THE KINETIC ANALYSIS AND COMPUTER MODELLING

OF

LIPOPROTEIN METABOLISM IN MAN

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TABLE OF CONTENTS

ABSTRACT

Plasma VLDL are a highly heterogeneous collection of particles in individuals with hypertriglyceridemia. This heterogeneity is seen metabolically through the complex kinetics that arise when labelled VLDL is used in turnover studies in these individuals. Previous studies have focused either on the the metabolism of apo B or triglyceride alone, and have not examined the metabolism of discrete subfractions within VLDL. This thesis examines the kinetic relationship between apo B and triglyceride metabolism in these subjects in VLDL, IDL and LDL by performing multiple label turnover studies simultaneously in four diverse type IV hyperlipoproteinemic subjects. Autologous ^{125}I-VLDL1 (S_f 60-400) and ^{131}I-VLDL2 (S_f 20-60) were co-reinjected with 2-^{3}H-glycerol into the subjects, and sequential samples of VLDL1, VLDL, IDL and LDL were isolated for determination of radioactivity in apo B and triglyceride. VLDL1 and VLDL2 were further separated into heparin-bound or unbound particles by the technique of heparin-Sepharose affinity chromatography.

Analysis of the kinetics of apo B in the unbound fractions in VLDL1 and VLDL2 in two subjects showed the presence of two pools of particles, one of which turned over more rapidly than the other. The kinetics of apo B in the bound fractions in VLDL1 and VLDL2 were, in contrast, dominated by a large slowly turning over pool of particles which resembled the kinetics of whole VLDL. Triglyceride specific radioactivity curves in both unbound and bound VLDL fractions exhibited a number of similar features including an initial rapid rise in specific radioactivity analagous to the rapid apo B pool and a broad peak. The kinetics of unbound and bound apo B fractions in VLDL supports the hypothesis of a partial precursor-product relationship existing

between these fractions. This observation further supports the hypothesis that the unbound fraction was richer in nascent-like particles, while the bound fraction contained more remnant particles. However, the similar kinetic characteristics of the triglyceride specific radioactivity curves in the unbound and bound fractions indicate that the bound fraction also contained a substantial proportion of nascent-like particles.

A multicompartmental model was developed to account for the kinetic data of both apo B and triglyceride in all fractions of VLDL and in IDL and LDL simultaneously. In the process of developing the model numerous assumptions were made. These include: 1. that the residence time of apo B and of triglyceride in a single compartment is the same; 2. that the composition of the unbound and bound VLDL and IDL and LDL fractions is heterogeneous with respect to both composition and metabolic behaviour and; 3. that within VLDL fractions there are populations of particles which turn over more rapidly than the liver triglyceride compartments, supporting the notion that the liver is the rate limiting step involved in triglyceride metabolism.

The VLDL section of the model comprises two parallel delipidation pathways which describe the metabolism of the more nascent-like and remnant particles which are found in both the unbound and bound VLDL fractions. The turnover rate of the more nascent-like particles was determined by the rapid fall of the unbound apo B specific radioactivity curve or was a function of the rapid rise of the VLDL triglyceride specific radioactivity curve. The slowly decaying tail of the bound apo B specific radioactivity curve defined the turnover rate of the remnant-like particles. To account for the observed precursor-product relationship between the unbound and bound fractions the parallel pathways were joined by a pathway converting unbound remnants to bound remnants. IDL and LDL were modelled as single delipidation

pathways similar to those of VLDL. The flow of apo B and triglyceride from one fraction to a denser fraction was assumed to occur via the remnant compartments. The input of new apo B and triglyceride however was assumed to enter the more rapidly turning over nascent-like compartments at the top of each delipidation pathway. Because problems arose in the analysis of the ^{125}I-VLDL1 data due to a rapid early transfer of VLDL1 radioactivity to VLDL2 at zero time it was not possible to estimate VLDL1 to VLDL2 conversion and hence determine direct input of apo B and triglyceride into VLDL2.

The main conclusions supported by the model are: 1. there is direct input of apo B and triglyceride into plasma at VLDL1, VLDL2, IDL and LDL levels; 2. heterogeneous triglyceride precursor pools leading to different rates of labelling of VLDL1 and VLDL2; 3. very substantial delipidation of nascent-like lipoprotein particles within each lipoprotein fraction; 4. early removal of particles of both VLDL1 and VLDL2; 5. higher than previously reported VLDL-triglyceride production and fractional catabolic rates and; 6. the inclusion in the model of the rapidly turning over triglyceride pool, identified in the heparin-unbound fraction, suggests that values for triglyceride production in man have been underestimated.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

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ABBREVIATIONS

VLDL	very low density lipoprotein
VLDL1	S_f 60-400 very low density lipoprotein
VLDL2	S_f 20-60 very low density lipoprotein
IDL	intermediate density lipoprotein
LDL	low density lipoprotein
HDL	high density lipoprotein
VHDL	very high density lipoprotein
apo A	apolipoprotein A
apo B	apolipoprotein B
apo C	apolipoprotein C
apo E	apolipoprotein E
EDTA	ethylenediaminetetra acetic acid
LPL	lipoprotein lipase
HTL	hepatic triglyceride lipase
LTP	lipid transfer protein
FCR	fractional catabolic rate
d.	density

CHAPTER 1

INTRODUCTION

1.1. Atherosclerosis: Significance and pathogenesis

Coronary heart disease is the major cause of death in Western society (Miller and Lewis, 1981). The usual underlying disease is atherosclerosis, which has a multifactorial pathogenesis. Of many risk factors, the abnormal metabolism of plasma lipids has received particular attention.

The first experimental evidence to suggest that lipids play a role in the development of atherosclerosis was provided by Anitschkow and Chalatow (1913), in which rabbits were fed a cholesterol-enriched diet. This finding had developed from the work of Ignatowski (1909), who produced the disease in these animals by feeding them meat, milk and eggs.

Lipids in atherosclerosis are thought to be derived from the blood, leaking into the arterial wall as a result of local mechanical (presumably haemodynamic) damage (Virchow, 1856). An alternative view of atherosclerosis was provided by Rokitansky (1852), who suggested that atherosclerosis resulted from deposition of formed elements from the blood on the lining of the arterial wall. These two views, which are not mutually exclusive, are still current as the lipid filtration theory (Poole and Florey, 1958; Adams et al, 1975) and the platelet-fibrin encrustation theory, as set out by Duguid (1946; 1960).

Epidemiological evidence has accumulated during the past three decades for an association between plasma lipoprotein levels and atherosclerosis in humans. Most of the studies relating plasma lipoproteins to atherosclerosis

have examined coronary heart disease, but strong relationships also exist between lipoproteins and the cerebral and peripheral vasculatures.

Although the pathogenesis of atherosclerosis is not completely understood, it is known that elevation of plasma lipoprotein levels correlates with the formation of atheromatous lesions rich in cholesteryl esters and other lipids. This simplistic description needs to be refined to take into account the complex patterns of plasma lipoproteins, the integral structure of the arterial wall, the diversity of cell types represented and the dynamics of lipoprotein metabolism at the cellular level.

1.2. Plasma lipoproteins

Macheboeuf (1929), at the Pasteur Institute in the 1920's, was the first to isolate and identify a plasma lipoprotein, a macromolecular complex of lipid and protein. Over the ensuing period investigators recognized that plasma lipoproteins could be divided into two major classes, alpha-1 and beta on the basis of their electrophoretic mobility (Blix et al, 1941). Cohn et al (1946) then separated these two classes of lipoproteins using ethanol/water mixtures at low temperatures. Interest in lipoproteins greatly increased in the 1950's with the realization by many investigators that elevated levels of plasma lipoproteins correlated with premature cardiovascular disease (Keys et al, 1950; Gofman et al, 1954). The subsequent work of Gofman et al (1954) led to the subdivision of plasma lipoproteins according to density.

Plasma lipoproteins were further characterized when Hatch and Lees (1968) showed that lipoproteins separated by paper electrophoresis correlated with those separated by differences in density. This, and other systems were then utilized by Fredrickson et al (1968) to study hyperlipoproteinemia and led

to the definition of five lipoprotein phenotypes, a sixth being subsequently added. They are summarized in Table 1.

Table 1. Hyperlipoproteinemia phenotype definitions and their association with genetic and other disorders[a]

Phenotype	Common name	Laboratory definition	Associated with genetic disorders	Conditions associated with secondary hyperlipoproteinemia
Type I	Exogenous hyperlipemia	Hyperchylomicronemia and absolute deficiency of LPL or PHLA Cholesterol raised Triglycerides greatly increased	Familial LDL deficiency Apo C-II deficiency	Dysglobulinemia, pancreatitis, poorly controlled diabetes mellitus
Type IIa	Hypercholesterolemia	LDL increased Cholesterol increased Triglycerides normal	Familial hypercholesterolemia LDL receptor abnormal Familial combined hyperlipidemia Polygenic hypercholesterolemia	Hypothyroidism, acute intermittent porphyria, anorexia nervosa
Type IIb	Combined hyperlipidemia	LDL increased VLDL increased Cholesterol increased Triglyceride increased	Familial hypercholesterolemia Familial combined hyperlipidemia	Hypothyroidism, acute intermittent porphyria, anorexia nervosa
Type III	Dysbetalipoproteinemia	Floating B-lipoproteins VLDL cholesterol/VLDL triglyceride > 0.35 Apo E_2 homozygote on isoelectric focusing Cholesterol increased Triglyceride increased	Familial dysbeta-lipoproteinemia	Diabetes mellitus, hypothyroidism, dysglobulinemia
Type IV	Endogenous hyperlipemia	VLDL increased Cholesterol normal or increased Triglyceride increased	Familial hypertriglyceridemia Familial combined hyperlipidemia	Glycogen storage disease, diabetes, nephrotic syndrome, renal failure, ethanol abuse
Type V	Mixed hyperlipidemia	Chylomicrons and VLDL increased LDL present but reduced Cholesterol increased Triglycerides greatly increased	Familial hypertriglyceridemia Familial combined hyperlipidemia	Poorly controlled diabetes mellitus, glycogen storage disease, hypothyroidism, nephrotic syndrome, dysglobulinemia, pregnancy, estrogen administration

[a] (Gotto, 1984)

1.3. Major lipoprotein classes

Gofman et al (1954) using a background density of salt found that plasma lipoproteins fell into four major classes called chylomicrons ($d < 0.93$ g/ml), very low density lipoproteins (VLDL, $0.93 < d < 1.006$ g/ml), low density lipoproteins (LDL, $1.006 < d < 1.063$ g/ml) and high density lipoproteins (HDL, $1.063 < d < 1.21$ g/ml). Based upon studies of the conversion of VLDL to LDL a new intermediate lipoprotein stage termed intermediate density lipoprotein (IDL, $1.006 < d < 1.019$ g/ml) was defined (Shore and Shore, 1962). The LDL class is now defined within a narrower range ($1.019 < d < 1.063$ g/ml). A summary of the physical properties of human plasma lipoproteins is in Table 2.

Table 2. Physical Properties of Human Plasma Lipoproteins[a]

	Electrophoretic definition	Particle size	Molecular weight	Density	S_f[d]
		nm		g/ml	
Chylomicrons*	Remains at origin[b]	75-1200	~400,000K	<0.93	>400
VLDL*	Pre-β lipoproteins	30-80	10-80,000K	0.93-1.006	20-400
β-VLDL	β-Lipoproteins	50-60		0.93-1.006	12-20
IDL	Slow Pre-β[c]	25-35	5-10,000K	1.006-1.019	12-20
LDL*	B-Lipoproteins	18-25	2,300K	1.019-1.063	0-12
Lp(a)	Slow Pre-β[c]	25-26	~500K	1.050-1.080	
HDL_2	α-Lipoproteins	9-12	360K	1.063-1.125	4-9[e]
HDL*					
HDL_3	α-Lipoproteins	5-9	175K	1.125-1.210	0-4[e]
HDL_c	between α and pre-β	20		1.060-1.090	

* Major human plasma lipoprotein species

[a] Smith et al 1983.

[b] On paper.

[c] On agarose.

[d] Svedberg flotation (S_f) units (10^{-13} cm/sec/dyne/g) in salt density 1.063 g/ml.

[e] S_f in salt density 1.21 g/ml.

1.3.1. Chylomicrons

Chylomicrons and very low density lipoproteins (VLDL) serve as the major transport vehicles for triglyceride within the circulation. These lipoproteins are collectively designated the 'triglyceride-rich' lipoproteins (TRL) since triglyceride is their major component.

Chylomicrons are intestinally derived lipoproteins which enter the circulation via the lymphatic system. They are normally only present in the plasma after a meal containing fat has been eaten. Plasma is clear of chylomicrons in the fasting normolipidemic individual. Chylomicrons are large particles, ranging in size from 15-250 nm (Schaefer et al, 1978). Table 3 describes the general composition of chylomicrons, and the other major lipoproteins, in terms of their major lipid components. Chylomicrons are predominantly all lipid, only 2 percent of their mass being protein. They contain predominantly long chain fatty acids (as triglycerides) with small amounts of cholesterol and phospholipid (Zilversmit, 1965; Alaupovic et al, 1968). Apo B_{48}, apo A_1 and apo A_4 are probably synthesized with the chylomicron particle while apo C and apo E attach to the particle in the lymph (Glickman and Green, 1977; Wu and Windmueller, 1978; Schonfeld et al, 1978). Following release into the circulation chylomicrons transfer apo A_1 and apo A_4 to HDL in exchange for more apo C and apo E (Havel et al, 1973; Imaizumi et al, 1978). Table 4 describes the apolipoprotein composition of chylomicrons and the other major lipoproteins.

Table 3. Major Constituents of Human Plasma Lipoproteins[a]

	Chylomicron	VLDL	IDL[b]	LDL	HDL
			%[c]		
Apolipoprotein	2	8	15	21	50
Phospholipid	5	12	19	22	25
Cholesteryl Ester	3	17	27	39	15
Free Cholesterol		9	10	12	4
Triglyceride	84	50	27	5	4
Free Fatty Acid	6	3	2	1	2

[a] From Schaefer et al 1978, Skipski et al 1967, Scanu and Hughes 1962, Nozaki et al 1986.

[b] S_f 12-20

[c] For each lipoprotein

Table 4. Characteristics of Plasma Apolipoproteins in Normal Fasting Humans[a].

	Plasma concentration		Distribution in lipoproteins					Major tissue source	Molecular weight
			Chylo	VLDL	IDL	LDL	HDL		
	mg/dl	mol%[b]			%[c]				
Apo A_1	130	43	6-9	+[d]	+	1	62	Liver, intestine	28,016
Apo A_2	40	22	4-6	+	+	+	20	Liver, intestine	17,414
Apo A_4			13-30				4	Liver, intestine	44,465
Apo B_{48}			12-22					Intestine	264,000
	80	5							
Apo B_{100}				25-37	30-40	91		Liver	515,000
Apo C_1	6	9	8	4-17	+	+	3	Liver	6,630
Apo C_2	3	3	14	7-17	+	+	1	Liver	8,900
Apo C_3	12	13	28	32-39	+	3	4	Liver	8,800
Apo D	10	5	+	+	+	+	3		22,000
Apo E_2									
Apo E_3	5	2	+	13-20	+	3	2	Liver	34,145
Apo E_4									
Apo F							1		

[a] From Schaefer et al, 1978; Kostner and Holasek, 1972; Brown et al, 1969; Havel and Kane, 1973; Osborne and Brewer, 1977; Suenram et al, 1979; Gotto et al, 1986.

[b] Based on total plasma concentration.

[c] For each lipoprotein.

[d] present in trace amounts.

1.3.2. Very low density lipoproteins

Very low density lipoproteins, the other member of the TRL family, are predominantly hepatically derived. Unlike chylomicrons which transport exogenous lipid from the intestine to the circulation, VLDL transport endogenous lipid, mainly triglyceride, from the liver to cells in the periphery.

VLDL represent a heterogeneous group of particles ranging in size from 30-80 nm. This heterogeneity with respect to physical properties and chemical composition has been demonstrated by centrifugation, electrophoretic and chromatographic techniques (Patsch et al, 1978; Kuchinskiene and Carlson, 1982; Pagnan et al, 1977; Trezzi et al, 1983). Attempts to elucidate the metabolism of VLDL have seen further subfractionation of VLDL into groups of various sized particles. Using Svedberg flotation rates (S_f) it is possible to subfractionate VLDL, and also IDL and LDL, into different sized particle groups. Table 2 describes the S_f rates of lipoproteins and the corresponding density ranges at which they can be isolated.

Unlike chylomicrons which contain apo B_{48}, human hepatic VLDL contain only the B_{100} form of apo B (Kane, 1983). In addition to apo B_{100}, rat and mouse livers are able to synthesize the B_{48} form of the apo B peptide (Sparks and Marsh, 1981; LeBoeuf et al, 1983). Despite the heterogeneous nature of VLDL each particle contains a constant mass of apo B (Havel, 1980). Tables 3 and 4 describe the composition of VLDL in terms of lipid and apolipoprotein content respectively. The composition of VLDL is variable due to variation in particle size. Large VLDL carry a greater proportion of triglyceride relative to cholesterol than smaller VLDL.

The data in Tables 3 and 4 describe the composition of plasma VLDL particles. These circulating VLDL have been subject to lipid and apolipoprotein exchange processes between other VLDL, other lipoproteins and receptors.

Nascent or newly secreted VLDL could be expected to have a different composition to that of circulating plasma VLDL. In addition, the composition of VLDL in terms of apolipoprotein and lipid content would vary according to fasted or fed status (Wilcox and Heimberg, 1987).

1.3.3. Intermediate density lipoproteins

Lipoprotein particles in the density range 1.006-1.019 g/ml used to be included in the LDL range however metabolic studies have indicated that lipoproteins in this class should be considered a separate lipoprotein species (Shore and Shore, 1962; Eisenberg et al, 1973). Intermediate density lipoproteins are believed to include VLDL remnants, the transition products of VLDL lipolysis (Musliner et al, 1986). The physical properties and chemical composition of IDL is described in Tables 2-4.

1.3.4. Low density lipoproteins

Low density lipoproteins, and HDL, in contrast to the role played by chylomicrons and VLDL in the transport of triglyceride, are the major cholesterol carrying lipoproteins.

Low density lipoprotein transports approximately 60 percent of the cholesterol in man, of which 75 percent is esterified. LDL are responsible for the transport of cholesterol from hepatic sources to cells in the periphery. More than 50 percent of LDL particle mass is free cholesterol and cholesteryl ester (Table 3), and it is therefore probably the most atherogenic lipoprotein in human plasma (Kesaniemi et al, 1987). Its protein composition is nearly all apo B_{100} and contains only trace quantities of apo C and apo E (Table 4).

1.3.5. High density lipoproteins

High density lipoproteins, in contrast to LDL, are involved in the movement of cholesterol from extrahepatic tissues to the liver (termed 'reverse cholesterol transport'). HDL also serves as a reservoir of apoproteins necessary for the metabolism of triglyceride-rich lipoproteins. It is much richer in protein than the less dense lipoproteins, VLDL, IDL and LDL. The HDL molecule contains approximately 50 percent lipid and 50 percent protein (Table 3). HDL unlike the other lipoproteins does not contain any apo B (Suenram et al, 1979) (Table 4). Quantitatively the most important HDL lipid is phosphatidylcholine (also known as lecithin).

High density lipoprotein has been separated into two major subclasses, HDL_2 and HDL_3 (Table 2), based upon particle density. HDL levels have been shown to vary inversely with cardiovascular disease risk, most of this variation appears to be attributable to changes in HDL_2 levels (Anderson, 1978).

1.3.6. Other lipoproteins

1.3.6.1. Beta very low density lipoprotein

Beta very low density lipoprotein (β-VLDL), an abnormal form of VLDL remnants, is formed when there is hypersecretion of VLDL combined with the apo $E_{2/2}$ phenotype (Berman et al, 1978). The β-VLDL of Type III patients tend to be smaller than normal VLDL and on analytical ultracentrifugation occupy the lower part (i.e., S_f 20-60) of the VLDL spectrum. β-VLDL particles, which as their name suggests exhibit β migration, have a higher ratio of cholesterol to triglyceride than normal VLDL or VLDL remnants (>0.42 vs 0.03-0.22) (Brown et al, 1983; Hazzard et al, 1972). β-VLDL is rich in apo E of abnormal structure,

the E_2 isoform, and has an apo E/Apo C ratio up to four times that of normal VLDL (1.05 vs 0.27) (Kushwaha et al, 1985).

1.3.6.2. Lipoprotein (a)

Lipoprotein (a) (Lp(a)) a variant of LDL, was discovered by Berg in 1963 as an antigen in the blood of certain individuals (Berg, 1963). It can be isolated on a density gradient between LDL and HDL at a density of 1.050-1.125 g/ml (Ehnholm et al, 1972). Lp (a), in addition to containing apo B_{100} also contains small amounts of apo (a) which is linked to apo B by a disulphide linkage (Fless et al, 1986). While the lipid moiety of Lp (a) is similar to that of LDL, Lp (a) has greater protein content, reflected by the presence of apo (a) (Kostner, 1986). McLean et al (1987) have recently shown that apo (a) is a deformed relative of plasminogen, the precursor of plasmin which dissolves fibrin clots.

The amount of LP (a) in plasma varies from undetectable to nearly 100 mg/dl (Albers et al, 1977), and there appears to be no correlation between the concentration of Lp(a) and LDL in plasma (Brown and Goldstein, 1987). High levels of Lp(a) are strongly associated with atherosclerosis and when combined with elevated LDL levels the risk of atherosclerosis rises five-fold (Armstrong, 1986).

1.3.6.3. Lipoprotein-X

Lipoprotein-X (Lp-X) appears in the plasma during cholestatic liver disease which results in hypercholesterolemia and hyperphospolipidemia (Seidel and Walli, 1986). The density range at which Lp-X can be isolated is 1.035-1.063 g/ml. This lipoprotein is characterized by a high content of phospholipids (66%) and free cholesterol (23%) and a small amount of protein (6%). About 60 percent of the protein is albumin which occupies the core of

the particle. Apo C_1, C_2 and C_3 and to a lesser extent apo D are the major apoproteins present in Lp-X. No other apoproteins are present.

Despite the absence of apo B and apo E, Lp-X has been shown to reduce both VLDL and chylomicron remnant uptake by perfused livers and isolated hepatocytes. Because Lp-X does not bind to hepatocytes it causes an efflux of hepatic cholesterol thereby activating HMG-CoA reductase and as cellular cholesterol levels rise the LDL receptor is down regulated (Seidel and Walli, 1986). The net result is therefore an accumulation of remnant particles, a reduction in conversion of lipoproteins to LDL, and an elevation in plasma cholesterol levels.

1.4. Lipoprotein structure

In 1979, Edelstein et al (1979) extending earlier models for chylomicrons and VLDL proposed a general structure for normal plasma lipoprotein particles in which neutral lipids, cholesteryl esters and/or triglycerides, are separated from the external aqueous environment by a surface monolayer consisting of apolipoproteins and the polar lipids, mainly phospholipid. The distribution of lipids between the surface and core is a function of their phase behaviour, thus it is possible to find significant amounts of cholesterol in the core and cholesteryl esters and triglycerides in the surface monolayer (Gotto et al, 1986).

All lipoprotein particles are spherical in shape, with the exception of newly synthesized or nascent lipoprotein particles. HDL newly synthesized by the liver are disk-like in shape upon secretion (Hamilton et al, 1976), however when some of its cholesterol is converted to cholesteryl ester, through the action of the enzyme lecithin:cholesterol acyltransferase (LCAT), the particle

assumes a spherical appearance. A certain proportion of neutral lipid may therefore be necessary to maintain the spherical structure of lipoproteins.

The surface coat or monolayer of HDL has a thickness of 1.4 nm (Morrisett et al, 1977), and contains the apolipoproteins and phospholipid head groups, the fatty acyl tails of which are orientated towards the centre of the lipoprotein. Unlike phospholipids which have a distinct polar head and neutral tail, apolipoproteins have amphipathic α-helices, the hydrophobic amino acid residues orientated towards the core, and the hydrophilic regions on the surface side of the lipoprotein (Assmann and Brewer, 1974; Brewer, 1981; Jackson et al, 1976; Segrest et al, 1979). Gotto et al (1986) have speculated that the role of the polar amino acid residues is to keep the apolipoproteins at the surface of the lipoprotein particle. This would facilitate apolipoprotein transfer between other lipoproteins and may be crucial to other specific functions of apolipoproteins, such as enzyme activation and receptor interaction.

1.5. Apolipoproteins and their metabolic functions

The apolipoproteins fall into two categories, those which are water soluble and which exchange between lipoproteins, and those, specifically apo B, which are water insoluble and remain fixed with a lipoprotein throughout its lifetime (Bilheimer et al, 1972).

Using different methods and approaches a variety of apolipoprotein nomenclature schemes have been proposed. The major schemes are based on elution position following column chromatography (Sata et al, 1972; Scanu et al, 1969), carboxly terminal amino acids (Shore and Shore, 1972; Brown et al, 1970), and component polypeptides of lipoprotein families (Alaupovic, 1968; Alaupovic et al, 1972). The scheme most commonly used is that of Alaupovic

(1968; Alaupovic et al, 1972) which uses the ABC system of naming the different apolipoproteins.

1.5.1. Apolipoprotein B

Human apolipoprotein B (apo B) is a glycoprotein that occurs in two forms, designated apo B_{100} and apo B_{48}, based upon their relative mobilities in SDS polyacrylamide gels (Kane et al, 1980). Apo B_{100} is a single polypeptide with a molecular weight of approximately 510 kDa (Olofsson et al, 1987) produced in the liver, and is an obligatory constituent of VLDL, IDL, and LDL (Mahley et al, 1984). Apo B_{48} however is approximately 48 percent the molecular weight of apo B_{100}, and is found in chylomicrons and their remnants (Mahley et al, 1981; Breslow, 1988) Apo B_{48} is also found in VLDL secreted from rat and mouse livers. Apo B_{48} represents the N-terminal half of apo B_{100} (Protter et al, 1986). In addition to apo B_{100} and apo B_{48} two other apo B associated proteins, apo B_{76} and apo B_{24}, have been observed in the presence of proteases (Cardin et al, 1984). The significance of these two proteins and their presence in vivo has not been determined (Fisher and Schumaker, 1986). More recently however other variants of apo B have been studied. Apo B_{38} (or B_{40}) and B_{95} have been reported as truncated variants of B_{100} which cause hypobetalipoproteinamia (Scott et al, 1988; Krul et al, 1988). These variants lack their carboxyl termini which may be necessary for intracellular assembly and secretion of apo B-containing particles.

Unlike other apolipoproteins, apo B is insoluble in aqueous buffers except in the presence of detergents or denaturants such as SDS, urea or guanidine hydrochloride (Kane, 1983), where it exists as a stable dimer (Steele and Reynolds, 1979).

The concentration of apo B in normal human plasma is between 70-100 mg/100 ml, mostly in the LDL fraction (Albers et al, 1975; Schonfeld et al, 1974). It is an integral part of chylomicrons, VLDL, IDL, and LDL and in patients with the condition of abetalipoproteinemia, where apo B is absent in the plasma, no chylomicrons or VLDL are secreted (Gotto et al, 1971; Malloy et al, 1981). Apo B does not exchange between other lipoproteins, as do other apolipoproteins (Eisenberg et al, 1973), probably because of its insolubility. The mass of apo B in different VLDL, IDL, and LDL particles appears to be constant and independent of particle weight and of other lipid and protein constituents (Redgrave and Carlson, 1979; Schonfeld, 1983). This combined with its non-exchanging characteristics make apo B the ideal tracee for studying the metabolism of VLDL, IDL, and LDL.

Apolipoprotein B_{100} interacts with heparin and other sulfated glycosaminoglycans and it is possible that this may represent an important physiological mechanism for lipoprotein binding to receptors (Mahley et al, 1984). Specifically it may represent lipoprotein binding to endothelial surfaces, in association with lipolysis, or to the ground substance of the arterial wall in association with the development of atherosclerosis (Hollander, 1968). The interaction between apo B_{100} and heparin is influenced by salt concentration (Shelburne and Quarfordt, 1977) and should therefore be ionic. The bonds are probably established with the positively charged amino groups, lysine and arginine, of the apo B_{100} (Farooqui and Horrocks, 1984).

Apolipoprotein B_{100} is the ligand of primary physiological importance in the metabolism of LDL (Goldstein and Brown, 1977). Apo B_{100} is responsible for binding the LDL particle to the LDL receptor, also known as the apo B,E receptor, which is present in both extrahepatic and hepatic tissues. Beisiegel et al (1981) has demonstrated in extrahepatic cells that the apo B,E and LDL

receptors are identical. Approximately 70 percent of LDL is catabolized by the LDL receptor pathway, nearly all by the liver (Brown and Goldstein, 1983b). Evidence for the role of apo B_{100} in the binding of LDL to receptors, and to heparin, has been demonstrated by Weisgraber et al (1978) and Mahley et al (1979) who were able to neutralize the positive charges on the lysine groups and modify the arginine residues to abolish any interaction with the receptor, or heparin.

In contrast to apo B_{100}, apo B_{48} does not bind to the LDL receptor, and is not the determinant responsible for the uptake of β-VLDL by macrophages (Mahley et al, 1984). It may be that another receptor exists which is specific for the apo E ligand, and does not interact with apo B-only containing lipoproteins. Herz et al (1988) have recently isolated a large membrane protein which may be shown to bind lipoprotein particles containing apo E.

Despite the differences between apo B_{100} and apo B_{48}, monoclonal antibody studies have shown similarities between the two apolipoproteins; this is partial evidence that both proteins are product of the same gene (Young et al, 1986). Further studies have shown that apo B is encoded by a single gene on chromosome 2 (Higuchi et al, 1987). Recent studies have demonstrated that from this gene a single nuclear mRNA is synthesized, edited and processed into two separate mRNA's (Higuchi et al, 1988). A 14.1-kilobase apo B mRNA codes for apo B_{100}, and the second mRNA, which codes for apo B_{48}, contains a premature stop codon, generated by a single base substistuion. This substitution, cytosine to uracil at nucleotide 6538, converts the translated CAA codon coding for glutamine at residue 2153 in apo B_{100} to a premature in-frame stop codon (Higuchi et al, 1988) which codes for apo B_{48}. Although the mechanism for this C to U substitution is unknown, the introduction of this stop codon appears to be species- and organ-specific (Chen et al, 1987). In

addition, studies undertaken using fetal and adult human intestine have demonstrated a developmental effect in the pattern of apo B synthesis (Glickman et al, 1986). Intestine from fetuses at 11 weeks produced predominantly apo B_{100}, after 16 weeks both apo B_{100} and B_{48} were produced while adult intesine produced only apo B_{48}.

Although apo B_{100} and apo B_{48} are closely related antigenically it is thought that apo B_{48} represents the half of the apo B_{100} protein which is not involved in receptor binding (Kane, 1983; Marcel et al, 1983). Monoclonal antibody studies have also shown that lipids are important in determining the expression of the apo B epitope (Breslow, 1988), and that delipidation of apo B_{100} abolishes its reactivity with monoclonals to the receptor binding region. Other studies however have shown that apo B reactivity is masked in VLDL and receptor binding occurs only after lipolysis (Catapano et al, 1979).

1.5.2. Apolipoprotein E

Apolipoprotein E (apo E) was first recognised by Shore and Shore (1969) and designated apo E by Utermann et al (1975). Subsequently it was identified as a minor protein component of normal VLDL which was rich in arginine and was found to a greater extent in cholesteryl ester-rich VLDL (Shore and Shore, 1973). The distribution of apo E in the major lipoprotein classes is described in Table 4. Human plasma concentrations of apo E range from 2.5 to 5.0 mg/100 ml and can be as high as 20 to 60 mg/100 ml in patients with Type III hyperlipidemia (Assmann, 1982).

The synthesis of apo E occurs in a wide variety of tissues. In addition to the liver, which is the major site, synthesis occurs in the intestine, brain, kidney, adrenal gland, spleen, thymus, pancreas, stomach, gonads, and macrophages (Zannis et al, 1985; Weisgraber, 1985; Basu et al, 1981).

Apolipoprotein E is a single polypeptide chain glycoprotein which is composed of 299 amino acids (Mr = 34.2 kDa) (Rall et al, 1982). The cDNA sequence has confirmed this and has shown the presence of an 18 amino acid signal peptide which is co-translationally cleaved (Zannis et al, 1982; Zannis et al, 1984). Human plasma apo E has been shown to consist of several isoproteins which differ in size and/or charge (Zannis and Breslow, 1981). One-dimensional isoelectric focusing demonstrated genetic polymorphism in apo E (Utermann et al, 1977). However, it took two-dimensional electrophoresis to illustrate the genetic basis of all of the apo E phenotypes (Zannis et al, 1981). The complex apo E isoform patterns are due to multiple alleles acting at a single genetic locus, and to desialylation within the circulation.

Family studies show that the different gel patterns are the result of a single apo E gene locus with three common alleles (Zannis and Breslow, 1981). The alleles have been designated ε2, ε3, and ε4 and their gene products are the apo E_2, apo E_3, and apo E_4 isoforms respectively. The two common variants of apo E, apo E_2 and apo E_4, differ by single amino acid substitutions from the E_3 isoform (Rall et al, 1982). The most common phenotype present in the human population is apo $E_{3/3}$, which is present in approximately 50-70 percent of subjects, depending on their ethnic origin (Havel, 1982; Zannis and Breslow, 1981; Wardell et al, 1982).

Apolipoprotein E-containing lipoproteins may bind to heparin and other glycosaminoglycans through the same region of apo E that mediates interaction with the lipoprotein receptors (Mahley et al, 1984). Apo E can serve as a ligand for the LDL receptor (Bersot et al, 1976; Innerarity et al, 1978).

Apolipoprotein E binding to the LDL receptor is characterized by a much higher affinity compared to the binding of apo B (Mahley et al, 1984).

Apo E-containing HDL displays a 20-25 fold greater affinity for the apo B,E receptor than LDL (Innerarity and Mahley, 1978; Pitas et al, 1979). This greater affinity is probably due to apo E having the potential to form multiple interactions with the LDL receptor (Mahley and Innerarity, 1983) whereas LDL may form only one such interaction (Innerarity and Mahley, 1978; Pitas et al, 1980).

1.5.3. Apolipoprotein C_1, C_2, and C_3

The C apolipoproteins are represented by three low molecular weight proteins present in plasma lipoproteins, designated apo C_1, C_2, and C_3. They are present in all major lipoprotein classes, although they are predominantly found in chylomicrons, VLDL, and LDL (Table 4). Although the C apolipoproteins have different metabolic functions they share the common property of redistributing among lipoprotein classes (Nestel and Fidge, 1982). In the fasting state, the C apolipoproteins are mainly associated with HDL, however with the production of chylomicrons and synthesis of VLDL the C apolipoproteins redistribute to the surface of the triglyceride-rich lipoproteins. As the triglyceride of chylomicrons and VLDL is hydrolysed there is a net transfer of apo C's and other surface components back to HDL (Mahley et al, 1984).

The apo C proteins are synthesized principally in the liver, with a minor portion being produced in the intestine (Wu and Windmueller, 1979; Krause et al, 1981).

Apolipoprotein C_1 is the smallest of the C apolipoproteins, consists of only 57 amino acids (Jackson et al, 1974), and has a molecular weight of 6613. The plasma concentration of apo C_1 is approximately 6 mg/100 ml (Curry et al, 1981). In addition to its ability to bind lipid (Segrest et al, 1974) apo C_1 has been

shown to activate LCAT (Soutar et al, 1975), although not as efficiently as apo A_1.

Human apo C_2 is present in plasma at a concentration of between 3-5 mg/100 ml (Nestel and Fidge, 1982). It contains 79 amino acids and has a molecular weight of 8826 (Jackson and Holdsworth, 1986). Apo C_2 is able to bind phospholipid (Sparrow and Gotto, 1982), and activate LCAT (Jonas et al, 1984). Its main function, however is to activate the enzyme lipoprotein lipase (LPL) (LaRosa et al, 1970; Havel et al, 1970), which catalyses the hydrolysis of triglycerides in chylomicrons and VLDL. The physiological importance of apo C_2 in activating LPL has been established by the findings of patients with inherited apo C_2 deficiency, who are severely hypertriglyceridemic (Type I phenotype) and have functional LPL deficiency (Breckenridge et al, 1978).

Human apo C_3 is the most abundant of the C apolipoproteins, and has a plasma concentration of approximately 12 mg/100 ml (Nestel and Fidge, 1982). It consists of a single polypeptide chain of 79 amino acids (Jackson and Holdsworth, 1986) and has a molecular weight of 8746 (Brewer et al, 1974). Apo C_3 is a glycoprotein containing both galactose and galactosamine (Vaith et al, 1978), and either 0,1, or 2 mol of sialic acid (Brewer et al, 1974). The resultant isoproteins recognizable by isoelectric focusing are designated C_{3-0}, C_{3-1}, and C_{3-2}. It has been suggested that the presence of apo C_3 may modulate the uptake and metabolism of triglyceride-rich lipoproteins (Shelburne et al, 1980; Windler et al, 1980). In addition patients with both apo A_1 and apo C_3 deficiency have been shown to have low plasma triglyceride levels, and a rapid conversion of VLDL to LDL (Ginsberg et al, 1986; Norum et al, 1982). In vitro lipolysis of their VLDL was inhibited by added apo C_3. Wang et al (1985) have suggested that apo C_3 may act to reduce or block the activity of LPL.

1.5.4. Apolipoprotein A_1, A_2, and A_4

The A apolipoproteins are represented by three proteins, A_1, A_2, and A_4. Human apo A_1 and A_2 are mainly associated with HDL while apo A_4 is found mainly in the d. 1.21 g/ml infranate (Weisgraber et al, 1978). High density lipoprotein levels are inversely correlated with susceptibility to coronary artery disease, and the same association has also been demonstrated for apo A_1 (Breslow, 1988).

Human apo A_1 and A_2 are the major protein constituents of HDL, comprising approximately 90 percent of total HDL protein mass (Schaefer et al, 1985; Schaefer and Ordovos, 1986).

Apolipoprotein A_1 is the most abundant apolipoprotein present in fasting human plasma with a concentration of between 100-160 mg/100 ml (Assmann, 1982). Apo A_1, in addition to being a component of HDL is present on chylomicrons but is rarely found on chylomicron remnants, VLDL or their remnants, or LDL (Mahley et al, 1984). Apo A_1 is a protein of 243 amino acids with a molecular weight of 28,000 (Baker et al, 1974; Brewer et al, 1978). It is synthesized, principally in the liver and intestine, as preproprotein A_1. The pre and propeptides are removed co-translationally and after secretion from the liver respectively (Gordon et al, 1982; Law et al, 1983; Zannis et al, 1983). Six apo A_1 isoforms are found in plasma: isoproteins 2 and 3 are the proapo A_1 forms and constitute only 2 percent of circulating apo A_1, while isoproteins 4,5, and 6 are the lipid binding forms (Zannis et al, 1980; Ghiselli et al, 1983). Apo A_1 has been shown to bind lipid although its major function is to activate LCAT (Fielding et al, 1972). In vitro there is an absolute requirement for apo A_1 for LCAT activity.

Apolipoprotein A_2 is the second most abundant apolipoprotein present in HDL, has a plasma concentration of approximately 40 mg/100 ml (Assmann,

1982). Like apo A_1, apo A_2 is also synthesized as a preproprotein (Schaefer and Ordovos, 1986). Apo A_2 has a molecular weight of approximately 17,400, and is a dimer consisting of identical subunits of 77 amino acids linked by a disulfide bond (Brewer et al, 1972; Lux et al, 1972).

Apolipoprotein A_2 has a much stronger affinity for HDL than apo A_1 and is able to displace it from HDL (Lagocki and Scanu, 1980). Chung et al (1979) observed that apo A_2 was able to inhibit LCAT activity because of its ability to displace apo A_1. In addition apo A_2 has been shown to increase hepatic triglyceride lipase (HTG) activity in vitro by threefold (Jahn et al, 1981).

Apolipoprotein A_4 is an immunologically distinct apolipoprotein with a molecular weight of approximately 44,500 (Beisiegel and Utermann, 1979; Weisgraber et al, 1978). About 10-13 percent of chylomicron apoprotein and 24-30 percent of intestinal VLDL apolipoprotein is apo A_4 (Green et al, 1980). Most apo A_4 is synthesized in the intestine (Bisgaier and Glickman, 1983) and is transported by TRL's to the plasma. Its plasma concentration is approximately 16 mg/100 ml although fat feeding increase levels (Green et al, 1980). Apo A_4 is displaced by apo A_1, apo A_2, and apo C during lipolysis of TRL (Lefevre et al, 1986). The function of apo A_4 is a unknown, however it has been shown to activate LCAT (Steinmetz and Utermann, 1983) and may act as a ligand for a high-affinity hepatic receptor (Ghiselli et al, 1986).

1.5.5. Apolipoprotein D

Apolipoprotein D, a glycoprotein found predominantly in HDL, was originally referred to as "thin-line" polypeptide (Alaupovic et al, 1972; Kostner, 1974). Apo D is also found in trace quantities in all other lipoprotein classes. It has a molecular weight in the range of 32,000-34,000 (Chajek et al,

1978; Albers et al, 1981). Apo D is approximately 65-75 percent protein and 25-35 percent lipid, with phospholipid and cholesteryl ester being the main lipid components (McConathy and Alaupovic, 1976). Apo D is synthesized in most tissues including adrenal, kidney, liver, small intestine, and spleen (Breslow, 1988).

The functional role of apo D remains unclear however Kostner (1974) suggested that it may activate LCAT, and also play a role as a transfer protein in the movement of cholesteryl ester from HDL to VLDL and of triglyceride from VLDL and LDL to HDL (Chajek and Fielding, 1978). Subsequent studies (Morton and Zilversmit, 1982) however found that antisera to apo D did not remove cholesteryl ester transfer activity and that apo D did not co-purify with cholesteryl ester transfer activity.

1.5.6. Apolipoprotein F

Apolipoprotein F is an acidic apolipoprotein with a molecular weight of 28,000 found in HDL (Olofsson et al, 1977). Its plasma concentration is approximately 3 mg/100 ml with the major fraction found in HDL (75-80%) followed by LDL (15-20%) and traces in VLDL and VHDL (d > 1.21 g/ml) (McConathy and Alaupovic, 1986). The function of apo F is unknown.

1.5.7. Apolipoprotein G

Apolipoprotein G can be isolated from VHDL (d > 1.21 g/ml) by column chromatography (McConathy and Alaupovic, 1986). It has molecular weight of 72,000. The presence or function of this apolipoprotein in lipoproteins has not been established.

1.5.8. Apolipoprotein H

Apolipoprotein H is distributed throughout the different lipoprotein classes, although 75 percent is in the d > 1.21 g/ml density. Apo H has a mobility similar to that of apo E (Polz et al, 1981) and has a molecular weight of 54,000. Its plasma concentration is in the range of 16-30 mg/100 ml (Polz and Kostner, 1979). Nakaya et al (1980) have demonstrated that apo H may modulate the activity of LPL, and that the presence of apo H increases LPL activity when apo C_2 is present. More recent studies however have failed to demonstrate the apo H has any activating effect on heart lipoprotein lipase in the presence of apo C_2 (Borensztajn et al, 1986).

1.5.9. Apolipoprotein SAA

Apolipoprotein serum amyloid A (Apo SAA) is an acute-phase plasma protein which increases in concentration, from trace levels, in a variety of disease or stress states (Rosenthal and Franklin, 1975). Apo SAA circulates in the plasma as an apolipoprotein of HDL (Benditt and Eriksen, 1977). Two isotypes of apo SAA occur, apo SAA_1 and apo SAA_2, which are different at the N-terminus of the amino acid sequence. The molecular weights of apo SAA_1 and apo SAA_2 are 11,640 and 11,840 respectively (Bausserman et al, 1984). The function of apo SAA is unclear.

1.6. Lipoprotein synthesis and secretion

1.6.1. Triglyceride-rich lipoproteins

The intestine and the liver of all species, including human, are the major sites of apolipoprotein synthesis and secretion of triglyceride-rich lipoproteins (TRL) (Jackson et al, 1976; Bisgaier and Glickman, 1983). The

synthesis of TRL occurs within the intracellular membrane compartment of intestinal enterocytes and liver hepatocytes (Havel et al, 1980).

1.6.1.1. Chylomicrons

The fatty acids and 2-monoglycerol precursors of chylomicron triglyceride are taken up by the enterocyte after being transported to the cells by bile salt micelles (Westergaard and Dietschy, 1976). Subsequently the monoglycerides are re-esterified to triglyceride in the smooth endoplasmic reticulum (SER). The synthesis of triglyceride appears to occur independently of the glyceraldehyde 3-phosphate pathway, the predominant pathway for synthesis of triglyceride in the liver (Bell et al, 1981). As a consequence of little or no de novo synthesis of triglyceride in the enterocyte chylomicron triglycerides closely resemble those of dietary fat (Zilversmit, 1965). Unlike triglyceride the source of phospholipids for chylomicrons originates in existing pools of mucosal phospholipid (Ardivson and Nilsson, 1972). Dietary cholesterol is also incorporated into chylomicrons, mainly as cholesteryl esters synthesized by ACAT within the absorptive cells (Norum et al, 1983).

The non-polar esters of long-chain fatty acids are synthesized as are the phospholipids in the SER. From here the lipids are transported into the cisternal space (Higgins and Hutson, 1984). At this stage there is no association between lipid and apolipoprotein (Christiansen et al, 1983). Apolipoproteins are synthesized on attached ribosomes of the rough endoplasmic reticulum (RER). Intestinal cells synthesize apo B_{48}, apo A_1, apo A_4 and C apoproteins and perhaps apo E but only in minor quantities (Alexander et al, 1972; Marsh and Sparks, 1979; Windmueller and Wu, 1981; Imaizumi et al, 1978).

Proteins and lipid come together at the point where the RER and SER come into contact (Alexander et al, 1976; Claude, 1970). The nascent particle is

then formed as the lipid and protein are transported towards the Golgi apparatus (Bell-Quint and Forte, 1981). Upon reaching the Golgi, more phospholipid (Janero et al, 1984) and cholesterol may be added and the nascent particles are concentrated within secretory vesicles (Higgins and Hutson, 1984). These vesicles then appear to bud off from the distal end of the Golgi and fuse with the basolateral membrane of the cell, from where they are secreted into the lymph (Bisgaier and Glickman, 1983; Miller and Small, 1987).

The secretion of chylomicron particles is however dependent upon the synthesis and secretion of apolipoproteins, apo B in particular (Glickman et al, 1986). The administration of cyclohexamide, an inhibitor of protein synthesis, blocks the secretion of nascent particles. In the genetic disorder of abetalipoproteinemia, were there is a failure of both liver and intestine to incorporate apo B into lipoproteins, the result is an accumulation of triglyceride droplets in hepatocytes and enterocytes (Bisgaier and Glickman, 1983).

1.6.1.2. Very low density lipoproteins

The synthesis and secretion of VLDL is similar to that of chylomicrons, however there are some differences. VLDL triglyceride, unlike that of chylomicrons, is synthesised via the glyceraldehyde 3-phosphate pathway. The fatty acids which are incorporated into VLDL lipids are derived from multiple sources, namely de novo synthesis from acetyl-CoA units produced by carbohydrate utilization, plasma free fatty acids, and from hydrolysis of lipids transported to the liver by remnant lipoprotein particles (Hamilton et al, 1967; Wilcox et al, 1975). Cholesterol which is incorporated into nascent VLDL maybe derived from either plasma or de novo synthesis. Due to the low levels of ACAT activity in hepatic cells most cholesterol in nascent VLDL is not esterified

(Erickson and Cooper, 1980), however the proportion increases upon cholesterol feeding and increased ACAT activity (Dolphin, 1981; Guo et al, 1982).

The progression of lipid and apoproteins towards the Golgi apparatus in hepatic cells is the same as that in the enterocyte. From the Golgi apparatus secretory vesicles bud off, migrate to, and fuse with the membranes at the sinusoidal front, from where they have access to the plasma via the sieve-plate fenestrae of the sinusoidal membrane (Alexander et al, 1972).

Apo B is essential not only for chylomicron synthesis but also for the assembly of VLDL which are secreted from hepatic cells. Unlike intestinal cells which are able to produce both apo B_{48} and B_{100}, human hepatic cells produce only apo B_{100} (Edge et al, 1985). In the rat and mouse however, hepatic cells produce comparable amounts of both apo B_{48} and apo B_{100} (Wu and Windmueller, 1979; Sparks and Marsh, 1981).

Subcellular fractionation studies have demonstrated a marked size and compositional heterogeneity of the Golgi lipoprotein population (Dolphin, 1981; Howell and Palade, 1982). It has been assumed that these particles indicate immature precursors of VLDL that have yet to acquire all of their constituents (Howell and Palade, 1982) however this may help to explain the heterogeneous nature of the VLDL population present in plasma.

1.6.2. Low density lipoproteins

Low density lipoprotein is normally formed as a result of the catabolism of VLDL to IDL and subsequently to LDL (Reardon et al, 1978; Sigurdsson et al, 1975; Ginsberg et al, 1981). In addition, in normal subjects most of the LDL apo B is derived from the catabolism of VLDL and IDL. However in the rat (Fidge and Poulis, 1978), and pig (Huff et al, 1985) much of the LDL production may

not be derived from VLDL or IDL, and may therefore be secreted directly into the plasma. This may be supported by findings of both preformed and newly secreted LDL-like particles in the perfused rat (Fainaru et al, 1977) and pig (Nakaya et al, 1977) livers.

An alternate explanation to the direct synthesis of LDL is that apo B could pass through a rapidly turning over VLDL pool (Beltz et al, 1985), which is not traced by the use of exogenous labels, and be rapidly converted to particles of both LDL size and composition. This theory may help explain the high LDL apo B production rates which are generally greater than those of VLDL in subjects with severe and inherited hypercholesterolemia (Janus et al, 1980; Soutar et al, 1977).

1.6.3. High density lipoproteins

High density lipoproteins appear to be derived from liver and intestine, and some may form from the redundant surface material of chylomicrons and VLDL. Unlike TRL, nascent HDL have not been successfully identified within subcellular compartments or isolated from Golgi-rich fractions (Gotto et al, 1986), although it has been proposed that HDL may be formed by a process similar to that of VLDL (Melin et al, 1984). Evidence to support this has come from hepatic perfusates where nascent HDL particles have been observed (Marsh, 1974; Hamilton, 1984). HDL secreted by cultured rat hepatocytes are discoidal in shape and are composed of a bilayer of phospholipids, mainly lecithin (Hamilton et al, 1976). When LCAT is active nascent HDL particles are spherical, and resemble those in plasma (Eisenberg, 1984).

High density lipoproteins may also be formed within plasma from the surface material of chylomicrons and VLDL after lipolysis of triglycerides (Nicoll et al, 1980). In vitro demonstrations have shown that following

lipolysis of TRL spherical complexes of apo A or apo C and phospholipid can be isolated. These particles resemble discoidal HDL particles and incubation with LCAT results in the formation of spherical HDL-like particles (Eisenberg, 1984).

1.7. Lipoprotein metabolism

Plasma lipoproteins are in a dynamic state in the body, being continuously degraded and replaced by newly synthesized particles. Although the liver is now recognized as the major site of terminal catabolism of the apo B-containing lipoproteins it is within the circulation that lipoproteins undergo their initial stages of catabolism. These important stages involve the exchange of apoproteins between lipoproteins and interaction with various enzymes.

1.7.1 Chylomicrons

Chylomicrons begin to appear in the plasma within 1 hour after ingestion of fat and are mostly removed from the plasma within 5-8 hours. The half life of intravenously injected thoracic duct chylomicron triglycerides in the circulation is about 5 minutes (Nestel, 1964). The catabolism of chylomicrons proceeds in a two-step process. The first step involves the action of LPL leading to the formation of triglyceride depleted remnant particles. The second step involves the receptor mediated removal of remnant particles primarily by the liver.

Upon reaching the circulation chylomicrons undergo rapid modification, some components leaving and other joining the particle. Apo B_{48} remains an integral part of the chylomicron particle, apolipoproteins A_1, A_2 and A_4 are transferred to HDL and the lipoprotein free fraction (Imaizumi et

al, 1978). Apolipoproteins C_1, C_2, C_3 and E transfer to the chylomicron particle from HDL (Havel et al, 1973) and VLDL (Blum, 1982). Chylomicron triacylglycerides are hydrolysed by LPL, an extrahepatic enzyme attached to heparan sulphate-like molecules at the endothelial surface of extrahepatic tissues (Jackson, 1983). The resulting fatty acids and glycerol are rapidly cleared from the blood (Fredrickson et al, 1958). The hydrolysis of chylomicrons is inhibited in the absence of LPL (Kompiang et al, 1976) and there is also an absolute requirement for apo C_2 which activates LPL (LaRosa et al, 1970; Fielding and Havel, 1977).

When the chylomicron is nearly depleted of triglyceride, the lipid-poor remnant is released into the plasma and circulates to the liver for uptake (Redgrave, 1970; Bergman et al, 1971). Apo C_3 appears to have a regulatory role in remnant uptake by the liver in that it may counteract the LPL activating action of apo C_2 (Breckenridge et al, 1978; Wang et al, 1985), inhibit hepatic triglyceride lipase (HTL) (Kinnunen and Ehnholm, 1976), and prevent premature removal of the partly catabolized chylomicron particle (Windler et al, 1980; Shelburne et al, 1980). The presence of the C_3 apoprotein may alter the conformation of the apo E resulting in its inability to interact with the receptor (Windler and Havel, 1985). Thus the binding of the remnant particle appears to require the loss of apo C_3. Following triglyceride hydrolysis there is a transfer of the surplus surface material to both HDL and the lipoprotein free fraction. Only apo B_{48} and apo E do not dissociate from the remnant particle (Hui et al, 1981).

Hepatic triglyceride lipase may also be involved in the catabolism of partly hydrolysed remnant particles (Jackson, 1983). Numerous reports have demonstrated its role in the metabolism of VLDL remnants (Nicoll and Lewis, 1980; Murase and Itakura, 1981; Breckenridge et al, 1982; Goldberg et al, 1982;

Nozaki et al, 1986), and is therefore possible that HTL may also play a role in the catabolism of chylomicron remnants. The inhibition of HTL has been shown to cause the appearance of apo B_{48} in LDL suggesting that HTL is essential for remnant particle catabolism (Daggy and Bensadoun, 1986).

Removal of chylomicron remnants occurs in the liver via a high-affinity receptor mediated process. The receptor involved in chylomicron uptake, the chylomicron or apo E receptor, was isolated from canine and human hepatocytes (Hui et al, 1986). More recently however, Beisiegel et al (1988) observed that this receptor was in fact an ATPase protein which bound non-specifically to apo E containing lipoproteins. Since this observation however, Herz et al (1988) have sequenced a cell surface protein of 503 kDa which contains four copies of the ligand binding domain and the epidermal growth factor-precursor homologous region of the LDL receptor. It is the extravascular domain of the LDL-receptor related protein which contains the four cysteine-rich regions similar to those which are responsible for binding apo B and apo E to the LDL receptor (Yamamoto et al, 1984). Earlier studies suggest that apo E appears to be the ligand involved in binding chylomicron remnant particles to a "receptor" (Arbeeny and Rifici, 1984), and that apo B_{48} appears to play no role in the interaction (Hui et al, 1984).

Evidence for the removal of chylomicron remnants by a receptor has come from studies in Watanabe Heritable Hyperlipidemia (WHHL) rabbits in which there is a lack of LDL receptors (Kita et al, 1981). These studies have demonstrated that although VLDL remnants and LDL accumulate (Kita et al, 1982a), chylomicron clearance is not affected (Kita et al, 1982b).

Although the clearance of particles via this apo E requiring non-LDL receptoris a saturable process (Sherill and Dietschy, 1978; Cooper et al, 1982) several studies have shown that apo B_{48}-containing particles are cleared

rapidly and more quickly than apo B_{100}-containing particles (Sparks and Marsh, 1981; Nestel et al, 1983; Stalenhoef et al, 1984). This rapid clearance is probably due to the high affinity of apo E for the receptor. The clearance of remnants is however, inversely correlated with fasting triglyceride levels (Nestel, 1964), and this has led to speculation that impaired chylomicron triglyceride clearance is as a result of competition for the action of LPL (Brunzell et al, 1973) with VLDL particles (Grundy and Mok, 1976). Lipoprotein lipase is the rate limiting enzyme of the catabolism of human VLDL (Reardon et al, 1982a; Goldberg et al, 1982).

Unlike the LDL receptor this other putative receptor is not subject to feed-back inhibition and thus allows continued hepatic uptake of chylomicron particles and their cholesterol (Arbeeny and Rifici, 1984; Arbeeny et al, 1987). The effect of this is to suppress both hepatic cholesterol synthesis and the activity of the LDL receptor (Angelin et al, 1983). Cells other than hepatocytes have also been shown to take up particles in an unregulated manner. Macrophages for instance are easily loaded with both cholesterol and triglyceride from β-VLDL through a process mediated by apo E (Nestel et al, 1985).

1.7.2. Very low density lipoproteins

This section, and the IDL and LDL sections will deal with the general biochemical events which take place as lipoprotein particles are metabolized. An account of kinetics studies undertaken in these fractions in relation to their metabolism will be discussed in Section 1.8.4.

The initial step in the metabolism of VLDL is analagous to that of chylomicrons - binding to LPL, followed by hydrolysis of triglyceride. The rate of hydrolysis occurs has been found to vary inversely with the triglyceride

content of the particle (Streja, 1979; Gotto et al, 1986). In addition to the apo C and apo E which may be attached to the VLDL upon secretion from the hepatocyte extra apo C and apo E may be transferred to VLDL from HDL. Like chylomicron metabolism the C and E apoproteins regulate the rate at which delipidation and removal take place. LPL is required for the hydrolysis of triglycerides, as is apo C_2 the activator of LPL (LaRosa et al, 1970). Inhibition of hydrolysis by apo C_3 also appears to modulate VLDL catabolism (Brown and Baginsky, 1972). The result of VLDL triglyceride hydrolysis is the generation of VLDL remnants, which unlike their chylomicron counterparts may have one of several fates. A proportion of VLDL particles may be converted to LDL (Bilheimer et al, 1972) and another removed directly from the circulation (Havel, 1982).

Removal from the plasma of partly catabolized VLDL particles occurs via interaction with hepatic receptors. This receptor mediated uptake may occur via the LDL receptor (Brown et al, 1981) or by an apo E-requiring receptor which is also on the surface of the hepatocyte. VLDL and their remnants may also leave the circulation via interaction with endothelial cells (Baker et al, 1984), arterial smooth muscle cells (Bierman et al, 1973), and macrophages (Wang-Iverson et al, 1985).

As VLDL are hydrolyzed and lose both phospholipid and C apoprotein to HDL they become cholesteryl ester enriched. This cholesteryl ester is acquired via lipid transfer protein (LTP) (Zilversmit et al, 1975; Barter et al, 1982b), from HDL synthesized in the plasma by LCAT. Lipid transfer protein is secreted from the liver and is found in association with HDL_3 (Barter et al, 1981). The same protein also promotes the exchange and transfer of triglyceride and phospholipid (Rajaram and Barter, 1980; Morton and Zilversmit, 1982). In

addition, triglyceride may be transferred to LDL where it would be hydrolysed by HTL (Decklebaum et al, 1982).

1.7.3. Intermediate density lipoproteins

As VLDL triglycerides are hydrolysed by LPL, smaller, cholesterol enriched remnants, or IDL, are formed and are subsequently converted to LDL by mechanisms which are incompletely understood (Musliner et al, 1987), or are removed from the circulation. It has been proposed that HTL hydrolyses remaining IDL-triglyceride and possibly phospholipid (Goldberg et al, 1982; Nozaki et al, 1986) on the surface of hepatocytes. Several studies have shown that reducing HTL activity leads to an accumulation of IDL-like particles in rats (Murase and Itakura, 1981), and in cynomologous monkeys (Goldberg et al, 1982).

In vitro studies have shown that IDL is the best substrate for HTL (Nicoll and Lewis, 1980) and that HTL is essential for IDL metabolism. Reardon et al (1982b) concluded that HTL did not play a rate limiting role in IDL metabolism, however more recent studies have observed an inverse correlation between HTL activity and IDL concentration in hypertriglyceridemia (Nozaki et al, 1986). In subjects with familial hepatic lipase deficiency there is an accumulation of triglyceride and phospholipid rich remnant and HDL_2 particles (Breckenridge et al, 1982)

As VLDL are hydrolysed by LPL they not only lose triglyceride, phospholipid and apo C but also apo E. While the lipid loss from VLDL remnants may be a result of HTL (Havel, 1984), loss of apo E is probably also due to a reduction in particle size (Gotto et al, 1986).

Intermediate density lipoprotein not converted to LDL may be removed from the circulation via the LDL receptor (Mahley et al, 1984). The interaction

with the receptor is mediated through the interaction of its ligands, apo E and apo B. IDL have also been shown to bind to another "receptor" although the clearance of remnants by this process is not very efficient (Kita et al, 1982b).

1.7.4. Low density lipoproteins

Normal LDL particles are cholesteryl ester enriched and are deficient of all apoproteins except apo B_{100}. In man nearly all LDL apo B normally arises from the catabolism of VLDL and IDL. Although essentially derived from VLDL, the LDL population may be heterogeneous as a result of lipid transfers between it and other lipoprotein fractions (Krauss and Burke, 1982; Oschry et al, 1985). In hypertriglyceridemia some LDL become enriched in triglyceride and cholesteryl ester depleted resulting in marked heterogeneity of the LDL fraction (Fisher, 1983; Teng et al, 1983). The lighter LDL fraction, LDL_1 (mean d. $\approx$ 1.0405 g/ml), is triglyceride rich while LDL_2 (mean d. $\approx$ 1.0480 g/ml) is triglyceride poor and cholesteryl ester rich (Teng et al, 1983).

Where LTP activity is high there is a redistribution of cholesteryl ester to larger particles in exchange for triglyceride (Decklebaum et al, 1982). Triglyceride present in LDL is presumably hydrolysed by HTL. Triglyceride-rich LDL, which also contain apo E and apo C, have been shown to be cleared rapidly from circulation in hypertriglyceridemic (Ginsberg et al, 1985) and hyperchylomicronemic (Nestel et al, 1978) subjects. This increased clearance may however be a function of increased insulin secretion in these disorders (Oppenheimer et al, 1985).

Low density lipoprotein particles are removed from the circulation via both receptor and non-receptor mediated processes (Pittman et al, 1982). Two thirds of LDL cleared from plasma each day is via the LDL receptor (Brown et al, 1981; Brown and Goldstein, 1983b). In the absence of LDL receptors a large

fraction of LDL continues to be removed be hepatic tissues (Pittman et al, 1982), presumably in part by other receptors.

In addition to uptake by the LDL receptor, LDL or a modified LDL particle, may be bound and endocytosed by the acetyl LDL receptor. LDL may be modified by malondialdehyde, released during platelet aggregation (Fogelman et al, 1980), or oxidised in the presence of endothelial cells (Henriksen et al, 1983; Morel et al, 1984; Morton et al, 1986). Such modification results in the uptake by another receptor, the scavenger receptor (Mahley and Innerarity, 1983), which transforms macrophages, in vitro, into foam cells identical to those found in atherosclerotic plaques (Brown and Goldstein, 1983). In contrast to other modifications oxidation can and most likely does occur in vivo (Morel et al, 1987; Morton et al, 1986).

In contrast to both the acetyl LDL and scavenger receptors, the activity of the LDL receptor is a function of cellular cholesterol levels. As cellular cholesterol levels increase with the uptake of cholesterol-rich remnant particles LDL receptor number are decreased (Kovanen et al, 1981; Angelin et al, 1983).

1.7.5. High density lipoproteins

High density lipoproteins are central to the regulation of lipoprotein metabolism (Nestel, 1986), and are important in their role of reverse cholesterol transport. The basis for the metabolic properties of HDL is that all lipid and apoprotein components of HDL are exchangeable (Grow and Fried, 1978; Schaefer et al, 1982). HDL acts as a reservoir for apo C_2 which is transferred to VLDL where it serves as an activator of LPL. It is eventually returned to HDL (Schaefer et al, 1978). In addition, both phospholipid and free cholesterol are transferred from triglyceride-depleted VLDL to HDL (Patsch et

al, 1978b). Return of apo C to HDL permits efficient utilization of this apoprotein and occurs in parallel with the movement of phospholipid and free cholesterol to HDL. Apo E has also been shown to transfer to HDL following VLDL delipidation; thus HDL may also act as a pool for apo E (Gordon et al, 1983). HDL however is not the only source of apo E, since newly secreted VLDL already contain apo E synthesized in the liver (Johnson et al, 1983).

The reverse cholesterol transport function of HDL results from the fact that the major HDL apoprotein, apo A_1, is a cofactor for LCAT which esterifies free cholesterol by transferring an acyl group from phospholipid (Glomset, 1968). Therefore HDL can serve as both a source for esterified cholesterol and a sink for free cholesterol and phospholipids. The mechanism for movement of free cholesterol from cells to HDL has been proposed to be either simple diffusion or receptor mediated (Ose et al, 1981; Biesbroeck et al, 1983).

HDL_3, the smaller HDL particle with a lower free cholesterol to phospholipid ratio, is a better acceptor of free cholesterol than HDL_2 (Rudel et al, 1984). As HDL_3 acquire cholesteryl ester or triglyceride from chylomicrons they are converted to HDL_2 (Eisenberg, 1984). The larger particles are then recycled to the smaller species through transfer of core lipids to VLDL or LDL or by undergoing hydrolysis via HTL (Deckelbaum et al, 1986). Lipid transfer protein is responsible for lipid transfer to and from VLDL and LDL. Cholesteryl ester and triglyceride are exchanged on a mole for mole basis (Zilversmit, 1984). This process is fundamental to the transport of cholesterol through the circulation.

The removal of HDL is via a hepatic site distinct from the LDL and chylomicron remnant receptors (Eisenberg, 1984; Rifici and Eder, 1984). Rifici and Eder (1984) observed that apo A_1 was the ligand responsible for most HDL binding to hepatic cells, although apo A_2 will also bind HDL to this receptor

site. The greater apo A_1 composition of HDL_2 probably explains its increased rate of removal compared to HDL_3 (Fidge et al, 1980; Hazzard et al, 1984). Specific sites for both HDL apo A_1 and A_2 binding have been observed by Fidge and Nestel (1985).

High density lipoprotein binding studies in rats have demonstrated that the removal of cholesteryl ester from HDL is greater than that of HDL apoproteins (Glass et al, 1983), suggesting that cholesteryl ester may be delivered to the liver without degradation of the HDL particle. It would appear that in some cells HDL binding is not followed by internalization and that in some cells internalization is not followed by apoprotein degradation (Pittman and Steinberg, 1984; Kagami et al, 1984). Recent studies by Pittman et al (1987) have observed that removal of core lipids from HDL does not require either endocytosis or apoprotein-mediated binding to a receptor. Regardless of the mechanism of the interaction of HDL and hepatocytes, the data suggest that recycling of HDL particles occurs. HDL_3 obtains free cholesterol from various tissues and are thus transformed into cholesteryl ester-enriched HDL_2. HDL_2 interact with VLDL, IDL, and LDL resulting in an exchange of lipids. Triglyceride-rich HDL_2 are hydrolysed to HDL_3 and so the cycle continues. This series of events may explain the antiatherogenic role of HDL.

1.8. Kinetic studies of apolipoprotein B-containing lipoproteins

Studies carried out during the last thirty years have demonstrated that all plasma lipoproteins are metabolically related. Volwiler et al (1955) and Gitlin et al (1958) were the first to report that following injection of labelled VLDL into humans radioactivity appeared in LDL. They postulated from these experiments that part of the LDL fraction was derived from VLDL. At this time however little was understood about lipoprotein composition, function, and

metabolism and as a consequence the value of their studies was not appreciated until the early 1970's when epidemiological studies demonstrated the association between plasma lipid levels and atherosclerosis. Since then however numerous kinetic studies have been undertaken to provide a better understanding of lipoprotein metabolism.

The overall catabolism of VLDL, IDL, and LDL can be studied in vivo by monitoring the kinetics of the B apolipoprotein, the only apolipoprotein that remains with the particle throughout its life. In addition however simultaneous apo B and triglyceride kinetic studies provide a wealth of extra information about lipoprotein metabolism. The subsequent simultaneous analysis of the kinetics of different lipoprotein moieties provides additional information, not available from studies where only a single moiety is traced. Further information can be gained by the further separation of lipoprotein fractions into subfractions in an attempt to isolate metabolically discrete populations of particles.

1.8.1. Approaches to VLDL kinetics

Of the six hyperlipoproteinemic phenotypes all but two, Type I and IIA, presents high levels of plasma VLDL triglyceride. Undoubtedly the molecular basis for the expansion of the VLDL fraction will be shown to be diverse. One approach to the understanding of these disorders has been through the use of the kinetic analysis of lipoprotein metabolism. Previous studies have demonstrated that both increased production and impaired removal of lipoproteins are implicated in these disorders (Grundy, 1984). Results derived from such studies however depend upon the methods of analysis employed and the assumptions which are used in these analyses. Several multi-compartmental models have been published recently, based upon the nature of

the distribution and removal of labelled VLDL triglycerides, and or VLDL apo B. These models have provided important insights into the regulation of VLDL production and removal and represent a physiological counterpart to the knowledge generated at the cellular and molecular levels.

Three approaches have been used in the study of VLDL turnover: the injection of radiolabelled free fatty acids, injection of radiolabelled glycerol, and injection of radiolabelled VLDL, or the protein precursors of VLDL. The first two methods use precursors of triglyceride while the third uses either exogenously or endogenously labelled VLDL. The injection of labelled precursors offers the advantage that, in addition to generating kinetic data for the product, VLDL, it provides information about the precursors of VLDL lipid. In addition, the use of labelled precursors results in the labelling of products in proportion to their rates of synthesis and turnover. Analysis of both precursor and product data are often complex and require sophisticated mathematical analyses together with a host of assumptions about the system (Zech et al, 1979). A simpler approach is to analyze the kinetics of plasma VLDL alone and ignore the kinetics of the precursors (Farquhar et al, 1965) i.e. regarded as a bolus. The assumption behind this approach, as it applies to triglyceride kinetics, is that the catabolism of VLDL triglyceride is the rate limiting step in the turnover of triglyceride. This assumption, which is critical to the analysis of the data gathered in this project, has been disputed by Zech et al (1979) and by Baker (1984).

The use of radiolabelled VLDL as the tracer avoids some of the problems of analyzing precursor kinetics. Studies using radiolabelled VLDL-triglyceride harvested from a donor are however limited by low rates of incorporation of label into lipid, resulting in small doses for reinjection. This procedure has therefore been restricted to animal use where larger doses of labelled

precursor can be given. The exogenous labelling of VLDL overcomes the problem of the above methods. Exogenous tracers however do not yield any information about the precursors of the tracer and certain assumptions with respect to the integrity of the VLDL tracer must be made (Packard and Shepherd, 1985). In addition, exogenous labelling results in the labelling of particle sub-populations in proportion to their mass, not in proportion to their rate of synthesis. It is therefore possible that in some instances a tracer plasma disappearance curve may dominated by the kinetics of a slowly turning over population of particles, and that the kinetics of the more metabolically active fraction be obscured. The analysis of data gathered from the use of such tracers is complex and while some investigators have developed complicated models (Phair et al, 1976; Berman et al, 1978; Baker et al, 1983; Packard et al, 1984; Beltz et al, 1985) of VLDL metabolism some have used simpler methods such as monoexponential analysis of data or the area under the specific radioactivity curve. Inherant in the use of simple analytical procedures is the assumption that the VLDL pool represents a homogeneous group of particles. The results of this project and findings by others (Reardon et al, 1978; Reardon and Steiner, 1982; Nestel et al, 1983; Packard et al, 1984) suggest that this assumption is not correct.

Methods of exogenous labelling and reinjection of VLDL have been used extensively by many investigators (Kesaniemi et al, 1982). Recent studies by Beltz et al (1985) however have demonstrated that metabolic parameters derived using exogenous compared to endogenous labelling methods are different. In the three subjects they report VLDL apo B production and FCR were greater, while VLDL to LDL apo B conversion was lower when estimated using the endogenously labelled VLDL apo B kinetics. These findings alone suggest that the VLDL fraction is more kinetically heterogeneous than the

exogenously labelled VLDL data would show. Exogenous labelling will only label particle populations present in the sample to be labelled. If there are VLDL populations which turn over rapidly then these particles may not be labelled, and hence cannot be traced when the labelled material is reinjected.

In an attempt to understand lipoprotein metabolism researchers have subfractionated the entire lipoprotein spectrum into distinct groups, i.e. VLDL, IDL, etc. Such separations have been based upon physical and compositional properties rather than physiological function. However, given the heterogeneity which appears to exist within the various lipoprotein classes lipoproteins should be compared on the basis of their metabolic or functional characteristics. To date however, most lipoprotein research has used ultracentrifugation or electrophoretic separation techniques. In order to appreciate the diverse results obtained in studies of lipoprotein kinetics it is necessary to review the methods that have been used to isolate the lipoproteins.

1.8.2. Ultracentrifugal lipoprotein isolation

Because plasma lipoproteins have lower hydrated densities relative to other plasma proteins, sequential flotation ultracentrifugation (SFU) (Havel et al, 1955) has been the principal method used for their isolation and classification. This procedure, which uses discontinuous stepwise increases in solvent density results in the isolation of particles which are less dense than the background density. Although SFU results in excellent separation of lipoproteins of density > 1.21 g/ml, it is frequently used as a preliminary separation procedures to be followed by another method. Other purification techniques which can further fractionate SFU fractions may be based upon particle size, charge, protein type or particle density.

Two problems may however be associated with the SFU procedure, the first being the potential to lose some of the apolipoproteins, especially apo A_1, C and E. Loss of surface proteins may result in aggregation of TRL (Lossow et al, 1969). Secondly, SFU separates according to density only, and therefore it must be recognized that a fraction isolated using this technique may be heterogeneous with respect to composition, size, charge and metabolic properties.

Two other lipoprotein isolation procedures, equilibrium density gradient (DGU) and single vertical spin density gradient ultracentrifugation (SVS) are currently used for preparative isolation.

Both procedures use step density gradients through which lipoproteins move until they come into equilibrium with the solvent density. One spin will isolate all lipoprotein fractions and will provide a visual display of the continuous lipoprotein distribution. The main difference between DGU and SVS is the spinning time required to achieve separation. SVS uses a vertical spinning rotor and will separate lipoproteins in approximately one-tenth the time required by DGU. Although both these procedures are more involved than SFU these isolation methods are less likely to result in dissociation of apolipoproteins from lipoprotein particles (Chung et al, 1986) probably due to the decreased centrifugation time compared to SFU.

Packard et al (1984) used both SFU and a modified DGU procedure, cummulative flotation (Lindgren et al, 1972), in order to isolate lipoproteins in their metabolic studies. They observed that SFU failed to clearly separate IDL from LDL resulting in an apparently increased conversion of VLDL to LDL. They concluded from these studies that neither separation procedure produced metabolically homogeneous lipoprotein fractions. These observations suggest

that great care must be taken when comparing findings based upon different isolation procedures.

1.8.3. Affinity chromatography

1.8.3.1. Heparin-Sepharose affinity chromatography

In 1972 Iverius (1972) demonstrated the ability of heparin, bound to Sepharose, to bind β-lipoproteins at low salt concentration. Subsequently both apo B and apo E were shown to interact with heparin, through a process which can be counteracted by neutralization of the positive charge of arginine and lysine residues (Mahley et al, 1979). As other lipoproteins do not interact with heparin this technique provides a means for separating lipoproteins of similar hydrated density but different apolipoprotein content. This procedure has been used for lipoprotein studies in human (Shelburne and Quarfordt, 1977; Trezzi et al, 1983; Nestel et al, 1983; 1983; Floren, 1984) and in animal (Quarfordt et al, 1978; Skinner and Rooke, 1980; Borensztajn et al, 1985; 1986; Huff and Telford, 1984; Laplaud et al, 1987) studies.

Of the various apolipoproteins only apo B and apo E are known to interact with heparin and other proteogylcans. Ligand-blot studies of the binding of high-reactive heparin to peptides of apo E have indicated the presence of multiple heparin-binding sites (Cardin et al, 1986). This study and that of Mahley et al (1984) suggest that the heparin-binding sites may correspond to the apo E binding sites involved in interaction with the LDL receptor. It is therefore possible that the binding of these lipoproteins may be an important physiological mechanism for lipoprotein binding to endothelial surfaces or to the ground substance of the arterial wall.

Several recent apo B-kinetic studies have examined the metabolism of VLDL in heparin-bound (bound) and heparin-unbound (unbound) fractions

(Nestel et al, 1983; Huff and Telford, 1984). Both studies demonstrated that the unbound VLDL fraction had lower apo E/apo C and cholesterol/triglyceride ratios. Nestel et al (1983) also showed that the S_f 12-60 fraction contained a greater proportion of bound particles (based upon apo B protein concentration) than did the S_f 60-400 VLDL. In addition, the apo E/apo C ratios for both the bound and unbound fractions were greater in the S_f 60-400 fraction compared to the S_f 12-60 fraction.

1.8.3.2. Immunoaffinity chromatography

Another procedure, immunoaffinity chromatograpy, can be used for the separation of lipoproteins, also on the basis of their apolipoprotein content. Recent studies (Gibson et al, 1984; Yamada et al, 1986) have used this method to separate lipoproteins according to their apo E content. This method has also been applied to the separation of lipoproteins based upon the presence of other apolipoproteins (Cheung and Albers, 1982). In this study HDL particles were separated on the basis of their apo A_2 content.

Problems with immunoaffinity chromatography may arise as a result of non-specific binding to the immunoabsorbent. This can be particularly serious when separating lipoproteins on the basis of their apolipoprotein content (Gibson et al, 1985). A further problem with this technique involves the elution of particles bound to very high-affinity antisera. Removal of such lipoprotein may require the use of denaturing agents or high-salt buffers (Cautrecasas and Anfinsen, 1986) which may alter lipoprotein structure and metabolic function. A third problem with this technique, and one which also applies to heparin-Sepharose affinity chromatography, examines the availability of epitopes in the lipid-bound state. Particles rich in triglyceride appear to mask antigenic sites on certain of the apolipoproteins (Schonfeld et

al, 1979). The incomplete exposure of these epitopes may create artifacts, since selection of a particular population of particles may result. Such masking however may truly reflect the metabolic status of the lipoprotein particle, and thus the separation technique may be appropriate.

1.8.4. VLDL-IDL kinetics

This section will describe the kinetics of the apo B and triglyceride moieties of VLDL and IDL. Although the metabolism of these components is related they appear to be independent of each other (Melish et al, 1980; Steiner and Reardon, 1983).

Early VLDL-LDL studies demonstrated precursor product relationships between VLDL and LDL utilizing triglyceride kinetics (Quarfordt et al, 1971; Barter and Nestel, 1972), radiolabelled amino acids (Phair et al, 1975; Eaton et al, 1976), and radiolabelled VLDL (Eisenberg et al, 1972; 1973; Bilheimer et al, 1972; Phair et al, 1976; Sigurdsson et al, 1975; 1976a; 1976b). These studies concluded that the metabolism of VLDL to LDL proceeded in a stepwise manner corresponding to the delipidation of VLDL and IDL particles.

1.8.4.1. Triglyceride kinetics

Many VLDL triglyceride kinetic studies have been undertaken using labelled precursors of triglyceride, such as glycerol or palmitate which are incorporated endogenously into the triglyceride moiety. In 1972, Barter and Nestel demonstrated a precursor-product relationship between VLDL and LDL triglyceride and also proposed the heterogeneous nature of particles with in the VLDL fraction. Earlier studies had described the VLDL triglyceride pool as a single compartment (Baker and Schotz, 1964; Farquhar et al, 1965; Eaton et al, 1969; Quarfordt et al, 1970) and it was not until tracer data of VLDL apo B

became available (Phair et al, 1975) that the need for a delipidation pathway, a chain of compartments along which there was progressive hydrolysis of triglyceride, became obvious. This pathway was incorporated into the VLDL-triglyceride model of Zech et al (1979) (Figure 1). A major criticism of this model is that the turnover rates of the four compartments in the delipidation pathway, like those in the apo B model, are constrained to be equal, and in addition, the proportion of triglyceride hydrolysed from each compartment is the same for all compartments.

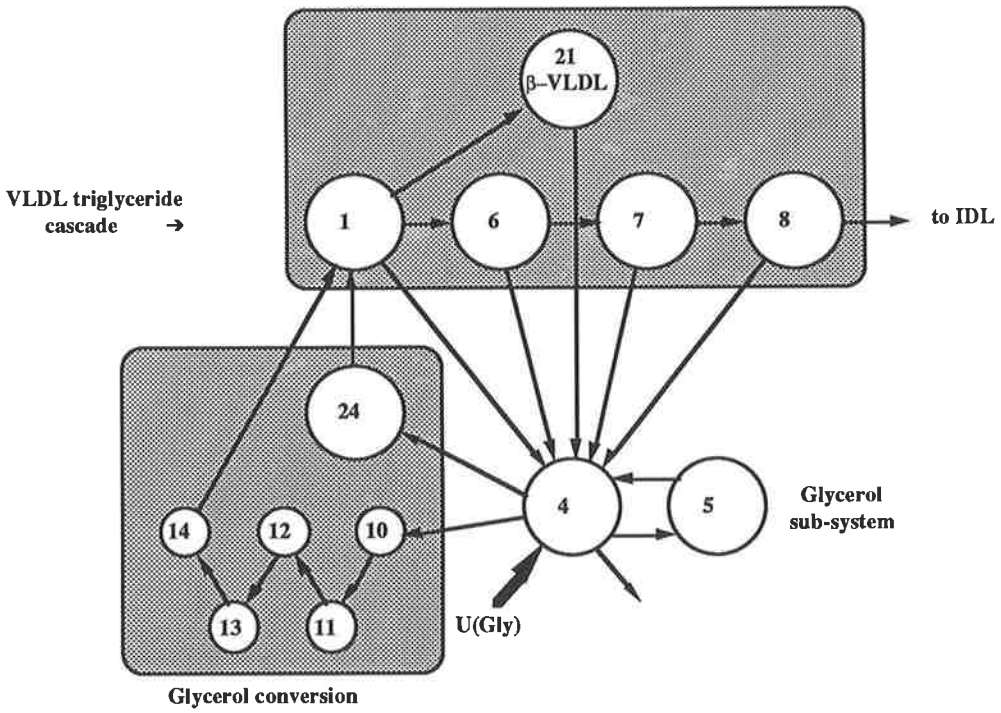


Figure 1. Modified version of the Zech et al (1979) VLDL triglyceride model. This model incorporates a glycerol subsystem (compartments 4 and 5), a glycerol conversion system (Compartments 10-14 and 24), and the VLDL triglyceride system. The apo B model of Berman et al (1978) had highlighted the need for a delipidation cascade which was subsequently incorporated into this model (Compartments 1,6,7, and 8). The pathways from the compartments in the cascade to compartment 4 represent the transfer of glycerol from VLDL to the plasma glycerol pool, a process which occurs during triglyceride hydrolysis. Compartments 10-14 inclusive represent the delay in the appearance of VLDL triglyceride often observed after glycerol is injected.

Such constraints imply that triglyceride hydrolysis proceeds at a constant rate. Studies using rabbits as a model have however shown that the rate of hydrolysis varies inversely with the triglyceride content of VLDL particles (Streja, 1979). Therefore the fraction of triglyceride hydrolysed and probably the turnover rate of each compartment in the chain should be independently determined by the data. That these parameters were constrained indicates that insufficient information was available to determine such values with confidence. To resolve such a problem more data, apo B turnover data or triglyceride data derived from multiple VLDL fractions should be collected to provide more information about the system for the model.

Following the injection of labelled glycerol, very low density lipoprotein-triglyceride specific radioactivity curves have been descibed as having four phases (Figure 2): an early rising phase, a plateau at the peak, a rapidly decaying phase, and a slowly decaying phase (Zech et al, 1979). While the plateau at the peak of the VLDL-triglyceride specific radioactivity curve can be explained by the delipidation cascade, the analysis and physiological interpretation of the early and later phases (1 and 3) of the curve is the subject of much debate (Baker, 1984).

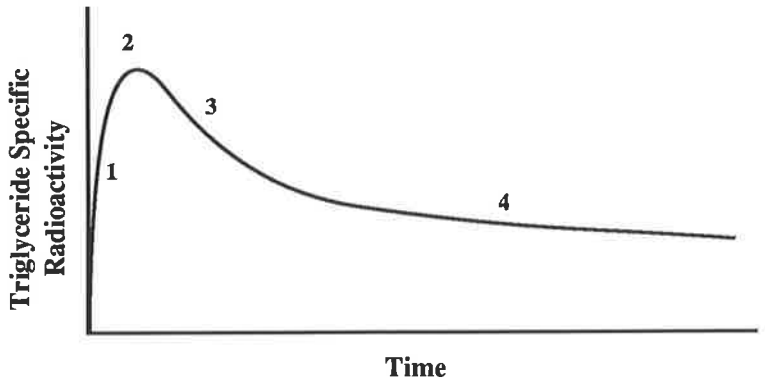


Figure 2. Typical VLDL triglyceride specific radioactivity curve after injection of labelled glycerol. Generally four phases are identified: an early rapidly rising phase (1), a plateau at the peak (2), a rapidly decaying phase (3), and a slowly decaying tail (4).

The conversion of glycerol or free fatty acid to plasma VLDL triglyceride is a complex multi-step process. Initial studies of triglyceride metabolism used labelled free fatty acids as triglyceride precursors (Carlson, 1960; Havel, 1961; Freidberg et al, 1961). In more recent studies however labelled glycerol has been used as the precursor for VLDL triglyceride (Farquhar et al, 1965; Reaven et al, 1965). Critical to the model proposed by Farquhar et al (1965) was the assumption that the turnover of liver triglyceride precursors was greater than that of the plasma VLDL triglyceride. Quarfordt et al (1970) however showed that liver triglyceride precursors and plasma VLDL triglyceride turnover rates in man were similar, and yet, in other studies where VLDL triglyceride has been reinjected in man (Havel and Kane, 1975), and in animals (Laurell, 1959; Havel et al, 1962; Baker and Schotz, 1964; Gross et al, 1967; Lipkin et al, 1978; Hannan et al, 1980) plasma triglyceride turn over is more rapid than that in liver (Figure 3). Malmendier and Berman (1978) also observed similar findings when they compared the decay curve of reinjected IDL triglyceride with the initial fall of the specific radioactivity curve of IDL triglyceride after labelled palmitate. If these observations are valid then the interpretation of a human VLDL triglyceride specific radioactivity curve, after glycerol, must be different to that made by Farquhar et al (1965). In non-human studies the initial rise to the triglyceride specific radioactivity curve represents the turnover rate of VLDL triglyceride while the falling slope must represent either liver triglyceride turnover or some other slowly turning over pool of triglyceride. Although predominantly derived from animal studies, observations that the turnover rate of the plasma VLDL triglyceride pool is greater than that of the liver suggest that triglyceride flux, and hence production, from the liver and into VLDL may be grossly underestimated.

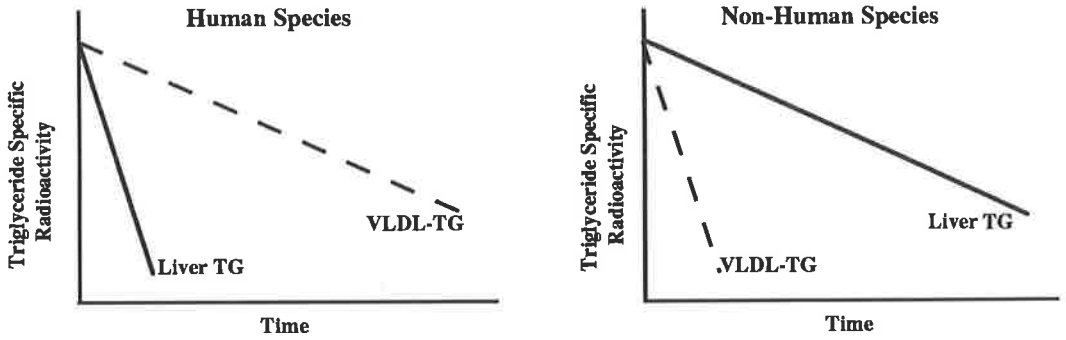


Figure 3. This figure illustrates the differences observed between rates of liver and VLDL triglyceride turnover in human and non-human species. Human studies have shown that the turnover rate of VLDL triglyceride is slower than that of liver triglyceride. This has been confirmed in only a few studies where the falling slope of the reinjected VLDL triglyceride specific radioactivity curve was the same as that observed for VLDL triglyceride after labelled glycerol. The rapid rise of the triglyceride specific radioactivity curve after glycerol was therefore attributed to the more rapidly turning over liver triglyceride pool. In non-human species however, where labelled VLDL triglyceride has been reinjected several studies have demonstrated that VLDL triglyceride turnover is rapid and faster than that of liver triglyceride.

In developing their VLDL triglyceride model, Zech et al (1979) have assumed that the rate limiting step in the turnover of triglyceride is in the plasma compartment. They have however recognized the need for a slowly turning over compartment within the liver which produces the tail of the VLDL triglyceride specific radioactivity curve. Melish et al (1980) developed a simpler model for VLDL triglyceride although their model was based upon similar assumptions to those used by Zech et al (1979).

Several early studies had demonstrated higher triglyceride specific radioactivities in the small-VLDL fraction (S_f 20-60) than that measured in the large-VLDL fraction (S_f 100-400) (Streja et al, 1977; Steiner and Ilse, 1981). Similar observations were also made in the simultaneous apo B and triglyceride studies of Steiner and Reardon (1983). These studies also demonstrated the direct input of triglyceride into the S_f 12-60 fraction. The conclusion to be drawn from these and apo B studies is that entry and exit of

both apo B and triglyceride can occur at any stage within the S_f 12-400 fraction, and probably also from within the LDL fraction.

Collectively these studies demonstrate that not all small-VLDL triglyceride is derived from large VLDL. That the triglyceride in small VLDL have a higher specific radioactivity than large VLDL may be explained by the secretion of nascent triglyceride from the liver directly into the circulation. An alternative may be that as large VLDL are synthesized they exchange lipids with intracellular lipid droplets resulting in a reduced VLDL-triglyceride specific radioactivity (Chao et al, 1986). Smaller VLDL particles would however exchange lipid to a lesser extent, and as consequence would have higher specific radioactivities.

1.8.4.2. Apolipoprotein B kinetics

Early approaches to VLDL apo B metabolism restricted particles to the vascular compartments from which VLDL was cleared by a first order process (Sigurdsson et al, 1976b; Packard et al, 1980). An extension of this model was devised to account for the bi-exponential nature of the VLDL apo B curve in hypertriglyceridemic subjects. Reardon et al (1978) proposed that this may represent the movement of VLDL particles between intravascular and extravascular compartments. They did however also suggest, as did others (Fisher et al, 1980; Packard et al, 1980), that the VLDL apo B curve may represent a heterogeneous collection of particles, each with its own metabolic characteristics. The decay curve of reinjected labelled lipoprotein apo B is therefore viewed as a composite in which catabolism is occurring simultaneously from two or more pools, each at its own rate (Packard et al, 1980; 1984). These concepts have been used in the development of compartmental models to account for apo B metabolism in both normolipidemic

and hyperlipoproteinemic subjects (Phair et al, 1976; Berman et al, 1978; Packard et al, 1984; Beltz et al, 1985).

Simultaneous studies of VLDL and LDL turnover by Reardon et al (1978) in hypertriglyceridemic subjects showed that not all VLDL was converted to LDL. Their findings observed that only 35% of VLDL apo B may be converted to LDL with the major loss of apo B occurring in the S_f 12-60 fraction. Normolipidemic subjects however appeared to convert all VLDL to LDL, although more recent findings have shown that even in normolipidemic subjects there can be considerable direct removal of particles prior to, and from the S_f 12-20 fraction (Packard et al, 1984).

In addition to the removal of particles prior to conversion to LDL the input of VLDL particles into small VLDL fraction has been demonstrated (Reardon et al, 1982; Fisher et al, 1980). Reardon et al (1982) showed that in subjects with type III hyperlipoproteinemia there was direct synthesis of VLDL particles which entered the S_f 12-60 fraction. This input would however appear to be confined to the small VLDL (S_f 20-60) fraction (Packard et al, 1984). Studies by Eaton et al (1983) demonstrated input into, not only the small VLDL, but also into IDL and LDL in hypertriglyceridemic subjects. These findings are consistent with the observation that the liver can secrete large or small VLDL, depending upon the need to transport either triglyceride or cholesterol (Schonfeld, 1970). This, together with information gathered from Golgi fractionation studies (Dolphin, 1981; Howell and Palade, 1982) suggests that the liver may be able to secrete particles throughout a wide range of the lipoprotein spectrum with different physical and metabolic characteristics, resulting in a truly heterogeneous population of lipoproteins.

That the VLDL fraction is metabolically heterogeneous has been demonstrated by reinjecting labelled VLDL fractions (Reardon et al, 1978;

Packard et al, 1984; Beltz et al, 1985). These studies have clearly highlighted the inadequacy of the Berman apo B model (Berman et al, 1978) (Figure 4) which does not make provision for either direct removal or input of apo B into the VLDL fraction, other than of large triglyceride-rich VLDL. The more recently developed model of Packard et al (1984) allows for both multiple inputs of apo B into VLDL and direct removal of particles at various stages within the VLDL. Although this demonstrates the value of multiple subfractions of VLDL it also shows that even within these narrow S_f ranges there is marked heterogeneity.

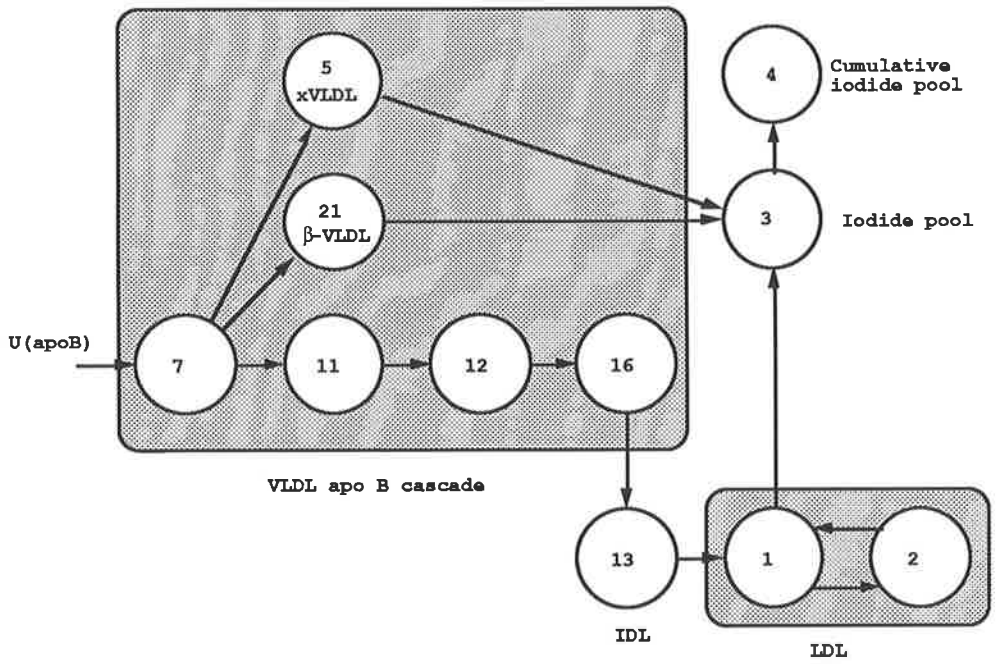


Figure 4. Apo B model proposed by Berman et al (1978) describing the metabolism of apo B through VLDL, IDL, and LDL fractions. This model does not allow for direct input of apo B into any lipoprotein fraction other than in Type III patients where input of apo B into IDL occurs independently of VLDL conversion. In addition this model does not permit the removal of apo B prior to conversion to IDL or LDL. As in the VLDL triglyceride model (Figure 1) the turnover rates of compartments within the cascade are equal. This suggests that within the VLDL fraction (Sf 20-400) each normal VLDL particle is metabolised at the same rate irrespective of apolipoprotein or lipid composition.

In addition, the differences observed between exogenously- and endogenously-labelled-VLDL apo B kinetics and metabolic parameters,

recently presented by Beltz et al (1985) (see Section 1.8.1), would support this conclusion.

1.8.5. LDL kinetics

Early LDL apo B studies used exogenously labelled autologous lipoproteins (Hurley and Scott, 1970; Langer et al, 1972; Sigurdsson et al, 1976). The kinetics of endogenously labelled LDL have also been studied by their incorporation of amino acids (Phair et al, 1975; Eaton et al, 1976). In only one study, that of Malmendier and Berman (1978), have the kinetics of apo B and triglyceride been studied simultaneously.

Langer et al (1972) first modelled LDL apo B using a two pool analysis commonly referred to as the Matthews analysis (Matthews, 1957). In their analysis they assumed that one compartment described intravascular and the other extravascular LDL apo B. By using only one intravascular compartment they assumed homogeneity of the LDL fraction. Malmendier and Berman (1978) however showed the need for two intravascular compartments to account for the kinetics of both apo B and triglyceride, thus demonstrating the heterogeneous nature of LDL. Earlier non-kinetic studies had also demonstrated the presence of multiple populations of particles within the LDL fraction (Adams and Schumaker, 1970; Hammond and Fisher, 1971). More recent studies have highlighted even greater heterogeneity in LDL (Krauss and Burke, 1982) and this is reflected in the most recent LDL apo B compartmental models (Foster et al, 1986; Teng et al, 1986; Zech et al, 1986).

Early kinetic studies suggested the existence of precursor-product relationships for LDL subspecies (Fisher et al, 1980; Malmendier and Berman, 1978). Zech et al (1986) have demonstrated this more recently and have produced a model which for both normal and hypercholesterolemic subjects

demonstrates the existence of pathways between LDL_1, the more bouyant, more triglyceride-rich LDL particle, and LDL_2. Their model predicts the step-wise delipidation of LDL_1 to form LDL_2. LDL_2 may then be reomved from the system or returned to the circulation as a bouyant LDL_1-like particle underscoring the physiological importance of cholesterol ester-triglyceride exchanges in the formation of dense LDL.

Early VLDL-LDL apo B studies observed that most LDL apo B was derived from the catabolism of VLDL (Eisenberg et al, 1973; Sigurdsson et al, 1975). More recent evidence however shows that in subjects with various forms of hyperlipoproteinemia LDL apo B production apparently exceeds that of VLDL (Janus et al, 1980; Eaton et al, 1983; Kissebah et al, 1984; Ginsberg et al, 1985). Kinetic studies have thus far failed to determine the source of the LDL which enters this fraction independent of VLDL catabolism. Although it has been proposed that some VLDL is secreted from the liver and is rapidly converted to LDL (Havel, 1984; Beltz et al, 1985; Huff et al, 1985). In tracer experiments such a rapidly metabolized pool of VLDL would not be isolated for exogenous labelling, and thus would not be seen to contribute radioactivity to the LDL fraction. Observations by others (Dolphin, 1981; Nestel et al, 1984) would however support the idea of LDL-size particles entering into the LDL fraction independent of any VLDL conversion. Endogenously labelled apo B kinetic studies also support the notion of direct input of LDL (Eaton et al, 1983; Phair et al, 1975).

Another explanation for direct synthesis of LDL is that when transport of lipoprotein triglyceride is low smaller hepatic particles are secreted (Nestel, 1986). This has been demonstrated by Huff et al (1985), who when they reduced cholesterol availability were able to show a reduction in direct secretion of LDL and an increase in both VLDL production and VLDL to LDL conversion.

1.9. Aims of the project

The aim of this project is to study simultaneously apo B and triglyceride metabolism in subjects with the relatively common disorder of hypertrigylceridemia. The main emphasis of this project however is on the metabolism of VLDL particles in these subjects. Discussion of the metabolism of IDL and LDL particles will however also be included.

Despite the marked heterogeneity of the disorder hypertriglyceridemia in general and of the VLDL fraction specifically several publications, notably Zech et al (1979), Fisher et al (1980), Eaton et al (1983), Packard et al (1984), and Beltz et al (1985) have described models in humans that share a number of similar characteristics and also significantly extend the earlier, simplier models.

Experiments were developed to produce kinetic data which allowed the testing of the following hypotheses: (1) that, in addition to the metabolism of VLDL1 to VLDL2, there is direct removal of VLDL1 particles (i.e. not converted to VLDL2) and the direct synthesis and secretion of smaller VLDL2 particles; (2) that the turnover of the liver triglyceride pool is rate limiting with respect to VLDL-triglyceride metabolism, and; (3) that the VLDL fraction represents a heterogeneous population of particles which can be further subfractionated to isolate physiologically distinct particles.

The aims will be addressed by using several approaches to derive the maximum amount of information from a series of lipoprotein turnover studies. Firstly, lipoproteins have been separated on their basis of their hydrated density characteristics (using ultracentrifugation) into large and small VLDL (VLDL1 S_f 60-400, VLDL2 S_f 20-60), IDL and LDL. Secondly, VLDL fractions have been further subfractionated on the basis of their ability to bind to heparin-Sepharose columns. Nestel et al (1983) have previously shown that VLDL

separated by this procedure yields particles that show precursor-product relationships. In addition, it has been suggested that separation of VLDL by this method may produce physiologically distinct particle populations (Cardin et al, 1986; Mahley et al, 1984). Thirdly, in all but one of the turnover studies the metabolism of apo B and of triglyceride have been studied simultaneously. Triglycerides were endogenously labelled with 2-^{3}H-glycerol and autologous VLDL were exogenously labelled with radioiodine (^{125}I-VLDL1, ^{131}I-VLDL2). These studies have provided comprehensive metabolic data on the triglyceride and apo B moieties of four separate VLDL species.

CHAPTER 2

METHODS

2.1. Laboratory procedures

2.1.1. Subjects

Four hypertriglyceridemic (type IV phenotype) subjects gave informed consent for the study. Their ages, body weights, heights, and plasma lipid profiles are presented in Table 5. Although all subjects present with type IV phenotype it is clear that there is considerable variation with respect to plasma triglyceride concentration between subjects. None of the subjects were being treated with drugs and in all, the hypertriglyceridemia was primary. To provide enough lipoprotein material to allow the measurement of multiple fractions of VLDL and IDL and LDL apo B and triglyceride, without having to obtain large plasma samples, only hypertriglyceridemic subjects were used in this study.

Table 5. Clinical data of subjects

Subject	Clinical State	Age	Sex	Weight	Height	Plasma Cholesterol	Plasma Triglyceride
		yr		kg	cm	mg/dl	
F	Type IV[a]	54	M	97.8	174	263.7 ± 17.3[b]	347.1 ± 39.4
K	Type IV	66	M	79.0	172	299.1 ± 21.6	870.2 ± 92.6
H	Type IV	61	M	70.5	160	229.8 ± 12.8	277.2 ± 35.4
J	Type IV	64	M	79.0	178	255.0 ± 16.1	436.0 ± 14.2

[a] According to classification of Fredrickson et al (1967).
[b] Mean ± SD

2.1.2. Chemical estimations

2.1.2.1. Protein

The protein content of lipoprotein and apolipoprotein samples was determined using the method of Lowry et al (1951). Folin-Ciocalteau reagent was obtained from BDH Chemicals (Melb., Aust.). Bovine serum albumin (Fraction V, Sigma, St Louis, U.S.A.) was used as a protein standard. Turbidity due to the presence of lipid was cleared by the addition of 100 µl 2.5% (w/v) sodium dodecyl sulfate (SDS) (Sigma, St Louis, U.S.A.) to the final mixture (Kashyap et al 1980). High salt concentrations were found to interfere with protein determinations, thus lipoproteins (IDL and LDL) were dialysed back to d. = 1.006 g/ml prior to the assay.

2.1.2.2. Lipids

Triglycerides and cholesterol were simultaneously estimated with a Technicon Auto-Analyser (AA-II) using Boehringer (Boehringer, Mannheim, U.S.A.) kits. Triglycerides were estimated by initial hydrolysis of glycerides to glycerol. The glycerol was reacted in three enzymic steps to liberate a proportional amount of NAD^+, which was measured spectrophotometrically. Cholesterol was estimated by a coupled reaction with cholesterol oxidase. The auto-analyser was calibrated with Precilip and Precilip E.L. reference sera (Boehringer, Mannheim, U.S.A.).

Lipoprotein and plasma triglycerides were also determined using the method of Neri and Frings (1973). Glycerides were saponified to glycerol, oxidised and condensed to 3,5-diacetly-1,4-di-hydrolutidine (Hantzsch Condensation) using sodium metaperiodate and acetylacetone with ammonia. This method employs the use of washed alumina (Al_2O_3) (BDH Chemicals, Melb., Aust.) (Brockman Grade I active neutral) to remove phospholipids, glycerol

and glucose from the isopropanol extract (Frings and Queen 1972). Mixtures containing zeolite, Lloyd reagent, $CuSO_4$ and $Ca(OH)_2$ have been utilized to remove phospholipids, glycerol and glucose from isopropanol extracts, however 1 g of alumina (washed with water to remove the 'fines' and dried at 100-110°C for 15-18 hrs) can be substituted for the 'zeolite' mixture. The advantages of the alumina over the zeolite mixture are numerous, the most important of these being its greater capacity to remove glycerol and glucose.

Triglycerides for specific activity determinations were estimated by extracting fatty acids using the procedure of Dole and Meinertz (1960). This method which separates fatty acids into two phases is based upon differences in the polarity of the fatty acids. Neutral fatty acids, such as glycerides, distribute predominantly in the upper non-polar (heptane) phase, while polar fatty acids remain in the lower (isopropanol/water) phase. Isopropanol and heptane were obtained from BDH Chemicals (Melb., Aust). Phospholipids, which have a tendency to hydrolyse (Hanahan 1960) may result in fatty acids moving from the lower to the upper phase. Aliquots of the upper phase should therefore, be removed quickly after formation of the two phases to prevent this fatty acid movement.

Triglycerides were extracted with Dole's solution containing isopropanol, heptane and H_2SO_4 (BDH Chemicals, Melb., Aust.) (40:10:1), (v/v/v). This was followed by the addition of heptane and water and mixed thoroughly. The upper phase was removed and dried under a stream of nitrogen. A volume of isopropanol was then added to each sample. Triglyceride concentrations were determined using the method of Neri and Frings (1973).

2.1.3. Pre-study diets

Pre-study dietary management is important to establish 'steady state' conditions and for the preparation of the tracer. VLDL isolated for exogenous labeling should be truely representative of the pool into which the tracer will later be reinjected, this is one of the basic tenets upon which tracer/tracee studies are based. It is debatable however as to whether it is possible or even correct to attempt to attain a steady state condition.

For four days prior to obtaining VLDL for iodination subjects were placed on a constant alcohol-free diet (30% energy as fat, 50% energy as carbohydrate and 20% energy as protein) aimed to reduce dietary fat particles. During this period, and over the course of the study subjects weights and plasma lipid concentrations were maintained.

2.1.4. Lipoprotein isolation

Plasma lipoproteins were isolated by sequential ultracentrifugation using the method of Havel et al (1955). For these procedures 50 Ti and 40 angle head rotors (Beckman Instruments, Palo Alto, CA) were spun in L5-65, L5-65B and L8-70 Beckman preparative ultracentrifuges (Beckman Instruments, Palo Alto, CA). Lipoproteins were separated for analysis into the following density fractions: VLDL d. < 1.006 g/ml (large VLDL S_f 60-400 (VLDL1) and small VLDL S_f 20-60) (VLDL2); IDL 1.006 < d. < 1.019 g/ml and; LDL 1.019 < d. < 1.063 g/ml. Lipoproteins of S_f > 400, if present, were removed by centrifugation at 20,000 x g for 30 minutes. Human VLDL for labelling and reinjection was isolated at S_f 60-400 and S_f 20-60. Plasma densities were adjusted with solid KBr (Ajax Chemicals, Sydney, Aust.), and salt solutions for overlaying contained NaCl (BDH Chemicals, Melb., Aust.), KBr, 1.0mM Na_2 EDTA (Ajax Chemicals, Sydney, Aust.) and were adjusted to pH 7.4 with tris(hydroxymethly)-methyl-amine)

(BDH Chemicals, Melb., Aust.). The conditions used to isolate the various lipoprotein fractions are described in Table 6. Each lipoprotein fraction was recentrifuged at its upper density, under the same conditions.

Table 6. Conditions used to isolate lipoproteins of various sizes from human plasma by ultracentrifugation.These conditions include salt density, centrifugal force and time. Most fractions may be denoted by Svedberg flotation units (10^{-13} cm/sec/dyne/g) at density = 1.063 g/ml with the exception of HDL which floats at a salt density of 1.21 g/ml. These conditions are as defined by Havel et al (1955) and Gustafson et al (1956).

Lipoprotein	Particles	Density[a]	Centrif. Force[b]	Time
	S_f	g/ml	g	hr
>	400	1.006	20,000	0.5
>	100	1.006	80,000	1.0
>	60	1.006	100,000	2.0
>	20	1.006	108,000	18.0
>	12	1.019	108,000	20.0
>	0	1.063	108,000	20.0
	HDL	1.210	108,000	24.0

[a] The infranate densities were adjusted by the addition of solutions of higher density according to the formula of Havel et al (1955). Standard density solutions were prepared by the addition of KBr to buffered saline. The densities were checked by piconometry.
[b] Average centrifugal force.

2.1.5. Lipoprotein iodination

Lipoprotein fractions were iodinated with ^{125}I or ^{131}I using the iodine-monochloride technique of McFarlane (1958) as modified for lipoprotein iodination by Bilheimer et al (1972) and Fidge and Poulis (1974). $Na^{125}I$ (1.0 mCi) and $Na^{131}I$ (1.0 mCi), carrier free, for iodination were obtained from the Radiochemical Centre, Amersham, England. Lipoproteins were iodinated at pH 10, to minimise incorporation of label into the lipid component, using 0.4M glycine (BDH Chemicals, Melb., Aust.) NaOH buffer. The amount of iodine monochloride (5 mM) added to the iodination mixture was calculated to give an

iodine/protein ratio of less than 1. A ratio close to 1 is important to minimise modification of the biological behaviour of the protein (Johnson et al, 1960), and to minimise lipid labelling. Lipoprotein bound iodine was separated from free iodine initially by gel filtration on a 0.9 * 25.0 cm column of Sephadex G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden), with 0.15M NaCl and 1.0mM Na_2 EDTA as the eluting buffer. This was followed by dialysis against numerous changes of buffered saline over a period of 4 hours.

Intramolecular distribution of radioisotope was determined in iodinated preparations. In preparations used in these studies 76-88% of the radioisotope bound to protein, with 11-20% being lipid bound and 1-4% remaining free. Precipitation of apoB from labelled VLDL doses revealed that between 25-32% of ^{125}I was in the large VLDL and 38-45% of ^{131}I was in the small VLDL.

2.1.6. Sterilization of lipoproteins for reinjection

Radioiodinated autologous lipoproteins for reinjection were passed through a 0.45μm Millipore filter (Millipore Corp., Mass., U.S.A.) after iodination. An aliquot of the lipoprotein was routinely selected for measurement of radioactivity and tested for the presence of bacterial endotoxins, the leading cause of pyrogenicity, using the Limulus kit (Mallinckrodt, St Louis). Rabbits were also used as a final test to ensure the absence of contamination.

2.1.7. Dietary procedures during studies

The nutritional state of subjects prior to and during lipoprotein turnover studies has a significant effect on the data generated and their interpretation. In general, researchers studying lipoprotein metabolism do not use similar dietary regimes. This often makes it difficult to compare the

metabolic parameters derived from different studies. In addition, it is important to maintain a steady state during the course of the study, although this is hard to achieve due to the many factors which can affect lipoprotein metabolism.

Prior to the reinjection of labelled VLDL subjects were fasted for 12 hours. During the 48-hour period of the study, the subjects who were ambulant ate very little fat (<5% energy) and a total of 85% of their required energy was derived from carbohydrate (80%) and protein (20%). This diet had been modified from that designed by Grundy et al. (1979) to minimise alimentary particles and VLDL over-production.

2.1.8. Dose administration and blood sampling

Iodinated autologous lipoproteins, 20-50 μCi ^{131}I S_f 20-60 and 40-80 μCi ^{125}I S_f 60-400, and 300 μCi 2-^{3}H glycerol were injected via an indwelling catheter placed in a forearm vein. All radioactive materials were injected as bolus doses. Potassium iodide was given daily, prior to, during and following the injection of radioiodinated lipoproteins, to prevent thyroidal uptake of radioiodine.

To permit blood sampling, an indwelling venous catheter was placed in the non-injected arm. Blood samples (15-30 ml), collected into 0.01% Na_2 EDTA tubes, were taken at frequent time intervals for the first 6 hours and then less frequently over the next 42 hours. Samples were kept at 4°C until plasma was separated by low speed centrifugation. Gentamycin sulphate (Sigma, St Louis, U.S.A.) (0.10 mg/ml) and 0.01% Na_2 EDTA was added to plasma samples which were kept at 4°C until further processed.

2.1.9. Heparin-Sepharose affinity chromatography

VLDL1 and VLDL2 were subfractionated by heparin-Sepharose affinity chromatography (Shelburne and Quarfordt, 1977) into two populations of particles; a heparin-bound (bound) and a heparin-unbound (unbound) fraction. Heparin Sepharose CL-6B (#17-0467-01) was obtained from Pharmacia (Pharmacia Fine Chemicals, Uppsala, Sweden). Heparin affinity columns (1.0 x 30.0 cm, Bio-Rad Econo-column, Bio-Rad Laboratories, Richmond) were equilibrated with 0.05 M NaCl/2 mM PO_4 pH 7.4 (starting buffer). The PO_4 solution was prepared by titrating 0.5 M $NaH_2PO_4.2H_2O$ (BDH Chemicals, Melb., Aust.) and 0.5 M $Na_2HPO_4.12H_2O$ (Ajax Chemicals, Sydney, Aust.). Samples containing between 2-3 mg of protein were dialysed against the starting buffer. The unbound fraction was eluted, with the starting buffer, at a flow rate of 20 ml/hr at 4°C, and collected in 2 ml fractions. After approximately 2 hours when the optical density of the fractions, measured at 280 nm, was zero a 0.8 M NaCl/2mM PO_4 buffer was used to elute the bound fraction. All fractions were measured at 280 nm and pooled into two groups. Measures of total protein showed recoveries generally greater than 85%. Although diluted after being separated on the column the concentration of the unbound and bound pooled samples was sufficient to permit apo B and triglyceride concentration and radioactivity determinations.

2.1.10. Lipoprotein delipidation

Preparations of lipoproteins were delipidated using the methanol, chloroform, diethyl ether (2:1:9), (v/v/v) method of Herbert et al (1973). Methanol, chloroform and diethyl ether were obtained from BDH Chemicals (Melb., Aust). At each step of the procedure samples were placed at -10°C for a period of 12 hours or more to improve delipidation efficiency and hence

protein recovery. Samples were centrifuged to pellet apolipoproteins and to enable removal of non protein phase. Some lipid rich samples required additional delipidation to separate lipid from protein. Following removal of the lipid phase protein samples were dried under a stream of nitrogen.

2.1.11. Quantification of apolipoprotein B

Apolipoprotein B for quantitation and specific activity determinations was precipitated from lipoprotein samples using a modification of the method of Egusa et al (1983). Comparison of this and the tetramethylurea (TMU) method for determining apo B concentration have shown no differences, except that the isopropanol method is simpler and less time consuming (Holmquist et al, 1975). Concentrated protein samples were diluted with saline and solutions with high salt content were dialysed against Na_2 EDTA-saline (d. 1.006 g/ml 0.1M EDTA, pH 7.4). Between 500-700 µg of protein, in about 1 ml, was mixed with an equal volume of 100% isopropanol (BDH Chemicals, Melb., Aust.). After vigorous mixing samples were incubated at room temperature overnight and centrifuged at 1000 x g for 30 minutes. The supernatant was aspirated and the pellet washed with a volume of 100% isopropanol. The pellet was recentrifuged and the supernatant removed. Samples were air dried and delipidated, using the method described in Section 2.1.10. This delipidation step was found be to essential when using lipemic samples in order to remove lipid labeled radioactivity. After delipidation samples were centrifuged, the supernatant removed, and the pellets air dried. Aliquots of 1N NaOH (Ajax Chemicals, Sydney, Aust.) were added to the pellet and the solution was incubated at 40°C until the pellet was dissolved. Protein content was determined using the procedure described in Section 2.1.2.1.

2.1.11.1. Preparation of antisera

Apo B was isolated from a washed LDL fraction (d. 1.030-1.040 g/ml) (Reardon et al 1981), and protein concentration determined. This narrow-cut of LDL was used as the standard in the assay procedure. An aliquot of the lipoprotein sample containing 1 mg of protein was mixed with 1 ml of Freund's adjuvant (Commonwealth Serum Labs., Melb., Aust.) and mixed using the double syringe technique. The antigen mixture was injected into multiple subcutaneous sites in a rabbit. After 2-3 weeks a booster injection was given. The rabbit was bled after antisera had reached a sufficiently high titer. Antisera was tested for monospecificity to apo B on immunodiffusion gels against other plasma proteins and apoproteins using the method of Ouchterlony (1958).

2.1.11.2. Immunoassays for apolipoprotein B concentration

Immunoassays for the determination of apo B concentration were carried out by the rocket immunoelectrophoretic procedure described by Reardon et al (1981). This method uses Lipase-TG (EC 3.1.1.3) (Calbiochemm Corp., La Jolla) to hydrolyse triacylglycerol in lipoprotein particles. Several workers (Schonfeld et al, 1979; Albers et al, 1980) have demonstrated that lipid in triglyceride-rich lipoproteins can mask the immunoreactivity of the B apoprotein. Due to the considerable heterogeneity in lipid composition across the lipoprotein spectrum it is important to ensure that lipid does not interfere with the measurement of the apoB. The effect of the lipase is to convert all standards and test lipoprotein particles to LDL-like particles, this ensures that the standard and test samples are immunochemically identical. This method of measuring apo B has been compared to that of Kane et al (1975) using the 1,1,3,3-tetramethylurea (TMU) procedure. Values obtained for the lipase-

incubated samples closely approximated those of the TMU method (Reardon et al, 1981).

Gels (1.0 mm in thickness) of 1% (w/v) agarose B (Pharmacia Fine Chemicals, Uppsala, Sweden) in Barbital buffer II (Bio-Rad Laboratories, Richmond), pH 8.6, and containing 1-2% (w/v) antiserum were prepared. Electrophoresis was carried out for 16 hr at a field strength of 8 V/cm. Gels were stained, with Coomasie Blue Brilliant R-250, according to Laurell (1972).

2.1.12. Specific radioactivity determinations

Apolipoprotein B and triglyceride specific radioactivity measurements were made on all lipoprotein fractions and subfractions.

2.1.12.1. Apolipoprotein B

The radioactivity of NaOH solublized apolipoprotein B was measured in a gamma scintillation counter (Packard Tri-Carb, 5500) which was standardised for simultaneous determination of ^{125}I and ^{131}I. ^{131}I crossover radioactivity which affects the apparent ^{125}I radioactivity count was determined and subtracted from ^{125}I radioactivity. All samples were counted for a minimum of 10 minutes. Radioactivity, both ^{125}I and ^{131}I, were corrected for decay back to the time of reinjection.

2.1.12.2. Triglyceride

To determine triglyceride specific radioactivities neutral lipids were extracted from samples and triglyceride concentrations were determined using the methods in Section 2.1.2.2. Aliquots of the isopropanol extract produced by this method were dried under nitrogen, redissolved in

scintillation fluid and counted (10 min) for radioactivity in a beta counter (LKB Scintillation Counter).

2.2. Kinetic analysis and modelling

Kinetic analysis and compartmental modelling are the studies of the turnover of particles and dissection of a system into distinct compartments respectively. The goal of the modelling process is to formulate a model which is a schematic for the physiological and biochemical process involved in a system.

Compartmental analysis is the mathematical theory for the behavior of compartments within a system. There are three objectives which can be achieved by using the application of compartmental analysis. The first objective is to develop a logical mathematical representation of the system being examined. Another is to develop and expand the theory of compartmental systems. The third objective of compartmental analysis consists of three phases: model specification, structural identifiability and parameter estimation. Model specification refers to the number of compartments within a model and the interconnections between compartments. Structural identifiability relates to whether the model can adequately describe the system under investigation and whether it is consistent with current ideas and views about the system. Parameter estimation is concerned with estimating the 'best' values for the model parameters (Anderson, 1983).

2.2.1. Compartmental description of a system

In addition to the field of lipoprotein metabolism, compartmental modelling has application in a variety of areas such as drug kinetics, studies of metabolic systems, analysis of ecosystems and chemical reaction kinetics.

Lipoprotein metabolism can be represented by a number of interlinked biological compartments which are involved in the metabolic process.

Compartments are described as an amount of material or group of particles which acts kinetically in a homogeneous distinct way. A compartment may not be an actual physical volume, such as a cell or organ, however the amount of some material in a physiological space, such as plasma, may be treated as a compartment. All particles in a compartment have the same probability of transition since within the compartment all particles are well mixed and considered indistinguishable by the system. Thus radiotracer introduced into a compartment will rapidly mix or interfuse with the tracee (Sheppard and Householder, 1951), so that at any time the ratio of tracer to tracee in each compartment is uniform throughout the compartment.

A compartmental system consists of one or more compartments, interconnected by pathways which permit the transition of particles between compartments. The transition from one compartment to another occurs by passing through some physical barrier or by undergoing some physical and/or chemical modification, such as the delipidation of VLDL particles. The rates of flow between compartments are called transfer rates.

When the size of compartments within a system is constant the system is said to be in a steady-state. Although the quantity of material or number of particles in a compartment is constant, there is continual turnover of particles. As particles leave a compartment they are replaced at the same rate by new particles. In the study of lipoprotein metabolism the attainment of steady-state requires good dietary management.

To determine whether a system is in steady-state tracer must be injected into the system and analysis of the relationships between compartments examined. When adding tracer to a system in steady-state, the amount injected

should be small enough so as not to alter the steady-state balance. The fact that compartments are assumed to be well mixed ensures that each compartment is a homogeneous mixture of tracer and tracee. It is also assumed that the kinetic behavior of the tracer is the same as that of the tracee in the compartment. A third assumption in steady-state is that upon entering a compartment tracer is instantaneously mixed throughout the tracee.

For the development of the mathematical theory of compartmental analysis the following relationship between tracer and tracee is expressed.

$$k = \frac{R}{Q} = \frac{r}{q} \qquad (1)$$

where k is the transfer rate constant, R and r are the transfer rates of the tracee and tracer respectively, and Q and q are the quantities of the tracee and tracer respectively in the compartment. This therefore states that for an ideal tracer the transfer rate constant for the tracer is equal to that of the tracee. From this we can mathematically define a general compartmental model. Figure 5 depicts the general two compartment model illustrating exchanges of material between compartments both in and outside the system (Brownell et al, 1968).

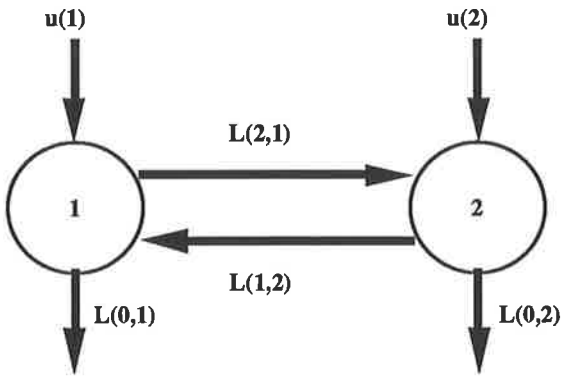


Figure 5. The general two pool model which allows for independent rates of entry and exit from each compartments and independent transfer rates between each compartment (Brownell et al, 1968).

2.2.2. The tracer equations

Radiotracers obey linear kinetics even in a non-steady state non-linear systems. In lipoprotein metabolism studies where steady-state is hard to achieve and lipoprotein metabolism is non-linear, tracers can be used to study a compartmental system. If however steady-state is achieved then the study of lipoprotein metabolism is made easier. Assuming steady-state and the assumptions stated above, tracer kinetics can be defined in terms of a number of linear subsystems (Berman and Schoenfeld, 1956). Thus for a steady-state system having n compartments, the rate of change of tracer in the i^{th} compartment is:

$$\frac{dq_i(t)}{dt} = \sum_{\substack{j=1 \\ j \neq i}}^{n} K_{ij}q_j(t) - K_{ii}q_i(t) \qquad (i = 1,2,\ldots,n) \qquad (2)$$

giving:

$$q_i(t) = \sum_{\substack{j=1 \\ j \neq i}}^{n} \frac{K_{ii}}{K_{ij}-K_{ii}} q_j(0) \left[e^{-K_{ii}t} - e^{-K_{ij}t} \right] \qquad (3)$$

where q_i is the amount of tracer in the i^{th} compartment at time t and $K_{ii}q_i(t)$ is the total amount of tracer leaving the i^{th} compartment per unit time (Berman and Schoenfeld, 1956). Under steady-state conditions all K_{ij} are constants (which depend only on certain parameters of the model) however when non steady-state or non-linear conditions apply K_{ij} may become time dependent. Applying the Laplace transformation, the system described in Equation (2) can be shown to have a multiexponential solution:

$$q_i(t) = \sum_{j=1}^{n} A_{ij} e^{-\lambda_j t} \qquad (i = 1,2,\ldots,n) \qquad (4)$$

where A_{ij} and λ_j are functions of the K_{ij} and can be derived from the experimental data. The kinetics of the tracer in a compartment system can therefore be reduced to a multiexponential model.

Estimates of the parameter values A_{ij} and λ_j can be obtained by the mathematical analysis of a plot (on semilogarithmic paper) of the quantity of tracer in a compartment as a function of time. By the conventional 'curve-peeling' technique, a minimal number of successive exponential terms $Ae^{-\lambda t}$ can be extracted from experimental data (Shipley and Clark, 1972) (Figure 6). A semilogarithmic plot of several values of q(t) vs. time can be fitted by a straight line. The slope of this line is usually most conveniently characterised by its half-life, $t_{1/2}$. The exponential constant, which in this case is also the transfer rate constant, k, is then obtained by use of the relationship:

$$k = \frac{\ln 2}{t_{1/2}} \qquad (5)$$

where ln2 is the natural logarithm of 2 (ln2 = 0.69315).

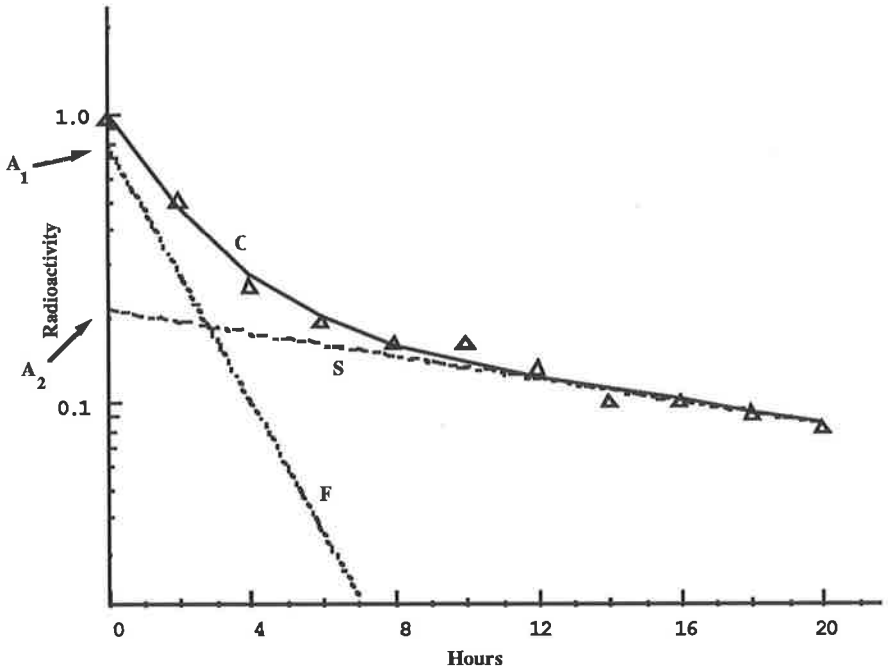


Figure 6. The solid line (C) represents the decay curve of a labelled material injected into plasma. Lines F and S represent the two exponential terms which can be extracted from the experimental data by curve peeling. The parameters A_1 and A_2 are defined by the intercept of these lines with the ordinate and the parameters λ_1 and λ_2 are defined by the slopes of these lines. The disappearance curve can therefore be represented by the multiexponential function:

$$A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$$

With some prior knowledge of the structure of the system the number of exponential terms obtained can be shown to approximate the number of compartments 'seen' at the sampling site (Berman and Schoenfeld, 1956; Smith and Mohler, 1976). In particular, for an n-compartment system sampled at the site of injection such as the blood, an estimate of (2n-1) parameters may be obtained; i.e. n λ_j's and (n-1)A_{ij}'s (Berman and Schoenfeld, 1956). If more than (2n-1) parameters are required to describe the system then it is necessary to sample at more than one site, such as an accumulating compartment. In lipoprotein studies, different lipoprotein classes are regarded as different

sampling sites, even though most lipoproteins are restricted to the vascular compartment. In addition, urine is frequently sampled for radioactivity because it acts as an accumulating compartment. As λj are the same for each compartment, each new site provides information for the estimation of an additional (n-1) parameters (A_{ij}'s) (Berman and Schoenfeld, 1956). Specific radioactivity (SA) is a function of the quantity of tracer (q(t)) and the mass of the tracee (Q). It is related to these variables in the formula below:

$$QSA(t) = q(t) \tag{6}$$

Since $K_1Q_1 = K_2Q_2$ in steady-state

$$K_{ij} = \frac{K_{0i} * Q_i}{Q_j} \tag{7}$$

It is the above equation which is crucial to the formulation of relationships between interconnected compartments and the distribution of radioactivity between these compartments at the time of reinjection. It is this relationship which has been used to assign the radioactivity of the injected bolus to compartments within the system. The specific radioactivity (SA) equations for compartment i are:

$$\frac{dSA_i(t)}{dt} = K_{0j}\, SA_j(0)\, e^{-K_{ij}} - K_{0i}SA_i(t) \tag{8}$$

and

$$SA_i(t) = \frac{K_{ii}}{K_{ij}-K_{ii}}\, SA_j(0)\, [\, e^{-K_{ii}} - e^{-K_{ij}}] \tag{9}$$

In Equations 3 and 9, radioactivity in compartment i starts at time zero with a value of zero, goes through a maximum at time t', and then approaches

zero as time approaches infinity. The time t' corresponding to the maximum radioactivity of the product is given by the formula:

$$t' = \frac{\ln K_{ij} - \ln K_{ii}}{K_{jj} - K_{ii}} \qquad (10)$$

This equation serves as a useful tool both in the analysis of complex compartmental problems and as a tool for predicting the likelihood of relationships between compartments.

2.2.3 Model formulation

While the mathematical relationships described above can be used to estimate the number of compartments involved in a system, they do not completely define how the compartments are interlinked. For example, the two exponential function described in Figure 6,

$$q = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \qquad (11)$$

can be described by five two-compartment models, each with a different spatial configuration of the K_{ij} parameters.

From the spectrum of kinetic models that are associated with this mathematical description of the system it is necessary to select one model which is consistent with known features of the system. The degree of complexity of this model is usually an indication of both the quality of the data gathered and the current knowledge and understanding of the system being modelled. If the data are extensive it may be possible to define a unique set of K_{ij} parameters for the system. On the other hand in the absence of sufficient data, the minimal number or simplest configuration of the K_{ij} set must be

chosen. Once the complexity of the model is defined, the K_{ij} values can be estimated from the relationship:

$$[K] = [A]\ [\lambda]\ [A]^{-1} \qquad (12)$$

where [A] is the n x n matrix of A_{ij}, for example

$$[A] = \begin{bmatrix} a_{11} & a_{1j} \\ & \\ a_{n1} & a_{nj} \end{bmatrix}$$

and each column is an eigenvector corresponding to a particular eigenvalue. [λ] is the n element diagonal matrix of the λ_j, or eigenvalue (Berman and Schoenfeld, 1956; Skinner et al, 1959; Berman et al, 1962a).

2.2.4 Modelling procedure: SAAM29/CONSAM29

Berman et al (1962b) developed a standard modelling program, SAAM (Simulation, Analysis and Modelling), to quantitatively relate the estimated parameter values to the experimental data. An updated form of this package, SAAM27 (Berman and Weiss, 1978) was originally programmed for a UNIVAC computer but has since been converted to a 256K DEC System K110, and subsequently to a DEC (Digital Electronics Corp.) VAX 11/780 computer. A conversational or interactive version of the program, CONSAM, developed by Boston et al (1981) permits rapid model development and increased control over modelling procedures. CONSAM the larger of the programs consists of over 300 subroutines and 65000 FORTRAN statements. Loading complete program into memory requires about one megabyte (1000K) of memory, however an overlay procedure facilitates reduction in core requirement to

approximately 100K. SAAM and CONSAM were used for the analysis of data collected during this project. Versions of SAAM/CONSAM were run on both a DEC VAX 11/785, running under VAX VMS, Version 4.1, and DEC MicroVAX II running under Ultrix, Version 1.2.

The operation of the SAAM program is based upon adjusting parameter values of a particular model until a 'best' fit to the experimental data is obtained. The program uses a common input format so that all model types can be expressed in terms of defined operational units. A summary of the more commonly used operational units of SAAM29 is presented in Table 7.

Each model can be defined in the input format by a set of parameter values which can be fixed, adjustable or dependent on other model characteristics, although not directly dependent on one another or other kinetic features of the system. The adjustable parameters permit the model to be fitted to experimental observations. These parameters, are identified as either linear or non-linear with respect to the model and are entered into the problem deck as initial estimates with upper and lower confidence limits. Information describing the parameters is inserted into the problem deck under the defined headings H LAM, H SIG, H KAP, H PAR, H INI, H STE and H DEP, although some of these headings are now redundant.

Experimental observations, QO(t), can be entered into the problem deck, together with the time and statistical weight of each observation under the heading H DAT (see Appendix A). The pattern of the model solution is also defined under this heading and the program can be instructed to calculate a specific set of theoretical values, QC(t), given a model, its parameter values and time of observation.

Table 7. A summary of the terms used most commonly in radiotracer kinetics with their SAAM29 equivalent (see Appendix A)

Terminology	Explanation	Related SAAM29 Tracer Terminology
M(J)	Steady state pool size for compartment j	Q_j
L(I,J)	Fraction of material in compartment j that will move to compartment i in unit time	K_{ij}
R(I,J)	Steady state flux of material from compartment j to compartment i	R_{ij}
F(J,T)	Model solution value for compartment j at time t	$q_j(t)$
U(J)	Steady state input into compartment j	
T	Independent time variable	t
QO(J,T)	Observed values of q_j at time t	$q_j(t)$
QC(J,T)	Theoretical values of q_j at time t	
SA	Specific Activity $q_j(t)/Q_j(t)$	SA
IC(J)	Value for q_j at time 0	$q_j(0)$
K(I)	Proportionality 'constant' to allow for variation in units in compartment j	

The general procedure used by SAAM to solve the system of equations which define a particular model and alter the model parameters so that they give the 'best' fit to the experimental observations can be summarized essentially in four main steps (Foster and Boston, 1983).

Step 1: The input file containing the parameters of the model and experimental data are interpreted by the program, invoking the internal machinery which defines a model solving path. Currently two methods, Runge-Kutta (Levy and Baggot, 1950) and Chu and Berman (Chu and Berman,

1974) are used for differential equation solving. The equations defining the model are built and buffers are established for retrieving parameter status information.

Step 2: Under a given set of initial conditions a model can be described by a response function $f_j(t)$ which relates a discrete number of parameter estimates x_i.

$$f_j(t) = f_j(x1,...xn,t) \qquad (13)$$

$$(j = 1,2,...n)$$

where n is the number of parameters. The program solves for $f_j(t)$ for nominated compartments and times. Thus for a linear kinetic model, the program solves the linear set of differential equations (see Equation 2)

$$f_j(t) = -K_{jj}f_j(t) + \sum_{\substack{i=1 \\ i \neq j}}^{n} K_{ji}f_i(t) \qquad (14)$$

$$(j = 1,...,n)$$

the above equation must be completed by appropriate initial conditions, i.e., by the quantities $f_j(0)$, j=1,2,..,n, representing the values of f_j at time t=0. In a more concise way we can write

$$\dot{f} = - Kf + f(t) \qquad (15)$$

or

$$f(0) = f_0 \qquad (16)$$

where

$$f = \begin{bmatrix} f_1 \\ f_2 \\ .. \\ f_n \end{bmatrix}$$

is the column vector formed by the variables of all components, f is the time derivative of f, and

$$K = \begin{bmatrix} K_{11} - K_{12} - .. - K_{1n} \\ -K_{21} + K_{22} - .. - K_{2n} \\ \cdots \quad \cdots \quad .. \quad \cdots \\ -K_{n1} - K_{n2} - .. + K_{nn} \end{bmatrix}$$

is the n*n matrix of all rates of elimination and of transfer. K_{ij} are the parameters of the model equivalent to x_i in Equation 13 are considered to be constant for each solution, and

$$f(t) = \begin{bmatrix} f_1(t) \\ f_2(t) \\ \cdots \\ f_n(t) \end{bmatrix}$$

is the column vector of all input rates, and

$$f_0 = \begin{bmatrix} f_1(0) \\ f_2(0) \\ \cdots \\ f_n(0) \end{bmatrix}$$

is the value of the column vector f when t=0.

Step 3: Linear combinations of the model solutions, QCk(t), are then assembled at time intervals specified by the SAAM deck. Thus:

$$QC_k(t) = \sum_{j=1}^{n} \sigma k_j f_j(t) \qquad (17)$$

$$(k = 1,2,...,n)$$

where 1 is the number of observation sites and σk_j are time independent linear coefficients.

For a given set of parameter estimates and initial conditions, the program solves the differential equations (Equation 13) which define the proposed model. The theoretical values QC(t) which are most like the experimental observations QO(t) at each time interval, are then calculated by adjusting the σk_j values. This is achieved by a least squares procedure. If the data are of the same weight:

$$[\sigma] = [f'f]^{-1} [f'QO] \qquad (18)$$

where the common subscript k has been dropped from all regressions. If a diagonal weight matrix, w, which represents the relative statistical weight of each observation is applied to the data points then:

$$[\sigma] = [f'wf]^{-1} [f'wQO) \qquad (19)$$

Step 4: The estimation of K_{ij} (which are non-linear parameters) in SAAM is facilitated with the aid of the Taylor series expansion. It is assumed that the observed quantity can be estimated from the sum of the directly calculated quantity (known as a zeroth iteration) and a linear perturbation to that quantity derived from alterations to the non-linear parameters. Thus:

$$QO_k = QC_k + \sum_{j=1}^{r} \frac{dQ0_k}{dx_j} \triangle x_j \qquad (20)$$

where x_j is one of the r non-linear adjustable parameters. Normal equations can be generated from (20) and the least squares solution for $\triangle x_j$ obtained. As QC is usually non-linear with respect to the variable parameters, x_j, it is

necessary to use an iterative procedure to obtain a least squares solution. Thus for unweighted data we find that:

$$[\triangle Q] = [\dot{Q}C\dot{Q}C]^{-1} [\dot{Q}C'\triangle x] \qquad (21)$$

where $[\triangle Q] = [QO - QC]$ whilst for weighted data, where weights are inversely proportional to the square of its standard deviation.

$$[\triangle x] = [\dot{Q}C'w\dot{Q}C]^{-1} [\dot{Q}C'w\triangle Q] \qquad (22)$$

The covariance matrix for parameter estimates is given by

$$[QC'wQC]^{-1} S^2 \qquad (22)$$

where S^2 is the residual variance about the model. The resulting matrix is the variance-covariance matrix which describes individual parameter variance estimates and correlation estimates between parameters.

2.2.5. Model assessment

The model solution produced by SAAM must then be appraised for its compatibility with the experimental data and there are a number of recognized measures in the SAAM output which are helpful in determining the acceptability of a particular least squares solution (Berman, 1963a). These include sums of squares for each component, and the model as a whole, the errors associated with each parameter estimation (standard deviation and fractional standard deviation) and an estimate of the correlation which exists between each estimated parameter.

2.2.5.1. Convergence

It is necessary to determine if a solution appears to have converged not

only to a least squares fit, but also to a logical fit. This may be indicated by visually comparing experimental and calculated functions, and by examining statistical information derived from the fit. Sums of squares for consecutive iterations should get smaller and error estimates for parameters should be within tolerance levels. In addition, to help accelerate convergence, correction factors (CONAB) are calculated within each iteration for the adjustable parameters. A rapid rate of convergence is suggested when CONAB is close to unity. Failure of the iterative procedure to converge suggests that, the data were inadequate with respect to noise, the variables were highly correlated, the initial estimates of the parameters were poor or that the model was too complex for reliable parameter estimation given the data presented.

2.2.5.2. Consistency

This occurs when the chosen model has sufficient freedom to adjust to the data. The accuracy of fit can be judged by the scatter of data about the calculated values. If the scatter of calculated to observed (QC/QO) values is random and close to unity then the fit is consistent. Systematic deviations from unity of QC/QO values suggest that the least squares fit is not consistent.

2.2.5.3. Model identifiability

The solution derived by SAAM/CONSAM may be consistent with the experimental data but is not unique. A unique solution will only arise if the observed data is error free. In the absence of this it is probable that many compartmental models, each with a different spatial arrangement, will fit the observed data equally as well as another model. To maximise the apparent identifiability of a model it must be consistent with other knowledge of the system and must provide parameter error estimates within tolerance ranges

(Cobelli et al, 1979). Providing extra information about the system being modelled will also help to improve model identifiability.

Perturbations of the system also provide information, if it is assumed that the changes produced by such perturbations are of the simplest form, i.e. a minimum number of parameters are influenced, then monitoring these changes may provide sufficient constraints to lead to a unique model (Berman, 1963b).

CHAPTER 3

RESULTS AND MODEL DEVELOPMENT

3.1. Lipoprotein composition

Table 8 summarises for the four subjects the plasma triglyceride, cholesterol and apolipoprotein B concentrations in VLDL, IDL and LDL. The VLDL data in Table 8 describe both VLDL1 (S_f 60-400) and VLDL2 (S_f 20-60) fractions. Triglyceride, apolipoprotein B and cholesterol concentrations in both VLDL fractions displayed the marked heterogeneity that is known to exist in VLDL and the heterogeneous disorder of type IV hypertriglyceridemia.

Table 8. Lipid and apoprotein B concentrations of lipoprotein fractions.

Subject	Lipoprotein Fraction	Triglyceride (TG)	Apo B	Cholesterol (Chol)	TG / Apo B	Chol / TG
			mg/dl			
F	VLDL1	81.6 ± 16.5[a]	6.8 ± 1.2	12.1 ± 1.7	12.0	0.15
	VLDL2	168.3 ± 33.4	13.7 ± 2.4	39.8 ± 4.2	12.3	0.24
	IDL	14.4 ± 4.1	7.0 ± 0.9	15.9 ± 1.4	2.1	1.10
	LDL	17.4 ± 2.3	85.4 ± 9.9	113.8 ± 11.2	0.2	6.54
K	VLDL1	506.9 ± 66.5	34.6 ± 5.2	136.2 ± 14.1	14.6	0.27
	VLDL2	211.8 ± 13.9	20.2 ± 1.9	71.6 ± 5.1	10.5	0.34
	IDL	33.1 ± 5.3	11.3 ± 1.6	21.7 ± 3.2	2.9	0.66
	LDL	27.9 ± 4.8	43.8 ± 5.8	52.3 ± 7.4	0.6	1.87
H	VLDL1	45.7 ± 10.1	7.6 ± 1.4	9.1 ± 1.2	6.0	0.19
	VLDL2	159.8 ± 24.8	27.8 ± 6.5	40.5 ± 5.4	5.7	0.25
	IDL	23.7 ± 4.9	11.1 ± 1.5	17.9 ± 2.1	2.1	0.76
	LDL	27.9 ± 2.1	90.7 ± 5.8	87.0 ± 5.8	0.3	3.12
J	VLDL1	105.2 ± 9.7	4.5 ± 1.2	14.6 ± 0.9	23.4	0.14
	VLDL2	248.6 ± 14.2	21.5 ± 3.3	46.9 ± 2.7	11.6	0.19
	IDL	17.1 ± 2.6	6.7 ± 0.9	11.3 ± 0.2	2.6	0.66
	LDL	16.7 ± 2.1	35.9 ± 5.1	72.7 ± 1.6	0.5	4.35

VLDL1, S_f 60-400; VLDL2, S_f 20-60

[a] Mean ± SD

The larger VLDL1 particles contained a greater amount of triglyceride, per apo B, than the smaller VLDL2 particles. Examination of the data in this table reveals that in three of the four subjects most of the apo B mass, and thus the majority of particles in the VLDL fraction were within the VLDL2 range, the exception being the most hyperlipidemic subject, subject K. The cholesterol/triglyceride ratios indicate, as expected (Nestel et al, 1983), that the VLDL2 fraction carried more cholesterol relative to triglyceride. The triglyceride/apo B ratios in the VLDL fraction were approximately five times those in the S_f 12-20 (IDL) fraction, indicating very significant delipidation between these two fractions. The LDL data were consistent with findings in hypertriglyceridemic subjects, showing normal cholesterol and apo B concentrations and relative triglyceride enrichment.

3.2. Heparin-Sepharose affinity chromatography

Heparin-Sepharose affinity chromatography of each VLDL fraction isolated two populations of particles which were either unretained (unbound) or retained (bound). Previous studies (Nestel et al, 1983; Huff and Telford, 1984) have characterized the unbound and bound fractions (see Section 1.8.3.1). The data in Table 9 describe the distribution of triglyceride and apolipoprotein B in the four subfractions. In both VLDL1 and VLDL2 the unbound fractions had higher triglyceride/apo B ratios than found in the bound fractions. This suggests that the bound fractions contained products of the unbound fraction. In all subjects, except for subject F, the triglyceride/apo B ratios of unbound and bound fractions in the VLDL1 fraction were greater than for the corresponding fractions in the VLDL2 fractions. It is however interesting to note that in all subjects the triglyceride/apo B ratio of the bound VLDL1 was less than that for the unbound VLDL2 fraction, suggesting that the latter was

Table 9. Triglyceride and apoprotein B concentrations of heparin-Sepharose fractionated VLDL.

Subject	VLDL Fract.	Unbound Fraction Triglyceride (TG)	Apo B	TG / Apo B	Bound Fraction Triglyceride (TG)	Apo B	TG / Apo B
		mg/dl			mg/dl		
F	VLDL1	30.6 ± 12.6[a]	2.0 ± 0.1	15.3	51.0 ± 25.3	4.8 ± 1.3	10.6
	VLDL2	56.1 ± 26.6	1.3 ± 0.8	43.1	112.2 ± 43.4	12.4 ± 3.1	9.0
K	VLDL1	381.4 ± 60.8	20.2 ± 5.8	18.9	125.5 ± 29.2	14.2 ± 3.3	8.8
	VLDL2	105.9 ± 12.2	7.6 ± 1.4	13.9	105.9 ± 15.4	12.5 ± 1.7	8.5
H	VLDL1	7.4 ± 2.7	0.65 ± 0.2	11.4	38.3 ± 4.5	7.0 ± 1.8	5.5
	VLDL2	80.2 ± 11.6	10.9 ± 3.2	7.4	79.6 ± 8.9	16.9 ± 3.3	4.7
J	VLDL1	67.6 ± 17.9	1.7 ± 0.2	39.7	37.6 ± 6.3	2.8 ± 0.9	13.5
	VLDL2	145.0 ± 18.1	7.6 ± 1.7	19.1	103.6 ± 14.6	13.9 ± 1.9	7.5

VLDL1, S_f 60-400; VLDL2, S_f 20-60

[a] Mean ± SD

not exclusively a product of VLDL1. On the basis of the apo B concentrations there appeared to be more particles in the bound than in the unbound fractions with one exception (subject K, VLDL1). A comparison of the unbound/bound apo B ratios (Table 10) shows that, except for subject H and perhaps subject J, the ratios were greater in the VLDL1 fraction, than in the VLDL2. An interpretation of this may be that, at least in subjects F and K, the smaller VLDL fraction contained a greater proportion of apo E-rich, apo C_2-poor remnant like particles. Both fractions (VLDL1 and VLDL2) therefore contained a population of both unbound and bound particles and, in subjects F and K (and J) as the particles became smaller, presumably as a result of hydrolysis, the proportion of bound particles increased.

Table 10. Unbound / Bound Mass Ratios in VLDL1 and VLDL2.

Subject	Apolipoprotein B		Triglyceride	
	VLDL1	VLDL2	VLDL1	VLDL2
	unbound/bound[a]		unbound/bound	
F	0.42	0.10	0.60	0.50
K	1.42	0.61	3.04	1.00
H	0.09	0.64	0.19	1.01
J	0.61	0.55	1.80	1.40

[a] ratio of heparin-unbound to heparin-bound lipoprotein mass

3.3. Lipoprotein kinetics

The following four sections will present the apo B and triglyceride kinetic data collected during the turnover studies carried out in the four subjects described above. In the figures which follow both the observed data and the model derived fit to the data are shown. During the course of the following sections there will, in addition to the presentation of results, be discussion of attempts to fit simple models to the data. The development of the final compartmental model used to fit the apo B and triglyceride data is discussed later in Sections 3.4.4 and 3.4.5.

3.3.1. Apolipoprotein B kinetics in VLDL1 and VLDL2

To study the metabolism of VLDL through to LDL autologous labelled ^{125}I-VLDL1 and ^{131}I-VLDL2 were injected together with a bolus of 2-^{3}H-glycerol. Subject J received only 2-^{3}H-glycerol due to pyrogen contamination of the labelled lipoprotein preparations. The apo B and triglyceride moieties were isolated and specific radioactivity curves were produced for both apo B and triglyceride in VLDL1, VLDL2, IDL, and LDL. Specific radioactivity curves of unfractionated VLDL1 apo B (Figure 7) were generally monoexponential,

although in subject H the VLDL1 decay curve was biexponential. Examination of the decay curves for the unbound and bound fractions (Figure 7) however were more revealing. The specific radioactivity curves for the unbound fraction were mono or biexponential, and decayed at a faster rate than that of the unfractionated VLDL1 curve. In subject H and to a lesser extent subject F, the dominant feature of the decay curve was a rapid fall ($t_{1/2} \approx 1$ hr) followed by a slowly decaying tail, the slope of which was comparable to that of the bound fraction. The curves for bound VLDL1, though largely monoexponential, closely resemble those for the whole VLDL1 fraction. In subject K however, there was an initial delay, or shoulder, before the specific radioactivity of the bound fraction curve fell, suggesting that the bound fraction may be the product of the more rapidly turning over unbound fraction. This observation, although not seen in other subjects might have been observed had the initial specific radioactivity of the bound fraction not been significantly less than that of the unbound fraction. If the specific radioactivities of the two fractions had been the same then a shoulder would have been observed in the decay curve of the bound fraction, assuming a precursor product relationship between the unbound and bound fractions. Theoretically the specific radioactivities of these fractions should have been the same at the time of reinjection; that they were observed to be different (Table 11) for the two fractions in all subjects suggests that mass distribution changes occured between the time when VLDL was isolated for labelling and the time of reinjection.

Apolipoprotein B specific radioactivity curves of ^{131}I labelled VLDL2 were qualitatively similar to those in the VLDL1 fraction. The average half-life of the VLDL2 bound fraction was marginally faster than that in the VLDL1 fraction. In the unbound VLDL2 however the fast component was slower than

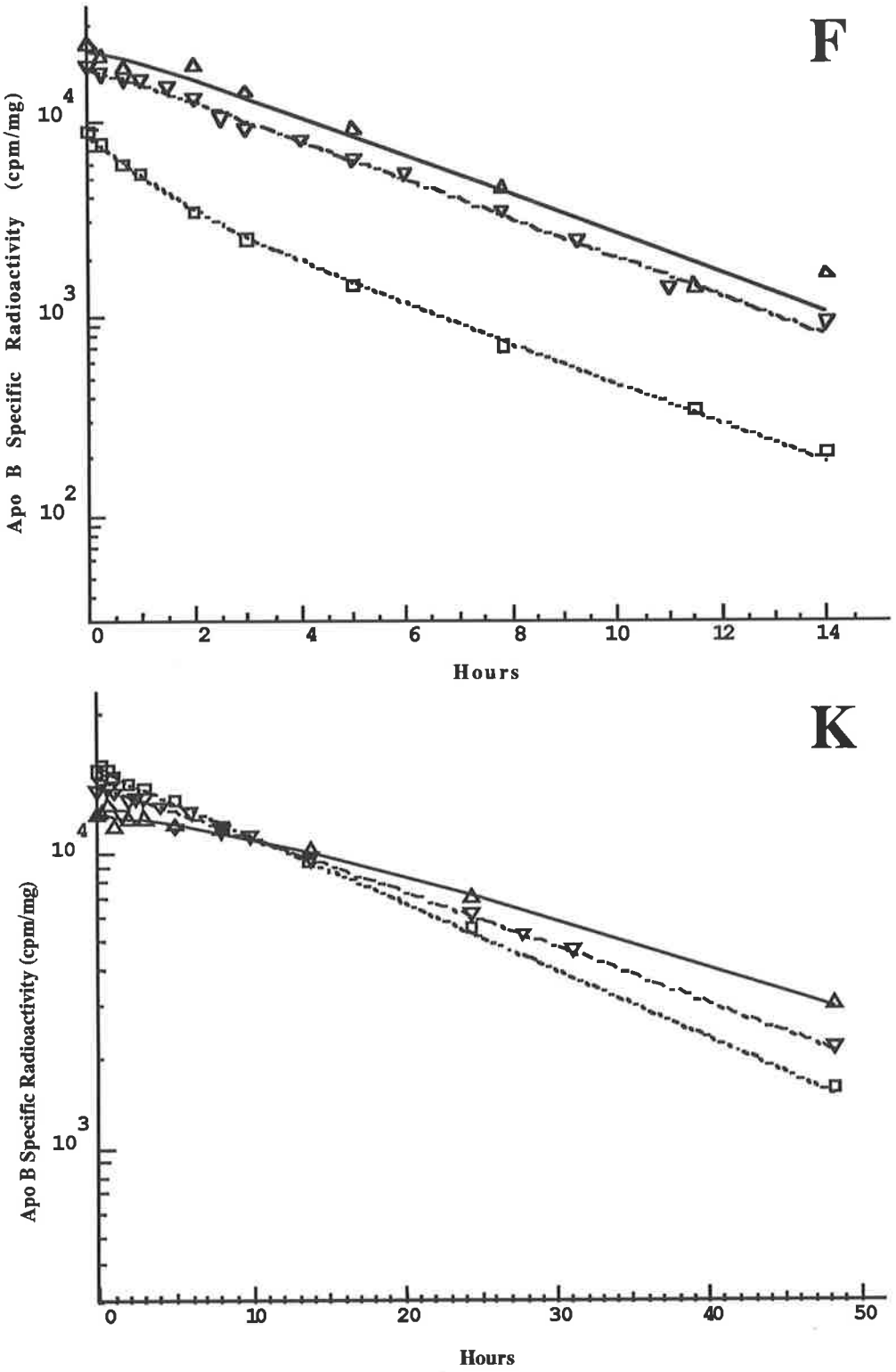


Figure 7. ^{125}I Specific radioactivity decay curves for apolipoprotein B of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL1 (Δ) for subjects F, K and H respectively following injection of unfractionated autologous ^{125}I-VLDL1. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5).

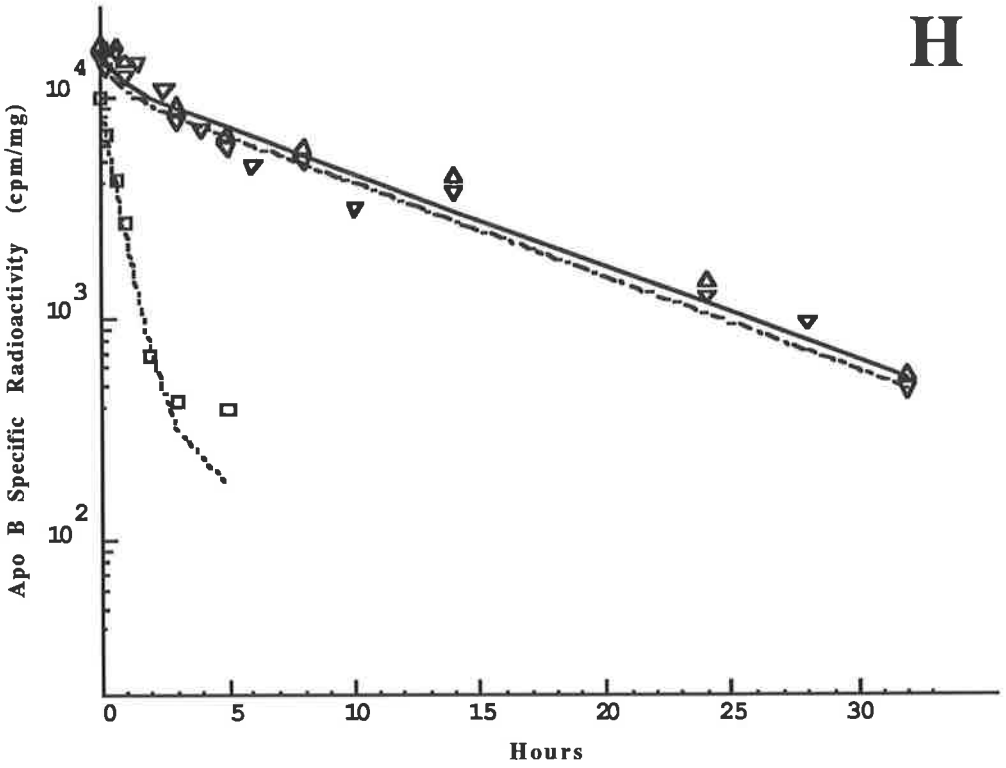


Figure 7. (cont'd)

that of the VLDL1 fraction, except in subject F where the unbound fraction decayed rapidly, while the slow component was faster than in the VLDL1 fraction. The bound VLDL2 specific radioactivity curve exhibited a more pronounced shoulder than seen with VLDL1 suggesting that this fraction may be a product not only of the VLDL2 unbound fraction but also of a component of VLDL1. Specific radioactivity curves of ^{125}I-VLDL2 (Figure 9) generated from the conversion of VLDL1 were also analysed and revealed average half-lives for both unbound and bound fractions that were similar to those of the corresponding ^{131}I-VLDL2 fractions. The ^{125}I bound VLDL2 curves did however show a more marked delay than observed in the ^{131}I labelled bound VLDL2 fraction. This observation may reflect a greater conversion of unbound to bound particles which are derived from the VLDL1 fraction than that which

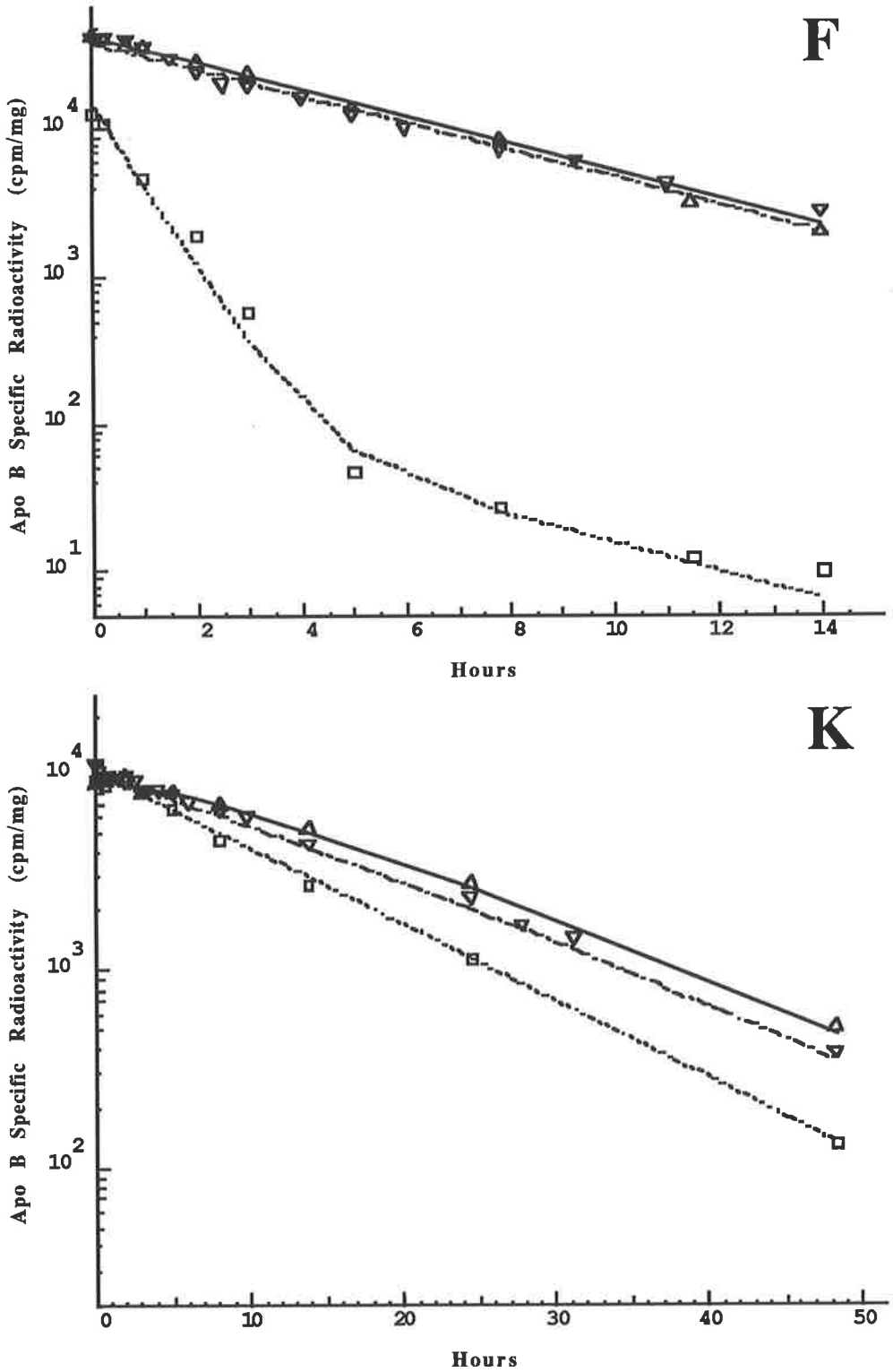
Table 11. Initial specific radioactivities of IDL, LDL and VLDL unbound and bound apo B fractions.

Subject	Fraction	Unbound		Bound		Unfractionated	
		^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
		cpm/mg (10^{-3})					
F	VLDL1	5.86		19.94		15.80	
	VLDL2	40.17	14.05	31.74	48.23	32.55	44.98
	Transfer[a]	**81.60%**		**80.40%**		**80.60%**	
	IDL					2.19	7.88
	LDL					0.07	0.06
K	VLDL1	19.54		13.79		17.15	
	VLDL2	32.10	10.3	17.05	10.31	22.75	10.31
	Transfer	**38.30%**		**51.90%**		**43.60%**	
	IDL					0.75	1.43
	LDL					0.12	0.15
H	VLDL1	8.38		16.83		16.12	
	VLDL2	48.50	23.4	40.92	47.20	43.90	37.86
	Transfer	**98.90%**		**85.40%**		**90.80%**	
	IDL					1.67	0.54
	LDL					0.07	0.04

VLDL1, S_f 60-400; VLDL2, S_f 20-60

[a] Transfer of ^{125}I radioactivity from VLDL1 to VLDL2 at time of injection would be observed in the VLDL2 fraction which contains a greater proportion of bound, remnant-like particles.

Figures 7 and 9 show that, at the time of reinjection (t=0), the ^{125}I-specific radioactivities of the VLDL2 fraction were greater than those of the VLDL1 (also shown in Table 11). The implication of these observations is that there was a rapid transfer of radioactivity and presumably VLDL1 mass from the VLDL1 fraction into the VLDL2 fraction. It would seem unlikely that this transfer cannot be entirely accounted for by the presence of artifacts in the reinjected VLDL1 dose.



Figures 8. ^{131}I Specific radioactivity decay curves for apolipoprotein B of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL2 (Δ) for subjects F, K and H respectively following injection of unfractionated autologous ^{131}I-VLDL2. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5).

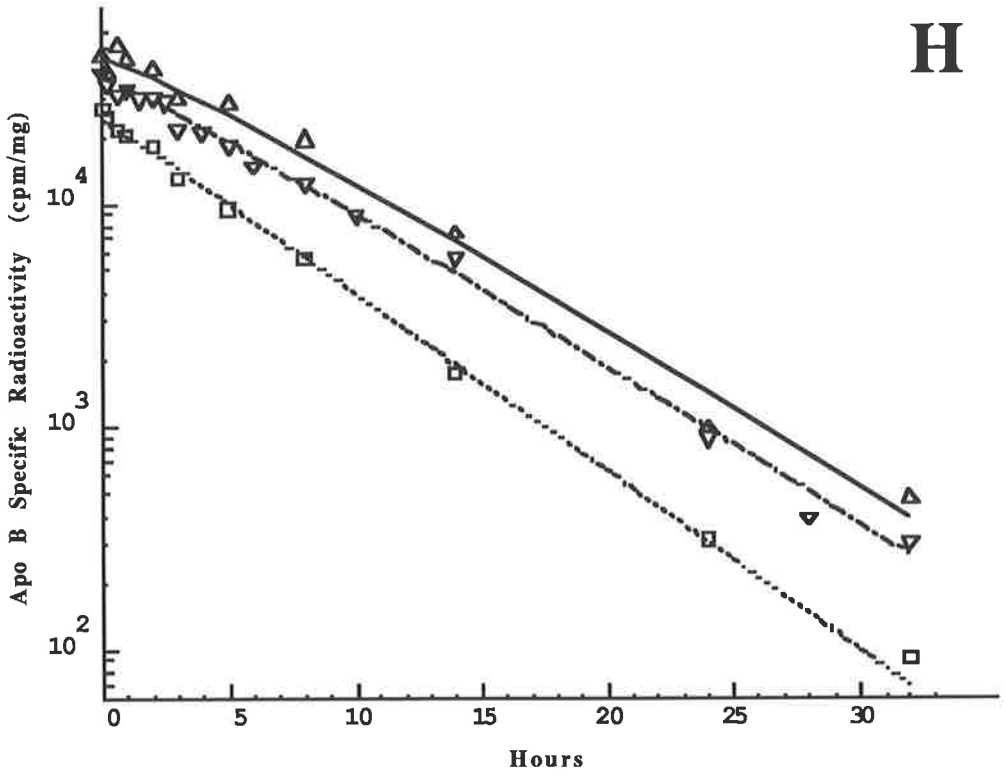
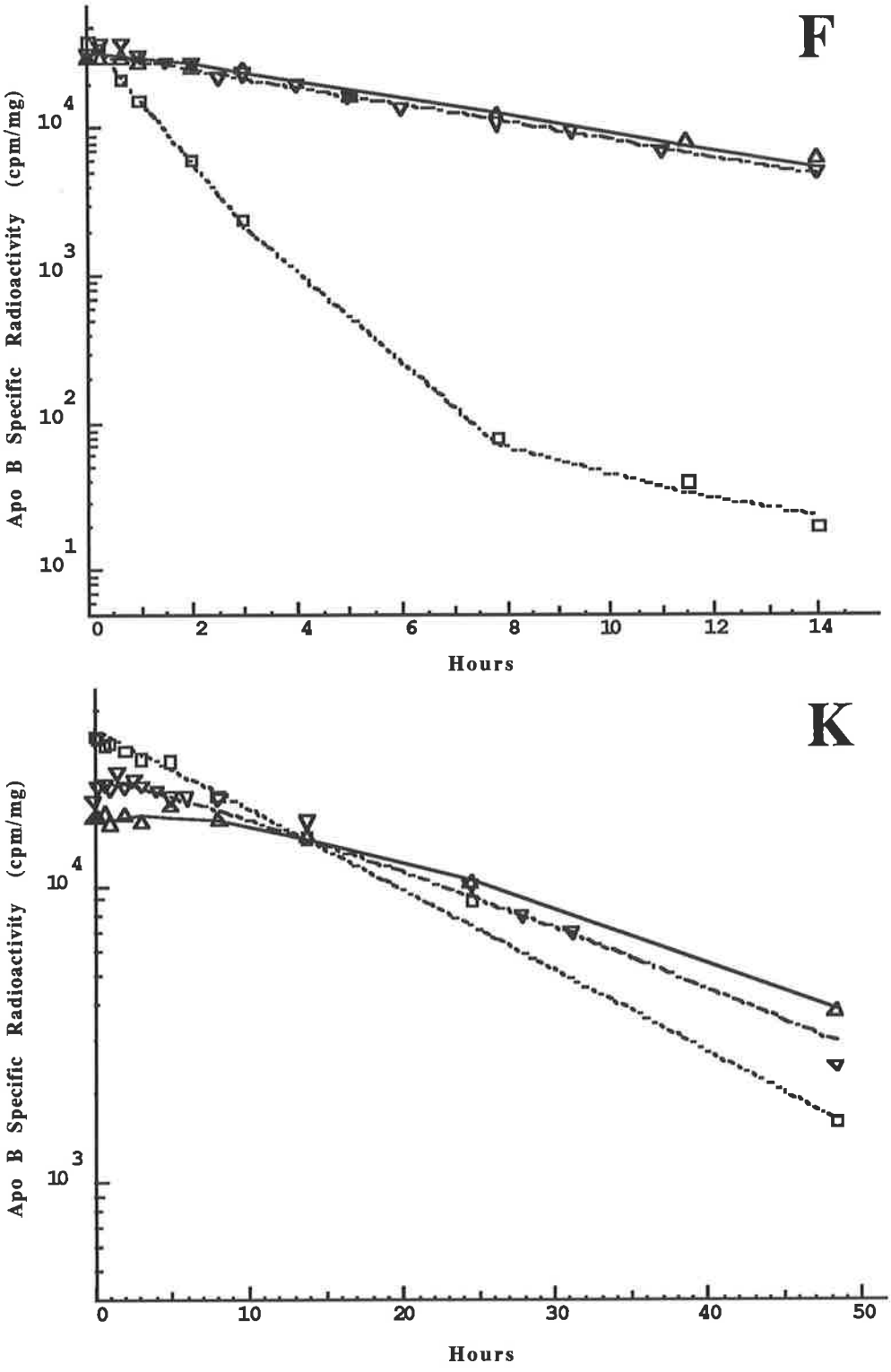


Figure 8. (cont'd)

The similarity of the ^{125}I and ^{131}I apo B decay curves in the VLDL2 fraction would suggest that those particles which have been derived from the VLDL1 fraction are not artifactual. This transfer may represent a rapid physiological remodelling of the VLDL1 particle which occurs after reinjection into the plasma compartment, an observation which was not observed in vitro. Despite this transfer, it was assumed that the ^{125}I tracer which remained in the VLDL1 fraction described the kinetics of true VLDL1 particles. In addition, it must recognised that this problem is limited to apo B data and not to triglyceride data, which is derived from the endogenous incorporation of ^{3}H-glycerol into VLDL triglyceride That the kinetics of apo B and triglyceride moieties in the VLDL1 fraction were modelled simultaneously, using the model developed for the VLDL2 fraction, suggests that use of the VLDL1-^{125}I tracer data was valid.



Figures 9. ^{125}I Specific radioactivity decay curves for apolipoprotein B of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL2 (Δ) for subjects F, K and H respectively following injection of unfractionated autologous ^{125}I-VLDL1. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5).

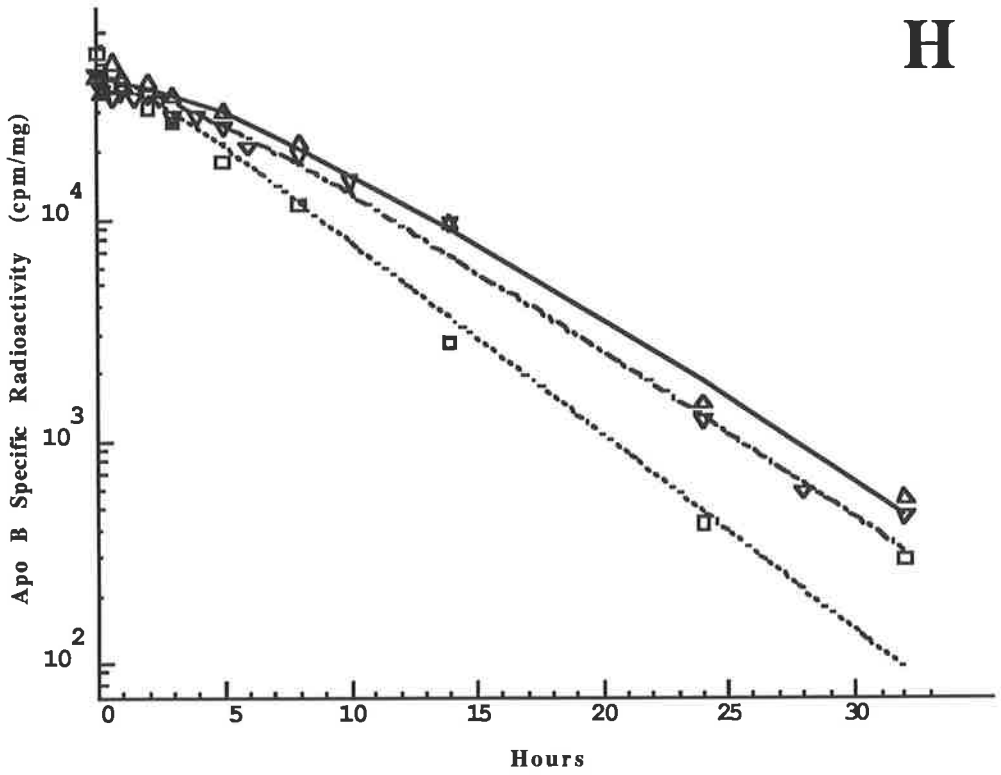


Figure 9. (cont'd)

3.3.2. Apolipoprotein B Kinetics in IDL and LDL

The apo B kinetics of the IDL and LDL fractions (Figures 10 and 11) revealed the transfer of label and presumably mass from the VLDL fraction to the IDL and subsequently LDL fraction. In each subject the IDL specific radioactivity curve exhibited an initial rise, reflecting the transfer rate of material from the VLDL fraction into IDL, followed by a slowly decaying tail. The average half-life of the IDL fraction was comparable to that of the slowly turning over component of the bound VLDL. Of interest was the observation that the ^{131}I labelled IDL reached a peak specific radioactivity earlier than that of the ^{125}I labelled IDL, indicating the less time involved in the conversion of VLDL2 to IDL compared to VLDL1. This observation suggests that little or none of the VLDL1 fraction is catabolised directly to the IDL density

bypassing the VLDL2 density range. This observation, is however somewhat dependent upon the nature of the rapid conversion or transfer of the ^{125}I labelled VLDL1. The observation that the intercepts for the IDL and to a lesser extent for the LDL specific radioactivity curves (Table 11) were not at zero suggests that ultracentrifugal separation of IDL and VLDL2 was not very efficient in the anglehead rotor or that because d=1.019 g/ml is purely an arbitrary division between VLDL and IDL, particles of comparable metabolic status may exist across this density boundary.

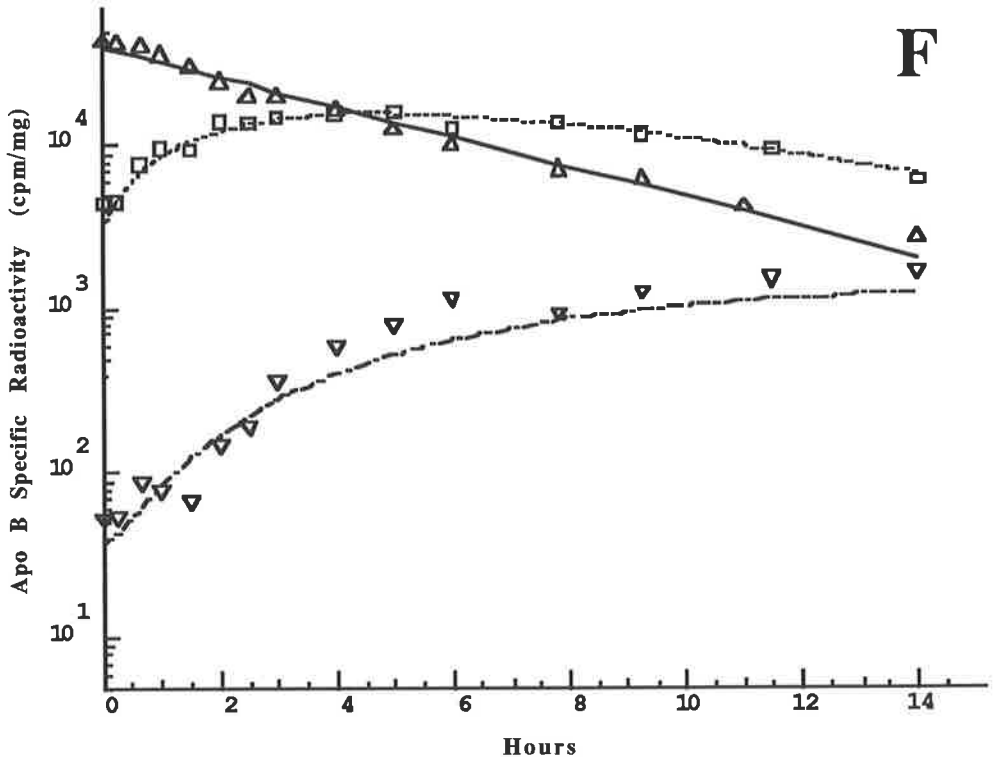


Figure 10. ^{131}I Specific radioactivity decay curves for apolipoprotein B of unfractionated VLDL2 (Δ), IDL (□) and LDL (∇) for subjects F, K and H respectively following injection of unfractionated autologous VLDL2. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5).

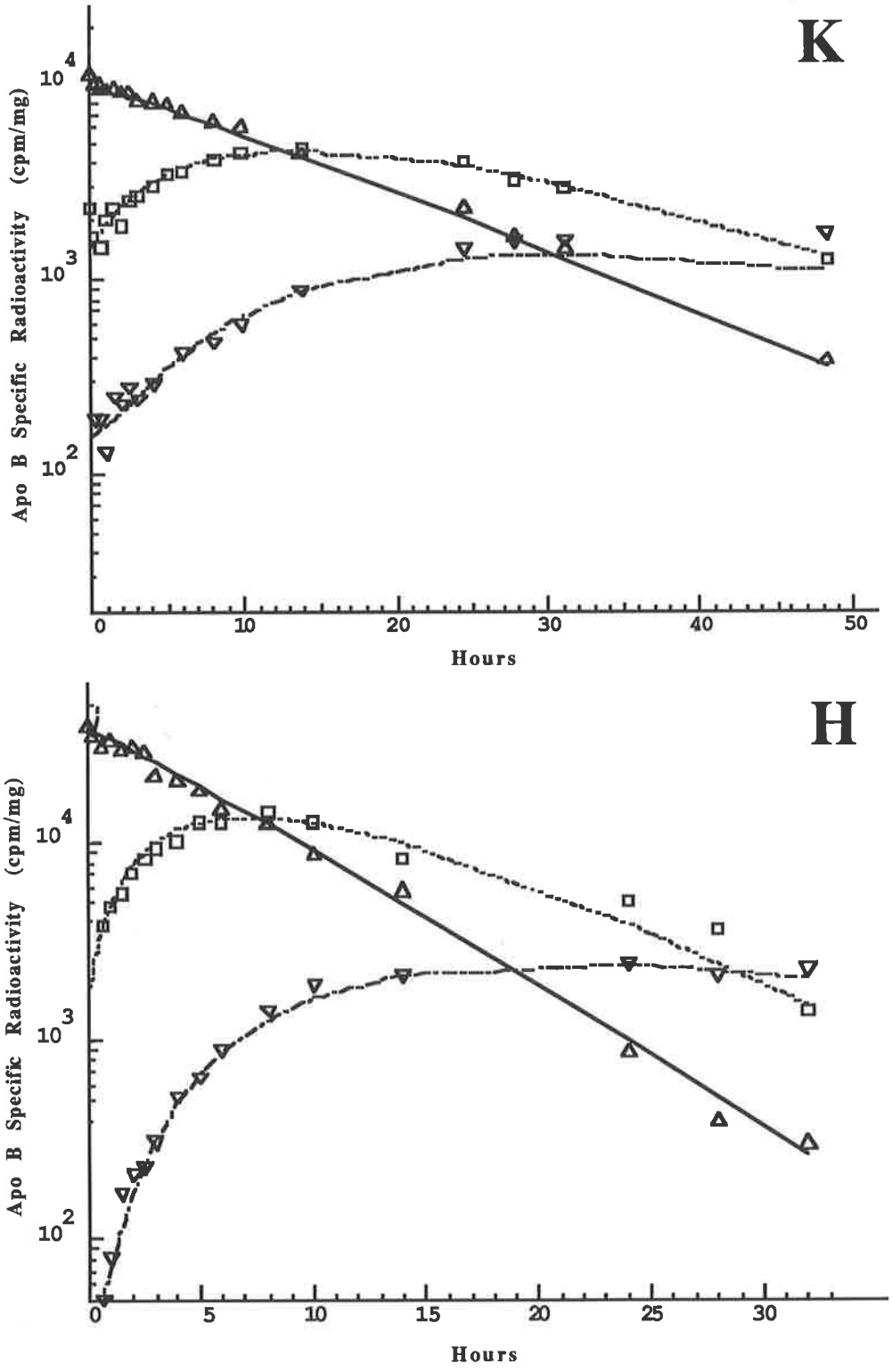


Figure 10. (cont'd)

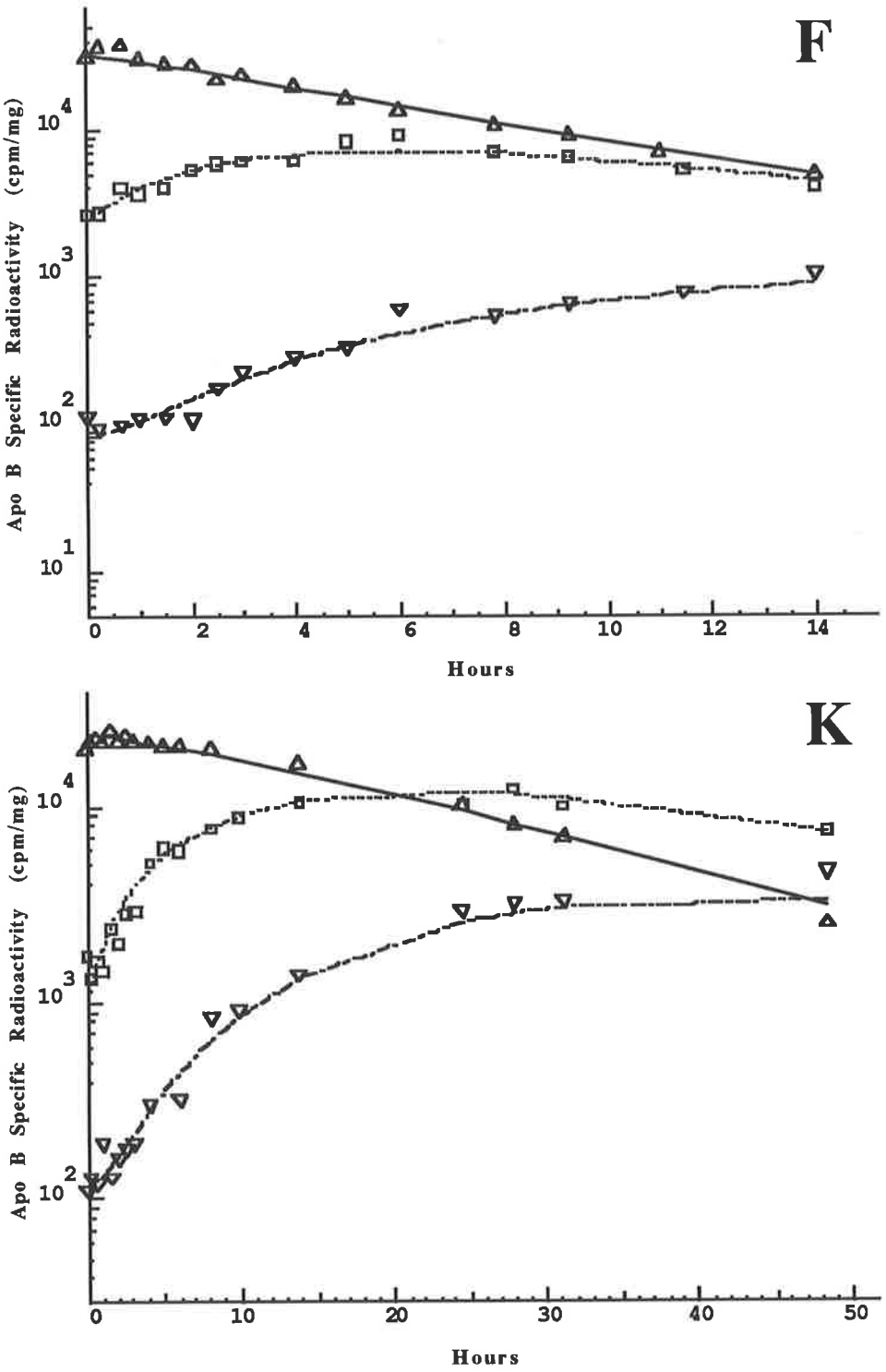


Figure 11. ^{125}I Specific radioactivity decay curves for apolipoprotein B of unfractionated VLDL2 (Δ), IDL (□) and LDL (∇) for subjects F, K and H respectively following injection of radiolabelled unfractionated autologous VLDL1. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5).

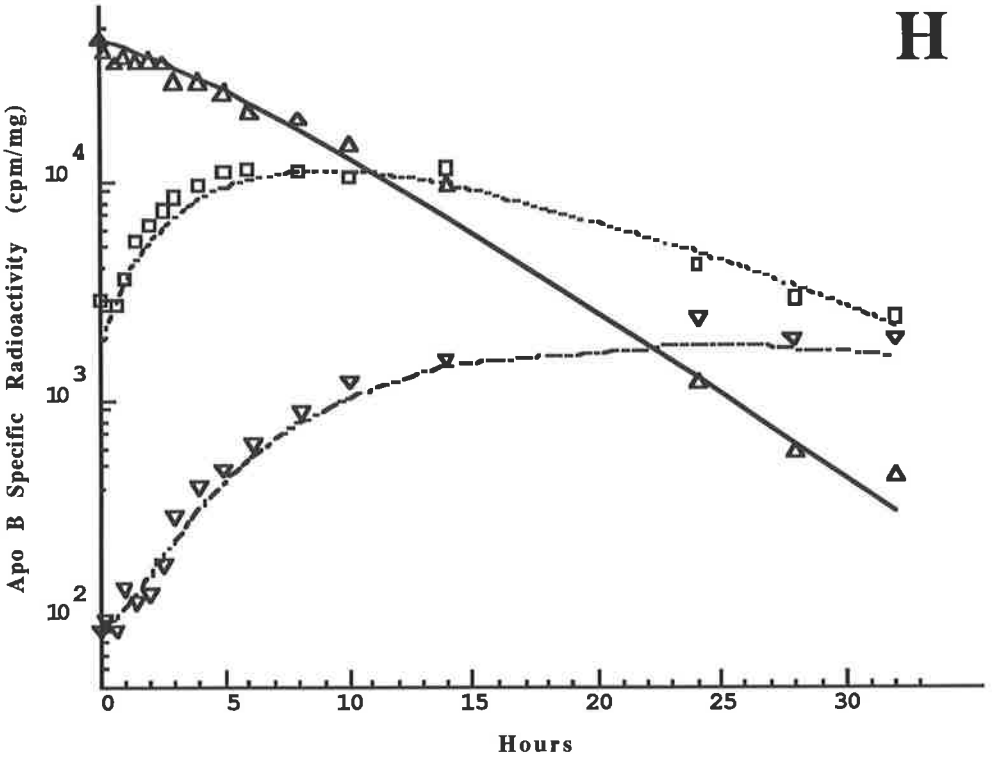


Figure 11. (cont'd)

3.3.3. Triglyceride Kinetics in VLDL1 and VLDL2

Very low density lipoprotein triglyceride kinetics were studied following the administration of a bolus of 2-^{3}H-glycerol, of which only a small fraction is incorporated into the triglyceride of lipoproteins. In both unfractionated VLDL1 and unfractionated VLDL2 triglyceride kinetics showed the same general features (Figures 12-14). Both curves exhibited a rapid initial rise in specific radioactivity which was followed by a relatively flat peak, and a subsequent decay as has been reported by others (Zech et al, 1979). In the VLDL1 of subject H and both VLDL fractions of subject J a slowly decaying tail was seen at later times. The tail which is often observed in VLDL triglyceride decay curves, and which is thought to represent a slow liver triglyceride precursor was not observed in subjects K and K or in the VLDL2 of H. The duration was too short in F to have detected this feature. The initial rise in

triglyceride specific radioactivity appeared to occur with minimal delay, which suggests that incorporation of glycerol into triglyceride and secretion within VLDL were quite rapid. In addition, the rate at which the VLDL1 and VLDL2 triglyceride specific radioactivity curves increased appeared to be the same. This is not consistent with the initial interpretation of the corresponding apo B kinetics that the bound fraction was derived from the unbound. In general the unbound triglyceride specific radioactivity curve attained a peak 2 to 3 hours after injection of labelled glycerol which was higher than that for the bound fraction. The peak specific radioactivity of the bound fraction was however higher than that of the unbound in the VLDL1 of subject K and VLDL2 of subject F. The specific radioactivity curve of the bound fraction peaked at the same time as that of the unbound except in subject F where the maximum was reached after that of the unbound fraction. The summation (weighted mean specific radioactivity) of both the unbound and bound curves thus resulted in the relatively long flat section at the peak of the unfractionated VLDL specific radioactivty curve. This portion of the curve most likely corresponds to the triglyceride delipidation process. After reaching its peak the unbound fraction specific radioactivity curve began to decay quite rapidly resulting in the curve falling below that of the bound fraction. The fall in triglyceride specific radioactivity of the bound fraction was relatively slow and the general shape of the curve suggested that the bound fraction was the product of the unbound fraction, although as indicated above, the nature of the initial rise implies the presence also of a directly secreted component.

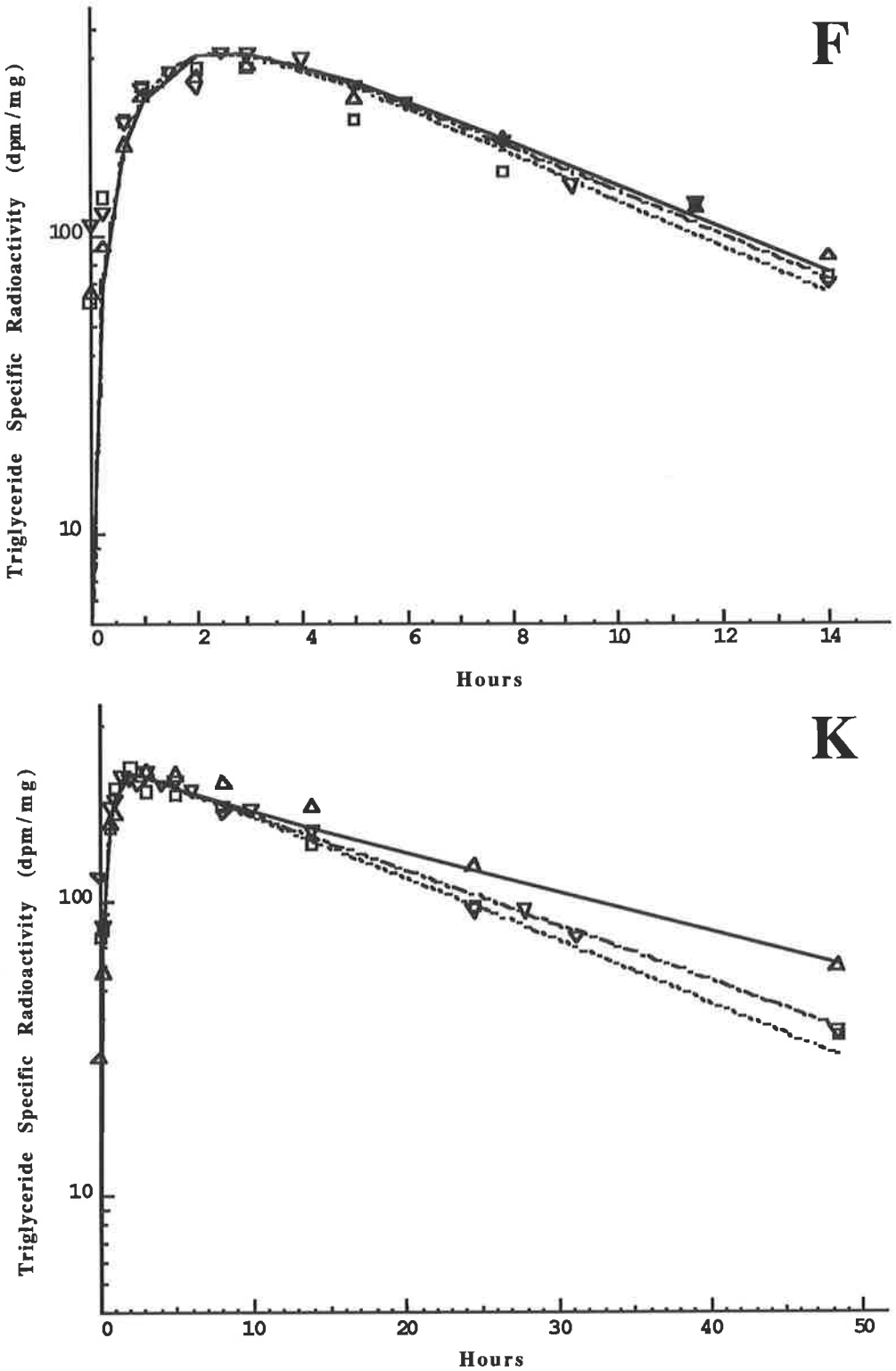


Figure 12. Triglyceride specific radioactivity decay curves of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL1 (Δ) for subjects F, K, H and J respectively. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5). The fits to the data were derived by coupling the ^{125}I apo B and triglyceride VLDL1 data.

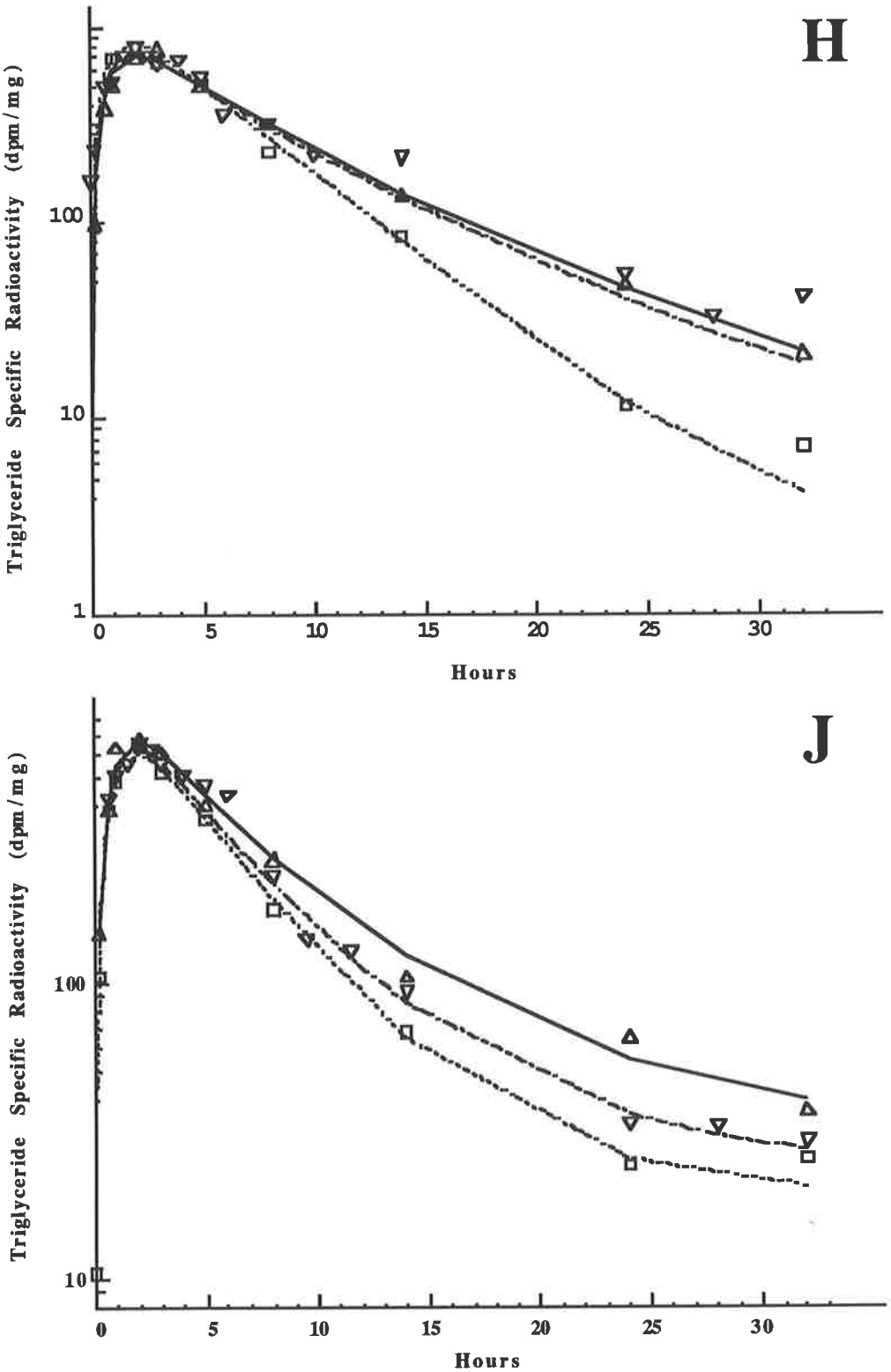


Figure 12. (cont'd)

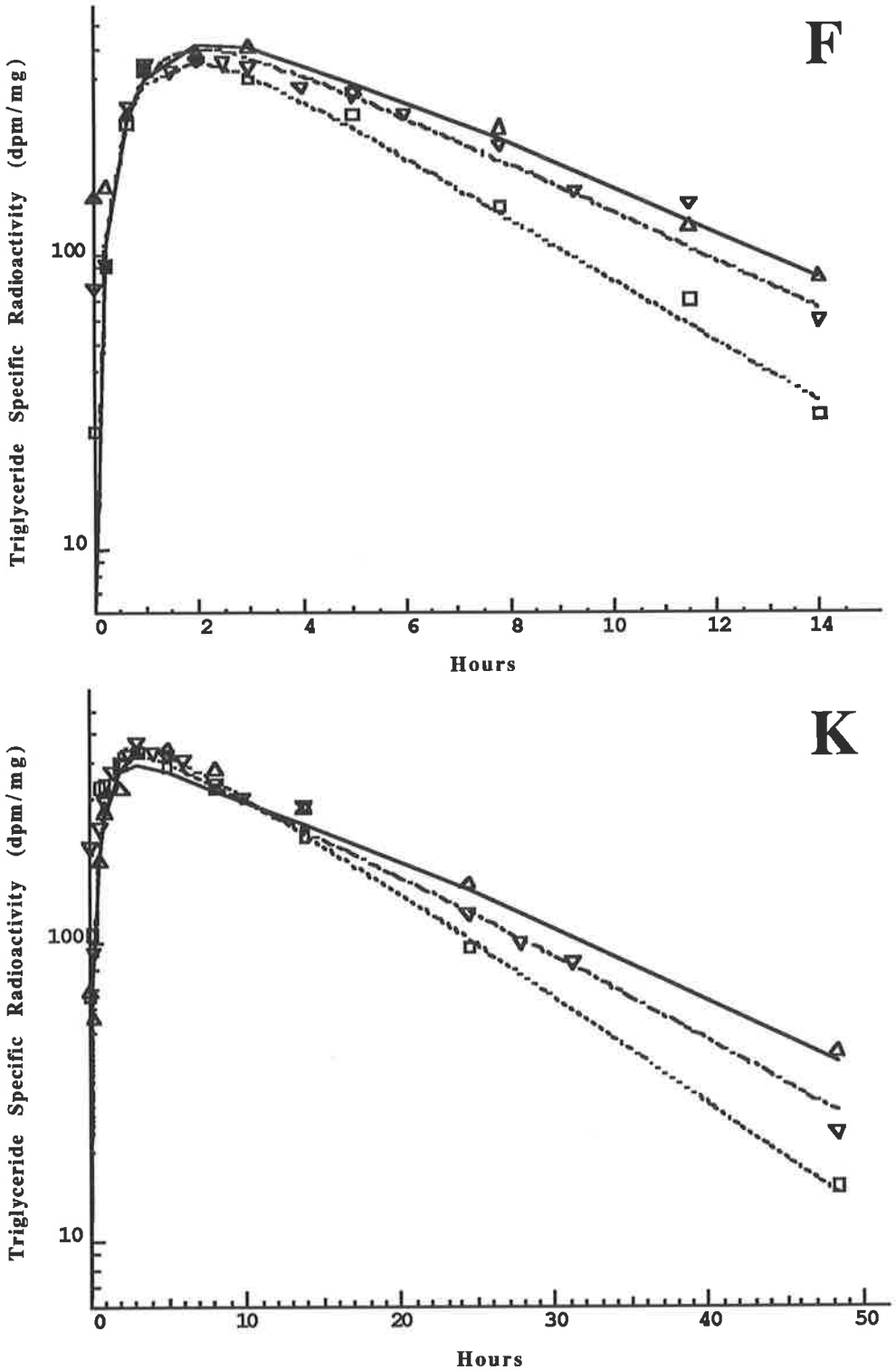


Figure 13. Triglyceride specific radioactivity decay curves of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL2 (Δ) for subjects F, K, H and J respectively. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5). The fits to the data were derived by coupling the ^{131}I apo B and triglyceride VLDL2 data.

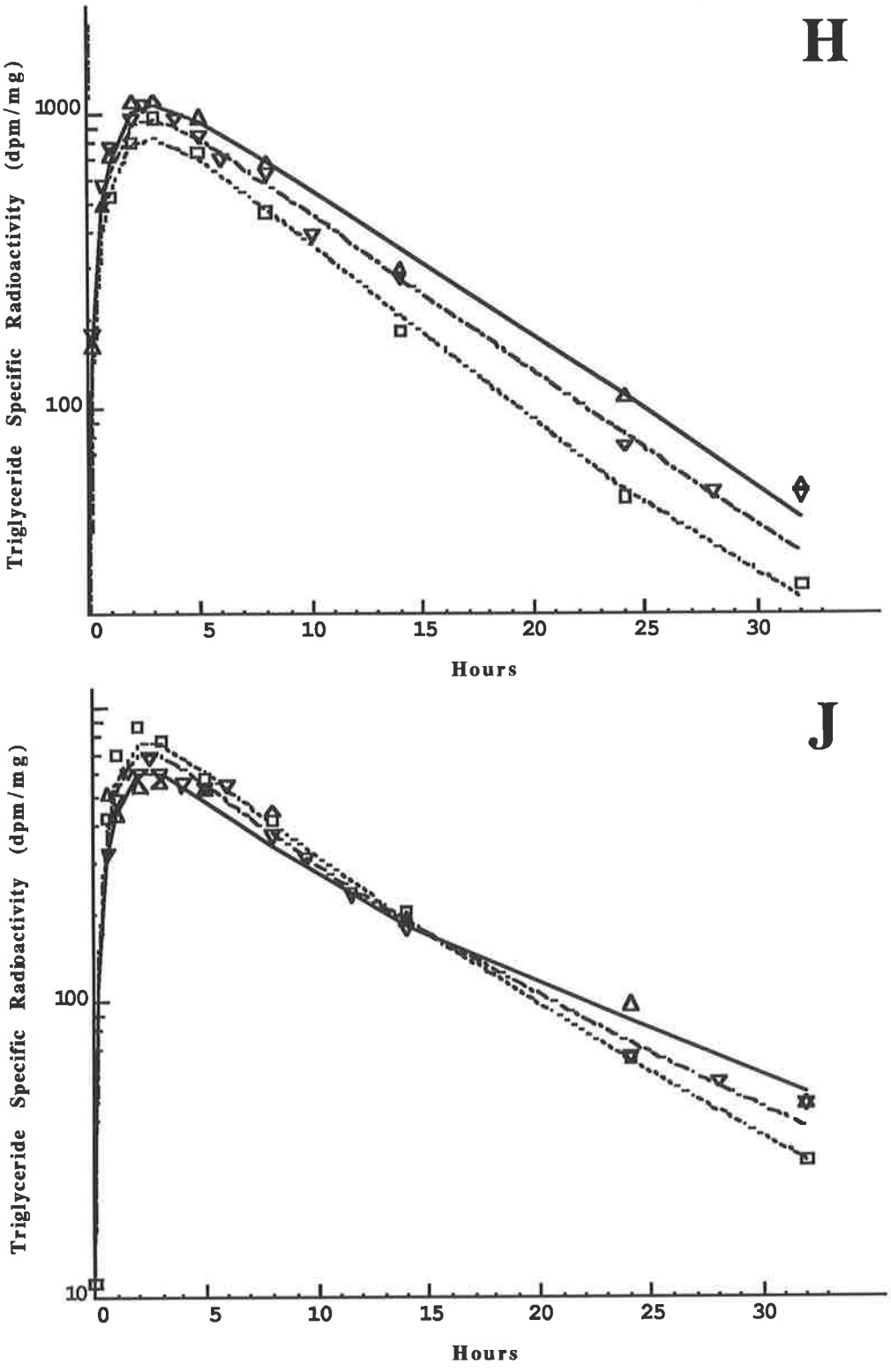


Figure 13. (cont'd)

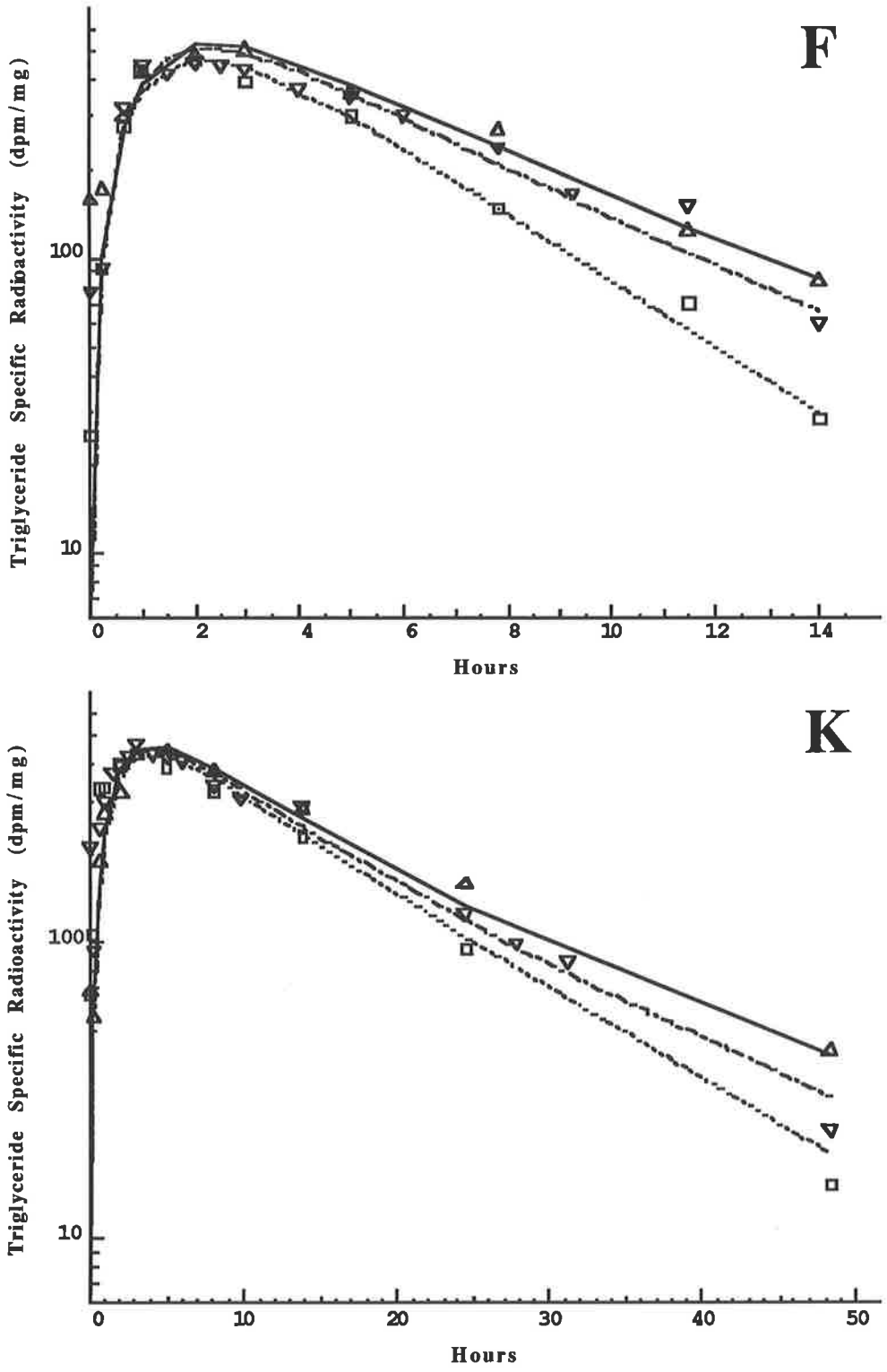


Figure 14. Triglyceride specific radioactivity decay curves of unfractionated (∇), heparin-unbound (□) and heparin-bound VLDL2 (Δ) for subjects F, K and H respectively. The symbols represent observations while the curves show the fits to the model (Figure 51, Section 3.5). The fits to the data were derived by coupling the ^{125}I apo B and triglyceride VLDL2 data.

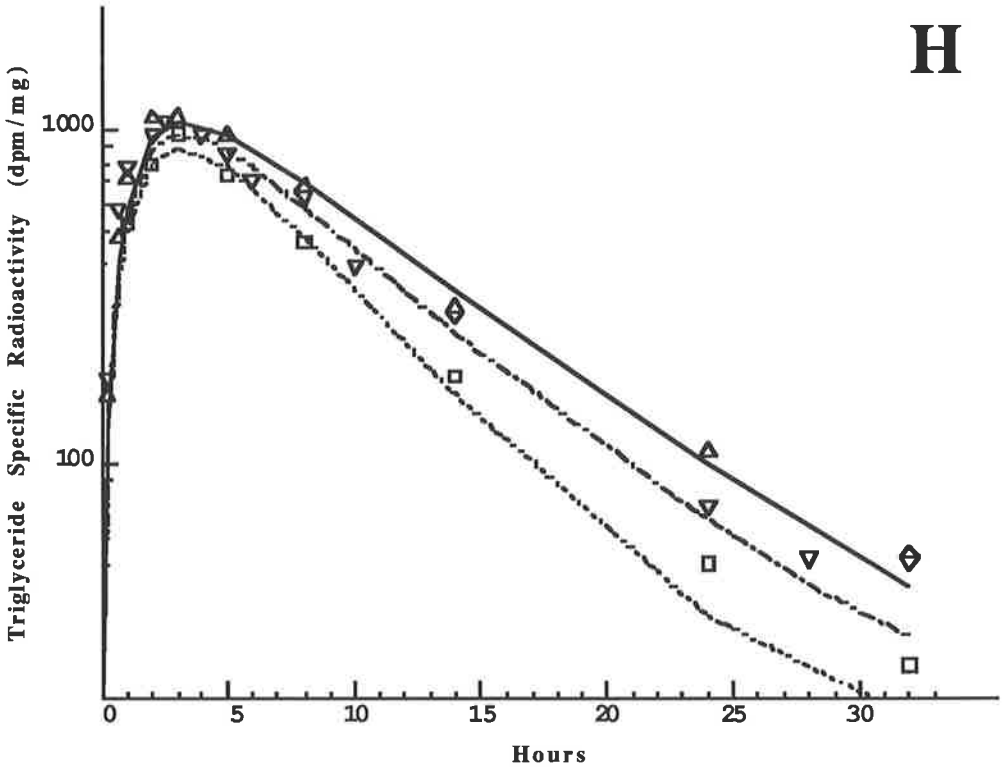


Figure 14. (cont'd)

Of major interest was the observation that the peak specific radioactivity within the VLDL2 fraction, in all subjects, was up to two times greater than that in VLDL1 (Figure 15, subject K), an observation which has also been observed by Streja et al (1977), Steiner and Ilse (1981) and Steiner and Reardon (1982). This implies that there may be independent input of new triglyceride, which has a greater specific radioactivity than that of the larger particles, into the VLDL2 fraction. Of interest also is the time to peak specific radioactivity of this fraction which was 0.25-1.0 hr after that in the VLDL1.

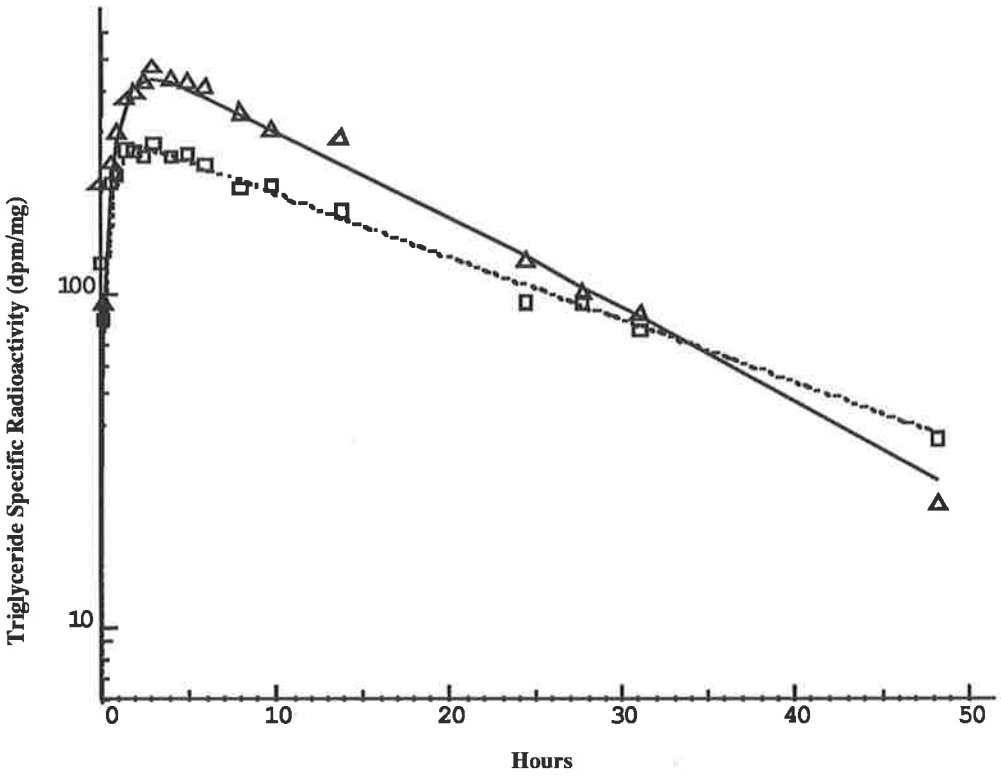


Figure 15. Triglyceride specific radioactivity decay curves of VLDL1 (□) and VLDL2 (Δ), for subject K. The symbols represent observations while the curves show the fits to the model. This figures demonstrates the higher specific radioactivity attained by the VLDL2 fraction.

3.3.4. Triglyceride Kinetics in IDL and LDL

Intermediate density lipoprotein triglyceride kinetics (Figures 16 and 17) generally exhibited the same features as the VLDL-triglyceride kinetics. There were however some differences, the rise of the specific radioactivity curve was not as rapid. The time to peak specific radioactivity was longer than that observed for the VLDL fractions, while the slowly decaying tail was comparable to that of the VLDL fractions. In subjects H and K peak IDL specific radioactivities occured at, and marginally after the point of crossover of the VLDL2 curve respectively. In subjects F and J however the IDL curves attained peak specific radioactivity prior to crossing the VLDL2 curve suggesting direct input of labelled triglyceride into the IDL fraction. The absence of an initial

rapid decay of the IDL curve suggests that this fraction is hydrolysed more slowly than the VLDL fractions. This, is however dependent upon the interpretation of the rise and fall of the triglyceride specific radioactivity curve. Low density lipoprotein triglyceride kinetics were similar to those of the IDL fractions. LDL specific radioactivity curves did however rise more slowly than did IDL and generally decayed at a slower rate. Like the VLDL and IDL curves there appeared to be no apparent delay in the initial rise of the LDL specific radioactivity curve. IDL and LDL triglyceride specific radioactivity curves attained peak specific radioactivity many hours before the IDL and LDL apo B curves reached their maximum, suggesting the direct input of triglyceride with high specific radioactivity into the IDL and LDL fractions.

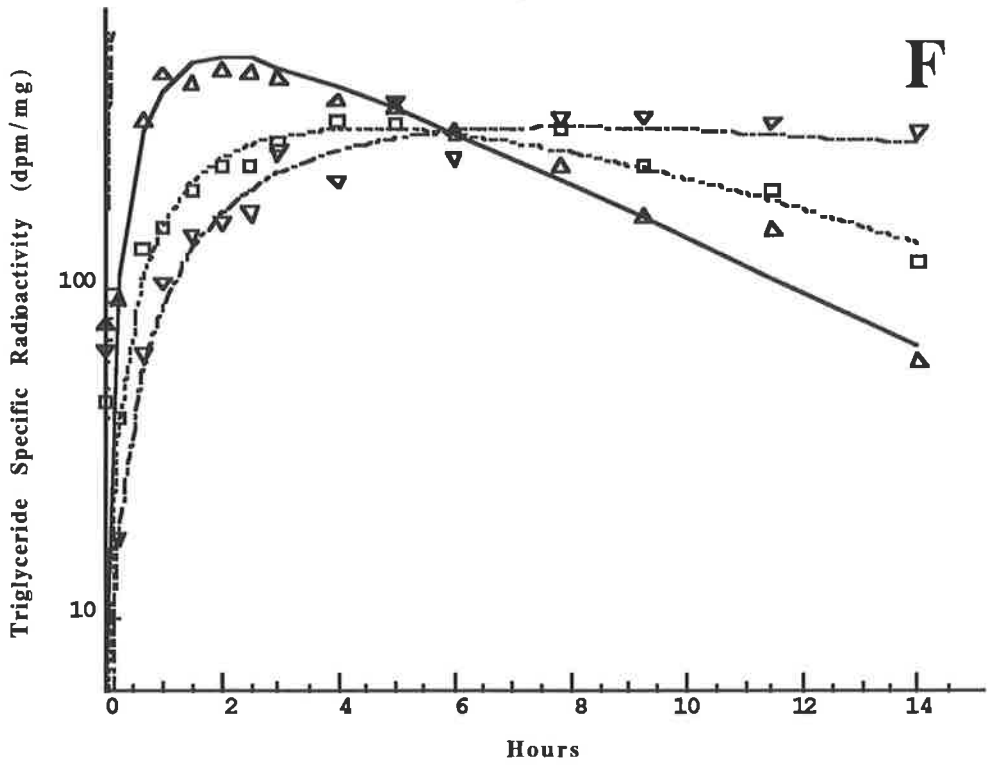


Figure 16. Triglyceride specific radioactivity decay curves of unfractionated VLDL2 (Δ), IDL (□) and LDL (∇) for subjects F, K, H and J respectively. The symbols represent observations while the curves show the fits of the model (Figure 51, Section 3.5) to the data. The fits to the data were derived by coupling the ^{131}I apo B and triglyceride data.

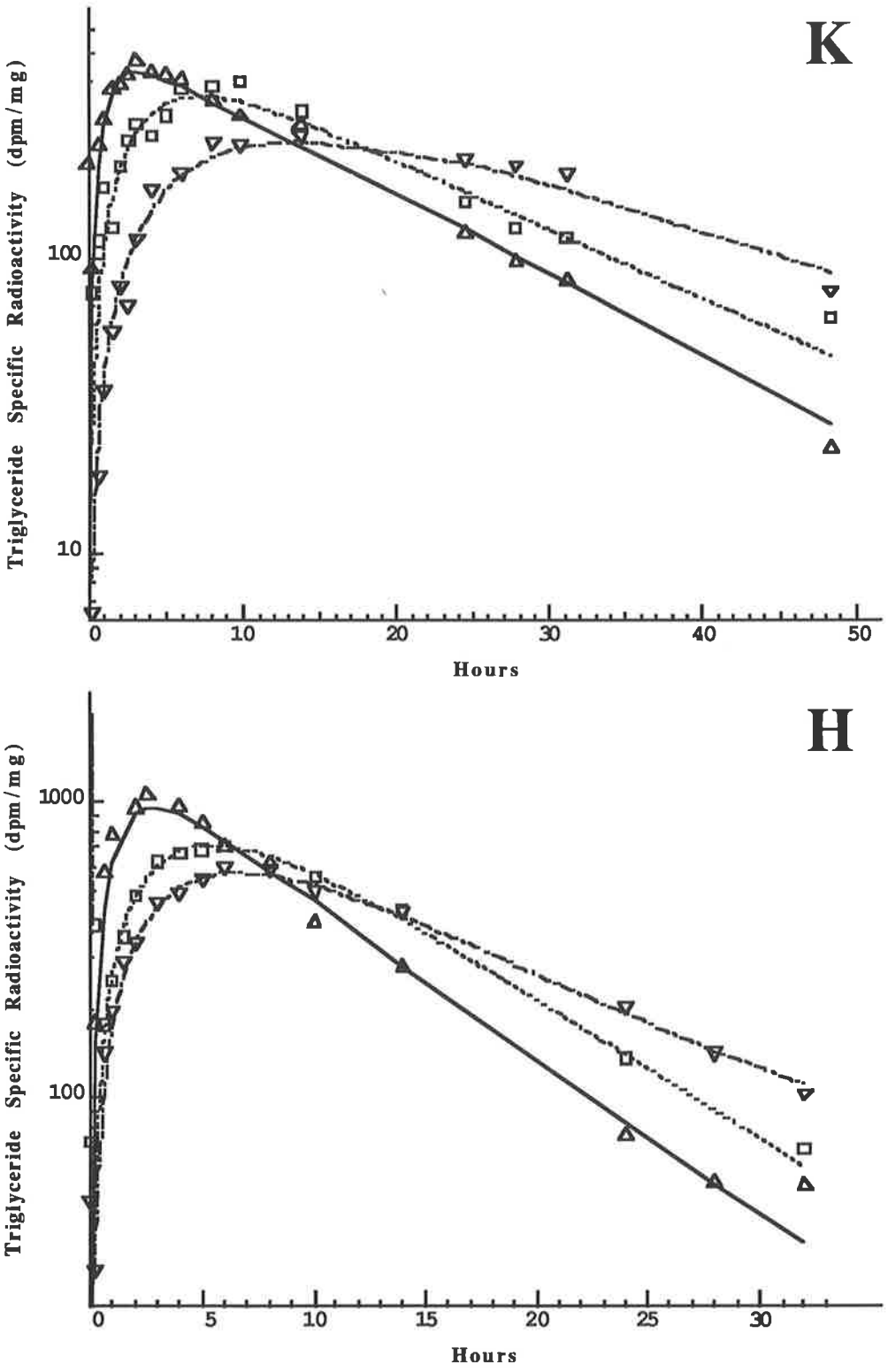


Figure 16. (cont'd)

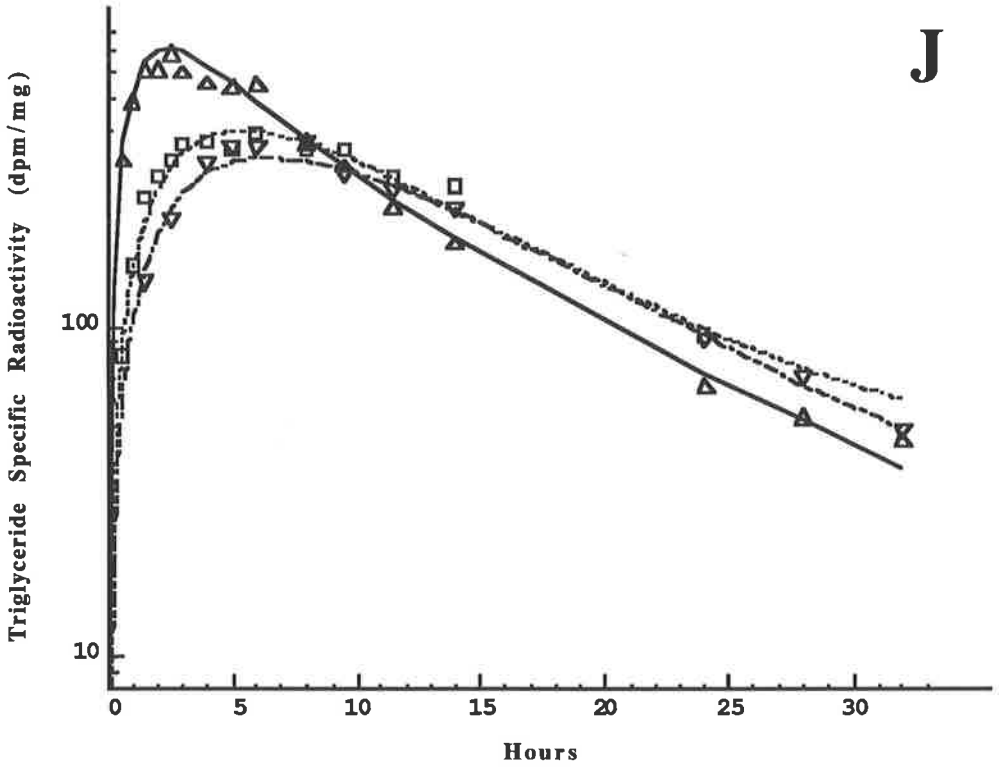


Figure 16. (cont'd)

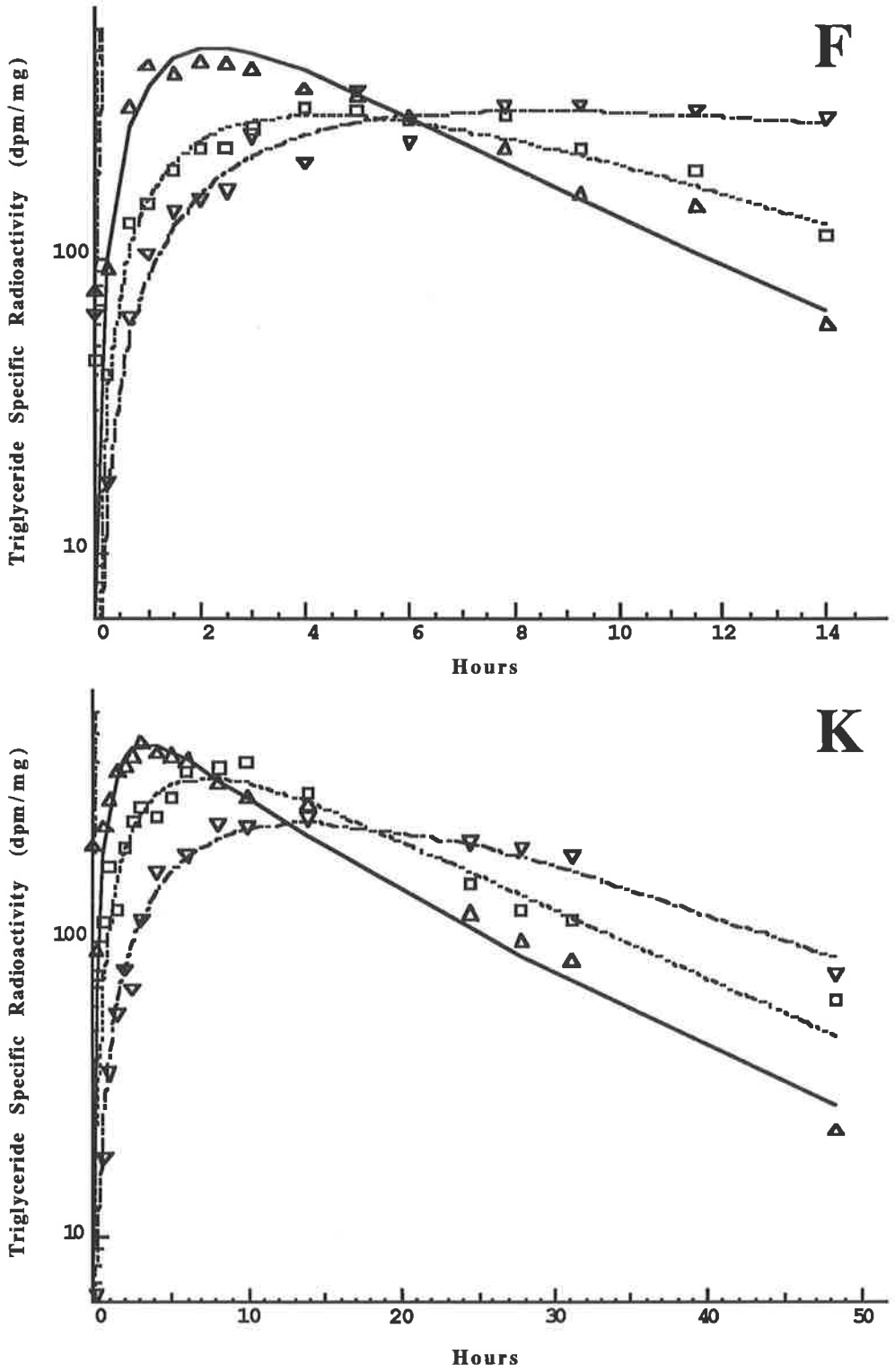


Figure 17. Triglyceride specific radioactivity decay curves of unfractionated VLDL2 (Δ), IDL (□) and LDL (∇) for subjects F, K and H respectively. The symbols represent observations while the curves show the fits of the model (Figure 51, Section 3.5) to the data. The fits to the data were derived by coupling the ^{125}I apo B and triglyceride data.

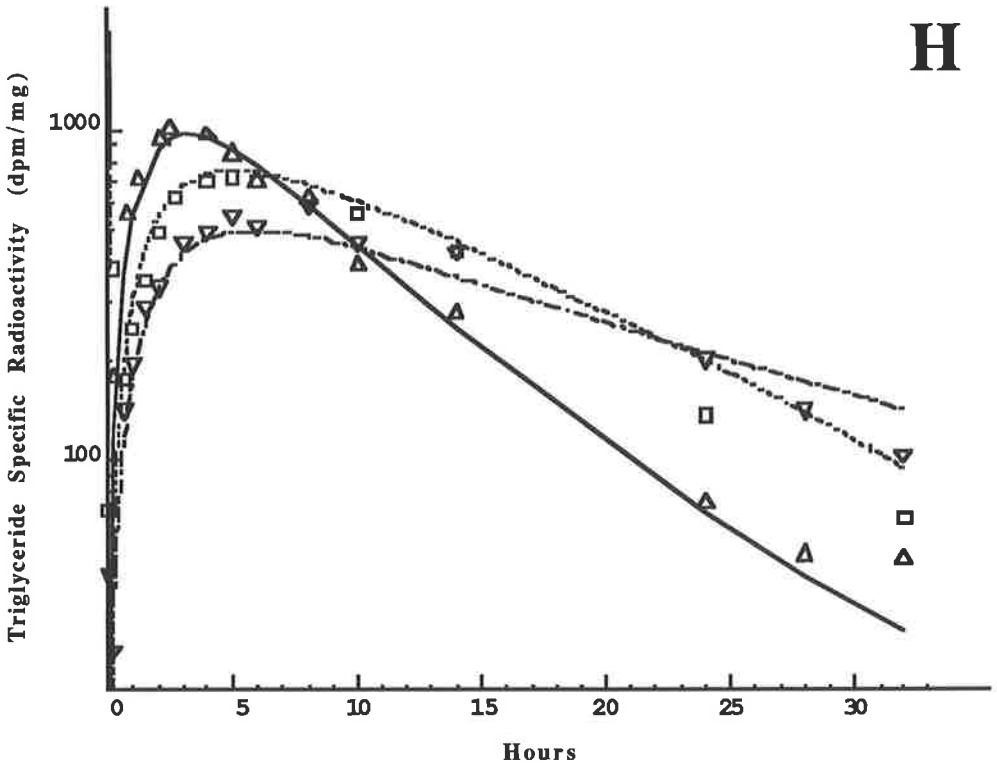


Figure 17. (cont'd)

3.4. Model development

To develop a model to account for the data observed in these studies a number of assumptions were made during the course of the modelling process. The first of these assumptions describes the relationship which exists between apo B radioactivity and apo B mass. This relationship, which holds only at zero time, the time of reinjection of labelled lipoproteins, is that apo B specific radioactivity is uniform within a labelled VLDL fraction, i.e. unbound VLDL1, or bound VLDL2. (It was not assumed that the initial specific radioactivities of the unbound and bound apo B fractions were the same.) This assumption permits relationships between apo B radioactivity and the rate constants connecting compartments to be defined. Assume that within a given labelled VLDL fraction there are two populations of particles, one of which is thought

to be the product of the other. The distribution of radioactivity between these populations is proportional to the size, or mass of each population such that,

$$\frac{IC(1)}{M(1)} = \frac{IC(2)}{M(2)}$$

where IC is radioactivity and M is mass. Under steady state conditions, rate constants between connecting compartments are inversely proportional to compartmental mass giving,

$$M(1) * L(2,1) = M(2) * L(2,2)$$

where L(2,1) is the rate constant describing transport from compartment 1 to compartment 2, and L(2,2) is the turnover rate constant of compartment B. Thus a relationship between radioactivity and rate constants can be defined,

$$IC(1) * L(2,1) = IC(2) * L(2,2)$$

This relationship permits the assignment of radioactivity to compartments and defines relationships between populations of particles which have not been physically separated.

Another assumption which has been used to develop a model for apo B and triglyceride metabolism relates to the residence time of apo B and triglyceride within the same compartment (Residence time is the inverse of fractional catabolic rate, FCR). This assumption constrains, or couples the residence time of apo B and of triglyceride within a given compartment to be equal. The FCR for both moities in a single compartment will therefore also be the same. Early kinetic studies represented the VLDL as a single compartment,

and as such the residence times of both apo B and triglyceride were equal. The physiological implication of equal residence times is that VLDL particles must be removed whole, without modification, from the circulation and are not able to return. Numerous studies however have demonstrated the need for hydrolysis of VLDL particles prior to removal from the circulation. Assuming that apo B and triglyceride kinetics are coupled and that within a model provision must be made for delipidation, compartments in series, referred to as a chain or a delipidation cascade can be used to account for the differences observed in the kinetics of these two moieties. Although the residence times of apo B and of triglyceride within a single compartment remain the same their residence times will be different for the chain. The residence time for VLDL triglyceride will be less than that for apo B. If, for example, compartments A and B are used to model VLDL apo B and triglyceride kinetics where (1) B is the product of A, (2) each compartment turns over at the rate of once per hour, (3) there is no loss of apo B from A, and (4) 25% of triglyceride in A is lost via hydrolysis then the residence times for VLDL apo B and triglyceride in the system (i.e. compartments A and B) will be 2.0 hr and 1.75 hr respectively. The residence times for apo B and triglyceride in the individual compartments are however the same.

As illustrated in Table 11 there was significant, rapid transfer of radioactivity from the VLDL1 to VLDL2 fraction at the time of reinjection of the labelled lipoproteins. Initially this study was designed to examine the conversion of VLDL1 to VLDL2, IDL and LDL but because of this complication it was not possible to determine the contribution of VLDL1 to apo B and triglyceride production. Accordingly, model development here focuses only upon the transformation and metabolism of VLDL2 apo B and triglyceride to IDL and LDL, and the relationships of unbound VLDL2 and bound VLDL2 to this

cascade. Despite this however the model developed for VLDL2 also described the apo B and triglyceride kinetics in VLDL1. Possible explanations for this rapid transfer will be discussed later.

3.4.1. Model 1. Precursor-product (VLDL)

Based solely upon the apo B kinetics it appeared that there was a precursor-product relationship between the unbound and bound fractions. The indication was that the bound fraction was derived from the unbound, and so a simple model (Figure 18) was tested. Previous studies in this laboratory (Nestel et al, 1983) had shown that when radiolabelled unbound VLDL was reinjected it was converted to the bound fraction. It was also noted that it was possible to have direct input of apo B into the bound fraction. The first VLDL model therefore comprised two pools, one of which accounted for the kinetics of the unbound fraction and one for the more slowly turning over bound fraction.

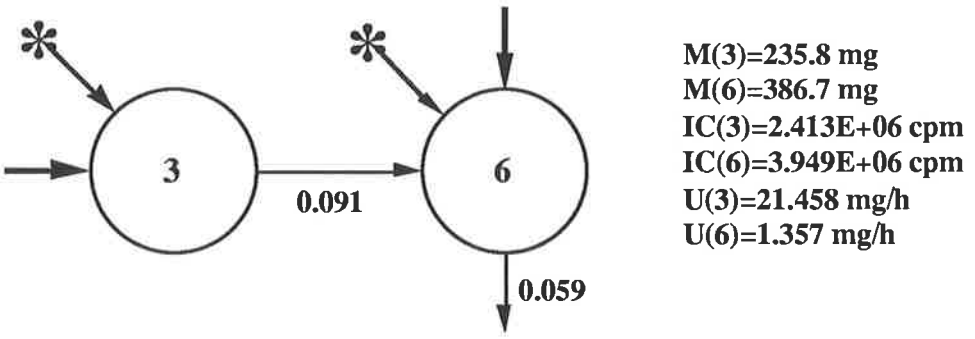


Figure 18. Two compartment model used to describe the kinetics of apo B in unbound (comp 3) and bound (comp 6) VLDL2 fraction in subject K. It was assumed that when labelled VLDL2 (unbound and bound) was reinjected radioactivity distributed in proportion to apo B mass in the unbound (M(3)) and bound (M(6)) pools. To satisfy the steady state conditions direct input of apo B into the bound fraction was required. L(i,j) h^{-1}.

A reasonable fit to the data was obtained using this simple model (Figure 19). This model however was not able to account for the shoulder observed in the decay curve of the bound fraction although, as will be shown later a more complex model provides a better fit. Based upon the fit of the model to the data we were able to hypothesize that the unbound fraction represented primarily nascent-like particles and the bound represented VLDL remnant particles.

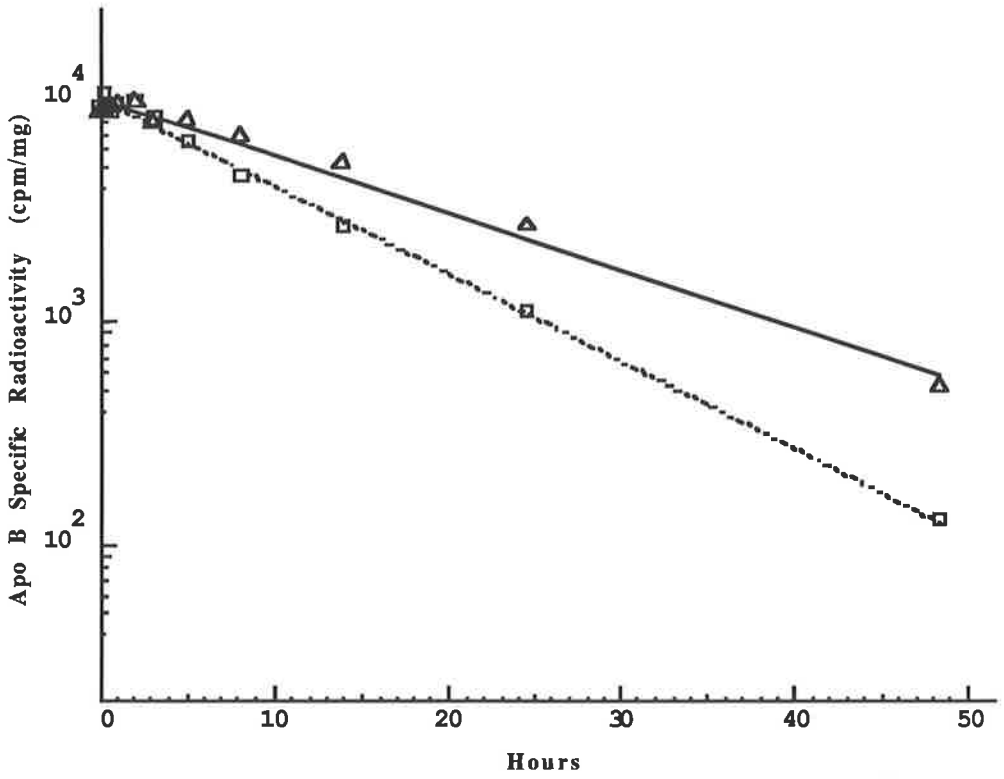


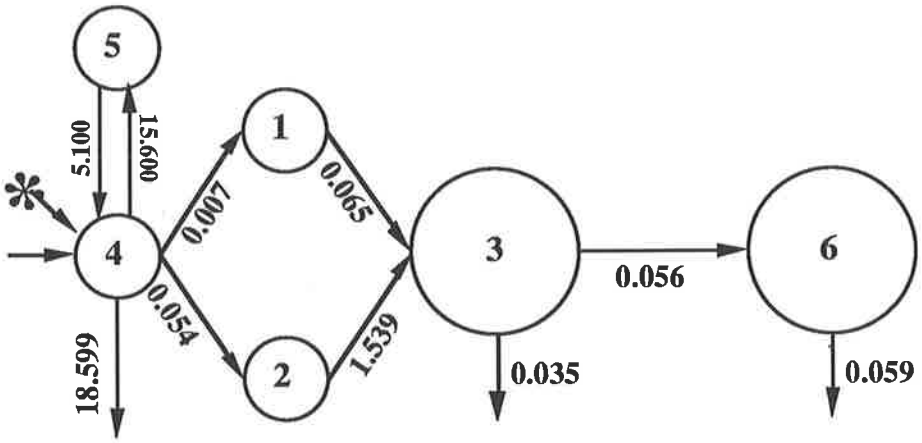
Figure 19. Fit of two compartment model (Figure 18) to unbound (□) and bound (Δ) ^{131}I VLDL2 apo B data of subject K.

To model the triglyceride kinetics it was assumed that the plasma glycerol model proposed by Malmendier et al (1974) would adequately account for glycerol kinetics in hypertriglyceridemic subjects. This is despite the fact that their model was developed in normolipidemic subjects. Malmendier et et (1974) proposed a two-pool model to account for glycerol injected into plasma.

They observed that more than 90% of the glycerol leaving the plasma compartment could be accounted for by conversion to glucose and CO_2. This glycerol subsystem was incorporated into the VLDL triglyceride model to account for glycerol kinetics and as a function to provide input into VLDL triglyceride synthesis compartments.

Several earlier studies by other workers (see Section 1.8.4.2) concluded that the rate limiting step in the metabolism of triglyceride was within the plasma compartment, and that the turnover rate of the liver triglyceride pool was faster that that of the VLDL. Zech et al (1979) included two conversion pathways in their VLDL triglyceride model (analagous to those in shown in Figure 2 (Section 1.8.4.2)), one of which had a turnover rate greater than that of VLDL-triglyceride. The first model proposed to account for the kinetics of unbound and bound VLDL2 triglyceride also used the assumption that liver triglyceride turnover was more rapid than that in plasma. Using the rate constants shown in Figure 18 to describe the turnover rates of the unbound and bound fractions, the model in Figure 20 was able to fit the unbound VLDL2 triglyceride data. Although this model was able to fit the triglyceride data of the unbound fraction (Figure 21) clearly the rate at which radioactivity entered the bound fraction was too slow to match the observed kinetics of the this fraction.

In subject K the VLDL2 triglyceride/apo B ratios of the unbound and bound fractions were 13.9 and 8.5 respectively. Thus for a particle to be converted from the unbound to bound form 38.5% of its triglyceride must be removed. The rate constant out of the unbound fraction ($L(0,3)=0.035\ h^{-1}$) (Figure 20) represents 38.5% of the turnover of the unbound compartment which must be hydrolysed to produce bound particles with their triglyceride/apo B ratio characteristics.



M(3)=3264 mg
M(6)=3264 mg
IC(4)=6.6E+08 dpm

Figure 20. Model of VLDL triglyceride metabolism incorporating glycerol exchange and conversion compartments (compartments 4 and 5, and 1 and 2 respectively). Compartments 3 and 6 represent the unbound and bound VLDL2 triglyceride fractions respectively. It was assumed that all new triglyceride entered the VLDL via the unbound compartment and that triglyceride was hydrolyzed from this compartment. The turnover rate of compartments 3 and 6 were the same as those of the respective compartments in the apo B model (Figure 18). L(0,3) represents that fraction of the triglyceride in comp. 3 which is hydrolysed prior to conversion into the bound form. L(i,j) h^{-1}.

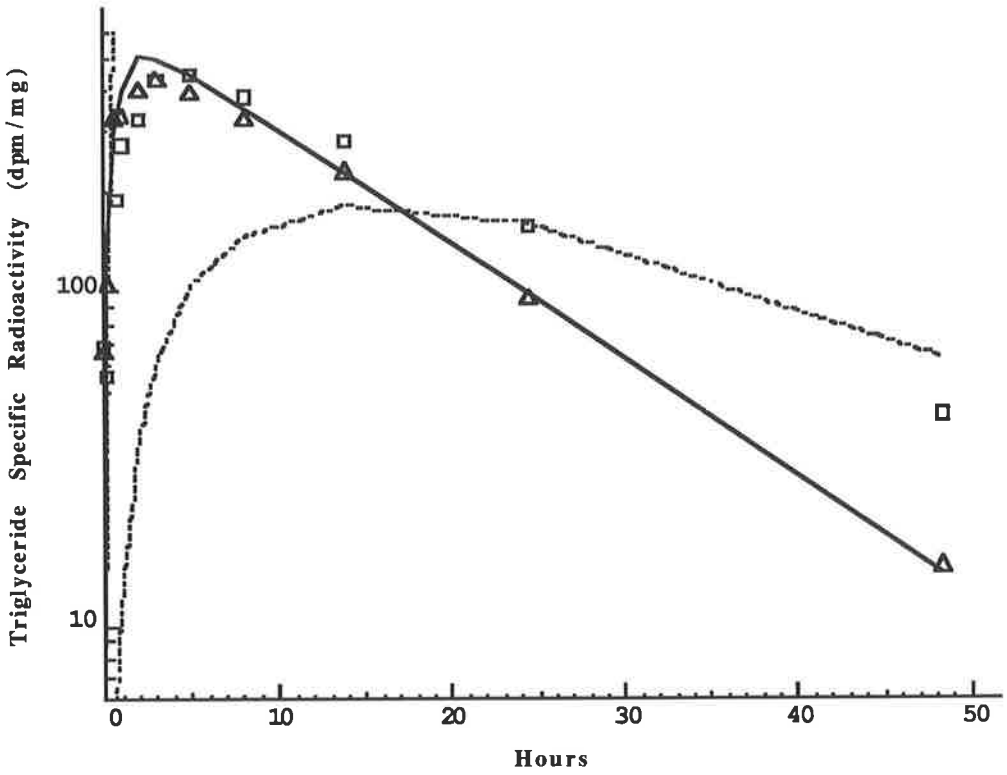


Figure 21. Fit of VLDL2 triglyceride model (Figure 20) to unbound and bound triglyceride data of subject K. Although the fit to the unbound fraction (Δ) is acceptable (zero convergence) the fit to the bound triglyceride data (□) is poor, as illustrated by the dashed line. In fitting these data it was assumed that the turnover of VLDL triglyceride was the rate limiting step in the metabolism of triglyceride, and that liver triglyceride turnover was more rapid.

3.4.2. Model 2. Slow liver, fast VLDL

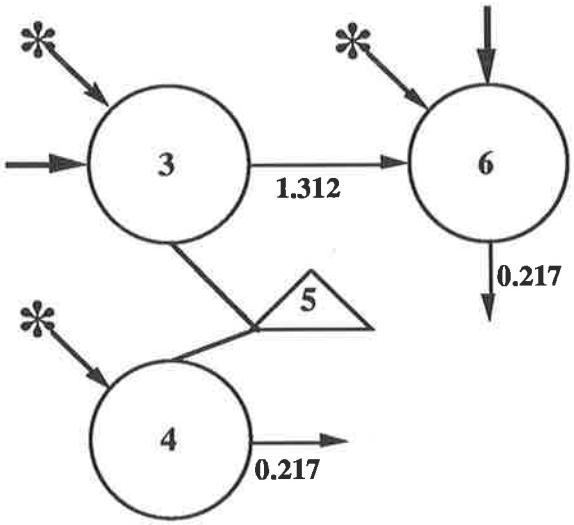
An alternate model was proposed to account for the triglyceride kinetics of the VLDL2 fraction. This model was based upon observations made by other workers, predominantly in non-human species, where they demonstrated that plasma VLDL-triglyceride turnover was more rapid than that of the liver (see Section 1.8.4.1). To propose a new model incorporating these ideas the apo B data was examined to look for evidence, such as a rapidly turning over component, that would support such a hypothesis.

The kinetics of the unbound VLDL2 apo B fraction of subject F (Figure 8) were dominated by a rapidly turning over component and by a minor, less

rapidly turning over component, comparable to the turnover rate of the bound fraction. Using these data, and the assumption that a precursor product relationship exists between the unbound and bound fractions a three compartment model was constructed to fit the apo B data (Figure 22). Three compartments were necessary to fit these data because of the presence of a slow component in the unbound fraction. It was assumed that the turnover rate of this component was equal to that of the bound fraction, and may therefore have represented minor contamination of the unbound fraction by the bound. Of the total mass associated with the unbound fraction the slow component represented less than one percent of this fraction, its impact on the type of model used was therefore negligible. Compared to subject K, the turnover rate of the unbound fraction in subject F was more than ten-fold greater (1.312 vs 0.091 h^{-1}). The turnover rate of the bound fraction was also greater.

The fit of this model to the VLDL2 apo B data of subject F is shown in Figure 23. Note the discrepancies in the initial specific radioactivity values of the unbound and bound fractions. Had these been closer at zero time than the bound curve may have exhibited a more pronounced delay prior to its decay.

In all subjects direct input of apo B into the bound fraction, other than that from the unbound fraction, was required to satisfy steady state conditions. This observation suggested that although the bound fraction appeared to be the product of the unbound fraction apo B was not derived exclusively from the unbound fraction.



M(5)=43.2 mg
M(3)=42.83 mg
M(4)=0.37 mg
M(6)=412.0 mg
IC(3)=6.726E+05 cpm
IC(4)=5.871E+03 cpm
IC(6)=1.546E+07 cpm
U(3)=56.188 mg/h
U(6)=33.274 mg/h

Figure 22. Three compartment model used to describe the kinetics of apo B in unbound (comp 3 and 4) and bound (comp 6) VLDL2 fraction in subject F. It was assumed that when labelled VLDL2 (unbound and bound) was reinjected radioactivity distributed in proportion to apo B mass in the unbound (M(3) + M(4)) and bound (M(6)) pools. To satisfy the steady state conditions considerable direct input of apo B into the bound fraction was required. L(i,j) h^{-1}.

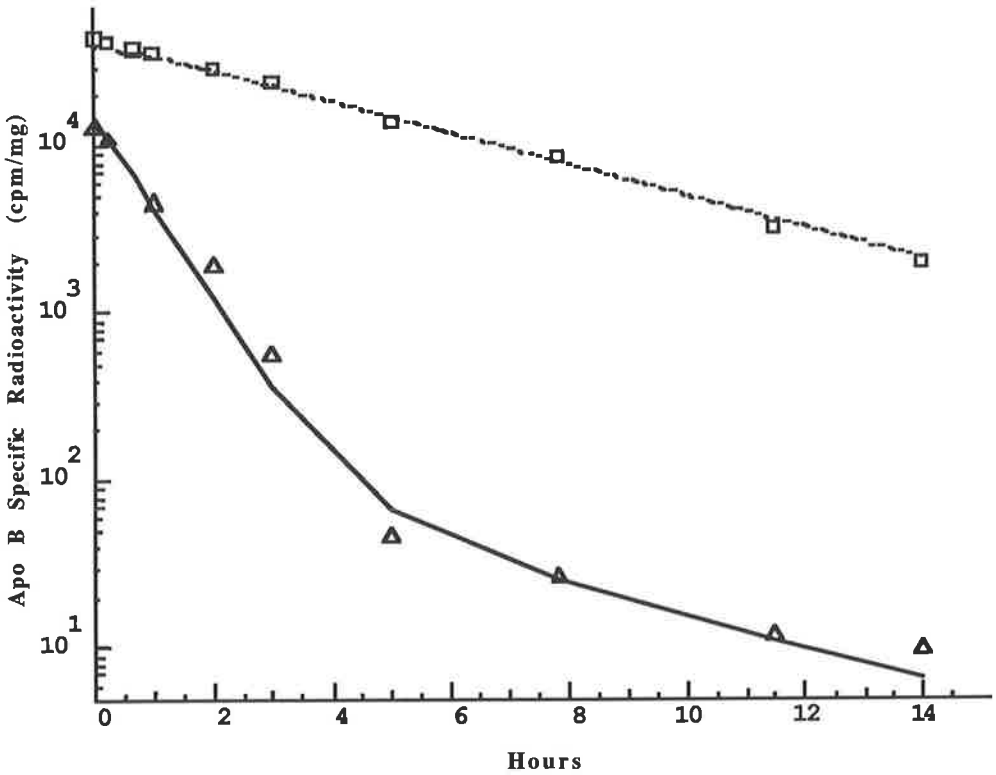


Figure 23. Fit of three compartment model (Figure 22) to ^{131}I unbound (Δ) and bound (□) VLDL2 apo B data of subject F following reinjection of labelled VLDL2.

In the previous triglyceride model (Figure 20) the turnover rate of the unbound VLDL2 triglyceride pool approximated the falling slope of the specific radioactivity curve. The rising slope of this curve however respresented the more rapidly turning over liver triglyceride compartment. In developing a new model to describe VLDL triglyceride kinetics it was assumed that the rate limiting step of triglyceride metabolism was in the liver (Figure 24). A new model was developed (Figure 24) where the rising slope of the triglyceride specific radioactivity curve is a function of the rapidly turning over unbound compartment and the falling part of the curve a function of the liver triglyceride compartment (comp. 2). Evidence of the slowly turning over liver triglyceride pool would appear as one of the later exponential functions, such as in the tail of the specific radioactivity curve, where evidence of the slow triglyceride synthesis pathway proposed by Zech et al (1979) is observed.

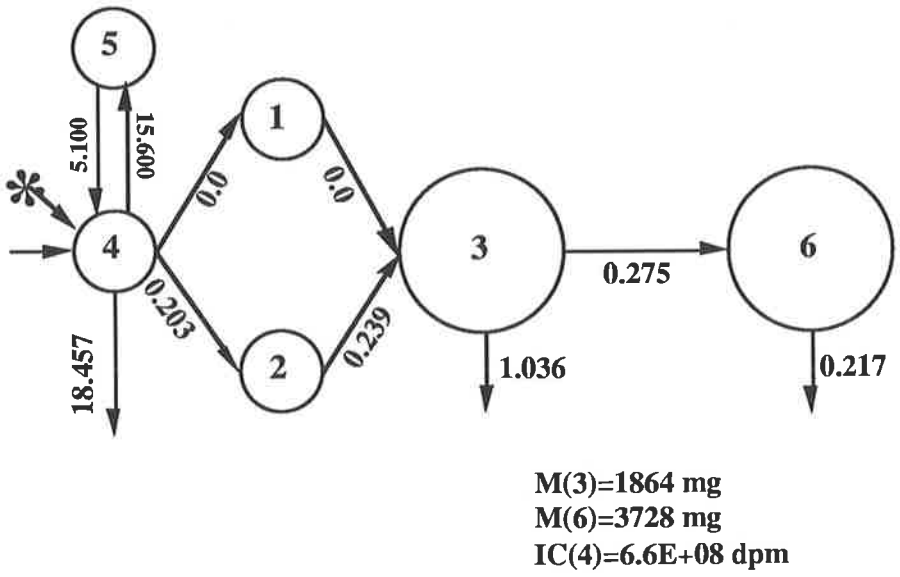


Figure 24. VLDL2 triglyceride model for subject F. In this model the unbound compartment (comp 3) turns over more rapidly than that of the liver compartment (comp 2) illustrating that the liver triglyceride compartment is the rate limiting step. The turnover rate of the bound fraction (comp. 6) is however comparable to that of the liver triglyceride compartment. L(i,j) h^{-1}.

Figure 25 shows the fit of the slow-liver model to VLDL2 triglyceride specific radioactivity data for subject F. Although the fit to the unbound data is good, once again the fit to the bound data is poor. This demonstrates that although the unbound VLDL2 triglyceride fraction turns over rapidly its turnover rate is not fast enough to produce the rapid rise observed in the specific radioactivity of the bound fraction. The faster turnover of the unbound fraction combined with the more rapid turnover of the bound fraction did however reduce the time to Tmax of the bound specific radioactivity curve. That the unbound data can be fit using this model demonstrates that either a fast-liver -> slow VLDL or slow-liver -> fast VLDL model can be used to fit such data. The physiological implications of using either model are significant and will be discussed later.

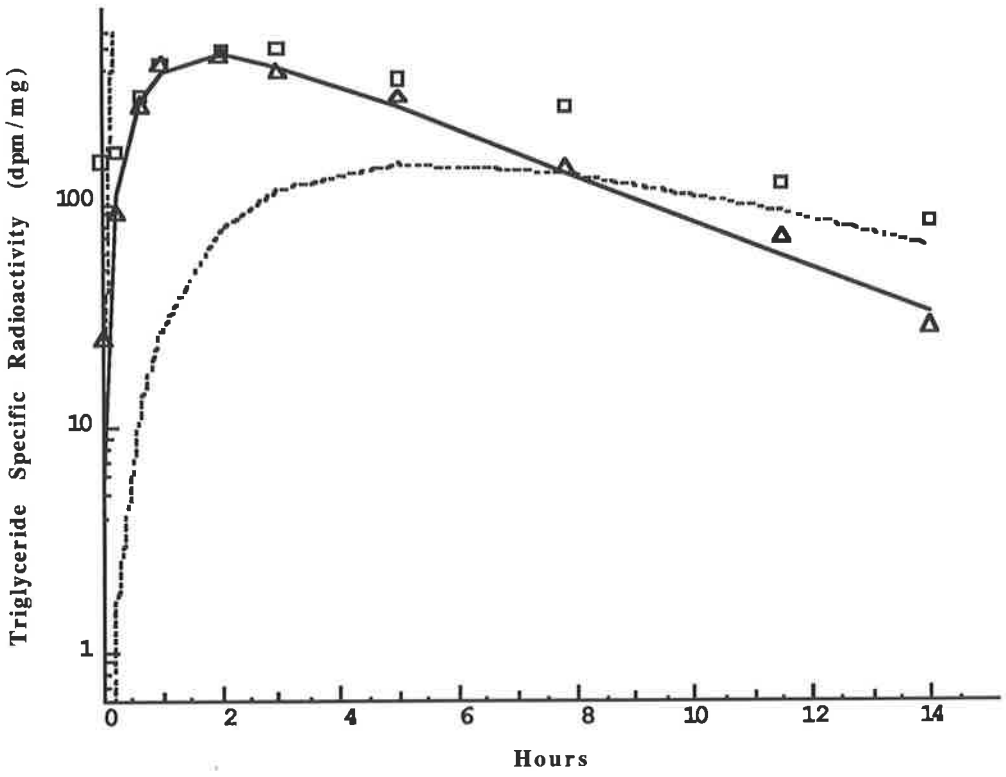


Figure 25. Fit of slow liver triglyceride model to unbound (Δ) and bound (□) VLDL2 triglyceride data of subject F. The dashed line represents the calculated fit to the bound data using the model in Figure 24.

Two features of the bound fraction kinetics prevented this model from fitting the data. The first being the near simultaneous rise in specific radioactivity of the unbound and bound fractions, and secondly the complex nature of the bound fraction curve after reaching its maximum specific radioactivity. The complex shape of the bound fraction's curve after peak specific radioactivity suggested that the bound fraction did not represent a homogeneous population of particles.

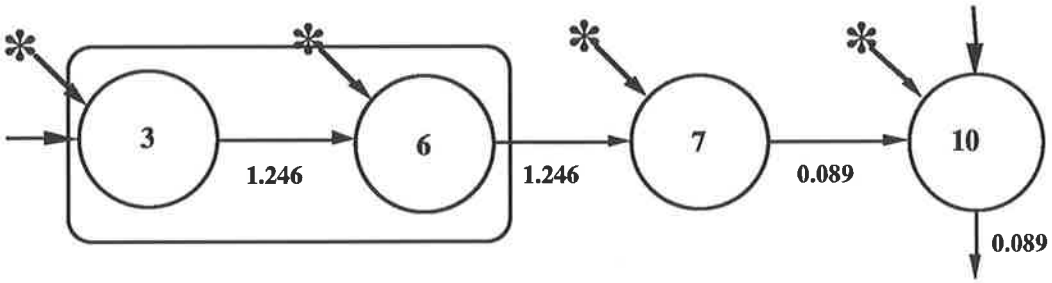
The presence of a rapidly turning over component in the unbound fraction was also observed in the earlier studies of Nestel et al (1983) in humans, and Huff and Telford (1984) who injected labelled human lipoproteins into minature pigs. Following the reinjection of labelled VLDL into humans both rapidly and slowly turning over components were identified in the VLDL apo B decay curves. In some subjects the unbound fraction decayed slowly while in others the decay was rapid. Assuming that the liver triglyceride conversion process is the rate limiting step in triglyceride metabolism there must be, within the unbound fraction a population of particles which turnover rapidly, at a rate comparable to that observed in subject F (approximately 1 h^{-1}). The fact that the postulated rapid component in the unbound VLDL2 was only seen in one of the three subjects could be readily explained in the unbound fraction in two of the other subjects, but not in subject F, contains a slowly turning over remnant-like fraction which is derived from the putative rapidly turning over more nascent like particles. In that case the kinetics of the rapidly turning over component would be masked. This concept is developed further in Model 3, below.

3.4.3. Model 3. Heterogeneous unbound VLDL

Taking the assumptions stated above, and the need to include the presence of a rapidly turning over population(s) of particles within the unbound fraction a new model was proposed to describe the kinetics of the unbound apo B fraction in subjects K and H. Although the decay of the unbound fraction in these subjects was mono-exponential and relatively slow compared to that of subject F, it is possible that this fraction may represent a heterogeneous population of particles, some of which turnover at a rate comparable to that of the unbound abo B specific radioactivity curve in F. In an experiment where a heterogeneous group of particles is labelled, and assuming uniform labelling, the kinetics of the most slowly turning over component will dominate while those of the more rapidly turning over components will be hidden. For this to be true however it is assumed that the slowly turning over fraction represents the largest fraction of the particle population. The model shown in Figure 26 describes the kinetics of the unbound fraction in subject K. This model, which assumes heterogeneity within the unbound fraction includes two rapidly turning over compartments (comps. 3 and 6) which, in series, are the precursors of the more slowly turning over unbound compartment (comp. 7), which in turn flows into a bound VLDL2 apo B compartment (comp. 10).

The notion of compartments in series is analagous to the delipidation chain in the Berman et al (1978) apo B model. Unlike their model however, two compartments in series represents the minimum number of compartments that may be present in the delipidation process which will fit the data. In addition, at least two compartments are required to maintain the coupling of the apo B and triglyceride data and therefore constrain equal residence times

for apo B and triglyceride in the same compartments while accounting for different residence times for these moieties in the unbound fraction overall.



M(3)=14.83 mg
M(6)=14.83 mg
M(7)=206.1 mg
M(10)=386.7 mg
IC(3)=1.505E+05 cpm
IC(6)=1.505E+05 cpm
IC(7)=2.906E+06 cpm
IC(10)=3.744E+06 cpm
U(3)=18.490 mg/h
U(10)=16.196 mg/h

Figure 26. Four compartment model used to describe the kinetics of apo B in unbound (comp 3,6 and 7) and bound (comp 10) VLDL2 fractions in subject K. Although not observed in the unbound decay curve it was assumed that the unbound fraction contained particles which turned over rapidly. The turnover rate of these compartments being determined by the rise of the triglyceride specific radioactivity curve. The turnover rate of compartment 7 was equal to the slope of the unbound apo B decay curve. It was also assumed that the turnover rate of the bound apo B fraction was the same as that of the compartment 7. This model allowed for the direct input of apo B into the bound fraction. L(i,j) h^{-1}.

To construct this model it was assumed that apo B radioactivity was distributed uniformly throughout the unbound fraction. This assumption, together with the relationship which exists between mass and the rate constants of connecting compartments enabled the following relationships to be defined relating radioactivity amd rate constants. For example,

$$IC(6)=IC(3)*L(6,3)/L(7,6)$$

$$IC(7)=IC(6)*L(7,6)/L(10,7)$$

where, $L(6,3)=L(7,6)$

and, $IC(3)+IC(6)+IC(7)=3.206E+06$ cpm

The fit of this model to the ^{131}I VLDL2 apo B of subject K is shown in Figure 27. The fit to both the unbound and bound fractions was better than that obtained by using the simpler precursor product model (Figure 18).

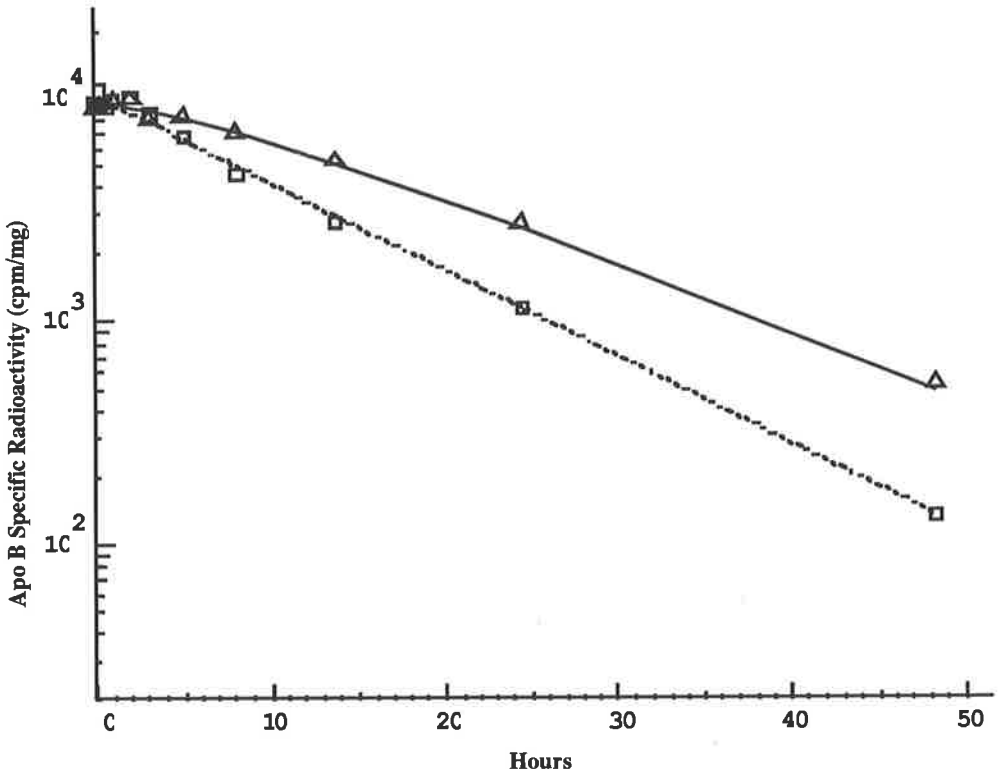


Figure 27. Fit of four compartment model (Figure 26) to unbound (□) and bound (Δ) VLDL2 apo B data of subject K, where the kinetics of the unbound fraction were described by the sum of three compartments, two of which turned over at a rate of 1.246 h^{-1}. The rapid turnover rate was defined by the rise of the VLDL2 triglyceride specific radioactivity data.

A three compartment model was fit to the unbound fraction despite the absence of any kinetic evidence to suggest the presence of more than one compartment. The inclusion of rapidly turning over compartments in the model was based upon the need to account for the rapid rise of the VLDL triglyceride specific radioactivity curve; assuming that the liver triglyceride compartments turnover more slowly that of VLDL. It is important to remember that during the development these models apo B and triglyceride data were

modelled simultaneously. As a consequence of this the turnover rates of compartments 3 and 6 in Figure 26 were defined by the function describing the rapid rise of the unbound VLDL2 triglyceride data.

Examination of the unbound and bound VLDL2 apo B decay curves in subjects H and K revealed that the unbound fraction decayed at a faster rate and mono-exponentially and as shown in Figure 18 the data could be modelled in such a way. Closer examination of these curves may however suggest that they decay at the same rate and that the differences observed in these curves may be a function of the precursor product relationship existing between these fractions. The model in Figure 26 incorporated this idea and as shown in Figure 27 the fit to the data was better than that in Figure 19 where the simpler model was used. Note the change in the turnover rate of the bound compartment between this and the simpler model (0.089 vs. 0.059 h^{-1}).

Figure 28 depicts the model used to fit the unbound VLDL2 triglyceride kinetics of subject K. This model which is coupled to that of Figure 26, the apo B model, incorporates two rapidly turning over compartments within the unbound fraction and one unbound VLDL2 triglyceride compartment (comp. 7) which turns over at the same rate as that of the bound fraction (comp. 10).

The following basic relationships between rate constants that define the coupled models (apo B and triglyceride) were assumed.

$$L(0,3)_{TG} + L(6,3)_{TG} = L(6,3)_{apo\ B}$$

$$L(0,6)_{TG} + L(7,6)_{TG} = L(7,6)_{apoB}$$

$$L(10,7)_{TG} = L(10,7)_{apoB}$$

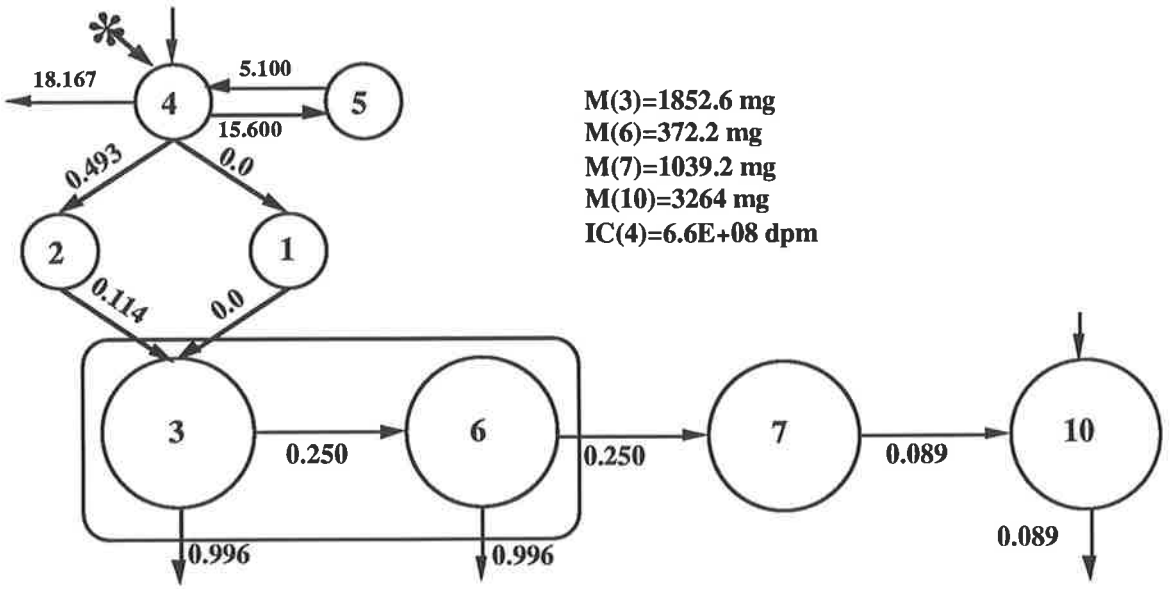


Figure 28. VLDL2 triglyceride model for subject K. In this model two unbound compartments (comp 3 and 6) turnover more rapidly than that of the liver compartment (comp 2) illustrating that the liver triglyceride compartment is the rate limiting step. The turnover rate of the bound fraction (comp. 10) is equal to that of the more slowly turning over unbound compartment (comp 7). The turnover rate of compartments 7 was determined by the kinetics of the unbound VLDL2 apo B data. L(0,3) and L(0,6) represent hydrolysis of triglyceride from compartments 3 and 6 respectively. It was assumed in this model that all triglyceride in compartment 7 was transported to the bound fraction. L(i,j) h^{-1}.

In the earlier model which was used to fit this data (Figure 20) one of the liver triglyceride compartments (comp. 2) turned over more rapidly than that of the unbound fraction. In this model however the turnover rate of this compartment was a magnitude slower than that of the first two compartments (comps. 3 and 6) describing the unbound fraction. It is important to note however that the turnover rate of compartment 7 (slow unbound) is comparable to that of the liver triglyceride compartment. This observation is of great significance and will be addressed later.

The fit of this model to the data is shown in Figure 29. The fit of this model to the unbound data was good, as it was in the other earlier models.

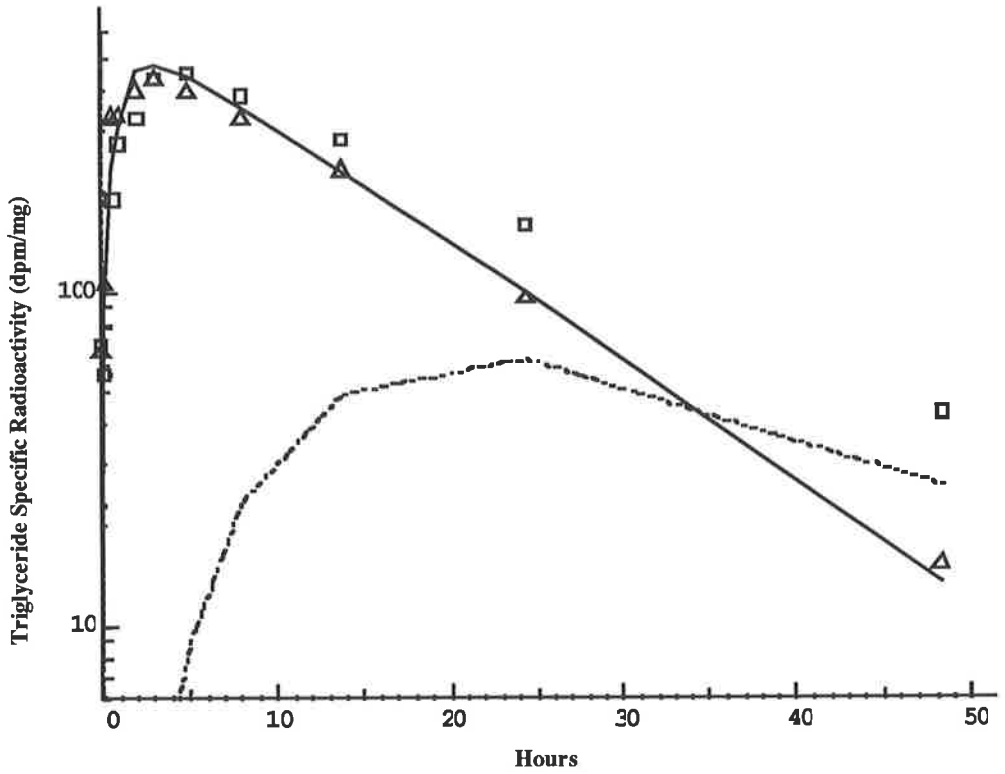


Figure 29. Fit of VLDL triglyceride model (Figure 28) to unbound (Δ) and bound (□) data of subject K. The dashed line represents the fit of the model to the bound data

Again it is clear that the use of a precursor product relationship between the unbound and bound triglyceride fractions will not fit the observed data (Figure 29). The fit to the bound fraction was worse when the more complex rather than when the simpler model (Figure 24) was used. Apparently the rate constant between the last of the unbound and the bound compartment as defined by the apo B data is too slow to permit a good fit fot he bound VLDL triglyceride fraction. In order to fit the bound triglyceride data there must be either a much more rapid transport into this fraction from the unbound than is predicted or like the unbound fraction the bound fraction must represent a heterogeneous population of particles, some of which turnover rapidly.

3.4.4. Model 4. Heterogeneous unbound and bound VLDL

Although the previous model was able to account for the kinetics of the unbound fraction, the single compartment describing the bound fraction resulted in a poor fit to the observed kinetics of the triglyceride moiety. Until this stage it has been assumed, based on the hypothesis of Nestel et al (1983) and the consistent precursor product relationships observed here for unbound and bound VLDL2 apo B, that the bound fraction was the product of the unbound fraction. However, such a model is clearly incompatible with the VLDL2 triglyceride kinetics in the unbound and bound fractions. Moreover, steady state transport rates into the bound fraction using such a simple model suggested that it could only be partially correct. Two further clues about the relationship between the unbound and bound fractions were taken from the studies of Nestel et al (1983) and Huff and Telford (1984). Following the reinjection of ^{125}I-labelled unbound VLDL into human subjects Nestel et al (1983) observed that the specific radioactivity of the bound fraction often started near that of the unbound and thereafter rose very rapidly suggesting that there was rapid, almost instantaneous conversion of unbound to bound particles through the exchange of an apoprotein, presumably apo C, between the two pools. Newly secreted particles may therefore contain both unbound and bound fractions. The proportion of each fraction may be dependent upon the rate of exchange of apo C and its availability in plasma. In addition, Huff and Telford (1984) injected ^{125}I-labelled unbound and ^{131}I-labelled bound VLDL into minature pigs, and although they described different FCR for the two fractions they did observe that the decay curves for the two fractions were biexponential (Figure 30). This observation suggested that not only the unbound fraction but also that the bound fraction represented a heterogenous

population of particles with respect to metabolic and probably physical and compositional properties.

In this regard there are several earlier studies. Thus, Nestel et al (1983) and Huff and Telford (1984) observed different apo E/apo C ratios between the unbound and bound fractions, the ratio being higher in the bound fraction. Nestel et al (1983) noted that this relationship was consistent with the unbound particles being the precursor of a more remnant-like bound particle, a relationship that was also supported by the kinetic data. More recently however Hui et al (1984) and Wilcox and Heinberg (1987) have observed that recent VLDL may be removed from the circulation shortly after secretion, and that the transfer of apo C_3 to these particles, presumably from HDL, diminishes this early uptake. According to this concept nascent-like particles could be found in either the bound or unbound fraction depending on the ratios of apo E/apo C in the particle.

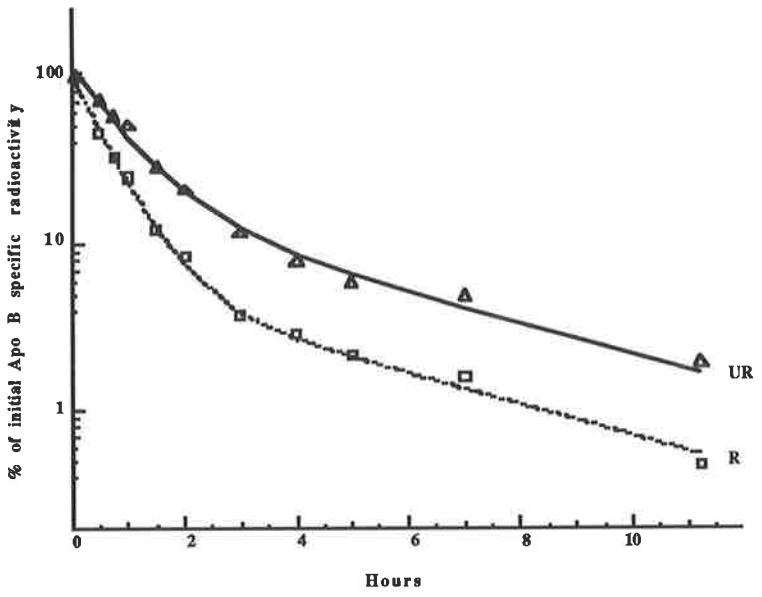


Figure 30. Disappearance curves of heparin-Sepharose fractionated apo B, following the injection into minature pigs of labelled unbound (UR) and bound (R) VLDL fractions (Huff and Telford, 1984).

A new model (Figure 31) was proposed to incorporate these observations with all of the assumptions previously stated. This model assumed that within the bound fraction, as in the unbound fraction, there are particles which turnover rapidly and that, in addition to being the product of the unbound fraction newly synthesized VLDL2 apo B and triglyceride enter directly into the bound fraction. As shown below this assumption relating to direct input into the bound VLDL2 fraction was necessary in order to fit the bound VLDL2 triglyceride kinetic behaviour. The model also allows for remnant-like particles to appear, after delipidation of the more-nascent particles, in both the unbound and bound fractions.

Data from subjects K and H is used to show how this new model fits the observed apo B and triglyceride specific radioactivity data. In addition to the figures described below Figures 7 to 17 show the observed data and the fit of this model, and the integrated model (Figure 51), to the observed data.

In this model the unbound fraction was modelled using three compartments, as in Figures 26 and 28. It was hypothesised that because the triglyceride specific radioactivity curve of the bound fraction increased at a rate equal to that of the unbound fraction there must, within the bound fraction, be particles with similar kinetic characteristics to those in the unbound fraction. Therefore, in the above model, it was assumed that the turnover rate of such particles was the same as that of the unbound fraction. In addition to the turnover rates of these compartments being the same it was assumed that the triglyceride/apo B ratios of unbound and bound compartments on the same level were the equal. It was also assumed that, for each VLDL fraction (unbound and bound), triglyceride was derived from the same pools.

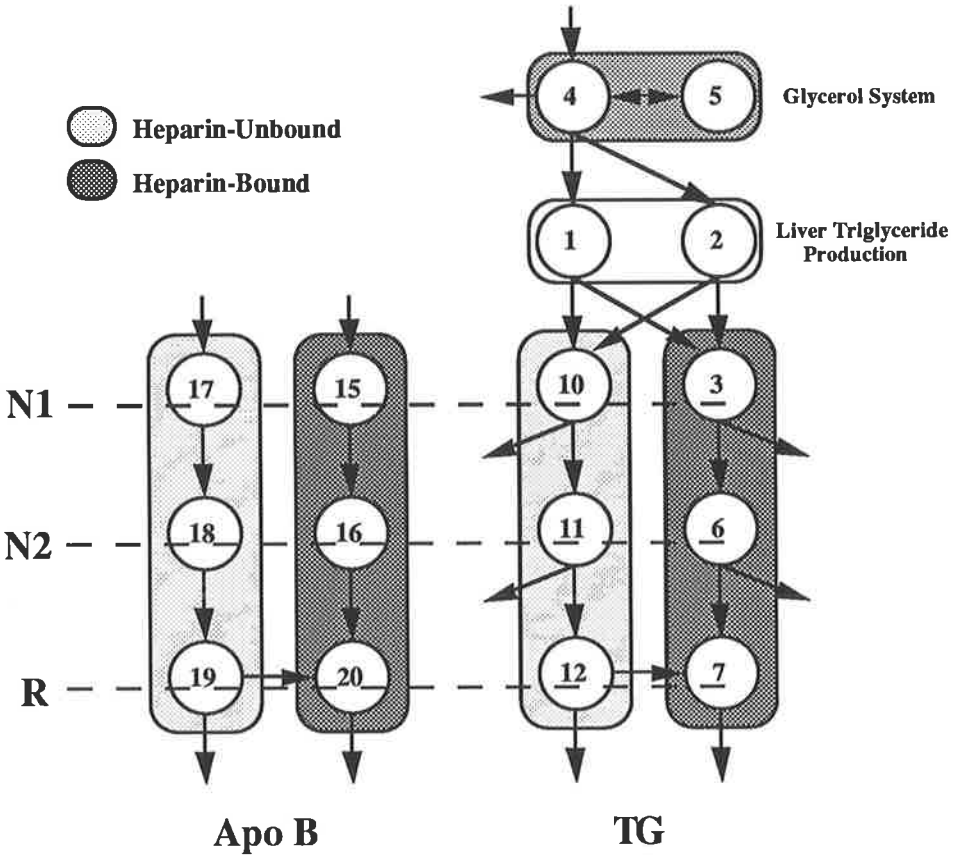


Figure 31. Proposed new VLDL apo B and triglyceride model. This model depicts two parallel delipidation pathways, one for each of the fraction, unbound and bound. N1, N2, and R represent the different stages of metabolism of VLDL particles. From a modelling perspective it was assumed that both apo B and triglyceride enter into both the unbound and bound fractions. No loss of apo B occurs prior to level R although delipidation of triglyceride occurs at the N and R level compartments. The arrows out of the R level compartments represent the sum of material which is converted to the next density level, lost directly from the plasma compartment and for triglyceride what is lost through the process of hydrolysis.

This new model can therefore be simply described as two delipidation pathways, one for each of the fractions (unbound and bound), which are connected via the slowly turning over compartments, and which was required to fit the bound apo B specific radioactivity data. This connection accounts for the net conversion of unbound to bound particles. As in the previous models the turnover rates for apo B and triglyceride in a given compartment are the coupled, such that,

$$L(18,17) = L(16,15) = L(0,10)+L(11,10) = L(0,3)+L(6,3)$$

$$L(19,18) = L(20,16) = L(0,11)*L(12,11) = L(0,6)+L(7,6)$$

$$L(0,19)+L(20,19) = L(0,20) = L(0,12)+L(7,12) = L(0,7)$$

In this VLDL model there are three levels of compartments. Levels N_1 and N_2, for which these compartments have the same rate constants, and the compartments on level R, these compartments turnover more slowly. Although not apparently the same the turnover rates of the unbound and bound R level compartments may be the same when the precursor product relationship between these two fractions is taken into consideration. Input of apo B into the model is via compartments 17 and 15, on level N_1, and loss via level R compartments. In the triglyceride section of the model however loss of triglyceride occurs at all levels while input of triglyceride is into compartments 10 and 3 on level N_1. The fraction of triglyceride hydrolysed from compartments on the same level was the same and, was also the same for the N_1 and N_2 level compartments, i.e.,

$$L(0,3) = L(0,10) = L(0,6) = L(0,11)$$

By imposing such constraints on the compartments in the bound fraction it was assumed that within the bound fraction there were particles with similar kinetic and triglyceride/apo B properties to those of the unbound fraction. Figures 32 and 33 show the VLDL2 triglyceride specific radioactivity curves for the unbound and bound fractions respectively in subject K. In addition, these figures describe the simulated kinetics of the individual compartments hypothesised to be found within each fraction. It is the

summation of these simulated functions which produces the observed specific radioactivity function. The specific radioactivity curves of the rapidly turning over N level compartments rise without delay and appear to reach a maximum between 2 and 4 hours. It is important to note the precursor product relationship between these curves, the fall of these curves although more rapid than that of the observed data, for both the unbound and bound data, does not reflect the rapid (approximately 1 per hour) turnover of these compartments. The rate at which these curves fall, of the order of 0.1 per hour, reflects the turnover rate of the fast liver triglyceride compartment, compartment 2. The turnover rate of the N level compartments however, is described by the rate at which the specific radioactivity curves of the N level compartments rise.

Included in these figures are the simulated curves for the R level compartments. Clearly these compartments have a slower turnover rate, as reflected by the time taken to reach maximum radioactivity. Note the extended peak of the R level compartment in the bound fraction. This characteristic can be attributed to the transport of triglyceride and hence radioactivity from the unbound to the level R bound fraction compartment.

Within the unbound and bound VLDL apo B fractions there are of course the equivalent rapidly and slowly turning over components. Figures 34 and 35 simulate the apo B specific radioactivity functions of the N and R level compartments in the unbound and bound VLDL2 of subject K. In both figures the kinetics of the rapidly turning over N level compartments can be seen. Only in the unbound fraction of subject F (VLDL1 and VLDL2), and that in the VLDL1 fraction of subject K was it possible to observe the kinetics of these rapidly turning over pools in the data.

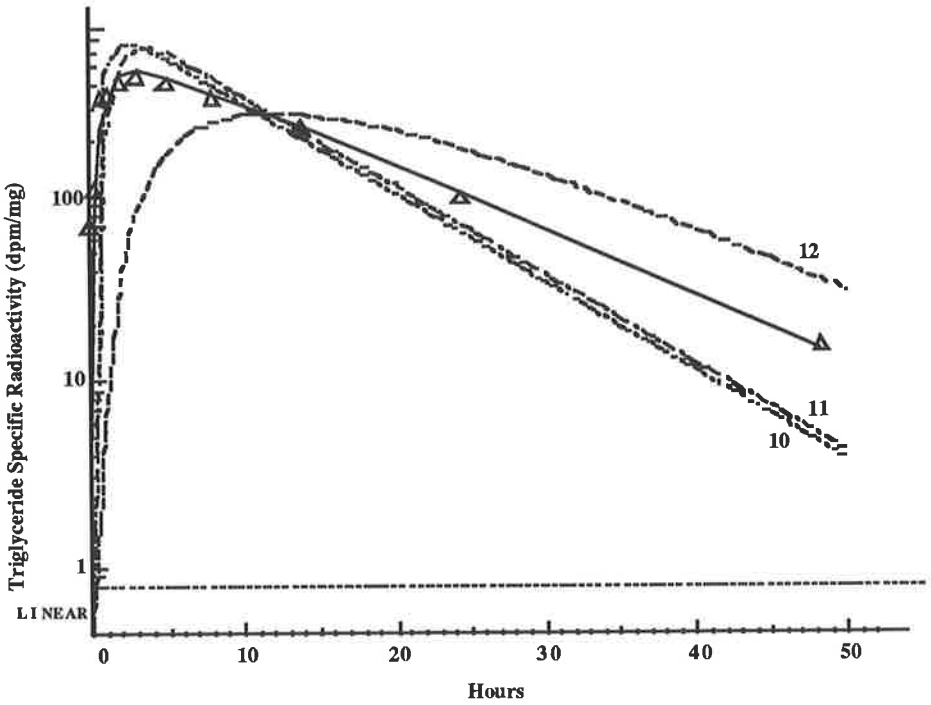


Figure 32. Observed VLDL2 unbound triglyceride specific radioactivity data (Δ) for subject K and model fit to data . The dashed lines represent simulated specific radioactivity functions for the N level (lines 10 and 11) and R level (line 12) compartments.

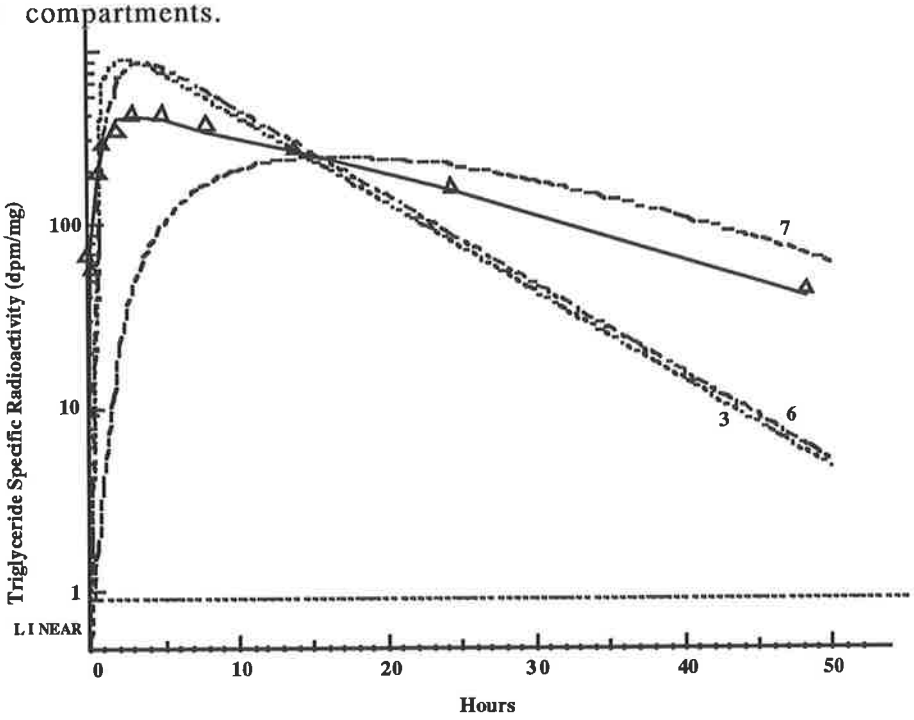


Figure 33. Observed VLDL2 bound triglyceride specific radioactivity data (Δ) for subject K and model fit to data. The dashed lines represent simulated specific radioactivity functions for the N level (lines 3 and 6) and R level (line 7) compartments.

In all other unbound apo B kinetic data the presence of a slowly turning over pool of particles obscured the kinetics of the fast components. It is clear in these figures that the N_2 level compartments are the product of N_1, this is illustrated by a delay prior to the fall of this curve. The slowly falling dashed line, which lies above the observed data curve, describes the kinetics of the R level compartments. Its proximity to the observed data highlights it dominance of the kinetics of these fractions compared to that of the N level compartments which represent only a small fraction of the VLDL.

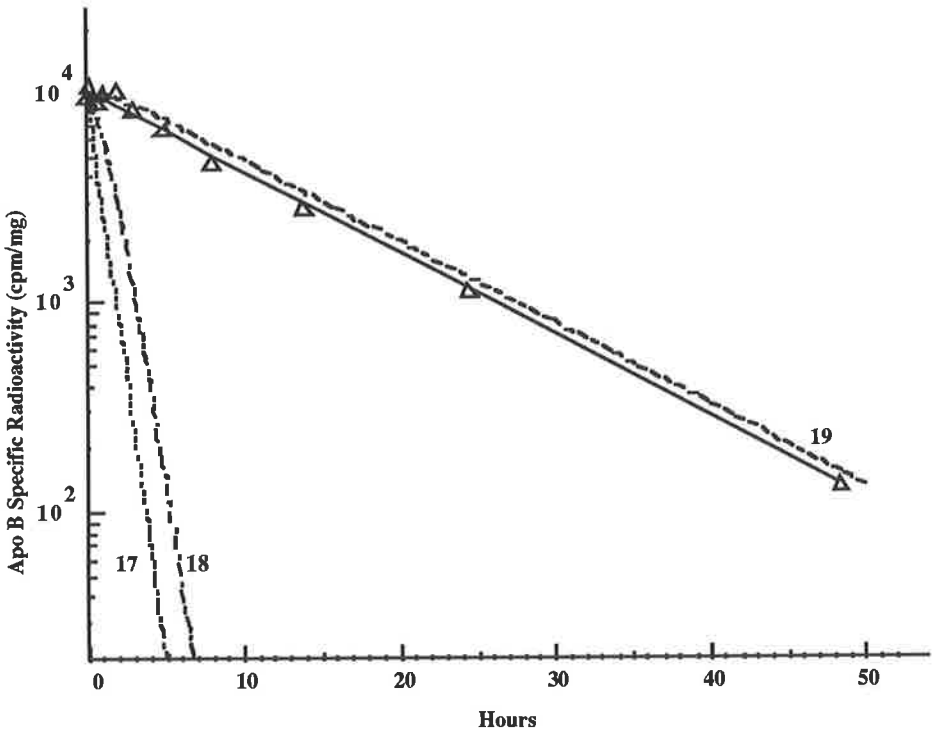


Figure 34. Observed VLDL2 unbound apo B specific radioactivity data (Δ) for subject K and model fit to data. The dashed lines represent simulated specific radioactivity functions for the N level (lines 17 and 18) and R level (line 19) compartments

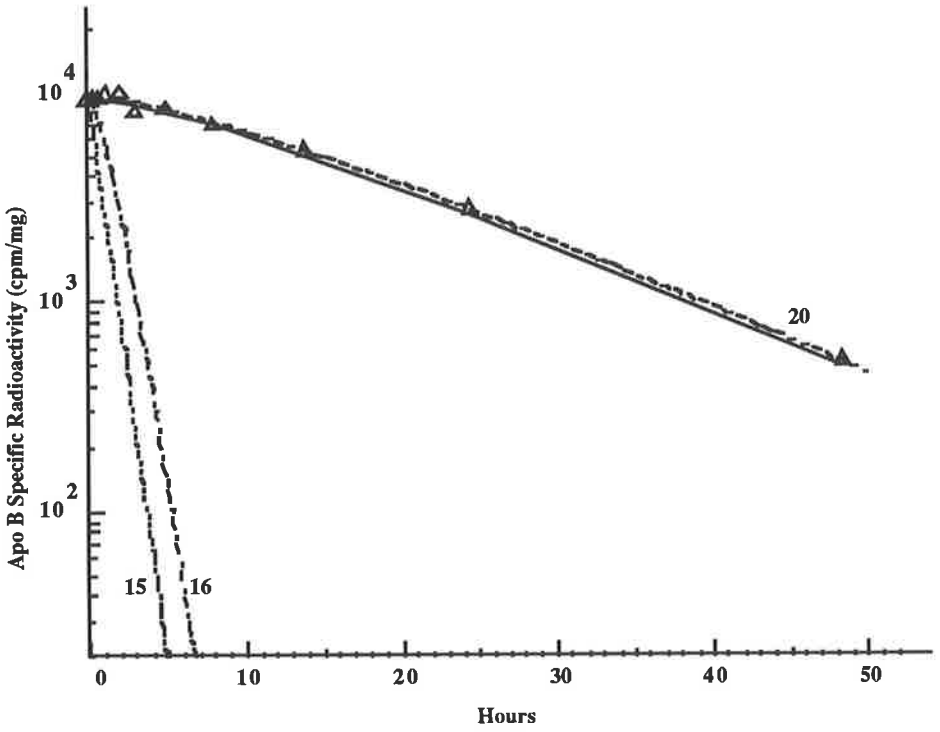


Figure 35. Observed VLDL2 bound apo B specific radioactivity data (Δ) for subject K and model fit to data. The dashed lines represent simulated specific radioactivity functions for the N level (lines 15 and 16) and R level (line 20) compartments

Although Figures 32 to 35 only simulate the kinetics of the N and R level compartments for apo B and triglyceride they demonstrate that the observed data can be represented by the sum of a series of functions with different kinetic characteristics. Added to this, it is important to understand how data derived from the reinjection of labelled lipoproteins is interpreted compared to that derived from the use of endogenous tracers.

For the VLDL1 fractions of subjects F and K, and the VLDL2 fraction of subject F a reduced model, consisting of only 2 levels of compartments was fit to the data. This reduced model (Figure 36) containing only level N_1 and R compartments was able to describe the data due to the presence of a rapidly turning over component in the unbound apo B fraction of these subjects.

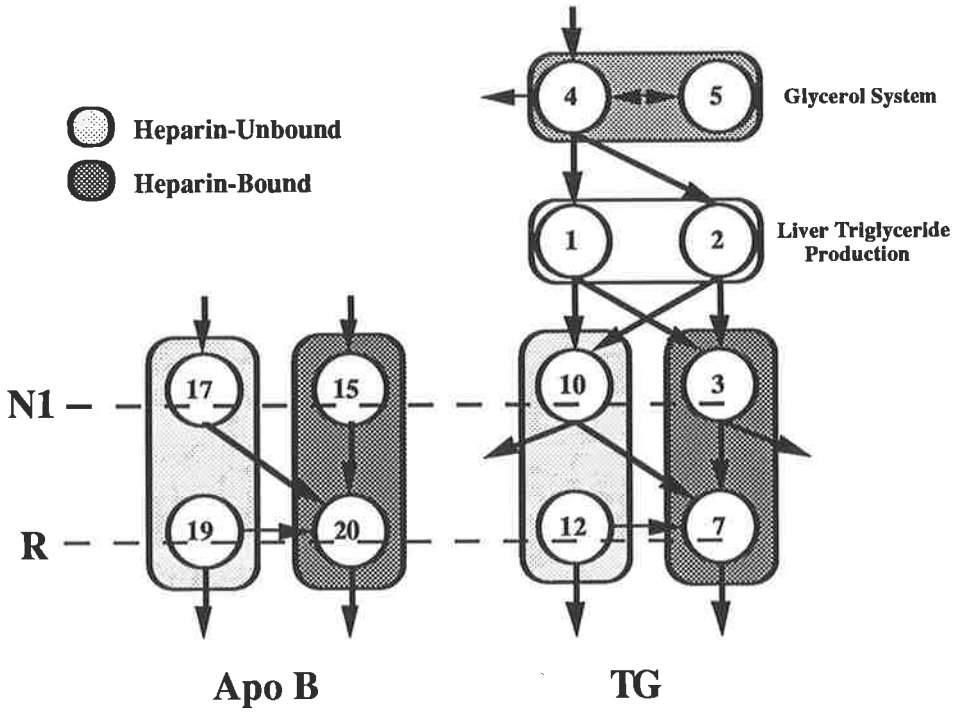


Figure 36. Reduced VLDL model used to fit VLDL data in subject F and the VLDL1 data of subject K. This model is essentially the same as that in Figure 31 except that in those fractions, where a rapidly turning over component was observed in the unbound apo B fraction the data could be fit using a model which did not contain the two compartment delipidation pathway. Unlike the model shown in Figure 31 where apo B and triglyceride are transported from the unbound N level to R level compartments this model shows that material is transported directly to the bound R level compartment.

The VLDL data from all subjects was fit using the models shown in Figures 31 and 36, where applicable. The kinetic and metabolic parameters derived from these fits are discussed in Section 3.6, following the development of the IDL and LDL models.

3.4.5. Model 4. IDL and LDL

Initially the specific radioactivity data of the IDL and LDL fractions was modelled using a single compartment for each fraction. It was assumed that IDL was the product of the slowly turning over bound VLDL2 compartment (comp. 20_B and 7_{TG}), and that LDL was the product of the IDL fraction. Using

these simple precursor product relationships a model was fit to the IDL apo B specific radioactivity data. This model assumed that apo B was derived from compartment 20. The fit to the data using this model was good (Figure not shown). To satisfy steady state conditions, direct input of apo B into the IDL fraction was required.

The corresponding simple model was used for the IDL triglyceride model in an attempt to fit the IDL triglyceride specific radioactivity data, which exhibited a rapid early rise, although not as rapid as that of the VLDL. This model (Figure 37), assumed that all VLDL triglyceride in compartment 7 (bound R level VLDL2 compartment) was transported to the IDL compartment, an assumption which prevents any delipidation of the VLDL2 particles prior to conversion to IDL. This model which was constrained by the slow kinetics of apo B in the IDL fraction was not able to fit the observed early rise of the IDL triglyceride curve (Figure 38).

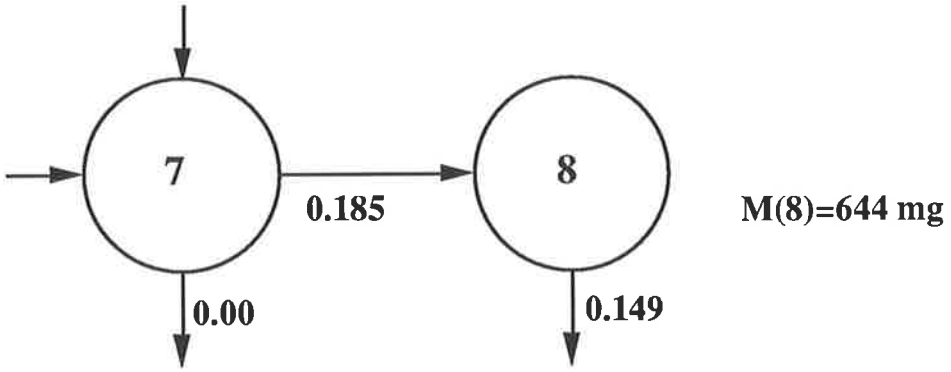


Figure 37. Simple model proposed to fit IDL triglyceride specific radioactivity data in subject H. Compartment 7 represents the slowly turning over bound VLDL pool, compartment 8 is the IDL triglyceride pool. Although only compartment 7 of the VLDL model is shown the complete VLDL model was used to describe the input function into the IDL fraction. It was assumed that no delipidation occured between compartments 7 and 8. Rate constants (h^{-1}) were derived from fitting the apo B data to this model.

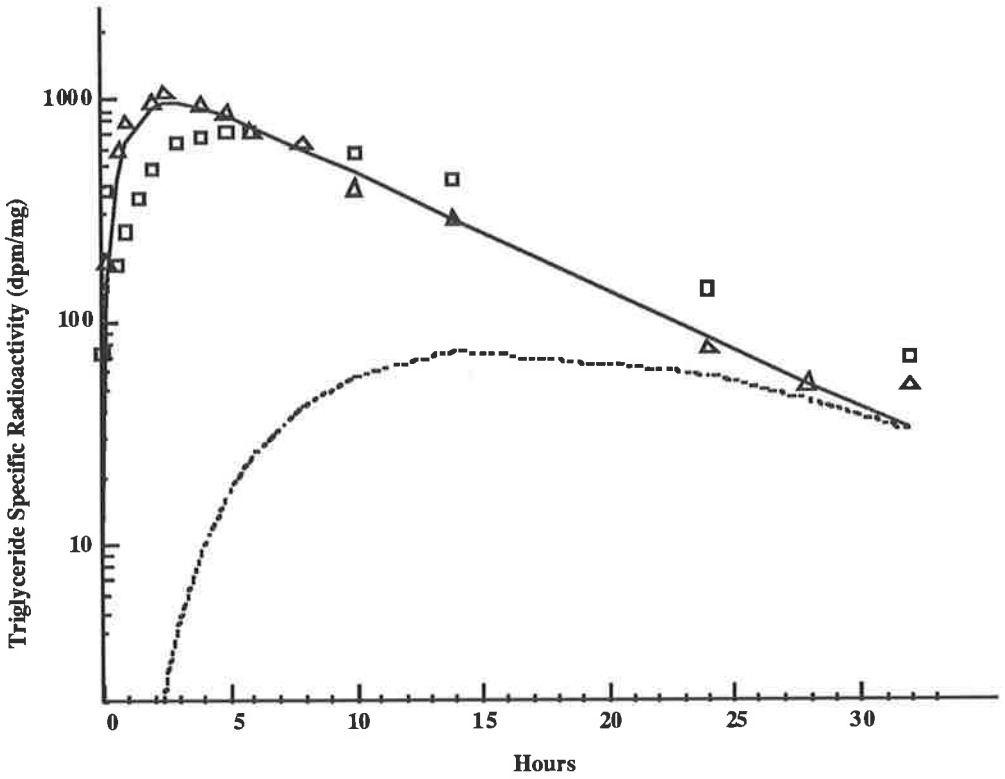


Figure 38. Fit of precursor product model to IDL triglyceride specific radioactivity curve for subject H. The solid line represents the fit of the VLDL model to the unfractionated VLDL2 triglyceride data (Δ), the dashed line is the fit of the precursor product model, above (Figure 37) to the IDL data (□).

Based upon observations made of the subfractions of VLDL and of the lipoprotein-triglyceride reinjection experiments of Malmendier and Berman (1978) it was hypothesised that the IDL fraction represented a heterogeneous population of particles some of which turned over rapidly. Others have already shown that the IDL density range contains a heterogeneous group of particles (Musliner et al, 1986), but only Malmendier and Berman (1978) have evidence to suggest this in relation to triglyceride metabolism. Following the reinjection of labelled IDL-triglyceride they observed the IDL-triglyceride decay curve was biexponential and that the turnover rate of the faster component was of the order of one per hour. The turnover rate of the slower component was an order of magnitude slower, at approximately 0.11 h^{-1}. These

turnover rates were estimated for a normo-lipidemic subject, and may therefore be somewhat slower in a hypertriglyceridemic subject. Although they observed this finding in the several subjects shown they did not analyse or discuss the IDL data. Using this information a new model was proposed to account of the kinetics of the triglyceride and apo B moieties of the IDL fraction. This model assumed that, as in the VLDL fractions, there were pools of particles which turned over more rapidly than other particles within the same fraction. The kinetics of the IDL apo B fraction suggested that most IDL particles were located in a large slowly turning over pool as there was no initial fall in the apo B decay curve prior to its rise. The new apo B model (Figure 39) allowed for the direct input of apo B into the IDL fraction via a quickly turning over compartment, in addition to that derived from the VLDL2 fraction. This model included two rapidly turning over compartments to act as a delipidation pathway through which triglyceride but not apo B could be removed. Like the VLDL model, the IDL and the LDL model (to follow) can also be thought of as containing N_1, N_2 and R level compartments. The N level compartments turning over more rapidly than on level R.

At the time of reinjection it was assumed that all IDL compartments contained labelled particles, suggesting that there was either rapid conversion of VLDL to IDL or that some IDL was isolated with VLDL2 and subsequently labelled. To set up the initial conditions for this model it was assumed that radioactivity was distributed between compartments in proportion to apo B mass.

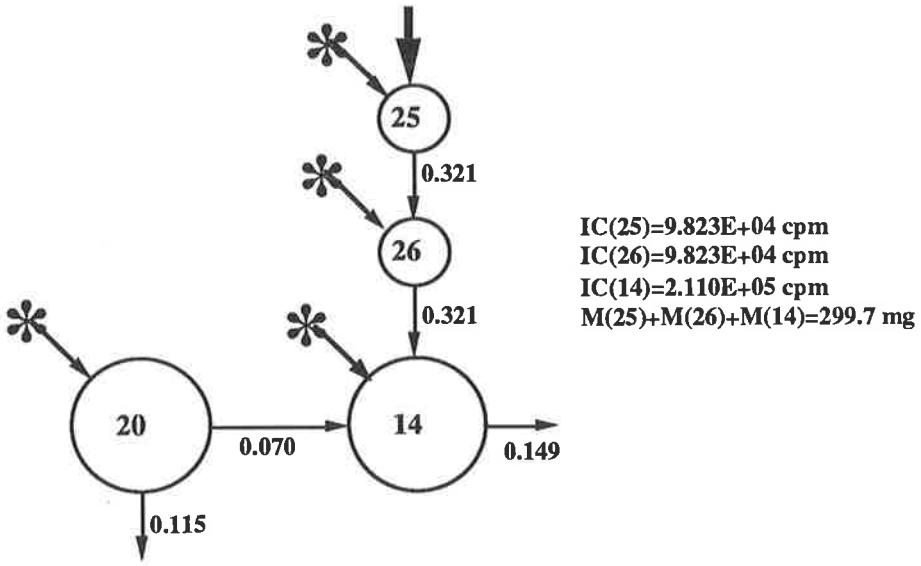


Figure 39. Proposed IDL apo B model showing transport of apo B from VLDL (comp. 20) and directly into IDL fraction. Compartments 25 and 26 represent a delipidation pathway and compartment 14 which turns over more slowly contains the bulk of the IDL particles. Although only compartment 20 of the VLDL model is shown the complete model was used to describe the function which fed IDL compartment 14. The rate constants L(26,25) and L(14,25) (h^{-1}) were defined by the rise of the IDL triglyceride data. The IDL apo B data defined the turnover rate of compartment 14.

Figure 40 illustrates the model used to describe IDL triglyceride kinetics in subject H. This model shows that triglyceride is derived from VLDL2 (only comp. 7 shown) and directly from the liver triglyceride conversion compartment (comp. 2). In all subjects it was assumed that there was no transport of triglyceride from the slow liver compartment (comp. 1) into the IDL or LDL compartments. Estimation of the contribution from this compartment could not be determined over the 48 hour time period of these studies. The direct synthesis of IDL triglyceride was assumed to be derived from compartment 2, the same compartment which feeds into VLDL2 and LDL, therefore implying that triglyceride entering these fractions is derived from the same source. The arrows out of compartment 2 represent transport to the VLDL2 unbound and bound fractions and to LDL.

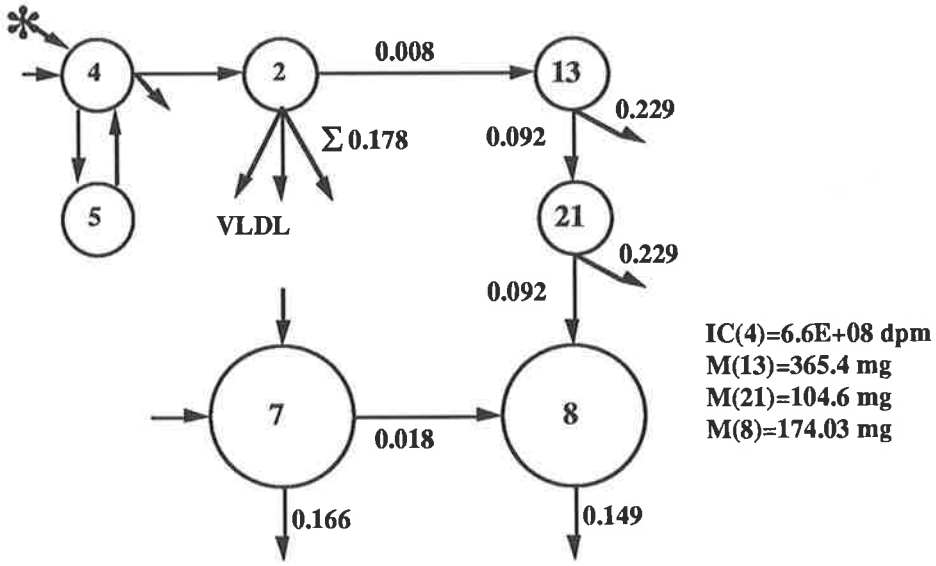


Figure 40. IDL triglyceride model incorporating delipidation pathway (comps. 13 and 21) which together with the VLDL compartment (comp. 7) feeds into compartment 8. IDL triglyceride is derived from VLDL and the fast liver triglyceride compartment (comp. 2). Only compartment 7 of the VLDL2 model is shown although the complete VLDL model was used to describe the function which fed the IDL fraction. Compartments 4 and 5 describe the glycerol subsystem. L(i,j) h^{-1}.

To fit the IDL triglyceride model it was necessary to assume that only 10% of the triglyceride in compartment 7 was transported to IDL, therefore 90% of compartment 7 must be hydrolysed prior to conversion of the particles to the IDL fraction. The value of 10% represents an arbitrary conversion rate. To fit the data however it was necessary to impose this constraint as it was possible to fit the IDL function without any transport from compartment 7 to the IDL. A conversion rate of more than 20% would have resulted in a triglyceride transport rate into the IDL fraction greater than that which the IDL fraction was capable of transporting. The contribution of radioactivity from the VLDL2 fraction to the early rise of the IDL function is small and only affects the IDL curve, in subject H after 10 hours. The 'hot' triglyceride derived from compartment 2 was solely responsible for the rapid rise of the IDL curve.

Figure 41 shows the fit of the IDL triglyceride model to subject H's IDL data. Although the IDL triglyceride curve crosses the VLDL2 curve at its peak this , as the model shows is not necessarily indicative of a precursor product relationship between these two fractions.

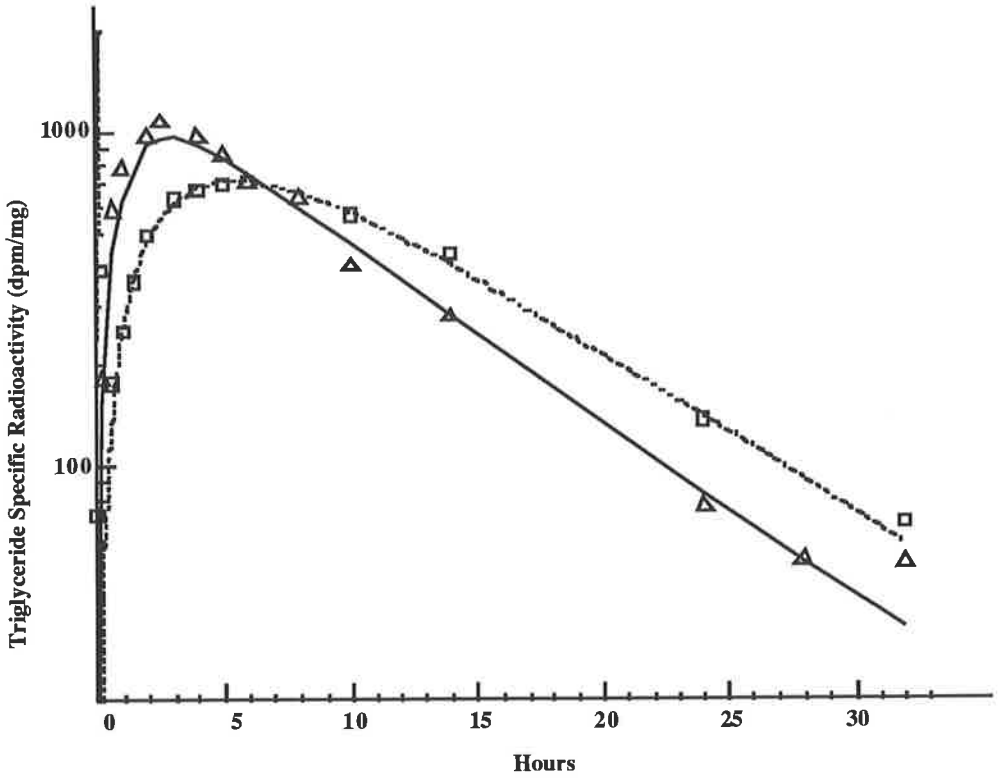


Figure 41. Fit of IDL triglyceride model (Figure 40), dashed line, to observed IDL triglyceride specific radioactivity data (□) in subject H. The solid line is the fit of the VLDL model to unfractionated VLDL2 data (Δ).

Figure 42 shows the observed IDL apo B specific radioactivity data for subject H, together with simulated specific radioactivity curves which describe the kinetics of the compartments used to model this data. The rapidly falling curves represent compartments 25 and 26 which turnover at approximately 0.3 per hour. The dashed curve lying above the observed IDL apo B data represents the specific radioactivity function of compartment 14. Its specific radioactivity rises as a result of receiving radioactivity from compartment 20 (VLDL).

Compartments 25 and 26 however derive apo B directly from another source ('cold') and as a consequence their specific radioactivity curves reflect only the loss of radioactivity from their compartments. The decay curve for compartment 26 does however have an initial delay, prior to its decay, due to the transfer of radioactivity from compartment 25.

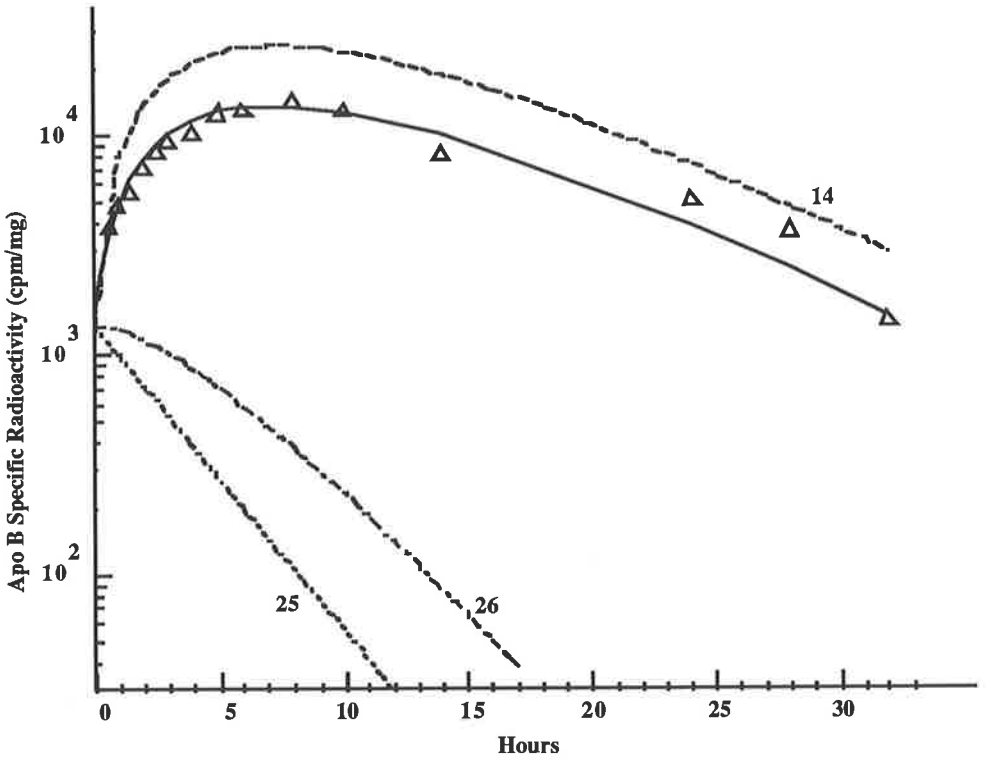


Figure 42. Observed IDL apo B specific radioactivity data (Δ) for subject H and model fit to data. The dashed lines represent simulated specific radioactivity functions for the N level (lines 25 and 26) and R level (line 14) compartments.

As a consequence of labelled IDL not being reinjected it was not possible to observe a clean plasma decay curve for this fraction. Therefore, analagous to the VLDL model, the initial rise of the triglyceride specific radioactivity data was used to estimate the turnover rate of the IDL N level compartments while the more slowly turning over IDL apo B data defined the turnover rate of compartment 14, level R. In addition to showing the fit of the IDL triglyceride

model to the IDL data Figure 43 describes the specific radioactivity functions of compartments 13, 21 and 8. These simulated curves are similar to those of the VLDL fraction although the rates at which the specific radioactivities of these compartments rise are slower.

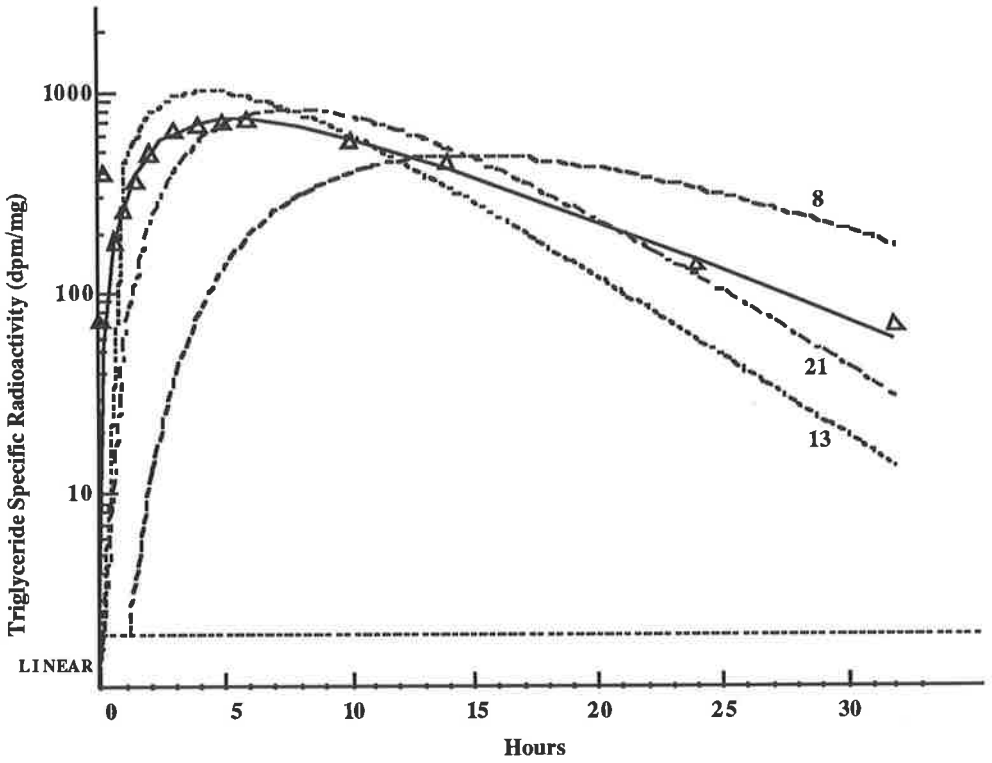


Figure 43. Fit of IDL model (solid line) to IDL triglyceride specific radioactivity data of subject H (Δ). Curves 13 and 21 are the simulated specific radioactivity functions of the N level compartments, compartments 13 and 21 respectively. Curve 8 is representative of the specific radioactivity function describing compartment 8.

LDL apo B and triglyceride specific radioactivity data were modelled in the same manner as that of the IDL fraction. Because of the short duration of these studies, less than 50 hours, only a single compartment was used initially to fit the LDL apo B data. The LDL model is however known to be more complex (Foster et al. 1986). Based upon the fit of the apo B data to a one compartment

model the turnover rate of the LDL fraction was determined. This rate, which varied between subjects, ranged from 0.015 to 0.040 per hour.

The first model used to fit the LDL triglyceride data of subject H is shown in Figure 44. Initially it was assumed that, analagous to the IDL triglyceride model all IDL triglyceride was transported to LDL. Clearly this model, like that initially used to describe IDL triglyceride kinetics did not describe the rapid rise observed in the LDL data (Figure 45).

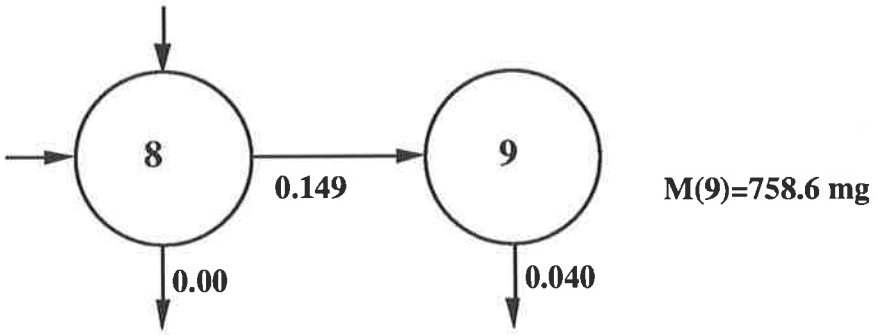


Figure 44. Simple model proposed to fit LDL triglyceride specific radioactivity data in subject H. Compartment 8 represents the slowly turning over IDL pool, compartment 9 is the LDL triglyceride pool. Although only compartment 8 of the IDL model is shown the complete VLDL and IDL models were used to describe the input function into the LDL fraction. It was assumed that no delipidation occured between compartments 8 and 9. Rate constants (h^{-1}) were derived from fitting the apo B data to this model.

Given that the kinetics of the LDL fraction were slow compared to VLDL and IDL, it was assumed that like the other fractions, the LDL fraction probably represents a heterogeneous population of particles, some of which turnover more rapidly than the bulk of the particles within the LDL fraction. This was also the conclusion of Malmendier and Berman (1978) who developed a two compartment model coupling the kinetics of LDL apo B and triglyceride moieties. Although their model was essentially a two compartment delipidation chain from which more than 90% of the triglyceride in the first compartment was hydrolysed, it demonstrated the heterogeneous nature of the LDL fraction.

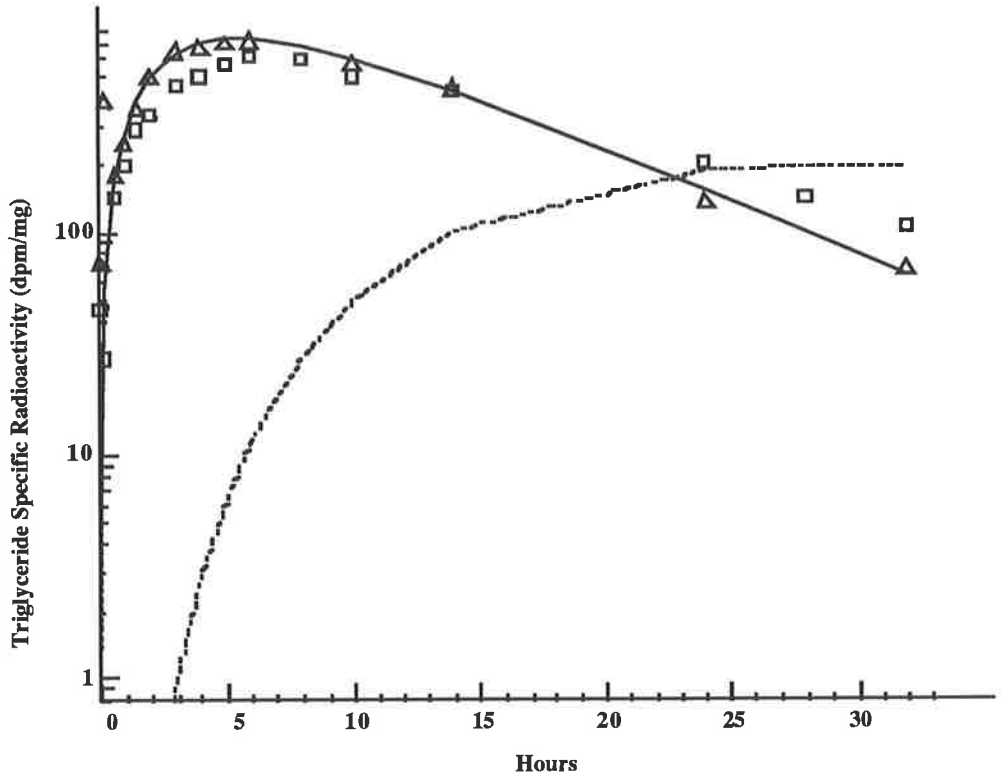


Figure 45. Fit of precursor product model to LDL triglyceride specific radioactivity curve for subject H. The solid line represents the fit of the IDL model to the IDL triglyceride data (Δ), the dashed line is the fit of the precursor product model, above (Figure 44) to the LDL data (□).

Unlike their model however the apo B and triglyceride models below (Figures 46 and 47 respectively) incorporate the delipidation part of the model differently. The model of Malmendier and Berman (1978) assumed that apo B and triglyceride were derived solely from IDL. The models presented here however allow for direct entry of apo B and triglyceride into the LDL fraction, in addition to that derived from IDL. Both models (Figure 46 and 47) were fit simultaneously to the LDL apo B and triglyceride data of subject H.

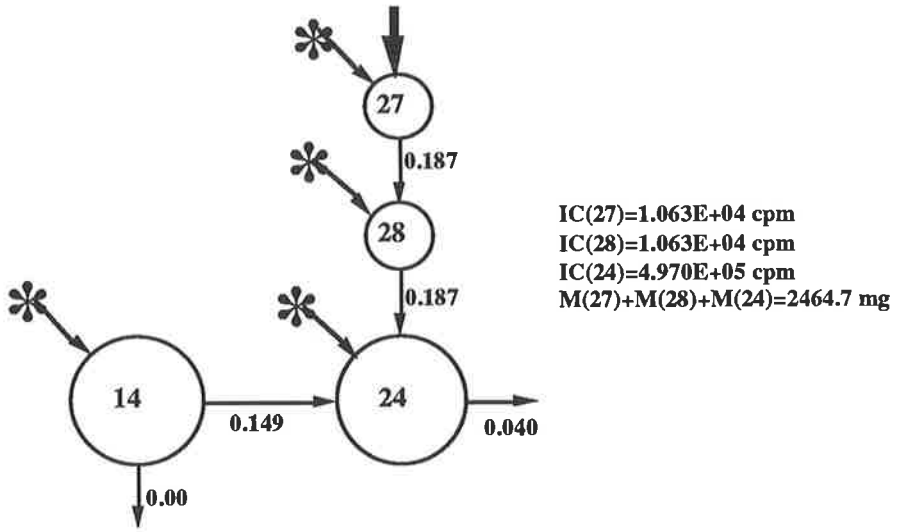


Figure 46. Proposed LDL apo B model showing transport of apo B from IDL (comp. 14) and directly into LDL fraction. Compartments 27 and 28 represent a delipidation pathway and compartment 24 which turns over more slowly contains the bulk of the LDL particles. Although only compartment 14 of the LDL model is shown the complete model was used to describe the function which fed LDL compartment 24. The rate constants L(28,27) and L(24,28) (h^{-1}) were defined by the rise of the IDL triglyceride data. The LDL apo B data defined the turnover rate of compartment 24.

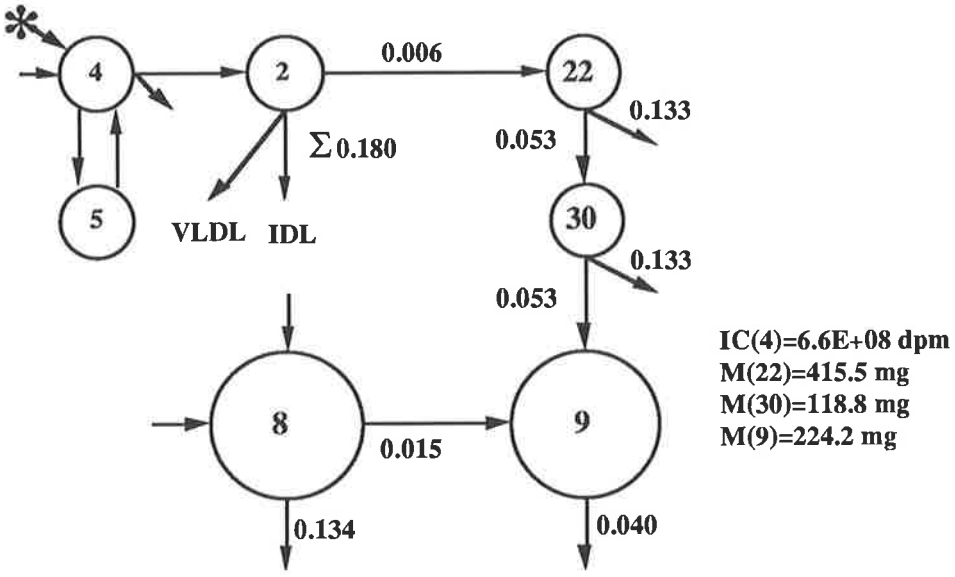


Figure 47. LDL triglyceride model incorporating delipidation pathway (comps. 22 and 30) which together with the IDL compartment (comp. 8) feeds into compartment 9. LDL triglyceride is derived from IDL and the fast liver triglyceride compartment (comp. 2). Only compartment 8 of the IDL model is shown although the complete VLDL and IDL model was used to describe the function which fed the LDL fraction. Compartments 4 and 5 describe the glycerol subsystem. L(i,j) h^{-1}.

Examination of the LDL rate constants in the apo B model, and in addition apo B transport rates from IDL, gives an indication as to the size of compartment 24 relative to that of compartments 27 and 28. In terms of apo B mass the combined mass of compartments 27 and 28 represents less than 5% of LDL. The effect the kinetics of these compartments have on the overall fit to the observed data is therefore negligible. In contrast however, the distribution of triglyceride in the LDL compartments shows that most LDL triglyceride is in compartment 22. Triglyceride which entered the LDL fraction via compartment 22 was derived from compartment 2, the same compartment which feeds triglyceride to the VLDL and IDL. The fraction of compartment 2 which is transported to IDL and LDL represents less than 5% of that which is transported to the VLDL. A good fit to the LDL triglyceride data was obtained (Figure 48). That the LDL curve reached its peak before crossing the IDL curve demonstrates the need for the direct input of 'hot' triglyceride into the LDL fraction. The good fit was only possible when it was assumed that LDL is partly the product of IDL and that LDL also receives triglyceride (and radioactivity) directly from compartment 2, the liver precursor compartment. The latter is necessary to account for the high LDL triglyceride specific radioactivity between 0 and 15 hours. It is important to note that compartment 2 was the source of 'hot' triglyceride for VLDL, IDL and LDL. The turnover rate of this compartments was therefore constant and it was the rapid turnover rate of the N level compartments that was responsible for the rise of the triglyceride curves in VLDL, IDL and LDL. To fit the LDL triglyceride data it was assumed that, as for VLDL to IDL conversion, 10% of IDL triglyceride was converted to the LDL fraction.

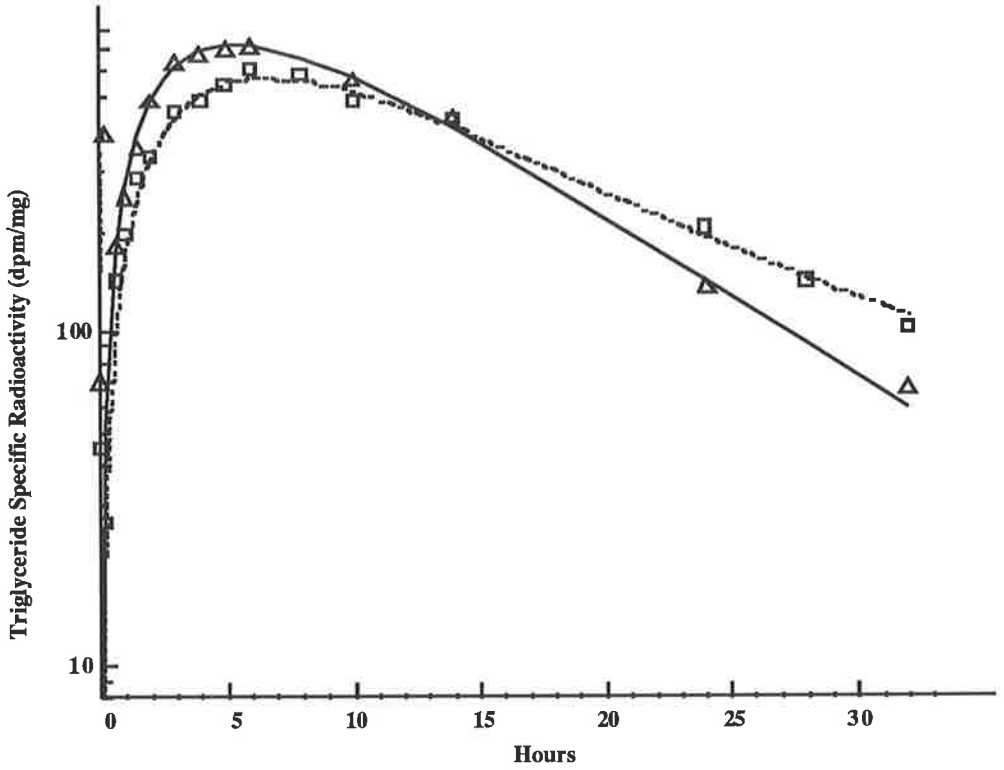


Figure 48. Fit of LDL triglyceride model (Figure 47), dashed line, to observed LDL triglyceride specific radioactivity data (□) in subject H. The solid line is the fit of the IDL model to the IDL triglyceride data (Δ).

The kinetic characteristics of apo B compartments 27 and 28, which were estimated by the rise of the LDL triglyceride specific radioactivity curve are shown in Figure 49 together with the simulated function of compartment 24 which approximates the observed data. Compared to the simulated IDl apo B curves the decay of the LDL curves is much slower. Curve 28 exhibits a marked delay prior to its decay as a result of the transfer of radioactivity from the other N level compartment, compartment 27.

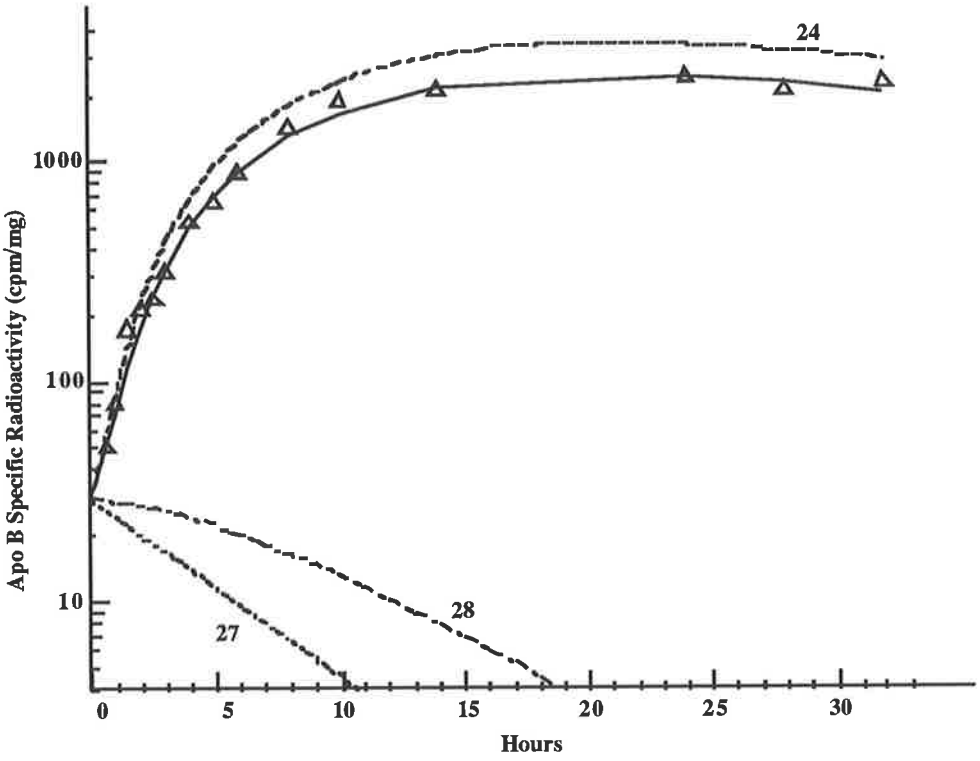


Figure 49. Observed LDL apo B specific radioactivity data (Δ) for subject H and model fit to data. The dashed lines represent simulated specific radioactivity functions for the N level (lines 27 and 28) and R level (line 24) compartments.

Figure 50 describes the simulated kinetics of the triglyceride containing compartments in the LDL model. Although the N level compartments in LDL display the same features as did the VLDL and IDL fractions clearly the rates at which these curves rise is slower than for the other fractions. In subject H the turnover rate of the N level compartments (comps. 22 and 30) was 0.187 h^{-1} which is approximately 60 % of that in the IDL fraction. The turnover rates of the R level compartments, compartments 24_B and 9_{TG}, are however much slower than those in IDL. Reliable estimates for the turnover rate of the LDL apo B compartment 24 could not be determined because of the lack of a decay component in the LDL curve. The turnover rate of compartment 24 were set as fixed parameters after reasonable fits to the apo B data were obtained.

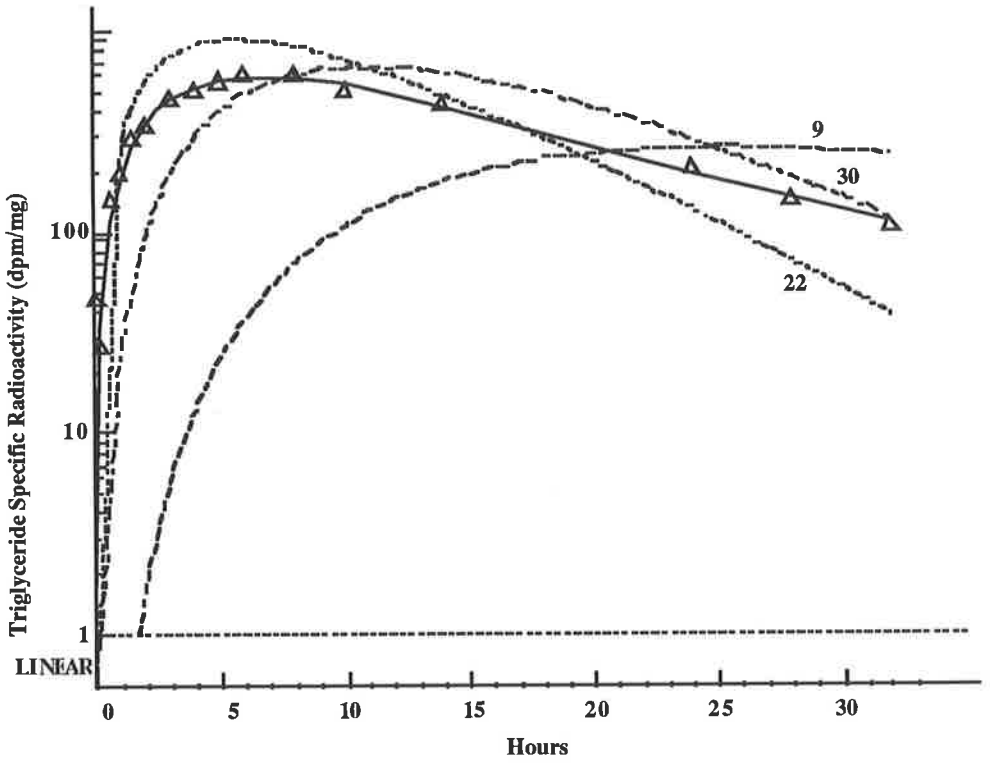


Figure 50. Fit of LDL model (solid line) to LDL triglyceride specific radioactivity data of subject H (Δ). Curves 22 and 30 are the simulated specific radioactivity functions of the N level compartments, compartments 22 and 30 respectively. Curve 9 is representative of the specific radioactivity function describing compartment 9.

3.5. Model 4. Integrated apo B and triglyceride model

From the separate models which have been described above comes an integrated model which describes the transport of apo B and triglyceride in VLDL2, IDL and LDL (Figure 51). Due to the rapid transfer of ^{125}I radioactivity from the VLDL1 to VLDL2 at or soon after the time of reinjection an integrated model incorporating the VLDL1 could not be developed. Although Figure 51 shows the presence of the VLDL1 model its relationship to the other fractions and how it may be interconnected could not been determined. Quantification of the pathway, if any, from VLDL1 to VLDL2 cannot be determined. In addition to this pathway there might be pathways directly from VLDL1 to IDL and LDL, the existence or otherwise of these pathways could not be determined.

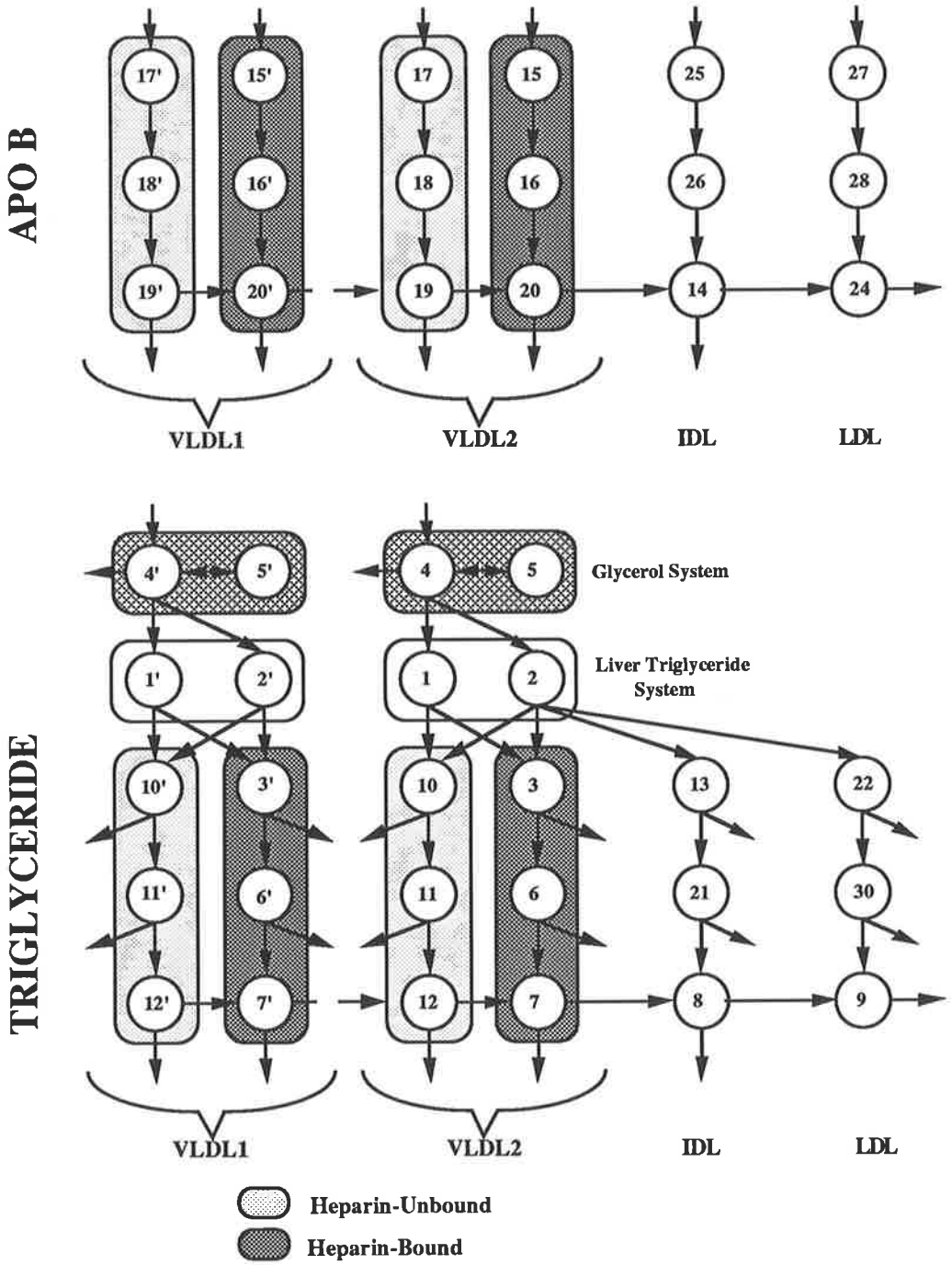


Figure 51. Integrated VLDL2, IDL and LDL apo B and triglyceride model. Although it was not possible to determine the relationship of the VLDL1 fraction to the VLDL2, IDL and LDL the VLDL1 model has been included. The arrows out of the triglyceride section of the model account for the delipidation of triglyceride. Those arrows leaving the remnant compartments however represent both removal of whole particles and delipidation. The arrows out of the apo B section describe removal of particles prior to conversion to the next density. Although VLDL2, IDL and LDL derive triglyceride from the same precursor system a different system was ued to fit the VLDL1 triglyceride data.

The SAAM input file which describes the model in Figure 51 is shown in Appendix A. This input file describes the relationships which exist between specific compartments and how the kinetics of the triglyceride and apo B data are coupled.

3.5.1. Kinetic and metabolic parameters

The VLDL2, IDL and LDL model was fit to all subject data, both ^{125}I and ^{131}I data, except for subject F where a reduced version of the model was used. Apo B and triglyceride specific radioactivity data were used simultaneously to derive all parameter estimates. Table 12 lists all the rate constants, together with error estimates, derived after fitting this model to the kinetic data. Examination of the error values, which are on average less than 10% suggest that the data collected support the proposed model. That these errors are low however does not imply that the proposed model uniquely describes the data in each of these subjects.

The values shown in Table 13 represent the sum of the L(i,j) for the model compartments shown in Figure 51. The values in this table describe the fractional turnover rates of the compartments on levels N1, N2 and R, where the values for N2 are the same as those of N1. Included in this table are estimates for turnover rates in the large VLDL fraction, VLDL1. Although the VLDL section of the model was developed using ^{131}I-VLDL2 data the same model was fit to the VLDL1 data. In addition, this model was fit to the ^{125}I data observed in the VLDL2, IDL and LDL fractions. That the metabolic parameters associated with the ^{131}I and ^{125}I VLDL2, IDL and LDL data were not dissimilar suggests that although most ^{125}I radioactivity, which was initially associated with the VLDL1, rapidly transferred from VLDL1 to VLDL2 at zero time the ^{125}I tracer in the VLDL2 and more dense fractions behaved like that of the ^{131}I

Table 12. Rate constants derived from fit of model (Figure 51) to ^{125}I and ^{131}I apo B and triglyceride kinetic data.

Rate Constant	Subject										
	F			K			H			J	
	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1	V2, I, L
	h^{-1}										
L(1,4)								0.047 (0.001)	0.208 (0.010)	0.374 (0.472)	
L(2,4)	0.241 (0.012)	0.512 (0.023)	0.631 (0.028)	2.488 (1.157)	0.946 (0.096)	0.966 (0.096)	0.253 (0.020)	0.329 (0.036)	0.849 (0.114)	0.431 (0.049)	1.213 (0.169)
L(0,4)	18.419 (0.018)	18.148 (0.018)	18.029 (0.036)	16.172 (1.164)	17.714 (0.089)	17.694 (0.088)	18.319 (2.180)	18.284 (0.037)	17.602 (0.106)	18.229 (0.049)	17.447 (0.157)
L(10,1)								0.003 (0.001)	0.003 (0.003)	0.005 (0.014)	
L(3,1)								0.001	0.001	0.004 (0.014)	
L(10,2)	0.068 (0.006)	0.081 (0.005)	0.076 (0.005)	0.074 (0.029)	0.049 (0.004)	0.057 (0.005)	0.040 (0.003)	0.158 (0.019)	0.075) (0.009)	0.170 (0.013)	0.126) (0.018)
L(3,2)	0.111 (0.004)	0.166 (0.005)	0.152 (0.004)	0.024 (0.009)	0.046 (0.004)	0.048 (0.004)	0.168 (0.006)	0.186 (0.021)	0.097 (0.009)	0.101 (0.008)	0.070 (0.011)
L(13,2)		0.007 (0.001)	0.006 (0.001)		0.004 (0.001)	0.004 (0.001)		0.027 (0.004)	0.008 (0.001)		0.003 (0.001)
L(22,2)		0.004 (0.001)	0.003 (0.001)		0.001 (0.001)	0.001 (0.001)		0.018 (0.003	0.006 (0.001)		0.002 (0.001)
L(0,3)	0.908 (0.011)	0.965 (0.034)	1.224 (0.041)	1.906 (0.179)	0.787 (0.066)	0.992 (0.088)	1.481 (0.090)	0.325 (0.045)	0.822 (0.100)	1.269 (0.120)	0.996 (0.045)
L(6,3)				0.384 (0.155)	0.128 (0.015)	0.254 (0.034)		0.300 (0.040)	0.274 (0.052)	0.213 (0.020)	0.249 (0.045)
L(7,3)	0.014 (0.011)	0.041 (0.006)	0.069 (0.011)				0.033 (0.006)				
L(0,6)				1.906 (0.179)	0.787 (0.066)	0.992 (0.088)		0.325 (0.045)	0.822 (0.100)	1.269 (0.020)	0.996 (0.045)

Table 12. (cont'd)

Rate Constant	F			K			H			J	
	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1	V2, I, L
					h^{-1}						
L(7,6)				0.384 (0.155)	0.128 (0.015)	0.254 (0.034)		0.300 (0.040)	0.274 (0.052)	0.213 (0.020)	0.249 (0.045)
L(0,7)	0.227 (0.004)	0.132 (0.005)	0.201 (0.004)	0.047 (0.002)	0.057 (0.002)	0.081 (0.002)	0.096 (0.004	0.182 (0.003)	0.166 (0.020)	0.222 (0.119)	0.081
L(0,10)	0.908 (0.011)	0.965 (0.034)	1.224 (0.041)	1.906 (0.179)	0.787 (0.066)	0.992 (0.088)	1.481 (0.090	0.325 (0.045)	0.822 (0.100)	1.269 (0.120)	0.996 (0.045)
L(11,10)				0.384 (0.155)	0.128 (0.015)	0.254 (0.034)		0.300 (0.040)	0.274 (0.052)	0.213 (0.020)	0.249 (0.045)
L(12,10)	0.014 (0.011)	0.041 (0.006)	0.069 (0.011)				0.033 (0.006)				
L(0,11)				1.906 (0.179)	0.787 (0.066)	0.992 (0.088)		0.325 (0.045)	0.822 (0.100)	1.269 (0.020)	0.996 (0.045)
L(12,11)				0.384 (0.155)	0.128 (0.015)	0.254 (0.034)		0.300 (0.040)	0.274 (0.052)	0.213 (0.020)	0.249 (0.045)
L(7,12)		0.139 (0.005)	0.212 (0.005)	0.007 (0.002)	0.063 (0.002)	0.089 (0.002)	0.096 (0.004)	0.202 (0.003)	0.185 (0.003)	0.222 (0.119)	0.032 (0.010)
L(8,7)		0.007 (0.001)	0.011 (0.001)		0.006 (0.001)	0.009 (0.001)		0.020 (0.001)	0.018 (0.002)		0.009
L(0,13)		0.725 (0.371)	0.756 (0.183)		0.219 (0.044)	0.213 (0.045)		0.123 (0.018)	0.229 (0.067)		0.201 (0.031)
L(21,13)					0.131 (0.045)	0.131 (0.056)		0.059 (0.088)	0.092 (0.001)		0.013 (0.127)
L(8,13)		0.592 (0.449)	0.561 (0.192)								
L(0,21)					0.219 (0.044)	0.213 (0.045)		0.123 (0.018)	0.229 (0.067)		0.201 (0.031)
L(8,21)					0.131 (0.045)	0.131 (0.056)		0.059 (0.088)	0.092 (0.001)		0.013 (0.127)

Table 12. (cont'd)

Rate Constant	F			K			H			J	
	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1 125_I	V2, I, L 125_I	V2, I, L 131_I	VLDL1	V2, I, L
					h^{-1}						
L(0,8)		0.169 (0.020)	0.186 (0.014)		0.060 (0.003)	0.071 (0.003)		0.106 (0.004)	0.134 (0.018)		0.071
L(9,8)		0.009 (0.001)	0.009 (0.001)		0.007 (0.001)	0.008 (0.001)		0.012 (0.001)	0.015 (0.002)		0.008
L(0,22)		0.068 (0.027)	0.108 (0.034)		0.053 (0.005)	0.048 (0.005)		0.064 (0.018)	0.134 (0.051)		0.126 (0.031)
L(30,22)					0.032 (0.007)	0.029 (0.009)		0.031 (0.057)	0.054 (0.001)		0.036 (0.127)
L(9,22)		0.055 (0.093)	0.080 (0.038)								
L(0,30)					0.053 (0.005)	0.048 (0.005)		0.064 (0.018)	0.134 (0.051)		0.126 (0.031)
L(9,30)					0.032 (0.007)	0.029 (0.009)		0.031 (0.057	0.054 (0.001)		0.036 ((0.127)
L(0,9)		0.015	0.015		0.045	0.045		0.040	0.040		0.043
L(16,15)				2.289 (0.208)	0.916 (0.068)	1.246 (0.106)		0.625 (0.073)	1.096 (0.205)	1.483 (0.141)	1.246
L(20,15)	0.922 (0.011)	1.006 (0.034)	1.314 (0.041)				1.326 (0.090)				
L(20,16)				2.289 (0.208)	0.916 (0.068)	1.246 (0.106)		0.625 (0.073)	1.096 (0.205)	1.483 (0.141)	1.246
L(0,20)	0.227 (0.004)	0.099 (0.007)	0.105 (0.010)	0.047 (0.002)	0.004 (0.003)	0.025 (0.003)	0.096 (0.004)	0.154 (0.003)	0.115 (0.012)	0.222 (0.119)	0.026
L(14,20)		0.039 (0.004)	0.106 (0.008)		0.059 (0.002)	0.064 (0.003)		0.048 (0.002)	0.070 (0.001)		0.064
L(18,17)				2.289 (0.208)	0.916 (0.068)	1.246 (0.106)		0.625 (0.073)	1.096 (0.205)	1.483 ((0.141)	1.246

Table 12. (cont'd)

Rate Constant	F			K			H			J	
	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1 ^{125}I	V2, I, L ^{125}I	V2, I, L ^{131}I	VLDL1	V2, I, L
					h^{-1}						
L(19,17)	0.922 (0.011)	1.006 (0.034)	1.314 (0.041)				1.326 (0.090)				
L(19,18)				2.289 (0.208)	0.916 (0.068)	1.246 (0.106)		0.625 (0.073)	1.096 (0.205)	1.483 (0.141)	1.246
L(0,19)				0.039 (0.001)							0.026
L(20,19)	0.227 (0.004)	0.138 (0.005)	0.212 (0.005)	0.007 (0.002)	0.063 (0.002)	0.089 (0.002)	0.096 (0.004)	0.202 (0.003)	0.185 (0.020)	0.222 (0.119)	0.064
L(26,25)					0.351 (0.085)	0.344 (0.096)		0.182 (0.100)	0.322 (0.034)		0.214 (0.151)
L(14,25)		0.124 (0.117)	1.318 (0.362)								
L(14,26)					0.351 (0.085)	0.344 (0.096)		0.182 (0.100)	0.322 (0.034)		0.214 (0.151)
L(0,14)		0.026 (0.024)	0.092 (0.005)								
L(24,14)		0.153 (0.024)	0.104 (0.005)		0.067 (0.003)	0.079 (0.003)		0.118 (0.004)	0.149 (0.007)		0.079
L(28,27)					0.085 (0.010)	0.078 (0.012)		0.094 (0.073)	0.187 (0.030)		0.162 (0.047)
L(24,27)		0.124 (0.117)	0.188 (0.070)								
L(24,28)					0.085 (0.100)	0.078 (0.012)		0.094 (0.073)	0.187 (0.030)		0.162 (0.047)
L(0,24)		0.015	0.015		0.045	0.045		0.040	0.040		0.043

Values in parentheses are ± SD

tracer. Within the VLDL fractions the N level compartments turned over at a rate of approximately once per hour, except in subject K where the turnover rate was greater than 2 per hour. The turnover rate of the VLDL1 and VLDL2 R level compartments was however much slower. between 0.5 and 0.2 per hour. Only in subjects F and K (VLDL1) was it possible to observe the kinetics of the N level compartment in the VLDL apo B data. In these subjects the slope of the fast component of the unbound apo B data was equal to that of the upswing of the triglyceride specific radioactivity curve. In the other subjects however, where the fast slope of the unbound apo B curve was not observed the turnover rate of the N level compartments was a function of the rapid rise of the triglyceride specific radioactivity curve.

Table 13 also lists, for each subject, the fractional turnover rates of the N and R level compartments in the IDL and LDL fractions. Except for subject F, the turnover rates for the IDL N level compartments are 20 to 30% of those in the VLDL fractions. The turnover rates of the R level compartments are however only marginally slower than the VLDL2 R level compartments. In subject F, the turnover rate of the N level compartments was the same as that in the VLDL2 fraction. The turnover rates of the N level compartments in the LDL fraction varied between 0.08 and 0.19 per hour. These values represented 20-60% of the rate of the equivalent IDL compartments. The turnover rates of the LDL R level compartments, which was fixed due to insufficient LDL data, ranged from 0.015 to 0.045 per hour. Overall the values in this table show that as lipoprotein density increases the fractional turnover rate of the N level compartments decreases, this trend however, excluding LDL, does not seem to apply to the R level compartments.

Table 13. Fractional turnover rates of compartments in VLDL, IDL, and LDL

Subject		VLDL1	VLDL2		IDL		LDL	
		^{125}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
					h^{-1}			
F	N	0.922 (0.011)	1.006 (0.034)	1.314 (0.041)	1.317 (0.117)	1.318 (0.362)	0.124 (0.117)	0.188 (0.070)
	R	0.227 (0.004)	0.138 (0.005)	0.212 (0.005)	0.179 (0.021)	0.196 (0.015)	0.015	0.015
K	N	2.289 (0.208)	0.916 (0.068)	1.246 (0.106)	0.351 (0.085)	0.344 (0.096)	0.085 (0.010)	0.078 (0.012)
	R	0.047 (0.002)	0.063 (0.002)	0.089 (0.002)	0.067 (0.003)	0.079 (0.003)	0.045	0.045
H	N	1.326 (0.090)	0.625 (0.073)	1.096 (0.205)	0.182 (0.100)	0.322 (0.034)	0.094 (0.073)	0.187 (0.030)
	R	0.096 (0.004)	0.202 (0.003)	0.185 (0.023)	0.118 (0.004)	0.149 (0.007)	0.040	0.040
J	N	1.483 (0.141)		1.246[a]		0.214 (0.151)		0.162 (0.047)
	R	0.222 (0.119)		0.089[a]		0.079		0.045

Values in parentheses are ± SD
[a] Values fixed from subject K ^{131}I-VLDL2

Examination of the distribution of apo B between the N and R level compartments (Table 14) clearly shows that more than two-thirds and generally more than 80% of lipoprotein apo B, and hence lipoprotein particles, were associated with the slowly turning over fraction in the VLDL1, VLDL2, IDL and LDL fractions (level R compartments).

Table 14. Distribution of apo B mass in VLDL1, VLDL2, IDL and LDL compartments[a]. (see Figures 31 and 51)

Subject		VLDL1	VLDL2		IDL		LDL	
		^{125}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
					%			
F	N1	16.65	12.11	13.86	36.78	1.11	5.11	3.23
	R	83.35	87.89	86.14	63.22	98.89	94.89	96.77
K	N1	1.80	4.03	4.21	1.12	2.38	16.05	15.18
	N2	1.80	4.03	4.21	1.12	2.38	16.05	15.18
	R	96.40	91.93	91.58	97.77	95.23	67.90	69.64
H	N1	33.66	14.97	8.91	14.78	9.01	17.17	9.40
	N2		14.97	8.91	14.78	9.01	17.17	9.40
	R	66.34	70.07	82.18	70.44	81.98	65.66	81.20
J	N1	9.20		4.89		7.87		10.11
	N2	9.20		4.89		7.87		10.11
	R	81.59		90.21		84.25		79.79

[a]Expressed as a percentage of the total mass in a given fraction associated with compartments on the same level, such that N1+N2+R=100%, except in subjec F and H (VLDL1 only) where N1+R=100% (see Figure 51).

In contrast, the distribution of VLDL triglyceride between the compartments at levels N1, N2 and R (Table 15) reveals that, except for subject K's VLDL1, most triglyceride was associated with the N level compartments, and in particular those on level N1. In the VLDL1 fraction of subject K, the most hyperlipidemic subject, most apo B mass (≈96%) was associated with the slowly turning over fraction (k=0.047 h^{-1}, Table 12). In three subjects between 30 and

45% of VLDL2 triglyceride was associated with the R level compartments, suggesting that in these subjects there was a large population of slowly turning over particles.

Within the IDL and LDL fractions most triglyceride, with the exception of subject F, was associated with the N level compartments. In subject F however, most triglyceride in the IDL and LDL compartments was associated with the slowly turning over R level compartments. Although there was considerable differences in the distribution of triglyceride across lipoprotein fractions for each subject there appeared to be no overall common pattern. Despite this however, the triglyceride/apo B ratio of the VLDL compartments (Table 16) reveal that there was progressive and significant delipidation of the N1 through to R compartments. Although the triglyceride/apo B ratios of the IDL and LDL compartments were less than those of the VLDL fractions the level of delipidation between the N1 and R level compartments was also high. Given that there was no loss of apo B prior to level R for any fraction the degree of delipidation between N1 and R ranged from 66 to 97% of the triglyceride entering each fraction. In all subjects the triglyceride/apo B ratio of the IDL N1 and N2 and LDL N1 level compartments was greater than that of the VLDL2 and IDL R level compartments respectively. The implication of this finding is that within the IDL and LDL fractions there are pools of particles with triglyceride/apo B ratios greater than or equivalent to particles isolated in less dense fractions.

It must be noted that the triglyceride/apo B ratios in the above table are calculated values based upon the kinetics of the unbound and bound VLDL, and IDL and LDL triglyceride and apo B fractions. In developing the VLDL section of the model it was assumed that the triglyceride/apo B ratio of the unbound and bound compartments on the same level were the same.

Table 15. Distribution of triglyceride mass in VLDL1, VLDL2, IDL and LDL compartments[a]. (see Figure 31)

Subject		VLDL1	VLDL2		IDL		LDL	
		^{125}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
					%			
F	N1	92.81	77.20	75.22	20.79	21.84	13.03	9.09
	R	7.19	22.80	24.78	79.21	78.16	86.97	90.19
K	N1	36.93	63.11	47.04	42.44	39.99	57.72	57.00
	N2	6.19	8.85	9.59	15.85	15.20	21.57	21.66
	R	56.88	28.05	43.37	41.71	44.81	20.71	21.34
H	N1	74.39	38.23	54.59	39.07	56.73	57.39	54.76
	N2		18.35	13.66	14.93	16.24	18.62	15.67
	R	25.61	43.32	31.75	46.00	27.02	23.99	29.57
J	N1	72.58		42.83		49.14		66.72
	N2	10.45		12.97		28.72		14.90
	R	16.96		44.19		22.14		18.38

[a]Expressed as a percentage of the total mass in a given fraction associated with compartments on the same level, such that N1+N2+R=100% (see Figure 51).

The triglyderide/apo B ratios of the unfractionated VLDL, and IDL and LDL fractions shown in Table 8 (Section 3.1) represent the weighted average of the ratios in Table 16.

Table 16. Triglyceride/apo B ratios of compartments in VLDL, IDL, and LDL integrated model. (see Figures 31 and 51)

Subject		VLDL1	VLDL2		IDL		LDL	
		^{125}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
					mg/mg			
F	N1	66.86	78.23	66.68	3.57	40.51	0.52	0.57
	R	8.36	3.19	8.28	1.85	1.63	0.19	0.19
K	N1	300.62	164.04	117.14	111.65	49.14	3.06	3.20
	N2	50.40	23.00	23.90	41.71	18.67	1.14	1.21
	R	8.65	3.20	4.97	1.25	1.38	0.26	0.26
H	N1	13.21	14.73	35.34	6.68	13.53	1.03	1.79
	N2		7.07	8.85	2.17	3.87	0.33	0.51
	R	2.31	3.57	2.23	1.19	0.71	0.11	0.11
J	N	185.66		125.17		14.72		3.15
	N2	26.74		25.10		8.60		0.70
	R	4.89		4.64		0.62		0.11

In addition to the delipidation of triglyceride between the N1 and R level compartments there was also significant delipidation between the R level compartments of VLDL2, IDL and LDL. As discussed earlier it was assumed that only 10 % of the triglyceride in the R level compartments (compt. 7, VLDL2 and 8, IDL) was transported to the R level compartments of the more dense fraction. The loss of triglyceride from these compartments was however associated with the direct loss of apo B, and hence particles, from the VLDL2 and IDL fractions. In subjects F and H only 40-50% of VLDL2 apo B was

converted to IDL, subject K converted more than 70% (Table 12). Only in the IDL fraction of subject F however was there loss of apo B prior to conversion to LDL. Thus in these subjects part of the apparent delipidation of triglyceride could be attributed to the direct loss of particles presumably to the liver.

Apo B and triglyceride production rates were determined for each subject (Table 17) in VLDL1, VLDL2, IDL and LDL fractions. Apo B production rates in the VLDL1 and VLDL2 fractions were comparable to those which have been observed by other workers (Sigurdsson et al, 1976b; Reardon et al, 1978; Janus et al, 1980). The higher apo B production rates for VLDL2 than VLDL1 in subjects F and J indicate that there were significant inputs, reflected by independent production values, of new material, both apo B and triglyceride, into VLDL2. In addition, there were also significant inputs, although of a smaller magnitude, of new material into the IDL density range in all subjects. This was not only reflected in the apo B kinetics but was also clearly evident with respect to triglyceride however at levels less than for the VLDL2 density range. In subjects K and H however VLDL1 apo B production exceeded that of VLDL2 thus making it impossible to determine an estimate of VLDL2 direct synthesis. Where VLDL2 apo B production is greater than that of the VLDL1 there clearly must be independent input of apo B into the VLDL2 fraction. The values in Table 17 however do not allow for loss of VLDL1 prior to its conversion to VLDL2 thus VLDL2 apo B direct input estimates must be viewed as minimum rather than maximum values. In addition the direct inputs of apo B into the IDL and LDL fractions does not take into account the possible conversion of VLDL1 directly to IDL or LDL.

Table 17. Apo B and triglyceride production in VLDL, IDL, and LDL

Subject	Lipoprotein Fraction		Apo B Pool Size	Apo B Total Prodn.	Apo B Direct Prodn.	Triglyceride Pool Size	Triglyceride Total Prodn.	Triglyceride Direct Prodn.
			mg	mg/kg/d	mg/kg/d	mg	mg/kg/d	mg/kg/d
F	VLDL1	^{125}I	226	10.51	10.51	2712	569.25	569.25
	VLDL2	^{125}I	455	13.62	≤13.62	5592	1065.53	≤1065.53
	VLDL2	^{131}I	455	20.34	≤20.34	5592	1356.14	≤1356.14
	IDL	^{125}I	232	6.45	2.59	478	34.35	32.18
	IDL	^{131}I	232	11.07	8.35	478	37.41	33.81
	LDL	^{125}I	2840	9.92	4.40	578	3.12	2.28
	LDL	^{131}I	2840	10.12	4.24	578	3.33	2.43
K	VLDL1	^{125}I	1066	13.36	13.36	15622	4012.72	4012.72
	VLDL2	^{125}I	622	6.99	≤6.99	6528	1146.49	≤1146.49
	VLDL2	^{131}I	622	9.92	≤9.92	6528	1162.86	≤1162.86
	IDL	^{125}I	348	6.95	0.41	1020	48.39	46.14
	IDL	^{131}I	348	7.97	0.87	1020	47.42	42.59
	LDL	^{125}I	1350	12.53	5.57	900	17.92	17.05
	LDL	^{131}I	1350	12.85	4.88	900	16.69	15.59
H	VLDL1	^{125}I	208	36.06	36.06	1242	476.27	476.27
	VLDL2	^{125}I	756	24.08	≤24.08	4360	354.79	≤354.79
	VLDL2	^{131}I	756	25.13	≤25.13	4360	888.08	≤888.08
	IDL	^{125}I	300	8.47	2.74	644	26.52	18.35
	IDL	^{131}I	300	12.49	2.96	644	45.59	40.03
	LDL	^{125}I	2465	22.04	13.56	758	14.95	13.94
	LDL	^{131}I	2465	27.25	14.76	758	27.33	26.45
J	VLDL1		144	5.97	5.97	3390	1108.22	1108.22
	VLDL2		694	12.86	≤12.86	8011	1610.43	≤1610.43
	IDL		216	6.40	1.30	551	27.60	19.17
	LDL		1158	12.01	4.53	538	18.05	14.26

Triglyceride production rates (Table 17), in contrast to those of apo B, were significantly higher, up to ten times, than those observed by other workers (Sigurdsson et al, 1976b) in subjects with the same phenotype and lipid profiles. In three subjects the production of VLDL2 triglyceride was greater than that in the VLDL1 fraction. However in subject K, the most hyperlipidemic subject, VLDL1 triglyceride production was greater than that in the VLDL2 fraction. In those subjects where VLDL2 triglyceride production exceeds that of the VLDL1 it demonstrates the importance of the VLDL1 fraction in terms of its capacity for triglyceride transport. In addition it suggests that there must be a significant input of new triglyceride into the VLDL2 fraction. In contrast to the high levels of triglyceride transport in the VLDL fractions production in the IDL and LDL fractions was low. Despite this however, Table 17 shows that, in comparison to the transport of triglyceride through and IDL or LDL fractions, the level of direct input of triglyceride into these fractions is very high. It was the input of this triglyceride which produced the rapidly rising IDL and LDL specific radioactivity curves observed in all subjects.

Fractional catabolic rates were determined for apo B and triglyceride in all fractions. Apo B FCR's in VLDL1 and VLDL2 fractions were comparable, although in subject H where the FCR of the VLDL1 fraction was five times greater than that of the VLDL2 fraction. Consistent with subject K being the most hyperlipidemic subject, this subject recorded the lowest VLDL1 and VLDL2 apo B FCR, both of which were significantly lower than the IDL FCR. Apo B FCR's in the IDL fraction were marginally faster than in VLDL2 suggesting that the IDL fraction, if competing for the same removal or catabolic processes as VLDL2, may be a better substrate than the VLDL2 particles. If however there is, within the VLDL2 and IDL fractions, particles which have similar metabolic

properties then the FCR's of VLDL2 and IDL fractions may be similar. Comparison of the apo B FCR's derived from the ^{125}I (initially labelling the VLDL1 particles) and ^{131}I labelled VLDL2 and IDL show that, in subject F, K and H, ^{131}I labelled particles turnover more rapidly. That there is a difference in the FCR's of these tracers may suggest that the particles have different metabolic fates.

Fractional catabolic rates for VLDL triglyceride were 2 to 10 times those of apo B, and were markedly greater than observed by other workers (Sigurdsson et al, 1976b). With the exception of subject H (^{125}I), VLDL triglyceride FCR's ranged from 0.58 to 1.13 per hour. In addition, IDL and LDL triglyceride FCR's were greater than those of the respective apo B moieties showing that, although these particles are metabolised more slowly than VLDL, there is considerable delipidation of triglyceride within these particles, and hence the increased FCR of the triglyceride moiety.

The triglyceride model of Zech et al (1979) was fit to the unfractionated VLDL2 triglyceride data of each subject. Table 19 describes both triglyceride production and FCR for the fit to the Zech model and that derived by using the model in Figure 51. This table highlights the different metabolic parameters which can be derived by using different models to analyse the same data. Clearly the Zech model provides estimates for both triglyceride production and FCR which are markedly lower than those derived using the new model. That Model 4 (Figure 51) results in increased triglyceride production, up to 4.5 times, can be accounted for by the inclusion of the rapidly turning over N level compartments in the model. These compartments were incorporated into the model because it was assumed that the turnover of the liver triglyceride pool was slower than that of the VLDL triglyceride pool and the observation of

a rapidly turning over population of particles in the unbound apo B fraction of two subjects.

Table 18. Fractional catabolic rates of VLDL, IDL, and LDL apo B and triglyceride

Subject		VLDL1	VLDL2		IDL		LDL	
		^{125}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	131I
					h^{-1}			
F	TG	0.855 (0.039)	0.776 (0.036)	0.988 (0.047)	0.292 (0.037)	0.318 (0.029)	0.022 (0.006	0.023 (0.004)
	apo B	0.189 (0.003)	0.122 (0.004)	0.182 (0.003)	0.113 (0.026)	0.194 (0.014)	0.014 (0.001	0.014 (0.001)
K	TG	0.845 (0.418)	0.578 (0.066)	0.586 (0.067)	0.156 (0.019)	0.153 (0.017)	0.065 (0.005	0.061 (0.005)
	apo B	0.041 (0.001)	0.037 (0.001)	0.052 (0.001)	0.066 (0.002)	0.075 (0.003)	0.031 (0.001	0.031 (0.001)
H	TG	1.126 (0.086)	0.239 (0.002)	0.598 (0.002)	0.121 (0.007)	0.208 (0.003)	0.058 (0.005	0.106 (0.001)
	apo B	0.509 (0.118)	0.094 (0.002)	0.097 (0.002)	0.083 (0.007)	0.122 (0.003)	0.026 (0.005	0.032 (0.001)
J	TG	1.076 (0.159)		0.947 (0.128)		0.189 (0.022)		0.103 (0.013)
	apo B	0.136 (0.061)		0.093		0.090 (0.007)		0.014 (0.001)

Values in parentheses are ± SD

Table 19. Comparison of VLDL2 triglyceride production and FCR values derived from Model 4 (Figure 51) and Zech et al (1979) model.

	Zech Model		Model 4	
Subject	Prodn.	FCR	Prodn.	FCR
	mg/kg/d	h^{-1}	mg/kg/d	h^{-1}
F	831.6	0.61	1356.1	0.99
K	701.1	0.35	1162.8	0.58
H	191.6	0.13	888.1	0.59
J	362.67	0.15	1610.4	0.95

Comparison of Model 4 with that of the apo B model proposed by Berman et al (1978) showed little difference. An explanation for this can be found by observing the distribution of apo B mass within the VLDL fractions (Table 14). Although the turnover rate of the nascent compartments is high compared to the rates estimated by the Berman model there is relatively little apo B mass in these compartments resulting in little or no effect on either apo B FCR or production.

3.5.2. VLDL and LDL triglyceride simulation studies

This section describes results derived from simulation studies which predict plasma VLDL, and LDL, triglyceride specific radioactivity curves following the reinjection of labelled VLDL-triglyceride. The shape of the predicted specific radioactivity curves is model dependent. This section examines the predicted triglyceride specific radioactivity functions generated using the Zech et al (1979) model and the model developed in Section 3.4.4. In addition, LDL-triglyceride simulation studies are presented.

Simulated functions can be derived by assuming that at time *t* a labelled precursor of VLDL triglyceride is administered. As this precursor is incorporated into VLDL triglyceride the specific radioactivity of triglyceride in the VLDL fraction increases, and then decreases as the labelled precursor is depleted and as a function of the metabolism and hydrolysis of VLDL. At different times throughout the course of the rise and fall of the VLDL triglyceride specific radioactivity function it is possible to determine, using a model, the amount of radioactivity present within with each compartment. Taking the radioactivity values of the different compartments at specific times, such as 1.5 hrs, it is possible to produce a simulated function which would be produced if labelled VLDL-triglyceride had been isolated at a specific time and then reinjected into a donor subject. If experiments can be performed which result in functions similar to those predicted by the simulation then the model is validated.

The early VLDL triglyceride reinjection studies of Farquhar et al (1965) showed that the decay curve of reinjected labelled VLDL triglyceride was slow relative to the rise of the VLDL curve after the injection of labelled glycerol or palmitate. On the basis of this observation they assumed that the turnover of the liver triglyceride compartment was faster than that of VLDL, and that the turnover rate of the VLDL triglyceride compartment was equal to the fall of the triglyceride curve after a bolus injection of labelled glycerol or palmitate. Based upon this information the Zech et al (1979) model was developed. Using the Zech model (Figure 2) a fit was obtained to the VLDL2 triglyceride specific radioactivity data of subject K (Figure 52). Included in this figure are three other panels (1.5, 3.0 and 8.0 hrs) which describe the simulated decay curves of reinjected labelled VLDL triglyceride isolated at 1.5, 3.0 and 8.0 hours after the injection of labelled glycerol. These times were selected because they

corresponded to the pre-T_{max}, T_{max} and post-T_{max} VLDL2 triglyceride specific radioactivity values of subject K. Clearly this figure shows that, irrespective of when the VLDL triglyceride was collected for reinjection, the slope of the VLDL decay curve was the same as the terminal slope of the VLDL triglyceride specific radioactivity curve after injection of glycerol, reflecting the turnover rate of the plasma VLDL triglyceride moiety. Using the new model however a fit was obtained to the same data (Figure 53) and, simulated decay curves were obtained for reinjected VLDL triglyceride collected at 1.5, 3.0 and 8.0 hours. It is clear from this figure that the triglyceride decay curve of the reinjected VLDL contains a rapidly falling component, the slope of which is equivalent to the function describing the turnover rate of the N level VLDL compartments. In the new model (Figure 51), unlike that of Zech et al (1979), it is assumed that the liver triglyceride compartment is rate limiting and that within the VLDL pool there are particles which turn over rapidly. In subject K the turnover rates of the liver precursor, N level and R level compartments were 0.110, 1.246 and 0.089 h^{-1} respectively. The terminal slopes of the reinjected VLDL triglyceride curves were the same as that of the falling VLDL curve after glycerol. The slopes of these curves ($k=0.089\ h^{-1}$) describe the turnover rate of the R level compartments rather than the more rapidly turning over liver precursor. In addition, it is important to observe that although the VLDL was collected before, during and after the maximum triglyceride specific radioactivity was reached all reinjected VLDL triglceride decay curves displayed a rapidly falling component. At the later times the proportion of slowly turning over particles increased from about 10% at 1.5 hours to 30% at 8 hours reflecting the movement of label, and hence conversion of particles, from the more rapidly N level to slowly turning over R level compartments. Clearly if VLDL were isolated after 10 hours and

reinjected it would be difficult to observe a rapid component in the decay curve.

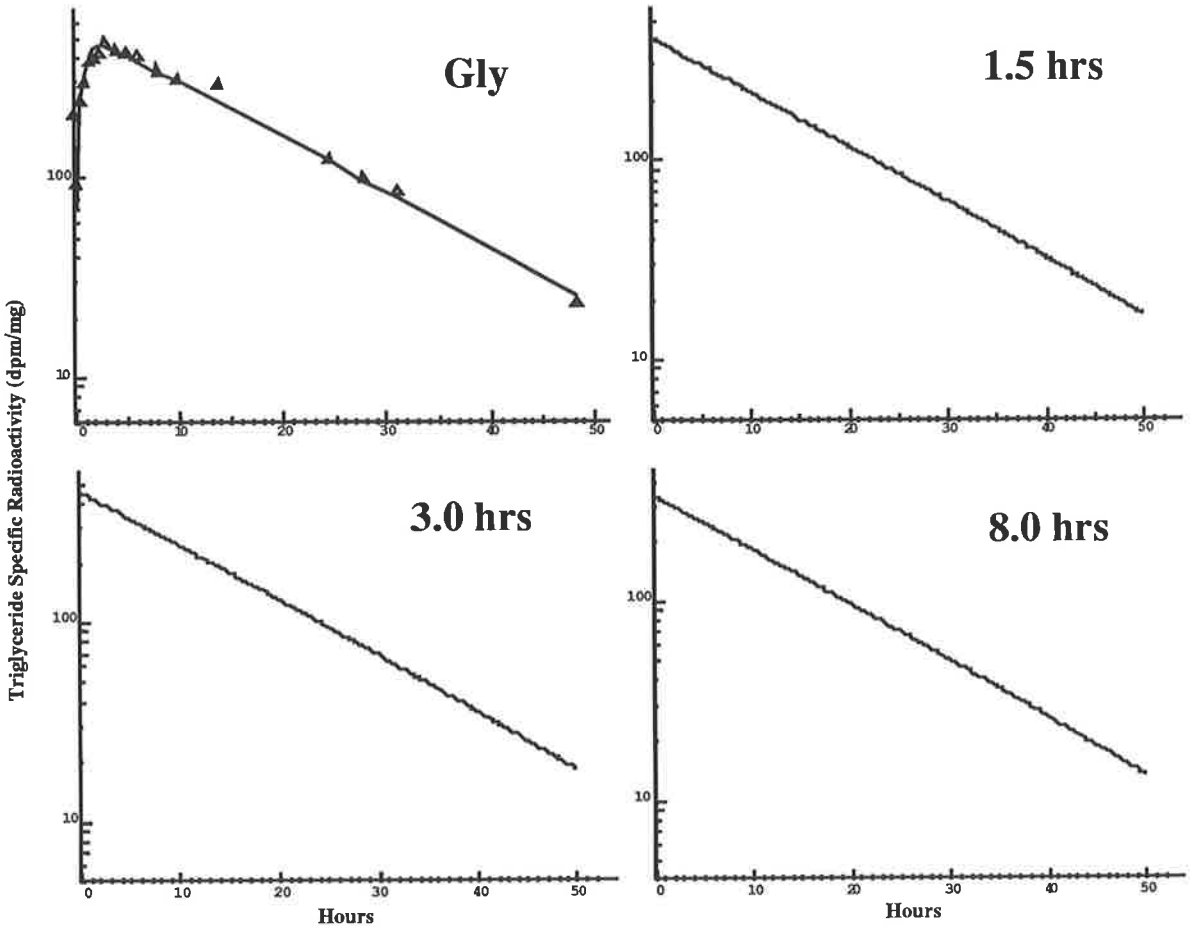


Figure 52. Fit of Zech et al (1979) (Figure 2) model to VLDL2 triglyceride specific radioactivity data of subject K (Gly). The other panels show simulated VLDL triglyceride decay curves of reinjected VLDL isolated at 1.5, 3.0 and 8.0 hours after the injection of labelled glycerol. Triglyceride decay curves of reinjected VLDL are the same as the terminal slope of the falling part of the VLDL curve after glycerol.

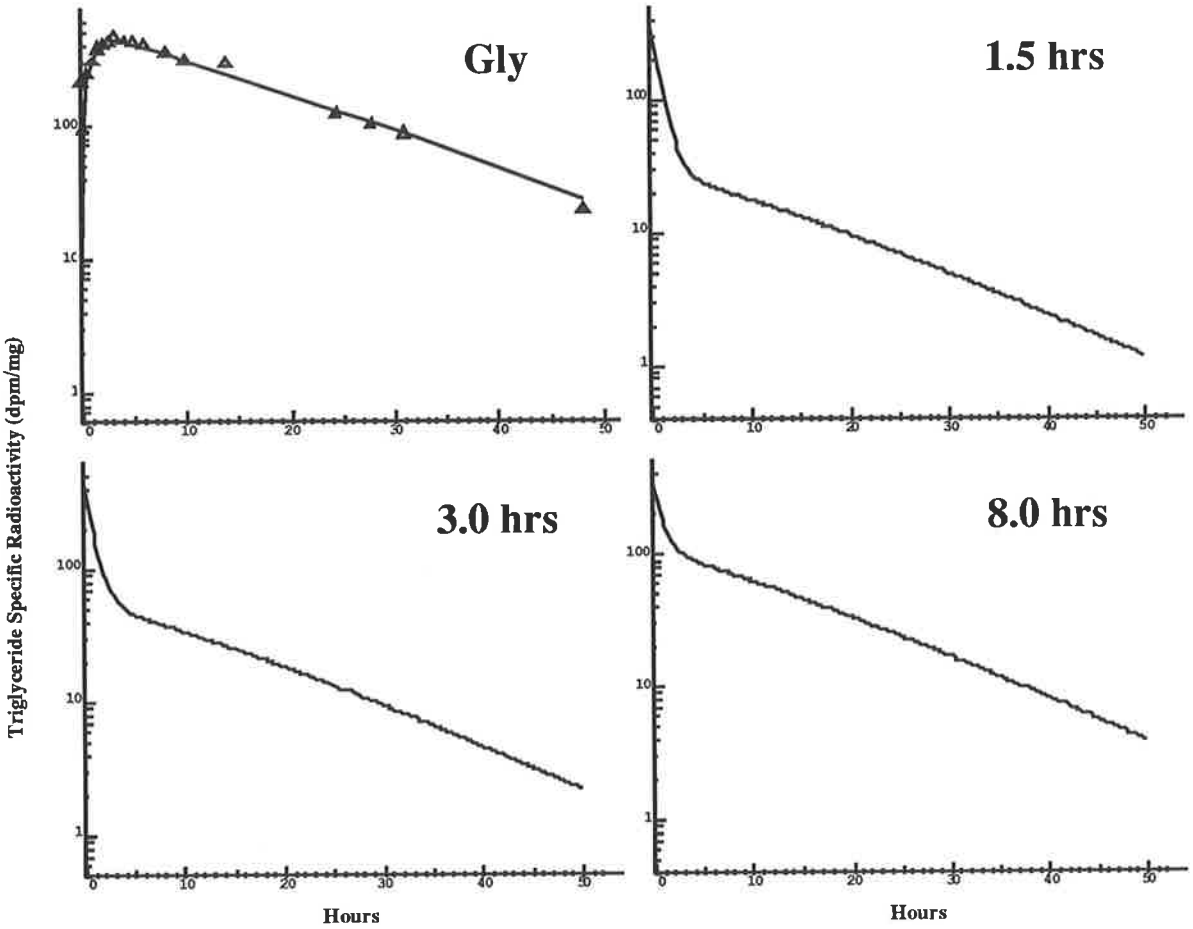


Figure 53. Fit of new model (Figure 51) to VLDL2 triglyceride specific radioactivity data of subject K (Gly). The other panels show simulated VLDL triglyceride decay curves of reinjected VLDL isolated at 1.5, 3.0 and 8.0 hours after the injection of labelled glycerol. Triglyceride decay curves of reinjected VLDL show presence of a rapidly turning over component in the VLDL fraction. The turnover rate of this fast component corresponds to the rapid rise of the VLDL curve after glycerol.

Malmendier and Berman (1978) examined the kinetics of LDL apo B and triglyceride simultaneously in normal and hyperlipidemic subjects. In addition to showing that the FCR for apo B and triglyceride in LDL were different they observed, although didn't discuss, a complex decay function for reinjected LDL triglyceride. Using the LDL model in Figure 52 a fit was obtained to subject K's LDL triglyceride specific radioactivity data (Figure 54).

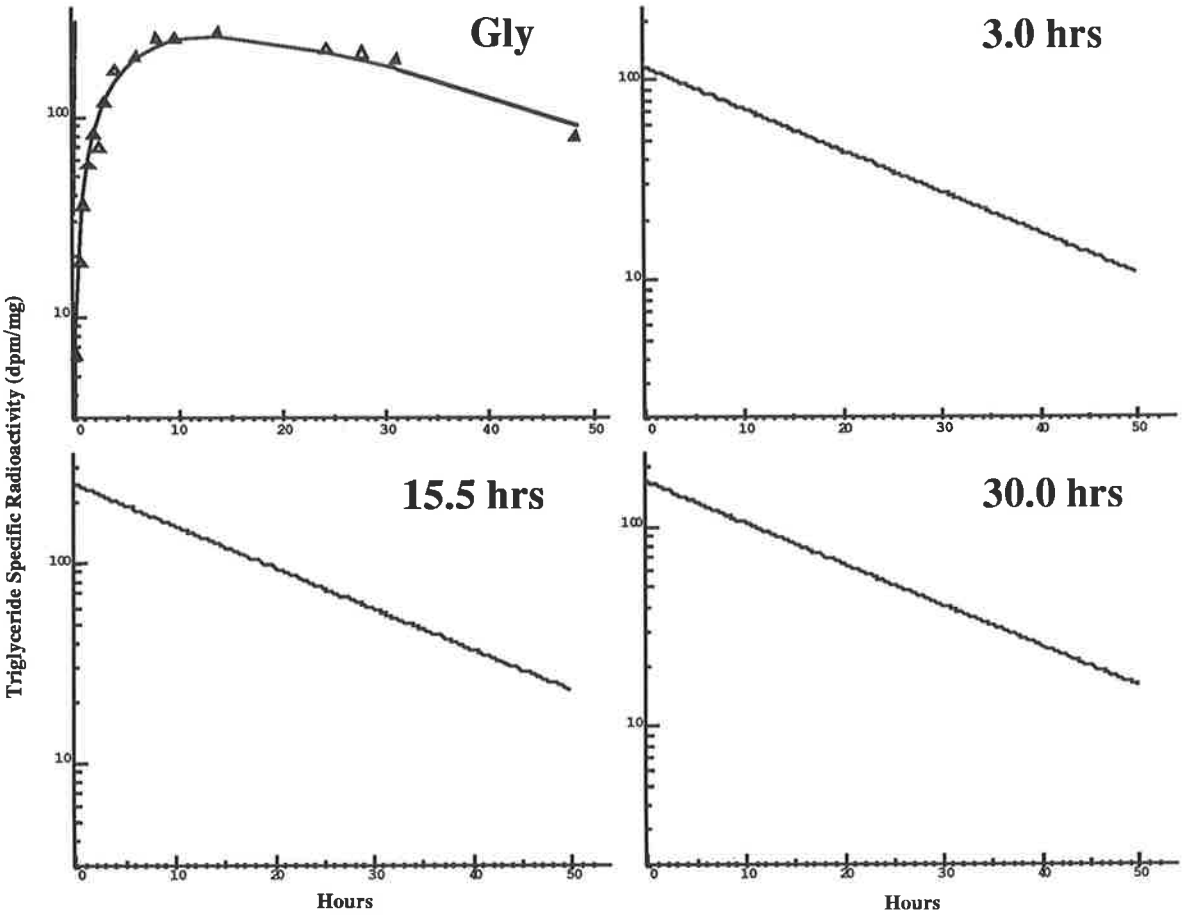


Figure 54. Fit of new model (Figure 51) to LDL triglyceride specific radioactivity data of subject K (Gly). The other panels show simulated LDL triglyceride decay curves of reinjected LDL isolated at 3.0, 15.0 and 30.0 hours after the injection of labelled glycerol.

Included in this figure are simulated curves describing the decay of the reinjected LDL triglyceride moiety isolated at 3.0, 15.5 and 30.0 hours, following injection of labelled glycerol. At each time (3.0, 15.5 and 30.0 hours) the decay curve was monoexponential and its slope was the same as that of the slowly turning over LDL R level compartment (turnover rate = 0.045 h^{-1}). In subject H however the decay curve of reinjected LDL triglyceride was biexponential in shape (Figure 55). This biexponential shape was a result of the marked differences in the turnover rates of the N and R level LDL compartments (0.187 and 0.040 h^{-1} respectively). The slope of the reinjected LDL triglyceride curve

decreased between 3.0 and 30.0 hours as label moved from the N level to more slowly turning over R level compartments. In addition to the transfer of label from the N to R level compartments, label was lost from the LDL N level compartments as a result of hydrolysis of triglyceride. Curve peeling of the 3.0 hour LDL triglyceride decay curve function would result in two functions, the faster of which represents the summer of the N level LDL compartments. Unlike the turnover rates of the VLDL N level compartments, the turnover rate of the LDL N level compartments were similar to those of the liver precursor compartment.

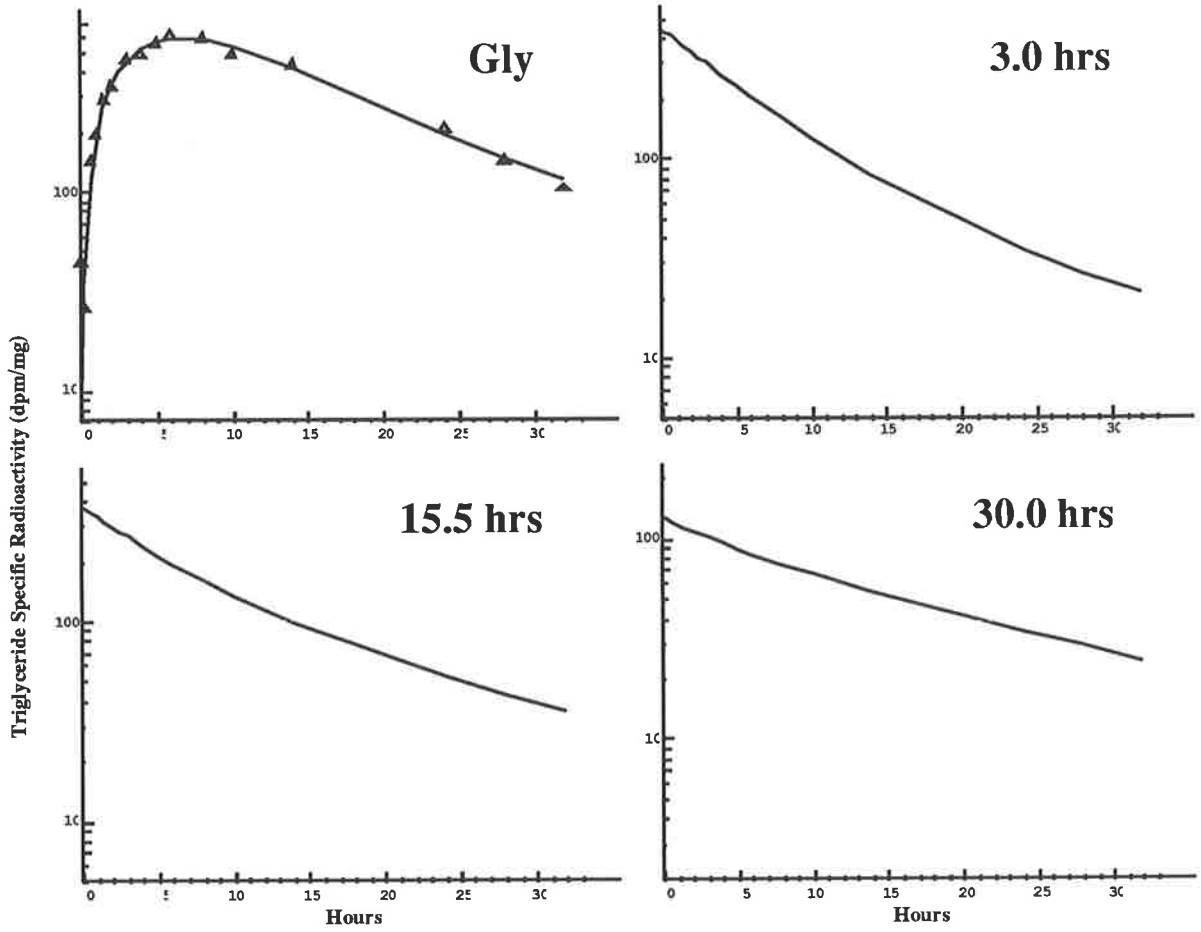


Figure 55. Fit of new model (Figure 51) to LDL triglyceride specific radioactivity data of subject H (Gly). The other panels show simulated LDL triglyceride decay curves of reinjected LDL isolated at 3.0, 15.0 and 30.0 hours after the injection of labelled glycerol.

CHAPTER 4

DISCUSSION

Despite the physical and chemical evidence that human VLDL and LDL are heterogeneous, especially in hypertriglyceridemics, most early studies of their metabolism, for the sake of simplicity, assumed homogeneity of one or both classes. The early studies of Sigurdsson et al (1975) which examined the conversion of VLDL ($d < 1.006$ g/ml) to LDL ($1.006 < d < 1.063$ g/ml) showed that in normal subjects there was a precursor product relationship between the two fractions. Following these studies others (Reardon et al, 1978; Reardon and Steiner, 1982; Fisher et al, 1980; Nestel et al, 1983; Packard et al, 1984) have subfractionated these species to reveal new levels of complexity in this metabolic scheme. The studies presented in this thesis describe the metabolism of apo B and triglyceride simultaneously in multiple lipoprotein fractions in four diverse type IV subjects. In addition, the metabolism of these moieties within two subfractions of VLDL was studied.

4.1. Experimental design and model structure

When developing a new lipoprotein model it is usually necessary to make certain assumptions about the system and the components within that system which are being modelled. Assumptions are necessary when either physicochemical information is missing or there is insufficient data to resolve certain pathways. For a model to be of physiological significance it should include all available information. It is however often difficult to incorporate the ideas and findings reported by other workers which are generally not made under identical experimental conditions. During the course of

developing a new integrated apo B and triglyceride model numerous assumptions were made about the system under investigation. The studies presented in this thesis were designed to test a number of hypotheses. These hypotheses and the assumptions which were critical to the development of a new model are discussed in the following sections. The observations made in these studies had a major impact upon the analysis of the data and has provided fresh insights and indicated new directions that may be taken in future kinetic studies of VLDL metabolism in humans.

4.1.1. Hepatic triglyceride precursor

Determining whether or not the liver or VLDL triglyceride pool represents the rate limiting step in the metabolism of triglyceride has been the subject of much debate for many years. The early human studies concluded that the rate limiting step was the VLDL triglyceride pool (Friedberg et al, 1961; Farquhar et al, 1965). In the one study of Havel and Kane (1975) however they observed that following the injection of labelled VLDL triglyceride the decay curve of this moiety was rapid. All the animal studies which have examined the clearance of VLDL triglyceride have also concluded that the liver represents the rate limiting step (Laurell, 1959; Havel et al, 1962; Havel, 1968; Gross et al, 1967; Lipkin et al, 1978; Hannan et al, 1980;). Studies from Steiner's laboratory (Steiner and Ilse, 1981), which showed striking similarities between VLDL triglyceride kinetic behavior in humans and lower animals in which the liver triglyceride precursor compartments are known to be rate-limiting, strengthened this assumption. Based upon these findings it was hypothesized that in humans, as in animals, the rate limiting step was the liver precursor compartment. To test this hypothesis it was assumed that within the VLDL fraction it would be possible to isolate particles which turned

over and which were able to account for the rapid rise observed in the VLDL triglyceride specific radioactivity curve.

Interpretation of the rise and fall in the triglyceride specific radioactivity curve in this analysis differs from that made by Zech et al (1979). Their model implied that the rise in the curve reflected a quickly turning over triglyceride pool within the liver through which glycerol was rapidly incorporated into VLDL-triglyceride which was then secreted into the plasma, accounting for the rapid upswing in the triglyceride specific radioactivity curve. In this analysis however, this rapid rise was interpreted in a different manner. Using the kinetics of the unbound apo B fraction, in subjects F and H, where the decay of the apo B specific radioactivity curve was rapid, the turnover rate of the most-nascent particles within the VLDL fraction was found to approximate that of the rise in the triglyceride curve. Interpretation of this rise, therefore, is that it represents the turnover rate of the recently secreted VLDL particles, rather than the input of newly synthesized triglyceride into the VLDL. From this it could be suggested that hepatic triglyceride pools turn over slowly and represent the rate limiting step involved in the metabolism of VLDL triglyceride. This has also been the conclusion of much earlier experiments in various animal species (Hannan et al, 1980; Baker, 1984) in which hepatic triglyceride turnover was measured directly and the plasma triglyceride or plasma-VLDL triglyceride turnover rate was measured independently either by reinjection of labelled VLDL-triglyceride or by injection of Triton WR1339. These earlier studies showed that the turnover of plasma triglyceride occurred at a rate faster than that which would be obtained from an analysis of the decay curve of labelled plasma triglyceride after injection of either labelled free fatty acids or glycerol into triglyceride. These studies have shown that the turnover rate of

"nascent" VLDL-triglyceride approximates the rate of rise in the VLDL-triglyceride specific radioactivity after injecting labelled glycerol, as predicted from the earlier animal studies and as implied by the studies of Havel and Kane (1975).

Furthermore examination of the early rise in the triglyceride specific radioactivity curves in the unbound and bound fractions revealed that both curves rose simultaneously. This is indicative of both fractions containing nascent triglyceride and that the liver is either secreting two types of particle, one unbound and one bound, or that the liver secretes one particle, which upon exposure to the plasma alters its apoprotein complement rendering it either unbindable (low apo E/apo C ratio) or bindable.

Another important observation was that the turnover rates of VLDL remnant particles and the hepatic triglyceride precursor were similar. Comparison of the turnover rates of these pools shows that in subject H the terminal slope of the VLDL2 triglyceride specific radioactivity curve reflects the turnover rate of the liver triglyceride compartments. In subject F however, the terminal slope of the VLDL2 triglyceride curve describes the turnover rate of the remnant particles. In this subject the turnover rate of the liver precursor is masked by the more slowly turning over remnant pool. This changing relationship may have led earlier workers to conclude, on the basis of VLDL reinjection studies, that hepatic synthesis and secretion is faster than the turnover of plasma VLDL triglyceride. The observation of the rapidly turning over nascent particles clearly demonstrates that although the turnover of the hepatic and remnant pools are comparable the hepatic pool is rate limiting with respect to the nascent particles.

4.1.2. VLDL1 to VLDL2 conversion

To test the hypothesis that large VLDL are converted to small VLDL, and the relationship between the unbound and bound VLDL1 and VLDL2 fractions, it was assumed that by reinjecting homologous ^{125}I-VLDL1 and ^{131}I-VLDL2 into each subject it would be possible to determine (1) the conversion of VLDL1 to VLDL2, (2) the direct removal of VLDL1 and, (3) the level of direct input of apo B and triglyceride into the VLDL2 fraction.

Although this assumption is still valid as an experimental approach, and indeed has been used successfully by other workers (Reardon et al, 1978; Packard et al, 1980; Packard et al, 1984), its application led to data that could not been integrated into the new model. Following the injection of ^{125}I-VLDL1 in each of 3 hypertriglyceridemic subject, there was an almost immediate transfer of a substantial fraction of injected tracer into the unbound and bound VLDL2 fractions (Table 11). This totally obscured the kinetics of any apo B-containing particles derived from VLDL1 if they were being transformed, as one would expect, to smaller VLDL2 particles. The rapid conversion of VLDL1 to VLDL2, which was evident within 3 minutes following injection, could have been an artifact of VLDL1 ultracentrifugal isolation and labelling; however, it was not seen when VLDL1 particles were labelled, incubated with plasma, and then separated again into VLDL1 and VLDL2 fractions. Essentially all of the radioactivity remained with the VLDL1 particles. Therefore, in each of the hypertriglyceridemic subjects studied, the VLDL1 particles were being transformed into VLDL2 particles by a very rapid process which may or may not be physiological. Nevertheless, it was possible to see from the incorporation of ^{3}H-glycerol into triglyceride of the unbound and bound VLDL1 fractions, that nascent VLDL1 particles were being introduced into the

circulation at rates comparable, or faster, to the formation of nascent VLDL2 particles.

4.1.3. Heparin-Sepharose affinity chromatography

The studies of Nestel et al (1983) revealed a precursor product relationship between the unbound and bound VLDL fractions separated by heparin-Sepharose affinity chromatography. It was therefore hypothesized that the separation of VLDL using this technique would isolate nascent and remnant-like particles. If it is correct that the hepatic triglyceride precursors represent the rate limiting step in triglyceride metabolism, then within the VLDL1 and VLDL2 fractions there must be at least two distinct populations of particles. One population of particles would have a high turnover rate to account for the rapid rise of the VLDL triglyceride specific radioactivity, the other population would turn over more slowly, the kinetics of which would be observed in the unfractionated VLDL or bound apo B decay curve. Shelburne and Quarfordt (1977), Nestel et al (1983) and, Huff and Telford (1984) have all used heparin affinity chromatography to separate VLDL into two classes of particles, one of which is capable of binding to heparin (bound), and is richer in cholesterol, triglyceride depleted and, has a greater apo E/apo C ratio than the class that does not bind (unbound). It is possible that the binding of lipoproteins to heparin may reflect the physiological process of ligand-receptor interaction Mahley et al (1984).

Fielding and Fielding (1986) have recently also isolated, by heparin-Sepharose chromatography, two populations of VLDL particles which contained either both apo E and apo B or only apo B. This may have physiological significance since two similar populations of particles, isolated by immunoaffinity chromatography, in rabbits, show strikingly dissimilar

metabolic fates (Yamada et al, 1986). Although affinity and immunoaffinity chromatography have been used to separate VLDL these methods may not separate particles according to the same criterion. Evidence from other studies (Nestel et al, 1983; Huff and Telford, 1984) however, suggests that both bound and unbound fractions contained apo E, although in a differing proportion to apo C.

The studies reported here, like those of Nestel et al (1983) and Huff and Telford (1984), demonstrate that heparin affinity chromatography did not cleanly separate the VLDL fractions into two populations of particles. That the unbound and bound VLDL fractions represented a heterogeneous population of particles was evident in the decay curve of the unbound apo B fraction in subjects F and K. Evidence of the heterogeneous nature of the bound fraction was hypothesized by the presence of the rapid rise of the triglyceride specific radioactivity curve of the bound VLDL fraction and by the observations of Huff and Telford (1984) who traced the decay of labelled human bound VLDL in minature pigs. Eventually it will be necessary to establish whether the subfractions represent metabolically discrete populations and whether the kinetically derived pools reflect physiologically the VLDL delipidation pathway.

4.1.4. Rapid unbound-VLDL fraction

The studies of Nestel et al (1983) revealed that in approximately one third of the subjects they studied, the decay of the unbound VLDL apo B fraction was rapid. It was hypothesized therefore, that the separation of VLDL by heparin-Sepharose would reveal, in at least some subjects, the rapid FCR predicted for nascent particles if the liver triglyceride precursor compartment is rate limiting. Of the three subjects injected with labelled apo B

subjects F and K displayed a rapid component in the unbound decay curve. In contrast to subject F, only in the VLDL1 fraction of subject K was the presence of a rapid component observed. In subject H and the VLDL2 fraction of subject K the rapid component was presumably masked by the presence of slowly turning over, remnant-like particles which are derived from the nascent precursors in the VLDL fraction. Clearly in these subjects heparin-Sepharose did not separate nascent from remnant particles or in the separation procedure there was contamination of the unbound fraction by bound particles. Such contamination may have resulted during the process of isolating VLDL1 and VLDL2 under ultracentrifugation. Several studies have shown that ultracentrifugation of lipoproteins at high g-forces causes compositional changes and loss of apo E and apo A_1 (Herbert et al, 1975; Fainaru et al, 1977b). If apo E was lost from some of the particles within the bound fraction during ultracentrifugation then following heparin chromatography these particles would not bind to the column and hence would be pooled with the unbound particles. In the event of this happening the kinetics of the nascent-like unbound particles would be masked by the more slowly turning over remnant particles. The degree of masking would however be dependent upon the extent of contamination by the "bound" particles.

The observation of the rapidly rising triglyceride specific radioactivity curve in the bound VLD fraction also demonstrated that within these fractions there are rapidly turning over nascent particles. That the kinetics of these particles are not seen in the decay curve of the bound apo B fraction can be explained by the presence, within this fraction, of the more slowly turning over remnant particles.

4.1.5. Model assumptions

In the course of developing a model to account for the kinetics of both the apo B and triglyceride moieties the following assumptions were made. One of these assumptions which has been used by other modellers (Malmendier and Berman, 1978; Phair et al, 1975; Zech et al, 1979; Le et al, 1982) is that the residence times of apo B and triglyceride within a single compartment are the same. The early triglyceride studies represented the VLDL triglyceride pool as a single compartment (Baker and Schotz, 1964; Eaton et al, 1969; Farquhar et al, 1965; Quarfordt et al, 1970; Shames et al, 1970), there by implying that the residence time of VLDL-triglyceride was the same as that of apo B. The studies of Phair et al (1975), using labelled leucine as a precursor of VLDL apo B observed a broadening of the plasma VLDL specific radioactivity at its peak. To explain this observation a chain of compartments (also known as a delipidation cascade) were used to model this feature. The delipidation cascade was used subsequently to explain why, in general, the residence time of VLDL triglyceride is less than that of the apo B moiety within this fraction. Although the delipidation cascade is able to account for the apparent differences in the residence of VLDL apo B and triglyceride, it maintains equal residence times for each moiety within a single compartment.

In addition, it was assumed that, at zero time, the radioactivity within the reinjected VLDL was distributed in proportion to the apo B mass both in bound and unbound fractions, and in proportion to the pools of particles which existed within these fractions. Thus, the apo B specific radioactivity of these injected particles were assumed to be equal. This assumption which has been used by many workers was tested in the studies of Packard and Shepherd (1985). They observed that the specific radioactivity of apo B across the VLDL spectrum was constant regardless of size of density of the

particle. More recently however, Le et al (1986) suggested that the differential labelling of VLDL subpopulations may account for the area under the IDL apo B specific radioactivity curve being greater than that of the VLDL precursor.

Examination of the apo B specific radioactivities of the bound and unbound fractions of the recipient subjects (i.e., after mixing of the injected tracer with the endogenous pools) were not always equal at zero time. It was assumed that these initial differences, also observed by Nestel et al (1983), reflected a change in the distribution of apo B between the two fractions during the time the tracer was prepared and the time it was injected into the subjects. That the initial specific radioactivities of the unbound and bound fractions were less similar in subjects F and H, compared to subject K, may be a reflection of the smaller and more rapidly turning over VLDL fractions within these subjects.

Changes in the distribution of particles between the unbound and bound fractions can affect interpretation of the kinetic data. In subjects F and H, where the initial specific radioactivities of the unbound apo B fractions were lower than those of the bound fractions, it was not clear that a precursor product relationship existed between these two fractions. Theoretically, where the specific radioactivity of the precursor starts off lower than that of the product the specific radioactivity curve of the product will not rise, and will at best exhibit a delay prior to its decay. In subject K however, the initial specific radioactivity of the unbound fraction was greater than or equal to that of the bound fraction. In this instance evidence for a precursor product relationship between the unbound and bound fractions was shown by the presence of a delay in the bound decay curve prior to its fall. Nestel et al (1983) reinjected labelled unbound VLDL, and although at early times the specific radioactivity

of the bound fraction was near that of the unbound, their studies showed more clearly the precursor product relationship between the unbound and bound fractions.

4.2. Integrated VLDL, IDL and LDL model

Although derived from four diverse type IV subjects, a single model (Figure 51) has been developed to describe the kinetics of VLDL, IDL and LDL apo B and triglyceride. In subject F however, a reduced model (Figure 36), based upon the same assumptions and physiologically interpretation, was fit to the data. The main features of both models are; (1) direct input of lipoprotein particles (apo B and triglyceride) of different hydrated densities into the plasma, mainly from the liver; (2) heterogeneous triglyceride precursor pools resulting in different rates of labelling of VLDL1 and VLDL2; (3) substantial delipidation of all nascent-like particles; (4) removal of VLDL2 particles prior to conversion to IDL and; (5) very high rates of VLDL triglyceride production, which appeared greater in VLDL2 than VLDL1, except in subject K. The model was simultaneously able to account for the VLDL apo B and TG kinetic data.

The technique of modelling both moieties within VLDL concurrently has been undertaken by relatively few workers (Beltz et al, 1985; Melish et al, 1980; Steiner and Reardon, 1983), despite the insights and benefits which can be obtained. To date only one study has examined and modelled the kinetics of LDL apo B and triglyceride simultaneously (Malmendier and Berman, 1978). Furthermore, the rising slope of the VLDL-triglyceride specific radioactivity curve has not previously been used to provide information about the rate constant of the nascent plasma VLDL particles in human subjects, an approach used in earlier quantitative studies of plasma triglyceride turnover in animals (Baker and Schotz, 1964; Baker, 1984).

4.2.1. VLDL model

The VLDL model (Figure 51) developed in this thesis consists of three sub-systems: glycerol, glycerol-to-triglyceride conversion, and VLDL. The VLDL1 and VLDL2 sub-systems were assumed to be identical in structure, based upon kinetic similarities of apo B and triglyceride in the corresponding fractions of each; however, they differ in that VLDL1 is transformed in part into VLDL2. As noted previously the appearance of ^{125}I from VLDL1 into VLDL2 was so rapid it was impossible to model the conversion of VLDL1 to VLDL2. Ignoring the contribution of VLDL1 to smaller particles the radioactivity remaining in VLDL1 was used to postulate a model in the same manner as for VLDL2. The analysis of the VLDL1 data is difficult to interpret without the additional information needed to elucidate the significance and impact of the rapid initial transformation of VLDL1 to VLDL2. Hence, as noted earlier, analysis and model development have focused upon the VLDL2 fraction.

The VLDL model was able to account for the marked differences among the subjects in the kinetic behavior of labelled apo B in the VLDL1 and/or VLDL2 unbound fractions, namely, the presence (or absence) of a dominant rapid component having the approximate rate constant predicted from labelled TG data for the nascent-like particles. Similar variations among subjects can also be seen in the data presented earlier by Nestel et al. (1983). Thus, in some subjects, nascent particles in the unbound fraction were assumed to be converted to unbound remnant particles and then to bound remnant particles (comps $17 \rightarrow 18 \rightarrow 19 \rightarrow 20$ for apo B and $10 \rightarrow 11 \rightarrow 12 \rightarrow 7$ for triglyceride), in which case the kinetic behavior of the unbound nascent-like particles would be masked in the unbound fraction. In other subjects (F and VLDL1 of H), the flow of unbound nascent particles was assumed to be directly

to bound remnant particles (comps 17→20 and 10→7), in which case the nascent-like particles would dominate the apo B kinetic behavior of the unbound VLDL fraction.

The nascent pool of particles was subdivided into two compartments to depict a minimal delipidation chain. Thus, the 6-compartment model consisting simply of nascent and remnant particles linked as 2- or 4-compartment chains could account for these kinetic differences in these subjects. It is possible however that the VLDL2 (or VLDL1) dilipidation chain is more complex or that it could be resolved into more sub-fractions.

The VLDL model as shown in Figure 51 permits the entry of nascent-like particles into both the unbound and bound fractions. Nestel et al (1983) however, observed that immediately following reinjection of labelled unbound VLDL apo B the specific radioactivity of the bound fraction was comparable to that of the unbound fraction, suggesting that upon exposure to the plasma there was a rapid remodelling of some particles, presumably involving the exchange of apo E or apo C with other lipoproteins. From this observation it could be inferred that only one type of particle may be secreted from the liver, presumably in the bound form, and that through apoprotein exchange is converted to the unbound form. The rate at which such a process would take place has not been determined.

These studies revealed that both bound and unbound VLDL fractions contained particles which behaved kinetically either as nascent or as partly catabolized lipoproteins. This could possibly be attributed to rapid metabolic interconversion of bound and unbound particles associated with the transfer of apo C. The in vivo remodelling of lipoproteins is a continuous process and involves mass transfer of apo E and apo C between HDL and VLDL (Tam and Breckenridge, 1987). Although within the VLDL fraction there may be apo E-

rich and apo E-poor particles, it is thought that apo E is not transported between these particles (Ginsberg, 1986). Studies with hepatocytes have shown that newly secreted VLDL are either apo C deficient (Davis et al, 1979) and/or rich in apo E relative to apo C (Wilcox and Hiemberg, 1987). Berry et al (1981) suggested that nascent VLDL may be rapidly removed by the liver; this process may involve the binding of apo E to receptors (Hui et al, 1985). Windler and Spaeth (1985) suggested that VLDL undergo compositional changes upon contact with plasma, resulting in a diminished affinity for hepatic receptors. Recent work (Windler and Havel, 1985) has shown that the transfer of apo C (especially apo C_3) to nascent VLDL reduces receptor affinity and thus retards early removal. Subsequent delipidation of the particle and remodelling which includes the loss of apo C, exchange with HDL of triglyceride for cholesteryl ester, and change in the conformation of apo B, restore the binding ability of the particle to receptors (Bradley et al, 1984). The appearance of newly secreted VLDL in both the unbound and bound fractions is therefore consistent with the early metabolism of VLDL. A high apo E/apo C ratio in both nascent and remnant particles may allow a proportion of the former and the bulk of the latter to bind to heparin by a process that resembles their physiological binding to the LDL receptor.

The triglyceride/apo B ratios were invariably higher in the unbound than in the bound fractions. Although this is consistent with a preponderance of newly secreted VLDL in the unbound fraction, both the unbound and bound fractions contained a proportion of slowly turning over remnant-like particles. The biexponential nature of the apo B specific radioactivity curve in the unbound fraction is consistent with other data indicating the unbound particles include partly catabolized VLDL (Nestel et al, 1983). Although generally cleared rapidly, the failure of the remnant particles in these

hypertriglyceridemic subjects to be cleared may be a reflection of their failure to attain the necessary conformation to bind to receptor, a disturbance recently reported in type V hyperlipoproteinemia (Nestel and Billington, 1987). In addition, the contamination by chylomicron remnants, which have been shown to be present in most forms of hypertriglyceridemia (Aviram et al, 1985; Cortner et al, 1987), of the VLDL fractions may mask the more rapid clearance of the VLD remnant particles.

Although all type IV hyperlipoproteinemics the apo B and triglyceride kinetics observed in each subject were quite distinct. The VLDL apo B kinetics of subject F showed the rapidly turning over unbound and slowly turning over bound fractions. Subject H (VLDL1 only) also displayed this feature. Subject F however was quite unique in that not only was the rapid unbound apo B fraction present but this subject showed that there was direct input into and direct removal, prior to conversion to IDL, from VLDL2. Although subjects K and H lost VLDL2 directly it could not be determined, from the apo B data, the extent of direct input of particles into the VLDL2 fraction. In these subjects the triglyceride data provided evidence that there was direct input into VLDL2 to account for the higher specific radioactivity of the VLDL2 fraction compared to VLDL1 and in subject H the large VLDL2 triglyceride transport. The apo B and triglyceride kinetics displayed in subject K, whose VLDL1 mass was mostly in the VLDL1 fraction, were different from those of the other subjects. Possibly the very low VLDL1 apo B FCR of this subject may be accounted for by the presence of chylomicron remnants which have been found throughout the lipoprotein spectrum (Cortner et al, 1987). Chylomicron remnants within this fraction have been shown to be catabolized slowly in type V hyperlipoproteinemics (Nestel and Billington, 1987). Although subject J was not injected with labelled VLDL his triglyceride specific radioactivity and apo

B mass data was fit using the same model. The turnover rates of the remnant VLDL, IDL and LDL remnant compartments of subject J were set using the rate constants derived from subject K. Lipid profiles for subject K and J were similar as were the characteristics of the triglyceride specific radioactivity curves. The VLDL1 data of subject J however was fit independently of any apo B data. As a consequence the errors associated with the fit of this data were greater than in those subjects where apo B data was used.

4.2.1.1. Apo B production and catabolism

The data for the apo B kinetics display the features reported recently by others (Packard et al, 1984; Beltz et al, 1985), although this analysis of the separated bound and unbound fractions has provided additional insight into the complexity of the catabolic processes. Whereas the fractional removal rates of whole VLDL1 and VLDL2 are relatively slow as observed by others in hypertriglyceridemic subjects (Packard et al, 1984; Beltz et al, 1985; Reardon et al, 1978) there is a rapidly catabolized fraction represented by a proportion of the unbound particles within VLDL. Other workers have postulated the existence of such a pool of rapidly removable larger VLDL particles (Beltz et al, 1985).

The apo B kinetics also confirmed the multiple inputs and exits involving different populations of VLDL. Although Reardon et al (1978) were the first to demonstrate, in hypertriglyceridemic subjects, a high rate of shunting of VLDL out of the circulation without conversion to IDL, this metabolic pathway has now been demonstrated to occur also in normal subjects to the extent of about 30% (Kesaniemi et al, 1985). It is more likely to occur with larger particles that contain more apo E than with smaller particles (Gianturco et al, 1983) and is therefore consistent with the affinity of apo E for

hepatic receptors (Bradley et al, 1984) and the preferential interaction of VLDL from hypertriglyceridemic subjects with the LDL receptor (Gianturco et al, 1982). It is worth noting that up to 70% of VLDL2 particles were removed presumably by this process. The metabolic channeling of particles may be predetermined by the nature of the secreted particle. In the rabbit, VLDL that contain both apo E and apo B are preferentially shunted back to the liver, whereas those which contain only apo B appear destined for more extensive delipidation and conversion to LDL (Yamada et al, 1986). Despite the lower apo E content of the IDL fraction direct shunting of IDL without conversion to LDL did occur, however only in subject F, presumably through the interaction of the apo B with the LDL receptor. In general however, IDL particles probably compete with LDL for the LDL receptor, but for IDL to bind to the receptor it probably requires further delipidation, and hence conversion to an LDL particle.

Most of the VLDL apo B mass and thus most lipoprotein particles were found within the VLDL2 fraction, except in subject K. These particles comprised more bound than unbound particles, had a higher cholesterol/triglyceride ratio and a lower triglyceride/apo B ratio than those in the VLDL1 fraction. This suggests that the VLDL2 fraction contained more remnant particles. However, the values for input of new apo B and triglyceride into the VLDL2 fraction clearly showed that this fraction also contained a large number of non-remnant particles. Evidence of there being a substantial quantity of nascent particles in the unbound VLDL2 fraction is also supported by the higher triglyceride/apo B ratio in the unbound VLDL2 than in the bound VLDL1 fractions.

The model also required direct input of VLDL2 particles as well as of VLDL1 particles (Table 17). In subjects F and J the VLDL2 production calculated

from ^{131}I was greater than the VLDL1 values. Although subject J, did not receive ^{131}I-VLDL2, direct secretion of VLDL2 would be required to account for the significantly greater transport of apo B through the VLDL2 fraction than that which passes through VLDL1. The production rates calculated for total VLDL apo B were of the order reported by others for hypertriglyceridemic subjects (Reardon et al, 1978; Kesaniemi et al, 1985) which are greater than for normolipidemic subjects (Packard et al, 1984).

Multiple input and exit pathways for VLDL particles of different sizes have been reported in all recently modelled systems of VLDL metabolism in man. Although the concept of direct secretion of smaller VLDL particles has been criticized on the basis that a rapidly turning over precursor particle can be missed by exogenous labelling techniques (Beltz et al, 1985) this should not apply to the analysis of endogenously labelled lipoproteins. It is therefore noteworthy that Fisher et al. (1980) using ^{3}H-leucine as a precursor label for apo B, reported rapid, simultaneous rates of labelling of large and small VLDL. Furthermore, they noted major biosynthetic inputs even into IDL in hypertriglyceridemic subjects (Fisher et al, 1980). Eaton et al (1983) who used [^{75}Se] selenomethionine as the precursor label observed major inputs into both the initial and final VLDL pools (within a four pool cascade) in both hypertriglyceridemic and normolipidemic subjects. A massive input into the largest initial kinetic pool of the VLDL was a hallmark of hypertriglyceridemia (Eaton et al, 1983). This is highly relevant to the possible failure of models based on exogenously labelled VLDL to identify this major influx of large VLDL, especially since Eaton et al (1983) had also found that these particles were least likely to be catabolized to IDL.

Such findings are nevertheless in line with the models developed by Beltz et al (1985) and by Packard et al (1984) who integrated data from the

kinetics of several exogenously labelled lipoprotein species. Both models consider multiple inputs at the beginning or near the end of the VLDL delipidation cascade, although the conclusion reached in one study was that a variable and flexible number of VLDL pools could explain the data without involving a need for multiple inputs (Beltz et al, 1985). However Packard et al (1984) drew attention to the metabolic channeling of particles within the spectrum of smaller VLDL, with those derived from the catabolism of larger VLDL having a different fate from those newly secreted; only the latter were destined for conversion to LDL.

Most models also incorporate multiple exits (Packard et al, 1984; Beltz et al, 1985; Yamada et al, 1986). In general the extent to which VLDL particles are catabolized fully to LDL is inversely related to their size (Packard et al, 1984) and apo E content (Yamada et al, 1986).

It is nevertheless important to note that direct measurements of net rates of secretion and uptake of VLDL across the splanchnic bed lead to different conclusions. Turner et al (1981) have measured the trans-splanchnic metabolism of labelled S_f 20-60 and of S_f 100-400 lipoproteins and observed uptake only of the former and direct secretion only of the latter.

Analysis of the ^{125}I and ^{131}I tracers in the VLDL2 fraction revealed different kinetics. Examination of the values for apo B FCR (Table 18) show a higher FCR for the directly iodinated VLDL2 than for the labelled VLDL1. Clearly this observation, which has also been noted by Packard et al (1984), reinforces the existence of lipoprotein heterogeneity within a given density range. That the FCR of the directly labelled VLDL2 was greater than that of VLDL2 derived from VLDL1 suggests that two groups of particles have different metabolic fates.

4.2.1.2. Triglyceride production and catabolism

A major extension in the interpretation of VLDL triglyceride kinetics was made possible by the additional data obtained from the heparin fractionation. Both VLDL1 and VLDL2 triglyceride kinetics exhibited features that have been described by Zech et al (1979); an initial rise, an early fast decay and, a late slow decay tail. The VLDL model (Figure 51) incorporated several features of the model that was constructed by Zech et al (1979), the first being the glycerol subsystem which was determined by Malmendier et al (1974) and the other being a modification of the free fatty acid to VLDL-triglyceride conversion subsystem proposed by Shames et al (1970). Since the triglyceride data did not display an appreciable delay prior to the rise in plasma-VLDL triglyceride specific radioactivity the delay component present in the Zech model was not used.

The data presented in this thesis have also described more fully the delipidation which converts nascent triglyceride-rich VLDL into triglyceride-depleted remnants. The broad peak of the bound fraction reflected the delipidation process within the bound fraction and, in addition, the conversion of unbound to bound particles. This was also observed in the bound apo B fractions which showed a pronounced delay prior to the fall in specific radioactivity. Apo B turnover experiments, which use exogenously labelled VLDL, do not, in general, show this delipidation process clearly unless the VLDL have been extensively subfractionated or frequent samples are collected during the first 15-30 minutes.

Although the delipidation process is primarily a function of lipoprotein lipase activity, a secondary process, the transfer of triglyceride from VLDL to other lipoproteins such as HDL (Barter et al, 1982b) may not be insignificant. Despite having observed rapid labelling of HDL triglyceride following the

injection of glycerol (unpublished data) these results would suggest that synthesis of newly secreted HDL triglyceride, rather than an exchange of core lipids between VLDL and HDL, which would occur at a slow rate, would be responsible for the HDL triglyceride kinetics, therefore reducing the importance of the transfer process as a way of removing triglyceride from VLDL.

The Zech et al (1979) model made provision for the delipidation of VLDL, but constrained the fraction delipidated in each pool to be the same and also the turnover rate of each pool. The new model shows that the fraction of each pool that is delipidated varies and thus should not be fixed. Clearly the reason for this constraint in the Zech model was an identifiability problem. The presence of additional data, like that obtained in these studies, may have allowed for the more accurate determination of compartment turnover rates.

The higher triglyceride/apo B ratio in the unbound VLDL2 fraction than in the bound VLDL1 had already suggested that independent input of new triglyceride into the VLDL2 fraction was likely. Nevertheless the ratios in the corresponding unbound fractions showed that in VLDL1 to be higher. The liver may therefore be secreting at least two different types of particles, the VLDL1 being more triglyceride enriched than the VLDL2. In addition, estimates of the VLDL precursor specific radioactivities showed that for the small VLDL the precursor triglyceride pool had approximately ten times the activity than that of the large VLDL precursor. In all but one subject the VLDL2 triglyceride pool was greater than that in VLDL1 demonstrating that the increased specific radioactivity of the VLDL2 fraction was not a function of pool size. There may therefore be different precursor pools in the liver or different mechanisms may be involved in the production of large and small VLDL. Large VLDL may undergo a longer assembly process and may derive

triglyceride from some slowly exchanging pools which are not labelled to any extent, there by effectively diluting the specific radioactivity of the VLDL1 fraction. Streja et al (1977) and Steiner and Reardon (1983) had also observed that the smaller VLDL attained a greater specific radioactivity than did the larger VLDL, an observation which has been confirmed in these studies.

The absolute production rates for VLDL triglyceride (Table 17) obtained in these subjects with the model are considerably higher than reported by others in type IV hypertriglyceridemia. Published production rates for triglyceride vary considerably, depending on the method by which the values have been calculated (Sigurdsson et al, 1976b). Many values, for the d < 1.006 g/ml (S_f >20), are around 200-400 mg/kg/day (Sigurdsson et al, 1976; Reardon et al, 1978;] et al, 1974) although Boberg et al (1972) reported an average of 983 mg/kg/day for hypertriglyceridemic subjects. The values, for VLDL1 triglyceride alone, are generally greater than those obtained by others measuring whole VLDL-triglyceride production. In the VLDL2 fraction triglyceride production values ranged from 888-1610 mg/kg/day, thus making whole VLDL-triglyceride production values 3-7 times those calculated by others. Clearly this suggests that VLDL-triglyceride production is much greater than has been calculated previously. Comparison of VLDL2 triglyceride transport with that derived using the Zech et al (1979) model reveals that the new model predicts both significantly greater triglyceride transport and higher fractional catabolic rate (Table 19). The reason for the higher values lies in the prediction and observation of the rapidly turning over population of particles, which represent a substantial proportion of the triglyceride mass within each VLDL fraction. Despite these high production rates the amount of triglyceride which passes through the bound remnant pool (compartment 7), of the VLDL fraction, is quite low. The level of lipolysis

that VLDL undergo is very high, leaving only a small fraction to reach the bound remnant pool.

4.2.2. IDL and LDL model

Early apo B turnover studies concluded that most LDL was derived from the catabolism of VLDL (Eisenberg et al, 1973; Sigurdsson et al, 1975). Like the early studies of VLDL metabolism it was assumed that the LDL fraction represented a homogenous population of particles. More recent studies however have shown that both IDL and LDL are heterogenous by size, density, composition and metabolic behaviour (Krauss and Burke, 1982; Packard et al, 1984; Musliner et al, 1985; 1987; Foster et al, 1986; Vega and Grundy, 1986; Teng et al, 1983; 1986).

To study the metabolic behaviour of apo B in IDL and LDL it is generally accepted that, in addition to tracing the conversion of VLDL into IDL and subsequently LDL, it is necessary to follow the metabolism of reinjected labelled LDL. Only when VLDL and LDL are reinjected is it possible to describe the heterogeneity within the less bouyant fraction. Although no subject received labelled IDL or LDL it is clear from the triglyceride specific radioactivity data that within these fractions there is considerable heterogeneity. The triglyceride specific radioactivity curves of IDL and LDL also support the notion of direct input of triglyceride, independent of that derived by conversion of VLDL and IDL.

The IDL and LDL models developed in this thesis are based upon many of the same assumptions which were used to develop the VLDL model. The structure of these models (Figure 51), like that of the VLDL shows a delipidation chain through which the new IDL and LDL enter and a subsequently delipidated. In addition to the direct input of apo B and

triglyceride it was assumed the IDL and LDL were derived from the conversion of VLDL2 and IDL respectively. It is important to note however that the conversion of particles from one density class to another is via the remnant compartments (comps 20→14→24, and 7→8→9). Within the IDL and LDL models it was assumed there were nascent and remnant-like particles. Nascent particles enter the IDL and LDL density ranges directly and are quickly delipidated to yield remnant particles. The rate at which the conversion of these nascent particles occurred was generally less than 30% of that in the VLDL fraction. The turnover rate of the LDL nascent compartments was less than that of the IDL. Evidence for the existence of such particles in IDL and LDL comes from the triglyceride specific radioactivity data within these fraction. Because labelled IDL and LDL were not reinjected the apo B kinetics of these fractions did not provide evidence to support the presence of quickly turning over nascent particles. The IDL and LDL apo B decay curves observed in these studies reflect the turnover rate of the rate limiting component. Although there may be quickly turning over pools of particles within IDL and LDL, the kinetics of such particles would be masked by the more slowly turning over particles.

Few studies have examined the metabolism of apo B within the IDL fraction. Although most LDL apo B models include an extravascular compartment to account, in part, for the biexponential nature of the LDL apo B decay curve the turnover studies reported here were carried out for a period of only two days. Generally LDL apo B turnover studies are carried out for a period of 10 to 14 days. As a consequence a slowly exchanging extravascular compartment was not incorporated into the model and therefore values for LDL FCR and production will be different to those determined using Mathew's (Mathews, 1957) or a more complex analysis.

Initially it was assumed that IDL apo B and triglyceride were derived from VLDL2 and LDL from IDL. It was however, not possible to fit the observed IDL and LDL triglyceride specific radioactivity curves using this assumption. This simple precursor-product approach produced fits that did not describe the rapid rise of radioactivity in both the IDL and LDL fractions. Although triglyceride and cholesteryl ester transfer between VLDL and IDL or LDL could account for transfer of radioactivity from VLDL to IDL and LDL it seems unlikely that such a mechanism would be rapid enough to predict the observed data. It was therefore assumed that labelled triglyceride must enter directly into the IDL and LDL fractions. This triglyceride was derived from the same precursor pools which supplied the VLDL2 fraction. To account for the rapid rise of the IDL and LDL triglyceride curves it was assumed that within each fraction there were quickly turning over pools of particles, like those in the VLDL fraction, and a slowly turning over remnant pool, the turnover rate of which was defined by the apo B kinetics. The turnover rate of the IDL and LDL nascent compartments was defined by the rise of the triglyceride specific radioactivity curves.

Only one previous study has examined the kinetics of apo B and triglyceride simultaneously within LDL (Malmendier and Berman, 1978). This study examined the conversion of apo B and of triglyceride from IDL to LDL. To date no studies have examined the kinetics of these moieties simultaneously in IDL. Although Malmendier and Berman (1978) included a two-pool LDL delipidation chain their model did not take into account the rapid rise in the LDL triglyceride specific radioactivity curve observed following glycerol. In addition, although they presented data describing IDL triglyceride kinetics they did not model this system.

4.2.2.1 Apolipoprotein B and triglyceride production and catabolism

Until recently it was thought that all LDL was derived from VLDL. However the simultaneous measurement of VLDL and LDL apo B transport revealed that, in hypertriglyceridemic subjects, some VLDL was removed directly from the circulation (Reardon et al, 1978). This removal was assumed to occur from the IDL density range. In these studies the IDL fraction was defined within the narrow range of S_f 12-20 although this fraction has been defined to represent particles of S_f 12-60.

Using the IDL model, described in the previous section, the transport of apo B and triglyceride through this fraction was determined. It is however important to note that estimates of turnover derived in this and the LDL fraction cannot be accurately determined without IDL or LDL reinjection data. Values for apo B and triglyceride production are therefore minimum values only. The reinjection of labelled IDL and LDL apo B would demonstrate the kinetic heterogeneity that others have shown exists within the fractions. (Fisher et al, 1980; Packard et al, 1984; Foster et al, 1986; Teng et al, 1986).

In the three subjects who received labelled VLDL2 between 40% and 70% of these particles were converted to IDL. In addition to input from the VLDL2 fraction there was considerable input of apo B into this fraction from another source. Of the total apo B turnover within IDL between 30 % and 70% was derived from non-VLDL2 sources. It is conceivable that in addition to direct input some IDL was derived from the VLDL1 fraction.

Because of the transient nature of the IDL fraction the transport of apo B through this fraction is rarely discussed. Berman et al (1978) represented the IDL fraction as a single compartment, and as such assumed that it represented a homogeneous population of particles. More recent studies

however (Beltz et al, 1985) however have shown wide variation among IDL apo B specific radioactivity curves. Although Beltz et al (1985) and Packard et al (1985) have used two compartment models to describe the IDL fraction these models may be an over simplification of the true nature of this IDL fraction. Although the composition of IDL has been measured in different hyperlipidemic subjects, profiles generated following the isolation of IDL by density gradient ultracentrifugation or gel filtration chromatography fail to reveal the presence of a single well defined peak. It is likely therefore, that the particles contained within the S_f range 12-20 may contain small VLDL, VLDL remnants and large bouyant LDL. If this is the case then separation of particles based upon size and density clearly does not isolate homogeneous fractions of particles.

The IDL model developed in this study allowed for the direct removal of IDL particles prior to conversion to the LDL density range. Only in subject F was there removal of IDL however, in subjects K and H all IDL was transported to the LDL fraction. In addition to the transport from IDL the apo B kinetics of all subjects demonstrated that the direct input of apo B into LDL was required. Accurate estimates of the input of apo B however could not be determined for the reasons discussed above. The clearance of LDL apo B from the subjects who received labelled VLDL was consistent with observations shown by others in similar subjects (Ginsberg et al, 1985; Packard et al, 1980). The rapid removal of LDL in hypertriglyceridemic subjects may be a function of their unusual composition (Fainaru et al, 1986). The rabbit studies of Yamada et al (1986) showed that the clearance of apo E-rich LDL, isolated using immunoaffinity chromatography, was greater than that of apo E-deficient LDL.

In contrast to the high levels of triglyceride transport in the VLDL fractions triglyceride transport in IDL and LDL represented approximately 5%

of that measured in VLDL2. Although there is significant amounts of triglyceride within these fractions the low levels of production may be a function of the slow kinetics which are observed in the apo B decay curves of both fraction. It is the kinetics of the slowest component which are observed in such decay curves. Is conceivable therefore, that within the IDL and LDL fractions there are particles which turn over more rapidly. The presence of such particles would, assuming there represent a significant proportion of particles within the fraction, result in increased transport of apo B and triglyceride through IDL and LDL.

Although both IDL and LDL fractions required direct input of apo B and triglyceride into each fraction to satisfy steady state conditions and the initial rise of the IDL and LDL triglyceride specific radioactivity curves the source of these particles could not be determined. Of the total turnover of apo B in IDL and LDL between 30 % and 60% of this was derived from non VLDL sources. Generally in normolipidemic individuals 50-100% of LDL is derived from VLDL (Kesanemi et al, 1987). Numerous studies in humans (Beltz et al, 1985; Janus et al, 1980, Kesanemi et al, 1985) and animals (Huff and Telford, 1985; Goldberg et al, 1983) have suggested that not all LDL is derived from VLDL. Beltz et al (1985) hypothesized the existence or a rapidly turning over pool of newly secreted VLDL which are rapidly converted to LDL following lipolysis. If such a pool exists then it would normally not be isolated for labelling hence total VLDL production would be underestimated. An alternative to this hypothesis is the direct secretion from the liver of IDL and LDL sized particles (Kesanemi et al, 1985). Recently however Goldberg et al (1988) has demonstrated that by inhibiting LPL the production of LDL independently of VLDL conversion was abolished. Such findings would support the notion of a rapidly turning over pool of nascent particles within the VLDL density range.

The IDL and LDL models developed here predict the presence of nascent-like particles within the IDL and LDL fractions. Observation of the triglyceride/apo B ratios of the compartments within the IDL and LDL fractions revealed that the ratios in the nascent compartments were greater than those of the remnant compartments in the VLDL fraction (Table 16). That such particles, with high triglyceride/apo B ratios, could be present in the IDL and LDL fractions suggests an inconsistency. The recent study of Goldberg et al (1988)) may therefore provide an explanation for the observed triglyceride kinetics within IDL and LDL. That initial rise in triglyceride specific radioactivity of the IDL and LDL nascent compartments is similar to that observed in the VLDL fraction may be suggestive of the secretion of large triglyceride rich nascent VLDL particles, some of which are rapidly hydrolysed to IDL and LDL particle size and density. Such nascent particles may be preferred substrate for LPL (Marzetta et al, 1989). The hydrolysis of these particles would result in loss of triglyceride and surface components, including apo E and apo C (Johnson et al, 1983) and an increase in hydrated density. The rise in the specific radioactivity of the IDL and LDL fractions may therefore be as a result of the rapid hydrolysis of nascent VLDL particles rather than the direct secretion of IDL and LDL particles. The triglyceride/apo B ratios of such particles could therefore be expected to approximate that measured within the whole IDL and LDL fraction.

4.3. Lipoprotein reinjection studies

Although no VLDL or LDL triglyceride reinjection studies were performed, Section 3.5.2 presents simulated studies which predict the kinetics of the triglyceride moiety within these fractions. The early studies of Friedberg et al (1961) and Farquhar et al (1965) determined that plasma VLDL

triglyceride metabolism was the rate limiting step in triglyceride metabolism. These studies observed that the turnover rate of VLDL triglyceride was equal to the terminal slope of the triglyceride decay curve after glycerol or palmitate. Since these studies many workers have observed in non-human species (Laurell, 1959; Baker and Schotz, 19864; Havel et al, 1962; Gross et al, 1967; Hannan et al, 1980) a rapid turnover rate for VLDL triglyceride, and have concluded that the terminal slope of the triglyceride curve reflects the slowly turning over liver precursor compartments. Havel and Kane (1975) reinjected VLDL triglyceride into human subjects, their findings corroborated those experiments performed in non-human species. Despite these observations, and the animal studies, the Zech et al (1979) VLDL triglyceride model incorporated the findings of the Friedberg et al (1961) and Farquhar et al (1965) studies. Unlike the Zech model the model developed here assumes that the liver is rate limiting, and as such predicts significantly greater estimates for VLDL triglyceride production and turnover.

A serious methodological problem noted in the study of Friedberg et al (1961) was that labelled VLDL isolated from a donor was stored for 4 days at 4°C prior to reinjection. During this period it is likely that exchange of triglyceride between the more- and less-rich triglyceride particles would have resulted in uniform specific radioactivity of the particles. Following the reinjection of these particles the VLDL triglyceride decay curve would have reflected the turnover rate of the slowly turning over remnant particles present in the VLDL fraction

If the assumption of Farquhar et al (1965) is correct then following the reinjection of labelled VLDL triglyceride the decay curve of the triglyceride moiety will be monoexponential and will be the same as the terminal slope following the injection of glycerol. If however the liver is rate limiting then

following injection of labelled VLDL triglyceride there should be an initial rapid decay followed by a more slowly decaying tail. The slope of the tail will describe the turnover rate of the slowly turning over remnant particles.

The time at which labelled VLDL is isolated from the donor is of importance. If VLDL is isolated 10 or more hours after the reinjection of labelled glycerol then upon reinjection the presence of a rapid component may not be observed. After 10 hours, in addition to extensive delipidation, and hence loss of radioactivity, most nascent particles will have been converted to remnants, the kinetics of which are slow.

Upon reinjection of labelled LDL triglyceride Malmendier and Berman (1978) observed, although didn't discuss, the initial fall of the LDL triglyceride curve. The simulated decay curve of reinjected labelled LDL triglyceride in subject H also displayed similar characteristics. That LDL triglyceride decay curves are not monoexponential suggests heterogeneity within the LDL fraction and that, based upon these studies these are particles within the LDL fraction which exhibit different kinetic characteristics.

CHAPTER 5

SUMMARY

Lipoproteins are macromolecular complexes of lipid and protein. Although their role in the transport of lipid to and from the periphery is generally understood, uncertainty surrounds the many mechanisms which result in hyperlipoproteinemia. The results presented in this thesis address a number of these unsolved questions through a series of experiments of novel design. Mechanistic hypotheses describing metabolic events were tested through development of a new model to account for the data. The major findings were: 1. evidence of increased heterogeneity within VLDL, IDL and LDL, and particularly within the VLDL fractions; 2. the presence of a rapidly turning over population of particles within VLDL; 3. significantly higher VLDL triglyceride production rates and FCR's than those calculated using the Zech et al (1979) model; and 4. evidence to support that liver-triglyceride turnover is rate limiting compared to the triglyceride moiety of some subpopulations of VLDL particles.

The key to many of these findings lay in the use of heparin-Sepharose affinity chromatography which was used to subfractionate VLDL particles in order to separate VLDL into nascent and remnant-like populations. However, this procedure did not cleanly separate nascent from remnant particle populations. The plasma disappearance curves of labelled unbound apo B suggested that the unbound fraction was partially contaminated with bound particles. To describe the VLDL fraction as being composed of particles which are either in the unbound or bound form is therefore an over simplification. Ideally, the use of more physiological procedures would permit the collection

of multiple discrete fractions. Nevertheless, the combination of heparin-Sepharose chromatography and ultracentrifugal separations did provided enough fractions and the definition of their interrelationships.

These studies have demonstrated the presence of a rapidly turning over population of particles in the unbound VLDL fraction in at least some subjects. It is possible that a better separation of VLDL would identify this rapidly turning over fraction in most people. Although the rapidly turning over unbound apo B fraction was not observed in all subjects, modelling the apo B and triglyceride data supported the presence of such components. This aspect will require further model development based on labelled apo B and/or triglyceride reinjections together with the isolation of more discrete multiple VLDL fractions. As demonstrated in these studies, it will not be possible to observe the presence of quickly turning over particles without observing the kinetics of particles within subfractions.

The reinjection of labelled VLDL-triglyceride may also support the presence of a rapidly turning over component within the VLDL fraction. However, as the simulation studies (Section 3.5.2) have shown, the identification of the rapid component is dependent upon the time at which VLDL is harvested for reinjection. Ethical considerations, such as the use of large amounts of radioactivity, may prevent such studies from being undertaken in human subjects. As a consequence studies such as these should be performed in non-human species whose lipid metabolism closely resembles that of humans. As an alternative to radioactive tracers, the use of stable isotopes may permit reinjection experiments to be performed in human subjects.

In addition to the reinjection of labelled VLDL-triglyceride, the observation that the specific radioactivity of VLDL2 triglyceride is always

greater than that of the VLDL1 fraction needs to be addressed. This observation suggests that the source of triglyceride precursors for VLDL1 and VLDL2 may be different. Although this point could not be fully addressed in these studies because of the rapid conversion of labelled-VLDL1 apo B to VLDL2 at the time of reinjection, the model developed here demonstrates that VLDL2 triglyceride precursors have a higher specific radioactivity than those which feed into VLDL1.

Although many researchers have demonstrated the existence of the 'direct' input of apo B lipoprotein species in the VLDL2, IDL and LDL density range, this has not been demonstrated for the triglyceride moiety. My studies suggest that, in addition to the direct input of apo B into all fractions, there is direct input of triglyceride. The source of triglyceride entering into IDL and LDL was assumed to be the same as that which supplies the VLDL2 fraction. The presence of this input alone, however, did not account for the observed triglyceride specific radioactivity curves in the IDL and LDL fractions. The model accounts for this feature by proposing that within the IDL and LDL fractions there were particles which, like those in the VLDL fractions, turned over more rapidly than the bulk of the particles within these fractions. The presence of these particles in the IDL and LDL fractions was not, however, supported by the apo B data in these fractions. In addition, although modelled in such a way, it could not be determined if such rapidly turning over particles are secreted directly into the IDL and LDL fractions, or if such particles are derived from large VLDL with high triglyceride specific radioactivity which are rapidly hydrolysed to IDL- or LDL-like sized particles following secretion.

Although the triglyceride specific radioactivity data suggested heterogeneity within IDL and LDL fractions, further experiments need to be

undertaken in which labelled IDL and LDL apo B are reinjected. Only by reinjecting these species, and with the collection of urine radioactivity data, will it be possible to construct a model which will describe the heterogeneity present within these fractions. By simply observing the kinetics of IDL or LDL following the reinjection of labelled VLDL it is not possible to accurately describe production levels or the degree to which new lipoprotein particles may enter directly into these fractions.

Determining the source of particles which are thought to enter directly into the more dense fractions, as opposed to those that pass via sequential conversion to a higher density, is currently a topic of significant interest. While studies using endogenous labels for apo B have suggested the presence of direct input of apo B into IDL and LDL, lipoprotein metabolism in humans is complicated by the interaction of LPL, HTL and LTP. To obtain a better understanding of the direct input of lipoprotein particles, studies need to be undertaken where the effect of LPL and LTP can be negated. Studies in which double and triple labels can be used, in combination with the separation of lipoprotein fractions into multiple subclasses, will also elucidate some of the mechanisms involved in lipoprotein metabolism. In addition, further studies to examine the effect of diets, drugs and genetic factors need to be performed. These factors may grossly alter lipoprotein metabolism and the spectrum of particles which are secreted by the liver.

In conclusion, while addressing a number of specific issues and resulting in data which allowed the testing of various hypotheses, this thesis raises many new questions. The new model which has been developed, is the simplest construct which will describe the data. Further studies will test various aspects of the model, and some will result in modifications to the model.

In due process, new physiological insights into the metabolism of lipoproteins and the factors which result in hyperlipoproteinemia will be identified.

APPENDIX A

SAAM input file describing the model used to fit all apo B and triglyceride turnover data. This input file describes the relationships which exist between compartments and how the turnover rates of individual compartments containing apo B and triglyceride are coupled.

```
A SAAM30        K131V2IL
2  35   25
3  5
4  2      1
H PAR
C
C             20-60 K APO B AND TG DATA
C                      131-I
C    FOLLOWING INJECTION OF LABELLED VLDL AND GLYCEROL
C    IT IS ASSUMED THAT THE RATES OF TURNOVER FOR A SINGLE
C    COMPARTMENT ARE THE SAME FOR BOTH TG AND APO B
C    ALL RATE CONSTANTS ARE EXPRESSED PER UNIT HOUR
C
C     INITIAL CONDITIONS FOR GLYCEROL IN C4
C
   IC(4)     6.6E+08
C
C     EXCHANGE RATE BETWEEN C4 AND C5 (GLYCEROL COMPS)
C
   L(4,5)    5.1
   L(5,4)    15.6
C
C     SUM RATE OUT OF C4 (EXCL L(4,5)
C
   P(25)     18.66
C
C     P(25)=LOSS OF GLYCEROL + GLYCEROL TO VLDL ETC
C
   P(25)=L(2,4)+L(0,4)
C
C     FRACTION CONVERTED TO COMP2 (LIVER)
C
   L(2,4)=P(2)*P(25)
   P(2)       5.170000E-02  1.550600E-02   1.395540E-01
C
C     PATHWAYS FROM LIVER (C2) TO UNB AND BD VLDL TG
C
   L(10,2)    5.688000E-02  2.278600E-02   2.050740E-01
   L(3,2)     4.817000E-02  1.796267E-02   1.616640E-01
C
C     PATHWAYS FROM LIVER (C2) TO IDL AND LDL TG
C
   L(13,2)    4.436000E-03  1.666667E-03   1.500000E-02
   L(22,2)    1.374000E-03  1.333333E-04   1.200000E-02
```

```
C
C     TURNOVER RATE OF COMP 3 (MOST NASCENT)
C     COMPS 3,6,7 REPRESENT BOUND VLDL TG
C     COMPS 10,11,12 REPRESENT UNBOUND VLDL TG
C     TURNOVER RATE OF 3,6,10, AND 11 ARE THE SAME
C     PROPORTION OF TG LOST FROM EACH IS ALSO SAME
C
    P(3)=L(0,3)+L(6,3)
    L(0,6)+L(7,6)=P(3)
    L(0,3)=L(0,6)
    L(0,10)+L(11,10)=P(3)
    L(0,11)+L(12,11)=P(3)
    L(0,10)=L(0,3)
    L(0,11)=L(0,3)
C
C     TURNOVER RATE OF MOST NASCENT COMPS
C
    P(3)       1.246400E+00  4.171866E-01   3.754680E+00
    L(0,3)=P(5)*P(3)
C
C     FRACTION HYDROLYSIS P(5)
C
    P(5)       7.961000E-01  2.603467E-01   1.000000E+00
C
C     UNBOUND TO BOUND REMNANT VLDLTG
C
    L(7,12)=P(21)
C
C     TURNOVER RATE OF REMNANT VLDL TG COMPS (P21)
C
    L(0,7)+L(8,7)=P(21)
    P(21)      8.970000E-02  2.887000E-02   2.598300E-01
    L(0,7)=P(8)*P(21)
C
C     FRACTION OF VLDL TG LOST FROM VLDL NOT
C     PASSING TO IDL COMPS
C
    P(8)       9.000000E-01
C
C     IDL TG COMPS 13, 21, 8
C     TURNOVER RATE OF REMNANT IDL TG COMP (P(26)
C
    L(0,8)+L(9,8)=P(26)
    P(26)      7.911000E-02  2.995585E-02   2.696027E-01
C
C     FRACTION OF IDL TG LOST FROM IDL NOT
C     PASSING TO LDL COMPS
C
    L(0,8)=P(10)*P(26)
    P(10)      9.000000E-01
C
C     RATE OF TURNOVER OF COMPS IN IDL DELIPIDATION
C     PATHWAY (P13) FOR COMPS 13 AND 21
C
```

```
   L(21,13)+L(0,13)=P(13)
C
C     FRACTION TG HYDROLYSIS (P20) FROM IDL
C
   L(0,13)=P(20)*P(13)
   L(8,21)+L(0,21)=P(13)
   L(0,21)=L(0,13)
   P(13)      3.437000E-01  0.000000E+00   1.000000E+00
C
C     SOMETIME NECESSARY TO CONSTRAIN HYDROLYSIS
C     OF IDL AND LDL NASCENT COMPS TO BE THE SAME
C
   P(20)=P(30)
C
C     RATE FOR TURNOVER OF REMNANT LDL COMP
C
   L(0,9)=P(27)
   P(27)      4.500000E-02
C
C     LDL TG COMPS 22, 30, 9
C     RATE OF TURNOVER OF COMPS IN LDL DELIPIDATION
C     PATHWAY (P23) FOR COMPS 22 AND 30
C
   L(30,22)+L(0,22)=P(23)
   P(23)      7.840000E-02  0.000000E+00   1.000000E+00
C
C     FRACTION TG HYDROLYSIS (P30) FROM LDL
C
   L(0,22)=P(30)*P(23)
   L(0,30)=L(0,22)
   L(0,30)+L(9,30)=P(23)
   P(30)      6.199000E-01  2.603467E-01   1.000000E+00
C
C
C     APO B SECTION OF THE MODEL
C
C     UNBOUND VLDL APO B COMPS 17, 18, 19
C     BOUND VLDL APO B COMPS 15, 16, 20
C
C     INITIAL CONDITIONS FOR BOUND VLDL APO B
C
   IC(15)+IC(16)+IC(20)=P(56)
   P(56)      3.662464E+06
C
C     INITIAL CONDITIONS FOR NASCENT BD VLDL COMPS
C     ARE THE SAME (EQUAL TURNOVER RATES)
C
   IC(15)=IC(16)
C
C     RADIOACTIVITY IN PROPORTION TO MASS
C
   IC(16)=IC(20)*P(21)/L(20,16)
C
C     INITIAL CONDITIONS FOR UNBOUND APO B COMPS
```

```
C
   IC(17)+IC(18)+IC(19)=P(55)
   P(55)      2.408246E+06
   IC(17)=IC(18)
   IC(19)=IC(18)*L(19,18)/L(20,19)
C
C     INITIAL CONDITIONS FOR IDL APO B
C
   IC(14)+IC(25)+IC(26)=P(57)
   IC(25)=IC(26)
   P(57)      4.774250E+05
   IC(14)=(IC(25)*L(14,26))/P(26)
C
C     INITIAL CONDITIONS FOR LDL APO B
C
   IC(24)+IC(27)+IC(28)=P(58)
   P(58)      2.116690E+05
   IC(27)=IC(28)
   IC(24)=(IC(28)*L(24,28))/P(27)
C
C     TURNOVER RATES OF VLDL UNB AND BD APO B COMPS
C
   L(19,18)=P(3)
   L(16,15)=P(3)
   L(18,17)=P(3)
   L(20,16)=P(3)
C
C     TURNOVER RATE OF REMNANT VLDL APO B COMPS
C
   L(20,19)=P(21)
C
C     FRACTION OF VLDL APO B LOST NOT PASSING
C     TO IDL APO B
C
   L(0,20)+L(14,20)=P(21)
   L(0,20)=P(11)*P(21)
   P(11)      2.843000E-01  6.420280E-02   5.778252E-01
C
C     IDL APO B COMPS 14, 26, AND 25
C     TURNOVER OF NASCENT IDL APO B COMPS
C
   L(14,26)=P(13)
   L(26,25)=L(14,26)
C
C     FRACTION OF IDL APO B LOST NOT
C     PASSING TO LDL
C
   L(0,14)+L(24,14)=P(26)
   L(0,14)=P(12)*P(26)
   P(12)      0.000000E+00
C
C     LDL APO B COMPS 24, 28, 27
C     TURNOVER OF NASCENT LDL APO B COMPS
C
```

```
   L(28,27)=P(23)
   L(24,28)=P(23)
C
C     TURNOVER RATE OF REMNANT LDL APO B COMP
C
   L(0,24)=P(27)
C
H STE
C
C     STEADY STATE MASS INFORMATION (MG TOTAL)
C
C     MASS UBD AND BD VLDL TG
C
   M(10)+M(11)+M(12)=3264
   M(3)+M(6)+M(7)=3264
   M(3)       1.238004E+03                    5000
   M(10)      1.832065E+03                    5000
   U(3)       1.543048E+03                    5000
   U(10)      2.283486E+03                    5000
C
C     MASS IDL TG
C
   M(8)+M(13)+M(21)=1020
   U(13)      1.401824E+02                    1000
C
C     MASS LDL TG
C    M(9)+M(22)=900
   U(22)      5.133763E+01                    1000
C
C     MASS UBD AND BD VLDL APO B
C
   M(17)+M(18)+M(19)=235.8
   M(15)+M(16)+M(20)=386.7
   M(15)      1.135999E+01                    100
   M(17)      1.483466E+01                    100
   U(15)      1.415909E+01                    100
   U(17)      1.848992E+01                    100
C
C     MASS IDL APO B
C
   M(14)+M(25)+M(26)=348.2
   U(25)      2.861800E+00                    100
C
C     MASS LDL APO B
C
   M(24)+M(27)+M(28)=1350
   U(27)      1.607168E+01                    100
C
C
H DAT
C
C     ALL TG DATA IN SPECIFIC RADIOACTIVITY
C                 (DPM/MG TG)
C     ALL TIMES ARE EXPRESSED IN HOURS
```

```
C
C     FUNCTIONS DESCRIBING VLDL, IDL AND
C               LDL TG DATA
C
X G(8)=(F(3)+F(6)+F(7))/3264
X G(9)=(F(10)+F(11)+F(12))/3264
X G(12)=(F(3)+F(6)+F(7)+F(10)+F(11)+F(12))/6528
X G(22)=(F(8)+F(13)+F(21))/1020
X G(23)=(F(9)+F(22)+F(30))/900
C
108 G(8)                                        FSD=.10
C
C     BOUND VLDL TG
C
              0
              .03             67.1
               ..              ..
               ..              ..
              48.38           42.4
109 G(9)                                        FSD=.10
C
C     UNBOUND VLDL TG
C
              0
              .03             65.3
               ..              ..
               ..              ..
              48.38           14.9
112 G(12)                                       FSD=.10
C
C     UNFRACTIONATED VLDL TG
C
              0
              .03             66.8
               ..              ..
               ..              ..
              48.38           22.9
C
122 G(22)                                       FSD=.10
C
C     IDL TG
C              0
              .03
               ..              ..
               ..              ..
              48.38           63
C
123 G(23)                                       FSD=.10
C
C     LDL TG
C
              0
              .03             24.9
               ..              ..
```

```
              ..                ..
            48.38             77
C
C     ALL APO B DATA IN SPECIFIC RADIOACTIVITY
C               (CPM/UG *1000)
C     DATA *1000 TO CONVERT FROM UG TO MG
C
C     FUNCTIONS DESCRIBING ALL APOB DATA
C
X G(10)=(F(15)+F(16)+F(20))/386.7
X G(11)=(F(17)+F(18)+F(19))/235.8
X G(13)=(F(15)+F(16)+F(17)+F(18)+
   F(19)+F(20))/622.5
X G(14)=(F(14)+F(25)+F(26))/348.2
X G(24)=(F(24)+F(28)+F(27))/1350
C
110 G(10)                     *1000          FSD=.10
C
C     BOUND VLDL APO B
C
              0
              0.03            9.03
               ..              ..
               ..              ..
              48.38           .52
111 G(11)                     *1000          FSD=.10
C
C     UNBOUND VLDL APO B
C
              0
              .03             9.39
               ..              ..
               ..              ..
              48.38           .13
113 G(13)                     *1000          FSD=.10
C
C     UNFRACTIONATED VLDL APO B
C
              0
              .03             11.29
               ..              ..
               ..              ..
              48.38           .38
C
114 G(14)                     *1000          FSD=.10
C
C     IDL APO B
C
              0
              .03             2.31
               ..              ..
               ..              ..
              48.38           1.28
C
```

```
124 G(24)                    *1000          FSD=.10
C
C     LDL APO B
C
              0
              .03
               ..              ..
               ..              ..
              48.38           1.71
C
C
C
101
C       BD VLDL TG INPUT (MG/KG/D)
    G(1)=U(3)*24/79.0
C       UBD VLDL TG INPUT (MG/KG/D)
    G(2)=U(10)*24/79.0
C       SUM VLDL TG INPUT (MG/KG/D)
    G(3)=G(1)+G(2)
C       DIRECT IDL TG INPUT (MG/KG/D)
    G(4)=U(13)*24/79.0
C       DIRECT LDL TG INPUT (MG/KG/D)
    G(6)=U(22)*24/79.0
C       BD VLDL APO B INPUT (MG/KG/D)
    G(31)=U(15)*24/79.0
C       UBD VLDL APO B INPUT (MG/KG/D)
    G(32)=U(17)*24/79.0
C       SUM VLDL APO B INPUT (MG/KG/D)
    G(33)=G(31)+G(32)
C       DIRECT IDL APO B INPUT (MG/KG/D)
    G(34)=U(25)*24/79.0
C       DIRECT LDL AP0 B INPUT (MG/KG/D)
    G(35)=U(27)*24/79.0
C       VLDL TG FCR (/H)
    G(41)=(U(3)+U(10))/6528
C       IDL TG FCR (/H)
    G(42)=(R(8,7)+U(13))/1020
C       LDL TG FCR (/H)
    G(43)=(R(9,8)+U(22))/900
C       VLDL APO B FCR (/H)
    G(44)=(U(15)+U(17))/622.5
C       IDL APO B FCR (/H)
    G(45)=(R(14,20)+U(25))/348.2
C       LDL APO B FCR (/H)
    G(46)=(R(24,14)+U(27))/1350
C       IDL TG TURNOVER (MG/KG/D)
    G(51)=(U(13)+R(8,7))*24/79.0
C       LDL TG TURNOVER (MG/KG/D)
    G(52)=(U(22)+R(9,8))*24/79.0
C       IDL APO B TURNOVER (MG/KG/D)
    G(61)=(U(25)+R(14,20))*24/79.0
C       LDL APO B TURNOVER (MG/KG/D)
    G(62)=(U(27)+R(24,14))*24/79.0
```

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