.21	±0.38	±27.7

TABLE I-14 (contd.)

Name	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
TS	0.16	1473	0.22	688	0.17
CB	0.22	1114	0.22	435	0.1
WB	0.39	819	0.22	592	0.2
MW	0.19	986	0.21	176	0.17
MD	0.25	1434	0.24	118	0.22
mean	0.24	1165	0.22	402	0.17
± s	±0.08	±254	±0.01	±224	±0.04

TABLE I-15:

Calculated compartment masses and rate constants in apoE2/2 homozygotes

Name	M(1)	k(0,1)	k(2,1)	M(2)	k(4,2)	k(6,2)
AY	38	0	2.08	3	24	0.66
MB	41	0	5.66	10	24	0.11
GS	68	0.77	2.99	11	19.01	0.11
EW	57	0.41	4.25	14	17.33	0.13
mean	51	0.3	3.75	9.5	21.1	0.25
± s	±12.2	±0.32	±1.35	±4.03	±3.0	±0.24
PM	50	2.84	11.32	23	24	0.09

Name	M(4)	k(0,4)	k(8,4)	k(9,4)	k(11,4)	M(5)
AY	92	0	0.67	0.14	0.03	104
MB	194	0	0.9	0.29	0	6
GS	146	0.03	0.83	0.44	0.08	59
EW	202	0.09	0.92	0.08	0.1	14
mean	159	0.03	0.83	0.24	0.05	45.8
± s	±44	±0.04	±0.10	±0.14	±0.04	±39.2
PM	176	0.43	2.3	0	0.46	52

Name	k(0,5)	k(7,5)	k(10,5)	M(6)	k(0,6)	M(7)
AY	0	3.2	0	7	0.3	192
MB	0	15.3	0	4.6	0.23	93
GS	0	7.99	0	4	0.29	255
EW	0	14.2	0.17	6.4	0.28	118
mean	0	10.2	0.04	5.5	0.28	165
± s	0	±4.9	±0.07	±1.24	±0.03	±64
PM	0	5.13	0	4	0.47	53

Name	k(0,7)	k(10,7)	M(8)	k(0,8)	k(11,8)	M(9)
AY	1.21	0.52	13	4.8	0	41
MB	0.43	0.56	34	4.56	0.64	151
GS	1.24	0.61	70	1.73	0	169
EW	0.49	1.2	137	1.36	0	95
mean	0.84	0.72	63.5	3.11	0.16	114
± s	±0.38	±0.28	±47.1	±1.58	±0.28	±50
PM	0	5.04	270	1.5	0	0

TABLE I-15 (contd.)

Name	k(0,9)	M(10)	k(0,10)	M(11)	k(0,11)
AY	0.31	417	0.24	23	0.13
MB	0.37	217	0.24	135	0.16
GS	0.38	520	0.3	84	0.14
EW	0.17	378	0.38	67	0.3
mean	0.31	383	0.29	77.3	0.18
± s	±0.08	±109	±0.06	±40.1	±0.07
PM	0	282	0.94	261	0.31

TABLE I-16: FSD of rate constants (apoE3/3)

	NC	CD	MF	EK	MM
k(0,1)	0.423	0.164	0.035	0.016	0.034
k(2,1)	0.026	0.051	0.031	0.046	0.023
k(4,2)	0.166	0.239	0.157	0.098	0.087
k(6,2)	0.137	0.212	0.138	0.089	0.087
k(0,6)	0.012	0.022	0.008	0.01	0.013
k(0,4)	0.133	0.058	0.099	0.362	0.028
k(0,5)	0.266	0.082	0	0.063	0.669
k(8,4)	0.057	0.093	0.043	0.051	0.031
k(0,8)	0.144	0.282	0.149	0.073	0.081
k(7,5)	0.136	0.055	0.215	0.054	0.173
k(0,7)	0	0	0	0	0
k(9,4)	0.044	0.084	0.172	0.04	0.073
k(0,9)	0.015	0.029	0.038	0.015	0.015
k(11,8)	0.059	0.107	0.059	0.034	0.036
k(0,11)	0.017	0.041	0.144	0.011	0.014
k(10,7)	0.11	0.044	0.178	0.056	0.092
k(0,10)	0.037	0.022	0.059	0.028	0.038
k(10,5)	0	0	0	0	0.194
k(11,4)	0	0	0.125	0	0.368
mean	0.094	0.083	0.087	0.055	0.108
± s	±0.105	±0.081	±0.068	±0.078	±0.157

TABLE I-17: FSD of rate constants (apoE4/4)

	TS	CB	WB	MW	MD
k(0,1)	0.019	0.027	0.022	0.009	0
k(2,1)	0.022	0.023	0.027	0.029	0.034
k(4,2)	0.086	0.115	0.08	0.073	0.111
k(6,2)	0.092	0.115	0.082	0.078	0.094
k(0,6)	0.017	0.012	0.019	0.011	0.008
k(0,4)	0.123	0.027	0	0.108	0.026
k(0,5)	0	0	0	0	0
k(8,4)	0.031	0.051	0.069	0.034	0.083
k(0,8)	0.066	0	0.179	0.075	0.125
k(7,5)	0.047	0.096	0.109	0.073	0.062
k(0,7)	0	0	0		0
k(9,4)	0.135	0.069	0.041	0.054	0.098
k(0,9)	0.067	0.027	0.011	0.021	0.023
k(11,8)	0.033	0.08	0.046	0.047	0.143
k(0,11)	0.018	0.045	0.014	0.021	0.024
k(10,7)	0.037	0.034	0.091	0.047	0.032
k(0,10)	0.023	0.038	0.047	0.026	0.018
k(10,5)	0	0	0	0	0
k(11,4)	0	0.126	0	0.187	0.063
mean	0.043	0.047	0.044	0.05	0.05
± s	±0.04	±0.041	±0.047	±0.045	±0.045

TABLE I-18: FSD of rate constants (apoE2/2)

	AY	MB	GS	EW	PM
k(0,1)	0	0	0.094	0.214	0.085
k(2,1)	0.028	0.014	0.024	0.021	0.021
k(4,2)	0.172	0.134	0.108	0.076	0.088
k(6,2)	0.162	0.132	0.101	0.076	0.08
k(0,6)	0.005	0.009	0.006	0.005	0.006
k(0,4)	0	0	2.369	0.389	0.187
k(0,5)	0	÷	0	0	0
k(8,4)	0.119	0.088	0.069	0.032	0.026
k(0,8)	0.134	0.103	0.098	0.038	0.019
k(7,5)	0.039	0.294	0.081	0.452	0.121
k(0,7)	0.05	0.442	0.077	0.371	0
k(9,4)	0.029	0.026	0.037	0.074	0
k(0,9)	0.009	0.012	0.012	0.05	0.017
k(11,8)	0	0.083	0	0	0
k(0,11)	0.036	0.023	0.031	0.027	0.016
k(10,7)	0.027	0.108	0.038	0.077	0.155
k(0,10)	0.016	0.119	0.029	0.06	0.054
k(10,5)	0	0	0	1.234	0
k(11,4)	0.181	0	0.056	0.04	0.038
mean	0.056	0.093	0.174	0.168	0.046
± s	±0.063	±0.114	±0.533	±0.293	±0.059

TABLE I-19:

Apolipoprotein B metabolism in apoE3/3 homozygotes

VLDL (Sf 60-400)	direct synth. [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to VLDL2 [pools/d]
NC	418	50	0.48	7.87
CD	426	47	2.13	6.94
MF	1022	78	6.43	6.67
EK	726	41	13.28	4.43
MM	872	91	3.98	5.6
mean	693	61	5.26	6.3
± s	±240	±20	±4.47	±1.18

VLDL (Sf 20-60)	direct synth. [mg/d]	flux from VLDL1 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to IDL&LDL [pools/d]
NC	393	394	164	1.58	3.22
CD	659	326	230	2.26	2.04
MF	174	520	205	0.84	2.55
EK	190	182	75	1.91	3.04
MM	259	510	208	1.65	2.04
mean	335	386	176	1.65	2.58
± s	±179	±125	±55	±0.47	±0.49

IDL (Sf 12-20)	direct synth. [mg/d]	flux from VLDL2 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to LDL [pools/d]
NC	0	528	242	0.57	1.62
CD	0	469	405	0.16	1.01
MF	0	484	389	0.26	0.98
EK	0	228	204	0.42	0.7
MM	0	380	353	0.28	0.8
mean	0	418	319	0.34	1.02
± s		±106	±81	±0.14	±0.32

TABLE I-19 (contd.)

LDL (Sf 0-12)	direct synth. [mg/d]	flux from IDL&VLDL2 [mg/d]	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
NC	0	391	1495	0.26	811
CD	97	404	2000	0.25	1307
MF	0	422	2141	0.2	1196
EK	95	143	950	0.25	1063
MM	0	326	1294	0.25	1131
mean	38	337	1576	0.24	1102
± s	±47	±102	±442	±0.02	±166

TABLE I-20:

Apolipoprotein B metabolism in apoE4/4 homozygotes

VLDL (Sf 60-400)

	direct synth. [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to VLDL2 [pools/d]
TS	657	85	4.15	3.58
CB	384	39	4.57	5.29
WB	474	105	2.53	1.98
MW	461	21	18.05	3.91
MD	139	17	0	8.16
mean	423	53	5.86	4.58
± s	±168	±35	±6.3	±2.08

VLDL (Sf 20-60)

	direct synth. [mg/d]	flux from VLDL1 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to IDL&LDL [pools/d]
TS	324	304	250	0.3	2.22
CB	245	206	125	1.1	2.5
WB	180	208	170	0.01	2.28
MW	207	82	55	0.32	4.95
MD	344	139	79	1.15	4.95
mean	260	188	136	0.58	3.38
± s	±64	±75	±69	±0.46	±1.29

IDL (Sf 12-20)

	direct synth. [mg/d]	flux from VLDL2 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to LDL [pools/d]
TS	0	554	466	0.24	0.95
CB	0	309	355	0.07	0.8
WB	0	387	272	0.33	1.1
MW	0	272	191	0.18	1.23
MD	0	373	203	0.1	1.73
mean	0	379	297	0.18	1.16
± s		±97	±103	±0.09	±0.32

TABLE I-20 (contd.)

LDL (Sf 0-12)

	direct synth. [mg/d]	flux from IDL&VLDL2	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
TS	0	441	2161	0.2	981
CB	93	289	2100	0.18	817
WB	0	298	1417	0.21	654
MW	0	237	1215	0.2	668
MD	0	370	1581	0.23	483
mean	19	327	1695	0.2	721
± s	±37	±71	±375	±0.02	±168

TABLE I-21:

Apolipoprotein B metabolism in apoE2/2 homozygotes

VLDL (Sf 60-400)	direct synth. [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to VLDL2 [pools/d]
AY	79	38	0	2.08
MB	232	41	0	5.66
GS	255	68	0.77	2.99
EW	265	57	0.41	4.25
mean	208	51	0.3	3.75
± s	±75	±12	±0.32	±1.35
PM	708	50	2.84	11.32

VLDL (Sf 20-60)	direct synth. [mg/d]	flux from VLDL1 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to IDL&LDL [pools/d]
AY	333	79	206	0.01	1.99
MB	92	232	215	0	1.5
GS	471	203	220	0.02	3.04
EW	201	242	238	0.08	1.78
mean	274	189	220	0.03	2.08
± s	±142	±65	±12	±0.03	±0.58
PM	266	566	258	0.3	2.91

IDL (Sf 12-20)	direct synth. [mg/d]	flux from VLDL2 [mg/d]	plasma pool [mg]	fract. rate direct catab. [pools/d]	fract. rate transfer to LDL [pools/d]
AY	0	407	250	1.23	0.64
MB	0	323	283	0.88	0.26
GS	0	656	498	1.01	0.31
EW	0	401	345	0.75	0.41
mean	0	447	344	0.97	0.41
±s		±125	±95	±0.18	±0.15
PM	0	671	325	1.25	0.82

TABLE I-21 (contd.)

LDL (Sf 0-12)	direct synth. [mg/d]	flux from IDL [mg/d]	plasma pool [mg]	LDL-FCR [pools/d]	total apo B synth. [mg/d]
AY	0	164	438	0.24	412
MB	0	74	358	0.21	324
GS	0	168	596	0.28	726
EW	0	161	439	0.37	466
mean	0	142	458	0.28	482
± s		±39	±86	±0.06	±150
PM	0	347	549	0.63	974

Table I-22: Plasma lipids and lipoproteins in control and in homozygous FH subjects

Subject	Sex	Age	Body Weight	Plasma Triglyceride	Plasma Cholesterol	Cholesterol in			Current Therapy
						VLDL	LDL	HDL	
		yr	*kg*				*mmol · l⁻¹*		
N_1	M	36	71	1.71	4.96	0.79	3.14	1.01	
N_2	F	56	51	0.94	3.76	0.33	1.66	1.78	
N_3	F	28	77	0.55	3.71	0.30	1.95	1.46	
N_4	M	45	79	2.01	6.19	0.99	3.96	1.23	
N_5	M	29	85	2.00	6.55	1.08	4.37	1.10	
FH_1	M	15	53	3.54	14.15	3.43	8.70	0.69	Plasmapheresis
FH_2	M	21	50	2.87	14.10	1.47	11.98	0.65	Plasmapheresis
FH_3	M	14	59	2.25	12.30	0.7	10.80	0.90	Cholestyramine
FH_4	M	10	40	1.95	14.63	0.83	12.95	0.85	Cholestyramine
FH_5	M	44	70	1.93	16.75	0.92	15.43	0.83	
FH_6	F	15	50	0.85	10.90	(0.1)[a]	10.00	0.73	Portacaval shunt/plasmapheresis
FH_7	M	25	66	0.80	11.50	(0.1)[a]	10.20	1.20	Portacaval shunt/plasmapheresis

[a]Assessment of VLDL cholesterol in subjects FH_6 and FH_7 is at the limit of detection.

Table I-23: Computed compartment masses and rate constants for normal and FH subjects

Subject	M_1[a]	$k_{0,1}$[b]	$k_{2,1}$	M_2[c]	$k_{3,2}$	$k_{0,2}$	M_4	$k_{0,4}$	$k_{8,4}$	$k_{9,4}$	$k_{11,4}$	M_5	$k_{0,5}$	$k_{7,5}$	$k_{10,5}$
N_1	79	3.7	6.5	53	8.6	1.0	42	0.8	7.6	2.3	0.0	52	0.0	7.6	0.0
N_2	18	0.0	16.2	16	18.0	0.2	44	0.7	3.6	0.4	1.9	23	1.7	4.3	0.0
N_3	9	17.7	8.4	9	7.4	1.1	2	16.0	14.0	14.0	0.0	36	0.0	7.2	0.0
N_4	108	1.1	3.7	43	8.4	0.8	93	1.2	1.1	0.4	1.2	80	0.0	2.1	2.4
N_5	115	5.0	3.2	42	8.2	0.3	77	1.0	3.1	0.1	0.2	85	0.0	2.8	0.0
Mean ± SD	66 ± 44	5.5 ± 6.4	7.6 ± 4.7	33 ± 17	10.1 ± 4.0	0.7 ± 0.4	52 ± 32	3.9 ± 6.0	5.9 ± 4.6	3.4 ± 5.3	0.7 ± 0.8	55 ± 24	0.3 ± 0.7	4.8 ± 2.2	0.5 ± 1.0
FH_1	108	3.7	6.0	69	7.2	2.2	83	2.9	3.0	0.1	0.0	108	0.0	3.4	0.3
FH_2	72	4.4	4.1	45	2.4	4.2	81	0.1	1.2	0.0	0.0	59	0.0	3.6	0.0
FH_3	110	1.2	2.4	24	1.4	9.6	23	0.1	1.3	0.0	0.0	532	0.6	1.0	0.7
FH_4	28	3.7	2.8	12	0.48	5.8	63	0.0	0.0	0.1	0.0	261	1.2	2.2	0.3
FH_5	59	3.6	7.7	21	14.4	7.7	35	0.0	4.3	0.0	4.3	71	0.1	1.3	1.7
Mean ± SD	75 ± 31	3.3 ± 1.1	4.6 ± 2.0	34 ± 20	5.2 ± 5.2	5.9 ± 2.6	38 ± 24	0.6 ± 1.1	2.0 ± 1.5	0.04 ± 0.05	0.9 ± 1.7	206 ± 178	0.4 ± 0.5	2.3 ± 1.1	0.6 ± 0.6
FH_6	9	1.8	24.0	18	10.0	2.1	9	0.0	17.8	0.0	1.3	13	0.0	0.8	0.3
FH_7	8	3.6	18.0	13	9.6	0.7	9	1.9	10.8	0.0	1.9	13	0.5	3.5	1.6
Mean	9	2.7	21.0	16	9.8	1.4	9	1.0	14.3	0.0	1.6	13	0.3	2.2	1.0

Masses (M_i) refer to the numbering of sub-compartments given in Fig. 1. Rate constants K_{ij} indicate transfer from sub-compartment j to sub-compartment i.

[a] Masses in mg.

[b] Rate constants (d^{-1}).

[c] $M_3 = M_2$; $k_{4,3} = k_{3,2}$ was a constraint in the model.

Table I-23 (continued)

Subject	M_6[a]	$k_{6,6}$[b]	$k_{6,6}$	M_7	$k_{6,7}$	$k_{10,7}$	M_8	$k_{6,8}$	$k_{11,8}$	M_9	$k_{6,9}$	M_{10}	$k_{6,10}$	M_{11}	$k_{6,11}$
N_1	66	0.8	0.0	115	0.5	2.9	66	1.53	3.38	160	0.6	680	0.49	1070	0.21
N_2	2	1.9	0.0	33	0.0	3.0	145	0.34	0.74	33	0.6	215	0.46	420	0.46
N_3	9	1.1	0.0	73	0.0	3.6	7	1.40	1.80	15	1.4	636	0.41	74	0.16
N_4	29	1.2	0.0	78	1.9	0.2	76	1.0	0.48	92	0.4	1380	0.15	728	0.20
N_5	12	1.0	0.0	219	0.0	1.1	270	0.23	0.65	7	1.0	1412	0.20	1171	0.20
Mean ± SD	24 ± 23	1.2 ± 0.4	0.0	104 ± 63	0.5 ± 0.7	2.2 ± 1.3	113 ± 90	0.90 ± 0.53	1.41 ± 1.09	61 ± 58	0.8 ± 0.4	865 ± 463	0.34 ± 0.14	693 ± 407	0.25 ± 0.11
FH_1	273	0.37	0.18	179	0.12	1.9	912	0.0	0.27	185	0.30	2994	0.13	2704	0.090
FH_2	302	0.50	0.12	513	0.05	0.37	184	0.17	0.36	144	0.25	3081	0.084	1563	0.084
FH_3	290	0.67	0.12	787	0.38	0.26	88	0.0	0.36	172	0.20	6166	0.12	532	0.060
FH_4	127	0.47	0.1	2175	0.05	0.21	0	0.0	0.0	47	0.37	5016	0.17	0	0.0
FH_5	166	0.96	0.0	770	0.0	0.24	618	0.24	0.0	0	0.0	8586	0.11	1600	0.072
Mean ± SD	232 ± 71	0.6 ± 0.2	0.1 ± 0.06	885 ± 681	0.1 ± 0.1	0.6 ± 0.7	360 ± 348	0.08 ± 0.10	0.20 ± 0.16	110 ± 73	0.22 ± 0.13	5174 ± 2086	0.12 ± 0.03	1279 ± 939	0.06 ± 0.0
FH_6	62	0.56	0.05	301	0.08	0.13	547	0.27	0.02	2	1.3	3230	0.10	357	0.057
FH_7	27	0.36	0.0	1110	0.02	0.18	473	0.03	0.17	0	0	4246	0.12	718	0.13
Mean	43	0.46	0.03	721	0.05	0.16	510	0.15	0.1	1	0.7	3738	0.11	538	0.09

Table I-24: *Plasma Lipids, Lipoproteins, and Clinical Data from Individuals with Different Apo B Xba I Genotype*

Subject	Genotype	Plasma		LDL-cholesterol	LDL-apoprotein	Clinical data
		Cholesterol	Triglyceride			
		mmol/liter			*mg/dl*	
1	X1X1	8.10	1.95	5.90	193	Angina
2	X1X1	7.74	1.24	5.99	192	Xanthelasmata
3	X1X1	6.89	1.66	4.93	149	Normal
4	X1X1	8.49	3.24	6.28	211	Corneal arcus
5	X1X1	7.08	1.43	5.28	179	Normal
n = 5	Mean±1 SD	7.76±0.59	1.88±0.65	5.73±0.47	187±19	
6	X1X2	8.09	1.20	5.76	151	Normal
7	X1X2	8.79	1.90	6.63	204	Normal
8	X1X2	7.12	2.60	4.80	173	Normal
9	X1X2	6.97	1.80	4.99	167	Angina
10	X1X2	8.09	2.31	6.01	174	Myocardial infarction in mother (age 55)
11	X1X2	6.85	0.83	3.76	156	Normal
12	X1X2	7.05	2.14	4.75	135	Normal
13	X1X2	8.53	2.15	6.58	186	Myocardial infarction in brother (age 54)
n = 8	Mean±1 SD	7.69±0.72	1.87±0.55	5.41±0.94	168±20	
14	X2X2	8.33	2.23	5.46	253	Arcus
15	X2X2	8.56	2.76	6.20	175	Myocardial infarction in father (age 45)
16	X2X2	6.36	0.82	3.79	146	Normal
17	X2X2	8.00	3.11	5.62	176	Normal
18	X2X2	7.03	1.68	4.88	160	Xanthelasmata
19	X2X2	8.09	2.57	5.84	192	Normal
n = 6	Mean±1 SD	7.73±0.78	2.32±0.77	5.30±0.78	184±34	

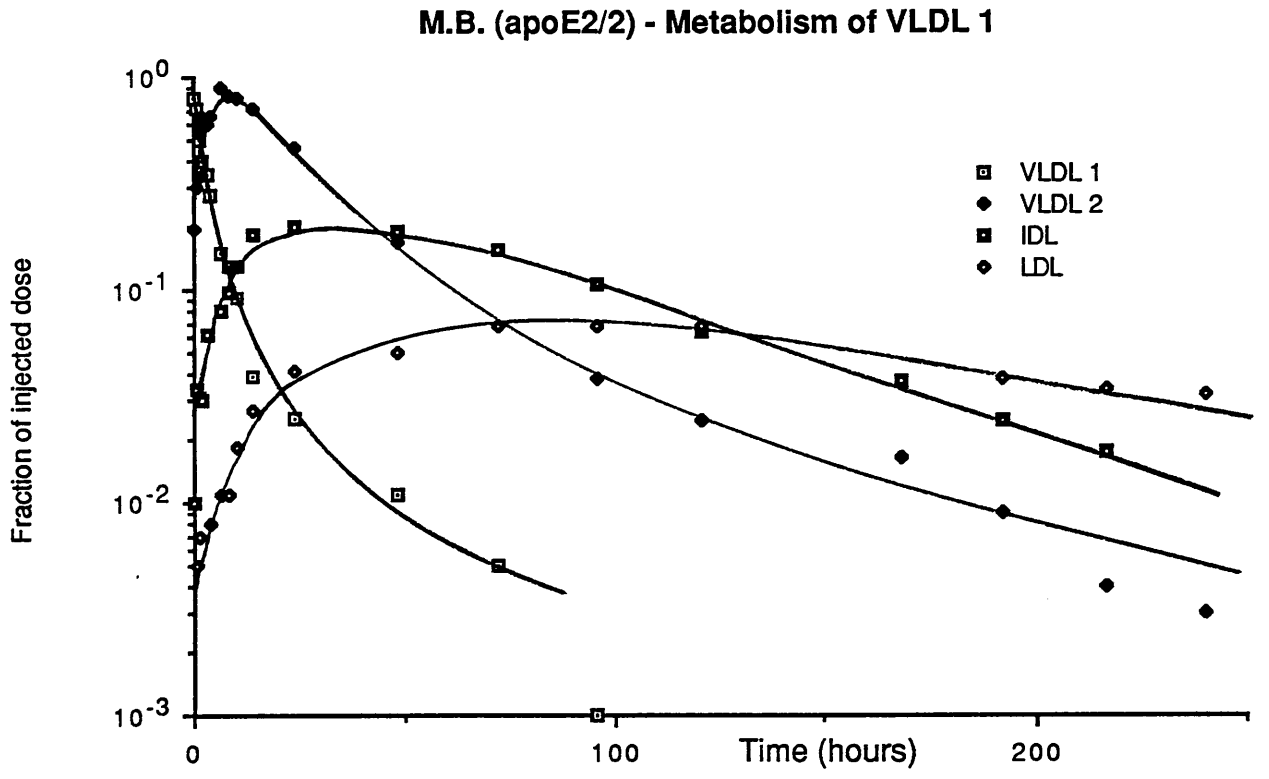
All values given in the table are means of at least three independent determinations. Analysis of variance showed no significant differences in plasma lipid parameters between any of the three groups (X1X1, X1X2, or X2X2).

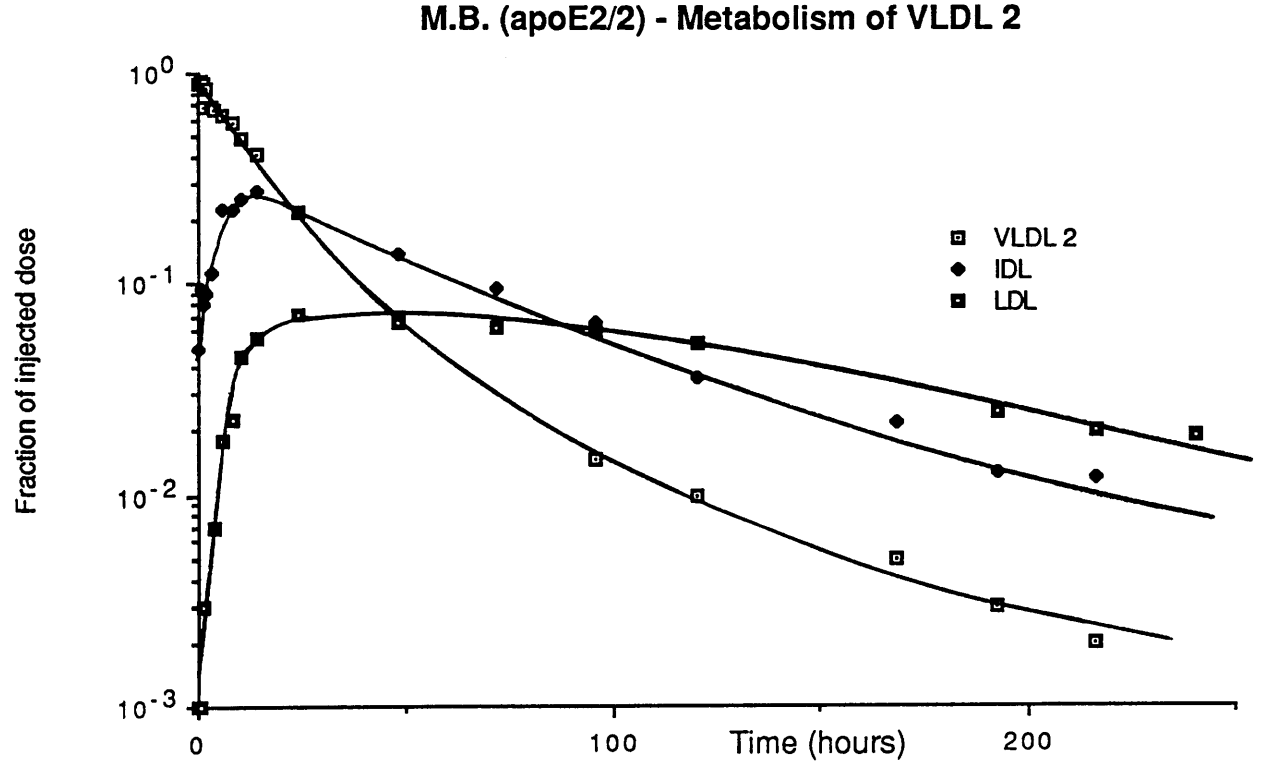
Table I-25: *LDL-Kinetic Parameters and Xba I Genotype*

Subject	Sex	RFLP genotypes			Fractional catabolic rate		Synthesis*	Absolute‡ receptor-mediated catabolic rate
		Xba I	Eco RI	Msp I	Total	Receptor mediated		
					pools/d		*mg/kg per d*	
1	F	X1X1	R1R1	M1M2	0.248	0.088	19.10	6.80
2	F	X1X1	R1R2	M1M2	0.256	0.101	19.70	7.80
3	F	X1X1	R1R1	M2M2	0.270	0.079	16.10	4.71
4	F	X1X1	R1R1	M1M2	0.282	0.069	23.80	5.82
5	M	X1X1	R1R2	M1M2	0.297	0.072	21.27	5.16
$n = 5$			Mean±1 SD		0.271±0.020	0.082±0.013	19.99±2.84	6.06±1.25
6	F	X1X2	R1R2	M1M2	0.219	0.078	13.23	4.71
7	F	X1X2	R1R1	M2M2	0.229	0.087	18.69	7.10
8	F	X1X2	R1R2		0.252	0.022	17.40	1.50
9	F	X1X2	R1R2	M1M2	0.251	0.044	16.80	2.90
10	F	X1X2	R1R1	M1M2	0.241	0.070	16.77	4.87
11	F	X1X2	R1R2	M1M2	0.218	0.084	13.60	5.20
12	M	X1X2	R1R2	M1M2	0.280	0.114	15.12	6.16
13	M	X1X2	R1R1	M2M2	0.205	0.056	15.25	4.16
$n = 8$			Mean±1 SD		0.237±0.024	0.069±0.028	15.86±1.89	4.58±1.77
14	F	X2X2	R1R1		0.218	0.020	22.10	2.00
15	M	X2X2	R1R1	M2M2	0.197	0.067	13.79	4.69
16	F	X2X2	R1R1	M2M2	0.182	0.042	10.60	2.50
17	F	X2X2	R1R1	M2M2	0.265	0.054	18.66	3.80
18	F	X2X2	R1R1	M2M2	0.217	0.070	13.90	4.50
19	F	X2X2	R1R1	M2M2	0.250	0.059	19.20	4.53
$n = 6$			Mean±1 SD		0.222±0.031	0.052±0.019	16.38±4.29	3.67±1.15
Analysis of variance		X1X1:X1X2;X2X2			$P < 0.025$	NS	NS	$P < 0.050$
		X1X1:X2X2			$P < 0.025$	$P < 0.025$	NS	$P < 0.010$

No significant differences could be detected when genotypically different groups (R1R1 versus R1R2; M1M2 versus M2M2) were compared by analysis of variance. * The synthetic rate is equal to the product of the total FCR and the plasma LDL pool (LDL concentration × plasma volume). ‡ The absolute receptor-mediated catabolic rate is the product of the receptor-mediated FCR and the plasma LDL pool.

FIGURES I-1 to I-15


M.B. (apoE2/2) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
Time (hours)
200


M.B. (apoE2/2) - Metabolism of VLDL 2
Fraction of injected dose
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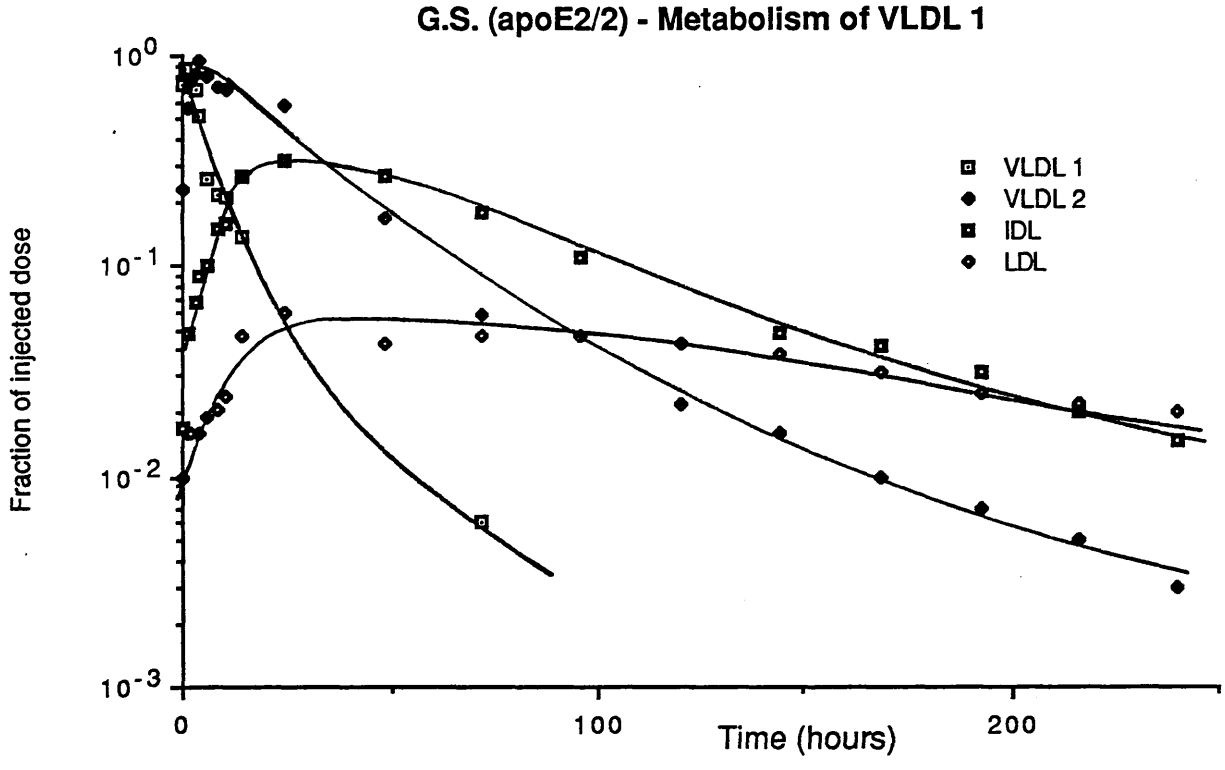
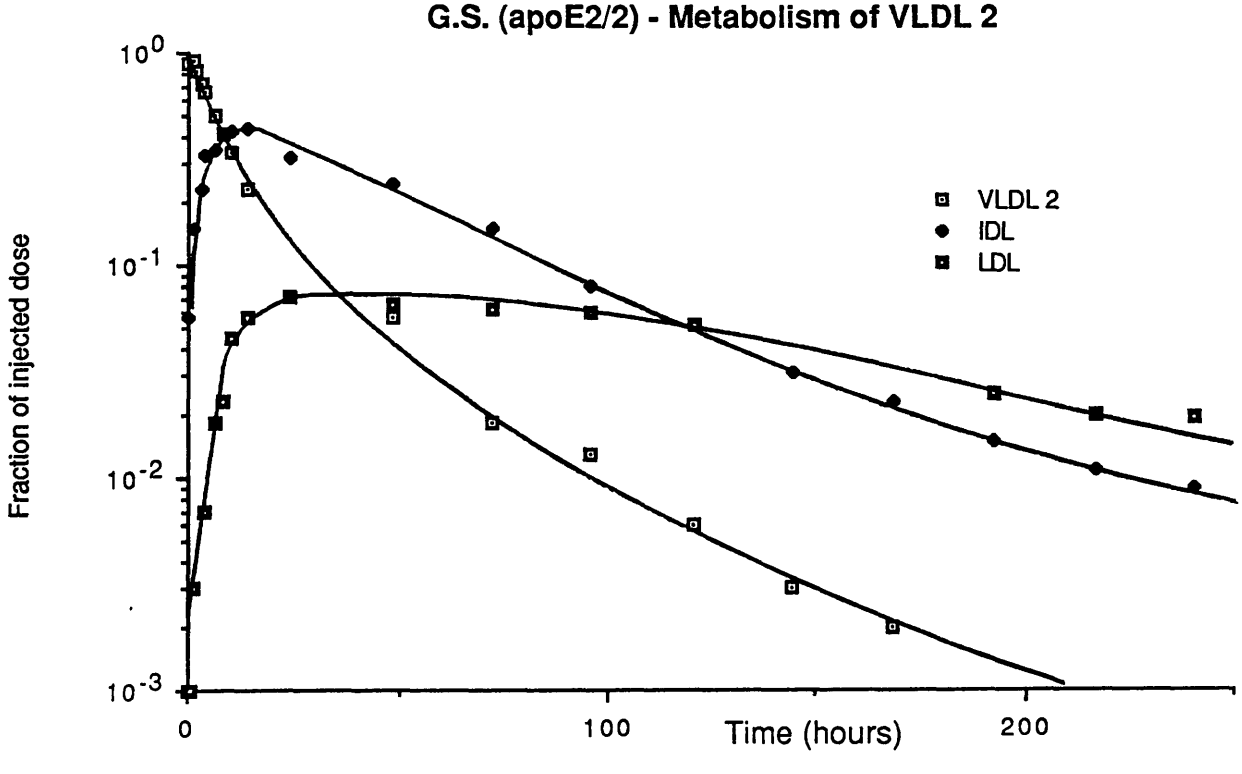
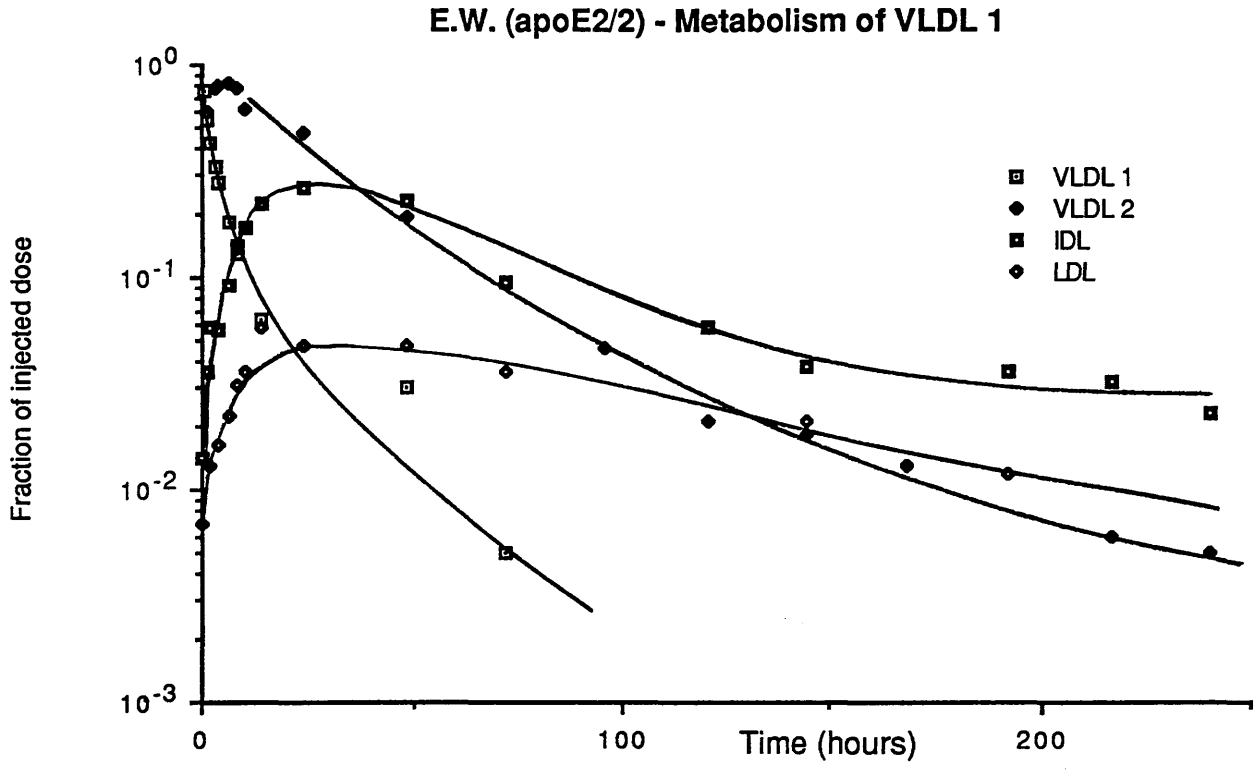
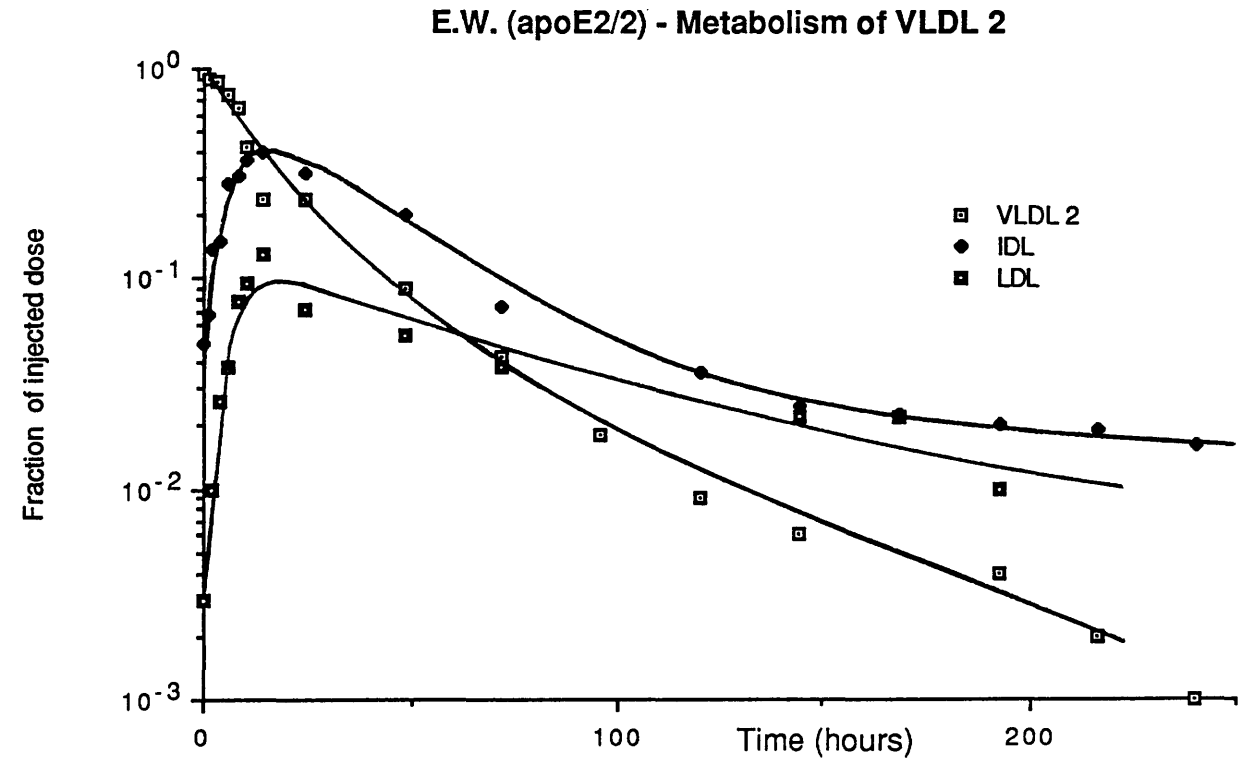
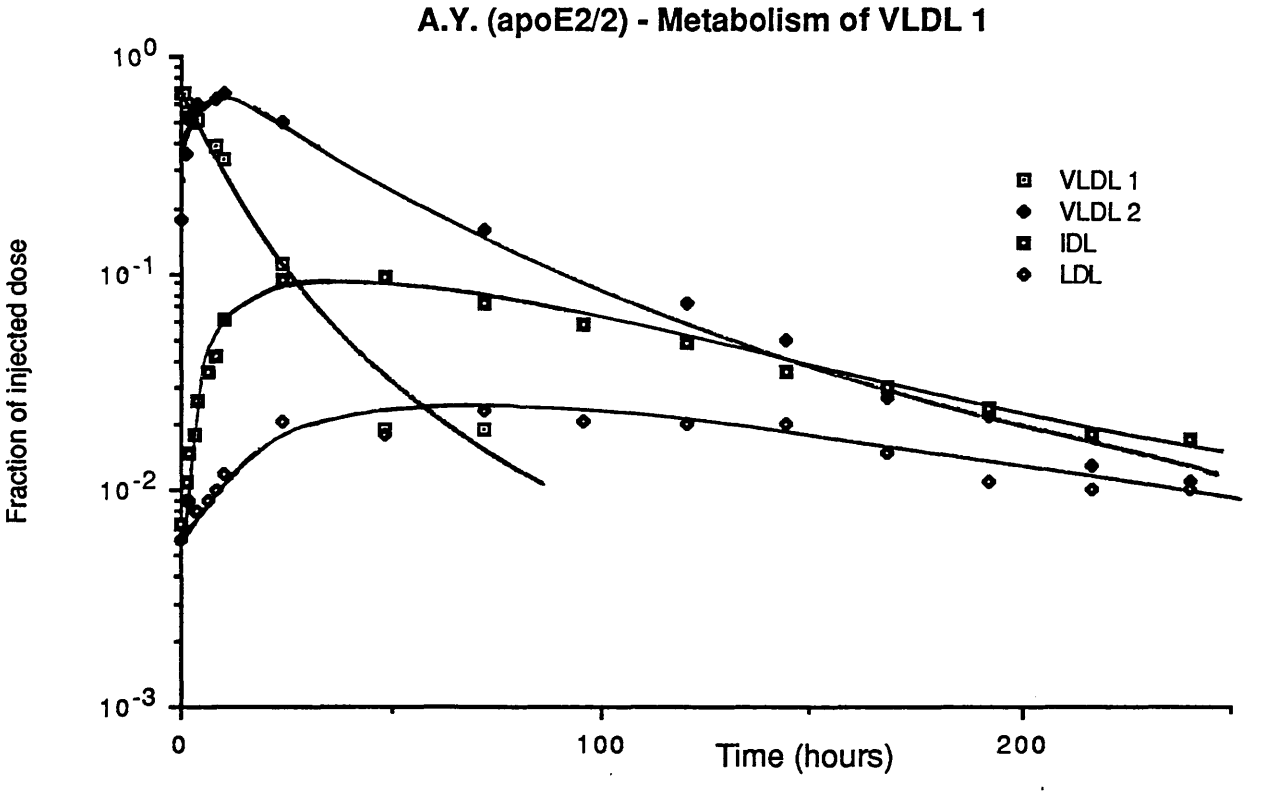
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
Time (hours)
200

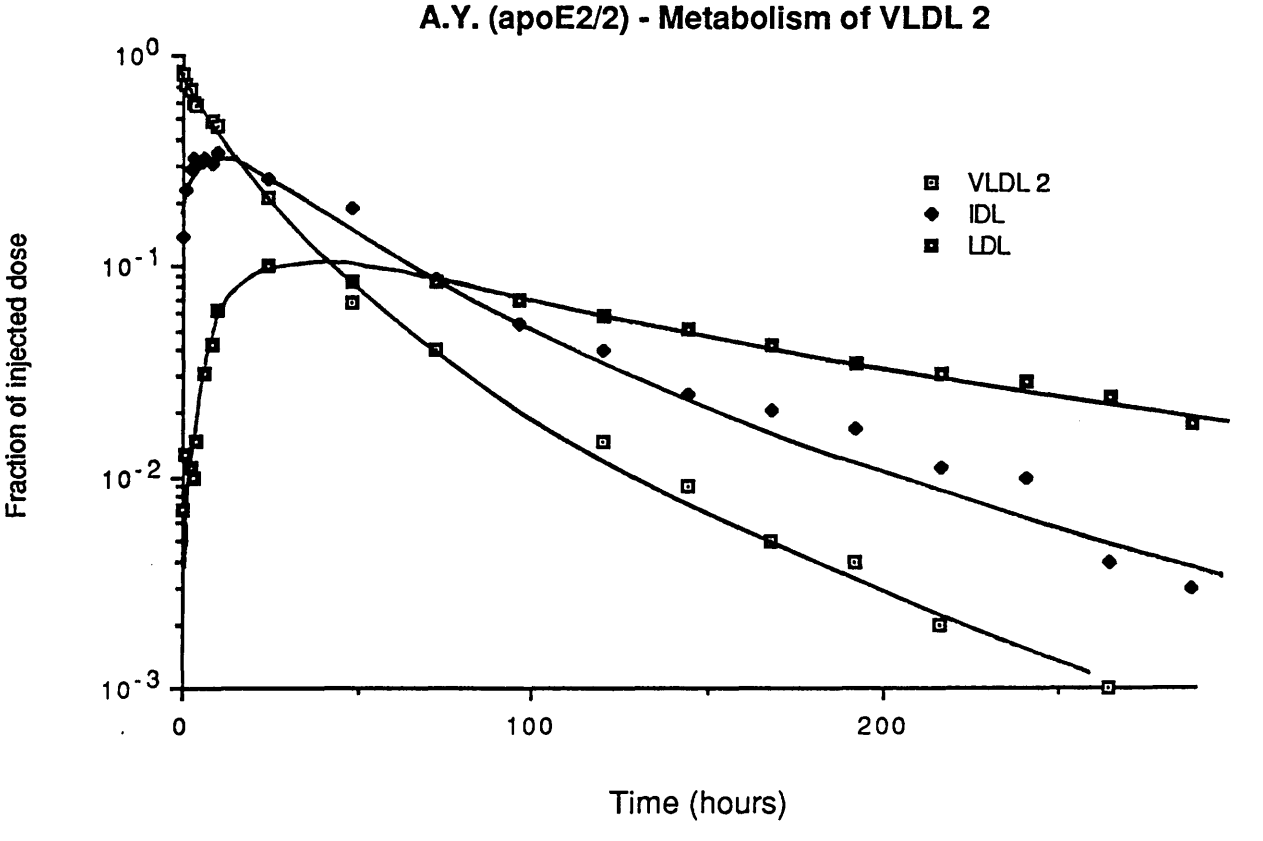

G.S. (apoE2/2) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)
VLDL 1
VLDL 2
IDL
LDL

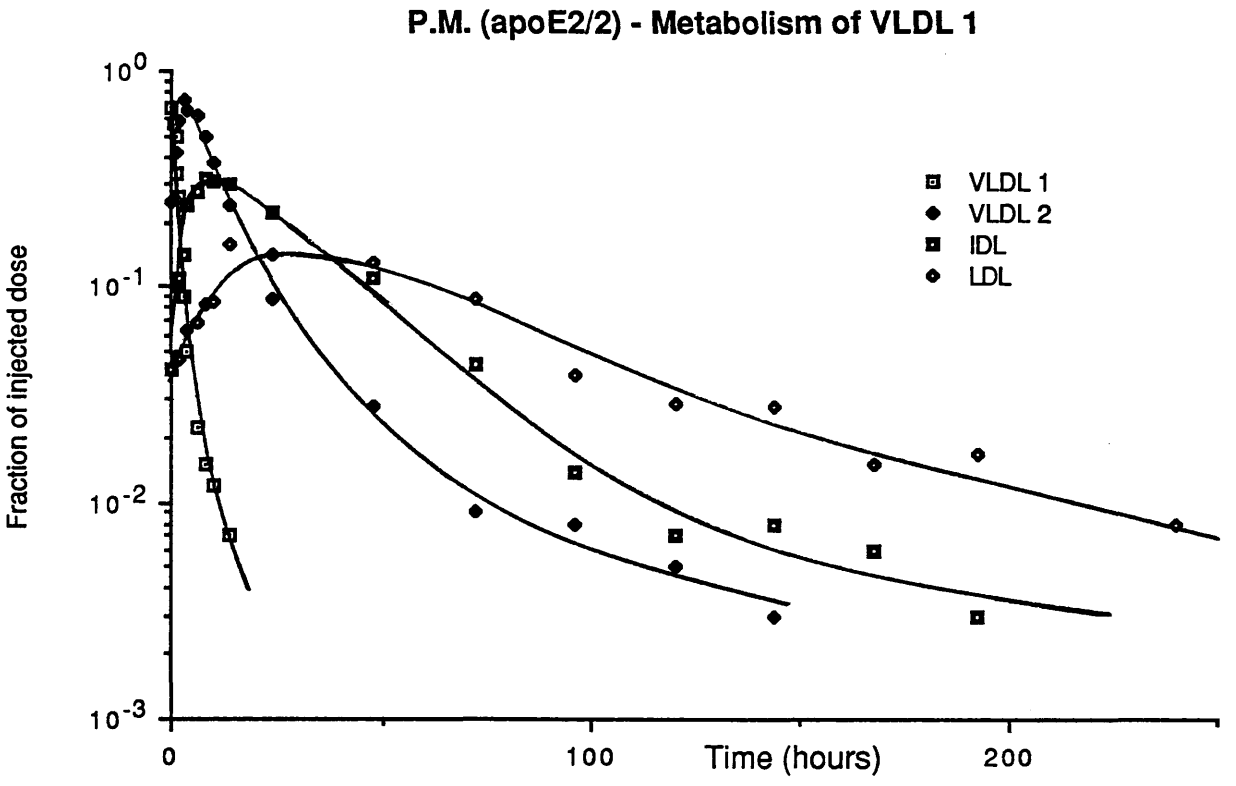

G.S. (apoE2/2) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)
VLDL 2
IDL
LDL


E.W. (apoE2/2) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)
VLDL 1
VLDL 2
IDL
LDL


E.W. (apoE2/2) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)
VLDL 2
IDL
LDL


A.Y. (apoE2/2) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)


A.Y. (apoE2/2) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)


P.M. (apoE2/2) - Metabolism of VLDL 1
Fraction of injected dose
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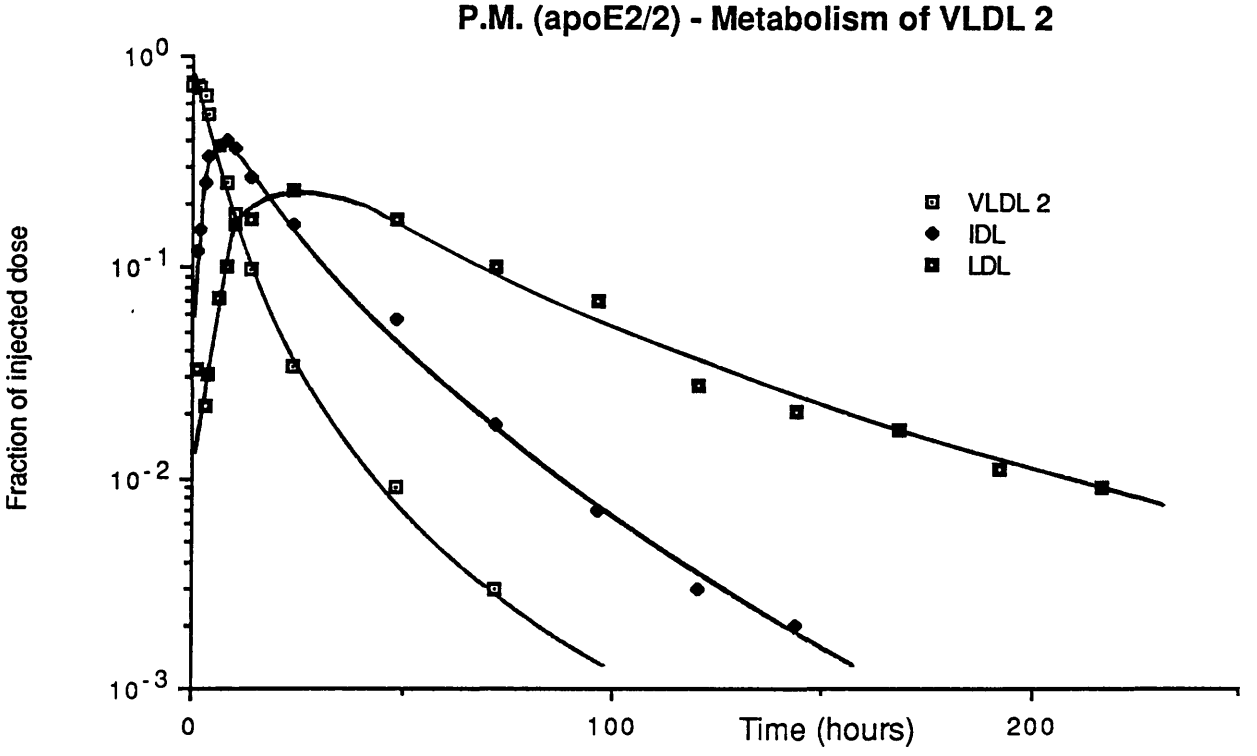
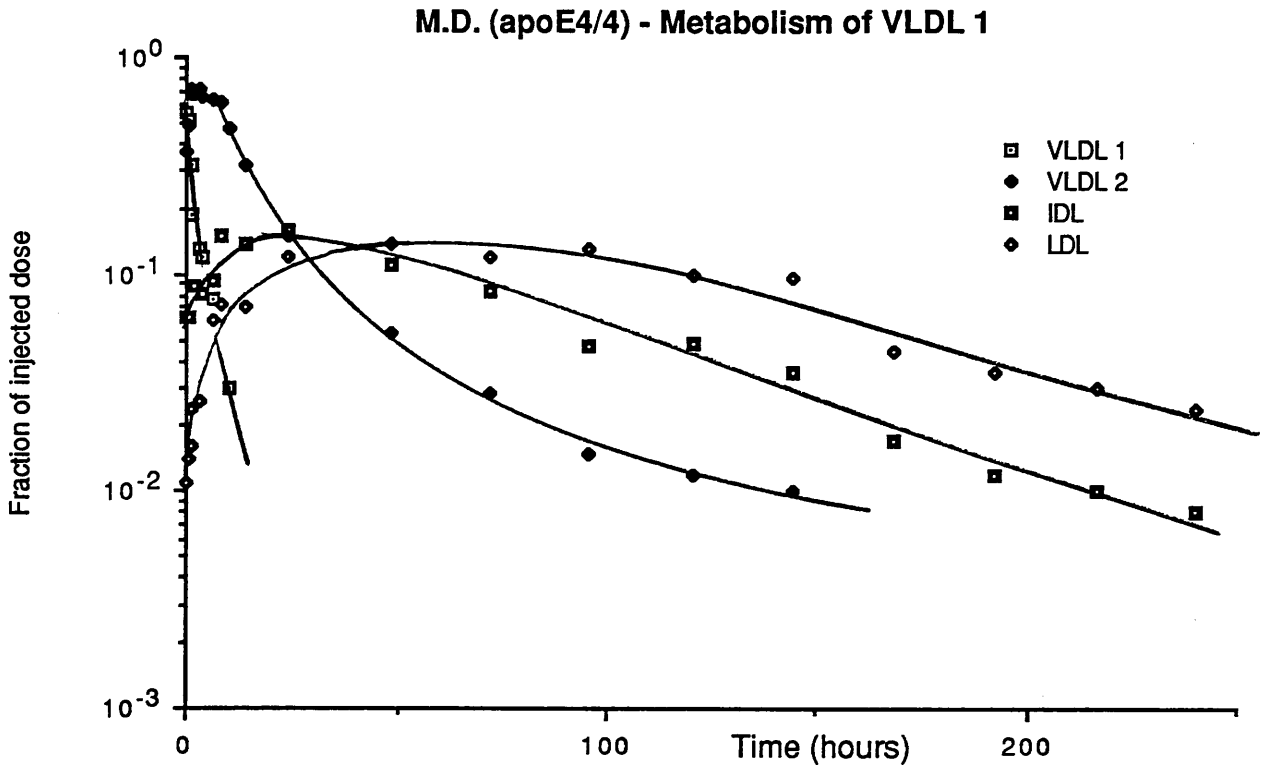
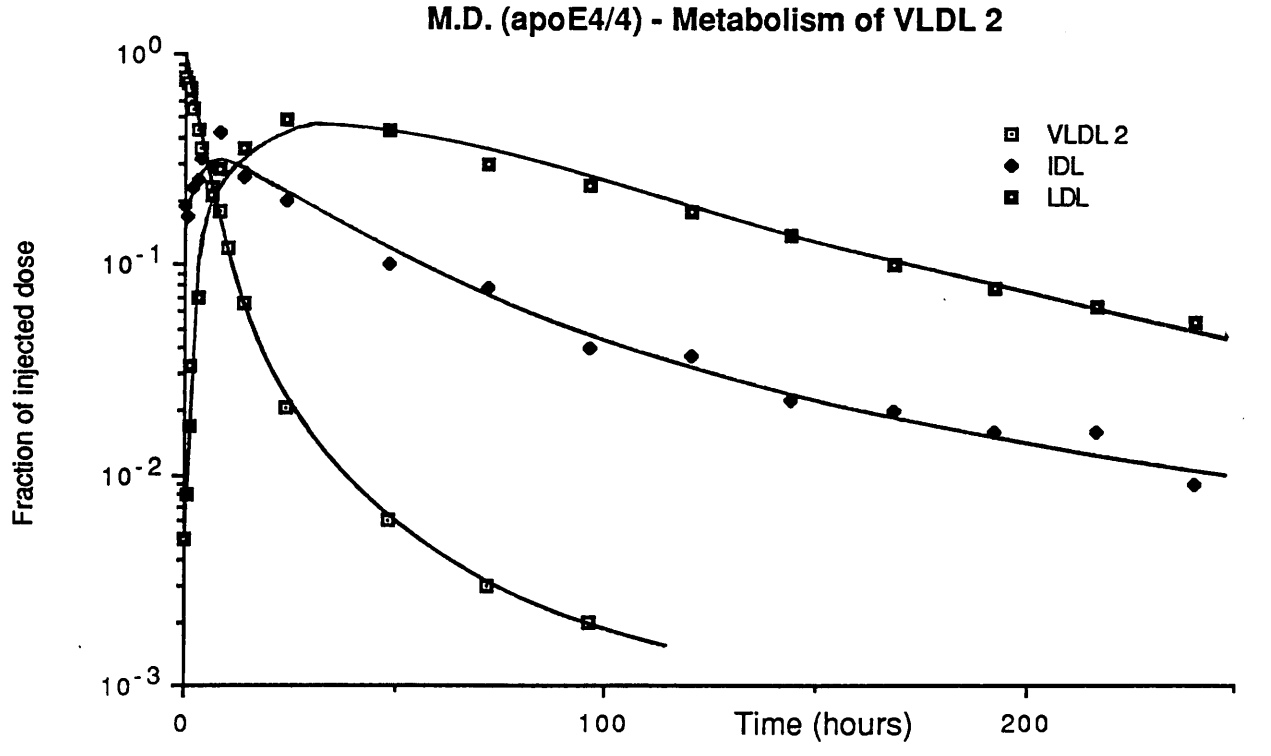
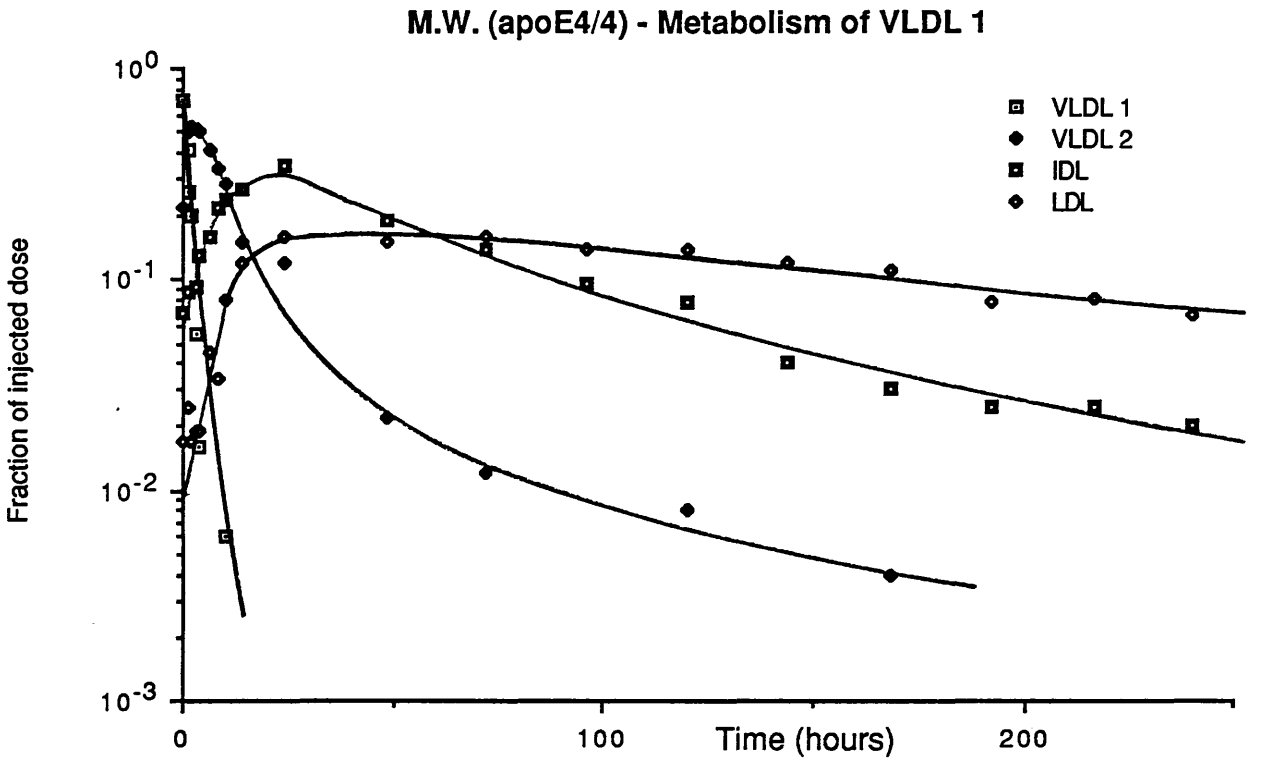
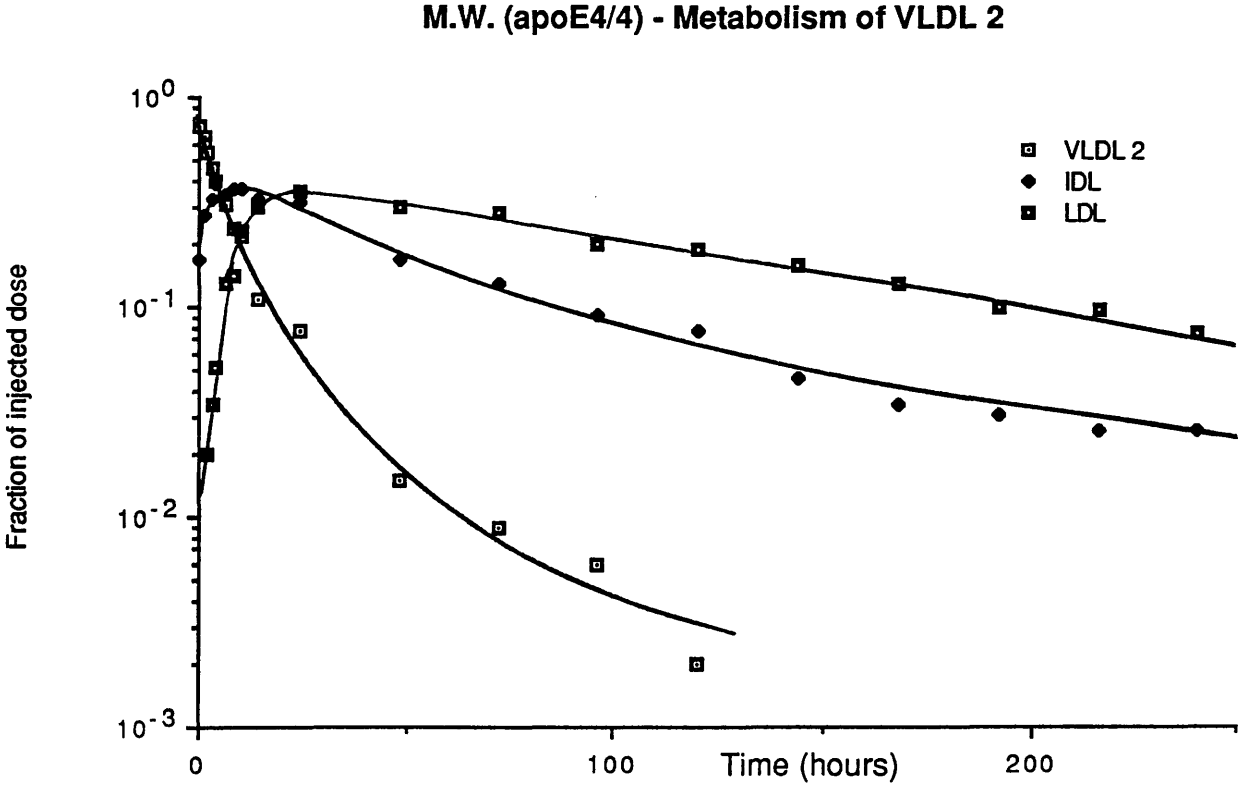
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)

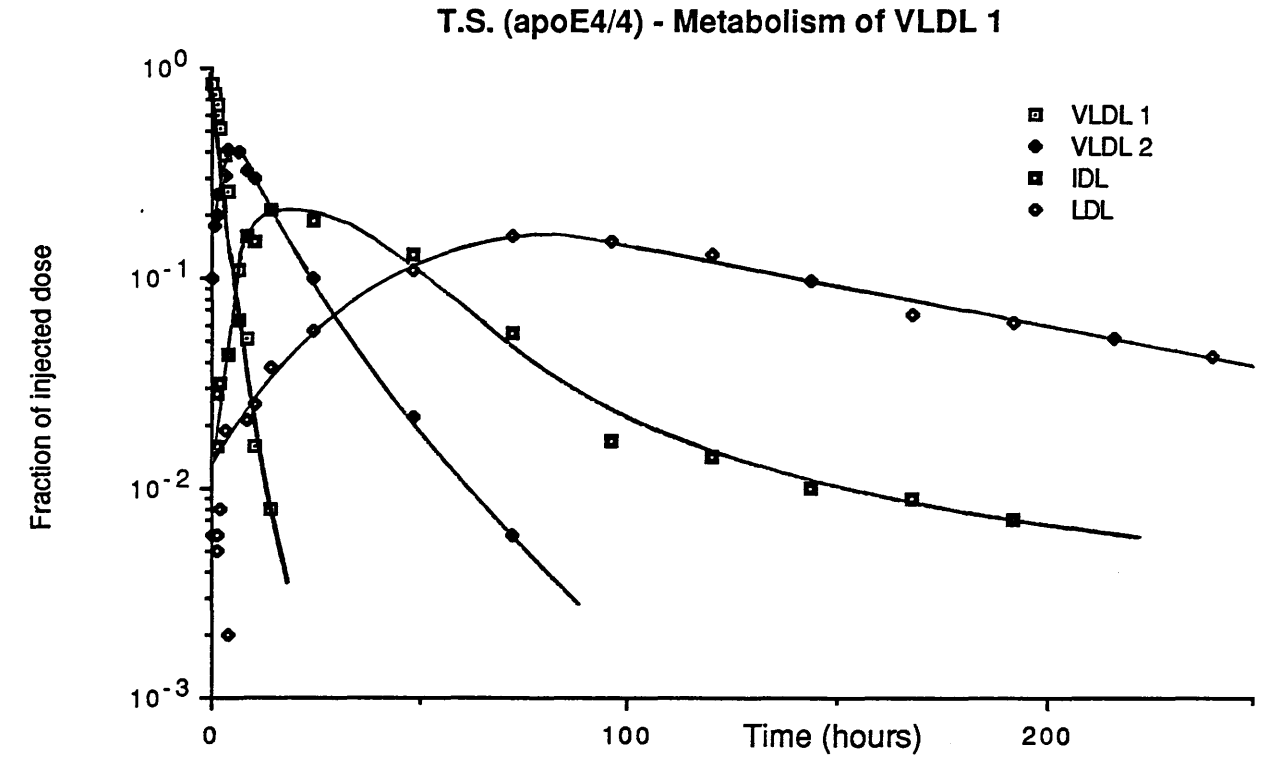

P.M. (apoE2/2) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)

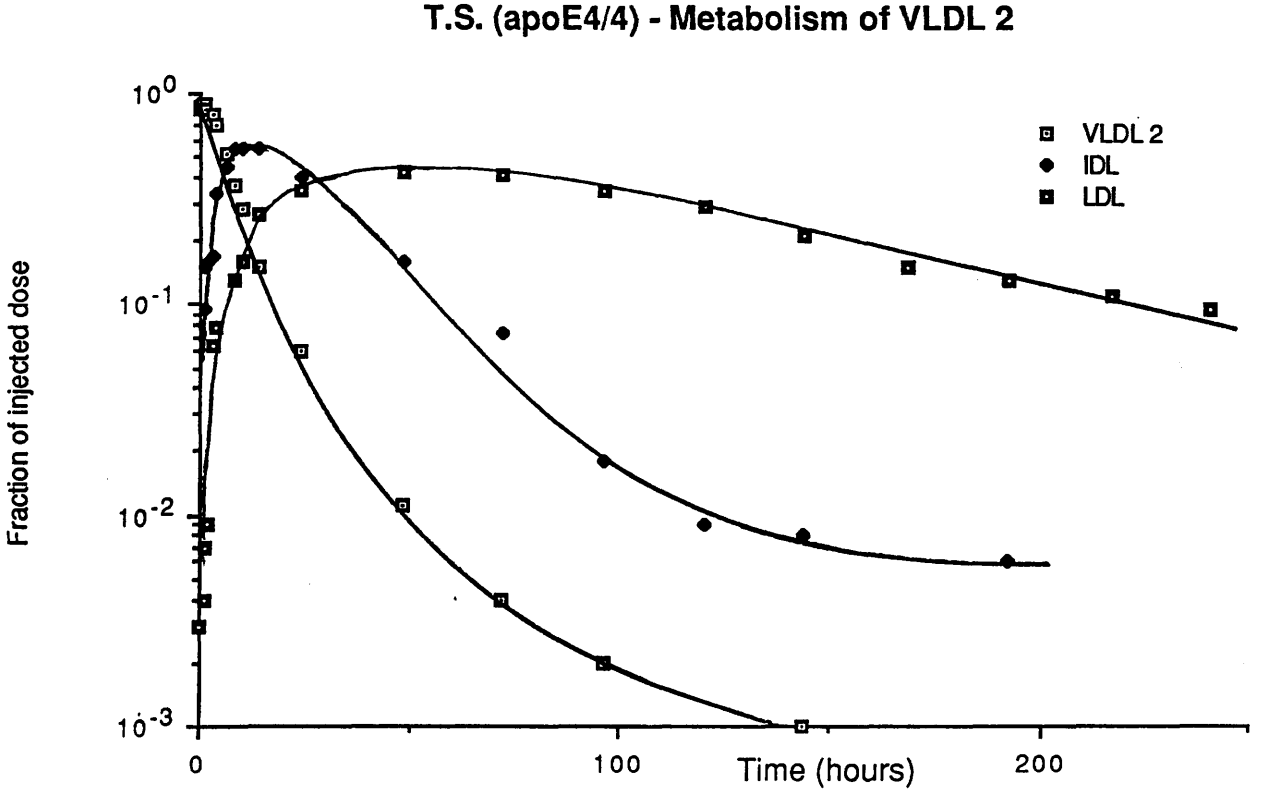

M.D. (apoE4/4) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)

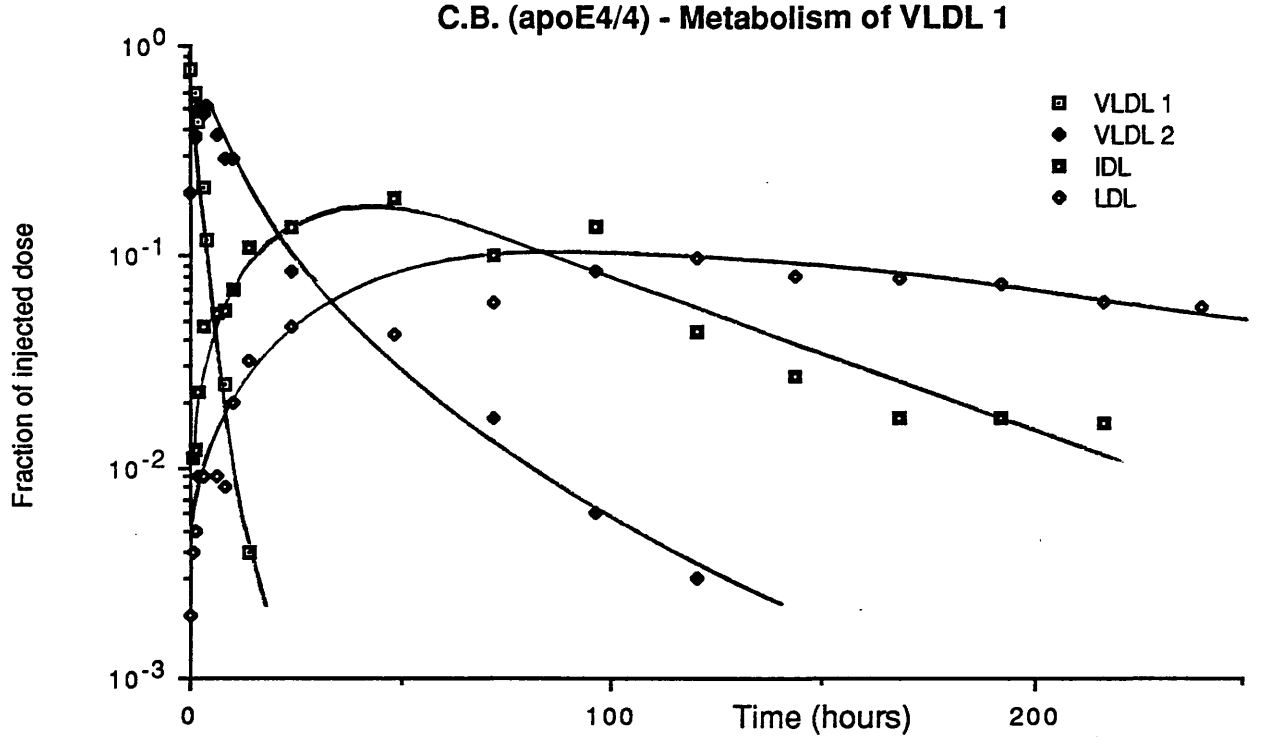

M.D. (apoE4/4) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)

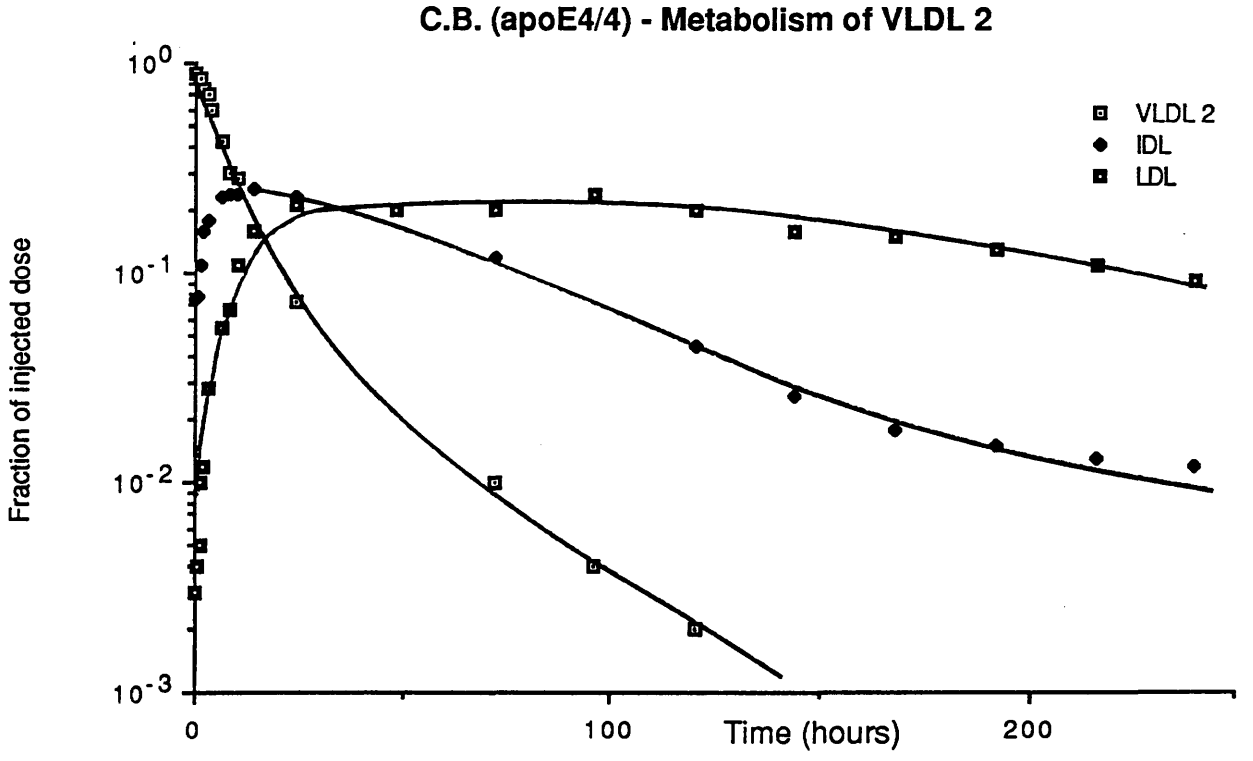

M.W. (apoE4/4) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)

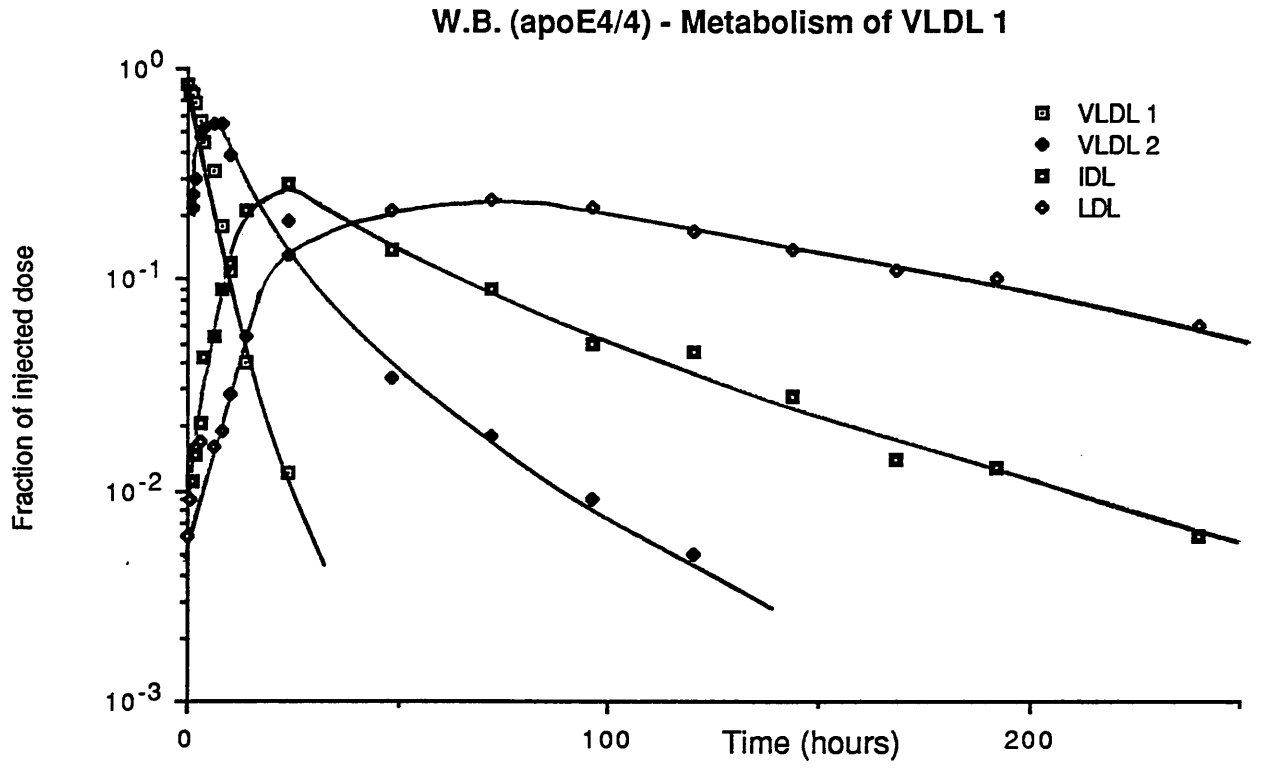

M.W. (apoE4/4) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)

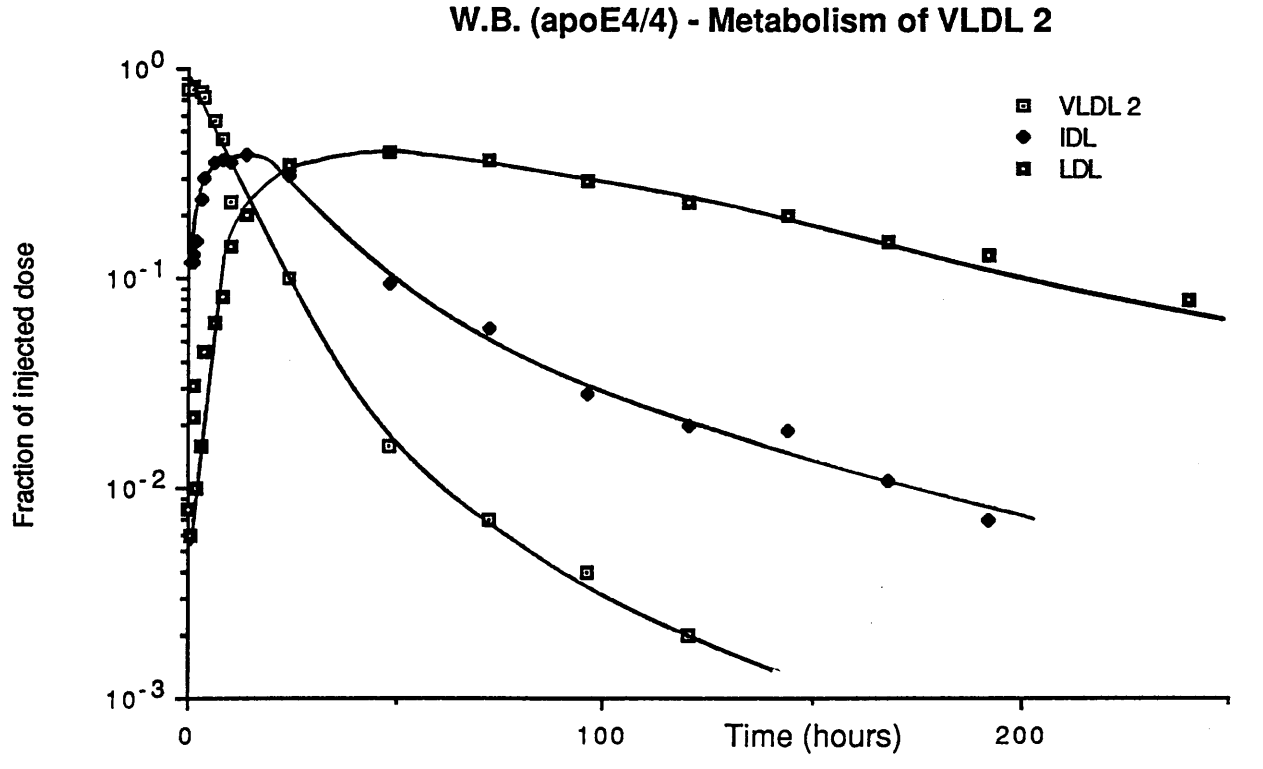

T.S. (apoE4/4) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)

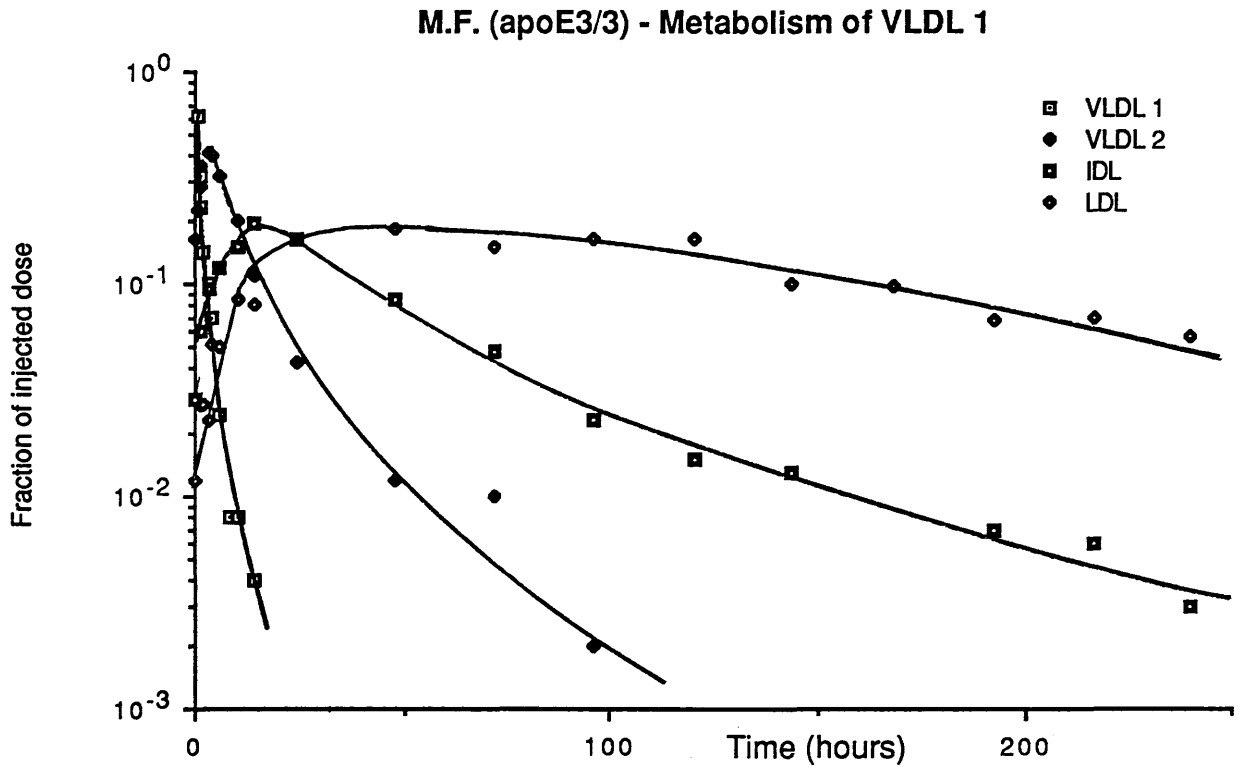

T.S. (apoE4/4) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)

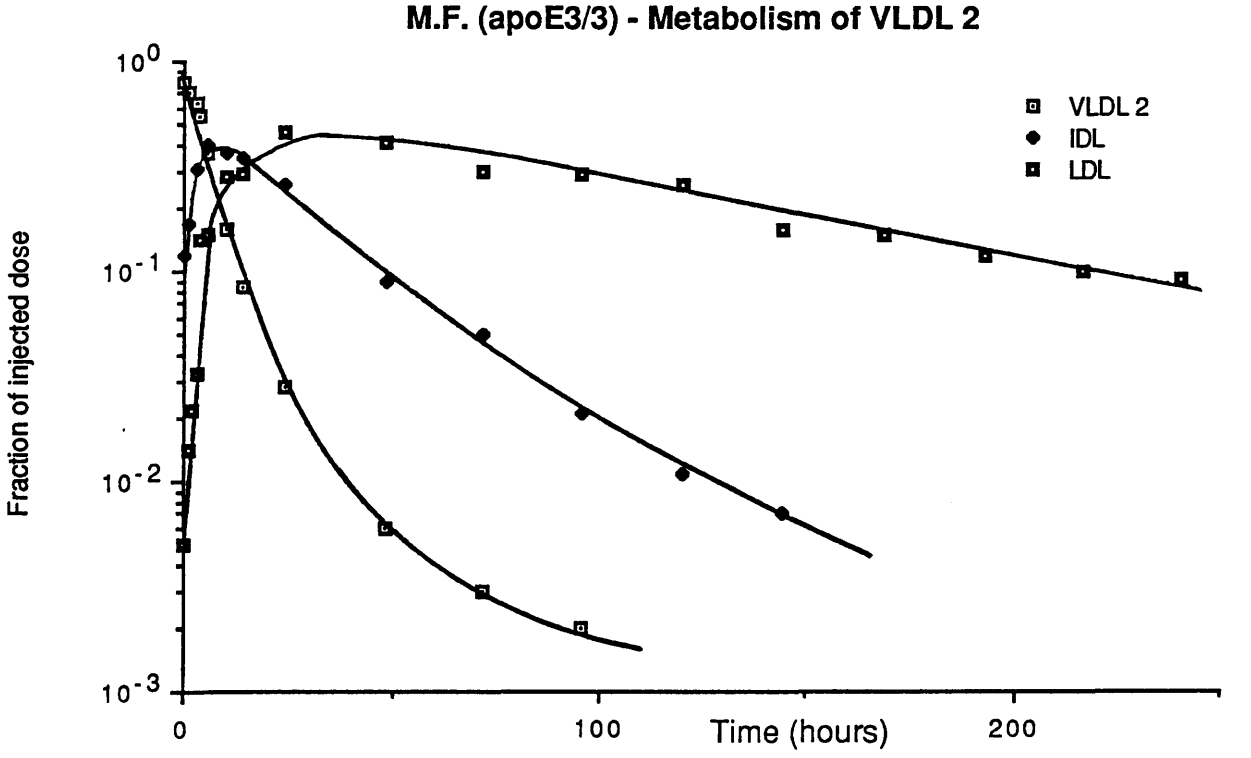

C.B. (apoE4/4) - Metabolism of VLDL 1
VLDL 1
VLDL 2
IDL
LDL
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)

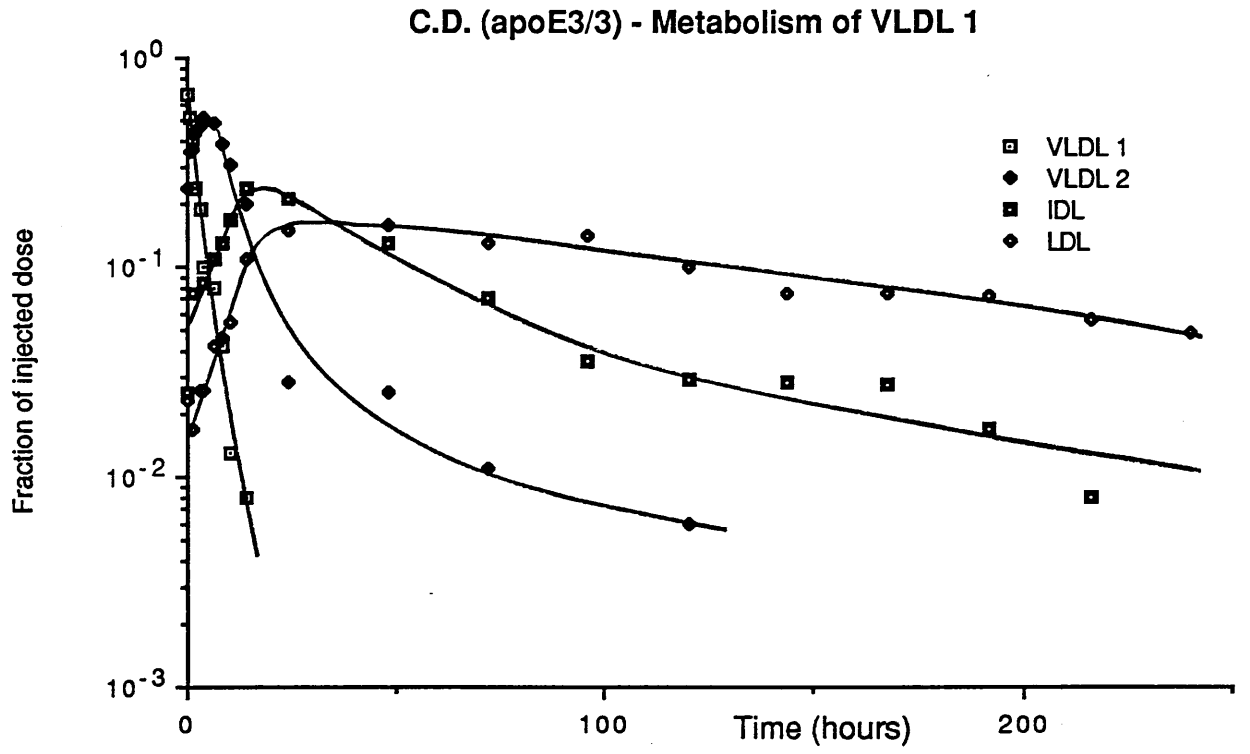

C.B. (apoE4/4) - Metabolism of VLDL 2
VLDL 2
IDL
LDL
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)

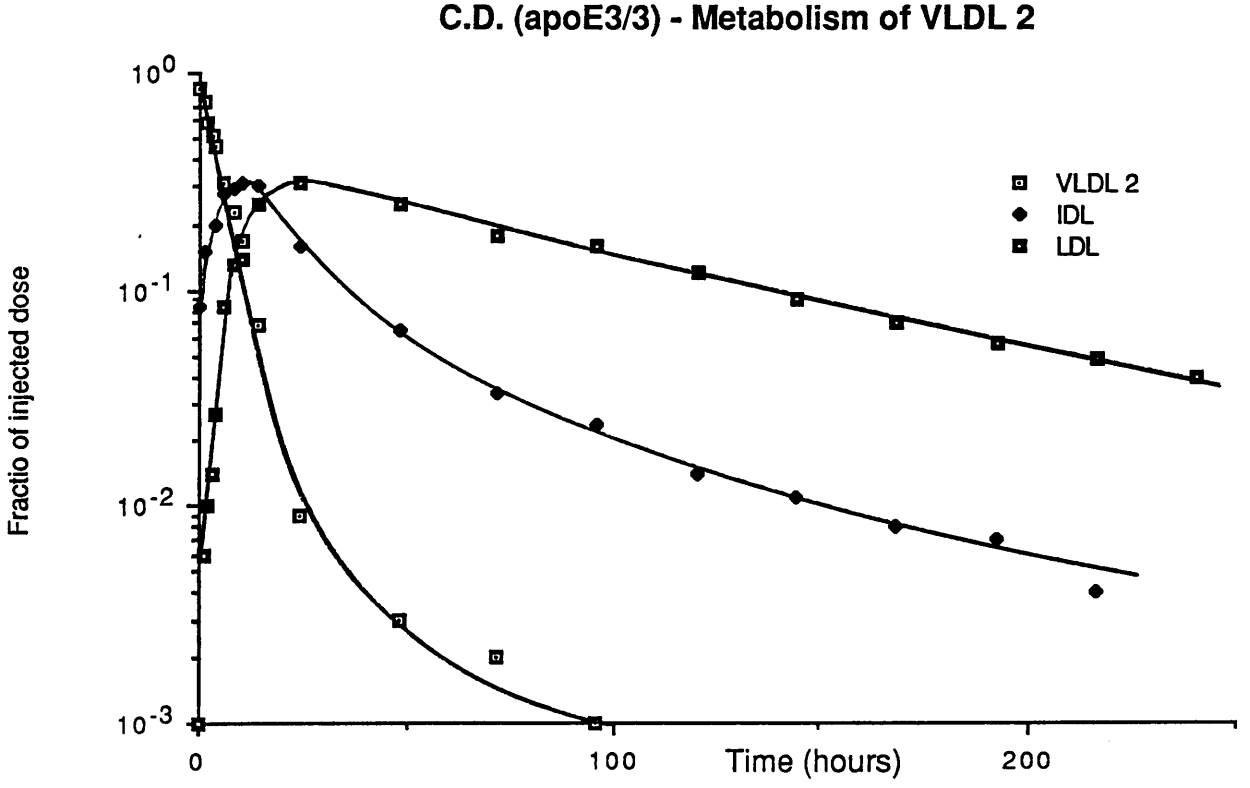

W.B. (apoE4/4) - Metabolism of VLDL 1
VLDL 1
VLDL 2
IDL
LDL
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)


W.B. (apoE4/4) - Metabolism of VLDL 2
VLDL 2
IDL
LDL
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
Time (hours)


M.F. (apoE3/3) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)


M.F. (apoE3/3) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)


C.D. (apoE3/3) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
200
Time (hours)


C.D. (apoE3/3) - Metabolism of VLDL 2
Fractio of injected dose
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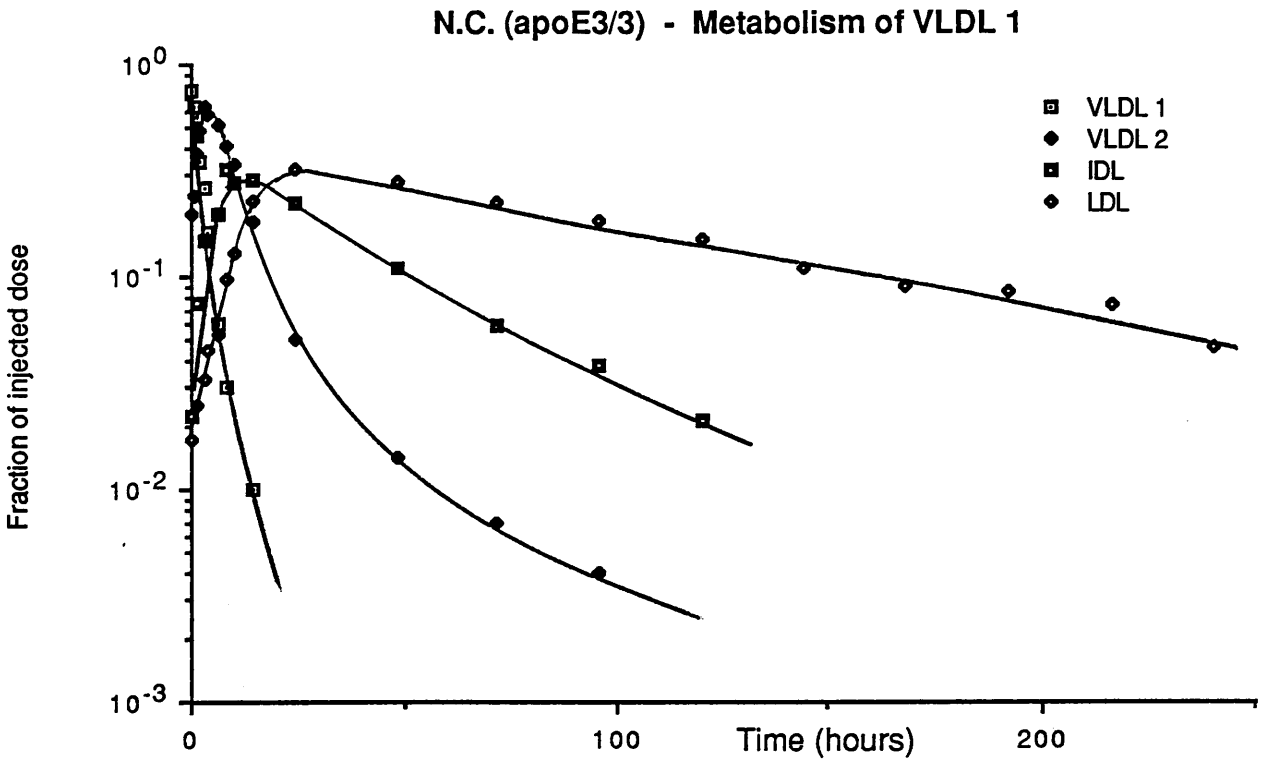
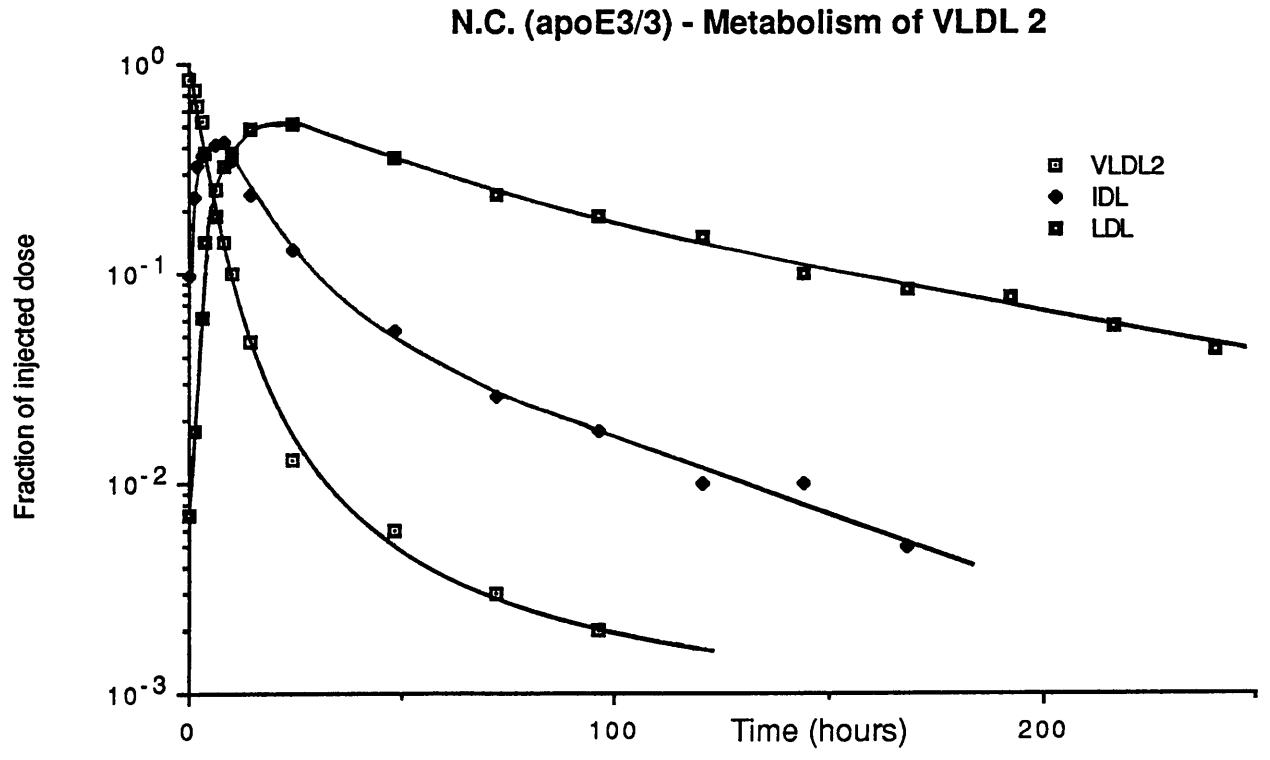
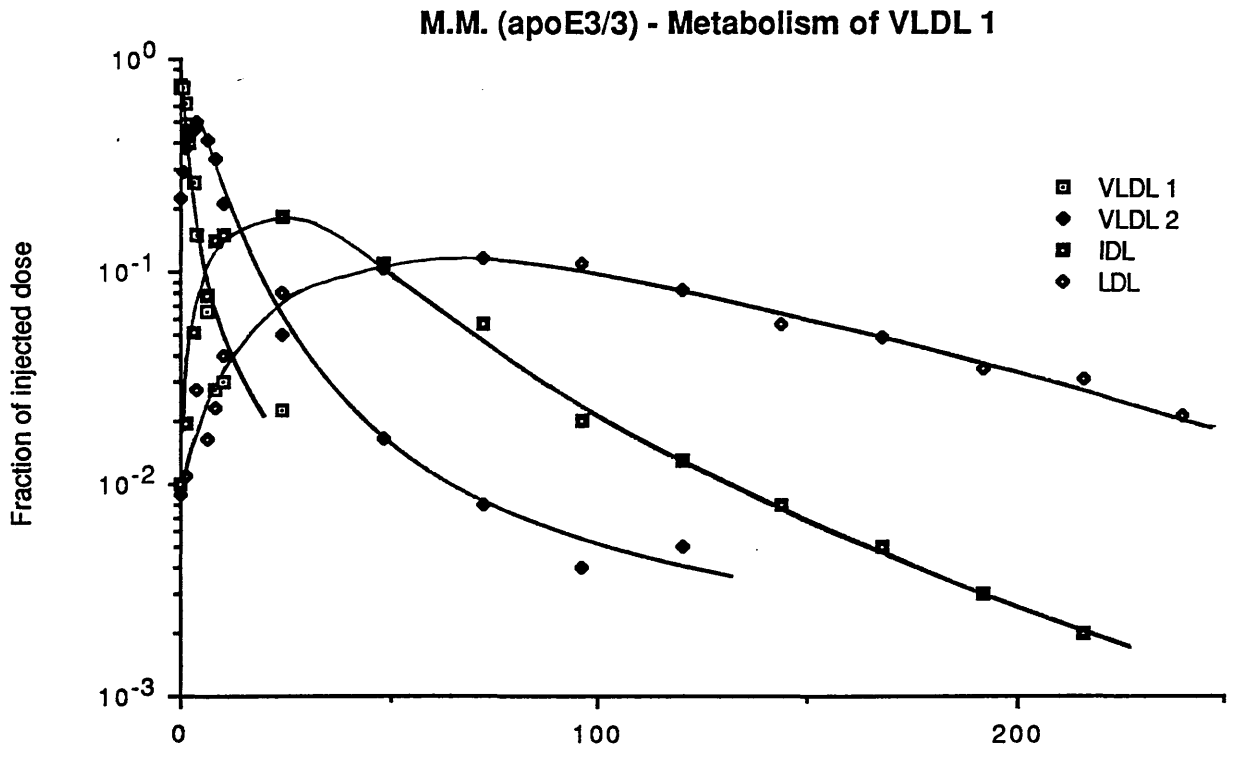
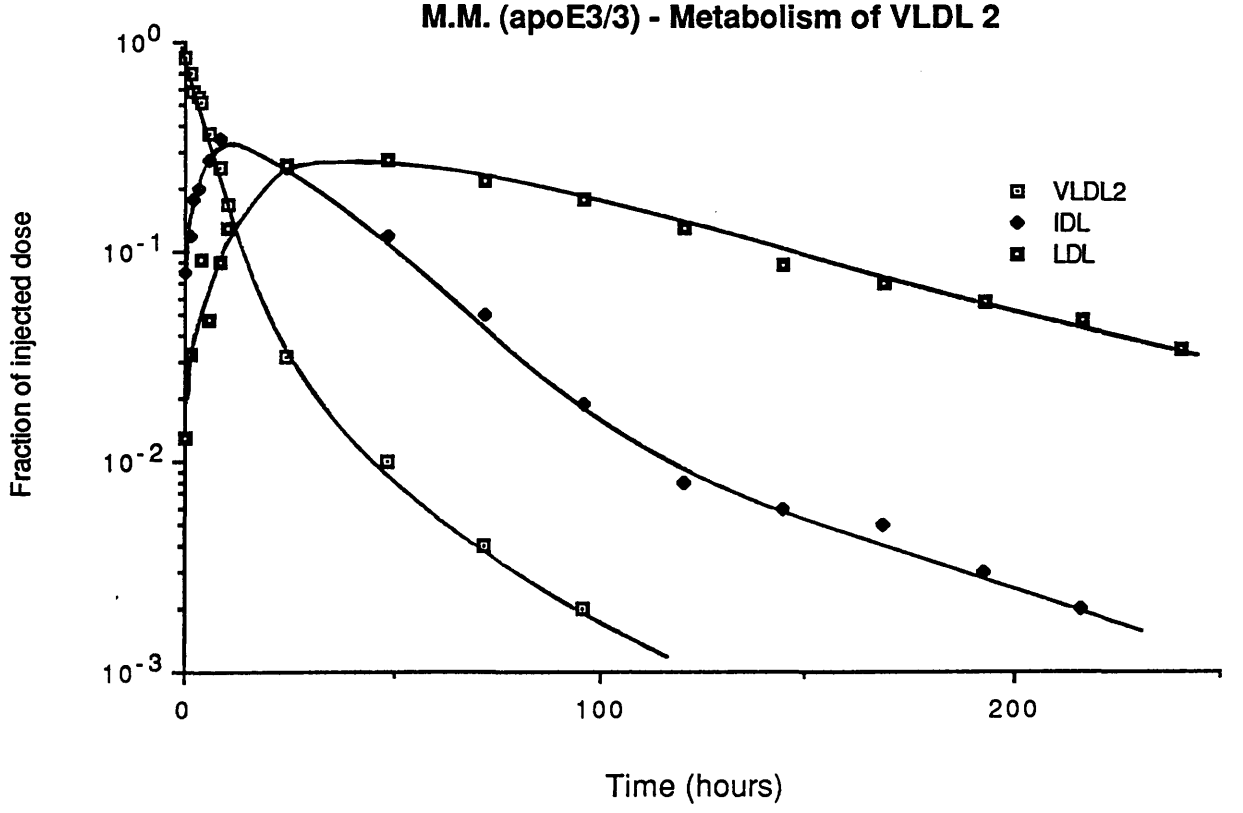
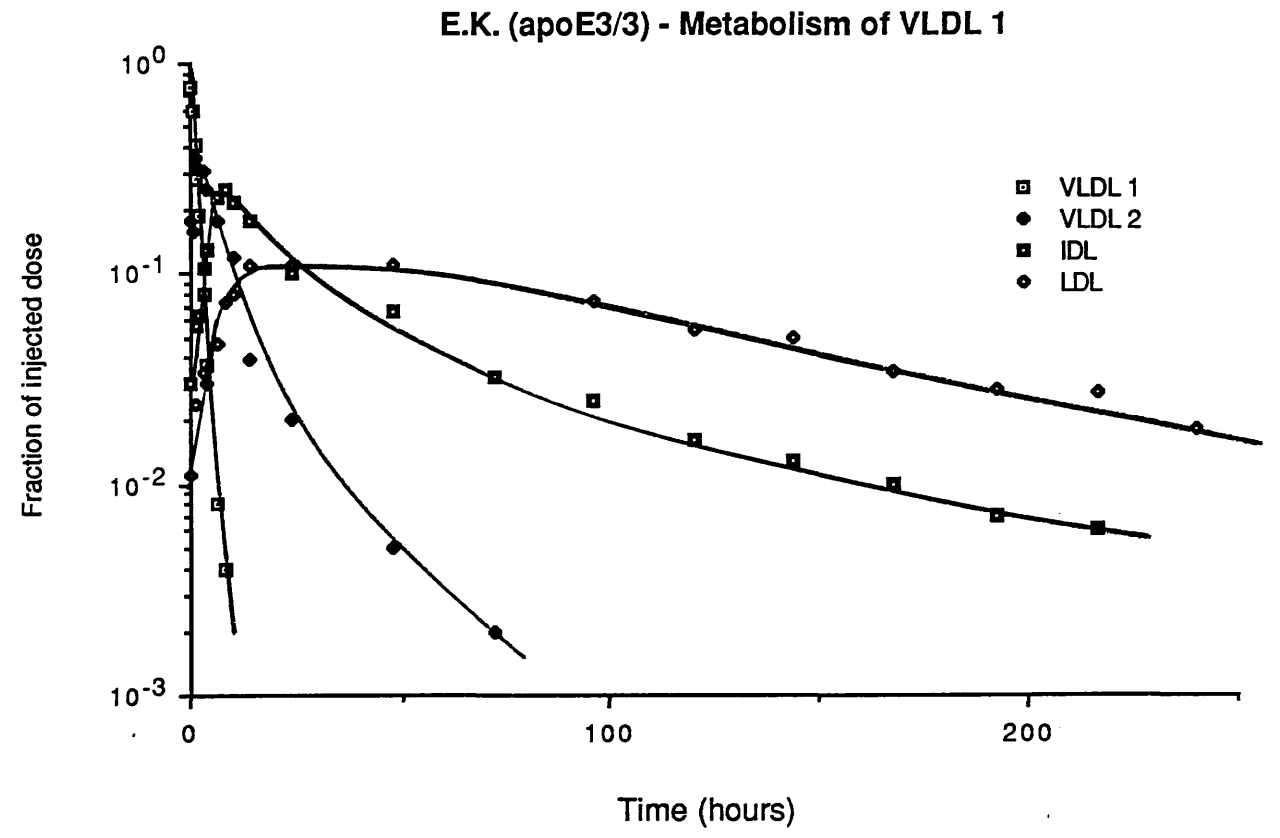
10^{-1}
10^{-2}
10^{-3}
VLDL 2
IDL
LDL
0
100
200
Time (hours)

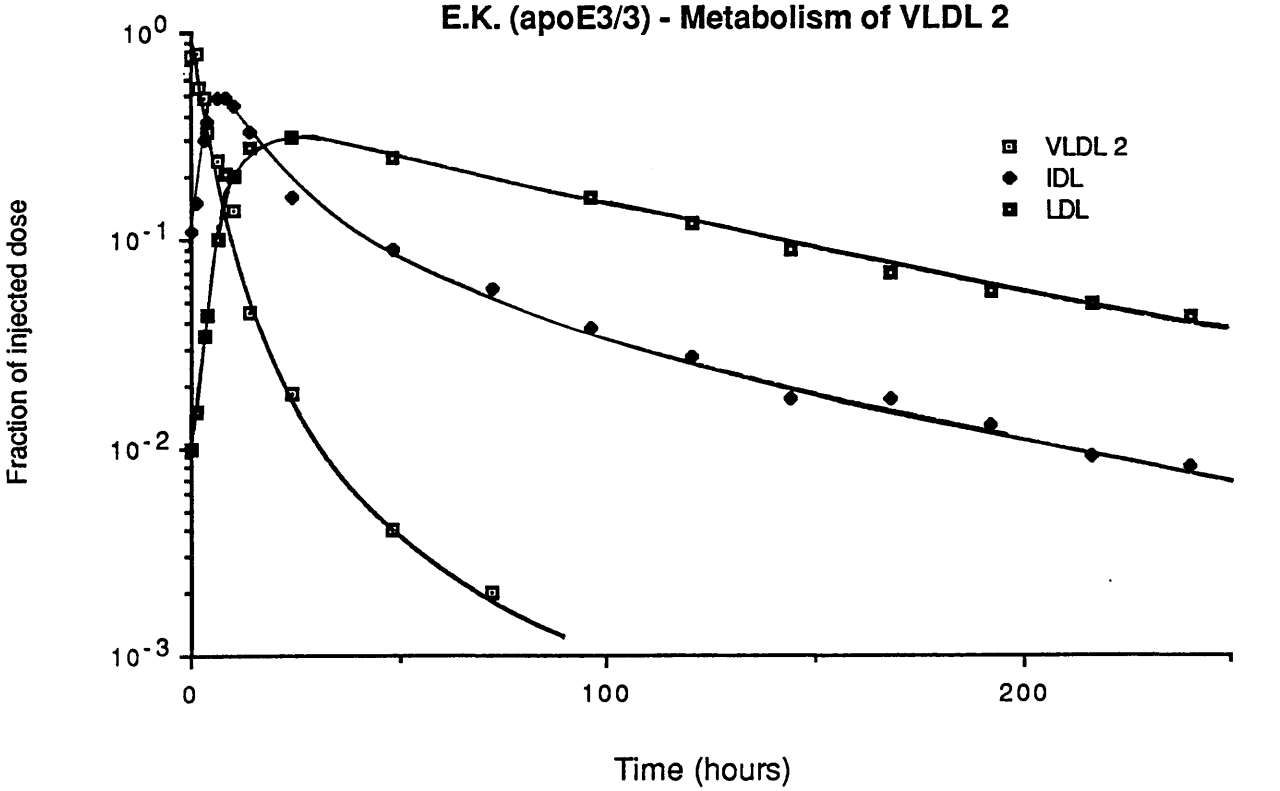

N.C. (apoE3/3) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL 1
VLDL 2
IDL
LDL
0
100
Time (hours)
200


N.C. (apoE3/3) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
VLDL2
IDL
LDL
0
100
Time (hours)
200


M.M. (apoE3/3) - Metabolism of VLDL 1
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
VLDL 1
VLDL 2
IDL
LDL


M.M. (apoE3/3) - Metabolism of VLDL 2
Fraction of injected dose
10^0
10^{-1}
10^{-2}
10^{-3}
0
100
200
VLDL2
IDL
LDL
Time (hours)





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THE METABOLISM OF LARGE AND SMALL VERY LOW DENSITY LIPOPROTEINS IN NORMAL AND DYSLIPOPROTEINEMIC STATES

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James Shepherd MD PhD
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Royal Infirmary
Glasgow G4 OSF
UK

Introduction

Plasma very low density lipoproteins (VLDL) are an heterogeneous population of particles isolated in the density interval 0.95-1.006 g/ml (Sf 20-400). The largest, least dense VLDL are rich in triglyceride while smaller species have increased contents of cholesterol and protein. Apolipoprotein B is the major protein constituent of VLDL. When labeled with iodine it acts as a useful tracer of the particle's metabolism.

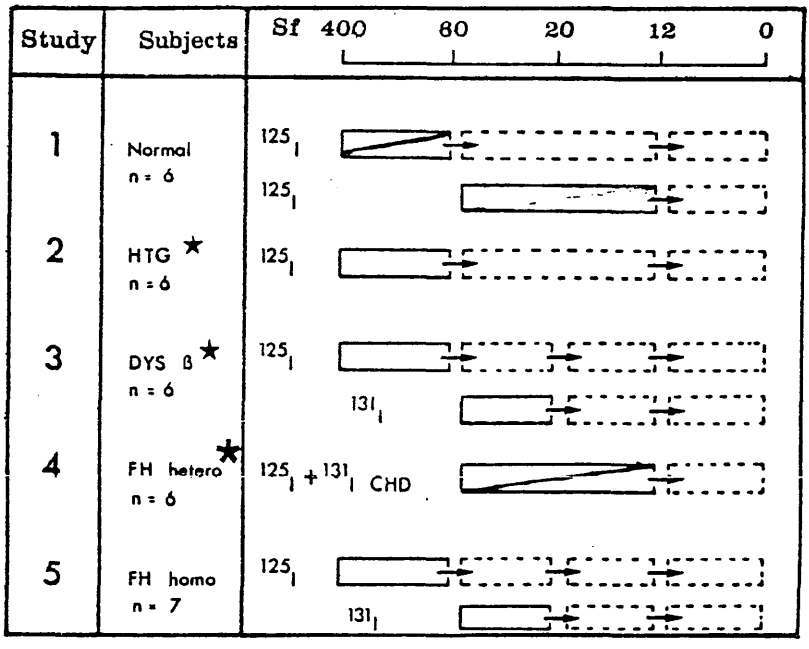
Normal individuals given a bolus injection of autologous ^{125}I-VLDL transfer more than half of the labeled apo B into low density lipoprotein (d 1.019-1.063 g/ml; Sf 0-12) via an intermediate species (IDL, d 1.006-1.019 g/ml; Sf 12-20) (1,2). That is, a precursor-product relationship exists between VLDL and denser apo B containing lipoproteins. In dyslipoproteinemic states, however this link is broken and other novel routes of apo B synthesis and catabolism appear (3,4). These conclusions drawn from early metabolic studies are based on the thesis that VLDL and its catabolic products can be viewed as homogeneous entities whose behavior within a given density interval is uniform. However, recent structural investigations of VLDL, IDL and LDL reveal the presence of multiple species which may exhibit individual rates of formation and breakdown. VLDL, for example, contains particles of varying lipid and apoprotein compositions. Some, the smaller species, are readily assimilated by cells via receptor mediated processes while larger VLDL are resistant to these effects (5). On the other hand, lipid exchange interactions seem to occur more readily with large triglyceride-rich VLDL (6). It is clear then that numerous mechanisms other than simple triglyceride hydrolysis are responsible for the remodelling and degradation of VLDL and that to begin to understand these we must dissect the apo B containing lipoproteins into relatively homogeneous sub-populations whose metabolic properties can be defined with some certainty. Since it is not yet clear what the basis of such a separation technique should be, we have as a first approximation used cumulative flotation ultracentrifugation (7) to separate lipoproteins on the basis of density and size. This procedure has been used to follow the metabolic properties of large (Sf 60-400) and small (Sf 20-60) VLDL in normal and hyperlipidemic states.

VLDL metabolism in normals

Cell culture studies have shown that hepatocytes can synthesise and secrete particles covering a broad size range (8), and in vivo it has been observed that apo B is inserted into VLDL throughout the Sf 20-400 distribution (9). The key question raised by Fisher (10) is whether such heterogeneous nascent particles ever attain metabolic equilibrium in the circulation, or alternatively are they catabolised via different metabolic channels depending on their origin? In a preliminary series of investigations (Study 1, Table 1) the transit of B protein was followed from large VLDL through its smaller delipidation products to LDL. Little of the radioactivity which was originally associated with large VLDL reached LDL. In light of that, the Sf 20-400 VLDL spectrum was fractionated in an attempt to define more clearly the origins and fates of its constituent particles.

Table 1 APOLIPOPROTEIN B METABOLISM

EXPERIMENTAL PROTOCOL

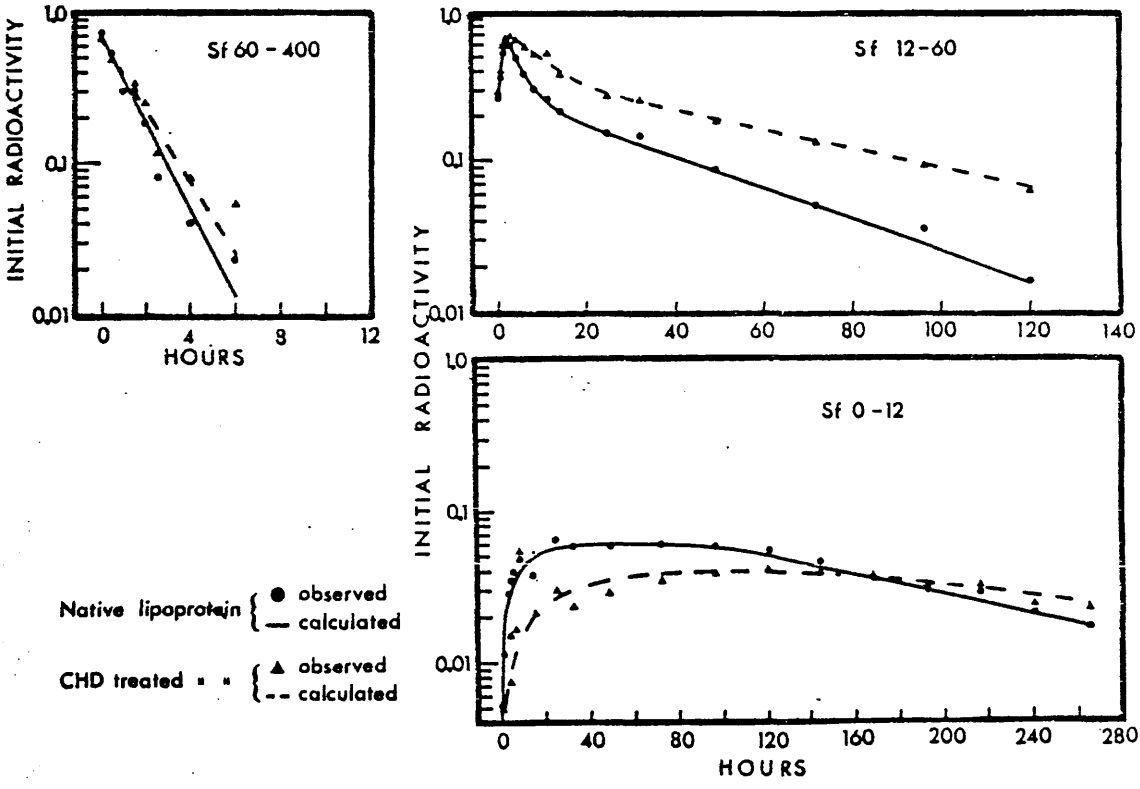


★ ± bezafibrate
★ ± cholestyramine

Within a few hours of injection of ^{125}I-labeled Sf 60-400 VLDL into normolipemic subjects, the tracer transferred virtually quantitatively to the Sf 12-60 flotation interval. Thereafter, however, it failed to progress into Sf 0-12 LDL (11). We concluded on the basis of this observation that Sf 12-60 remnants of VLDL lipolysis must be subject to direct clearance from the circulation, possibly by a process which involves the agency of cell membrane receptors. This hypothesis was tested by modifying the arginine residues on the B protein of the VLDL tracer in order to interfere with any potential receptor interaction.

The modification did not change the rate at which large triglyceride rich particles were converted to smaller remnants (Figure 1), a metabolic step which is thought to depend on lipoprotein lipase activity, but it did retard the clearance of the Sf 12-60 particle from the circulation (12). So, receptors do seem to have an involvement in remnant catabolism. Some of the B protein in the tracer (about 10%) appeared in LDL, but at a rate which was also delayed by arginine modification. This raises the question of another, possibly separate role for receptors in mediating the conversion of Sf 12-60 remnants of VLDL metabolism to LDL.

Figure 1 Transit of native and 1,2 cyclohexanedione-modified apo B from VLDL (Sf 100-400) through IDL (Sf 12-100) to LDL (Sf 0-12)



Clearly, most LDL cannot originate from large VLDL but presumably is derived from smaller particles of Sf 20-60. When these were labeled directly, the majority transferred into LDL (11). So, the Sf 12-60 density range contains a mixture of lipoproteins only some of which are destined to make LDL and within this interval there are particles whose fate depends on their pedigree; that is, there appears to be metabolic channelling within the VLDL flotation interval.

VLDL metabolism in hypertriglyceridemia

The protocol devised to examine B protein metabolism in Study 1 was extended to hypertriglyceridemic individuals (Study 2, Table 1). In this group, large VLDL was catabolised more slowly than normal, with a consequent increase in its plasma concentration. Bezafibrate corrected the defect and reduced the VLDL pool size by 70% (13). Interestingly, the drug did not alter the rate of catabolism of the remnants generated from these large triglyceride-rich particles, although it did diminish the catabolic rate of LDL and expand its circulating mass (Table 2).

Table 2 EFFECTS OF BEZAFIBRATE ON APOLIPOPROTEIN B METABOLISM IN HYPERTRIGLYCERIDEMIA*

	Fractional Catabolic Rate (pools/d)		
	Sf 100-400	Sf 12-100	Sf 0-12
Control	7.0±7.5	1.23±0.55	0.47±0.25
Bezafibrate	22.9±24.0	0.98±0.38	0.35±0.12
paired t test	<0.05	NS	<0.05

* n=6

It is apparent therefore that fibrates can exert various effects at different points on the Sf 0-400 metabolic cascade. Their known stimulatory influence on lipoprotein lipase accords with the suggestion (see above) that this enzyme governs the clearance rate of large triglyceride rich VLDL. But the lack of effect on the rate of Sf 12-60 remnant catabolism, a process which in fact was not abnormal in the hypertriglyceridemic individuals indicates that lipase is not critical in their catabolism. Presumably their removal was mediated by "receptors" as suggested for the normolipemic group examined in Study 1.

The response of LDL to fibrate therapy was investigated in greater detail in another group of hypertriglyceridemic volunteers. It transpired that the drug suppressed the hyperactivity of catabolic processes operating independently of the LDL receptor which apparently were responsible for the low circulating LDL levels in this group (14). As a result, during treatment, more LDL became channelled into the receptor route.

VLDL metabolism in dysbetalipoproteinemia

Apolipoprotein E (Apo E) exists in three major isoforms, designated E_2, E_3 and E_4. Apo E_3, the wild type protein, is common in the population, while the rarer E_2 and E_4 appear to have arisen by point mutations (15). Recent studies have shown that the isoforms exert an influence on B protein metabolism in that subjects homozygous for E_4 have higher plasma LDL levels while in E_2 homozygotes this parameter is reduced (16). When an as yet unknown second stimulus is applied to the latter group, frank Type III hyperlipoproteinemia ensues (15), with characteristic compositional anomalies in VLDL and accumulation of IDL in the plasma. Previous investigations have shown that the conversion of VLDL through IDL to LDL is slower in these individuals (17). We set out to reinvestigate the problem further as outlined in Table 1, Study 3. Large and small VLDL of Sf 60-400 and 20-60 were radiolabeled and their metabolic fate followed in six subjects before and during bezafibrate therapy (18). The rate of catabolism of large VLDL was slow in these subjects and, as noted for other hypertriglyceridemic subjects (Table 2) accelerated during fibrate therapy. This effect, coupled with the reduction in B protein synthesis which accompanied administration of the drug, led to an 80% decrement in the circulating level of Sf 60-400 apolipoprotein B (Table 3).

Treatment also reduced the input of apo B into small VLDL but did not increase the characteristically slow rate of catabolism of these particles in the Type III subjects. So again, bezafibrate had diverse effects at different points in the metabolic cascade. Interestingly, although treatment reduced LDL catabolism by up to 50%, as it had done in the hypertriglyceridemic subjects (Table 2), we did not record a rise in the plasma concentration of the fraction. This was because therapy had simultaneously suppressed LDL synthesis by promoting direct clearance of IDL from the circulation. Such an effect might be due to increased hepatic B/E receptor activity.

Apolipoprotein B metabolism in Familial hypercholesterolemia

Familial hypercholesterolemia (FH) derives from defective expression of the LDL receptor on cell membranes and, in consequence, there is failure to clear the lipoprotein normally from the circulation. Not only does this lead to an increase in the level of LDL in the plasma but there are also increments in VLDL and IDL (19). Studies 1-3 (Table 1) provide evidence that the B/E receptor appears to be involved in VLDL and IDL metabolism as well as that of LDL. Two additional investigations (Studies 4 and 5) were designed to follow the metabolic consequences of partial or complete receptor deficiency. FH heterozygotes, who express only half of the normal LDL receptor complement have high circulating levels of intermediate (Sf 12-60) lipoproteins, partly because they can convert them only slowly to LDL. Treatment with cholestyramine reduced their plasma concentration by promoting their catabolism.

In a more detailed assessment the metabolism of large and small VLDL apo B was examined in a group of 7 subjects homozygous for the FH defect. Here, large VLDL underwent normal conversion to smaller remnants which accumulated in the circulation because their subsequent turnover was defective. Two distinct patterns of metabolic behavior characterised the smaller VLDL fraction. Some subjects converted VLDL

Table 3 THE EFFECTS OF BEZAFIBRATE ON APOLIPOPROTEIN B METABOLISM IN LARGE AND SMALL VLDL OF TYPE III HYPERLIPOPROTEINEMIC SUBJECTS*

	Apo B in large VLDL Sf 60-400			Apo B in small VLDL Sf 20-60		
	Synthetic rate ($mg.kg^{-1}.d^{-1}$)	Plasma concentration (mg/dl)	Fractional catabolic rate (pools/d)	Synthetic rate ($mg.kg^{-1}.d^{-1}$)	Plasma concentration (mg/dl)	Fractional catabolic rate (pools/d)
Control	10.8±3.0	15.5±5.9	2.0±0.9	13.8±1.2	30.8±8.1	1.2±0.3
Bezafibrate	4.7±0.9	2.9±0.9	4.5±1.4	9.7±2.1	14.6±2.5	1.66±0.35
paired t test	<0.005	<0.01	<0.05	<0.01	<0.02	NS

* n=6

apo B through IDL to LDL at a very slow rate but in sufficient quantity to account for all apo LDL synthesis. In others there was rapid transmission of some VLDL apo B radioactivity directly into LDL but this pathway contributed little to the LDL B protein mass. It was therefore necessary to postulate a direct influx of apolipoprotein B into the IDL or LDL density interval in such individuals. So, receptor deficiency seems to be associated with (a) reduced direct catabolism of remnants derived from large VLDL; (b) slow B protein transit from VLDL though IDL to LDL; (c) prolonged residence of LDL in the circulation. The severe impairment of IDL to LDL conversion in these subjects provides strong supportive evidence that the receptor might be involved in this process.

Conclusions

The kinetic studies described above indicate that VLDL (Sf 20-400) can be viewed as containing at least three separate entities. Large triglyceride rich VLDL are found at the upper (least dense) end of the spectrum. The formation of these is probably favored in carbohydrate feeding and familial hypertriglyceridemia. The delipidation of these large VLDL mainly by the action of lipoprotein lipase results in the formation of remnants in the Sf 12-60 density interval. It is possible that the size of the remnant formed depends on the activity of lipase. If this enzyme is functioning normally then the residence time of VLDL is short and there is only a limited opportunity for cholesteryl ester to be incorporated by exchange from HDL into the VLDL core (20). Reduced lipase activity, in contrast prolongs the circulation time of large VLDL and favors the enrichment of the particle in cholesteryl esters. This then results in larger remnants in the VLDL density range. Little of this apo B transits the delipidation cascade to LDL and in fact it appears to have a metabolism analogous to that of gut-derived chylomicrons ie the formation of nascent triglyceride-rich particles which are lipolysed to remnants and cleared directly from the plasma via receptor-mediated pathways. Most LDL comes from small VLDL secreted into the Sf 20-60 density interval. The initial catabolic rate of these particles is slower than that of the larger species and from the results of type III and FH studies appears to involve both the 'B/E' (LDL) receptor and apolipoprotein E. In one extreme case of homozygous FH we observed that the conversion of small VLDL, through IDL to LDL took 9 days to complete. There is evidence to suggest that the formation of small VLDL is favored in familial combined hyperlipidemia and in dietary cholesterol supplementation and this may to a degree explain the increased levels of LDL associated with these conditions.

The further analysis of VLDL metabolism requires that methods are devised to permit the separation of metabolically distinct species that are at present co-isolated by size or density fractionation. One likely approach will be to prepare subfractions of differing apoprotein content by immunoaffinity chromatography and test their metabolic properties both in vitro and in vivo.

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Übersicht

Very Low Density Lipoprotein Apolipoprotein B Metabolism in Humans

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Summary. The human plasma lipoproteins encompass a broad spectrum of particles of widely varying physical and chemical properties whose metabolism is directed by their protein components. Apolipoprotein B_{100} (apo B_{100}) is the major structural protein resident in particles within the Svedberg flotation range 0–400. The largest of these, the very low density lipoprotein (VLDL), rich in triglyceride, are metabolised by sequential delipidation through a transient intermediate density lipoprotein (IDL) to cholesterol-rich low density lipoproteins (LDL). Several components contribute to the regulation of this process, including (a) the lipolytic enzymes lipoprotein lipase and hepatic lipase (b), apolipoproteins B, CII, CIII and E, and (c) the apolipoprotein B/E or LDL receptor. Lipoprotein lipase acts primarily on large VLDL of Sf 60–400. Hepatic lipase on the other hand seems to be critical for the conversion of smaller particles (Sf 12–60) to LDL (Sf 0–12). Although most apo B_{100} flux is directed to the production of the delipidation end product LDL, along the length of the cascade there is potential for direct removal of particles from the system, probably via the actions of cell membrane receptors. This alternative pathway is particularly evident in hypertriglyceridaemic subjects, in whom the delipidation process is retarded.

VLDL metabolism shows inter subject variability even in normal individuals. In this regard, apolipoprotein E plays an important role. Normolipidaemic individuals homozygous for the apo E_2 variant exhibit gross disturbances in the transit of B protein through the VLDL-IDL-LDL chain.

Key words: VLDL-LDL conversion in normal and hyperlipoproteinaemic subjects – Multicompartmental modelling – Metabolic channelling

Apolipoprotein B is unique in several respects. It is larger than most proteins, and, to date, is the longest sequenced polypeptide. Moreover, it occurs in two forms in the plasma [24]. The larger, called apo B_{100} is found in very low and low density lipoproteins (VLDL and LDL) and derives from synthesis in the liver. It comprises a single chain of 4536 amino acids [7] and is responsible for maintaining the structural integrity of its parent lipoproteins. The other variant, approximately one half the size of apo B_{100}, has been designated apo B_{48} [24]. It constitutes the structural polypeptide in chylomicrons and is synthesised exclusively in the intestine. It is in fact a truncated form of hepatic apo B in which translation has been terminated at amino acid 2152 [7]. These two apo B variants differ in one important respect in that B_{100} encapsulates a binding site for the "LDL" or "apo B/E" receptor in its C terminal half. This has been deleted in B_{48} and therefore the chylomicron does not bind to the LDL receptor [22].

When the apolipoprotein B-100 containing lipoproteins are isolated from plasma they constitute a spectrum ranging in density from 0.95 to 1.063 kg/L ie with Svedberg flotation coefficients of Sf 0–400. The least dense particles are triglyceride rich (Table 1) with a low content of cholesterol and protein – the latter comprising apo B-100, apo C and apo E. With increasing density (decreasing

Abbreviations: apo B, C, E = Apolipoprotein B, C, E; CETP = Cholesteryl ester transfer protein; FCH = Familial combined hyperlipidaemia; FH = Familial hypercholesterolaemia; FHTG = Familial hypertriglyceridaemia; HDL = High density lipoprotein; HL = Hepatic lipase; IDL = Intermediate density lipoprotein; LDL = Low density lipoprotein; LpL = Lipoprotein lipase; RFLP = Restriction fragment length polymorphism; Sf = Svedberg flotation coefficient; VLDL = Very low density lipoprotein; WHHL = Watanabe heritable hyperlipidemic

Table 1. Composition of apoliprotein B containing subfractions in normal subjects

Lipoprotein Subfraction	Triglyceride	Free cholesterol	Esterified cholesterol	Phospholipid	Protein
	gram/100 grams, mean ± 1SD				
Sf 60–400 $VLDL_1$	56.2 ± 4.8	1.7 ± 2.3	16.0 ± 4.3	17.0 ± 1.4	9.1 ± 2.4
Sf 20–60 $VLDL_2$	35.1 ± 4.0	8.1 ± 1.4	21.1 ± 5.9	21.4 ± 2.4	14.4 ± 1.6
Sf 12–20 IDL	12.4 ± 2.0	11.2 ± 2.3	33.4 ± 4.8	23.9 ± 1.3	19.1 ± 2.3
Sf 0–12 LDL	5.1 ± 0.2	13.5 ± 1.5	34.8 ± 2.2	23.0 ± 1.6	23.6 ± 1.6

flotation rate) the lipoproteins become triglyceride depleted and enriched in cholesterol, cholesteryl ester and protein. The composition of the protein component alters so that apo C and apo E are lost while apo B becomes dominant. This spectrum can be viewed as a "delipidation cascade" in which the less dense Sf 100–400 VLDL are hydrolysed to form denser intermediate lipoproteins (IDL) and then finally LDL. A number of enzymes participate in this remodelling process including lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyl transferase [14]. Other proteins too such as cholesteryl ester transfer protein and the B/E receptor are important. The details of how these lipoprotein transformations occur have been recently reviewed in this Journal [14]. In the following discussion we focus on the quantitative aspects of this delipidation sequence and how it differs in normal and hyperlipidaemic subjects.

VLDL – LDL Conversion in Normals

The first investigations of the metabolic fate of trace-labelled VLDL in man demonstrated [18] that radioactivity initially present in Sf 10–200 "VLDL" was rapidly transferred to the Sf 3–9 LDL density interval. Later with appreciation of the protein heterogeneity in VLDL, apo B was specifically examined and found to be the moiety that was conserved in this process [13] in that all LDL apo B in the plasma could be attributed to the delipidation of VLDL. Sigurdsson et al. [42] initially quantified this conversion and found that in normals not only did all "LDL" (d 1.006–1.063 g/ml) come from VLDL but in addition all of the VLDL was catabolised to LDL. This rather strict precursor-product relationship was later shown to be not altogether correct in that while the majority of VLDL apo B did appear in the 1.006–1.019 kg/L density range (ie IDL), in normals a smaller proportion of this ultimately became LDL [23]. The transient intermediate, IDL, is short lived and of low concentration in most subjects but can be substantially elevated in certain dyslipidaemias. Further investigations of the VLDL-IDL-LDL metabolic cascade have revealed that there are multiple sites of entry and exit which can only be quantified using computer-based multicompartmental modelling techniques. These not only permit the calculation of apoprotein flux rates but also allow for the testing of quantitative hypotheses regarding the physiology of the VLDL-LDL conversion.

Berman et al. [2] were the first to formulate a mathematical model describing the VLDL-LDL conversion. This includes features required to explain both apo B, and apo C kinetics (Fig. 1a). In normal individuals, input of newly synthesised material occurred into the largest triglyceride-rich VLDL, which was converted through a chain of compartments (the delipidation cascade) to IDL. The VLDL spectrum also contained a slowly metabolised species (termed β-VLDL) which did not contribute to IDL or LDL. The latter were modelled as single compartments in which IDL was restricted to the plasma space. Any model of this kind should allow for not only the behaviour of the apoproteins but also for that of the major VLDL lipid, triglyceride. Such a scheme (Fig. 1b) has been proposed by Beltz and colleagues [1]. It differs from the original model in that it (1) permits a variable delipidation chain length (2) proposes an extravascular IDL sub-compartment derived from very large, rapidly catabolised VLDL and (3) allows slowly metabolised "remnant" VLDL to contribute to LDL production.

In order to test some of the hypotheses implied in these models, we have used two approaches. First, VLDL (Sf 20–400) was split into two fractions, $VLDL_1$ (Sf 60–400) and $VLDL_2$ (Sf 20–60) on the basis of the results of a number of metabolic studies in which the behaviour of many discrete subfractions of VLDL were examined (Fig. 2).

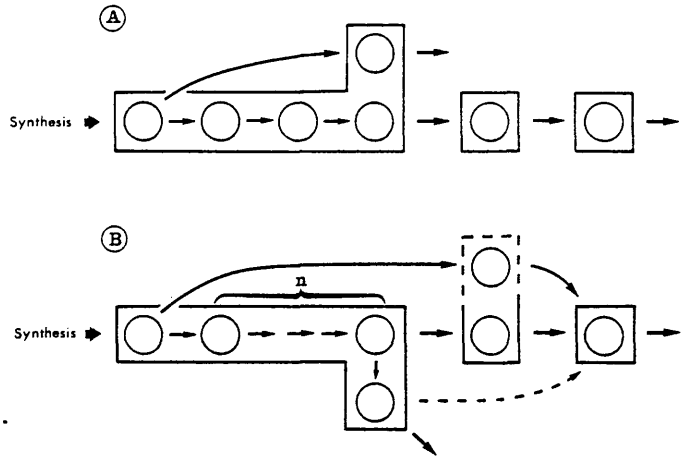

Fig. 1. Development of mathematical models describing VLDL-LDL conversion. **A** Original model proposed by Berman et al. [9] in which VLDL is a single chain delipidation cascade feeding IDL and LDL. A compartment is included in VLDL to represent a slowly metabolised species. **B** This modified model by Beltz et al. [10] permits variable delipidation and the possibility of LDL production from rapidly catabolised large VLDL via an extravascular IDL pool

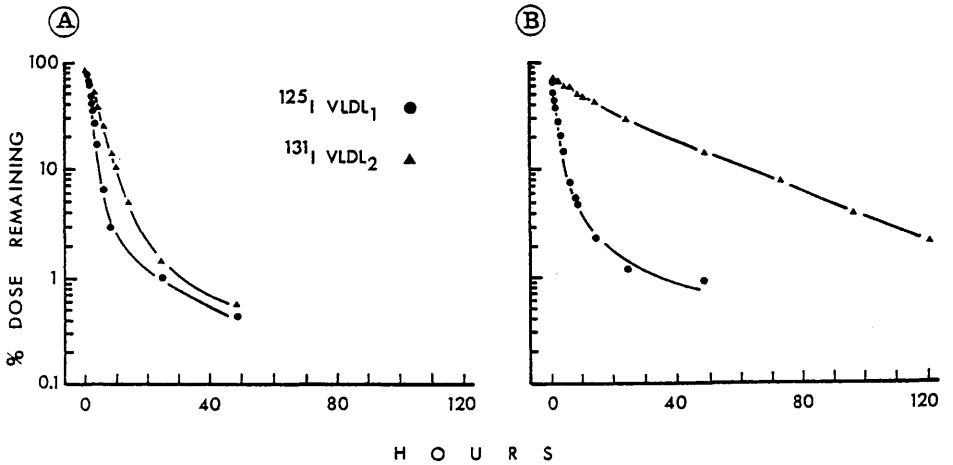

Fig. 2. Plasma decay curves of trace labeled large and small VLDL in **A** a normolipidaemic subject and **B** an homozygous FH individual. Note that the clearance of large VLDL is the same in both individuals. The impact of the LDL receptor defect is on the smaller VLDL species

From these it was clear that the denser more cholesterol rich $VLDL_2$ were in certain circumstances, like homozygous familial hypercholesterolaemia, metabolised at rates that were distinct from that of the triglyceride rich $VLDL_1$. This has also proved to be the case in type III hyperlipidaemic patients [37] and hepatic lipase deficiency (Th Demant, J Shepherd, CJ Packard, unpublished observations). In the second approach, $VLDL_1$ was modified chemically with 1,2 cyclohexanedione in order to block potential interaction of the tracer or its metabolic products with lipoprotein receptors [36]. This study indicated that receptors had no role in the initial lipolysis of triglyceride rich $VLDL_1$ but were important in later stages where IDL was converted to LDL or catabolised directly from the plasma. Amalgamation of these results produced the working model, shown in Figure 3, which forms a useful basis for the consideration of apolipoprotein B kinetics in both normal and hyperlipidaemic subjects. The flux of B protein through this system in normal individuals is enumerated in Figure 4. It was necessary to postulate that there was direct synthesis of apo B into both large and small VLDL. Two thirds of this material was transmitted through to IDL and LDL while the remainder generated a "remnant" pool which was cleared slowly from the circulation, probably via receptors. One important finding of these investigations which is demonstrated in Figure 4 is the subcompartmentalisation of IDL and LDL. This was needed to allow for the observation that apo B associated with the small VLDL tracer appeared more rapidly and in greater amount in IDL and

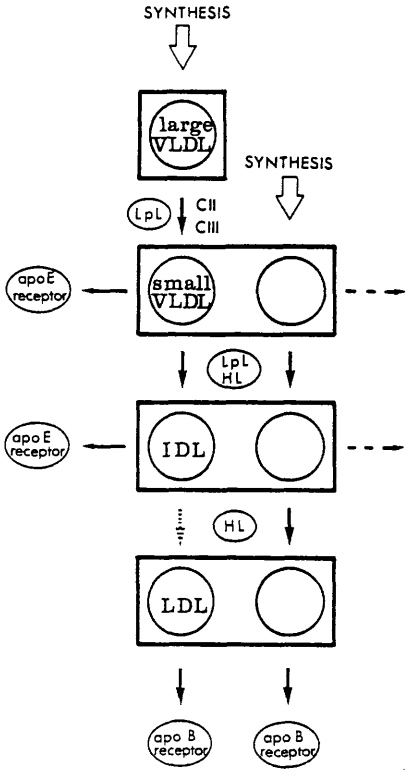

Fig. 3. Metabolic scheme outlining VLDL metabolism. De novo input of large and small VLDL feeds delipidation chains that lead to IDL and LDL. Parallel processing pathways within these fractions account for their metabolic heterogeneity. Initial delipidation is thought to depend on the activity of lipoprotein lipase and the small molecular weight regulatory C apolipoproteins. Further down the chain, hepatic lipase plays an increasingly important role. Direct receptor mediated catabolism is permitted at multiple points along the cascade. This may be governed by the presence of B or E proteins on the particles

LDL than apo B from large VLDL. That is, metabolic channels are present in the VLDL-LDL conversion process so that the fate of an apo B containing lipoprotein depends to a certain extent on its pedigree. Indeed, since VLDL is such an heterogeneous mixture of particles we ought to expect metabolic heterogeneity in its products, IDL and LDL. The recognition of this phenomenon is an exciting development in our understanding of the structure and function of these lipoproteins [32, 16].

Quantitative investigations of the rates of transport of apo B through the VLDL-IDL-LDL cascade reveal how much of each species is made and indicate its probable precursor. However, further work is needed to elicit the mechanisms involved in these transformations. The study of pathological conditions where a specific component is impaired allows us to build a picture of the key proteins, enzymes and receptors that are involved in such a scheme (Fig. 3).

VLDL-LDL Conversion in Hypertriglyceridaemia

Early studies of hypertriglyceridaemic subjects [38] have shown that these individuals make more VLDL apo B than is required for LDL synthesis. We have seen above that about 30–50% of IDL apo B in normal individuals does not reach LDL. So, the cascade from VLDL to LDL must allow for direct catabolism at multiple sites along its length. The nature of these catabolic mechanisms is not completely clear nor is it known what causes a particle to take the route of direct catabolism rather than be subject to further delipidation. One possibility, suggested by in vitro studies, is that if a VLDL particle has a prolonged residence in the plasma it may acquire too much cholesteryl ester in its core to permit it to shrink to the size of LDL. In support of this view, it has been shown that VLDL subfractions from normal subjects may be hydrolysed in the test-tube to LDL-like particles [8]. Large VLDL from hypertriglyceridaemics on the other hand seems to be unable to be lipolysed

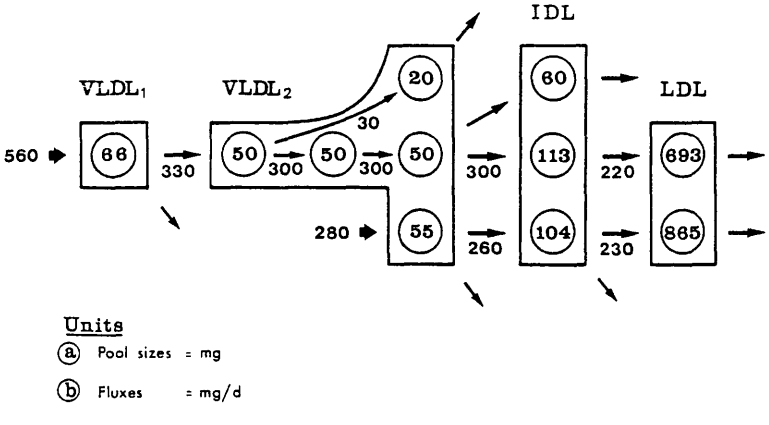

Fig. 4. Multicompartmental model describing the flux of apolipoprotein B through the cascade system in a normal subject

sufficiently to form LDL in vitro and in vivo would constitute a fraction of VLDL that fails to transit the delipidation cascade [34]. Metabolic studies from our laboratory have shown that hypertriglyceridaemic patients, in common with normals, catabolise $VLDL_1$ to smaller remnants within the $d < 1.006$ kg/L density interval but these fail to progress to IDL and LDL. In contrast smaller VLDL (Sf 20–60) is a much better precursor of LDL [35]. This aspect of VLDL-LDL conversion (Fig. 3) offers an explanation of a number of findings. Kissebah et al. [25] divided hypertriglyceridaemic subjects into those with familial hypertriglyceridaemia (FHTG) and those who had familial combined hyperlipidaemia (FCH). VLDL in the former tends to be larger and more triglyceride-rich than normal while the lipoprotein fraction in the latter disorder has a composition and size similar to that of control subjects. VLDL apo B and triglyceride is overproduced in the FHTG subjects but LDL synthesis is normal, suggesting that it is $VLDL_1$ that is being generated by the liver. On the other hand, FCH is associated with an excess of both VLDL and LDL synthesis and so it may be postulated that in this situation it is $VLDL_2$ rather than $VLDL_1$ that is being elaborated. A similar explanation may be offered for other situations in which VLDL and LDL behave discrepantly. Carbohydrate feeding to normal individuals leads to increased triglyceride (VLDL) levels and decreased LDL [41], whereas fish oils cause a decrease in VLDL and a rise in LDL [48]. It can be postulated that on the former diet, larger VLDL are synthesised which are poor LDL precursors while the opposite is true in the latter.

The mechanisms responsible for the conversion of large and small VLDL to IDL and LDL are partly understood. Lipoprotein lipase (LpL) situated on the capillary endothelium, is responsible for the removal of triglyceride from triglyceriderich particles. In vitro studies of the suitability of different lipoprotein fractions as substrates for this enzyme have demonstrated that chylomicrons and larger VLDL are better than the smaller denser lipoproteins. Conversely the other membrane bound lipase released into post-heparin plasma – hepatic lipase (HL) – shows particular affinity for smaller VLDL and IDL [33] suggesting that these two enzymes may have complementary roles in the delipidation cascade. Metabolic studies in hypertriglyceridaemia provide further evidence for this. Classically, LpL deficiency is associated with the accumulation of chylomicrons. However recent investigations [47] have demonstrated that large apo B-100 containing VLDL also accumulate. These particles are triglyceride-rich, and when trace-labelled and re-injected into LpL deficient subjects fail to be degraded to IDL and LDL. LpL must therefore be the key rate controlling step in the conversion of large to small VLDL (Fig. 3). Of course, triglyceride hydrolysis is not the only process involved in this conversion. The whole particle must be remodelled with loss of surface components (phospholipid, apo C and free cholesterol) to HDL and acquisition of cholesteryl ester by the action of cholesteryl ester transfer protein CETP [15, 49]. The activity of the enzyme is modulated by hormones, particularly insulin [39] and it can be activated by hyperlipidaemic drugs such as bezafibrate. If the latter is given to hypertriglyceridaemic subjects an increased rate of clearance of large VLDL is observed [44] but catabolism of smaller VLDL and IDL is not affected. The small molecular weight apoproteins CII and CIII appear to have opposing effects on the activity of this enzyme and on VLDL triglyceride hydrolysis. Apo CII is an essential cofactor for LpL action and if it is absent or defective [4] a clinical picture similar to primary LpL deficiency (Type I) results with the accumulation of VLDL and chylomicrons. Equally rare individuals who have a genetic lesion which causes an absence of CIII from the plasma have low triglyceride levels and VLDL catabolism is accelerated above normal [17]. These findings from studies of inherited disorders together with the observed inverse correlation between the CII/CIII ratio and VLDL triglyceride levels [6] in other subject groups suggests a strong influence of these apoproteins on LpL activity.

It is strange that despite an absence of functional LpL, Type I individuals can convert "VLDL apo B" to IDL and LDL at approximately normal rates [33]. A possible explanation for this paradox is that when whole VLDL is trace labelled most of the B protein is present in smaller VLDL. Its conversion to denser lipoproteins is not critically dependent on LpL. Rather, this component of the delipidation process seems to depend on the activity of hepatic lipase. Preliminary studies in our laboratory of VLDL metabolism in a patient with hepatic lipase deficiency indicate that while the catabolism of $VLDL_1$ to $VLDL_2$ is unimpaired, the transfer of apo B through $VLDL_2$ to IDL and LDL is diminished. In fact, in this individual normal LDL was virtually absent from the plasma. These results are in accord with animal experiments in which antibody-induced inhibition of HL leads to accumulation of small VLDL and IDL and a fall in LDL [19]. If it is postulated that IDL-LDL conversion involves hepatic lipase

then this activity must be located in the liver, a contention supported by examination of lipoprotein flux across the splanchnic bed. Turner et al. [52] found that while there was evidence for secretion of large (Sf 100–400) $VLDL_1$ from the liver there was no detectable uptake of this lipoprotein fraction. On the other hand, radio-iodinated lipoproteins of Sf 12–60 ($VLDL_2$ and IDL) were extracted from the circulation by the splanchnic bed and about half of the radioactivity reappeared in the hepatic vein as LDL (Sf 0–12). Therefore, this enzyme occupies a pivotal role in the transformation of apo B containing lipoproteins in the lower part of the delipidation cascade.

VLDL-LDL Conversion in Dysbetalipoproteinaemia

So far we have introduced two enzymes (LpL and HL) and two apoproteins (CII and CIII) as key components in the VLDL-LDL conversion. Partial or complete deficiency of these moieties has a profound impact on the regulation of the delipidation process. One further condition that is associated with primary hypertriglyceridaemia is Type III hyperlipidaemia (dysbetalipoproteinaemia). Individuals with this disorder appear to have inherited a double defect. They possess mutant apolipoprotein E [54] whose lipoprotein receptor binding properties are compromised by the substitution of cysteine for arginine at position 158 in the polypeptide chain [31, 58]. In addition another gene predisposing to hyperlipidaemia seems to be necessary to produce the elevated lipid levels. About 1% of the population possess the apo E mutation (E_2/E_2) present in the Type III condition. A further 2% are homozygous for a mutation at a separate site in which cysteine is substituted by arginine ($E_{4/4}$) at position 112. These aberrant E proteins influence the levels of the apo B-containing lipoproteins in plasma [12, 55, 56, 57]. Normolipidaemic individuals with the $E_{2/2}$ phenotype have lower plasma apo B and LDL cholesterol than the 60% of subjects who express the normal E_3/E_3 wild type pattern. An $E_{4/4}$ individual, conversely, tends to express higher plasma apo B and LDL cholesterol levels. The explanation for this relationship is not known although it has been postulated [56] that the $E_{2/2}$ mutation leads to decreased uptake of chylomicron remnants and their associated cholesterol by the liver. As a result, the liver expresses more LDL receptors in order to fulfil its sterol requirements. LDL catabolism is increased and plasma levels fall. An alternative hypothesis is that individuals with $E_{2/2}$ produce less LDL from VLDL precursors because the protein is essential for this process [11]. More VLDL apo B would then be channelled into remnants which would accumulate in the plasma. There is support from metabolic studies for both hypotheses. For example, Type III patients have a characteristically low rate of conversion of VLDL to LDL [37, 53] which fails to respond to fibrate-induced activation of LpL. In fact, although fibrates do lower the lipid levels in these individuals, they fail to rectify the basic distortion of their lipoprotein spectrum. Relatively high levels of VLDL remnants and IDL persist. A similar pattern underlies the lipoprotein profile in normolipidaemic E_2/E_2 individuals. Recent studies in our laboratory examined the flux of apo B through the plasma of normal individuals of defined apo E phenotype. Compared to E_3/E_3 subjects an E_2/E_2 individual expresses slower VLDL and IDL decay rates and higher levels of these

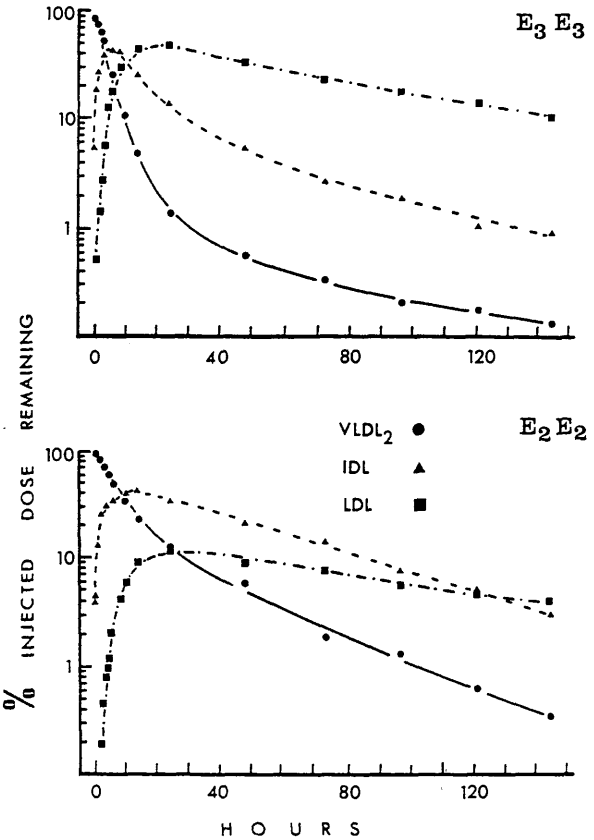

Fig. 5. Plasma decay curves describing the transit of apo B from small VLDL through IDL and LDL in subjects homozygous for the E_2 and E_3 proteins. Autologous trace labeled small VLDL was injected and its flow into IDL and LDL followed over the course of 150 hours. The pool sizes of small VLDL, IDL and LDL for the E_3/E_3 subject were 148, 205 and 1280 mg respectively; and for the E_2/E_2 individual, 294, 426 and 710 mg

fractions, and a reduced conversion to LDL (Fig. 5). The catabolic rate of the product LDL is no different from normal.

As noted above, type III individuals treated with fibrates retain cholesteryl ester rich VLDL in their plasma suggesting, as was found in our metabolic study, that the lipoprotein class still contained a high proportion of VLDL 'remnants' despite the successful hypolipidaemic therapy. These drugs act mainly to reduce overall VLDL synthesis without accelerating the characteristically slow catabolic rate of small VLDL seen in these individuals [37]. In contrast two other agents, oestrogen [28] and mevinolin [10], correct the lipid composition of VLDL in type III, presumably by facilitating clearance of the 'remnant' population. These drugs are known to upregulate hepatic lipoprotein receptors and may well act to overcome the inefficient interaction between $E_{2/2}$ containing lipoproteins and receptors. The exception to this rule seems to be the apo E deficient patient reported by Schaefer et al. [40] who failed to respond appropriately to oestrogen therapy. This observation indicates the need for some apo E, however defective, to mediate VLDL remnant removal.

VLDL-LDL Conversion in Hypercholesterolaemia

Familial hypercholesterolaemia results from a partial or complete deficiency of the LDL or B/E receptor. This protein, present on the membranes of most cells in the body, is able to bind LDL and internalise it. The lipoprotein is delivered to secondary lysosomes where its cholesterol is released into the cell to meet structural and metabolic requirements [5, 20]. This receptor-mediated pathway is autoregulated. Knowledge of its operation is the key to our understanding of how LDL levels are controlled in man. Early studies of the FH condition focused on the gross increase in LDL cholesterol and the impact that this had an atherosclerosis. More recently it has become appreciated that the B/E receptor has a much wider role in apolipoprotein B metabolism. Its absence affects not only LDL but also VLDL and IDL. The discovery of a mutant strain of rabbits (the Watanabe Heritable Hyperlipidaemic – WHHL – rabbit) that lack functioning LDL receptors, provided a model for the detailed study of hepatic secretion and interconversion of lipoproteins in the receptor deficient state. The marked hypertriglyceridaemia in WHHL animals was the first indication that lack of the receptor might have an impact on the clearance of VLDL as well as LDL [3, 27]. These animals, like FH patients, metabolised chylomicrons normally [26]. So their increased plasma triglyceride could not be attributed to an inability to clear dietary fat from the circulation. Trace labeled VLDL was retarded in its clearance from the rabbits' plasma [27] and the lipoprotein therefore accumulated there. This phenomenon did not seem to mirror the situation in humans. In an early metabolic study, Soutar and her colleagues [45, 46] reported normal VLDL apo B turnover rates and normal plasma triglyceride levels in a group of homozygous FH subjects. The picture is further confused if VLDL-LDL conversion is examined. The human studies indicated that LDL production exceeded by up to 2 fold the VLDL catabolic rate and consequently direct input of apo B into LDL had to be postulated [45]. Evidence gained from perfusion studies on the WHHL rabbit, however indicated that the liver made only VLDL [21]. No lipoproteins of LDL density were found in the perfusate medium. The measured increase in LDL synthesis in the rabbit was attributed to a reduction in direct VLDL catabolism and an increase in its conversion to LDL; whereas normal rabbits transferred about 8% of VLDL-B to LDL this value was increased to 40% in receptor-deficient animals [59]. The discrepancy between the animal model and humans is not fully resolved. However, we have recently re-examined the situation in a group of seven FH homozygotes in whom we investigated the metabolism of large $VLDL_1$ and small $VLDL_2$ (Th Demant, J Shepherd, CJ Packard, unpublished observations). A number of interesting findings emerged. First, the conversion of $VLDL_1$ to $VLDL_2$ was unimpaired by the lack of receptors, consistent with the role of LpL as the mechanism responsible for this step (Fig. 3). Small (Sf 20–60) VLDL metabolism, on the other hand, was grossly abnormal (Fig. 2). Both the clearance of remnants from this density interval and the rate of delipidation to IDL and LDL were inhibited. We observed that the FH subjects with the highest triglyceride levels oversynthesised apo B and derived most of their LDL from VLDL precursors. Other FH patients did not derive all LDL from VLDL and in these subjects (as in those studied by Soutar et al. [50] de novo LDL synthesis had to be invoked in order to account for the observed plasma LDL mass.

New Horizons in Apolipoprotein B Metabolism

Recombinant DNA technology provides a powerful new tool for the investigation of the role of genetics in the regulation of lipoprotein metabolism. The umbrella term "normality" which

encompasses plasma cholesterol levels ranging from 2.5–6.5 mmol/l and triglyceride from 0.5–2.5 mmol/l needs to be redefined since it clearly incorporates a spectrum of individuals with widely varying lipid metabolism. Some studies on the effects of apoprotein polymorphism such as that described for apo E above have been completed. Mutation in this protein alone has been estimated to account for 16% of the phenotypic variance in LDL cholesterol [43]. Another variation at the gene level has been described for apo B. By digestion with the endonuclease XbaI a restriction fragment length polymorphism (RFLP) in the apo B gene can be detected that appears to correlate with the LDL cholesterol level [29, 50]. The mechanism of this effect is not clear but initial investigations [9] indicate that alterations in the B protein may result in its perturbed receptor binding and catabolism. Given the importance of lipoprotein-receptor interactions, such polymorphisms may have a number of consequences which impinge on VLDL-LDL conversion. Other methods for detecting variation in apo B structure using monoclonal and polyclonal antibodies [30, 51] have been published. The application of these techniques should allow us to subdivide "normal" individuals into groups whose metabolism can be subjected to vigorous scrutiny to determine those factors responsible for the regulation of the system.

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Catabolic Rate of Low Density Lipoprotein Is Influenced by Variation in the Apolipoprotein B Gene

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Abstract

This study examines the potential influence of genetic variation on the metabolism of LDL. Restriction fragment length polymorphisms (RFLP) of the gene coding for apo B were identified using the endonucleases Xba I, Eco RI, and Msp I in a group of 19 subjects with moderate hyperlipidemia. There was a significant association between the Xba I polymorphism and the total fractional clearance rate (FCR) of LDL. The individuals with the *X1X1* genotype had, on average, a 22% higher FCR ($P < 0.025$) than those with the genotype *X2X2* (*X2* allele = presence of Xba I cutting site). This difference was attributable to increased clearance by the receptor-mediated pathway of LDL catabolism. In this group of subjects, there was no association of LDL kinetic parameters and RFLPs of the LDL receptor gene or the AI- CIII- AIV gene cluster. The data suggest that variation in apo B itself, presumably acting through variable binding to the LDL receptor, makes a significant contribution to the rate of catabolism of LDL.

Introduction

Apo B, the major protein component of LDL, contains the binding site for the LDL receptor and consequently plays a pivotal role in the metabolism of the lipoprotein, by facilitating its cellular uptake and degradation (1, 2). In recent years, attention has focused on the receptor as the mediator of cholesterol homeostasis in the body, and we now know that a variety of mutations in this protein may produce gross disturbances in plasma LDL levels (3, 4). Now, with the isolation of the gene coding for apo B (5), we are able to use the techniques of molecular biology to analyze the contribution that the ligand might make to alterations in lipoprotein metabolism.

DNA probes for the human apo B gene have recently been isolated (6–9) and a number of common restriction fragment length polymorphisms (RFLP)[1] have been described (9–11). One of these, an RFLP detected using the restriction enzyme Xba I, has been shown in normal individuals to be associated with variations in plasma cholesterol and triglyceride (12–14). The same polymorphism has also been reported to represent an independent risk factor for ischemic heart disease (15), although there is not universal agreement on this point (16). In this study, we use three polymorphisms of the apo B gene locus to examine the relationship between variation in the apo B gene and the metabolism of LDL in a group of individuals with moderate hyperlipemia.

Methods

Subjects. Volunteers aged between 40 and 60 yr were identified as hyperlipidemic during an opportunistic screening program in the city of Glasgow. Those detected were given dietary advice designed to correct their lipid abnormality and reassessed after 2 mo. At this point, potential sufferers from familial hypercholesterolemia were diagnosed on the basis of recognized criteria (17) and excluded if they had tendon xanthomata or a first-degree relative (particularly a child) with raised LDL cholesterol. Two subjects with total cholesterol values of 8.5 and 8.3 mmol/liter were rejected on this basis. None of the remaining 19 unrelated Caucasian subjects (4 male, 15 female; Table I) who failed to respond adequately to the diet, had a strong family history of premature cardiovascular disease (i.e., more than one affected first-degree relative aged < 55 yr). Two presented with xanthelasmata, two with corneal arcus, and two with angina of effort (Table I). Their plasma cholesterol levels were, on average, 7.69±0.73 mmol/liter and their diet was maintained during the evaluation of LDL metabolic parameters. 60 mg potassium iodide was given thrice daily for 3 d before and 2 wk after the study to prevent thyroidal sequestration of radioiodide. Biochemical tests showed that none of the subjects suffered from hepatic, renal, or endocrine disease and none had overt ischemic heart disease. All subjects gave informed consent to the study, which was approved by the Ethical Committee of Glasgow Royal Infirmary.

Metabolic studies. LDL turnover was assessed in the patients using a protocol described elsewhere (18, 19). Briefly, autologous LDL (d = 1.03–1.05 kg/liter) was prepared by rate zonal ultracentrifugation (20) and divided into two aliquots, which were labeled separately with ^{125}I and ^{131}I (21). The latter was then subjected to modification with 1, 2-cyclohexanedione, which blocks the arginyl residues on its protein moiety (22) and provides a tracer of receptor-independent LDL metabolism (18, 19). Such modification prevents interaction of the lipoprotein with the receptor and denies it access to the receptor-dependent degradation pathway. Plasma clearance of each tracer was followed over a 2-wk period and the radioactive decay curves were constructed and analyzed using the SAAM 29 computer program (23). This gave fractional clearance rates (FCRs) for the native and chemically modified LDL that were used to obtain values for total, receptor-independent, and, by difference, receptor-mediated catabolism of the lipoprotein (18, 19). Plasma apo LDL concentrations were determined from calculations based on serial LDL cholesterol measurements and on compositional data derived from analyses of the isolated lipoprotein (24). The absolute clearance rate for LDL apoprotein was then calculated as the product of the total FCR and the plasma LDL pool (i.e.,

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1. *Abbreviations used in this paper:* FCR, fractional clearance rate; RFLP, restriction fragment length polymorphism.

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apo LDL concentration times the plasma volume). This parameter is commonly expressed per kilogram of body weight, and under the steady state conditions of the study equals the synthetic rate of the protein. Separate absolute clearance rates can also be calculated for the receptor-dependent and independent routes as the product of the apo LDL pool and the FCR can be determined for each pathway.

DNA analysis. Blood was collected into 2.0 mg/ml K_2 EDTA and stored at −20°C until analysis. DNA was prepared from these specimens by the Triton X100 lysis method (25), and a 5.0-μg aliquot was digested using a panel of enzymes (Eco RI, Xba I, Pvu II, Xmn I, and Nco I) at 2–10 U of enzyme per microgram of DNA according to the supplier's instructions (Anglian Biotech, Colchester, England).

The fragments generated in each digest were separated by agarose electrophoresis and transferred to Hybond filters (Amersham Corp., Amersham, England) by Southern blotting.

Polymorphisms of the apo B gene were detected (Fig. 1): (*a*) by hybridizing the two Xba I digest fragments designated *X1* (8.6 kb) and *X2* (3.5 kb), with the 3.5-kb probe pABC3.5 (11); (*b*) in an Eco RI digest by hybridizing the two fragments, *R1* (10.5 kb) and *R2* (12.5 kb), with the cDNA probe pAB3 (11); and (*c*) after digestion with Msp I and probing with PH2 (a 2-kb Hind III fragment subcloned from an apo B genomic recombinant). Multiple hybridizing fragments could be demonstrated (11). Those 2.6 kb and larger were designated *M1* and those 2.2 kb and smaller were designated *M2*. Polymorphisms of the LDL receptor gene were detected using a 1.9-kb Bam HI cDNA (26, 27) after digestion with either Pvu II or Nco I.

Apolipoprotein AI/CIII/AIV gene cluster polymorphisms were identified using (*a*) a 2.2-kb Pst I fragment (28) of the apo AI gene after Xmn I digestion and (*b*) a 1.0-kb Pvu II fragment (29) of the C-III gene after Pvu II digestion.

All probes were labeled with ^{32}P dCTP at a specific activity of 800 Ci/mmol (Amersham Corp.) by a random oligonucleotide priming method (30). The hybridization, filter washing, and autoradiographic procedures are described elsewhere (28).

Statistical analysis. Statistical analysis was carried out by the Minitab program (State College, PA). A one-way analysis of variance was performed to test the null hypothesis that kinetic variation was not associated with genetic variation detected by the different RFLPs. The *F* statistic was employed to test the significance of differences between the genotypes. We considered significance to be at the 0.05 level.

Results

The 19 subjects could be divided into three groups on the basis of the polymorphisms detected using the Xba I endonuclease (Table I). There was no difference in body weight or mean age, nor in the plasma concentrations of cholesterol, triglyceride, LDL cholesterol, or apoprotein between those with the genotype *X1X1* (absence of cutting site) and those with the genotype *X2X2*. There were also no significant differences in these parameters when the subjects were grouped according to the polymorphisms detected with the Msp I and the Eco RI enzymes. Similarly, the gross composition of LDL was not altered in individuals of different Xba I genotype (Table II).

However, the metabolic properties of LDL did differ between the genotype groups. The catabolic rate of LDL apoprotein measured as the fraction of the plasma pool catabolized each day (FCR), was significantly higher in individuals with the *X1X1* genotype compared with those with *X2X2* ($F = 9.18$; $P < 0.025$). The subjects with the genotype *X1X2* had an intermediate mean FCR. Simultaneous with the injection of native lipoprotein, subjects received a tracer of cyclohexanedione-treated LDL that permitted the estimation of receptor-dependent versus receptor-independent removal (Table III). This revealed that the difference in overall catabolism was due to an increase in the fraction and amount of LDL degraded by the receptor route. Subjects of *X1X1* genotype exhibited a 58% higher receptor-mediated FCR than those with the genotype *X2X2* ($F = 9.08$; $P < 0.025$) and cleared 65% more LDL protein through this pathway. No such difference was observed in the fraction of LDL degraded by receptor-independent mechanisms. Likewise, when the synthetic rate of LDL apoprotein was calculated, no significant association with genotype was observed, although this parameter was highly variable within the groups.

There was a weak association between LDL apoprotein clearance rate and the apo B polymorphism detected using Msp I (Table IV), but the differences did not reach statistical significance. In the 19 subjects examined, LDL kinetic parameters were not significantly different in individuals with different RFLP genotypes of the LDL-receptor gene or the AI-CIII-AIV gene cluster (data not shown).

Discussion

The individuals examined in this study had diet-refractory hyperlipidemia that arose from a combination of oversynthesis

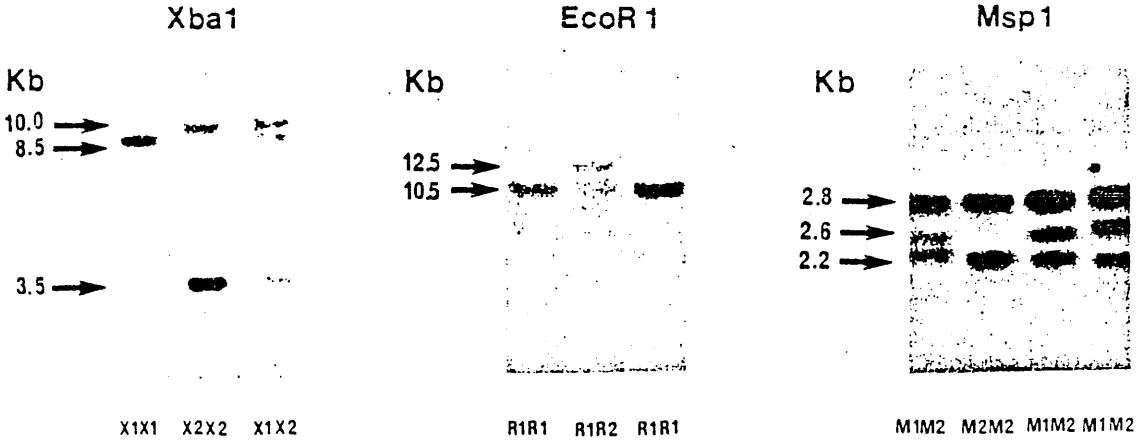

Figure 1. Southern blot analysis of the Xba I, Eco RI, and Msp I polymorphism of the apo B gene. 5 μg of DNA from three individuals is shown. The Msp I polymorphism is a length variation due to different numbers of copies of a 14-bp repeat sequence in the 3′ flanking region of the gene (36).

Table I. Plasma Lipids, Lipoproteins, and Clinical Data from Individuals with Different Apo B Xba I Genotype

Subject	Genotype	Plasma		LDL-cholesterol	LDL-apoprotein	Clinical data
		Cholesterol	Triglyceride			
		mmol/liter			*mg/dl*	
1	X1X1	8.10	1.95	5.90	193	Angina
2	X1X1	7.74	1.24	5.99	192	Xanthelasmata
3	X1X1	6.89	1.66	4.93	149	Normal
4	X1X1	8.49	3.24	6.28	211	Corneal arcus
5	X1X1	7.08	1.43	5.28	179	Normal
$n = 5$	Mean±1 SD	7.76±0.59	1.88±0.65	5.73±0.47	187±19	
6	X1X2	8.09	1.20	5.76	151	Normal
7	X1X2	8.79	1.90	6.63	204	Normal
8	X1X2	7.12	2.60	4.80	173	Normal
9	X1X2	6.97	1.80	4.99	167	Angina
10	X1X2	8.09	2.31	6.01	174	Myocardial infarction in mother (age 55)
11	X1X2	6.85	0.83	3.76	156	Normal
12	X1X2	7.05	2.14	4.75	135	Normal
13	X1X2	8.53	2.15	6.58	186	Myocardial infarction in brother (age 54)
$n = 8$	Mean±1 SD	7.69±0.72	1.87±0.55	5.41±0.94	168±20	
14	X2X2	8.33	2.23	5.46	253	Arcus
15	X2X2	8.56	2.76	6.20	175	Myocardial infarction in father (age 45)
16	X2X2	6.36	0.82	3.79	146	Normal
17	X2X2	8.00	3.11	5.62	176	Normal
18	X2X2	7.03	1.68	4.88	160	Xanthelasmata
19	X2X2	8.09	2.57	5.84	192	Normal
$n = 6$	Mean±1 SD	7.73±0.78	2.32±0.77	5.30±0.78	184±34	

All values given in the table are means of at least three independent determinations. Analysis of variance showed no significant differences in plasma lipid parameters between any of the three groups (X1X1, X1X2, or X2X2).

and defective catabolism. Most had LDL apoprotein synthetic rates (Table III) in excess (31–33) of normal values (11–13 mg/kg per d), whereas their total LDL FCRs lay between the values observed (32, 33) in controls (0.35±0.06 pools/d) and those (18, 31) in familial hypercholesterolemia heterozygotes (0.19±0.046 pools/d) that have only a partial complement of LDL receptors. They also exhibited reduced receptor-mediated FCRs that varied from 9 to 39% of the total. This is lower than the 50% we have previously observed in normolipidemic controls (33, 34) because of the general inverse relationship between plasma LDL concentration and receptor activity (31). Note also that there are differences seen in the estimated contribution of the receptor pathway when alternative methods of modifying LDL are used. For example, Kesaniemi et al. (35) reported much higher values for receptor-mediated clearance using glucosylated LDL. The relative merits of the different approaches have been discussed in detail (34).

Table II. LDL Composition in Individuals with Different Apo B Xba I Genotype

Genotype	LDL composition				
	Free cholesterol	Esterified cholesterol	Triglyceride	Phospholipid	Protein
	g/100 g				
X1X1 ($n = 5$)	9.29±0.81*	36.93±2.77	8.06±0.99	20.52±0.35	25.21±1.15
X1X2 ($n = 8$)	9.25±0.77	38.26±1.93	7.02±1.28	21.43±1.29	23.91±0.72
X2X2 ($n = 6$)	8.42±0.80	37.64±1.34	7.26±0.73	20.97±0.58	25.69±1.73

No significant differences were present in composition between any of the three groups.
* Mean±1 SD.

Table III. LDL-Kinetic Parameters and Xba I Genotype

Subject	Sex	RFLP genotypes			Fractional catabolic rate		Synthesis*	Absolute‡ receptor-mediated catabolic rate
		Xba I	Eco RI	Msp I	Total	Receptor mediated		
					pools/d		*mg/kg per d*	
1	F	X1X1	R1R1	M1M2	0.248	0.088	19.10	6.80
2	F	X1X1	R1R2	M1M2	0.256	0.101	19.70	7.80
3	F	X1X1	R1R1	M2M2	0.270	0.079	16.10	4.71
4	F	X1X1	R1R1	M1M2	0.282	0.069	23.80	5.82
5	M	X1X1	R1R2	M1M2	0.297	0.072	21.27	5.16
$n = 5$			Mean±1 SD		0.271±0.020	0.082±0.013	19.99±2.84	6.06±1.25
6	F	X1X2	R1R2	M1M2	0.219	0.078	13.23	4.71
7	F	X1X2	R1R1	M2M2	0.229	0.087	18.69	7.10
8	F	X1X2	R1R2		0.252	0.022	17.40	1.50
9	F	X1X2	R1R2	M1M2	0.251	0.044	16.80	2.90
10	F	X1X2	R1R1	M1M2	0.241	0.070	16.77	4.87
11	F	X1X2	R1R2	M1M2	0.218	0.084	13.60	5.20
12	M	X1X2	R1R2	M1M2	0.280	0.114	15.12	6.16
13	M	X1X2	R1R1	M2M2	0.205	0.056	15.25	4.16
$n = 8$			Mean±1 SD		0.237±0.024	0.069±0.028	15.86±1.89	4.58±1.77
14	F	X2X2	R1R1		0.218	0.020	22.10	2.00
15	M	X2X2	R1R1	M2M2	0.197	0.067	13.79	4.69
16	F	X2X2	R1R1	M2M2	0.182	0.042	10.60	2.50
17	F	X2X2	R1R1	M2M2	0.265	0.054	18.66	3.80
18	F	X2X2	R1R1	M2M2	0.217	0.070	13.90	4.50
19	F	X2X2	R1R1	M2M2	0.250	0.059	19.20	4.53
$n = 6$			Mean±1 SD		0.222±0.031	0.052±0.019	16.38±4.29	3.67±1.15
Analysis of variance		X1X1:X1X2:X2X2			$P < 0.025$	NS	NS	$P < 0.050$
		X1X1:X2X2			$P < 0.025$	$P < 0.025$	NS	$P < 0.010$

No significant differences could be detected when genotypically different groups (R1R1 versus R1R2; M1M2 versus M2M2) were compared by analysis of variance. * The synthetic rate is equal to the product of the total FCR and the plasma LDL pool (LDL concentration × plasma volume). ‡ The absolute receptor-mediated catabolic rate is the product of the receptor-mediated FCR and the plasma LDL pool.

It is difficult to make a definitive diagnosis of familial hypercholesterolemia in individuals with moderately elevated cholesterol levels using available techniques. Clinical criteria remain the best guide, but even here, lack of available family history may cloud the issue. In this study, commonly accepted exclusion criteria were set for individuals with familial hypercholesterolemia (see Methods). However, it is important to note in interpreting the data that these criteria are not absolute.

Although individuals with familial hypercholesterolemia were excluded from the study and all of the subjects had similar plasma lipid and lipoprotein levels, the FCR for LDL var-

Table IV. LDL Kinetic Parameters and Eco RI and Msp I RFLP Genotypes

Genotype	Fractional catabolic rate		Synthesis*	Absolute‡ receptor-mediated catabolic rate
	Total	Receptor mediated		
	pools/d		*mg/kg per d*	
Eco RI-RFLP				
R1R1 $n = 12$	0.234±0.031	0.063±0.019	17.33±3.70	4.62±1.50
R1R2 $n = 7$	0.253±0.029	0.074±0.032	16.73±3.01	4.78±2.07
Msp I-RFLP				
M1M2 $n = 9$	0.255±0.027	0.080±0.020	16.20±6.87	5.49±1.39
M2M2 $n = 8$	0.227±0.032	0.064±0.014	15.77±3.00	4.50±1.28

*‡ For definitions see Table III.

ied by up to 50%. This variability was related to the apo B genotype of the individual. The group of five subjects with genotype *X1X1* had a 22% higher total FCR than the six who had genotype *X2X2*. More detailed examination of the cause of this difference, using a receptor-blocked LDL tracer, revealed that the difference was due to an increased flux through the receptor pathway. Both the proportion of the plasma LDL apoprotein pool and the amount cleared via receptors was significantly elevated in *X1X1* individuals. It is unlikely that these observations can be explained by changes of the constitutive activity of the receptor. Rather, they indicate that variations in the structure of the ligand LDL are responsible. Our data suggest that apo B produced in *X2X2* individuals has a perturbed structure that diminishes its ability to interact with the receptor on cell membranes. Since it is known that only one B protein is present on each particle, this hypothesis also implies that individuals who are heterozygous for the polymorphism would produce two forms of LDL (one receptor active, the other relatively inactive) and would express an intermediate FCR (Table III).

The DNA sequence change that creates the Xba I restriction site occurs at the third base of the codon for threonine 2,488 in apo B (36). No amino acid change results, and so it is unlikely that the Xba I polymorphism itself is functionally significant. Rather, this site is probably in linkage disequilibrium with an important change elsewhere in the coding region. One possibility is that the important mutation lies in the putative receptor binding site (5), i.e., between amino acids 3,147–3,157 or 3,351–3,367, a region close to the Xba I cutting site.

The findings presented here also suggest a mechanism for the association of Xba I genotype and plasma cholesterol in the normal population (12–14). In subjects with normal lipid levels, there is a strong relationship between the receptor-mediated FCR and LDL concentration (31). The observed higher plasma cholesterol in individuals of *X2X2* versus *X1X1* genotype thus might be explained by the production of a relatively receptor-inactive apo B in the former group that would lead to accumulation of LDL in the circulation. Where the influence of synthesis becomes predominant, as in our present cohort of hypercholesterolemic patients, this relationship with plasma LDL concentration would be diminished. It is not yet clear whether the metabolic changes described in this study relate to the higher incidence of ischemic heart disease reported in one study to be associated with the *X1* allele (15). However, if the trend toward higher LDL apoprotein synthesis in the *X1X1* group (Table IV) is confirmed in subsequent studies, then it may point to the importance of LDL flux and plasma concentration as risk markers for ischemic heart disease.

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Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein

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Abstract Hepatic lipase deficiency produces significant distortion in the plasma lipoprotein profile. Particles with reduced electrophoretic mobility appear in very low density lipoprotein (VLDL). Intermediate density lipoprotein (IDL) increases markedly in the circulation and plasma low density lipoprotein (LDL) levels fall. At the same time there is a mass redistribution within the high density lipoprotein (HDL) spectrum leading to dominance in the less dense HDL_2 subfraction. The present study examines apolipoprotein B turnover in a patient with hepatic lipase deficiency. The metabolism of large and small very low density lipoproteins was determined in four control subjects and compared to the pattern seen in the patient. Absence of the enzyme did not affect the rate at which large very low density lipoproteins were converted to smaller particles within this density interval (i.e., of VLDL). However, subsequent transfer of small very low density lipoproteins to intermediate density particles was retarded by 50%, explaining the abnormal accumulation of VLDL in the patient's plasma. Despite this, intermediate density particles accumulated to a level 2.4-times normal because their subsequent conversion to low density lipoprotein has been almost totally inhibited. Consequently, the plasma concentration of low density lipoprotein was only 10% of normal. On the basis of these observations, hepatic lipase appears to be essential for the conversion of small very low density and intermediate density particles to low density lipoproteins. The pathways of direct plasma catabolism of these species were not affected by the enzyme defect. In vitro studies were performed by adding purified hepatic lipase to the patient's plasma. This did not modify the size of HDL_2 significantly, but resulted primarily in triglyceride hydrolysis in the less dense apolipoprotein B-containing particles.—**Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd.** Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* 1988. **29:** 1603–1611.

Supplementary key words VLDL • HDL • apoB • HDL_2 • HDL_3

The lipolytic degradation of circulating triglyceride-rich lipoproteins involves simultaneous loss of core triglycerides and coat phospholipid from the particles. Two key enzymes, located on the endothelial surfaces of capillary beds, are believed to participate in this process (1). Lipoprotein lipase, the better characterized of the two, is associated primarily with adipose tissue and skeletal muscle. It shows particular affinity for larger lipoprotein particles (2) like chylomicrons and very low density lipoprotein (VLDL) with Svedberg flotation rates (S_f) greater than 100. Hereditary absence of the enzyme therefore results in accumulation of such particles in the circulation, leading to the phenotypic lipoprotein disorder called Type I hyperlipoproteinemia (3).

The function of the other enzyme, hepatic lipase, synthesized and secreted by hepatocytes, is less well documented (1). It possesses both triglyceride hydrolase and phospholipase activities in vitro (4) and has a higher affinity for smaller, denser lipoprotein particles (2). Animal studies (5, 6) have suggested that its actions may be directed primarily at the metabolism of small VLDL, intermediate density lipoprotein (IDL), and high density lipoprotein (HDL). Antibody infusions (5), designed to inhibit the enzyme, lead, within a few hours to the accumulation of S_f 20–100 VLDL and S_f 12–20 IDL in plasma, with a concomitant reduction in circulating low density lipoprotein (LDL). At the same time, the mass of phospholipid within the HDL_2 density interval (1.063–1.125 kg/l) increases (5, 6). However, it has also been proposed that hepatic lipase

Abbreviations: S_f, negative sedimentation coefficient at d 1.063 kg · l^{-1} and 26°C; VLDL, very low density lipoproteins, d < 1.006 kg · l^{-1}; IDL, intermediate density lipoproteins, d 1.006–1.019 kg · l^{-1}; LDL, low density lipoproteins, d 1.019–1.063 kg · l^{-1}; TMU, 1,1,3,3-tetramethylurea; HDL, high density lipoproteins, d 1.063–1.210 kg · l^{-1}; HDL_2, high density lipoprotein subfraction 2, d 1.063–1.125 kg · l^{-1}; HDL_3, high density lipoprotein subfraction 3, d 1.125–1.210 kg · l^{-1}; LCAT, lecithin:cholesterol acyltransferase; FFA, free fatty acids.

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is involved in the elimination of HDL_2 constituents by the liver (7–10). Recently two pairs of brothers, one Canadian (11) and one Swedish (12), were reported to have a deficiency of postheparin hepatic lipase activity. The lipoprotein profile in all four patients was distorted, with the accumulation of small VLDL (β-VLDL) and HDL_2, but it was not clear from the observations whether the enzyme acted primarily on VLDL or HDL. Since both of these particles are linked metabolically, disturbances in the metabolism of one would have an impact on the other, possibly via the agency of plasma lipid transfer activities. The present report describes in vivo and in vitro studies on the lipoproteins of one of the Swedish patients.

MATERIALS AND METHODS

Subjects

The four subjects who formed the control group in this study were healthy males aged 32–65 years. Routine clinical and laboratory screening revealed no evidence of cardiologic, renal, hepatic, endocrine, or metabolic disease. Plasma lipid and lipoprotein levels were determined according to the Lipid Research Clinics protocol (13).

The hepatic lipase-deficient patient (GP, age 63 years), one of two affected Swedish brothers, has been described in detail elsewhere (12). He is an $apoE_3/E_4$ heterozygote. At the time of this study he had a moderate hypertriglyceridemia without any increase in VLDL cholesterol (Table 1) and with virtually immeasurable postheparin hepatic lipase but normal lipoprotein lipase activity. His general health remains good. Specifically, there was no clinical evidence of central or peripheral vascular disease. All subjects gave their informed consent to the study which followed the guidelines of the Ethical Committees of Glasgow Royal Infirmary and the Karolinska Hospital, Stockholm.

In vivo studies

Isolation and characterization of plasma lipoproteins was performed using two ultracentrifugation techniques. The cumulative flotation ultracentrifugation procedure, a modification (14) of the method of Lindgren, Jensen, and Hatch (15) was employed to isolate apolipoprotein B-containing particles in four subfractions: S_f 60–400 (large VLDL), 20–60 (small VLDL), 12–20 (IDL), and 0–12 (LDL). The detailed methodology is described in earlier publications (14,16). Since the majority of the apolipoprotein B-containing lipoproteins lie within the density interval 1.006–1.063 kg · l^{-1}, this material was also subjected to continuous gradient analysis by the rate zonal ultracentrifugation method of Patsch et al. (17). To facilitate characterization of GP's profile, a marker of radioiodinated normal LDL (18) was added to his plasma specimen prior to analysis. The HDL profile in this patient has already been shown to be abnormal by ultracentrifugation (19) as well as polyacrylamide gel electrophoresis (12). It was reexamined by the rate zonal separation procedure (17).

Fractions isolated by the cumulative or rate zonal ultracentrifugation procedure were analyzed to determine their contents of free and esterified cholesterol, triglyceride, phospholipid, and protein as outlined elsewhere (20).

Kinetic studies

Total VLDL of density less than 1.006 kg · l^{-1} was prepared in a Beckman Ti 60 rotor (Beckman Instruments, Palo Alto, CA) by ultracentrifugation of 250 ml of fasting plasma for 18 hr at 10°C and 40,000 rpm. The lipoprotein was aspirated, pooled, and its density was increased to 1.182 kg · l^{-1} by addition of solid NaBr (0.384 g · ml^{-1}). A discontinuous salt gradient from density 1.0988 kg · l^{-1} to 1.0588 kg · $l^{-1}$was constructed over 2.0-ml aliquots of the VLDL in an SW40 rotor, and the preparation was centrifuged according to a modification (14) of the procedure of Lindgren et al. (15) to isolate large and small VLDL frac-

TABLE 1. Plasma lipids and lipoproteins in control subject and a hepatic lipase-deficient subject

Subjects	Total Triglyceride	Total Cholesterol	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL
			mmol/l		
N1[a]	1.71 ± 0.38[b]	4.96 ± 0.54	0.79 ± 0.21	3.14 ± 0.35	1.01 ± 0.13
N2	2.55 ± 0.71	6.23 ± 0.79	1.02 ± 0.25	4.09 ± 0.50	1.36 ± 0.19
N3	0.94 ± 0.25	3.76 ± 0.40	0.33 ± 0.17	1.66 ± 0.32	1.78 ± 0.24
N4	2.00 ± 0.75	6.55 ± 1.00	1.08 ± 0.54	4.37 ± 0.83	1.10 ± 0.22
Mean	1.80 ± 0.58	5.38 ± 1.10	0.81 ± 0.29	3.32 ± 1.06	1.31 ± 0.30
HL(-)[c]	2.82 ± 0.49	5.10 ± 1.05	0.75 ± 0.41	2.18 ± 0.29	1.96 ± 0.25

[a]Normal subjects 1 to 4.
[b]Mean ± SD, n = 5.
[c]Hepatic lipase-deficient patient GP.

tions of S_f 60–400 and 20–60, respectively. These fractions were than labeled with ^{131}I and ^{125}I by a modification (21) of the Macfarlane ICl procedure (22). Labeling efficiency varied between 5 and 15% and the conditions used gave less than 1 mol of iodine per 300,000 daltons of B protein as described previously (16). The labeled tracers were sterilized by membrane filtration (0.45 μm Amicon filters (Amicon Corp., Bedford, MA)) prior to reinjection into the donor. Preparation time overall was less than 48 hr. The tracers were administered at 8:00 AM after an overnight fast and, in order to minimize chylomicron production, the subjects received a hypocaloric fat-restricted (less than 5 g) diet during the first day of the study. Plasma samples were collected at frequent intervals over the first 72 hr and then daily in the fasting state for 14 days. Plasma from each time point was used to isolate large and small VLDL, IDL, and LDL by the method outlined above (14, 16). These lipoproteins were treated with 1,1,3,3-tetramethylurea (TMU) as described elsewhere (16) to prepare apolipoprotein B, whose specific activity was calculated following direct measurement of protein (16) and radioactivity content. The apolipoprotein B pool circulating with each of these lipoprotein fractions was determined by replicate analyses of plasma samples collected intermittently throughout the turnover study. Losses during centrifugation were corrected by comparing the total cholesterol mass recovered in all four fractions with the $d < 1.063\ kg \cdot l^{-1}$ cholesterol content of the subject's plasma determined by the standard Lipid Research Clinics protocol (13). Further correction was made for possible B protein loss during selective TMU precipitation by comparing the apolipoprotein B recovered at the end of the procedure with the values calculated as the difference between total and TMU-soluble (apolipoproteins E and C) protein contents (23).

Kinetic analysis

The radioactivity associated with the B protein present in each fraction was calculated from the apolipoprotein B specific activities and the individual pool sizes. These were expressed as a percentage of the total B protein radioactivity present in the plasma 10 min after injection and the resulting values were used to construct decay curves which were analyzed by the SAAM 29 multicompartmental modeling program (24). The model that was employed is described elsewhere (16). Its main features allow for: *a*) apolipoprotein B input at the level of both large and small VLDL and LDL; *b*) stepwise delipidation of VLDL following the concept of Berman et al. (25); and *c*) parallel pathways of a B protein processing from small VLDL through IDL to LDL.

Rate constants were determined and, in combination with B protein pool sizes, were used to calculate flux ratios and steady-state synthetic input.

In vitro studies

These studies were performed in Sweden. The methods for lipid analysis, lipoprotein separations and isolations, and polyacrylamide gradient gel electrophoresis have all been described (12). Blood taken from the fasting subject was put into chilled EDTA-tubes kept on ice and plasma was recovered within 30 min by low speed centrifugation at 2°C. Hepatic lipase was isolated in Lund from postheparin plasma of healthy volunteers by repeated heparin-Sepharose chromatography (26) and shipped on dry ice to Stockholm. The enzyme preparation had a hepatic lipase activity of 9.2 U/mg protein (1 unit representing the release of 1 μmol fatty acid per min at 37°C) and was devoid of lipoprotein lipase activity, as determined by specific assays using radiolabeled trioleoylglycerol emulsions as substrate (27). Incubations were performed in triplicate in the dark at 37°C (to prevent auto-oxidation) for the indicated periods of time and were terminated by putting the incubation vessels on ice. Two series of incubations were performed. In one series whole plasma was used while in the other we used plasma from which VLDL and LDL had been removed by tube slicing after preparative ultracentrifugation at d 1.063 $kg \cdot l^{-1}$. Before use, the preparation was dialyzed against 0.15 M NaCl and its original volume was restored with the same solution (28). To the incubation mixtures were added either 0.15 ml of enzyme solution or 0.15 ml of 0.15 M NaCl per ml of plasma.

Two identical studies performed 1 year apart gave the same results. In the first, the enzyme activity was 135 mU/ml (27); in the second, not reported here, the activity was 225 mU/ml. Enzyme activity was assayed the day before the experiment.

RESULTS

The lipid and lipoprotein profiles of the control subjects and of the hepatic lipase-deficient patient (GP) are shown in Table 1. The hypertriglyceridemia in GP, whose plasma VLDL cholesterol concentration was normal, is explained by the finding noted earlier (11, 19) that the majority of the triglyceride was present in LDL and HDL (1.42 and 0.64 $mmol \cdot l^{-1}$, respectively). These lipoproteins were enriched in triglyceride (cholesterol/triglyceride ratio in LDL was 1.5, versus 6.0–16.0 in normals; in HDL the ratio was 3.0, versus 5.0–16.0 in normals). On the other hand, the cholesterol/triglyceride ratio in GP's VLDL was high (1.1 versus 0.4–0.8 in normals).

Compositional analysis of the four apolipoprotein B-containing lipoprotein subfractions is presented in Table 2. The large and small VLDL in GP had normal triglyceride contents but were reduced in cholesteryl esters and enriched

TABLE 2. Compositions of apoB-containing lipoproteins in hepatic lipase deficiency

	Triglyceride	Cholesteryl Ester	Free Cholesterol	Phospholipid	Protein
			g/100 g (n = 3)		
$VLDL_1$[a]	62.2 ± 3.1[b,c]	9.3 ± 0.5	3.9 ± 1.8	14.6 ± 3.4	10.0 ± 0.2
	(56.2 ± 4.8)	(16.0 ± 4.3)	(1.7 ± 2.3)	(17.0 ± 1.4)	(9.1 ± 2.4)
$VLDL_2$	34.6 ± 0.9	12.7 ± 2.2	13.3 ± 2.6	23.4 ± 1.2	16.0 ± 0.6
	(35.1 ± 4.0)	(21.1 ± 5.9)	(8.1 ± 1.4)	(21.4 ± 2.4)	(14.4 ± 1.6)
IDL	27.9 ± 1.7	13.5 ± 3.7	12.7 ± 2.0	24.7 ± 1.2	21.0 ± 0.8
	(12.4 ± 2.0)	(33.4 ± 4.8)	(11.2 ± 2.3)	(23.9 ± 1.3)	(19.1 ± 2.3)
LDL	23.6 ± 1.4	24.5 ± 4.9	7.1 ± 0.2	26.4 ± 1.2	18.6 ± 4.9
	(5.1 ± 0.2)	(34.8 ± 2.2)	(13.5 ± 1.5)	(23.0 ± 1.6)	(23.6 ± 1.6)

Values in parentheses are from normolipidemic control subjects.
[a]$VLDL_1$, S_f 60-400 lipoproteins; $VLDL_2$, S_f 20-60 lipoproteins; IDL, S_f 12-20 lipoproteins; and LDL, S_f 0-12 lipoproteins.
[b]Mean ± SD.
[c]Each fraction was isolated from GP on three occasions and assayed as described in Methods.

in free cholesterol. However, the IDL and LDL fractions were quite abnormal in composition. Both fractions were enriched in triglyceride at the expense of cholesteryl ester. The phospholipid, protein and free cholesterol contents were unchanged. In the lipase-deficient subject, most VLDL apoB (Table 3) resided at the denser end of the spectrum (i.e., S_f 20-60); and the level of IDL apoB was raised severalfold.

Tracers of large and small VLDL were used to investigate the origins of the above abnormal distributions. The results are presented in **Fig. 1** and Table 3. The distribution of apolipoprotein B in the normal subjects reflected their lipoprotein pattern (Table 1) in that most of its mass was found in the LDL density interval. In contrast, most B protein in GP was associated with IDL. The normal subjects synthesized about 1000 mg of total VLDL apolipoprotein B each day, 70% of which appeared first in the large S_f 60-400 component. Approximately half of this protein was metabolized through IDL to LDL, the remainder leaking out of the delipidation cascade at the levels of large and small VLDL and IDL. GP produced 570 mg of total VLDL apolipoprotein B, most of which was secreted with small VLDL particles. That portion of protein that appeared first in large VLDL was transferred to the smaller VLDL range (S_f 20-60) at a normal rate. The mass of B protein in GP's small VLDL pool was normal. However its fractional clearance rate (Table 3; Fig 1) was retarded and its rate of transfer to the IDL fraction was delayed. On the other hand, its direct catabolism from the circulation was at least as high as normal. Despite the reduced input of protein from VLDL, the plasma pool of IDL was elevated 2.4-times, principally because the plasma clearance of this fraction was so slow (Table 3, Fig. 1). The transfer of B protein from IDL to LDL occurred only at about 5% of normal, although it should be noted that, as in the case of VLDL, the rate of direct removal of IDL apolipoprotein B from the circulation (0.25 pools · d^{-1}) was not reduced. A small amount of IDL apolipoprotein B did transfer into

TABLE 3. Apolipoprotein B metabolism in normal subjects

	Large VLDL ApoB				Small VLDL ApoB				
			Fractional Rate					Fractional Rate	
Subject	Synthesis	Plasma Pool[a]	Direct Catabolism	Transfer to $VLDL_2$	Synthesis Direct	Synthesis from $VLDL_1$	Plasma Pool[a]	Direct Catabolism	Transfer to IDL
	mg/day	*mg*	*pools/day*		*mg/day*	*mg/day*	*mg*	*pools/day*	
N1NC	802	79	3.7	6.5	393	512	246	0.36	3.3
N2CD	710	80	2.9	6.0	467	478	226	1.32	2.0
N3MQ	290	18	0.0	16.2	137	290	100	0.70	2.8
N4TS	954	115	5.0	3.2	238	372	258	0.35	1.9
Mean ± SD	689 ± 246	73 ± 35	2.9 ± 1.8	8.0 ± 4.9	259 ± 91	413 ± 88	208 ± 63	0.68 ± 0.39	2.5 ± 0.6
HL(-)	91	9	1.8	8.3	480	74	265	1.08	0.97

[a]Plasma pool derived from steady-state analysis using SAAM 29 program. This agrees with the observed apolipoprotein B pool to within ± 15%.

the LDL fraction (Fig. 1, Table 3) which was degraded at a low normal fractional clearance rate. Table 2 indicates that this LDL was not normal in composition, and indeed from rate zonal ultracentrifugation of the IDL/LDL lipoproteins in GP's plasma (Fig. 2) it is clear that there was no discrete LDL peak. The small amount of material isolated as "LDL" by cumulative flotation ultracentrifugation probably represents the denser component of the IDL spectrum.

In vitro studies

Incubation of whole plasma from the hepatic lipase-deficient patient for 24 hr with or without added purified hepatic lipase resulted in the formation of about 1 mmol of cholesteryl ester per liter of plasma in both cases (Table 4), very close to earlier reported values (12). A corresponding decrease of phospholipids, the donor of the fatty acid in the esterification reaction, occurred in the two incubations. The percentage of plasma cholesterol that was esterified increased from 51% — a lower than normal value as pointed out before (12) — to 67%. These incubation-induced changes are due to lecithin:cholesterol acyltransferase (LCAT) activity as both α- and β-LCAT activity are normal in hepatic lipase deficiency (12).

The plasma triglyceride concentration remained unchanged during the control incubation, while the addition of hepatic lipase resulted in a decrease of the triglyceride content of about 0.5 mmol · l^{-1}.

The incubations of the VLDL- and LDL-depleted plasmas, which contain HDL as the major lipoprotein class, resulted in changes similar to those observed for whole plasma (Table 4). The cholesteryl ester concentration rose by 0.7 mmol · l^{-1} whether or not hepatic lipase was present. The decrease in phospholipids was of the same order.

The hepatic lipase caused a slight decrease in the triglyceride content of HDL (0.1 mmol · l^{-1}). At the same time the concentration of free fatty acids (FFA) increased by 0.14 mmol · l^{-1} as compared to 0.08 mmol · l^{-1} without added lipase. This latter increase is due to release of FFA from lecithin during the LCAT reaction (29).

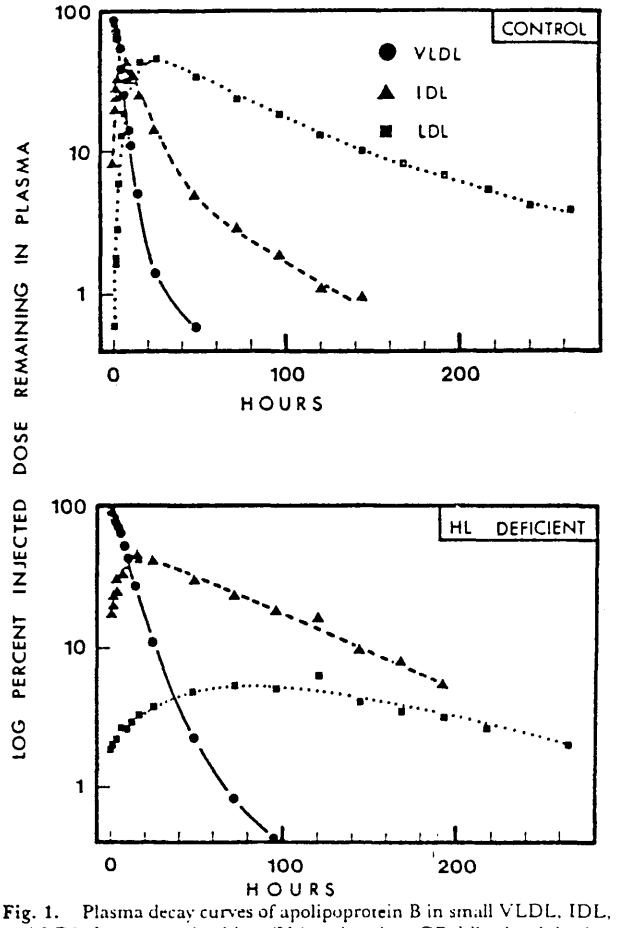

Fig. 1. Plasma decay curves of apolipoprotein B in small VLDL, IDL, and LDL from control subject (N1) and patient GP following injection of ^{125}I-labeled small VLDL. Symbols represent observed data and lines represent the computer-derived fit.

The HDL particles of GP's plasma migrate exclusively as large HDL_2 particles on gradient gel electrophoresis (12). In conformity, when GP's HDL was examined using the standard rate zonal method (Fig. 3), the majority of particles were associated with the HDL_2 density interval. HDL_3 was virtually absent. The size of the HDL particles of this

and in a hepatic lipase-deficient subject (HL(-))

IDL ApoB				LDL ApoB			
		Fractional Rate					
Synthesis from $VLDL_2$	Plasma Pool[a]	Direct Catabolism	Transfer to LDL	Synthesis Direct	Synthesis from IDL + $VLDL_2$	Plasma Pool[a]	Fractional Catabolic Rate
mg/day	*mg*	*pools/day*		*mg/day*	*mg/day*	*mg*	*pools/day*
817	337	0.77	1.65	0.0	556	1750	0.32
445	420	0.09	0.90	279	379	2630	0.25
275	210	0.32	0.98	0.0	290	635	0.46
499	496	0.15	0.85	90	423	2650	0.20
509 ± 196	366 ± 106	0.33 ± 0.27	1.10 ± 0.33	92 ± 114	412 ± 96	1916 ± 824	0.31 ± 0.10
262	886	0.25	0.05	0.0	43	215	0.21

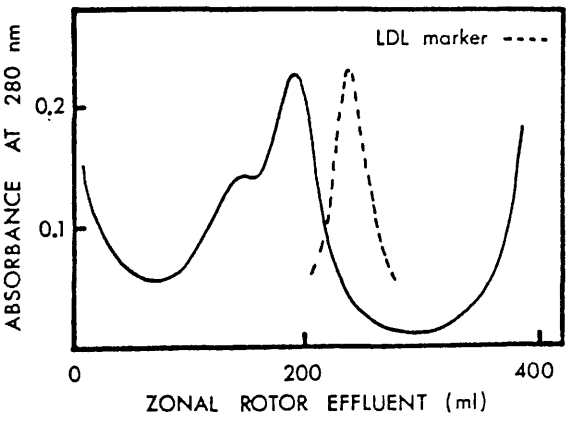

Fig. 2. Rate zonal ultracentrifugation profile of d 1.006-1.063 kg · l^{-1} lipoproteins in patient GP. The radioactive marker indicates the flotation characteristics of LDL prepared from a control subject.

hepatic lipase-deficient patient was not modified by the incubation with hepatic lipase (**Fig. 4**).

DISCUSSION

Intravenous heparin administration triggers the release into the plasma of two lipases which are distinct in their structure and function (1, 30). The better characterized lipoprotein lipase plays a primary role in the metabolism of large triglyceride-rich lipoproteins. Its deletion, therefore, results in the accumulation in the circulation of gut-derived chylomicrons containing apolipoprotein B-48- and B-100-containing large VLDL of hepatic origin (31). Endogenously labeled VLDL triglyceride is cleared slowly from the plasma of such patients (32). However, Nicoll and Lewis (2) have shown that lipoprotein lipase cannot be solely responsible for the catabolism of these particles since, even in its absence, VLDL (d < 1.006 kg · l^{-1}) is converted to IDL, albeit at a reduced rate. Clearly the other lipase in plasma must contribute, and indeed these authors (2) and others (33) have demonstrated that it is able to use small VLDL and IDL as efficient substrates for triglyceride hydrolysis. Certainly its action is inversely correlated with the levels of IDL in plasma (34); and, as indicated from the antibody inhibition experiments of Goldberg et al. (5), abolition of its activity causes an acute increase in the circulating mass of small VLDL and IDL with a reduction in LDL in cynomolgus monkeys. Such studies would implicate hepatic lipase in the VLDL delipidation cascade although the literature is not altogether consistent in this regard since Reardon, Sakai, and Steiner (35), in a study of the metabolism of large and small VLDL, were led to the conclusion that hepatic lipase has no role to play here. Certainly, hepatic lipase seems to have little activity against VLDL in vitro (Nilsson-Ehle, P., unpublished data). Most of the available evidence (7-10) suggests that the high density fraction, particularly HDL_2, is the preferred substrate for the enzyme. The recent discovery of hepatic lipase-deficient patients allows us to make a direct assessment of the mode of action of the enzyme. Our earlier studies (12) suggested that the disease was associated with delayed clearance of both VLDL and HDL with the resultant accumulation of β-VLDL and HDL_2.

The patient GP synthesized VLDL apolipoprotein B at about half of the rate seen in controls. Most (80%) of the material which he produced comprised smaller particles of S_f 20-60. This abnormal pattern of secretion, favor-

TABLE 4. Effects of incubation at 37°C for various times on lipid concentrations in whole plasma and VLDL- and LDL-depleted plasma from case GP, with and without added hepatic lipase (HL)

		Concentration of[a]							
		Phospholipids		Cholesteryl Ester		Triglycerides		Free Fatty Acids	
	Incubation Time	Without HL	With HL	Without HL	With HL	Without HL	With HL	Without HL	With HL
	hr	*mmol/l plasma*							
Whole plasma	0		4.86	2.85	2.79	4.22	3.96		
Whole plasma	0.5	4.51	4.92	2.87	2.83	4.16	4.28		
Whole plasma	1.5	4.53	4.73	2.93	2.92	3.98	4.10		
Whole plasma	6	4.55	4.16	3.13	3.18	4.13	3.46		
Whole plasma	24	4.12	3.90	3.81	3.68	4.24	3.62		
Depleted plasma	0	1.77	1.83	0.83	0.81	0.82	0.82	0.10	0.12
Depleted plasma	0.5	1.97	1.85	0.86	0.82	0.90	0.86	0.15	0.15
Depleted plasma	1.5	1.85	1.81	0.91	0.88	0.85	0.82	0.14	0.19
Depleted plasma	6	1.75	1.63	1.03	1.03	0.87	0.71	0.15	0.20
Depleted plasma	24	1.44	1.40	1.47	1.47	0.82	0.71	0.18	0.26

[a]Mean values; triplicate incubations.

ing smaller particles, is responsible for the apparent cholesterol enrichment which we noted in the total VLDL (Table 1, ref. 12). The rate at which the large VLDL in GP was converted to smaller remnants was normal, in accord with our previous suggestion that this process is entirely lipoprotein lipase-dependent. Subsequent processing of the smaller VLDL, however, occurred at about one-half of the normal rate. This seems, therefore, to be the first point at which hepatic lipase plays a significant part in the delipidation process. Certainly the decrease in the observed fractional rate of VLDL to IDL conversion cannot be attributed to an expansion of the VLDL pool since this was obviously not present in GP (Table 3). The most remarkable defect in GP's apolipoprotein B metabolism was the virtual complete block in particle transfer between IDL and LDL. This resulted in a major increase in the circulating mass of IDL and a 90% decrease in LDL. In fact, examination of GP's LDL zonal profile raises doubts about the existence of any true (i.e., "normal") LDL in this patient. What was identified by the cumulative flotation procedure may in fact have been contaminating IDL. Thus, we would conclude that GP largely or entirely lacks the ability to form normal LDL. The IDL that accumulated in his plasma is removed at a rate reminiscent of LDL, and earlier studies from this laboratory (36) suggest that a receptor pathway may be implicated in the process. Indeed, examination in Table 3 of the fractional rates of direct removal of any of the four B protein-containing particles indicates that there was no defect in these pathways. Thus, lipoprotein and hepatic lipases are discrete in their activities. Lipoprotein lipase focuses on large VLDL catabolism and also contributes approximately 50% to the conversion of smaller VLDL to IDL. Hepatic lipase, on the other hand, while sharing

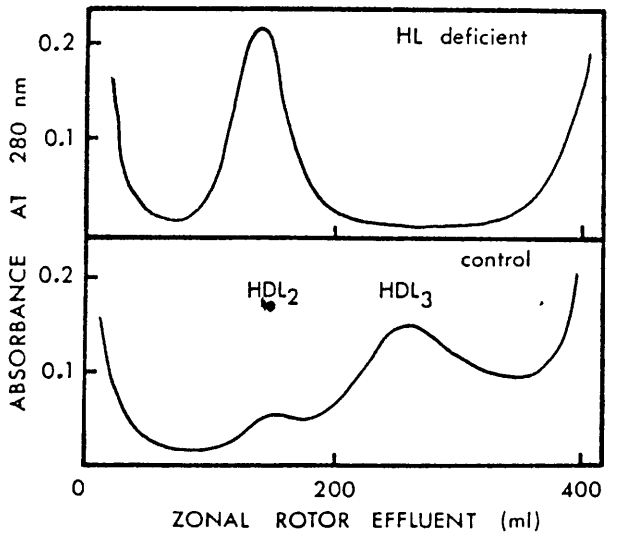

Fig. 3. Rate zonal ultracentrifugation profile of HDL from a control subject and patient GP.

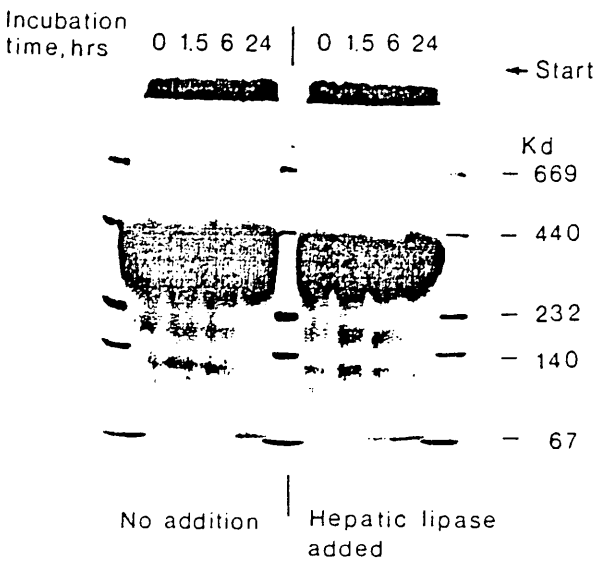

Fig. 4. Polyacrylamide gradient gel electrophoresis of total plasma lipoprotein isolated after incubation of plasma from patient GP at 37°C with or without added purified hepatic lipase. Marker proteins as in ref. 12. Note *1*) the presence of large HDL particles in the region corresponding to molecular masses of 300–400 kDa as the totally dominating HDL species, and *2*) the lack of effect of incubation with or without the lipase on HDL particle size.

in the latter process, is almost entirely responsible for catalysis of the conversion of IDL to LDL. Extrapolation from this suggests that such a process must occur in the liver, in agreement with published transhepatic catheterization studies (37). The mechanism itself, though still speculative, may well also involve the agency of receptors since it is known that individuals with homozygous familial hypercholesterolemia also accumulate IDL in the plasma (38). A separate, though possibly linked, function for the enzyme relates to the interconversion of HDL subspecies. Hepatic lipase has been proposed to be the principal enzyme involved in removing phospholipid and triglyceride from HDL, and individuals with low hepatic lipase activities have been found to have higher HDL_2/HDL_3 ratios (7). Again, what is remarkable in this patient is the virtual absence of HDL_3, as recorded by rate zonal ultracentrifugation (Fig. 4) or nondenaturing gradient gel electrophoresis (12). The HDL that accumulates is triglyceride-rich. According to current concepts, this lipid comes from triglyceride-rich particles in the circulation and its transfer is mediated by cholesteryl ester transfer protein. It has been suggested that HDL_2 phospholipids would normally be hydrolyzed by hepatic lipase with simultaneous shrinkage of the particle to the size of HDL_3 (9). Indeed, Rao et al. (39) have observed that heparin infusions in lipoprotein lipase-deficient patients lead to an acute mass transfer from HDL_2 to HDL_3. However, our in vitro studies failed to confirm this suggestion. When plasma from GP was incubated with purified hepatic lipase, no decrease in HDL_2 particle size was observed and what triglyceride hydrolysis did take place

seemed to result from an action of the enzyme on VLDL and LDL. But, this must be viewed with caution since it is possible that the composition of GP's HDL_2 is abnormal and the particle may have decreased affinity for the enzyme.

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Apolipoprotein B metabolism in homozygous familial hypercholesterolemia

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Abstract This report describes the metabolism of apolipoprotein B-containing lipoproteins in seven familial hypercholesterolemic (FH) homozygotes and compares the results to the values obtained from five healthy control subjects. The concentration, composition, and metabolism of large, triglyceride-rich very low density lipoproteins ($VLDL_1$, S_f 60–400) were the same in the control and FH groups, indicating that this component of the VLDL delipidation cascade was unaffected by the absence of receptors. In contrast, familial hypercholesterolemic small $VLDL_2$ (S_f 20–60) was enriched with cholesterol and depleted in triglyceride. Moreover, its plasma concentration was elevated as a result of an increase in its synthesis and a defect in the removal of a remnant population within this density interval. The latter accounted for up to 50% of the total mass of the fraction. Onward transfer of apolipoprotein B (apoB) from small VLDL through intermediate density lipoprotein (IDL) to low density lipoprotein (LDL) was retarded, suggesting that receptors were involved in this supposedly lipase-mediated event. IDL and LDL concentrations increased up to fourfold above normal in the plasma of the FH patients due partly to the delay in maturation and partly to defective direct catabolism. We conclude that the LDL receptor plays multiple and important roles in the metabolism and transformation of apoB-containing particles in the S_f 0–400 flotation interval. —James, R. W., B. Martin, D. Pometta, J. C. Fruchart, P. Duriez, P. Puchois, J. P. Farriaux, A. Tacquet, T. Demant, R. J. Clegg, A. Munro, M. F. Oliver, C. J. Packard, and J. Shepherd. Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. *J. Lipid Res.* 1989. 30: 159–169.

Supplementary key words $VLDL_1$ (S_f 60–400) • $VLDL_2$ (S_f 20–60) • LDL receptor • IDL • LDL

Familial hypercholesterolemia (FH) is a common metabolic disorder caused by defective expression of the gene that codes for low density lipoprotein (LDL) receptors on cell membranes. Affected individuals accumulate the lipoprotein in their plasma where it predisposes to tissue sterol deposition (xanthomatosis) and premature vascular disease. The trait shows autosomal codominant inheritance and therefore occurs in heterozygous and homozygous forms. Within these two subdivisions there is a broad spectrum of clinical presentations which is thought to result from multiple potential mutations affecting the structure of the receptor protein. FH is therefore a clinically useful umbrella term for a group of conditions, all of which reflect a defect in LDL receptor activity (1).

Early clues to the etiology of the disease came from a series of metabolic studies that showed that its characteristic feature, hypercholesterolemia, arises primarily from delayed clearance of LDL from the circulation (2, 3). However, in some patients, oversynthesis of LDL apoprotein (apoLDL) also seems to contribute to the expanded plasma LDL pool (3). This intriguing finding led to more detailed investigations designed to locate the source of LDL overproduction. In normal subjects, most if not all apoLDL comes (4) from the lipolysis of very low density lipoprotein (VLDL). During this process the VLDL apolipoprotein B (apoB) moiety is conserved and constitutes a marker of the fate of the lipoprotein particle. Soutar, Myant, and Thompson (5) used this information to trace the flux of B protein from VLDL to LDL in FH, and concluded that such individuals synthesized insufficient VLDL to account for the mass of LDL in their circulation. It followed that direct secretion of LDL, possibly by the liver, might be a feature of FH homozygosity. The discovery of a spontaneous mutation (6) in the LDL receptor protein in rabbits (the Watanabe Heritable Hyperlipemic rabbit) provided a means of addressing this question. In contrast to the human studies, analysis of apolipoprotein B transit from VLDL to LDL in the animal model sug-

Abbreviations: S_f, negative sedimentation coefficient at d 1.063 $kg \cdot l^{-1}$ and 26°C; $VLDL_1$, very low density lipoproteins, S_f 60–400; $VLDL_2$, very low density lipoproteins, S_f 20–60; IDL, intermediate density lipoproteins, S_f 12–20; LDL, low density lipoproteins, S_f 0–12; HDL, high density lipoproteins; apoB, apolipoprotein B; FH, familial hypercholesterolemia.

TABLE 1. Plasma lipids and lipoproteins in control and in homozygous FH subjects

Subject	Sex	Age	Body Weight	Plasma Triglyceride	Plasma Cholesterol	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL	Current Therapy
		yr	*kg*			*mmol · l⁻¹*			
N_1	M	36	71	1.71	4.96	0.79	3.14	1.01	
N_2	F	56	51	0.94	3.76	0.33	1.66	1.78	
N_3	F	28	77	0.55	3.71	0.30	1.95	1.46	
N_4	M	45	79	2.01	6.19	0.99	3.96	1.23	
N_5	M	29	85	2.00	6.55	1.08	4.37	1.10	
FH_1	M	15	53	3.54	14.15	3.43	8.70	0.69	Plasmapheresis
FH_2	M	21	50	2.87	14.10	1.47	11.98	0.65	Plasmapheresis
FH_3	M	14	59	2.25	12.30	0.7	10.80	0.90	Cholestyramine
FH_4	M	10	40	1.95	14.63	0.83	12.95	0.85	Cholestyramine
FH_5	M	44	70	1.93	16.75	0.92	15.43	0.83	
FH_6	F	15	50	0.85	10.90	(0.1)[a]	10.00	0.73	Portacaval shunt/plasmapheresis
FH_7	M	25	66	0.80	11.50	(0.1)[a]	10.20	1.20	Portacaval shunt/plasmapheresis

[a]Assessment of VLDL cholesterol in subjects FH_6 and FH_7 is at the limit of detection.

gested (7) that there was no requirement for direct LDL secretion, a proposal supported (8) by perfusion experiments that showed that WHHL rabbit livers elaborate VLDL but not LDL. However, the experimental animal did demonstrate that defective receptor activity resulted in perturbations in both VLDL and LDL metabolism; and Soutar, Myant, and Thompson (9) have also reported that intermediate density lipoprotein (IDL) metabolism is altered in the human deficiency state. Thus, receptor deficiency has wider consequences for apoB metabolism than was first appreciated. The present study shows that the LDL receptor has multiple roles in VLDL, IDL, and LDL apoB metabolism.

METHODS

Subjects

Five normolipemic and seven homozygous FH individuals participated in the study. Their plasma lipid and lipoprotein profiles are presented in Table 1. The normal subjects were recruited from the general population of Glasgow via a coronary screening program. All were healthy individuals receiving no drug therapy. Specifically, none of the participants showed evidence of hepatic, renal, or endocrine dysfunction on the basis of biochemical and hematological screening.

Subject FH_1 was born of an Italian father and Swiss mother. Both parents were heterozygous for FH. One brother died at the age of 14 of myocardial infarction and another, although alive, is severely hypercholesterolemic. FH_1 originally presented with xanthomata of the Achilles tendons and of the extensor tendons of hands and knees. Arcus senilis was evident and there were cholesterol deposits in his natal cleft. Triple bypass grafting had been performed 3 years prior to the study because of coronary insufficiency. His fibroblasts exhibited less than 15% of normal LDL receptor activity as determined by standard procedures (10).

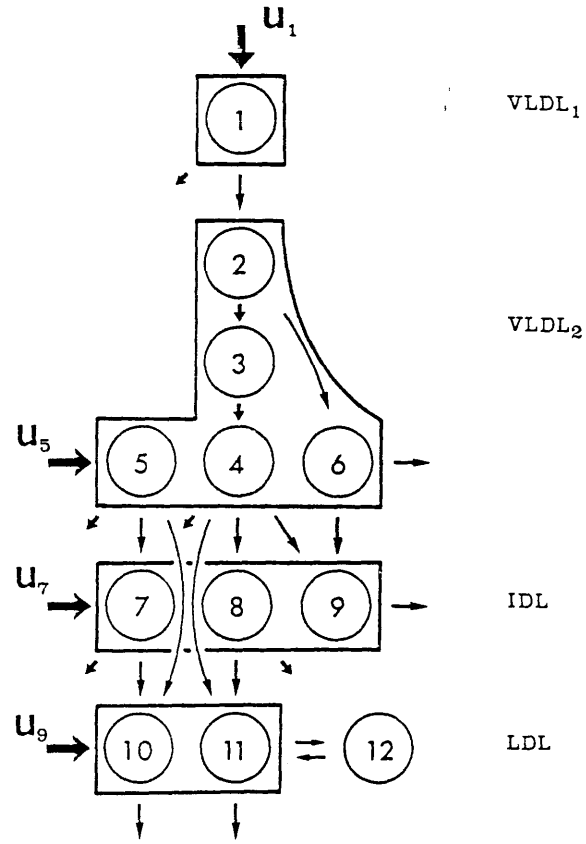

Fig. 1. Multicompartmental model describing the kinetics of $VLDL_1$, $VLDL_2$, IDL, and LDL. The parameters U_1, U_5, U_7, and U_9 represent de novo input of apoB. Exchange between compartments 11 and 12 was fixed at $k_{12,11}$ = 0.05 pools · d^{-1} and $k_{11,12}$ = 0.13 pools · d^{-1}.

TABLE 2. Compositions of apolipoprotein B-containing subfractions in control and in homozygous FH subjects

Subfraction	Subject[a]	FC	CE	TG	PL	Protein
		g/100 g				
$VLDL_1$	A	5.1 ± 2.5	14.9 ± 3.5	53.6 ± 3.9	14.9 ± 1.6	12.2 ± 4.4
$VLDL_1$	B	9.4 ± 0.4	21.8 ± 0.9	35.2 ± 2.7	19.3 ± 1.4	14.3 ± 0.8
$VLDL_1$	C	1.7 ± 2.3	16.0 ± 4.3	56.2 ± 4.8	17.0 ± 1.4	9.1 ± 2.4
$VLDL_2$	A	9.6 ± 3.2	34.5 ± 2.6	19.2 ± 2.8	19.6 ± 1.3	17.2 ± 3.2
$VLDL_2$	B	9.5 ± 0.1	34.0 ± 4.2	22.8 ± 3.6	18.6 ± 0.6	15.3 ± 0.1
$VLDL_2$	C	8.1 ± 1.4	21.1 ± 5.9	35.1 ± 4.0	21.4 ± 2.4	14.4 ± 1.6
IDL	A	10.5 ± 3.1	38.2 ± 2.0	6.5 ± 1.3	20.2 ± 2.2	24.5 ± 4.5
IDL	B	10.0 ± 0.6	42.6 ± 2.1	4.8 ± 1.4	21.3 ± 2.3	21.4 ± 0.6
IDL	C	11.2 ± 2.3	33.4 ± 4.8	23.6 ± 1.4	23.9 ± 1.3	19.1 ± 2.3
LDL	A	8.0 ± 2.8	37.5 ± 4.2	5.3 ± 1.5	19.0 ± 1.9	30.2 ± 3.2
LDL	B	8.6 ± 0.1	43.9 ± 1.8	2.2 ± 0.6	11.9 ± 1.4	22.5 ± 0.1
LDL	C	13.5 ± 1.5	34.8 ± 2.2	5.1 ± 0.2	23.0 ± 1.6	23.6 ± 1.6

[a] A, homozygous FH (n = 5); B, homozygous FH + portacaval shunt (n = 2); C, controls (n = 5).

Subjects FH_3 and FH_4 are brothers of Scottish origin. Both parents had plasma LDL cholesterol concentrations in excess of the reference values for their community, and the boys had widespread tendon xanthomata and subcutaneous cholesterol deposits in their gluteal fold. Their fibroblast LDL receptor activity was less than 30% of normal. Signs and symptoms of cardiovascular dysfunction were absent.

The clinical and biochemical features of subjects FH_6 and FH_7 are presented in earlier reports (11, 12). FH_6 is Moroccan and FH_7 French. Cell culture studies performed in the laboratory of Goldstein and Brown (10) showed that their fibroblasts expressed virtually no normal LDL receptor binding activity. Both patients had undergone portacaval shunt surgery in 1975. Their routine liver function tests remain normal.

FH_5 is a 44-year-old Englishman with widespread tendon xanthomata and severe left carotid artery stenosis. He is refractory to all lipid-lowering drug therapy including sequestrant resins. His three children are hypercholesterolemic. Fibroblast and lymphocyte assays (13) failed to reveal any detectable receptor activity.

FH_2, a Ugandan subject of Indian extraction, was found to be severely hypercholesterolemic in childhood. He exhibited widespread tendon xanthomata and had coronary artery bypass surgery for occlusive disease approximately 6 years ago. His lymphocytes expressed less than 10% of normal receptor activity (13).

Therapy

FH_1, FH_2, FH_6, and FH_7 had been receiving biweekly plasmapheresis, and FH_3 and FH_4 were prescribed cholestyramine. These interventions were discontinued at least 6 weeks prior to initiation of the kinetic studies outlined below.

Protocol

The procedures that were used to examine the kinetics of apoB metabolism are detailed elsewhere (14, 15). Briefly, one unit of plasma was obtained from each fasting subject by plasmapheresis and used to prepare total VLDL ($d < 1.006$ kg·l^{-1}) by ultracentrifugation in a Beckman Ti60 rotor for 24 hr at 50,000 rpm (4°C). The supernatant VLDL was collected and its density was increased to 1.118 kg·l^{-1} by the addition of solid NaCl (170 mg/ml of VLDL solution). A 2-ml aliquot of this preparation was layered over a 0.5-ml cushion of d 1.182 kg·l^{-1} NaBr solution in a Beckman SW 40 rotor tube and a discontinuous salt gradient from d 1.0988–1.0582 kg·l^{-1} was constructed above it (16). The rotor was subjected to centrifugation at 39,000 rpm for 1 hr and 38 min at 23°C and decelerated without braking. $VLDL_1$ of S_f 60–400 was removed in the top 1.0 ml of solution which was replaced with 1.0 ml of d 1.0588 kg·l^{-1} solution before continuing with the separation. $VLDL_2$ (S_f 20–60) was then isolated from the top 0.5 ml of the gradient following centrifugation at 18,500 rpm for 15 hr and 41 min at 23°C. The S_f 60–400 and S_f 20–60 VLDL fractions were labeled with ^{131}I and ^{125}I, respectively, by the procedure of Bilheimer, Eisenberg, and Levy (17), and subsequently sterilized by membrane filtration (0.45 μm Amicon filters, Amicon Corp., Bedford, MA). Fifty μCi (approximately 0.5 mg VLDL protein) of each was then injected into the bloodstream of the respective donor. The tracers were routinely administered at 8:00 AM, and throughout the first day of the turnover the subjects were given a diet that contained less than 5 g of fat but their normal intake of carbohydrate and protein. This approach was designed to minimize chylomicron production and has been used in a number of previous studies (14, 15). Venous blood samples were collected at frequent intervals over the first 72 hr and thereafter on a daily basis. S_f 60–400 and S_f 20–60 VLDL were isolated directly from the plasma specimens at each time point, following the centrifugation schedule outlined above. Additionally, intermediate density lipoprotein (IDL, S_f 12–20) and LDL (S_f 0–12) were pre-

TABLE 3. Apolipoprotein B metabolism in normal and

Subject	Large VLDL ApoB (S_f 60–400)				Small VLDL ApoB (S_f 20–60)				
			Fractional Rate					Fractional Rate	
	Synthesis	Plasma Pool	Direct Catabolism	Transfer to $VLDL_2$	Synthesis Direct	Flux from $VLDL_1$	Plasma Pool	Direct Catabolism	Transfer to IDL
	mg/d	*mg*	*pools/d*		*mg/d*		*mg*	*pools/d*	
N_1	802	79	3.7	6.5	393	512	246	0.36	3.3
N_2	290	18	0.0	16.2	137	290	100	0.70	2.3
N_3	237	9	17.7	8.4	262	77	65	0.52	4.7
N_4	520	108	1.1	3.7	361	403	289	0.51	1.1
N_5	954	115	5.0	3.2	238	372	258	0.35	1.9
Mean ± SD	560 ± 280	66 ± 44	5.5 ± 6.4	7.6 ± 4.7	278 ± 91	331 ± 145	191 ± 91	0.49 ± 0.13	2.8 ± 1.2
FH_1	1045	108	3.7	6.0	390	648	660	0.57	1.11
FH_2	614	72	4.4	4.1	214	297	536	0.30	0.65
FH_3	398	110	1.2	2.4	1200	265	896	0.55	0.64
FH_4	181	28	3.7	2.8	983	73	475	0.83	1.23
FH_5	674	59	3.6	7.7	217	462	310	0.53	0.73
Mean ± SD	582 ± 289	75 ± 31	3.3 ± 1.1	4.6 ± 2.0	601 ± 411	350 ± 193	563 ± 192	0.56 ± 0.17	0.88 ± 0.24
FH_6	226	9	1.8	24.0	13	210	120	0.28	1.41
FH_7	165	8	3.6	18.0	74	137	75	0.28	1.41
Mean	196	9	2.7	21	44	174	98	0.36	1.64

pared by subjecting the SW 40 rotor contents to two further 23°C centrifugation steps of 39,000 rpm for 2 hr and 34 min and 30,000 rpm for 21 hr and 10 min, respectively. IDL was removed from the top of the gradient in a volume of 0.5 ml and LDL in a 1.0-ml aliquot.

Each lipoprotein fraction was treated with 1,1,3,3 tetramethylurea (18) and the resulting insoluble pellet was extracted with organic solvents (chloroform–methanol 1:1, vol/vol) to isolate apolipoprotein B prior to determination of its specific activity by previously published procedures (14). The mean apolipoprotein B present in each fraction derived from fasting blood specimens was used to calculate the plasma concentration of B protein in that density interval. To compensate for potential losses in the precipitation procedure, an independent estimate of this parameter was obtained as the difference between the total protein and tetramethylurea-soluble protein content of each fraction (18). Compositional analysis of the fractions (S_f 60–400, 20–60, 12–20, and 0–12) was performed by methods reported elsewhere (14, 15). This information permitted correction for losses incurred during centrifugation, viz: the cholesterol content of all four fractions was summed and the resulting value was compared with the total apoB-associated cholesterol (total cholesterol minus HDL cholesterol) measured by the standard Lipid Research Clinics methodology (19). Calculated losses of apoB during centrifugation were of the order of 10–15%.

Apolipoprotein B kinetic analysis

Kinetic analysis of the data was performed using the SAAM 29 program (20). Total apoB radioactivity decay curves and the mass of B protein associated with each lipoprotein fraction were used to derive rate constants and protein fluxes. The model (**Fig. 1**) is a development of an earlier published version (14):

1) Large VLDL apoB ($VLDL_1$, S_f 60–400) behaves as a single species which decays monoexponentially in both normal and FH subjects. This is either catabolized directly or transferred to the $VLDL_2$ (S_f 20–60) range.

2) Within the S_f 20–60 lipoproteins there is an arrangement akin to that described by Berman et al. (21). Some apoB enters a catabolic cascade and is converted to IDL (S_f 12–20) while other material is diverted into a slowly metabolized remnant species (compartment 6, Fig. 1).

3) There is input of newly synthesized apoB into the S_f 20–60 density range. This is required because not all of the S_f 20–60 apoB mass can be accounted for by transport from large VLDL, and when large (S_f 60–400) and small (S_f 20–60) VLDL are labeled separately the kinetics of appearance of these tracers in IDL and LDL apoB is different. Usually the radioactivity derived from labeled small VLDL appears more quickly in these denser fractions and accounts for a higher proportion of their mass. Provision is made for this phenomenon by incorporating in the model parallel pathways leaving small VLDL and appearing in IDL and LDL.

4) In the IDL range it was necessary to postulate the existence of a slowly metabolized species (compartment 9, Fig. 1).

in homozygous familial hypercholesterolemic subjects

IDL ApoB (S_f 12–20)					LDL ApoB (S_f 0–12)				
			Fractional Rate						
Synthesis Direct	Flux from $VLDL_2$	Plasma Pool	Direct Catabolism	Transfer to LDL	Synthesis Direct	Flux from IDL + $VLDL_2$	Plasma Pool	Fractional Catabolic Rate	Total B Synthesis
mg/d		*mg*	*pools/d*		*mg/d*		*mg*	*pools/d*	*mg/d*
0.0	817	337	0.77	1.65	0.0	556	1750	0.32	1195
0.0	275	210	0.32	0.98	0.0	290	635	0.46	427
0.0	305	95	0.32	2.87	0.0	273	710	0.39	499
0.0	312	245	1.07	0.23	0.0	359	2110	0.17	881
0.0	499	496	0.15	0.85	9.0	423	2650	0.20	1282
0.0	441 ± 204	277 ± 134	0.52 ± 0.34	1.3 ± 0.9	18 ± 36	380 ± 102	1571 ± 788	0.31 ± 0.11	857 ± 349
0.0	668	1278	0.06	0.46	0.0	628	5698	0.11	1435
0.0	349	842	0.11	0.30	134	256	4644	0.084	962
0.0	577	1047	0.32	0.23	140	636	6748	0.11	1738
0.0	586	2222	0.06	0.21	315	543	5046	0.17	1479
94	241	1388	0.11	0.24	396	453	10186	0.083	1381
19 ± 38	484 ± 161	1355 ± 473	0.13 ± 0.096	0.29 ± 0.09	197 ± 141	503 ± 140	6464 ± 1992	0.11 ± 0.03	1399 ± 250
51	174	848	0.21	0.02	224	62	3587	0.080	514
163	140	1613	0.02	0.18	304	323	4964	0.128	706
107	157	1231	0.12	0.1	264	193	4276	0.10	610

5) LDL was distributed between two plasma compartments (compartments 10 and 11), only one of which was permitted to equilibrate with the extra vascular space. This was necessary to accommodate the observation that there were differential rates of appearance and removal of LDL apoB depending on whether the protein was derived from large or small VLDL.

The model provided an acceptable fit to the observed data in both the control and FH subjects, and the calculated masses derived from the kinetic analyses were within 20% of the measured values.

Ethical considerations

All subjects (or their parents) gave informed consent to the study which met the requirements of the Ethical Committee of each host institution. They were prescribed KI (60 mg thrice daily) for 3 days prior to and 1 month after isotope administration in order to minimize thyroidal sequestration of radioiodide.

RESULTS

Plasma lipids and lipoproteins

In familial hypercholesterolemia, plasma LDL cholesterol is characteristically elevated several-fold (1) while HDL concentrations are generally low (Table 1). Plasma triglyceride varies, and may be increased above normal. Portacaval shunt surgery is known to lower circulating VLDL (1, 5) and presumably was responsible for the low triglyceride concentrations recorded in FH_5 and FH_7.

Table 2 presents the mean compositional analyses of VLDL, IDL, and LDL subfractions in the control and FH groups. The composition of S_f 60–400 lipoproteins differed in the FH subjects only in their content of free cholesterol ($P < 0.02$). The smaller VLDL (S_f 20–60), on the other hand, was enriched in cholesteryl esters and depleted in triglyceride ($P < 0.005$ in each case) as was IDL. The depletion of free cholesterol in FH LDL ($P < 0.005$) probably resulted from the prolonged residence time of the particles in the plasma, increasing their exposure to lecithin:cholesterol acyltransferase.

Apolipoprotein B kinetic analysis

The metabolism of apolipoprotein B in the FH homozygotes not only differed substantially from normal in several major respects (Table 3) but also showed considerable variation within the group itself. Radioactive apoB initially present in large (S_f 60–400) $VLDL_1$ was metabolized rapidly in both FH and normal subjects (Fig. 2 and Fig. 3, respectively), at rates that were not significantly different (fractional clearance in controls = 13.1 ± 7.5 pools · d^{-1} versus 7.9 ± 2.7 pools · d^{-1} in the FH group). The majority of this B protein transferred into the S_f 20–60 small $VLDL_2$ density interval, at which point the two groups showed divergent metabolic patterns. The clearance of small VLDL apoB in the FH

group was substantially delayed, resulting in a slow appearance of radioactivity in IDL and ultimately in LDL. This transfer, usually complete within 24 hr in control subjects, took 120–140 hr in the FH homozygotes, and examination of the metabolic behavior of directly labeled small VLDL apoB showed a similar pattern. The data obtained from the two tracers (^{131}I-labeled VLDL apoB (S_f 60–400) and ^{125}I-labeled $VLDL_2$ apoB (S_f 20–60)) were merged in the SAAM program to give a single set of kinetic parameters which permitted quantitation of the rates of transport of the B protein through these density classes (Table 3 and Table 4).

The parameters describing the metabolism of large S_f 60–400 VLDL apoB in the group of five medically treated FH subjects (FH_1–FH_5, Tables 3 and 4) were not significantly different from those of the controls. Both the rates of synthesis and catabolism and the plasma pool of apoB in the particles present in this density interval were normal. About 60% of this large VLDL transferred to the smaller, denser S_f 20–60 range. The remainder was removed directly from the plasma compartment. In the normal group, this transfer accounted for 54% of the B protein mass present in the S_f 20–60 interval. The rest of the $VLDL_2$ apoB came from direct synthesis, presumably by the liver. The magnitude of the de novo input of $VLDL_2$ apoB in the FH group was on average twice normal, but Table 3 shows that this was not a universal phenomenon but rather was the result of excessive B protein synthesis into S_f 20–60 VLDL in FH subjects 3 and 4. The 2.9-fold increase in the mass of $VLDL_2$ B protein in the FH subjects derived primarily from delayed clearance. The latter appeared to arise from two abnormalities. First, the rate of transfer of B protein out of $VLDL_2$ into IDL was only 31% of normal (Table 3). Secondly, more of the $VLDL_2$ apoB was diverted into a slowly metabolized remnant population (Fig. 1, compartment 6). In the control group, only 7% of the material in compartment 2 was diverted from the delipidation cascade into remnants, whereas 50% took this route in FH subjects 1 to 5. The mean computed mass of apoB in the remnant population was 232 mg in the FH subjects versus 24 mg in the normals (Table 4, M_6). This expansion was not due solely to increased remnant formation. Remnant removal was also defective in the FH group, $k_{0,6}$ being reduced by 50% in the latter.

FH subjects 1–4 showed no requirement for direct synthesis of apoB into IDL. They, as did the controls, derived this fraction entirely by transfer from VLDL. The flux of

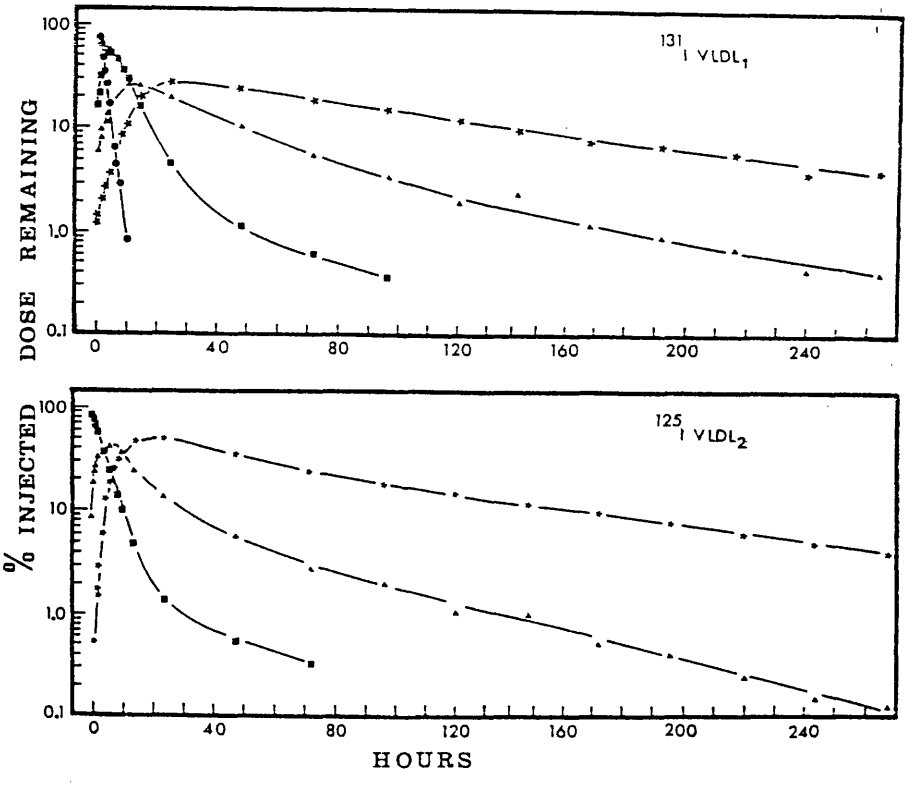

Fig. 2. Apolipoprotein B radioactivity decay curves of ^{131}I-labeled $VLDL_1$ (S_f 60–400) and ^{125}I-labeled $VLDL_2$ (S_f 20–60) in control subject N_1. Values are expressed as percent of initial dose. $VLDL_1$ (●), S_f 60–400; $VLDL_2$ (■), S_f 20–60; IDL (▲), S_f 12–20; LDL (*), S_f 0–12.

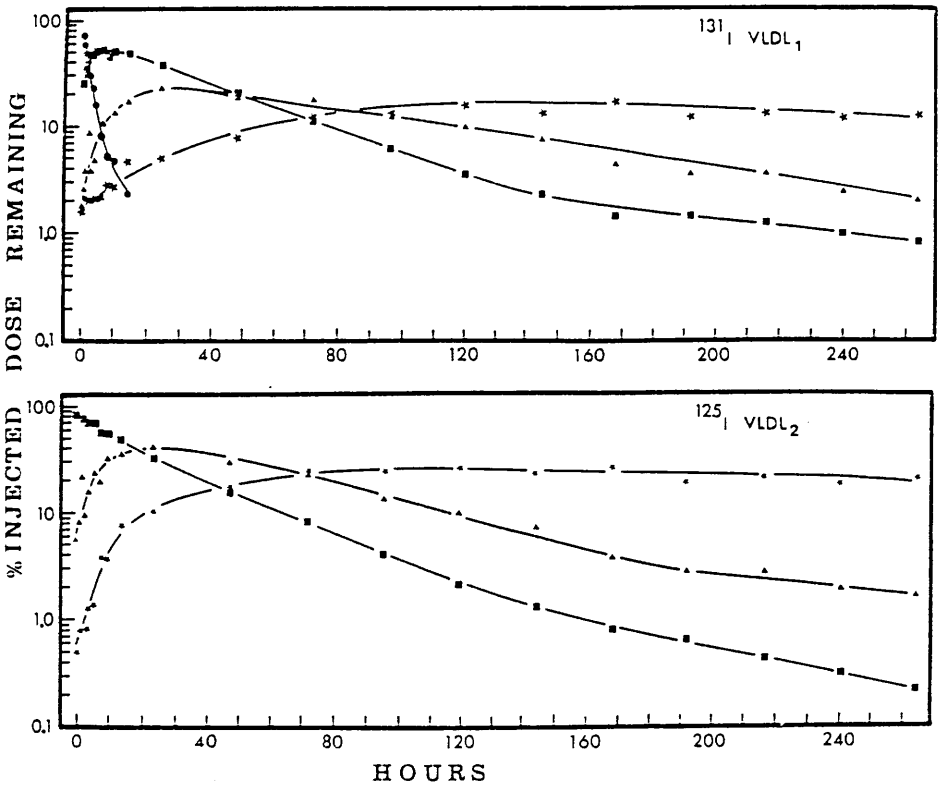

Fig. 3. Apolipoprotein B radioactivity decay curves of ^{131}I-labeled VLDL$_1$ (S_f 60–400) and ^{125}I-labeled VLDL$_2$ (S_f 20–60) in subject FH$_2$. Values are expressed as percent of initial dose. VLDL$_1$ (●), S_f 60–400; VLDL$_2$ (■), 20–60; IDL (▲), S_f 12–20; LDL (*), S_f 0–12.

B protein from small VLDL to IDL in all of the FH subjects was essentially normal. So, the marked (4.9-fold) increase in circulating IDL resulted from a major reduction in its rate of catabolism in FH. The fractional rates of direct removal and transfer of this lipoprotein to LDL were significantly retarded (by 72%, $P < 0.02$, and 78%, $P < 0.05$, respectively). However, despite the decrease in fractional transfer, the flux of IDL apoB into the LDL density interval was normal or even slightly increased, at a mean value of 503 mg · d^{-1}. In most FH subjects, in addition to B protein transfer from IDL, it was necessary to specify direct LDL apoB input to account for the observed circulating mass of LDL. Total LDL production from both sources was increased to 700 ± 176 mg · d^{-1} in the FH group compared to 398 ± 116 mg · d^{-1} in the controls ($P < 0.02$). However, decreased B protein catabolism also contributed to the 4.1-fold expansion of the FH LDL apoB pool. The fractional catabolism of apoB from this lipoprotein was 0.11, versus 0.31 pools · d^{-1} in the controls ($P < 0.005$).

The data from the two portacaval shunt subjects FH$_6$ and FH$_7$ were handled separately since they were distinctly different from the others in the group (Table 3). They showed a substantial reduction in the rate of large VLDL apoB synthesis compared to both the controls and the other FH patients. Moreover, the fractional clearance rate from this pool was high and so its plasma concentration was very low. This was also true for small VLDL apoB whose rate of production either from large VLDL or by direct synthesis was reduced. Shunt surgery, however, did not seem to affect the distribution of B protein within small VLDL in that remnants again accumulated to about 45% of the total mass (cf M_5, Table 4, with the pool of small VLDL, Table 3). Interestingly, the rate of remnant removal ($K_{0,6}$, Table 4) was not apparently affected by surgery, whereas the fractional transfer of VLDL$_2$ apoB to IDL appeared to be higher (Table 3) even though the net amount transferred was low. The IDL apoB pools in FH$_6$ and FH$_7$ were as high as in the other patients in the group. Again, defective catabolism played a major role in generating this phenomenon, although in both of these subjects it was necessary to invoke direct IDL synthesis in order to account for the total mass of apoB in the fraction. The pattern was repeated in the LDL density interval. Expansion of the pool resulted primarily from defective catabolism although increased

TABLE 4. Computed compartment masses and rate constants for normal and FH subjects

Subject	M_1[a]	$k_{0,1}$[b]	$k_{2,1}$	M_2[c]	$k_{3,2}$	$k_{6,2}$	M_4	$k_{0,4}$	$k_{8,4}$	$k_{9,4}$	$k_{11,4}$	M_5	$k_{0,5}$	$k_{7,5}$	$k_{10,5}$
N_1	79	3.7	6.5	53	8.6	1.0	42	0.8	7.6	2.3	0.0	52	0.0	7.6	0.0
N_2	18	0.0	16.2	16	18.0	0.2	44	0.7	3.6	0.4	1.9	23	1.7	4.3	0.0
N_3	9	17.7	8.4	9	7.4	1.1	2	16.0	14 0	14.0	0.0	36	0.0	7.2	0.0
N_4	108	1.1	3.7	43	8.4	0.8	93	1.2	1.1	0.4	1.2	80	0.0	2.1	2.4
N_5	115	5.0	3.2	42	8.2	0.3	77	1.0	3.1	0.1	0.2	85	0.0	2.8	0.0
Mean ± SD	66 ± 44	5.5 ± 6.4	7.6 ± 4.7	33 ± 17	10.1 ± 4.0	0.7 ± 0.4	52 ± 32	3.9 ± 6.0	5.9 ± 4.6	3.4 ± 5.3	0.7 ± 0.8	55 ± 24	0.3 ± 0.7	4.8 ± 2.2	0.5 ± 1.0
FH_1	108	3.7	6.0	69	7.2	2.2	83	2.9	3.0	0.1	0.0	108	0.0	3.4	0.3
FH_2	72	4.4	4.1	45	2.4	4.2	81	0.1	1.2	0.0	0.0	59	0.0	3.6	0.0
FH_3	110	1.2	2.4	24	1.4	9.6	23	0.1	1.3	0.0	0.0	532	0.6	1.0	0.7
FH_4	28	3.7	2.8	12	0.48	5.8	63	0.0	0.0	0.1	0.0	261	1.2	2.2	0.3
FH_5	59	3.6	7.7	21	14.4	7.7	35	0.0	4.3	0.0	4.3	71	0.1	1.3	1.7
Mean ± SD	75 ± 31	3.3 ± 1.1	4.6 ± 2.0	34 ± 20	5.2 ± 5.2	5.9 ± 2.6	38 ± 24	0.6 ± 1.1	2.0 ± 1.5	0.04 ± 0.05	0.9 ± 1.7	206 ± 178	0.4 ± 0.5	2.3 ± 1.1	0.6 ± 0.6
FH_6	9	1.8	24.0	18	10.0	2.1	9	0.0	17.8	0.0	1.3	13	0.0	0.8	0.3
FH_7	8	3.6	18.0	13	9.6	0.7	9	1.9	10.8	0.0	1.9	13	0.5	3.5	1.6
Mean	9	2.7	21.0	16	9.8	1.4	9	1.0	14.3	0.0	1.6	13	0.3	2.2	1.0

Masses (M_i) refer to the numbering of sub-compartments given in Fig. 1. Rate constants K_{ij} indicate transfer from sub-compartment j to sub-compartment i.
[a]Masses in mg.
[b]Rate constants (d^{-1}).
[c]$M_3 = M_2$; $k_{4,3} = k_{3,2}$ was a constraint in the model.

TABLE 4. (continued)

Subject	M_6[a]	$k_{0,6}$[b]	$k_{9,6}$	M_7	$k_{0,7}$	$k_{10,7}$	M_8	$k_{0,8}$	$k_{11,8}$	M_9	$k_{0,9}$	M_{10}	$k_{0,10}$	M_{11}	$k_{0,11}$
N_1	66	0.8	0.0	115	0.5	2.9	66	1.53	3.38	160	0.6	680	0.49	1070	0.21
N_2	2	1.9	0.0	33	0.0	3.0	145	0.34	0.74	33	0.6	215	0.46	420	0.46
N_3	9	1.1	0.0	73	0.0	3.6	7	1.40	1.80	15	1.4	636	0.41	74	0.16
N_4	29	1.2	0.0	78	1.9	0.2	76	1.0	0.48	92	0.4	1380	0.15	728	0.20
N_5	12	1.0	0.0	219	0.0	1.1	270	0.23	0.65	7	1.0	1412	0.20	1171	0.20
Mean ± SD	24 ± 23	1.2 ± 0.4	0.0	104 ± 63	0.5 ± 0.7	2.2 ± 1.3	113 ± 90	0.90 ± 0.53	1.41 ± 1.09	61 ± 58	0.8 ± 0.4	865 ± 463	0.34 ± 0.14	693 ± 407	0.25 ± 0.11
FH_1	273	0.37	0.18	179	0.12	1.9	912	0.0	0.27	185	0.30	2994	0.13	2704	0.090
FH_2	302	0.50	0.12	513	0.05	0.37	184	0.17	0.36	144	0.25	3081	0.084	1563	0.084
FH_3	290	0.67	0.12	787	0.38	0.26	88	0.0	0.36	172	0.20	6166	0.12	532	0.060
FH_4	127	0.47	0.1	2175	0.05	0.21	0	0.0-	0.0	47	0.37	5046	0.17	0	0.0
FH_5	166	0.96	0.0	770	0.0	0.24	618	0.24	0.0	0	0.0	8586	0.11	1600	0.072
Mean ± SD	232 ± 71	0.6 ± 0.2	0.1 ± 0.06	885 ± 681	0.1 ± 0.1	0.6 ± 0.7	360 ± 348	0.08 ± 0.10	0.20 ± 0.16	110 ± 73	0.22 ± 0.13	5174 ± 2086	0.12 ± 0.03	1279 ± 939	0.06 ± 0.03
FH_6	62	0.56	0.05	301	0.08	0.13	547	0.27	0.02	2	1.3	3230	0.10	357	0.057
FH_7	27	0.36	0.0	1140	0.02	0.18	473	0.03	0.17	0	0	4246	0.12	718	0.13
Mean	43	0.46	0.03	721	0.05	0.16	510	0.15	0.1	1	0.7	3738	0.11	538	0.09

direct synthesis was again contributory. The fractional catabolic rates of apoB in IDL and LDL did not appear to be affected by shunt surgery.

DISCUSSION

VLDL comprises a structurally heterogeneous spectrum of particles whose properties are the subject of continuing research. In normal individuals most VLDL is in the denser S_f 20–60 interval (18). Familial hypertriglyceridemic subjects, on the other hand, possess increased amounts of larger particles (14, 15, 18, 22) with greater triglyceride content, and a similar distribution can be induced in normal individuals by carbohydrate feeding (23). The size of the nascent VLDL may govern its subsequent metabolic behavior in the plasma. Large particles in the flotation range S_f 100–400 undergo rapid hydrolysis via lipoprotein lipase, acquiring in the process cholesteryl ester by transfer from HDL. Such particles, in fact, are the favored acceptors of sterol ester (24). The remnants that come from their lipolysis remain within the VLDL spectral distribution, although at its denser end. Here they resist further hydrolysis and are thought to be catabolized as a unit via receptor-mediated mechanisms. They therefore make little contribution to the production of LDL. The latter appears to come from smaller, denser VLDL which is secreted directly by the liver (14, 15). The extent to which remnants contribute to the total VLDL population can be assessed from the cholesteryl ester/triglyceride ratio in that fraction (25). Where they are abundant, as in Type III hyperlipoproteinemia, the ratio is increased. This compositional abnormality was found in the small VLDL and IDL of our FH patients, indicating that B protein metabolism was grossly perturbed in this group of subjects.

FH subjects 1–5 possessed large VLDL that was essentially normal both in terms of composition and metabolism. In particular, the rate of conversion of large to small VLDL, mediated by lipoprotein lipase, was not influenced by the receptor defect. The impact of the disease only became apparent at the level of small VLDL. A higher proportion of B protein in this fraction was channelled into slowly metabolized remnants, which presumably accounted for the elevated cholesteryl ester/triglyceride ratio found in FH S_f 20–60 $VLDL_2$. The mechanism underlying this phenomenon is not clear, although it may be postulated that the prolonged plasma residence time of small VLDL which followed from retardation of the whole delipidation process might expose the particle to increased cholesteryl ester transfer from HDL, thereby limiting its potential for further lipolysis. Defective catabolism of the remnants ($k_{0,6}$, Table 4) also played a part in their accumulation in these receptor-deficient patients. This observation suggests that the LDL receptor may be implicated in this process. The calculated 10-fold increase in the circulating VLDL remnant population in FH (M_5, Table 4) accounted for about one-half of the expanded S_f 20–60 VLDL pool. The remainder derived from an increment in direct synthesis of small VLDL, particularly evident in subjects FH_3 and FH_4 (M_5, Table 4).

An unexpected finding of this study, evident from the decay profiles (Fig. 3), was that the fractional rate of transfer of B protein from small $VLDL_2$ through IDL to LDL was very slow. These delipidation steps are thought to be mediated by lipases, and recent results from animal and human studies (26, 27) implicate hepatic lipase in the process. It is not immediately obvious why the VLDL–LDL conversion should be so slow in these LDL receptor-deficient subjects. Several possibilities exist. First, the delipidation pathway may be saturated, limiting the processing of apoB to about 500 $mg \cdot d^{-1}$, and reducing its fractional rate of transfer through the cascade. However, this would imply (Table 3) that the conversion process is virtually saturated in normal subjects also, and would not explain why patients FH_2, FH_3, and FH_7 exhibit such a low B protein flux from VLDL to LDL. A second alternative is that the greatly increased IDL and LDL pools act by product inhibition to suppress hepatic lipase activity. Or thirdly, it may be postulated that LDL receptors and lipase act cooperatively in the conversion process. It is known that the majority of LDL receptors are found in the liver (28) as is a lipase which expresses high affinity for smaller VLDL and IDL (29); and transhepatic measurements indicate that this organ is the most likely site of IDL–LDL conversion (30). Hepatic lipase is reported to be located on the surface of sinusoidal cells (31), which themselves are a particular variety of endothelial cells. The latter are known from tissue culture studies to express LDL receptors (32), although at confluence they do not necessarily participate in lipoprotein internalization and degradation, but may merely bind and release the particles (33). It is therefore possible that in the liver they assist lipolysis, bringing small VLDL and IDL into contact with hepatic lipase whose hydrolytic actions lead to the production of LDL. Subsequent loss of apolipoprotein E from the particle would reduce its affinity for the receptor and facilitate its release back into the circulation. Such a mechanism is, of course, highly speculative although it does explain the slow VLDL–LDL conversion that is seen in dysbetalipoproteinemia, in familial hypercholesterolemia, and in hepatic lipase deficiency (34).

As might be expected in FH homozygotes, direct catabolism of apoB-containing particles was retarded. LDL was cleared at one-third of the normal rate, presumably by receptor-independent mechanisms. The data presented in Table 3 and in an earlier publication by Soutar and her colleagues (9) show that IDL catabolism

is similarly retarded and indicate that receptors must play a role in the process. We have already shown (35) that this is likely to be the case in an experiment in which treatment of VLDL or IDL with 1,2 -cyclohexanedione, an agent that blocks potential interaction of the lipoprotein with receptors, slowed both the direct catabolism of IDL and its conversion to LDL. Thus the consequences of receptor deficiency are as profound for IDL as for LDL. Most of the differences observed between the normal and FH homozygotes are so marked that it is unlikely they can be explained by the necessary variation in age and body weight between the two groups. The influences of these factors, however, should be borne in mind when differences between the two groups are small.

A number of studies (3, 5) have indicated that homozygous FH is associated with overproduction of LDL. Table 3 shows that this also occurred in FH subjects 1-5 in whom total B protein synthesis was on average 63% higher than in controls ($P < 0.025$). The mechanism responsible for this effect is unknown, although recent in vitro experiments have linked the catabolism of LDL with the control of apoB synthesis. Cultured hepatoma cells, starved of the lipoprotein, show enhanced apoB mRNA production which is inhibited by addition of LDL to the culture medium (36). Extrapolation to the in vivo situation would suggest that failure of LDL to be assimilated by the liver via the receptor pathway may derepress apoB production in that organ.

We have already commented on the variability in apoB metabolism which was apparent in our seven homozygous FH subjects. Patient FH_1, who exhibited the highest plasma triglyceride levels, secreted excessive amounts of apoB into VLDL and showed no requirement for direct LDL synthesis. These results are reminiscent of the situation prevalent in Watanabe rabbits (7, 8). However, in our subjects with lower plasma triglyceride levels, an increasingly higher proportion of B protein was secreted directly into LDL. Clearly, the rabbit model is not applicable here.

If we assume that prior to shunt surgery FH subjects 6 and 7 resembled the other five in this study, then it may be surmised that the portacaval anastomosis primarily inhibits triglyceride synthesis, limiting secretion of VLDL into the plasma. In this situation, more apoB appears in the triglyceride-depleted IDL and LDL particles. The procedure itself seems to make no impact on the rates of catabolism of VLDL remnants, IDL or, as reported earlier, of LDL (9).

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APOLIPOPROTEIN B METABOLISM

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INTRODUCTION

Apolipoprotein B-100 (apo B) containing lipoproteins, isolated from plasma, constitute a spectrum ranging in density from 0.95 to 1.063 kg/L ie with Svedberg flotation coefficients of Sf0-400. The least dense particles are triglyceride rich with a low content of cholesterol and protein - the latter comprising apo B-100, apo C and apo E. With increasing density (decreasing flotation rate) the lipoproteins become triglyceride depleted and enriched in cholesterol, cholesteryl ester and protein. The composition of the protein component alters so that apo C and apo E are lost while apo B becomes dominant. This spectrum can be viewed as a "delipidation cascade" in which the less dense Sf 100-400 VLDL are hydrolysed to form denser intermediate lipoproteins (IDL) and then finally LDL. A number of enzymes participate in this remodelling process including lipoprotein lipase, hepatic lipase and lecithin:cholesterol acyl transferase. Other proteins too such as cholesteryl ester transfer protein and the B/E receptor are important. In the following discussion we focus on the quantitative aspects of this delipidation sequence and how it differs in normal and hyperlipidaemic subjects.

METHODS

The approach used to investigate apo B metabolism involved the isolation of two subfractions of VLDL, $VLDL_1$ (Sf 60-400) and $VLDL_2$ (Sf 20-60) and their trace labelling with ^{131}I and ^{125}I respectively. Following re-injection into the donor, transit of apo B through $VLDL_1$, $VLDL_2$, IDL (Sf 12-20) and LDL (Sf 0-20) was followed by repeated purification of the B protein in these fractions at frequent time intervals and determination of its

specific activity (1,2). Total radioactivities were calculated as the product of the specific activity and measured mass for each lipoprotein fraction. These data were then used in multicompartmental analyses using the SAAM program (3) to construct a metabolic model and to generate estimates for kinetic rate constants. The model employed is shown in Figure 1.

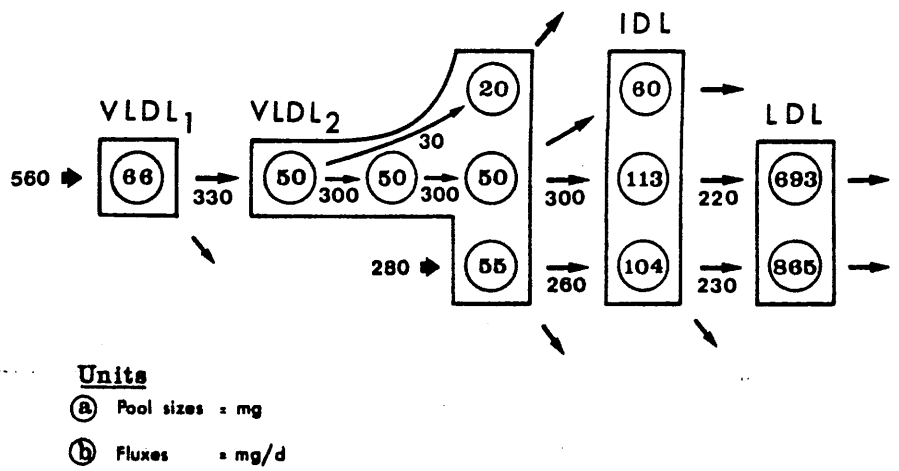

Fig.1: Multicompartmental model describing the flux of apolipoprotein B through the cascade system in a normal subject.

RESULTS AND DISCUSSION

Early work in normolipaemic individuals suggested a rather simple precursor-product relationship between VLDL and LDL (4). However, recent studies from our laboratory and from other workers (5,6) have revealed a complex pathway with multiple inputs and exits. The flux of B protein through this system in normal

individuals is quantified in Figure 1. It was necessary to postulate that there was direct synthesis of apo B into both large and small VLDL. Two thirds of this material was transmitted through to IDL and LDL while the remainder generated a "remnant" pool which was cleared slowly from the circulation, probably via receptors. One important finding of these investigations was the requirement for subcompartmentalisation of IDL and LDL. This was needed to allow for the observation that apo B associated with the small VLDL tracer appeared more rapidly and in greater amount in IDL and LDL than apo B from large VLDL. That is, metabolic channels are present in the VLDL-LDL conversion process so that the fate of an apo B containing lipoprotein depends to a certain extent on its pedigree. Since VLDL is such an heterogeneous mixture of particles we ought to expect such metabolic heterogeneity in its products, IDL and LDL. The recognition of this phenomenon is an exciting development in our understanding of the structure and function of these lipoproteins (6).

Quantitative investigation of the rates of transport of apo B through the VLDL-IDL-LDL cascade reveal how much of each species is made and indicate its probable precursor. However, additional work is needed to elicit the mechanisms involved in these transformations. Further insight into the role of certain enzymes and receptors in the VLDL to LDL conversion comes from studies of human mutants.

VLDL apo B metabolism in familial hypercholesterolaemia (FH)

Familial hypercholesterolaemia results from partially or completely defective expression of the LDL or B/E receptor. This protein, present on the membranes of most cells in the body, is able to bind LDL and internalise it. Early studies of FH focused on the gross increase in LDL cholesterol that accompanies it and the impact that this had on atherosclerosis. More recently it has become appreciated that the B/E receptor has a much wider role in apolipoprotein B metabolism. Its absence affects not only LDL but also VLDL and IDL. Soutar et al (7) demonstrated a delayed clearance of IDL apo B in subjects homozygous for FH. We have recently reexamined the situation in a group of seven FH

homozygotes in whom we investigated the metabolism of large $VLDL_1$ and small $VLDL_2$ (Th Demant, J Shepherd, C J Packard, unpublished observations). A number of interesting findings emerged. First, the conversion of $VLDL_1$ to $VLDL_2$ was unimpaired by the lack of receptors. Small (Sf20-60) VLDL metabolism, on the other hand, was grossly abnormal. Both the clearance of remnants from this density interval and the rate of delipidation to IDL and LDL were inhibited. Those FH subjects with the highest triglyceride levels oversynthesised apo B and derived most of their LDL from VLDL precursors. This process was inadequate in normotriglyceridaemic FH individuals who (like those studied by Soutar et al. (8)) appeared to make and secrete LDL de novo.

VLDL apo B metabolism in lipase deficiencies

Two lipolytic enzymes - lipoprotein lipase and hepatic lipase - are believed to contribute to the delipidation of triglyceride rich particles. Recently we have had the opportunity of conducting VLDL apo B metabolic studies in these deficiency states. In the absence of lipoprotein lipase the conversion of $VLDL_1$ to $VLDL_2$ is severely impaired as suggested from previously published work (9). The transfer of apo B between these two VLDL fractions occurs at only 10% of the normal rate and the $VLDL_1$ apo B pool is greatly increased. Trace labelled $VLDL_2$ however behaves in a different fashion. Its conversion to IDL and LDL is near normal, a finding that implies that lipoprotein lipase is not required for these steps (Th Demant, C J Packard, J Shepherd, unpublished). Rather, this component of the delipidation process seems to depend on the activity of hepatic lipase.

Studies in our laboratory of VLDL metabolism in a patient with hepatic lipase deficiency indicate that while the catabolism of $VLDL_1$ to $VLDL_2$ is unimpaired, the transfer of apo B through $VLDL_2$ to IDL and LDL is diminished. In fact, normal LDL was virtually absent from the plasma of this individual in accord with animal experiments which showed that antibody-induced inhibition of HL leads to accumulation of small VLDL and IDL and a fall in LDL (10). If it is postulated that IDL-LDL conversion involves hepatic lipase then this activity must be located in the liver, a

contention supported by examination of lipoprotein flux across the splanchnic bed. Turner et al. (11) found that while there was evidence for secretion of large (Sf100-400) $VLDL_1$ from the liver there was no detectable uptake of this lipoprotein fraction. On the other hand, radio-iodinated lipoproteins of Sf 12-60 ($VLDL_2$ and IDL) were extracted from the circulation by the splanchnic bed and about half of the radioactivity reappeared in the hepatic vein as LDL (Sf 0-12). Therefore, this enzyme occupies a pivotal role in the transformation of apo B containing lipoproteins in the lower part of the delipidation cascade.

New perspectives in apolipoprotein B metabolism

Common genetic variations in apolipoproteins B and E contribute significantly to the variation in plasma cholesterol in a population (12,13). Using the techniques of recombinant DNA technology, it is now possible to examine sub-sets of normolipaemic individuals to discover the genetic contribution to the wide diversity of blood lipid levels. One variation in the apo B gene is observed with the pABC cDNA probe following digestion with the XbaI endonuclease (12). A recent report (14) from our laboratory demonstrates that hypercholesterolaemic subjects who lack the cutting site (ie "X2X2" genotype) have a slower clearance of LDL from their circulation than those homozygous for the allele with the cutting site ("X1X1" genotype). The fractional catabolic rate for apo LDL in these groups was 0.22 $\pm$ 0.03 pools/day and 0.27 $\pm$ 0.02 pools/day respectively. We were careful to exclude subjects with FH and postulate that these differences are due to altered apo B in these individuals. Certainly this is associated with alterations in apo B conformation as detected by monoclonal antibodies (15). Further evidence to support this hypothesis comes from comparision studies performed in normal subjects (16).

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