DEVELOPMENT AND APPLICATION OF A NOVEL METHOD TO DETERMINE LARGE VERY LOW DENSITY LIPOPROTEIN ($VLDL_1$) KINETICS

by

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Author's Declaration

Unless otherwise indicated by acknowledgment or reference to published literature, the presented work in this thesis is the author's own and has not been submitted for a degree at another institution.

IQBAL ALSHAYJI ________________________________ Date______________

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To my Mother,

with the utmost love, respect and appreciation.

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List of Abbreviations

Apo	Apolipoprotein
ABCA1	ATP-binding cassette A1
ALT	Alanine aminotransferase
ARF-1	ADP ribosylation factor 1
CABG	Coronary artery bypass graft
CE	Cholesteryl esters
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHOL	Cholesterol
CM	Chylomicrons
CR	Clearance rate
CV	Coefficient of variation
CVD	Cardiovascular disease
DNL	*de novo* lipogenesis
ER	Endoplasmic reticulum
ERGIC	Endoplasmic reticulum-Golgi intermediate compartment
Exp	Exponential decay
FA	Fatty acid
FC	Free cholesterol
FCR	Fractional catabolic rate
FSR	Fractional synthetic rate
GC-MS	Gas chromatography mass spectrometry
HDL	High density lipoprotein
HL	Hepatic lipase
$HOMA_{IR}$	Homeostasis model assessment-estimated insulin resistance
HSPG	Heparan sulfate proteoglycans
IDL	Intermediate density lipoprotein
IL	Intralipid
IVFTT	Intravenous fat tolerance test
LCAT	Lecithin cholesterol acyltranferase

LDL	Low density lipoprotein
LDLR	LDL receptor
LLTP	Large lipid transfer protein
LPL	Lipoprotein lipase
LRP	LDL receptor-related protein
MI	Myocardial infarction
MTP	Microsomal triglyceride transfer protein
MW	Molecular weight
NEFA	Non-esterified fatty acid
PL	Phospholipids
PLTP	Phospholipids transfer protein
PR	Production rate
QC	Quality control
SAAM II	Simulation, Analysis And Modeling II
SD	Standard deviation
sdLDL	Small dense low density lipoprotein
SEM	Standard error of the mean
$\mathbf{S_f}$	Svedberg floatation rate
SR-BI	Scavenger receptor type B class I
SS	Steady State
TG	Triglycerides
TRL	Triglyceride-rich lipoprotein
VLDL	Very low density lipoprotein
VLDLR	VLDL receptor
$\dot{V}O_{2max}$	Rate of maximal oxygen uptake

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Figure 8.1: Production rates of [A] $VLDL_1$-TG and [B] $VLDL_1$-apoB and [C] Intralipid-TG clearance rate for all subjects (n = 23) according to apoE phenotypes: E2/E3 (n = 3), E3/E3 (n = 11) and E4+ (n = 9). $P > 0.05$ for differences between phenotypes. 180

Abstract

High concentrations of large very low density lipoproteins ($VLDL_1$) give rise to atherogenic dyslipidaemia, which is usually associated with insulin resistant conditions (e.g. obesity) and increases the risk for cardiovascular disease (CVD). Isotopic tracer methods for determining $VLDL_1$ kinetics are costly, time-consuming, labour intensive and need experience and skill to calculate the kinetic parameters. The aim of this thesis was to develop a simpler and cost-effective method of obtaining triglyceride-rich lipoproteins (TRL) kinetic data, based on the fact that chylomicrons (CM) or CM-like particles (e.g. Intralipid) compete with large $VLDL_1$ for the same lipoprotein lipase (LPL)-mediated catalytic pathway. From this method, it was possible to determine $VLDL_1$-triglyceride (TG) and -apolipoprotein (apo) B production rates and the Intralipid-TG clearance rate (as a surrogate measure of CM clearance). Kinetic data obtained from this method agreed with values and relationships obtained from stable isotope methods. The protocol is relatively quick, inexpensive, and transferable to non-specialist laboratories.

As a first application, the 'Intralipid method' was used to investigate the effects of hyperinsulinaemia and hyperglycaemia due to glucose ingestion on $VLDL_1$-TG and -apoB production rates and Intralipid-TG clearance rate. This showed that hepatic $VLDL_1$ production is suppressed in response to hyperinsulinaemia and that the change in Intralipid-TG clearance rate with hyperinsulinaemia correlated significantly with HOMA-estimated insulin resistance ($HOMA_{IR}$). In addition, alanine aminotranferase (ALT) concentrations (a marker for liver fat), within normal range, predicted the extent of hepatic $VLDL_1$ suppression.

Secondly, the Intralipid method was used to investigate the mechanisms responsible for the hypotriglyceridaemic effect of a moderate exercise session (120 min walking at 50% $\dot{V}O_{2max}$) in overweight/obese middle-aged men; the section of the population at high risk of CVD in whom exercise-for-health guidelines are targeted. This showed that the exercise-induced reduction in plasma TG was due to increased $VLDL_1$-TG and -apoB clearance, rather than decreased hepatic production. Exercise

also increased Intralipid-TG clearance rate, but to a lesser extent than $VLDL_1$, suggesting an increased affinity of $VLDL_1$ for LPL-mediated lipolysis post-exercise.

Taken together, the Intralipid method is a relatively simple, safe and cost-effective method to determine in $VLDL_1$-TG and -apoB production rates and Intralipid-TG clearance rates. It is also sensitive enough to detect physiological changes in TRL kinetics.

1. Introduction and Literature Review

1.1 Introduction

This chapter aims to establish a scientific rationale for the experimental studies described in this thesis. It starts with a brief overview of lipoprotein classification and metabolism. This is followed by an in-depth review of very low density lipoprotein (VLDL) metabolism and regulation, with special emphasis on the effects of elevated concentrations of VLDL in the formation of atherogenic dyslipidaemia. In addition, the effects of hyperinsulinaemia and moderate exercise on VLDL production are also discussed. Finally, methods used for measuring VLDL kinetics are reviewed.

1.2 Lipoprotein Metabolism

1.2.1 Classification and Properties of Lipoproteins

Lipids, being insoluble in water, need to be combined with proteins for transport in the blood. The resulting particles are *lipoproteins* and the protein moieties are known as *apolipoproteins (apo)* (Gurr *et al.*, 2002). The Lipoprotein particle consists of a hydrophobic core of triglycerides (TG) and cholesteryl esters (CE), surrounded by a monolayer surface of the amphipathic (both hydrophilic and hydrophobic) phospholipids (PL), small amounts of free cholesterol and proteins (Ginsberg *et al.*, 2005). Lipoproteins differ according to the ratio of lipid to protein within the particle as well as the proportions of lipids, they form a continuum of particles varying in composition, size, density and function (Gurr *et al.*, 2002; Ginsberg *et al.*, 2005). It is convenient, therefore, to classify plasma lipoproteins into different density classes and to separate and isolate them by ultracentrifugation (Gurr *et al.*, 2002). These are: chylomicrons (CM), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) (**Table 1.1**). It should be noted, however, that these classes are not homogenous and there is a wide variety of particle size and chemical composition within each class. The major apolipoproteins are described in **Table 1.2**.

Table 1.1: Composition and characteristics of the human plasma lipoproteins (Gurr *et al.*, 2002; Ginsberg *et al.*, 2005; Packard & Shepherd, 1997)

Class	Density ($g.ml^{-1}$)	Svedberg Floatation Rate (S_f)	Diameter (nm)	Apolipoproteins	Function	Composition (%)			
						Protein	TG	CHOL*	PL
Chylomicrons	<0.95	>400	80-1000	AI, AII, AIV, B-48, CI, CII, CIII, E	Transport of dietary fat	2	80-95	2-7	3-9
VLDL	0.95-1.006	20-400	30-80	B-100, CI, CII, CIII, E	Transport of endogenous fat	8	55-80	5-15	10-20
IDL	1.006-1.019	12-20	25-35	B-100, CI, CII, CIII, E	-	19	20-50	20-40	15-25
LDL	1.019-1.063	0-12	18-25	B-100	Transport of CHOL to periphery	22	5-15	40-50	20-25
HDL	1.063-1.210	–	5-12	AI, AII, AIV, CI, CII, CIII, D, E	Reverse transport of CHOL	40	5-10	15-25	20-30

* Free and esterified cholesterol (CHOL).

Table 1.2: Characteristics of the major apolipoproteins (Gurr *et al.*, 2002; Ginsberg *et al.*, 2005)

Apolipoprotein	MW	Major site of synthesis	Function
apo A-I	28 000	Liver, intestine	Structural component of HDL; activates LCAT
apo A-II	17 000	Liver, intestine	May inhibit HL activity; inhibits AI/LCAT
apo A-IV	44 500	Intestine	Activates LCAT; possibly facilitates transfer of apos between HDL and chylomicrons
apo A-V	39 000		Associated with lower TG levels; facilitates LPL
apo B-48	241 000	Intestine	Structural component of chylomicrons
apo B-100	513 000	Liver	Necessary for secretion of VLDL from liver; structural protein of VLDL, IDL and LDL; ligand for the LDL receptor
apo C-I	6 600	Liver	Activates LCAT; may inhibit hepatic uptake of chylomicrons and VLDL remnants; may inhibit CETP
apo C-II	8 800	Liver	Activates LPL (essential cofactor)
apo C-III	8 800	Liver, intestine	Inhibits LPL and hepatic uptake of chylomicrons and VLDL remnants
apo E	34 000	Liver (60-80%), other tissues including adipose tissue	Ligand for LDL receptor, LDL receptor-related protein and proteoglycans

Lipoproteins can also be classified according to their major lipids content into TG-rich lipoproteins (TRL), which include chylomicrons and VLDL, and cholesterol-rich lipoproteins, which include LDL and HDL (Sparks & Sparks, 1994).

Lipoprotein metabolism is a complex process to which new insights are continually being added. TRL metabolism is divided into the exogenous and endogenous lipid transport pathways. The exogenous pathway involves the delivery of dietary lipids (cholesterol, TG and PL) from the small intestine to the liver and peripheral tissues, while the endogenous pathway involves the delivery of lipids from the liver to peripheral tissue. As far as cholesterol metabolism is concerned, the exogenous and endogenous pathways are collectively known as forward cholesterol transport, whereas HDL metabolism is also known as the reverse cholesterol transport as it removes cholesterol from peripheral tissues.

1.2.2 Exogenous Lipoprotein Metabolism

After ingestion of a meal, dietary TG and cholesterol are absorbed into the small intestine cells where they are packed into nascent apoB-48-containing chylomicrons (Ginsberg *et al.*, 2005) with a Svedberg flotation rate (S_f) of >400 (Gurr *et al.*, 2002) (**Figure 1.1**). The newly synthesised chylomicrons enter the circulation through the lymphatic system and via the superior vena cava, where they acquire apoC-II, apoC-III and apoE (Ginsberg *et al.*, 2005). Chylomicrons also contain apoA-I and apoA-IV (Alaupovic, 1991). The TG content of chylomicrons is then hydrolysed by lipoprotein lipase (LPL) (section 1.2.5) situated on the capillary endothelium of predominantly adipose and muscle tissues (Fielding & Frayn, 1998; Goldberg, 1996). While apoC-II acts as activator for LPL (Olivecrona & Beisiegel, 1997), both apoC-III (Wang *et al.*, 1985) and apoE (Jong *et al.*, 1997) act to inhibit lipolysis by LPL. However, it is the balance of apoC-II and apoC-III that determines, in part, the efficiency with which LPL hydrolyses chylomicron TG (Ginsberg *et al.*, 2005; Chan *et al.*, 2008). The released non-esterified fatty acids (NEFA) from chylomicrons are either taken up by adipose tissues, re-incorporated into TG and stored, or by muscle tissue where they can be used for energy (Ginsberg *et al.*, 2005). Some dietary NEFA will also ‘spillover’ into the circulation (Barrows *et al.*, 2005). As a result of lipolysis, a new particle called ‘chylomicron remnant’ is formed with some attached LPL molecules (Saxena *et al.*, 1989). However, it should be noted that the majority of chylomicrons do not form small chylomicron remnants, rather they show signs of being marginated to the vascular endothelium and removed as very large chylomicron remnants long before they reach the size of VLDL (i.e. S_f 60-400 or the S_f 20-60 range) (Karpe *et al.*, 1997). Chylomicron remnants are relatively enriched in CE, both diet- and HDL-derived, which is transferred in exchange for TG, mediated by cholesteryl ester transfer protein (CETP) (Cooper, 1997; Ko *et al.*, 1994; Chung *et al.*, 2004). They are also enriched in apoE and apoB and depleted of apoA-I and apoCs (Cooper, 1997; Ginsberg *et al.*, 2005). The normal physiological function of chylomicron remnants is to return bile cholesterol to the liver by an enterohepatic circulation (Redgrave, 2004), while its residual TG content is an important source of hepatic fatty acids (FA), accounting for ~73% of newly synthesised hepatic VLDL in mice (Jung *et al.*, 1999).

Hepatic uptake of remnant particles (**Figure 1.2**) is thought to start when they are sequestered in the liver perisinusoidal space (space of Disse), where they undergo further processing by hepatic lipase (HL) and LPL (both of which may remain associated with the particle) and acquire apoE. LPL, HL and apoE can potentially serve as ligands for a group of hepatic scavenger receptors, which endocytoses the particles, leading to their lysosomal catabolism. Endocytosis of remnant particles occurs via the apoB/apoE-recognizing LDL receptor (LDLR) or LDL receptor-related protein (LRP) in association with heparan sulfate proteoglycans (HSPGs) or independently by proteoglycans that are secreted into the space of Disse (MacArthur *et al.*, 2007; Havel & Hamilton, 2004; Mahley & Ji, 1999).

Another member of the LDLR family that functions as a peripheral apoE-recognising remnant lipoprotein receptor is the VLDL receptor (VLDLR). It is expressed abundantly in FA-active tissues (heart, skeletal muscle and adipose) and binds TRLs but not LDL (Takahashi *et al.*, 2004). It is likely that VLDLR functions in concert with LPL and has been reported to play a major role in chylomicron metabolism by enhancing LPL-mediated TG hydrolysis in mice (Goudriaan *et al.*, 2004).

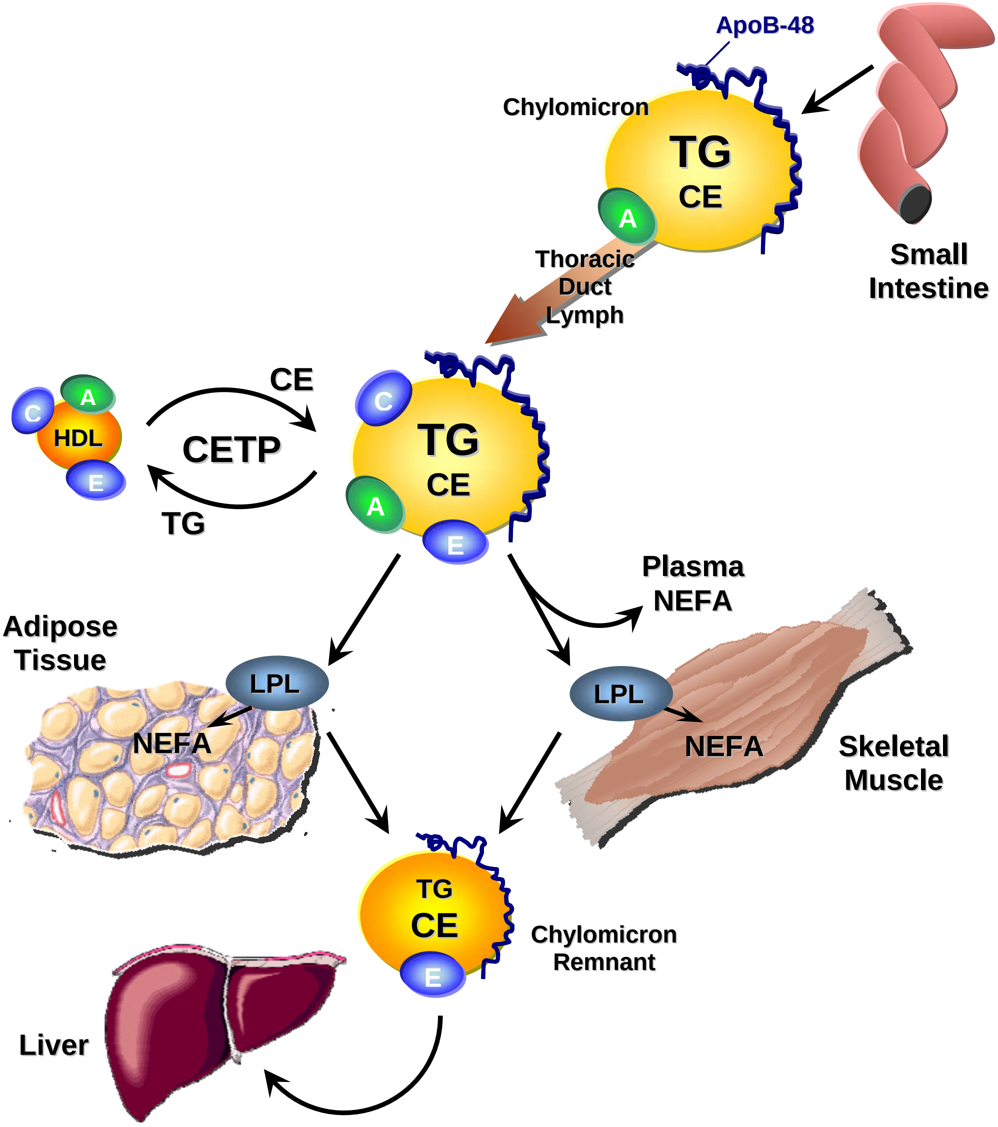


Figure 1.1: Chylomicron Metabolism. Dietary triglycerides (TG) and cholesteryl esters (CE) are absorbed into the small intestine and packed as chylomicrons, which also contain apoB-48 and apoA-I proteins. Chylomicrons are secreted into the circulation through the lymphatic system, where they acquire apoCs and E (apoC-II acts as an activator for the lipoprotein lipase enzyme (LPL) and apoE is needed for receptor recognition by the liver). Chylomicron-TG is hydrolysed by LPL situated on the capillary endothelium of mainly adipose tissues and skeletal muscles, providing the underlying tissue with non-esterified fatty acids (NEFA). Some dietary NEFA may 'spillover' in the circulation during hydrolysis by LPL. Chylomicron-TG is also removed from the particle in exchange for CE from high density lipoprotein (HDL). This is catalysed by the action of cholysteryl ester transfer protein (CETP). The result is a slightly smaller and CE-enriched particle called 'chylomicron remnant'. It is also depleted in apoCs and apoA-I. The majority of chylomicron remnants are removed from plasma long before they reach the size of VLDL particles range. Remnant particles are taken up by the liver by a number of receptors (see **Figure 1.2**).

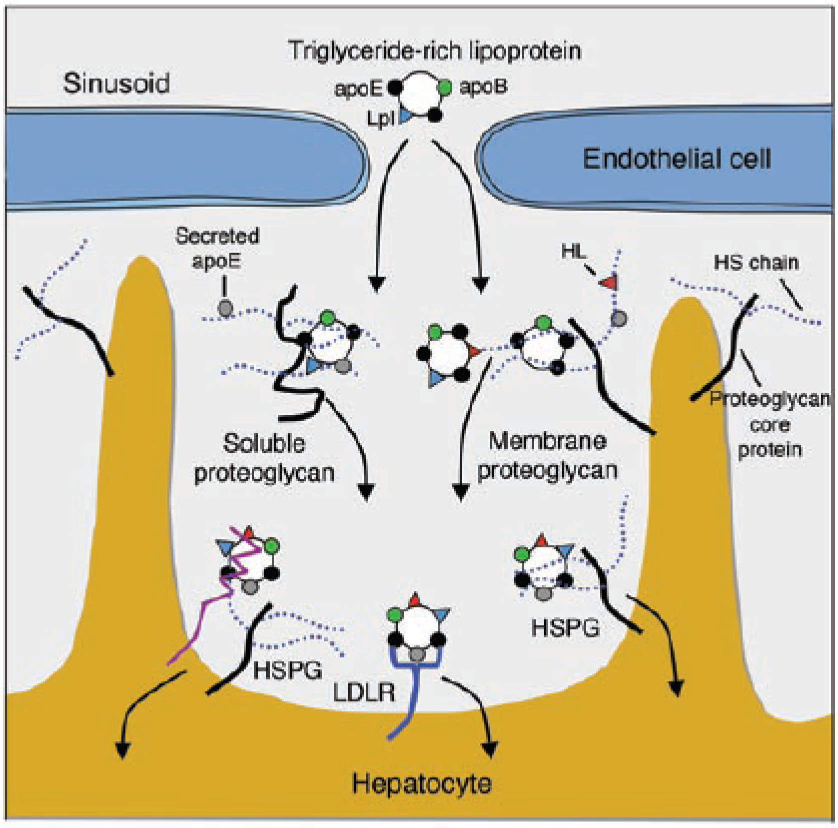


Figure 1.2: Model of possible hepatic uptake of TRL remnants. Hepatocytes and endothelial cells produce membrane-bound heparan-sulfate (HS) proteoglycans (HSPGs) and secrete proteoglycans into the space of Disse. After lipolytic processing of lipoproteins in the circulation by lipoprotein lipase (LPL; blue triangles), apoE-enriched (black circles) remnant lipoproteins enter the space of Disse through fenestrations in the endothelium. Remnant lipoproteins are thought to be sequestered near the hepatocyte cell surface via apoE-HS binding or lipase-HS bridging on secreted HSPGs. Lipoproteins are further processed in the space of Disse by transfer of soluble apoE (grey circles) and by hepatic lipase (HL; red triangles) bound via HS. ApoE, HL, and LPL can potentially serve as ligands of TRLs. Endocytosis of lipoprotein particles occurs via LDL receptor (LDLR; blue) or LDL receptor-related protein (LRP; purple) in association with HSPGs or independently by proteoglycans. [Figure and legend reproduced with permission from *Journal of Clinical Investigation* (MacArthur *et al.*, 2007).]

1.2.3 Endogenous Lipoprotein Metabolism

The major bulk of endogenous plasma TG is carried by VLDL, which is continually synthesised and secreted by the liver (Packard & Shepherd, 1997). The VLDL particle contains one molecule of apoB-100 (Elovson *et al.*, 1988) and approximately 5-25,000 TG molecules in its core (Bjorkegren *et al.*, 1998). It also contains apoCs and apoE (Packard & Shepherd, 1997). There are at least two major subclasses of VLDL particles: large TG-rich $VLDL_1$ (S_f 60-400) and smaller, more dense $VLDL_2$ (S_f 20-60), which have more cholesterol and a lower ratio of apoCs and apoE to apoB (Packard & Shepherd, 1997; Packard *et al.*, 1984). In normolipidaemic subjects, about 75% of the variation in plasma TG seems to be determined by the $VLDL_1$ concentration (Tan *et al.*, 1995). Once in the circulation, the TG content of $VLDL_1$ is rapidly removed by LPL-mediated hydrolysis (Karpe *et al.*, 2007) and the action of CETP, which facilitates the transfer of TG from TRLs (mainly VLDL) to HDL in exchange for CE from HDL to apoB-containing lipoproteins (VLDL, IDL and LDL) (Stein & Stein, 2005; Ko *et al.*, 1994). As $VLDL_1$ particles become progressively depleted in TG, they may either attain the same size and TG content as $VLDL_2$ particles, or continue its delipidation to form IDL. This is further aided by the lipolytic action of HL, which is also involved in the hydrolysis of IDL-TG. As a result, LDL appears as the terminal particle (Packard *et al.*, 1984). During the transition of VLDL to IDL and IDL to LDL, a portion of the surface PL, apoCs and apoE are transferred to HDL (Verges, 2005).

Approximately 10% of $VLDL_1$ particles undergo complete delipidation to LDL, while the remaining cease delipidation in the VLDL or IDL density range forming remnant particles that persist in the circulation for considerable periods before being removed from plasma (Packard & Shepherd, 1997; Packard *et al.*, 1984). On the other hand, almost all $VLDL_2$ particles will be effectively delipidated to LDL (Gaw *et al.*, 1995; Packard *et al.*, 1984). Similarly, not all IDL particles are delipidated to LDL, some are cleared by the liver (Packard & Shepherd, 1997) along with TRL remnant particles, possibly by the clearance pathway as chylomicron remnants (see **Figure 1.2**).

LDL is the main cholesterol-carrying lipoprotein in plasma with each particle containing one molecule of apoB-100. Generally about 65% of the total plasma cholesterol is carried in LDL in man (Wang & Briggs, 2004) and its major role is to supply tissues with cholesterol, which is paramount for every cell for the synthesis and maintenance of cell membranes. LDL particles are removed from plasma by the LDLR with LDL_2 particle exhibiting the highest affinity (Packard & Shepherd, 1997). While 70% of the LDLRs are located on hepatic cells, 30% are located on the other cells of the body (Verges, 2005).

1.2.4 HDL Metabolism

The movement of lipids via chylomicrons, VLDL, IDL and LDL provides the peripheral cells with FA (fuel) and cholesterol (necessary for synthesis and maintenance of cell membranes). While this 'forward' movement of TG is balanced by the metabolic consumption of NEFA as a source of fuel, there is no analogous (i.e. catabolic) mechanism for the metabolic disposal of cholesterol. This is accomplished by 'reverse' cholesterol transport by HDL particles (Tulenko & Sumner, 2002). Over the last several decades, epidemiological studies have revealed that levels of LDL-cholesterol are directly associated with risks of CVD, while an inverse relationship existed between HDL-cholesterol and the risk of the disease (Wang & Briggs, 2004).

HDL particles are synthesised and secreted, mainly by the liver, as nascent or lipid-poor disc-shaped particles (pre-β HDL) containing apoA-I and small amount of PL (Wang & Briggs, 2004; Tulenko & Sumner, 2002). This lipid-poor apoA-I avidly absorbs free cholesterol and PL from peripheral cells mediated by ATP-binding cassette A1 (ABCA1) transporter on the 'donor' cells plasma membrane (Wang & Briggs, 2004). The free cholesterol, being amphipathic, is absorbed onto the surface of the small HDL, which is progressively esterified by lecithin cholesterol acyltranferase (LCAT; bound to HDL and activated by apo A-I) and stored in the core of HDL (Tulenko & Sumner, 2002; Wang & Briggs, 2004). The continuous addition of cholesterol converts pre-β HDL to larger spherical HDL_3. Together with the action of phospholipid transfer protein (PLTP) which transfers phospholipids to HDL (mostly HDL_3) particles from VLDL/LDL (van Tol, 2002), HDL_3 is converted to the much larger HDL_2 particles (Wang & Briggs, 2004; Verges, 2005). This stage of cholesterol transfer from cells to the HDL_3 is mediated either by cell-surface receptors scavenger receptor class B type I (SR-BI) or passive diffusion, both distinct from that mediated by ABCA1 (Wang & Briggs, 2004). In addition, HDL accumulates apoC-II and apoE from VLDL and IDL, which serve as a reservoir of apolipoproteins, especially apoC-II which is needed for the activation for LPL (Tulenko & Sumner, 2002).

The TG content of HDL is hydrolysed by HL, while removal of cholesterol from HDL_3 particles occurs through CETP-mediated neutral lipid (TG and CE) exchange between HDL and apoB-containing lipoproteins. Because the bulk of cholesterol removal is accomplished by shuttling to these particles, their uptake by the liver is essential for the disposal of HDL cholesterol (Tulenko & Sumner, 2002; Wang & Briggs, 2004). The degradation of the larger HDL_2 occurs directly by CE-selective uptake by the liver mediated by SR-B1. The apoA-I from the degradation is either recycled for new HDL formation or cleared by the cubulin, a receptor highly expressed in kidney and yolk sac (Wang & Briggs, 2004).

Figure 1.3 summarises the endogenous lipoprotein metabolism.

1.2.5 Lipoprotein Lipase

Lipoprotein lipase (*EC* 3.1.1.34) is a key enzyme for the hydrolysis of circulating TG (Fielding & Frayn, 1998; Preiss-Landl *et al.*, 2002). It is synthesized by parenchymal cells, then transferred to the luminal surface of endothelial cells, where it is bound to HSPG (Goldberg, 1996). LPL is distributed in a variety of tissues, with the highest concentrations occurring in adipose tissue and muscle (Wong & Schotz, 2002) [cardiac and skeletal (Preiss-Landl *et al.*, 2002)]. However, substantial LPL activity is also detectable in differential macrophages, brain, placenta, lung, spleen, pancreatic β-cells and steroidgenic tissue (Preiss-Landl *et al.*, 2002). LPL functions to supply the underlying tissue with FAs derived from the TG-rich core of circulating chylomicrons and VLDL (Olivecrona *et al.*, 1997). However, chylomicrons have been shown to be the preferred substrate for LPL (Potts *et al.*, 1991; Fisher *et al.*, 1995), as they may bind to LPL with an affinity of 50 times higher than VLDL (Xiang *et al.*, 1999). In addition, it has been shown that about forty LPL molecules may act on a TRL particle simultaneously to achieve the rates of TG hydrolysis observed (Scow & Olivecrona, 1977). After hydrolysis of TG, the TG-depleted particle detaches along with some LPL molecules that may dissociate from the endothelium and leave attached to the remnant particle (Goldberg, 1996; Fielding & Frayn, 1998). There is a continual turnover of LPL at the endothelial site of action, with replacement of the dissociated LPL molecules by newly secreted molecules from within the tissue. LPL circulating attached to lipoprotein particles may play an important role in their eventual receptor-mediated uptake (Fielding & Frayn, 1998) (see **Figure 1.2**).

The apoC-II associated with the TRL particle acts as an activator of the enzyme (Goldberg, 1996; Olivecrona & Beisiegel, 1997) and apoE, which is a strong heparan-binding protein, anchors TRLs to the HSPG on the surface of the endothelial cells (Goldberg, 1996). In contrast, apoC-III is the principal plasma inhibitor of VLDL lipolysis; it inhibits LPL as well as interfering with remnant lipoprotein clearance (Shachter, 2001; Havel *et al.*, 1973).

The regulation of LPL is tissue-specific in such a way that its expression correlates highly with both the need for, and the uptake of, lipid fuels by the tissue. For instance, in the fed state, when energy is abundant, LPL is downregulated in skeletal muscle and heart and activated in white adipose tissue which facilitates the lipolysis of TRL-TG in the latter tissue, directing released NEFA for esterification and storage (Fielding & Frayn, 1998). On fasting, however, the situation is reversed, with suppression in adipose tissue and upregulation in muscle, increasing TRL-TG lipolysis in muscle, so that the resultant NEFAs are directed to the tissue in which they are needed as an oxidative fuel (Fielding & Frayn, 1998; Ruge *et al.*, 2005). Such changes in LPL expression are mediated predominantly through the action of hormones, such as insulin, glucocorticoid, and adrenaline (Mead *et al.*, 2002) as well as cytokines, fatty acids and glucose (Preiss-Landl *et al.*, 2002). Insulin is of particular interest because it plays a central role linking dyslipidaemia and insulin resistance, the defining feature of the metabolic syndrome (Howard, 1999). This will be discussed further in section 1.5.

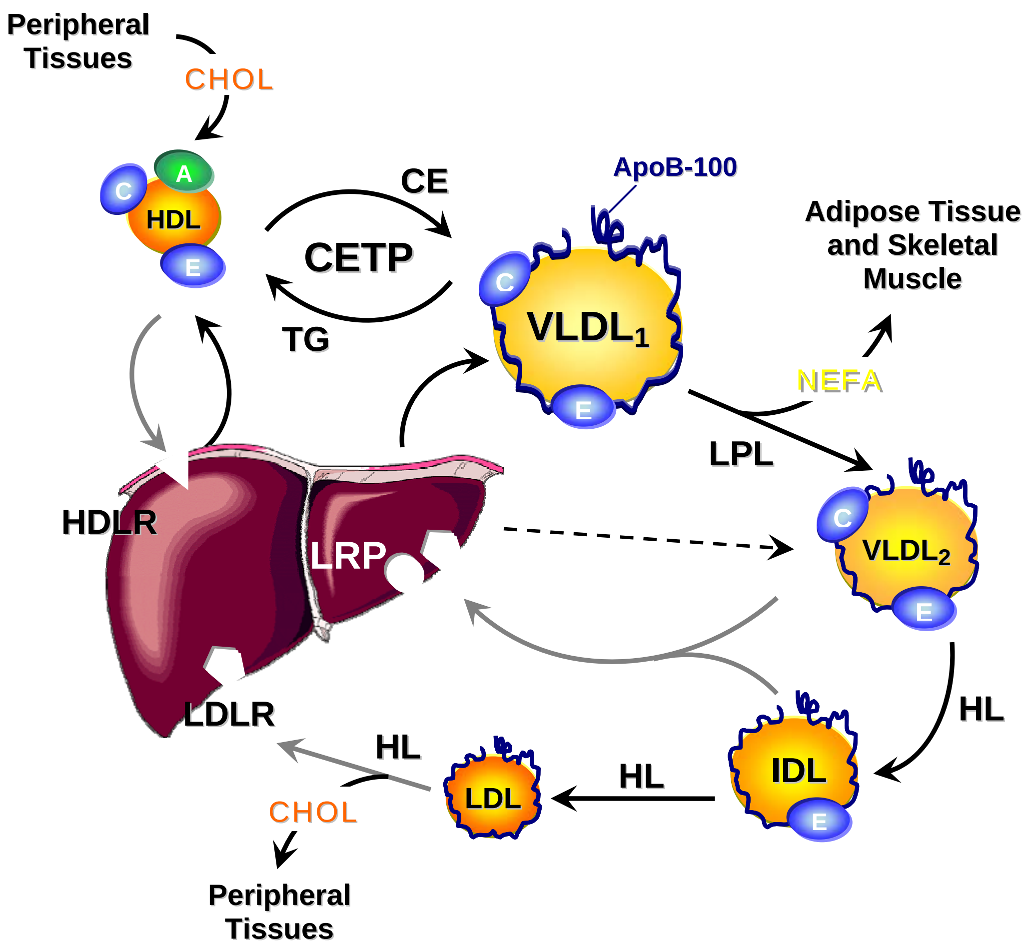


Figure 1.3: Overview of the general aspects of endogenous lipoprotein metabolism. The grey lines represent hepatic uptake of lipoproteins and lipoprotein remnants. The major bulk of endogenous triglycerides (TG) is secreted by the liver in the form of $VLDL_1$ particles, of which apoB-100 protein forms an integral part on the surface of the particle. Once in the circulation, TG in the core of $VLDL_1$ is hydrolysed by the action of lipoprotein lipase (LPL) which provides non-esterified fatty acids (NEFA) to adipose tissue and skeletal muscle. $VLDL_1$ also loses some of its TG content to HDL in exchange for cholesteryl esters (CE) via the action of cholesteryl ester transfer protein (CETP). The continual removal of TG from $VLDL_1$ gives rise to smaller $VLDL_2$, which is also produced by the liver. $VLDL_2$ is either taken up by the liver or delipidated further by the action of hepatic lipase (HL) to form IDL then LDL. Unlike other apolipoproteins, apoB-100 stays with the particles as they are delipidated. LDL is the main cholesterol (CHOL)-carrying lipoprotein which provides cholesterol to peripheral tissues. HDL, on the other hand, is also secreted by the liver and acts as a 'reverse' cholesterol carrier, from peripheral tissues to the liver. LDL is taken up by the liver by the apoB/E recognising LDL receptor (LDLR). Remnant (smaller) lipoprotein particles are also taken up by the liver in a similar manner to chylomicron remnants (Figure 1.2) by hepatic receptors, such LDLR and LDL receptor-related protein (LRP).

1.2.6 Integration of TRL Metabolism

In the fasted state, the continually secreted VLDL (particularly the larger $VLDL_1$) particles by the liver are the major substrates for LPL (Fisher *et al.*, 1995), providing endogenous fat to peripheral cells. However, after ingestion of a meal, the newly formed chylomicrons, being the preferred substrate for LPL (Potts *et al.*, 1991; Fisher *et al.*, 1995), will compete with $VLDL_1$ for the same LPL-mediated catalytic pathway (Brunzell *et al.*, 1973; Karpe *et al.*, 1993a). As the percent TG hydrolysed by LPL in TRL subfractions was chylomicrons > $VLDL_1$ > $VLDL_2$, it was proposed that increasing the size and TG content of a lipoprotein particle increases its susceptibility to hydrolysis by LPL (Fisher *et al.*, 1995). Another possible explanation for this preferential lipolysis of chylomicrons compared with VLDL might be due to transient changes in $VLDL_1$ composition in the postprandial state. Both $VLDL_1$ and $VLDL_2$ are enriched in cholesterol, apoE, apoC-I and apoC-III in the postprandial state, but depleted in apoC-II (Bjorkegren *et al.*, 1997). While apoC-II acts as activator for LPL (Olivecrona & Beisiegel, 1997), both apoC-III (Chan *et al.*, 2008) and apoE (Jong *et al.*, 1997) act to inhibit lipolysis by LPL.

As a result of this competition, the delipidation of $VLDL_1$ particles is delayed and their concentration is increased as they accumulate in plasma after the appearance of chylomicrons (Karpe *et al.*, 1993a) or chylomicron-like particles (Karpe & Hultin, 1995; Bjorkegren *et al.*, 1996) (**Figure 1.4**). Indeed, the postprandial increase in the TRL particle number is mainly accounted for by VLDL, particularly $VLDL_1$ (Karpe *et al.*, 1993a). $VLDL_2$ particles, on the other hand, are not affected by this competition and, in fact, their concentrations are often lowered or unchanged postprandially (Bjorkegren *et al.*, 1996; Karpe *et al.*, 2007). This is because, firstly, less $VLDL_1$ is converted into $VLDL_2$ and, secondly, $VLDL_2$ is not a particularly good substrate for LPL (Fisher *et al.*, 1995; Karpe *et al.*, 2007).

This competition and the preferential lipolysis by LPL between chylomicron-like particles and $VLDL_1$ form the basis of work described in this thesis.

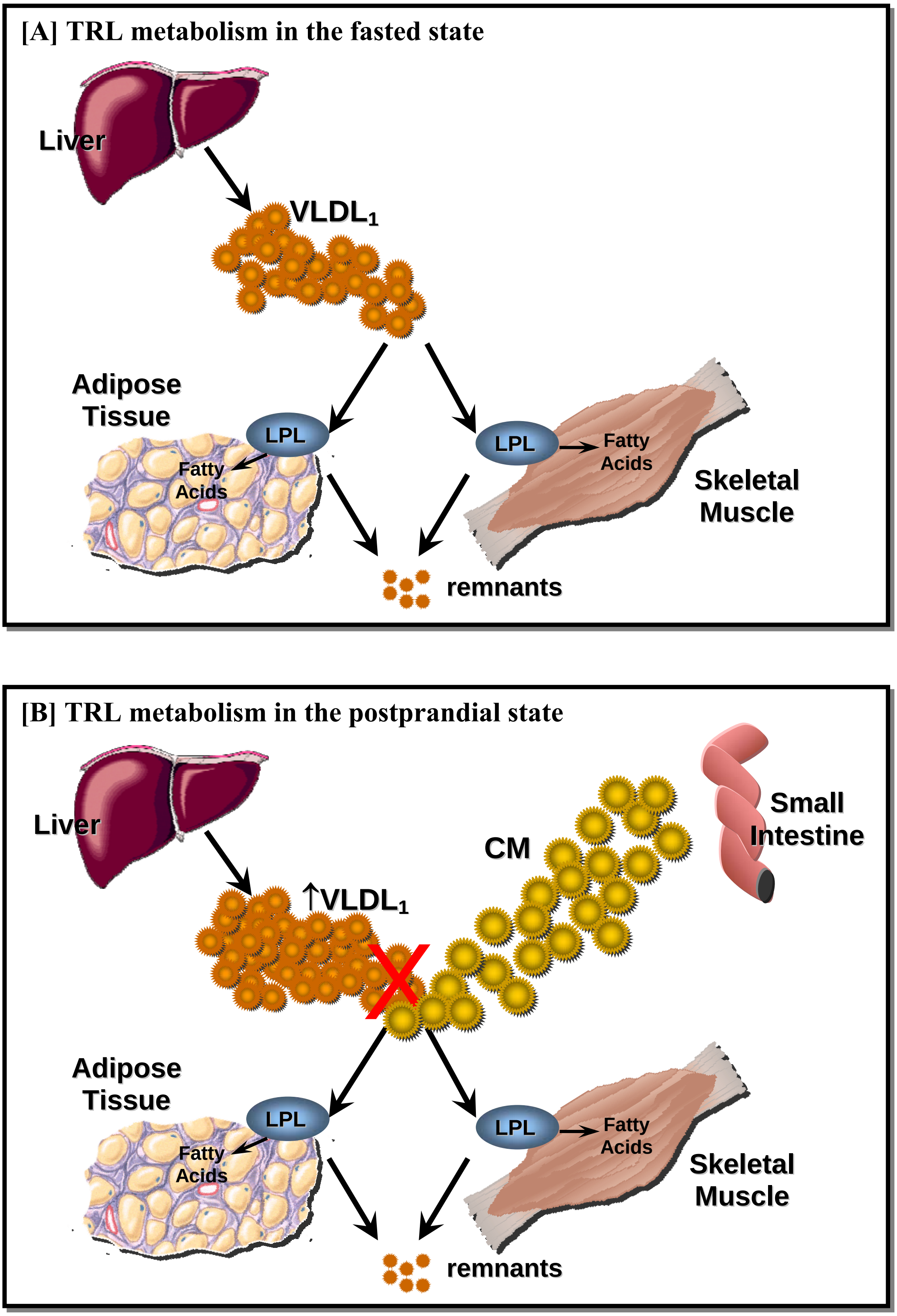


Figure 1.4: TRL metabolism in the [A] fasted and [B] postprandial state. $VLDL_1$ and chylomicrons (CM) are both cleared by lipoprotein lipase (LPL), situated on the capillary endothelium of adipose tissue and skeletal muscle. CMs are the preferred substrate for LPL. Thus, the presence of CM (or CM-like particles) inhibits $VLDL_1$ clearance by this pathway, causing $VLDL_1$ to accumulate in the circulation.

1.3 Hepatic Assembly and Secretion of VLDL

The assembly of VLDL in the liver involves a number of complex processes which are still not fully understood. VLDL assembly is believed to take place in two major steps within two different compartments of the cell, rough endoplasmic reticulum (ER) and Golgi apparatus, and involves three different particles, pre-VLDL, $VLDL_2$ and $VLDL_1$ (Olofsson & Borén, 2005). It commences during the synthesis of apoB which is an integral part of the VLDL structure.

1.3.1 Apolipoprotein B Structure

Unlike other plasma lipoproteins, apoB is an amphipathic high molecular weight glycoprotein which is insoluble in aqueous solutions (Segrest *et al.*, 2001; Kane, 1983). Because it binds irreversibly to TRLs and LDL, it is not transferable between lipoproteins (Kane, 1983; Davidson & Shelness, 2000). There are two forms of apoB in mammals, which are encoded by the same gene (Davidson & Shelness, 2000). The full-length apoB, apoB-100, consists of 4536 amino acids, whereas apoB-48 corresponds to 48% of the protein from the N-terminal (Davidson & Shelness, 2000). ApoB-48 is produced by a site-specific cytidine-to-uridine RNA editing reaction which converts a glutamine codon to a stop codon causing translational termination of (intestinal) apoB mRNA at residue 2152 (Davidson & Shelness, 2000; Olofsson & Borén, 2005). In humans, apoB-100 is expressed in the liver, forming VLDL, while apoB-48 is expressed in the small intestine, forming chylomicrons.

The apoB protein has a pentapartite structure consisting of one globular N-terminal structure, followed by two amphipathic β-sheets alternating with two amphipathic α-helical domains (NH_2-$\beta\alpha_1$-β_1-α_2-β_2-α_3-COOH) (Segrest *et al.*, 2001). The $\beta\alpha_1$ domain (the N-terminal 1000 residues) is a globular structure that bears structural homology with the lipid-binding pocket of microsomal TG transfer protein (MTP) (an ER luminal protein with lipid transfer activity – see below) and the lamprey vitellogenin (an egg yolk lipoprotein) (Segrest *et al.*, 2001). Thus, apoB, MTP and vitellogenin are considered members of the same gene family collectively known as large lipid transfer proteins (LLTPs). Although vitellogenin was presumed to be the ancestral member, recent evidence suggests that MTP may be the oldest LLTP family member (Shelness & Ledford, 2005).

The amphipathic β-sheet domains of apoB interact irreversibly with the neutral lipid core, while the α-helical domains, which are similar to those found in soluble apolipoproteins, may form strong but reversible binding to the lipoprotein surface (Davidson & Shelness, 2000). The β_2-region contains the LDL receptor-binding domain, needed for binding and hepatic endocytosis of LDL particles, and resembles the receptor binding domain of apoE, another LDLR ligand. As this domain is in the truncated C-terminal region of apoB-48, chylomicrons depend on apoE to bind to the LDLR (Segrest *et al.*, 2001).

1.3.2 Assembly of VLDL

The intracellular assembly of VLDL (**Figure 1.5**) starts in the rough ER during the biosynthesis of apoB, which is lipidated co-translationally; i.e. while the C-terminal portion of apoB is still being synthesised on the ribose of the ER, the N-terminal portion is translocated across the ER and is assembled as a small lipoprotein particle (Olofsson & Borén, 2005; Dashti *et al.*, 2007). ApoB interacts co-translationally with MTP, which catalyses the addition of lipids [in the order of TG > CE > diacylglycerol > cholesterol > PL (Rava *et al.*, 2005)] to the growing apoB molecule. This results in the formation of a primordial HDL-sized lipoprotein particle, pre-VLDL, which is retained in the cell by interaction with chaperones and other ER proteins (Olofsson & Borén, 2005; Shelness & Sellers, 2001). It has been recently shown that MTP is not required for the initiation of this step as the first N-terminal 1000 amino acid residue (apoB:1000) has the capacity to fold forming a PL-rich 'lipid-pocket', independently of the structural requirement and lipid-transfer activity of MTP (Manchekar *et al.*, 2004; Dashti *et al.*, 2007). It is currently unclear at which point in VLDL assembly that MTP is required. In addition to its lipid transfer activity, MTP facilitates the co-translational translocation of apoB across the ER lumen (Blasiole *et al.*, 2007) and prevents its degradation as apoB will be sorted to proteasomal degradation if it misfolds or is underlipidated (Fisher & Ginsberg, 2002; Hussain *et al.*, 2003). Pre-VLDL is lipidated further to become $VLDL_2$, or sorted to degradation. This conversion of pre-VLDL to $VLDL_2$ may explain why the MTP activity is needed for the secretion of apoB-100 after the translation is completed (Rustaeus *et al.*, 1998; Olofsson & Borén, 2005).

The apoB-100 containing $VLDL_2$ particle exits the ER at specific exit sites carried by two vesicle proteins: a GTPase referred to as SAR1 and a coat protein called coatamer protein II (COPII) (Gusarova *et al.*, 2003; Olofsson & Borén, 2005), which fuse to become ER-Golgi intermediate compartment (ERGIC). It is converted to *bona fide* $VLDL_1$ by post-translational acquisition of TG in the Golgi apparatus (Shelness & Sellers, 2001; Olofsson & Borén, 2005). This lipidation differs from that which gives rise to $VLDL_2$ and requires that apoB has reached the size of apoB-48. In addition, it is dependent on ADP ribosylation factor 1 (ARF-1), a small GTP binding protein which plays a role in the membrane trafficking between the ER and the Golgi apparatus (Asp *et al.*, 2000; Asp *et al.*, 2005). It has been suggested that this 'maturation' step of $VLDL_1$ may involve the formation of lipid droplets in the lumen of the secretory pathway, which fuse with apoB to form $VLDL_1$ (Olofsson & Borén, 2005). Further, this conversion of $VLDL_2$ to $VLDL_1$ seems an additional step for TG secretion from the liver as it is not necessary for the secretion of apoB (Olofsson & Borén, 2005). The liver has been shown to secrete both $VLDL_1$ and $VLDL_2$ particles (Packard *et al.*, 2000). Interestingly, this stepwise assembly and secretion of VLDL explains the time delay between the biosynthesis of apoB-100 and the major addition of lipids to the $VLDL_1$ particles, which was estimated as ~15 min (Adiels *et al.*, 2005a).

1.4 Regulation of VLDL Production

The secretion of VLDL has been shown to be mainly dependent on fatty acids and insulin.

1.4.1 Role of Fatty Acids

The liver has the capacity to store TG in order to accommodate plasma fatty acids, which are surplus to immediate energy requirements, thereby neutralising any potential 'lipotoxicity' to peripheral tissue. There are three potential sources of fatty acids which enter the hepatic TG storage pool: *de novo* lipogenesis (DNL), plasma NEFA [originating from adipose tissue and 'spillover' from dietary fat lipolysis (Barrows *et al.*, 2005)] and remnant lipoproteins (Gibbons *et al.*, 2000). A healthy liver uses different sources of FAs in the fasted and fed states (Barrows & Parks, 2006). It is estimated that during fasting, ~77% of VLDL-TG is derived from recycling adipose FAs and ~4% from DNL. With feeding, 44% come from adipose FAs, 15% from uptake of chylomicron remnants, 10% from dietary NEFA spillover into plasma and 8% from DNL (Barrows & Parks, 2006), with dietary FAs appearing in VLDL within 90 min of food intake (Heath *et al.*, 2003).

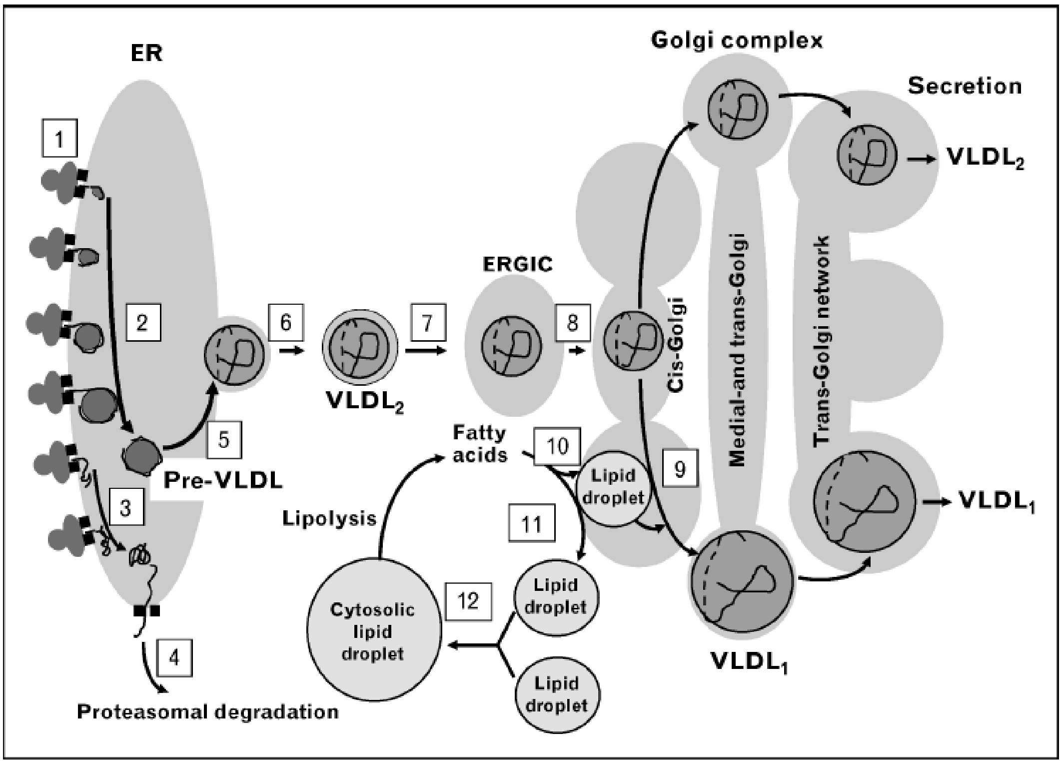


Figure 1.5: The Intracellular assembly of VLDL. (Numbers in parentheses refer to corresponding numbers in the figure.) The assembly process starts in the rough endoplasmic reticulum (ER) by the biosynthesis and concomitant (cotranslational) translocation of apoB-100 to the lumen of this organelle (1). ApoB-100 interacts cotranslationally with the microsomal triglyceride transfer protein (MTP) and is lipidated to form a primordial particle (pre-VLDL) (2). Alternatively, apoB-100 fails to be lipidated and misfolds resulting to degradation (3). The protein is unfolded and retracted to the cytosol, ubiquinated, and sorted to proteasomal degradation (3 and 4). Pre-VLDL is converted to $VLDL_2$ late in the ER compartment (5). $VLDL_2$ exits the ER at specific exit sites of this organelle by Sar1/Cop II vesicles (6), which fuse to become the ER–Golgi intermediate compartment (ERGIC) (7). ERGIC fuses with Cis-Golgi (8). In the Golgi apparatus, $VLDL_2$ is converted to $VLDL_1$ by the addition of a bulk load of triglycerides (9). This lipidation process differs from that which gives rise to pre-VLDL and $VLDL_2$. The formation of $VLDL_1$ may involve the formation of a lipid droplet in the lumen of the secretory pathway (10), the mechanism of which may follow that of cytosolic lipid droplets (11 and 12). The formation of the cytosolic droplets also involves a fusion step (12). [Figure and legend with permission from *Current Opinion in Lipidology* (Adiels *et al.*, 2006a).]

It has been suggested that this hepatic TG pool is metabolically active with a rapid turnover such that extracellular FAs entering the cell are either oxidised in the mitochondrion or esterified into TG in the ER and then transferred to the cytosolic storage pool. TG required for VLDL synthesis is recruited from this pool by a process of lipolysis to give FAs (Gibbons *et al.*, 2000). This may explain the findings of Malmstrom *et al.* (1999) who reported that acute NEFA flux is not accompanied by concomitant changes in the production of total VLDL-apoB (Malmstrom *et al.*, 1999). In fact, reducing NEFA concentrations using acipimox (an antilipolytic agent) caused a shift from $VLDL_1$ to $VLDL_2$ production, without affecting total VLDL-apoB production (Malmstrom *et al.*, 1998), suggesting an important role of fatty acid availability for the assembly and secretion of $VLDL_1$.

Conversely, earlier studies which did not isolate VLDL subfractions indicate that acutely raising plasma NEFA concentrations could increase both VLDL-TG and VLDL-apoB production (Lewis *et al.*, 1995), thus suggesting a role for FAs in the stimulation of TG and apoB synthesis. This assumption is supported by animal studies, where chronic administration of intravenous albumin-bound oleic acid doubled hepatic secretion of apoB-100 (without changes in apoB mRNA levels), suggesting that FAs might act as both a stimulus for TG synthesis, thereby driving assembly of VLDL, and as a 'signal' for apoB assembly (Zhang *et al.*, 2004b). Indeed, increased liver fat has been shown to increase the secretion of $VLDL_1$-apoB-100, and more – but not larger – $VLDL_1$ particles are secreted (Adiels *et al.*, 2006b).

1.4.2 Role of Insulin

Despite insulin's induction of lipogenesis in the liver, it acutely suppresses hepatic VLDL production in normal weight, healthy subjects (Lewis *et al.*, 1993) – specifically affecting the $VLDL_1$ subclass (Malmstrom *et al.*, 1998; Adiels *et al.*, 2007). This effect is partly attributed to its induced reduction of NEFA flux to the liver, thus decreasing substrate availability for VLDL assembly (Coppack *et al.*, 1994). However, other mechanisms are likely to play a role as this suppressive effect of insulin on $VLDL_1$ production persisted even when compared with the action of acipimox (which caused a similar reduction in NEFA) (Malmstrom *et al.*, 1998). Although these mechanisms are still elusive, some have been investigated. Insulin inhibits the secretion of apoB (Sparks & Sparks, 1994) and insulin signalling (via phospho-inositide 3-kinase) inhibits the maturation phase of VLDL assembly by preventing bulk lipid transfer to a VLDL precursor, thus enhancing the degradation of apoB (Brown & Gibbons, 2001). In addition, it inhibits transcription (Lin *et al.*, 1995) and upregulation (Wolfrum & Stoffel, 2006) of the MTP gene, although it has been suggested that this is unlikely to account for the acute effect of insulin on VLDL production due to the long half-life of the MTP protein (4.4 days) (Lin *et al.*, 1995; Blasiole *et al.*, 2007).

In contrast, chronic hyperinsulinaemia, caused by insulin resistance, is associated with increased production of VLDL (Malmstrom *et al.*, 1997a), specifically $VLDL_1$ (Adiels *et al.*, 2007). Possible mechanisms for this loss of the suppressive effect of insulin have been studied in a fructose-fed hamster model of insulin resistance (Taghibiglou *et al.*, 2000). These included increased cellular secretion of total and VLDL-TG, enhanced stability of nascent apoB, thereby reducing its post-translational degradation and increased MTP mass (Taghibiglou *et al.*, 2000). In addition, insulin resistance also leads to loss of the acute insulin-mediated inhibition of apoB secretion (Chirieac *et al.*, 2004). In humans, central obesity and an increased liver fat content, which are usually associated with insulin resistance conditions, have been associated with the lack of insulin-induced suppression of $VLDL_1$ production (Adiels *et al.*, 2007) (see below).

1.5 Defective TRL Metabolism and Atherogenic Dyslipidaemia

The concept of atherogenicity of TG and TRL, especially in the postprandial state, was first introduced almost 30 years ago by Zilversmit (Zilversmit, 1979). Since then, an increasing body of evidence has implicated hypertriglyceridaemia as an independent risk factor for cardiovascular disease (CVD), particularly coronary heart disease (CHD) (Cullen, 2000). TRL are thought to have a role in the development and progression of atherosclerosis (Tanaka *et al.*, 2001; Cullen, 2003) as TRL remnants have been found in human aortic atherosclerotic plaques (Nakano *et al.*, 2008). In addition, elevated concentrations of $VLDL_1$ particles are believed to start a chain of metabolic events that generate an 'atherogenic lipoprotein phenotype', also known as atherogenic dyslipidaemia.

1.5.1 Atherogenic Lipoprotein Phenotype

The atherogenic lipoprotein phenotype is a cluster of lipoprotein abnormalities associated with insulin resistant states (Ginsberg *et al.*, 2005), such as obesity (Bamba & Rader, 2007; Marsh, 2003; Chan *et al.*, 2004a), type 2 diabetes (Marsh, 2003; Taskinen, 2003) and the metabolic syndrome (Grundy, 2006; Kolovou *et al.*, 2005), and significantly contributes to increased risk of CVD (Avramoglu *et al.*, 2006; Watson *et al.*, 2003).

Such atherogenic dyslipidaemia manifests as increased concentrations of hepatic VLDL (causing hypertriglyceridaemia), small dense LDL (sdLDL), TRL remnants and low HDL concentrations (Grundy, 2006; Taskinen, 2003; Avramoglu *et al.*, 2006). Importantly, it is the excess hepatic production of $VLDL_1$ [the major determinant of plasma TG concentration (Hiukka *et al.*, 2005)] but not $VLDL_2$ particles (Adiels *et al.*, 2005b; Adiels *et al.*, 2006b) that is associated with insulin resistance, indicating that $VLDL_1$ and $VLDL_2$ are independently regulated (Gill *et al.*, 2004a; Packard & Shepherd, 1997).

1.5.2 Elevated Levels of $VLDL_1$, Insulin Resistance and Obesity

Hepatic overproduction of VLDL (particularly the large $VLDL_1$) has been associated with insulin resistance (Gill *et al.*, 2004a; Johanson *et al.*, 2004) and related conditions such as diabetes (Adiels *et al.*, 2005b), the metabolic syndrome (Grundy, 2006) and obesity (Mittendorfer *et al.*, 2003a). Of particular interest, abdominal obesity which is characterised by excess visceral and/or deep upper body subcutaneous adipose tissue accumulation and is strongly associated with obesity, insulin resistance and atherogenic dyslipidaemia (Després, 2007; Sniderman *et al.*, 2007). Because visceral fat is more lipolytically active than subcutaneous fat *in vitro* (Mauriege *et al.*, 1987; Arner, 1998; Rebuffe-Scrive *et al.*, 1989), it has been hypothesised that visceral fat accumulation results in a markedly increased flux of NEFA via the portal vein to the liver (Bjorntorp, 1990; Chan *et al.*, 2004a), thereby increase substrate availability for hepatic VLDL formation (Kissebah *et al.*, 1976). However, Nielsen *et al* (2004) demonstrated that although the release of NEFA from visceral fat depots into the portal vein increases with increasing visceral fat, it was much smaller than the amount derived from lipolysis of upper-body subcutaneous fat

(Nielsen *et al.*, 2004). Nevertheless, hepatic overproduction of $VLDL_1$ particles has been found to be driven by increased liver fat content in man (Adiels *et al.*, 2006b) and reduction in visceral adipose tissue is associated with decreased hepatic production of VLDL (Riches *et al.*, 1999). In addition, increased NEFA flux to the liver impairs hepatic extraction of insulin (Chan *et al.*, 2004a). Normally, insulin inhibits hepatic $VLDL_1$ production, in the fed state, but this effect is lost in insulin resistant conditions and high liver fat content, leading to increased hepatic $VLDL_1$ secretion (Adiels *et al.*, 2007).

Furthermore, the clearance of $VLDL_1$ and TRL particles and their remnants is impaired in insulin resistant conditions due to decreased LPL activity (Mead *et al.*, 2002), excess of apoC-III (an inhibitor of LPL) (Florez *et al.*, 2006) and failure to bind to LDLR and LRP (Packard, 2003) or suppression of LDLR (Mamo *et al.*, 2001).

1.5.3 VLDL$_1$ and the Generation of Atherogenic Dyslipidaemia

Whether due to hepatic overproduction or defective clearance, elevated levels of $VLDL_{1,}$ which give rise to a plasma TG concentration > 1.5 $mmol.l^{-1}$, are believed to start a sequence of events that result in the atherogenic lipoprotein phenotype (Packard, 2003; Taskinen, 2003). Increased proportion of large LDL is a marker of efficient lipolysis of TG, which, in effect, will lower fasting and postprandial levels of TRL (Griffin, 1997). However, the presence of $VLDL_1$ gives rise to LDL particles (βLDL) with altered apoB-100 conformation which impedes binding of these particles to LDLR. In addition, impaired clearance of $VLDL_1$ particles (see above) will result in their accumulation in the circulation. As a result, $VLDL_1$ and βLDL particles have a prolonged residence time in the circulation, thereby increasing their likelihood of remodelling by CETP (Packard, 2003), which is increased in activity in insulin resistant states (Guerin *et al.*, 2001). Under these conditions, CETP promotes the acquisition of TG by LDL and HDL in exchange for CE from HDL to $VLDL_1$ and LDL (Packard, 2003; Guerin *et al.*, 2001). These TG-enriched LDL and HDL particles become favourable substrates for HL resulting in a reduction in particle size. HL also has an enhanced activity in obesity and insulin-resistant states (Deeb *et al.*, 2003). This gives rise to (1) sdLDL with a prolonged residence time (~5 days) compared with the larger LDL derived from $VLDL_2$ and IDL, which has a residence time of ~2 days (Packard, 2003) and (2) small unstable HDL particles that are cleared rapidly from the circulation, resulting in low HDL cholesterol and apoA-I (Rashid *et al.*, 2003) and loss of its protective potential against CVD (Wang & Briggs, 2004). sdLDL is the most readily oxidised subfraction in the lipoprotein class (Tribble *et al.*, 1992), increasing its atherogenic potential. **Figure 1.6** shows a schematic diagram of the mechanisms involved in the formation of atherogenic dyslipidaemia.

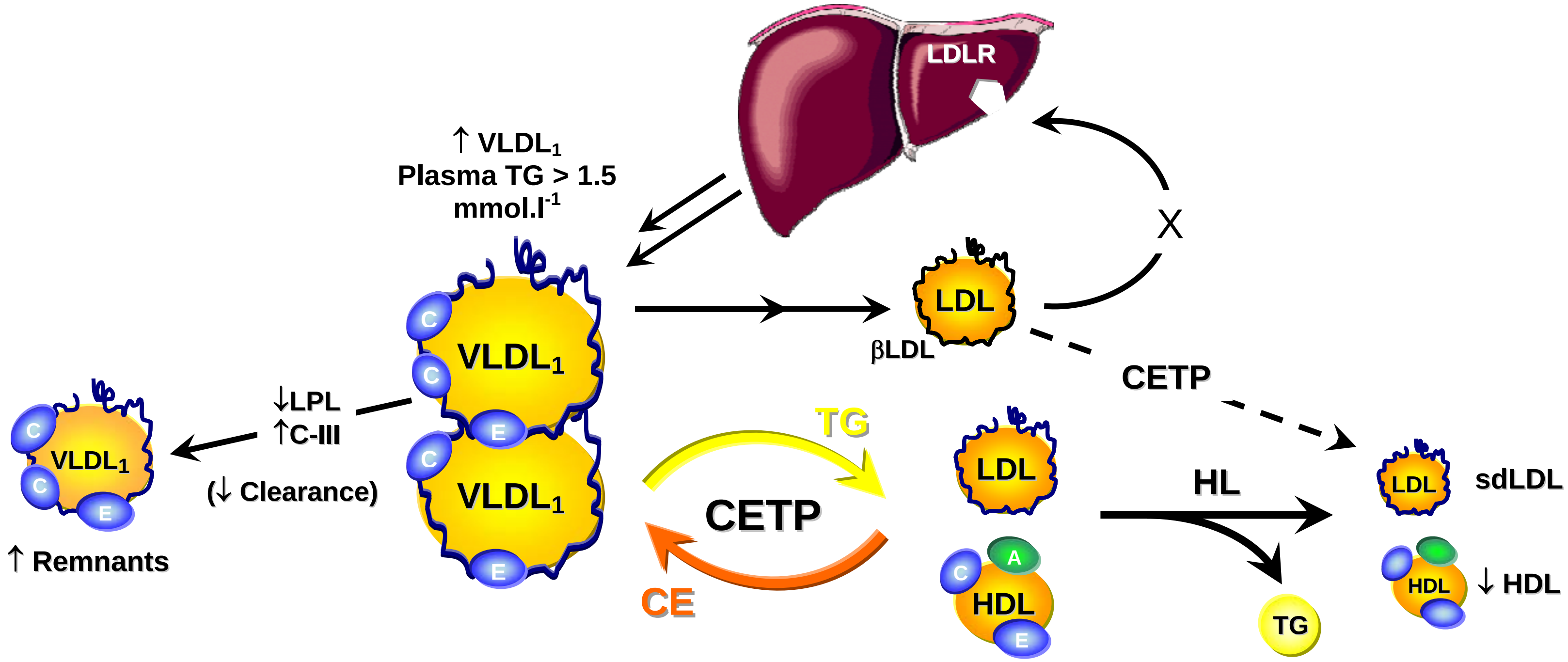


Figure 1.6: Schematic diagram of the mechanisms involved in the formation of atherogenic dyslipidaemia. Elevated concentrations of $VLDL_1$ particles, producing plasma triglyceride (TG) concentrations > 1.5 mmol.l^{-1}, are believed to start a chain of reactions to generate an atherogenic lipoprotein phenotype (hypertriglyceridaemia, small dense (sd)LDL and low HDL). $VLDL_1$ accumulates in the circulation due to hepatic overproduction, coupled with decreased clearance due to low lipoprotein lipase (LPL) activity and/or excess of apoC-III (an inhibitor of LPL). Lipolysis of $VLDL_1$ gives rise to βLDL particles which have an altered apoB confirmation and, thus, fail to bind well to the LDL receptor (LDLR). Consequently, $VLDL_1$ and βLDL have a prolonged residence time in plasma. This increases their likelihood of undergoing remodelling by cholesteryl ester transfer protein (CETP), which catalyses the transfer of TG from $VLDL_1$ to LDL and HDL in exchange for cholesteryl esters (CE). TG-enriched LDL and HDL become good substrates for hepatic lipase (HL) which hydrolyses the TG content of these particles. As a result, the atherogenic sdLDL and small unstable HDL particles are formed. sdLDL is readily oxidised, thus, increasing its atherogenic potential, while the smaller HDL particles are cleared rapidly from the circulation resulting in low HDL concentrations and losing its protective potential against CVD.

1.6 Effect of Exercise on TRL Metabolism

1.6.1 Exercise as a Therapy for Dyslipidaemia

Due to the well established role of TRLs in CVD in progression of atherosclerosis described above, interventions that have the potential to reduce fasting and/or postprandial TG concentrations have been investigated as valuable tools for lowering CVD risk. Exercise has long emerged as an affordable, non-pharmaceutical method of improving lipoprotein metabolism. Regular exercise training has been shown to reduce total TG [as well as VLDL-TG (Kraus *et al.*, 2002)] concentrations, increase HDL cholesterol in adult men and women (Kraus *et al.*, 2002; Kodama *et al.*, 2007) and increase LDL particle size (Kraus *et al.*, 2002; Altena *et al.*, 2006). In addition, exercise improves insulin sensitivity in men and women (Gill *et al.*, 2002b), sedentary overweight/obese subjects (Houmard *et al.*, 2004), hypertriglyceridaemic men (Zhang *et al.*, 2006) and subjects with the metabolic syndrome (Zhang *et al.*, 2007) and type 2 diabetes (Alam *et al.*, 2004). Certainly, sedentary lifestyle has been associated with increased risk of obesity, metabolic deterioration, type 2 diabetes, CVD, cancer and all-cause mortality (Slentz *et al.*, 2007). In fact, a recent review on exercise training in patients with atheromatous CVD has found it to be a 'true therapy', reducing mortality by 25-35%, reducing clinical manifestations and complications (rhythm problems, thrombosis) and improving physical capacity, reintegration and quality of life (Casillas *et al.*, 2007). The following section focuses mainly on the effect of exercise on TRL metabolism.

1.6.2 Impact of Endurance Training

Compared with their untrained peers, endurance-trained men have reduced levels of postprandial lipaemia (Cohen *et al.*, 1989; Ziogas *et al.*, 1997; Merrill *et al.*, 1989). In addition, a period of endurance training has been shown to decrease fasting and/or postprandial TG concentrations in normolipidaemic subjects (Weintraub *et al.*, 1989), overweight men and women with mild-to-moderate dyslipidaemia (Kraus *et al.*, 2002) and older men and women (Halverstadt *et al.*, 2007). However, evidence suggests that this TG-lowering effect of exercise is due to short-term metabolic responses to recent exercise which is lost after a period of detraining. For example, there was no difference between fasting or postprandial TG concentrations between endurance trained and untrained young adults (Herd *et al.*, 2000) or untrained middle-aged men (Tsetsonis *et al.*, 1997) after at least 2 days of inactivity, or after an exercise training programme when post-training measurements were made 60 h after the last exercise session (Herd *et al.*, 1998).

1.6.3 Impact of a Single Exercise Session

An increasing body of evidence shows that a bout of exercise (e.g brisk walking, jogging, cycling etc) reduces fasting and/or postprandial TG concentrations in different population groups including young adult men (Tsetsonis & Hardman, 1996b; Zhang *et al.*, 1998; Herd *et al.*, 2001) and women (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002a), middle-aged men (Gill *et al.*, 2001a; Gill *et al.*, 2001b) and women (Tsetsonis *et al.*, 1997), postmenopausal women (Gill & Hardman, 2000) and individuals with the metabolic syndrome (Zhang *et al.*, 2006) and type 2 diabetes (Tobin *et al.*, 2008), although responses are more heterogeneous in patients with type 2 diabetes (Gill *et al.*, 2007). The percentage reductions in plasma TG concentrations following exercise are broadly similar (15-25%) across these groups, however, subjects with higher TG concentrations (e.g. centrally obese men) had a greater decrease in postprandial TG compared with subjects with lower TG (Gill *et al.*, 2004b). Furthermore, a single session of exercise has shown to acutely decrease VLDL (Gill *et al.*, 2006) and VLDL-TG concentrations (Borsheim *et al.*, 1999; Magkos *et al.*, 2006; Morio *et al.*, 2004).

A session of exercise conducted before (Katsanos & Moffatt, 2004; Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a; Gill & Hardman, 2000; Gill *et al.*, 2001a; Gill *et al.*, 2001b) or after (Katsanos & Moffatt, 2004; Hardman & Aldred, 1995) ingestion of a fat meal has been reported to attenuate postprandial lipaemia. However, the weight of evidence (Zhang *et al.*, 2004a; Zhang *et al.*, 1998; Ferguson *et al.*, 1998; Borsheim *et al.*, 1999) suggests that the maximal TG-lowering effect of exercise appears to occur after a delay of approximately 12-18 h, rather than during or immediately post-exercise (Malkova & Gill, 2006). For example, Zhang and colleagues (Zhang *et al.*, 1998) reported a 21% greater reduction in postprandial TG when exercise was performed 12 h compared to 1 h prior to a meal and 49% lower than that performed after a meal. However, this TG-lowering effect seems to be ‘short-lived’ as exercising 24 h prior to the meal does not attenuate postprandial lipaemia (Zhang *et al.*, 2004a).

1.6.4 Energy Expenditure and Energy Deficit

The energy expended during exercise is a key determinant of the TG-lowering effect: the higher the energy expenditure, the bigger the reduction in postprandial lipaemia (Petitt & Cureton, 2003). For instance, doubling energy expenditure by either doubling exercise intensity for the same duration [60 *vs.* 30% of maximal oxygen uptake ($\dot{V}O_{2max}$) for 90 min] (Tsetsonis & Hardman, 1996a) or by doubling exercise duration at the same intensity (120 *vs.* 60 min at 50% $\dot{V}O_{2max}$) (Gill *et al.*, 2002a) essentially doubles the exercise-induced reduction to lipaemia. In addition, postprandial TG concentrations are similarly reduced by different exercise settings (e.g. 180 min of walking at 30% $\dot{V}O_{2max}$ *vs.* 90 min at 60% $\dot{V}O_{2max}$) when the same amount of energy is expended (Tsetsonis & Hardman, 1996b). Similarly, a 2-h cycling at 60% $\dot{V}O_{2max}$ significantly decreased VLDL-TG and -apoB concentrations (Magkos *et al.*, 2006), whereas 1-h exercise of the same intensity had no effect (Magkos *et al.*, 2007). Of note, recently, Gormsen *et al* (2006) reported a significant positive correlation between VLDL-TG production and resting energy expenditure, suggesting that it should be taken into account when VLDL-TG production comparisons between groups are made (Gormsen *et al.*, 2006).

Furthermore, the exercise-induced improvement in insulin sensitivity appears to be related to duration and intensity of exercise (Houmard *et al.*, 2004) and thus directly related to energy expenditure (Magkos *et al.*, 2008). It was proposed that exercise-induced changes in a homeostasis model assessment-estimated insulin resistance ($HOMA_{IR}$) are curvilinearly related to exercise energy expenditure with a threshold of ~3.77 MJ (900 kcal) for improvements in $HOMA_{IR}$ to be manifested (Magkos *et al.*, 2008). However, an inverse association was observed between the exercise-reduced changes in baseline (i.e. resting) $HOMA_{IR}$, suggesting that less exercise may be required to improve insulin sensitivity in insulin-resistant subjects than those with good insulin sensitivity at baseline (Magkos *et al.*, 2008).

The mechanisms by which exercise energy expenditure attenuates postprandial lipaemia are currently not known. Interestingly, dietary-induced energy deficit of similar magnitude to that induced by a bout of exercise session results in a much smaller TG reduction (Gill & Hardman, 2000). This implies that either the TG-lowering effect of exercise is not dependent on energy deficit and/or that exercise- and dietary-induced energy deficits are not metabolically equivalent. However, a recent study investigating the effects of exercise, with or without energy replacement, on fasting and postprandial TG metabolism, demonstrated that the exercise-induced TG-lowering effect was only evident with an accompanying energy deficit (Burton *et al.*, 2008). This suggests that dietary-induced and exercise-induced energy deficits elicit different effects on postprandial metabolism. This may be related to specific body tissues in which the energy deficits occur as exercise induces quantitatively larger muscle and hepatic substrate deficits than energy intake restriction (Burton *et al.*, 2008).

1.6.5 Intermittent versus Continuous Exercise

Current exercise-for-health guidelines recommend the accumulation of physical activity throughout the day (Haskell *et al.*, 2007). Thus, it is important to understand whether small sessions of exercise spread throughout the day would be as beneficial in lowering TG concentrations as a single prolonged session of exercise. A number of studies have been conducted to investigate the effects of intermittent and continuous exercise, such as 90-min session *vs.* three 30-min sessions (Gill *et al.*, 1998), 30-min *vs.* three 10-min exercise session (Murphy *et al.*, 2000; Altena *et al.*, 2004) and even 30-min continuous exercise *vs.* ten 3-min bouts performed throughout the day (Miyashita *et al.*, 2006). All these studies consistently demonstrated that intermittent patterns of physical activity are effective in lowering TG concentrations as long as sufficient energy is expended, independently of the duration of the individual sessions.

1.6.6 Potential Mechanisms Responsible for the Exercise-Induced TG Reduction

Concentrations of TRLs in the circulation reflect the balance between rate of appearance and rate of clearance of intestinally-derived chylomicrons and hepatically-derived VLDL. Thus, the reduction in plasma TG concentration following exercise could be due to disturbance in the balance of appearance and clearance rates of chylomicrons and/or VLDL.

1.6.6.1 Effect of Exercise on Chylomicron Metabolism

The fact that maximal reduction in postprandial lipaemia is observed after a delay of number of hours, seems unlikely that the exercise-induced TG reduction is due to a reduction in gastrointestinal blood flow leading to potentially slower gut absorption and secretion of chylomicrons (Malkova & Gill, 2006). This is supported by three pieces of evidence: (1) the peak time for chylomicron-TG concentrations is not delayed post-exercise (Gill *et al.*, 2001a; Gill *et al.*, 2006), (2) gastric emptying time, evident by peak time of ingested paracetamol concentration, is unaffected by prior exercise (Gill *et al.*, 2001a; Gill *et al.*, 2001b) and (3) chylomicron particle number is not affected by prior exercise (James *et al.*, 2007).

There is clear evidence that endurance-trained individuals have high clearance rates of chylomicron-like lipid emulsions compared with untrained peers (Cohen *et al.*, 1989; Podl *et al.*, 1994), which is likely to reflect the increased post-heparin plasma LPL activity (reflecting overall LPL activity from all body tissues) observed in them (Podl *et al.*, 1994; Kantor *et al.*, 1984). In addition, muscle LPL activity has been reported to increase over 200% in response to intense exercise sessions lasting for hours (Lithell *et al.*, 1984; Sady *et al.*, 1986).

On other hand, recent studies investigating the effects of a more moderate exercise on LPL activity are equivocal. While some studies reported a significant increase in plasma (Zhang *et al.*, 2002), muscle and adipose tissue LPL activity after a moderate session of exercise (Perreault *et al.*, 2004), others reported no significant difference in muscle (Herd *et al.*, 2001) or post-heparin plasma LPL activity (Gill *et al.*, 2003) or muscle LPL mass (Magkos *et al.*, 2006) post-exercise. Interestingly, however, in the absence of an exercise-induced effect on muscle LPL mass, a significant ~20% increase in plasma LPL concentrations has been observed (Magkos *et al.*, 2006). Similarly, a significant correlation between LPL activity and the exercise-induced changes in fasting and postprandial TG has been observed without an effect on post-heparin LPL activity post-exercise (Gill *et al.*, 2003).

1.6.6.2 Effect of Exercise on VLDL Metabolism

Studies investigating the effect of moderate exercise on postprandial lipaemia demonstrated that the lipoprotein class most affected by prior exercise is VLDL

(Malkova *et al.*, 2000), specifically $VLDL_1$, rather than chylomicrons (Gill *et al.*, 2001a; Gill *et al.*, 2006). However, little is known about the mechanisms that regulate VLDL concentrations (i.e. production and clearance rates) in response to moderate exercise. Certainly, there is no information available regarding the effect of exercise on large $VLDL_1$.

Malkova *et al* (2000) examined the influence of a prolonged session of prior exercise (running at ~60% $\dot{V}O_{2max}$) on postprandial extraction of TG across the leg (Malkova *et al.*, 2000). Although they found no significant increase in total, chylomicron- and VLDL-TG uptake across the leg, TG clearance (defined as uptake divided by arterial concentration) – a marker for the efficiency of TG removal - was greater following exercise. However, as absolute VLDL-TG uptake did not differ significantly between control and exercise conditions, it is unclear whether the lower VLDL-TG concentrations following exercise were due to increase efficiency of removal or a lower VLDL production rate (Malkova *et al.*, 2000).

Following exercise, there is an increase in circulating NEFA (Burton *et al.*, 2008), which, theoretically, can increase hepatic VLDL secretion. However, this is not the case, as there is no correlation between the change in NEFA concentration and VLDL-TG following exercise (Borsheim *et al.*, 1999). This may be explained by the concurrent increase in 3-hydroxybutyrate following exercise (Malkova *et al.*, 2000; Burton *et al.*, 2008), which a marker of hepatic fatty acid oxidation (Williamson & Whitelaw, 1978), suggesting a shift of the hepatic fatty acid flux towards β-oxidation and ketone body production and away from re-esterification and VLDL synthesis (Malkova & Gill, 2006).

Due to the fact that chylomicrons are the preferred substrate for LPL and their presence prevents VLDL clearance (Fisher *et al.*, 1995; Bjorkegren *et al.*, 1997), it has been hypothesised that moderate exercise likely to reduce hepatic VLDL production, rather than increase its clearance (Malkova & Gill, 2006). However, recent evidence suggests that a session of moderate exercise (e.g. walking or cycling for more than 1 h at 60% $\dot{V}O_{2max}$) increases VLDL-TG clearance rates (Magkos *et* *al.*, 2006; Tsekouras *et al.*, 2007) in healthy, lean, young men. In addition, Magkos *et al* also reported a decreased production of VLDL-apoB (reflecting the number of particles being produced) post-exercise (Magkos *et al.*, 2006), which may contribute to the exercise-induced reduction in VLDL concentrations. It should be noted, however, that these findings may not necessarily be applicable to overweight or centrally obese individuals (at which exercise-for-health guidelines are targeted), as this condition is (1) associated with hepatic overproduction of $VLDL_1$ due to their likely increased liver fat content (Adiels *et al.*, 2006b) and (2) impaired ability to suppress hepatic VLDL production due to their likely insulin resistance (Adiels *et al.*, 2007).

1.6.7 Effect of ApoE Phenotype

ApoE is a 299-amino acid glycoprotein which is an integral surface component of TRLs and some subclasses of HDL (Hatters *et al.*, 2006). It primarily functions as a ligand for receptor-mediated uptake of TRLs, but also modulates the activity of LPL, LCAT and CETP (Leon *et al.*, 2004). The apoE gene has three alleles (ε2, ε3, and ε4) that give rise to 6 different phenotypes (E2/2, E2/3, E2/4, E3/3, E3/4, and E4/4) (Davignon *et al.*, 1988), with the E3 isoform being the most common (Hagberg *et al.*, 2000). ApoE polymorphism has been shown to have a substantial influence on plasma lipids and lipoproteins, which could be explained by a number of mechanisms (Kolovou & Anagnostopoulou, 2007): (1) receptor-binding affinities of different apoE-containing lipoproteins, (2) dietary fat clearance, (3) differences in the clearance of LDL-apoB and (4) efficiency of intestinal cholesterol absorption.

In the general population, the E2 isoform is associated with elevated levels of TG and apoE compared with the E3 isoform (Hagberg *et al.*, 2000), which is caused by impaired clearance of remnant particles bearing the apo E2 isoform probably due to defective receptor recognition (Havel *et al.*, 1980). Homozygous E2/2 carriers can develop type III hyperlipoproteinaemia, which is characterised by the accumulation of chylomicron and VLDL remnants in fasting plasma (Kolovou & Anagnostopoulou, 2007). On the other hand, the apo E4 isoform is typically associated with low TG levels compared with the apo E3 individuals (Davignon *et al.*, 1988). This is consistent with the fact that apo E4 subjects clear chylomicron remnants into the liver more rapidly than E3/3 subjects and twice as fast as E3/2 subjects (Weintraub *et al.*, 1987).

In contrast, studies investigating the effects of apoE polymorphism on ($\dot{V}O_{2max}$), lipids and lipoproteins in response to endurance exercise training are conflicting. While a cohort study reported an improvement in lipid and lipoprotein profile in subjects homozygous for the E 3/3 , despite a small reduction in $\dot{V}O_{2max}$, compared with E 2/3 and E 3/4 (Thompson *et al.*, 2004), another reported no significant influence of apoE polymorphism on $\dot{V}O_{2max}$ in response to exercise (Leon *et al.*, 2004). Conversely, in a study with smaller subject number, increases in $\dot{V}O_{2max}$ (and HDL) in response to endurance exercise training, were in the order, apo E4 > apo E3 > apo E2 (Hagberg *et al.*, 1999).

However, to the best of the author's knowledge, there is only one study investigating the effect of apoE polymorphism on lipoprotein responses to moderate exercise. In a study including 38 men and 43 women, Gill *et al* (Gill *et al.*, 2002b) reported no significant differences between subjects possessing the E3/2, E3/3 and E4/3 phenotypes on the magnitude of the exercise-induced reduction in fasting or postprandial TG.

1.7 Measurements of TRL Kinetics

Lipoprotein metabolism is a complex system of tightly regulated and coordinated dynamic processes. Measurements of plasma lipid and lipoproteins concentrations provide useful, yet limited information – 'a snapshot of the various processes' (Packard, 1995), which do not reveal the dynamics of the system. This is achieved *in vivo* by the use of kinetic studies, in which labelled precursors are used to help characterise the metabolic pathways of lipoprotein metabolism (Packard, 1995; Barrett *et al.*, 2006). The system can be studied either in steady state (when the concentration of the substance of interest is constant) or in acute perturbation (non steady state, where a stimulus is applied and the return to steady state is monitored) (Packard, 1995).

There are basically two study protocols for investigating the metabolism of apoB-containing lipoproteins (i.e. $VLDL_1$, $VLDL_2$, IDL and LDL) (Demant & Packard, 1997). Lipoproteins can be tracer-labelled exogenously by isolating them from plasma, labelling them with radioactive substance (e.g. radioiodine). They are then re-injected into the donor subject and followed over time as they disappear from the plasma compartment. Alternatively, a stable isotope amino acid (e.g. D_3-leucine or D_5-phenylalanine) is injected, either as a bolus or as a primed constant infusion, and incorporated into the newly synthesised apoB protein serving as an endogenous label (**Table 1.3** shows the characteristics of each tracer and labelling method). In either case, multiple plasma samples are collected during the first 12 h of tracer injection and daily thereafter in the fasted state for up to 10-14 days. ApoB-containing lipoprotein fractions are then separated at each timepoint using cumulative gradient ultracentrifugation in salt solutions (Lindgren *et al.*, 1972) and apoB is isolated by selective precipitation (Egusa *et al.*, 1983). In the case of radioactive tracer studies, the specific activity is calculated by measuring the radioactive tracer in a scintillation counter, whereas in the stable-isotope tracer studies, tracer enrichment is determined by gas chromatography mass spectrometry (GC-MS) (Demant *et al.*, 1996). Multicompartmental modelling using the Simulation, Analysis And Modeling software (SAAM II; SAAM Institute, Seattle, WA) is used to calculate rates of production, delipidation and catabolism of apoB-containing lipoproteins (Packard *et al.*, 1995).

Although such methods provide valuable information about lipoprotein metabolism, they are difficult, time-consuming, expensive and labour-intensive. Furthermore, the laboratory manipulations are complex and require the use of specialised equipment (see below). [Comprehensive reviews of kinetic methods can be found by (Barrett *et al.*, 2006; Packard, 1995; Chan & Watts, 2006; Chan *et al.*, 2004b).]

1.7.1 Using Stable-Isotopes to Measure $VLDL_1$ and $VLDL_2$ Kinetics

Recently, Adiels and colleagues (Adiels *et al.*, 2005a) developed a multicompartmental model which permits the kinetics of apoB and TG to be assessed simultaneously in $VLDL_1$ and $VLDL_2$ fractions. The method involves administering D_3-leucine and D_5-glycerol as bolus injections in the fasted state. Several blood samples are taken before and for 8 h after the tracer injection while subjects are still fasting. Adiels *et al.* (Adiels *et al.*, 2005a) reported a significant linear correlation between TG and apoB production in $VLDL_1$ and $VLDL_2$, suggesting a coupling of the two processes governing the metabolism of these subfractions.

Table 1.3: Characteristics of tracers [modified from (Packard, 1995)].

Advantages and disadvantages of endogenous versus exogenous tracers	
Exogenous	**Endogenous**
Labels specific lipoprotein apos or lipoprotein subfractions	Labels all lipoprotein apolipoproteins
Labelled according to mass distribution	Labels according to production rate
Measures synthesis indirectly by inference from steady state calculation	Measures synthesis directly by tracer incorporation
Tracer does not recycle	Tracer may recycle
Urine data useful for assessing catabolism	Excretion data of little value
Radioactive versus stable-isotope endogenous tracers	
Radioactive	**Stable isotope**
Radiation hazard	Safe
Limited applicability	Applicable to all, including women and children
Limited repeatability	Can be repeated many times
Easy to measure	Difficult to measure with sufficient precision
Inexpensive apparatus and tracers	Capital cost high

For purposes of this thesis, it is essential to appreciate the complexity of the stable-isotope method, thus, **Figure 1.7** shows a schematic summary of the laboratory techniques involved, which were developed and are still used in the Vascular Biochemistry Department (Glasgow Royal Infirmary, University of Glasgow). Briefly, $VLDL_1$ and $VLDL_2$ fractions are separated from plasma by cumulative gradient ultracentrifugation (Lindgren *et al.*, 1972). ApoB protein is precipitated using absolute isopropanol and delipidated using ethanol-ether (Egusa *et al.*, 1983). Samples are then prepared (derivatised and fragmentised) for analysis of leucine and D_3-leucine enrichment in hydrolysed apoB protein and plasma amino acids using the GC-MS (Demant *et al.*, 1994). In order to determine glycerol enrichment in TG, the isopropanol and ethanol-ether supernatants are treated with zeolite to precipitate PL and obtain TG. Glycerol is then extracted by saponification using potassium hydroxide and ethanol (Witter & Whitner, 1972), then derivatised and fragmented for enrichement determination by the GC-MS (Beylot *et al.*, 1987).

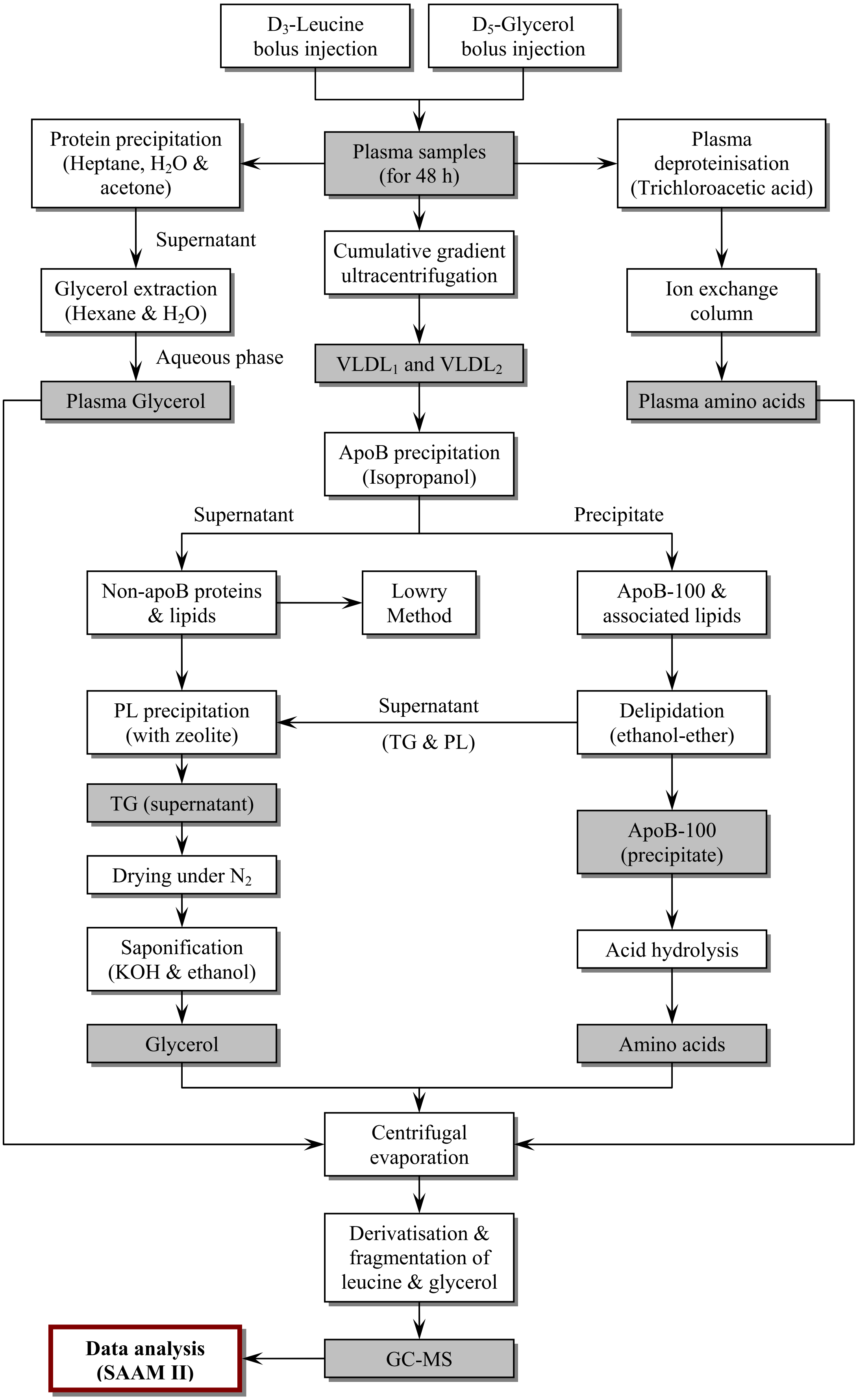


Figure 1.7: Schematic diagram of the stable-isotope laboratory protocol used to determine the kinetics of $VLDL_1$- and $VLDL_2$-TG and -apoB.

Based on the enrichment of leucine and glycerol in plasma, $VLDL_1$ and $VLDL_2$, and the known injected amounts of labelled leucine and glycerol, the kinetic parameters are determined using the modelling software SAAM II as previously described (Adiels *et al.*, 2005a). **Figure 1.8** shows the mathematical multicompartmental model used to assess the kinetics of glycerol and leucine in plasma and lipoprotein fractions, which is based on the apoB model originally described by Packard *et al* (Packard *et al.*, 1995).

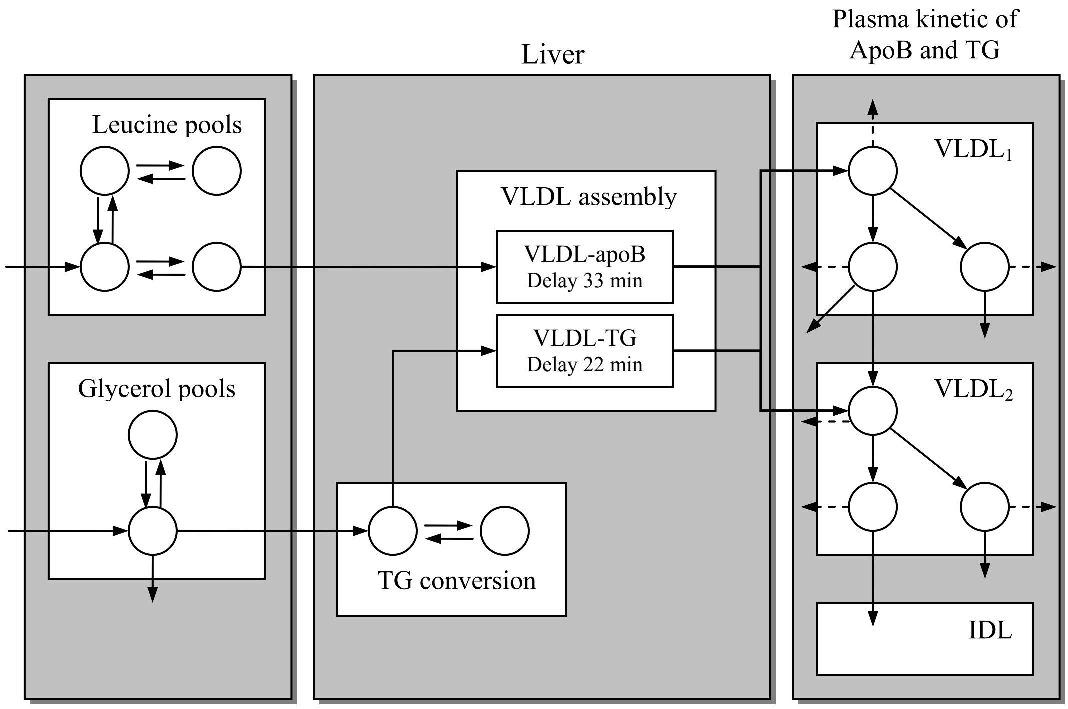


Figure 1.8: A diagram of the mathematical multicompartmental model (using SAAM II software) used to simultaneously determine the kinetics of apoB and TG in $VLDL_1$ and $VLDL_2$ after a bolus injection of stable isotopes D_3-leucine and D_5-glycerol [Figure from (Adiels *et al.*, 2005a)].

1.7.2 Chylomicron Kinetics

1.7.2.1 ApoB-48 Labelling with Stable-Isotopes

Kinetics of apoB-48 containing lipoproteins are difficult with only a few studies reported using endogenously labelled stable-isotope tracer (primed constant infusion of [5,5,5-$^2H_3$] leucine) (Welty *et al.*, 1999; Welty *et al.*, 2004; Lichtenstein *et al.*, 1992; Hogue *et al.*, 2007). This is because in the fasting state, apoB-48 concentrations are generally too low for protein enrichment to be detected, whereas they vary postprandially (along with other lipoproteins) which makes interpretation of tracer data very difficult (Barrett, 1998). However, these obstacles were first overcome by Lichtenstein *et al.* (1992) by providing small hourly feeds of identical composition to the subjects (over a 20 h period) to ensure an 'elevated' steady state of chylomicron concentrations. Recently, Bickerton *et al.* used a combination of stable isotope labeling to specifically label VLDL (using an intravenous infusion of [$^{2}H_2$]palmitate) and chylomicrons (using a test meal containing [U-^{13}C]palmitate) to quantify the TG extraction across human skeletal muscle and adipose tissue (Bickerton *et al.*, 2007). However, it was reported that rapid recirculation of the label leads to loss of chylomicron specificity (Heath *et al.*, 2003; Karpe *et al.*, 2007).

1.7.2.2 Retinol Palmitate

Another commonly used, non-stable isotope, method to measure chylomicron metabolism involves the ingestion of retinol with a fat load, which is esterified into retinyl palmitate and secreted into the intestinal lymph in the core of the chylomicron particle. The palmitate within the core remains with the particle as it undergoes lipolysis and becomes a remnant particle. Thus, production and clearance rates of chylomicrons can be determined by monitoring the concentrations of plasma palmitate or chylomicron-associated palmitate (Barrett, 1998). However, a major disadvantage of this approach is the exchange of core components between chylomicrons and other lipoproteins [e.g. 25% of retinyl palmitate may also be found in apoB-100 containing TRLs (Cohn *et al.*, 1993)].

1.7.2.3 Chylomicron-Like (Lipid) Emulsions

Intravenous lipid emulsions have been developed to supply patients with a balanced parenteral nutrition and to prevent or correct essential fatty acid deficiency. They are TG-rich particles that are modeled on the endogenous chylomicrons and consist of a TG core, traditionally derived from vegetable oils (e.g. soybean, safflower, coconut, olive). This TG is stabilised by a monolayer of PL derived from egg yolk (Olivecrona & Olivecrona, 1998; Carpentier & Dupont, 2000). However, unlike chylomicrons, lipid emulsions contain no cholesterol or apolipoproteins (Olivecrona & Olivecrona, 1998). Nevertheless, once in the circulation, these TG-rich particles rapidly acquire apoCs and apoE from plasma lipoproteins, mainly HDL (Iriyama & Carpentier, 1994); apoC-II is necessary for the action of LPL and apoE is important for receptor recognition.

Because the intravascular metabolism of these TG-rich particles resembles that of chylomicrons (Olivecrona & Olivecrona, 1998), they have been also used to study particular aspects of chylomicrons metabolism, both *in vitro* and *in vivo*. After binding of emulsion particles to LPL situated on the endothelial cells of adipose and muscle tissues, a substantial amount of the TG content of these particles is hydrolysed and the released NEFAs are either taken by the adjacent tissue or spilled into the circulation (Evans *et al.*, 1999; Carpentier & Dupont, 2000). However, evidence suggests that lipolysis and particle removal may be simultaneous rather than sequential mechanisms like chylomicrons; i.e. emulsion particles may disappear from blood before they have become TG-depleted remnants. This is because some remnants are not likely to leave the site of lipolysis (endothelial site) but be internalized together with HSPG/LPL or be delivered to adjacent specific lipoprotein receptors (Olivecrona & Olivecrona, 1998), including VLDLR (Carpentier & Dupont, 2000).

Intralipid® (soybean oil) and Liposyn® (safflower oil) are two widely used commercial lipid emulsions, with the major difference in composition is their α-linolenic acid content. While Intralipid contains 8%, Liposyn has only 0.5% (Byrne, 1982). Intralipid emulsions have been used in intravenous fat tolerance tests to study TG clearance rate (see below). Recently, Park and colleagues (Park *et al.*, 2000; Park *et al.*, 2001) have reported a new method to measure chylomicrons kinetics by labelling Liposyn with radioactive tracers.

Redgrave and Maranhao (1985) prepared experimental lipid emulsions which, unlike the commercial ones, contain triolein, cholesteryl oleate, cholesterol and egg phosphatidyl-choline (Redgrave & Maranhao, 1985). The particles are usually smaller than those in commercial emulsions and they contain no PL vesicles (Olivecrona & Olivecrona, 1998). These emulsions have also been used in chylomicron kinetic studies, where the emulsion was double-labelled with radioactive cholesteryl esters and TG. After intravenous injection into the subjects, determination of the plasma decaying curves of the labeled lipids allows the 2-step metabolism of chylomicrons to be followed (Oliveira & Maranhao, 2002).

1.7.2.4 Intravenous Fat Tolerance Test

The intravenous fat tolerance test (IVFTT) provides a simple measure of postprandial TG clearance. It was first introduced in the early 1960s by Carlson and Hallberg (Carlson & Hallberg, 1963) in dogs then in humans in 1965 (Hallberg, 1965). In 1972, Carlson and Rössner (Carlson & Rossner, 1972) provided an IVFTT protocol which involved the injection of a bolus dose of Intralipid (0.1 g TG per body mass) into a forearm vein, with blood being drawn from a contralateral antecubita vein at 5-min intervals for 40 min. The decline in plasma Intralipid-TG concentrations followed first-order kinetics (i.e. the clearance rate was directly proportional to the concentration). Initially, Carlson and Rössner measured Intralipid-TG concentrations indirectly in plasma by measuring plasma turbidity using nephelometry (Carlson & Rossner, 1972), and although Rössner later reported a good reproducibility of this method for up to 6 months (Rossner, 1982), Sady and colleagues found better reproducibility using enzymatic TG quantification (Sady *et al.*, 1986).

1.8 Summary and Objectives

This chapter attempted to briefly review current knowledge of lipid and lipoprotein metabolism, particularly TRLs. Special emphasis was placed on $VLDL_1$ assembly, metabolism, regulation and role in development of atherogenic dyslipidaemia. In addition, the role of exercise as a potential therapeutic option to reduce VLDL concentrations, in an attempt to improve the atherogenic lipoprotein phenotype, was discussed. Finally, the last section focused on the necessity of determining TRL kinetics; i.e. production and clearance rates, rather than concentrations, and the methods used to do so. Of note, despite the well-established heterogeneity of VLDL and the independent regulation of $VLDL_1$ and $VLDL_2$, the majority of kinetic studies, especially those aimed at investigating TG kinetics, have focused on total VLDL, rather than $VLDL_1$ and $VLDL_2$ separately. This is likely due to the difficulty in determining TG kinetics in VLDL subfractions compared with total VLDL. Although this obstacle has been recently overcome, the method still employed the use of stable-isotope tracers. Although these methods are considered the 'gold-standard' in kinetic studies and have been providing valuable information for a better understanding of lipoprotein metabolism, they are not widely available to most laboratories due to their complexity and high cost. Therefore, the aims of the present thesis are to develop and validate a relatively easy and cost-effective method of determining $VLDL_1$ kinetics and use it to investigate the effects of hyperglycaemia and hyperinsulinaemia as well as moderate exercise on $VLDL_1$ kinetics. This is important as elevated concentrations of $VLDL_1$, rather than $VLDL_2$, are believed to be responsible for the generation of atherogenic dyslipidaemia. In addition, it is essential to elucidate the mechanisms by which moderate exercise, a potential tool for lowering CVD risk, reduces plasma TG in obese/overweight middle-aged men; a typical population at which exercise-for-health guidelines are targeted.

2. General Methods

This chapter is divided into three main parts. The first part describes the employed experimental procedures, many of which are common to several studies. The second part explains a study conducted to compare between a chemical manual reaction and an antibody-based automated method for apoB determination in VLDL fractions. Finally, the third part describes a pilot study conducted to determine the concentration and dose of a glucose drink needed to sustain a steady hyperinsulinaemic and hyperglycaemic state.

2.1 Subject Recruitment

Apparently healthy subjects were recruited from within the University of Glasgow, Glasgow Royal Infirmary and Greater Glasgow area by personal contact as well as by local advertising using posters, web-based and radio announcements. At least one week prior to the study, volunteers were interviewed and a subject information sheet about the study, including possible risks and discomforts (Appendices A1 & A2), was given and explained to them. Volunteers were also encouraged to ask any questions before signing an informed consent (Appendices A1 & A2) and screened according to a health screen questionnaire (Appendices B1 & B2). Their resting arterial blood pressure was measured and a venous blood sample was collected to test for their liver, renal, and thyroid functions. For the glucose and exercise studies described in Chapters 4 and 5, subjects were also tested for fasting glucose. Common exclusion criteria were used as follows:

a. A history of known cardiovascular disease or abnormalities, including established CHD (e.g. MI, stroke, CABG), acute illness or active, chronic systemic disease
b. Uncontrolled hypertension (>160/90 mmHg on anti-hypertensive medication)
c. Taking any medication known to influence carbohydrate or lipid metabolism;
d. Anaemia (Hb <12 $g.dl^{-1}$ males, <11 $g.dl^{-1}$ females)
e. Current smoking (stopped for at least 6 months)
f. Frank diabetes (fasting blood sugar ≥7 $mmol.l^{-1}$) (Chapters 4 and 5)

g. Abnormal renal, liver or thyroid function tests
h. Allergy to soybeans
i. Taking part in another study (within the last 3 months)

Inclusion criteria were different for different studies. These will be discussed in Chapters 4 and 5. All study protocols were approved by the Research Ethics Committee of the North Glasgow University Hospitals NHS Trust.

2.2 Anthropometry

2.2.1 Height and Weight

The heights and weights of subjects were measured using a weighing machine (D. Brash and Sons; Glasgow, UK) with an attached graduated metal plate. Subjects stood barefoot with their heels together and wearing light clothing. Height (m) and weight (kg) measurements were made to the nearest 0.1 unit.

2.2.2 Waist and Hip Circumferences

Waist and hip circumferences were measured using a flexible, inelastic tape measure (Supralip®160, West-Germany). The waist circumference was measured on the horizontal plane midway between the costal margin and iliac crest with the abdominal muscles relaxed and the subject breathing shallowly. The hip measurement was made horizontally around the maximum circumference of the buttocks. The reported values are means of two to three measurements.

2.3 Blood Sampling

Venous blood was obtained via an indwelling cannula (Biovalve, 18G/1.2 mm, Vygon, France) placed in an antecubital vein, to which a 10 cm three-way stopcock (Connecta Plus 3, BD, Sweeden) was attached. The cannula was kept patent by flushing with non-heparinised saline solution (0.9% NaCl). Blood samples were collected directly in 10-ml tubes containing K_3EDTA as an anticoagulant (BD Vacutainer Systems, Plymouth, UK). Blood samples were then placed immediately in ice and centrifuged (GS-6KR, Beckman Instruments, Inc, California, US) within 15-30 min of collection for 15 minutes at 3000 rpm and 4^o C.

EDTA plasma was pipetted into aliquots of 200 μl in 0.5 ml Eppendorf tubes (Treff Lab, Switzerland) and 2 × 350 μl in Apex tubes (2 ml, Alpha Laboratory Ltd, UK) and frozen immediately at -70^o C, for subsequent analysis of insulin, NEFA, glucose and lipid profile. The remaining EDTA plasma was used for lipoprotein separation. This was either started on the same day of blood collection or the next morning. In case of the latter, plasma was stored overnight at 4^o C.

At the end of a successful lipoprotein separation, the remaining EDTA plasma was pipetted into aliquots of no less than 0.5 ml (to minimise any freeze drying effect) in 1.5 ml Eppendorf tubes (Treff Lab, Switzerland) and stored at -70^o C.

2.4 Blood Samples Analysis

Lipoprotein fractions and EDTA plasma were separated and analysed in the Vascular Biochemistry Department of Glasgow Royal Infirmary, University of Glasgow. The methods and techniques employed were taught to the author by researchers in the Vascular Biochemistry Department.

2.4.1 Separation of $VLDL_1$ and $VLDL_2$ Lipoprotein Fractions

$VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) fractions were isolated from plasma using a modification of the cumulative ultracentrifugation density gradient technique described by Lindgren *et al.* (Lindgren *et al.*, 1972).

The density of 2 ml of plasma was adjusted to d 1.118 $g.ml^{-1}$ by the addition of 0.341 g NaCl. This was carefully layered over a cushion of 0.5 ml d 1.182 $g.ml^{-1}$ solution in an ultraclear Beckman SW 40 ultracentrifugation tube (Beckman Instruments Inc., UK) which had been coated with polyvinyl alcohol (Holmquist, 1982); this allowed the solutions to gravity feed down the side of the tubes smoothly without disturbing the formation of the gradient. A discontinuous gradient was formed by overlayering d 1.0988 $g.ml^{-1}$ (1 ml), d 1.0860 $g.ml^{-1}$ (1 ml), d 1.0790 $g.ml^{-1}$ (2 ml), d 1.0722 $g.ml^{-1}$ (2 ml), d 1.0641 $g.ml^{-1}$ (2 ml) and finally d 1.0588 $g.ml^{-1}$ (2 ml). The density solutions were prepared from stock solutions 1.006 $g.ml^{-1}$ and d 1.182 $g.ml^{-1}$ of NaBr in 0.195M NaCl, 0.001% Na_2EDTA and their densities were measured to 3 decimal places in a Paar Scientific densitometer (model DMA 35). The centrifugation was carried out using a Beckman SW 40 rotor (Beckman Instruments Inc., UK) in a

Beckman L8-60M ultracentrifuge for 1.38 h at 39K rpm and 23^o C for separation of the $VLDL_1$ fraction. Rotors were decelerated without brakes and $VLDL_1$ was removed in the top 1 ml using a finely drawn glass Pasteur pipette. This volume was replaced by 1 ml of d 1.0588 g.ml^{-1} and tubes were recapped and placed back in the centrifuge overnight for separation of $VLDL_2$. Various run times and speeds required for $VLDL_2$ separation were previously calculated as shown in **Table 2.1**. At the end of the run, 0.5 ml of $VLDL_2$ fraction was aspirated in the same way as $VLDL_1$. Fractions were kept in tightly capped 2 ml Apex tubes (Alpha Lab Ltd., UK) at 4^o C for subsequent analysis.

Table 2.1: Various times and speeds required for the separation of lipoprotein fractions using Beckman L8-60M Ultracentrifuge and SW 40 rotor.

$VLDL_1$ (ω^2t 9.81)		**$VLDL_2$** (ω^2t 2.12)	
Time (h)	**RPM**	**Time (h)**	**RPM**
1.38	39K	12.03	21.1K
		14.52	19K
		15.41	18.5K
		16.34	18K
		17.31	17.5K
		18.08	17.2K
		20.58	16K

2.4.2 Spectrophotometric Assays

Plasma analyses were carried out using commercially available enzymatic colorimetric kits. Plasma glucose (Glucose hexokinase, Randox Laboratories Ltd, UK) and total and HDL cholesterol (CHOL and HDL-C, Roche Diagnostics, UK, respectively) were analysed in the fasted state. LDL cholesterol was calculated in the fasted state using the Friedewald equation [LDL cholesterol (mmol.l^{-1}) = total cholesterol (mmol.l^{-1}) – HDL cholesterol (mmol.l^{-1}) – (TG (mmol.l^{-1})/2.2) (Friedewald *et al.*, 1972)]. TG (TG, Roche Diagnostics Limited, UK), NEFA (NEFA C, Wako Chemicals, USA) and glycerol (GLY, Randox Laboratories Ltd, UK) were analysed at all time points.

In Chapter 5, PL (Phospholipids C, Wako Chemicals, USA) and FC (Free Cholesterol , Wako Chemicals USA) were measured in the $VLDL_1$ fraction in all time points. In addition, $VLDL_1$-apoC-II, -apoC-III, and -apoE were also measured in the fasted state using commercial automated trubidimetric immunoassay kits, supplied by Wako Chemicals.

All the above analyses were made by an automated clinical chemistry analyser (ILab^{TM} 600, Instrumentation Laboratory, USA) by Mrs. Josephine Cooney, Mrs. Elaine McDonald and Mrs. Elizabeth Murray (Department of Vascular Biochemistry, Glasgow Royal Infirmary).

Serum ALT concentrations were measured at screening (General Biochemistry Laboratory, Glasgow Royal Infirmary) using commercially available enzymatic colorimetric kit (Alanine Aminotranferase, Abbott Laboratories, USA).

2.4.3 Insulin ELISA

Insulin was measured in freshly frozen EDTA plasma using commercially available ELISA kits (Mercodia AB, Uppsala, Sweden). The method is a solid phase two-site enzyme immunoassay. The CV for the assay was < 4%.

2.4.4 Apolipoprotein E Phenotyping

ApoE phenotype was determined for each subject by Mrs Elizabeth Murray (Department of Vascular Biochemistry, Glasgow Royal Infirmary), by isoelectric focusing using Western blot techniques as described by Menzel (Menzel & Utermann, 1986) and Havekes (Havekes *et al.*, 1987).

2.4.5 Total Protein Determination (Lowry Method)

Total protein content in lipoprotein fractions was measured using a modified Lowry method (Lowry *et al.*, 1951). This method involved the addition of 1 ml of Biuret reagent [100 ml of 2% Na_2CO_3 in 0.1 M NaOH (w/v), 1 ml of 2% NaK Tartrate (w/v), 1 ml of 1% $CuSO_4$ (w/v), and 1 ml of 10% (w/v) sodium dodecyl sulphate (SDS)] to a total volume of 200 μl of sample (100 μl $VLDL_1$ + 100 μl of distilled water or 50 μl $VLDL_2$ + 150 μl distilled water). Samples were diluted or concentrated as required. One hundred microlitre of 1:1 Folins Ciocalteu reagent (Sigma-Aldrich Company Ltd., Irvine, Scotland) was then added and mixed immediately. After incubation at room temperature for 30-60 min, the developed colour intensity was measured at an optical density of 750 nm using a Beckman DU 70 Spectrophotometer. The protein concentration was calculated using a standard curve of known concentrations. Inter-assay precision was checked using 2 levels (100 µl and 200 µl) of human and bovine quality control (QC) materials. The coefficients of variation (CVs) for the low QC were (human: 1.8%, bovine: 1.3%) and high QC were (human: 1.0%, bovine: 1.4%).

2.4.6 Accuracy and Precision of Assays

The accuracy and precision of the automated assays described section 2.4.2, except for glycerol, were monitored using quality control sera (Wako QC abnormal and normal, Wako Chemicals GmbH, Denmark; distributed by Alpha Laboratories, UK). Glycerol quality control was obtained from Randox Laboratories Ltd, UK. In order to minimise intra-assay variation, all samples obtained for each subject were performed in the same analyser run for a given assay. The CVs for the assays were 2.9% for total cholesterol, 3.8% for TG, 2.8% for HDL-cholesterol, 5.2% for NEFA, 2.0% for glucose, 1.9% for glycerol, 2.2% for free cholesterol, and 3.8% for PL.

2.5 Bruce Protocol

All subjects recruited for the exercise study described in Chapter 5 underwent a modified (ACSM, 2006) Bruce protocol (Bruce *et al.*, 1973) under the supervision of a medical doctor: Dr Nicholas Barwell or Dr Lesley Hall, at the Institute of Diet, Exercise and Lifestyle (IDEAL) at the University of Glasgow. A modified twelve lead electrocardiographic (ECG) monitoring was conducted throughout the test and arterial blood pressure was measured at rest and immediately after a Bruce protocol during recovery.

2.6 Submaximal Exercise Test

In the exercise study described in Chapter 5, subjects undertook a submaximal exercise test to estimate the gradient necessary to elicit an intensity corresponding to 50% $\dot{V}O_{2max}$. The test consisted of a four-stage treadmill (Woodway Gmbh, Weil am

Rhein, Germany) walk (**Figure 2.1**) to determine the relationship between increasing gradient and the subjects' $\dot{V}O_2$ at their self-selected walking speed. $\dot{V}O_{2max}$ was estimated by extrapolation of the O_2 uptake/heart rate relationship up to the subject's predicted maximum heart rate: [220 – age] beat.min^{-1} (ACSM, 2006). Each stage was five minutes long with expired air samples being taken during the last minute of each stage. Heart rates were recorded during the expired air collections as were perceived rates of exertion using the Borg scale (Borg, 1973). The first stage of the test was on a level treadmill with gradient increasing by 2.0 to 3.0% in each subsequent stage. The increase in gradient for each stage was established on an individual basis based on the subject's heart rate response. The relationship between $\dot{V}O_2$ and treadmill gradient was determined for each subject and, together with the estimated $\dot{V}O_{2max}$, the gradient necessary to elicit an intensity corresponding to 50% $\dot{V}O_{2max}$ was calculated.

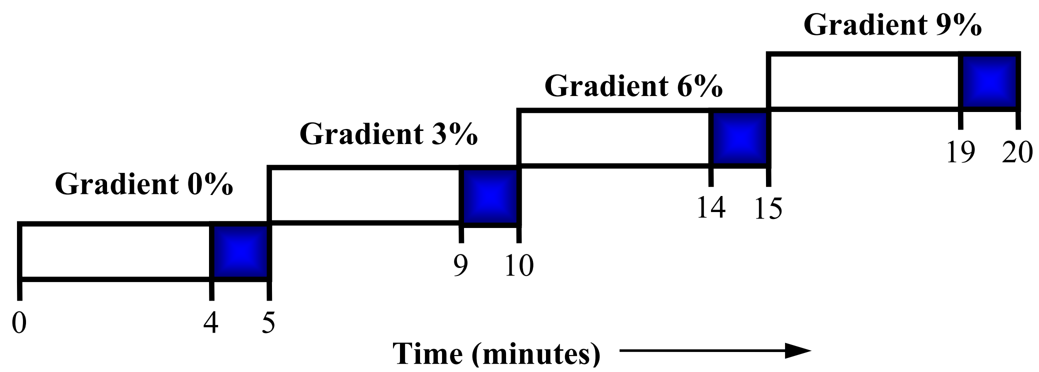


Figure 2.1: A schematic diagram of a 4-stage submaximal exercise test performed for subjects in Chapter 5. The blue block represents an expired air sample and heart rate measurements.

2.7 Monitoring of Heart Rate

The subject's heart rate (Chapter 5) was monitored continuously during exercise and the recovery period by short range telemetry (Polar S610i, Polar Electro, Finland).

2.8 Measurement of Oxygen Uptake and Carbon Dioxide Production

Oxygen uptake and CO_2 production in Chapter 5 were determined at rest before and during exercise. Samples of expired air were collected into 100 or 150 L Douglas bags. While wearing a nose clip, subjects breathed through a mouthpiece fitted to a lightweight large 2-way respiratory valve (2700 series, Hans Rudolph Inc. USA), which in turn was connected to a lightweight tube. The tubing was terminated at a two-way valve which opened and closed the Douglas bag. All equipment was supplied by Cranlea & Co. Birmingham, England.

An aliquot of expired air (measured using a flow meter) was removed from each Douglas bag to determine the fraction of O_2 and CO_2 using a gas analyser (Servomex 4100, Servomex Group Ltd., East Sussex, England). The analyser was calibrated before each use with certified reference gases (BOC Ltd, Surry, UK) and the reference gases were calibrated against a 'gold standard' reference gas to ensure consistency of results.

The remaining volume of expired air in each Douglas bag was measured by evacuation through a dry gas meter (Harvard apparatus, supplied by Cranlea & Co. Birmingham, England). The temperature of air in the Douglas bag was measured during evacuation using the same dry gas meter.

Barometric pressure was measured using a barometer and the measured expired gas volumes were corrected to standard temperature and pressure (STPD) for a dry gas using the universal gas equation. Inspired gas volumes were derived using the Haldane transformation (Consolazio *et al.*, 1963) and O_2 uptake, CO_2 production, minute ventilation, respiratory exchange ratio and the ventilatory equivalent for oxygen were calculated. Rates of substrate utilization expenditures were calculated via indirect calorimetry using the equations described by Frayn (Frayn, 1983) and energy expenditure was determined by multiplying the mass of substrates used by their respective energy densities.

2.9 Apolipoprotein B Measurements in $VLDL_1$ and $VLDL_2$ Fractions – Comparison of a Manual and an Automated Method

2.9.1 Introduction

In the Vascular Biochemistry laboratory, for kinetic studies, apoB is usually measured in lipoprotein fractions using a standardised manual method, which is applicable to VLDL, IDL and LDL fractions. ApoB is precipitated after the addition of an equal volume of absolute isopropanol (Egusa *et al.*, 1983). The supernatant is aspirated and its protein content is measured using a modified version of Lowry method (Lowry *et al.*, 1951). There is no apoB solubilisation in isopropanol and the precipitated apoB is virtually free of soluble apolipoproteins. ApoB protein concentrations are measured by the difference between total protein content in lipoprotein and the protein measured in supernatant. This method is standardised and widely used for the measurement of the relatively low concentrations of apoB in $VLDL_1$ and $VLDL_2$ fractions. However, it is laborious, time consuming and, being a manual technique, is subject to personal error.

Another method for measuring apoB concentrations is direct measurement using an automated *in vitro* immunoturbidimetric assay with the use of commercially available kits (see below). Being an automated method, the immunoturbidimetric assay saves time, effort and potentially improves reproducibility. However, these have been designed for apoB measurements in serum or plasma, where apoB concentrations are high and mainly represent apoB in the LDL fraction [85-90% of total apoB (Alaupovic, 1991)]. It was therefore unclear whether this method is suitable for the measurement of the low apoB concentrations in $VLDL_1$ and $VLDL_2$ fractions.

Therefore, the purpose of the present study was to compare the automated turbidimetry immunoassay method with the conventional precipitation one as an alternative method for the measurement of apoB protein in $VLDL_1$ and $VLDL_2$.

2.9.2 Materials and Methods

2.9.2.1 Specimens and Separation of $VLDL_1$ and $VLDL_2$ fractions

A total of 260 EDTA plasma samples were used to separate $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) fractions by a modification of the cumulative ultracentrifugation density gradient technique as previously described (section 2.4.1)

2.9.2.2 Isopropanol Precipitation Method (Egusa *et al.*, 1983)

All $VLDL_1$ and $VLDL_2$ samples were brought to room temperature and 0.5 ml of absolute isopropanol was added to 0.5 ml of each sample in glass tubes (13/100 glass tubes, Labco Ltd., Buckinghamshire, UK). After immediate vigorous mixing, samples were then incubated for at least 24 h at 4^o C, after which they were spun at 3000 rpm and 4^o C for at least 30 min. Immediately, the supernatant was carefully aspirated using a drawn-out Pasteur pipette, without drawing out any of the precipitated apoB.

Total protein content in the $VLDL_1$ and $VLDL_2$ fractions and the non-apoB protein content of the supernatant were measured using the modified Lowry method as described earlier (section 2.4.5).

2.9.2.3 Immunoturbidimetric Method

The apoB content of the same samples was measured directly using commercially available kits (WAKO Apolipoprotein B-HA, Wako Chemicals GmbH, Denmark; distributed by Alpha Laboratories). This method involved the specific combination of apoB in the sample with anti-human apoB antibodies in the reagent to yield an insoluble aggregate that causes increased turbidity. The degree of turbidity is measured optically using an autoanalyser (ILab™ 600, Clinical Chemistry System, Instrumentation Laboratory, USA). The method in Vascular Biochemistry has been optimised for apoB measurements in lipoprotein fractions by increasing the volume of sample used in the assay from 3 μl to 12 μl (results were then multiplied by a factor of 0.25). The supplied quality control was also diluted to account for the lower concentrations of apoB (WAKO 3 was diluted 1:5 to give a mean (range) concentration of 15.3 (12.3-18.4) $mg.dl^{-1}$, which is within the range of expected results).

2.9.2.4 Calculating ApoB %CV for Each Method

$VLDL_1$ and $VLDL_2$ were separated from two subjects and each fraction was pooled together. ApoB was measured in ten samples using the immunoturbidimetric method and the isopropanol-Lowry method. Means, SDs and CVs were calculated for each method.

2.9.3 Results

Figure 2.2 shows $VLDL_1$- and $VLDL_2$-apoB concentrations ($mg.dl^{-1}$) measured by the immunoturbidimetry method in 260 samples plotted against that measured by the isopropanol precipitation method with the line of equality; the line all points would lie on if the two methods always gave exactly the same measurement. The two methods correlate well for both fractions with R^2 (goodness-of-fit) of 0.88 for $VLDL_1$ and 0.93 for $VLDL_2$. However, the isopropanol method measured values as ~1.3 times higher than the immunoturbidimetric method for both $VLDL_1$ and $VLDL_2$ fractions. This was particularly apparent as the apoB concentrations increased.

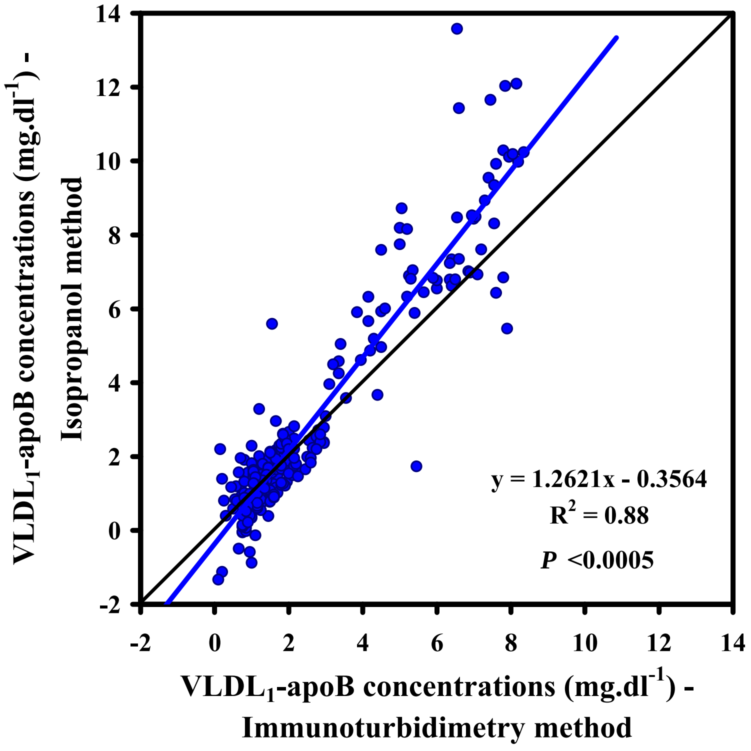
Figure 2.3 shows the percent difference between measurements by the two methods (isopropanol minus immunoturbidimetry) for each sample against their mean in both the $VLDL_1$ and $VLDL_2$ fractions. This is done to explicitly show extreme or outlying observations and any lack of agreement between the two methods (Bland & Altman, 1999). In addition, **Figure 2.3** shows the 95% limits of agreement estimated as mean percentage difference ± 2 SD for each fraction, which define the range within which most differences between measurements by the two methods will lie (Bland & Altman, 1999). This was: 0.8 ± 110.3% for $VLDL_1$ and 9.1 ± 66.4% for $VLDL_2$, giving rise to 95% limits of agreement between (-109.4% to +111.1%) for $VLDL_1$ and between (-57.3% to +75.5%) for $VLDL_2$. From **Figure 2.3**, a cut-off value of ~3.0 $mg.dl^{-1}$ could be made, above which measurements between the two methods appeared to show better agreement for both $VLDL_1$ and $VLDL_2$ fractions compared to values less than 3 $mg.dl^{-1}$, where deviation around the mean was more scattered. This is better illustrated in **Figure 2.4**, which shows the %difference (isopropanol minus immunoturbidimetry) in apoB concentrations plotted against the mean of two methods for values $\geq$ 3.0 $mg.dl^{-1}$. In this case, the deviation around the mean is evenly scattered, especially in the $VLDL_1$ fraction, and the 95% limits of agreement (mean ± 2SD) became smaller: between -33.3% and 73.7% (20.2 ± 53.5%, n = 65) for $VLDL_1$ and between -17.5% and 53.0% (17.7 ± 35.3%, n = 84) for $VLDL_2$. In addition, it is clear from **Figure 2.4A** that there are two outliers in the $VLDL_1$ fraction. Although excluding these two measurements did not influence the mean percentage difference, it reduced the SD value (20.7 ± 37.5%) and consequently, the 95% limits of agreement (-16.8 to 58.2%).

Finally, **Table 2.2** shows the mean $VLDL_1$- and $VLDL_2$-apoB concentrations, SDs and CVs measured 10 times for each method in $VLDL_1$ and $VLDL_2$ fractions using pooled samples. The apoB concentrations were lower in $VLDL_1$ compared to $VLDL_2$ and it is clear that the immunoturbidimetric method was more reproducible than the isopropanol method at such low concentrations (CV 3.4% vs. 9.9% for $VLDL_1$ and 1.4% vs. 3.2% for $VLDL_2$, respectively).

Table 2.2: Calculated mean, SD and CV for apoB concentrations measured 10 times for the same sample using the two methods

	$VLDL_1$-apoB ($mg.dl^{-1}$)		**$VLDL_2$-apoB ($mg.dl^{-1}$)**	
	Immunoturbidimetric method	Isopropanol method	Immunoturbidimetric method	Isopropanol method
Mean	3.74	2.07	6.96	5.65
SD	0.13	0.20	0.10	0.18
CV (%)	**3.38**	**9.88**	**1.39**	**3.19**

[A]



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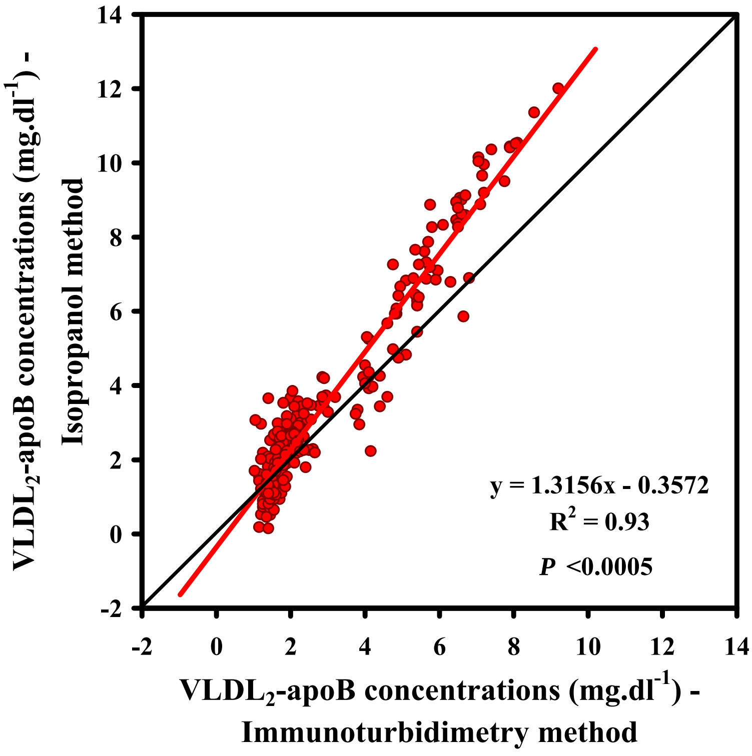
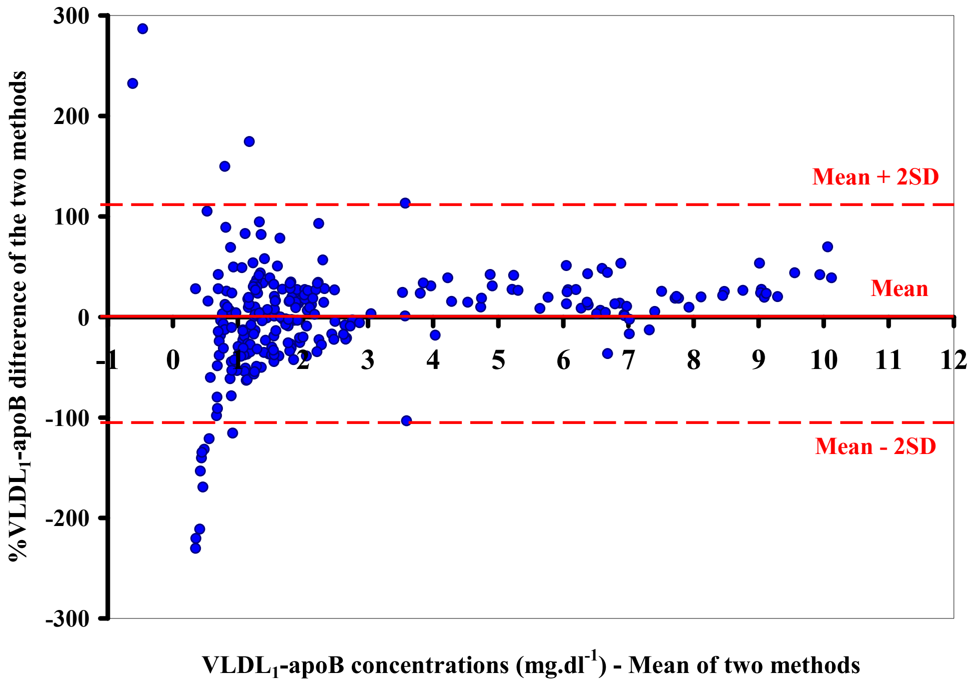


Figure 2.2: ApoB concentrations ($mg.dl^{-1}$, with linear-regression lines of 'best-fit' and equation of the line) in [A] $VLDL_1$ and [B] $VLDL_2$ fractions measured using an automated immunoturbidimetric method and by a manual isopropanol method with the line of equality. (n = 260)

[A]



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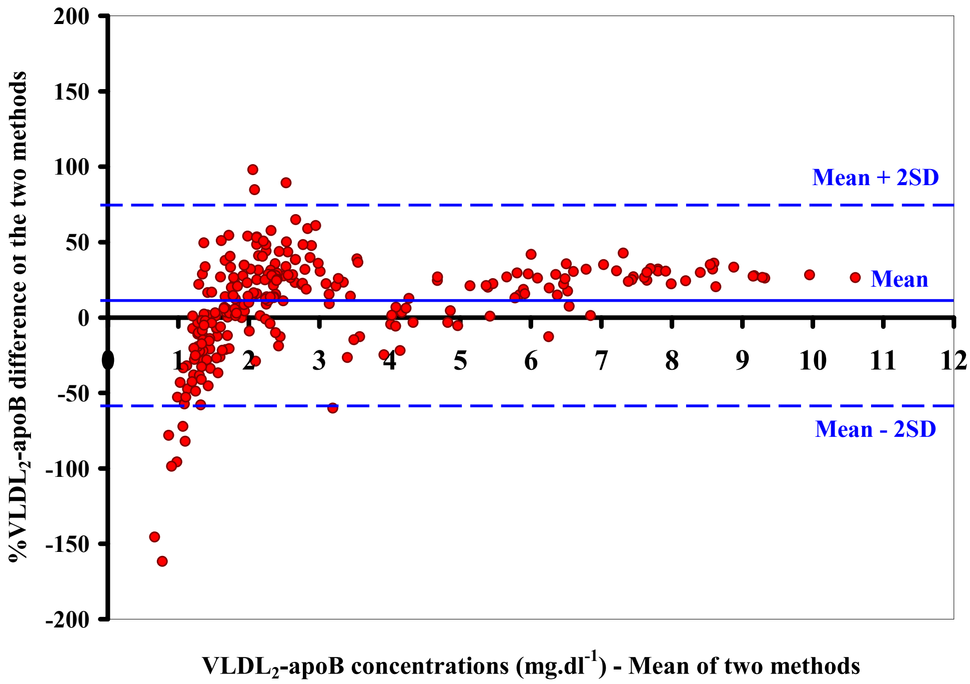
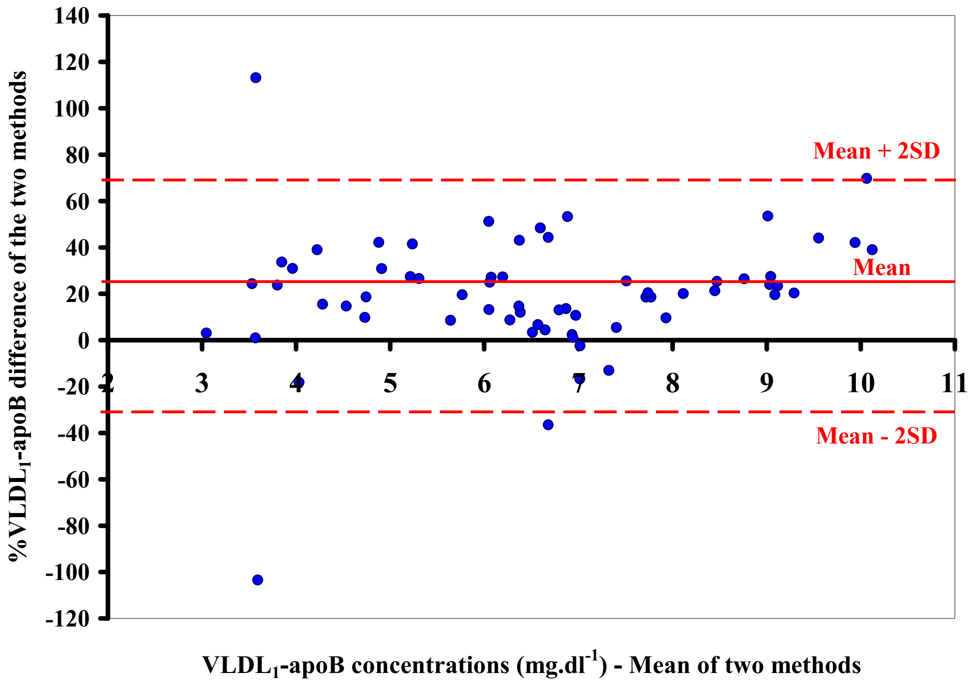


Figure 2.3: A scatterplot of the percent difference in apoB concentrations (mg.dl^{-1}) between the two methods (isopropanol minus immunoturbidimetry) against their mean in [A] **$VLDL_1$ and** [B] **$VLDL_2$, with 95% limits of agreement (mean ± 2SD): $VLDL_1$ (0.8 ± 110.2%) and $VLDL_2$ (9.1 ± 66.4%), n = 260.**

[A]



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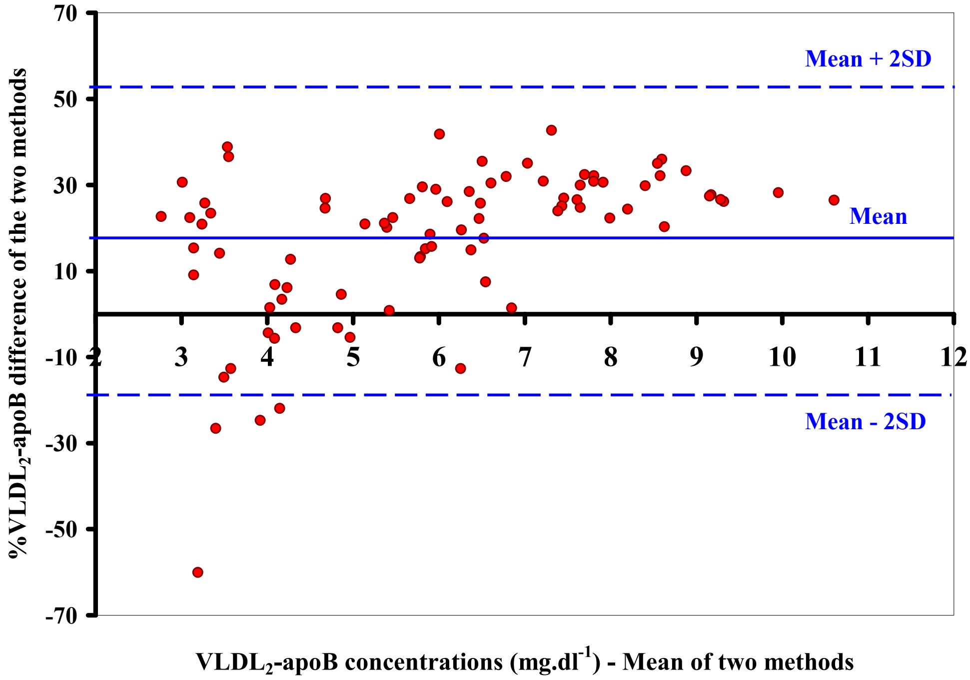


Figure 2.4: A scatterplot of the percent difference (isopropanol minus immunoturbidimetry) for apoB concentrations $\geq$ 3.0 mg.dl^{-1} against the mean of the two methods in [A] VLDL$_1$ and [B] VLDL$_2$, with 95% limits of agreement (mean ± 2SD): VLDL$_1$ (20.2 ± 53.5%, n = 65) and VLDL$_2$ (17.7 ± 35.3%, n = 85).

2.9.4 Discussion

Due to the low concentrations of apoB in VLDL fractions, the manual precipitation of apoB using isopropanol (Egusa *et al.*, 1983) has historically been the preferred choice, followed by a modification of another manual method (Lowry *et al.*, 1951) to determine the apoB protein concentrations in the fractions. Being a chemical method, this technique is not affected by the size of the lipoprotein particle, the possible masking of the apoB epitopes or changes in time over standardisation of commercial apoB assays. However, this method is time-consuming and requires a high degree of precision and skill in separating the non-apoB supernatant from the precipitated apoB protein, which may sometimes be difficult in cases of very high or very low apoB concentrations.

Conversely, apoB can be measured directly using an automated immunoturbidimetric assay, with the use of commercially available kits. The fact that this method is automated, makes it faster, allows for a minimal involvement of human error, and, consequently, better precision. One possible shortcoming of this immunological method is that it relies on detecting certain epitopes on the apoB particle and, therefore, has the disadvantage of being dependent on the manufacturer's choice of such epitopes, which could be subject to change at any time.

Because of the large number of samples per trial used in this thesis and the relative difficulty of using the manual isopropanol method for apoB measurements, the two methods were compared using 260 samples from the $VLDL_1$ and $VLDL_2$ fractions. This was done to assess the possibility of replacing the isopropanol precipitation method with the automated immunoturbidimetric method.

The present results show that the two methods agree well for both fractions with R^2 values of ~0.9. This is especially true for apoB concentrations $\geq$ 3.0 $mg.dl^{-1}$. Although the immunoturbidimetric method seems to give values ~20% lower than the isopropanol method, this is consistent and the deviation from the mean of the two methods still lies within the 95% limits of agreement. However, the immunoturbidimetric method allows better precision and reproducibility than the manual isopropanol method, with CVs of 3.4% and 1.4% (compared to 9.9% and 3.2%) in $VLDL_1$ and $VLDL_2$ fractions, respectively. In addition, being an automated method, the immunoturbidimetry allows the feasibility and the ease of analysing large number of samples in a relatively short period of time, compared to the isopropanol method.

In conclusion, it is inevitable to find some lack of agreement between different methods, but what matters is the amount by which methods disagree (Bland & Altman, 1999). The automated immunoturbidimetry method correlated consistently and reasonably well with the manual isopropanol precipitation method, with better precision, reproducibility and ease of use for measurements of multiple samples. Therefore, the immunoturbidimetric method was chosen to measure apoB concentrations in this thesis.

2.10 Glucose Pilot Study

The purpose of this pilot study was to determine whether it was possible to maintain a 'pseudo' steady state of high circulating glucose and insulin concentrations by ingesting small amounts of a glucose solution at frequent intervals. If this approach was feasible, it would represent a more straightforward and less invasive method of inducing hyperinsulinaemia and hyperglycaemia than invasively infusing glucose and/or insulin. A number of different patterns of glucose ingestion were investigated to determine an appropriate protocol, which will be used in the study described in Chapter 4.

2.10.1 Subjects and Methods

Four subjects (2 women and 2 men – age (range) 20-33 years; body mass 55-95 kg; BMI 22.6-28.1 $kg.m^{-2}$) were included in this study after giving informed consent. Subjects reported in the morning to the metabolic suite at the Institute of Diet, Exercise and Lifestyle, at the University of Glasgow, after a 12-h fast. Subjects were cannulated in an anticubital vein and after a 10-min interval a baseline EDTA fasting blood sample was drawn. A second baseline sample was taken 10 min later. All blood samples were placed immediately in ice. EDTA plasma was obtained after centrifugation for 10 min at 3000 rpm and divided into 5 aliquots in 1.5 ml Eppendroff tubes. Aliquots were stored immediately at -20^{o} C for subsequent analysis of glucose, NEFA and insulin concentrations as previously described in sections 2.4.2 and 2.4.3.

The glucose drinks were prepared by dissolving the appropriate weight of glucose powder (Thornton and Ross Ltd., Huddersfield, England) in hot water and making the required volume up with cold water. Approximately 5-6 ml of lemon juice per 100 ml of water were added for taste.

2.10.2 Protocol I

The first protocol was designed to introduce a glucose concentration of 30% (w/v) (15 g in 50 ml) every 15 min for 2 h. Two female subjects participated in this protocol (**Table 2.3**). Blood samples were drawn every 15 min.

Table 2.3: Characteristics of the two female subjects who participated in Protocol I

Subject	Age (y)	Body mass (kg)	BMI (kg.m^{-2})	HOMA
1	20	66.4	23.81	1.4
2	33	55	22.6	1.6

Figure 2.5 shows glucose, insulin and NEFA responses for the two subjects. Glucose concentrations increased slowly in subject 1 and immediately in subject 2 before declining close to baseline at the end of 2 h. Consequently, insulin concentrations increased in response to the glucose drink, but differently in the two subjects: while subject 1 had a delayed insulin response corresponding to glucose concentrations, subject 2 had a dramatic increase in insulin concentrations for the duration of the trial. However, none of the two subjects reached any steady state during 2 h. Similarly, NEFA concentrations declined slowly in subject 1 but dramatically in subject 2 over the course of trial in response to insulin. NEFA concentrations were only suppressed at the end of the 2 h.

Despite different responses in glucose and insulin concentrations, neither subjects reached a ‘pseudo’ steady state. Because one subject had a delayed response, it was clear that an initial bolus glucose drink is needed to induce a more rapid response. Also, because the other subject had an exaggerated insulin response to the glucose drink, it might be beneficial to decrease its dose, and possibly increase its frequency to account for the reduced concentration.

2.10.3 Protocol II

The second protocol started with a bolus drink of 20% glucose (30 g in 150 ml) followed by 20% glucose drink (5 g in 25 ml) every 10 min (instead of 15 min) for a period of 2 h. Blood samples were taken at 15-min intervals. One male subject participated in this protocol (aged 29 years, body mass 95 kg, BMI 28.1 kg.m^{-2} and HOMA 1.4).

Figure 2.6 shows glucose, insulin and NEFA responses during this protocol. The bolus glucose drink successfully increased glucose and insulin concentrations shortly after ingestion. However, the glucose drink, thereafter, was not sufficient to maintain a steady state of glucose and insulin as they started to decline after 90 min. In addition, the drink did not induce a desirable suppression in NEFA concentrations before the 2 h.

Therefore, a bolus glucose drink seemed essential to induce a faster and immediate response in glucose and insulin concentrations. However, a dose of 5 g glucose every 10 minutes did not seem sufficient to maintain elevated glucose and insulin concentrations. Furthermore, it would be helpful to increase the observation time of the study to investigate whether a steady state could be reached and/or maintained for a longer period of time.

2.10.4 Protocol III

In this protocol, glucose drink concentration and the observation time were increased. It started with 30% bolus glucose drink (30 g in 100 ml) followed by 60% glucose drink (15 g in 25 ml) every 15 min for 4 h. These doses were chosen as it became obvious from the previous two protocols that a stronger glucose drink might be needed to achieve the desired 'pseudo' steady state and suppress NEFA at a time shorter than 2 h. One subject participated in this protocol (aged 22 years, body mass 80.5 kg, BMI 27.8 kg.m^{-2} and HOMA 4.0).

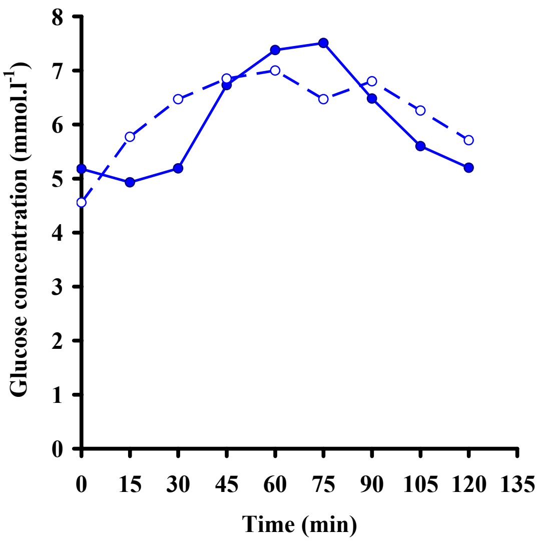
Figure 2.7 shows glucose, insulin and NEFA responses during the 4-h observation period for this protocol. Glucose and insulin concentrations increased quickly in response to the bolus glucose drink, which caused a desirable, fast reduction in NEFA concentrations. However, the glucose drink caused abrupt responses in both glucose and insulin concentrations. In addition, there was tendency for insulin concentrations to continually increase during the 4 h. It is uncertain, however, whether this increase is related to the subject's HOMA of 4.0, which may indicate insulin resistance, or whether it was caused by the increased glucose drink concentration.

2.10.5 Conclusion

From these protocols, it was obvious that (1) a bolus glucose drink was needed to trigger an immediate insulin response, (2) providing 5 g of glucose every 10 minutes was not sufficient to maintain elevated levels of glucose and insulin, while providing 15 g glucose every 15 minutes produced abrupt responses and (3) although NEFA concentrations started to decline immediately in response to insulin, it took about 60 min to be suppressed almost completely in the last protocol.

In conclusion, taking these findings into account, it was decided that the final protocol for the glucose ingestion would be; a bolus glucose drink of 25% (w/v) (30 g of glucose in 120 ml), followed by 25% glucose drink (10 g in 40 ml) every 15 min for the duration of the trial. The glucose ingestion protocol would start an hour prior to the Intralipid infusion described in Chapter 4 to ensure adequate suppression of NEFA concentrations.

[A]



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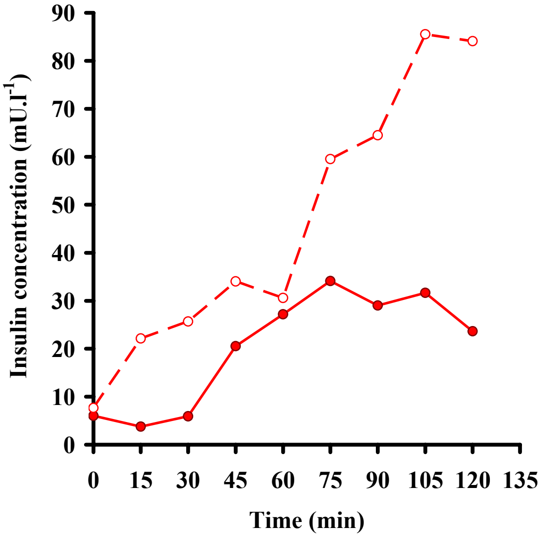

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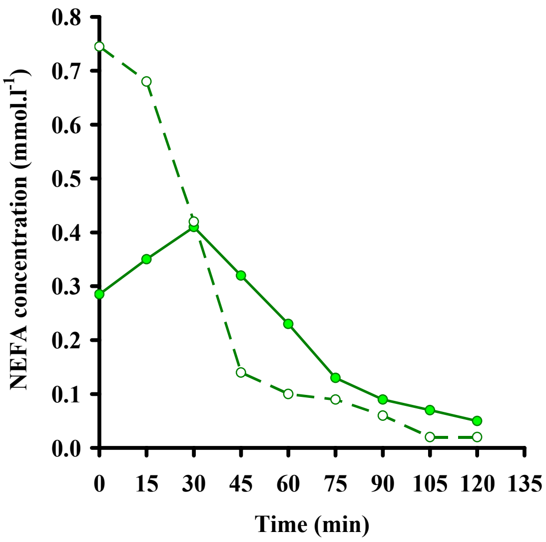


Figure 2.5: [A] glucose, [B] insulin and [C] NEFA response (n = 2) for the glucose pilot - Protocol I: 30% (w/v) glucose (15 g in 50 ml) every 15 min for 2 h. The solid lines represent subject 1, while the dotted lines represent subject 2.

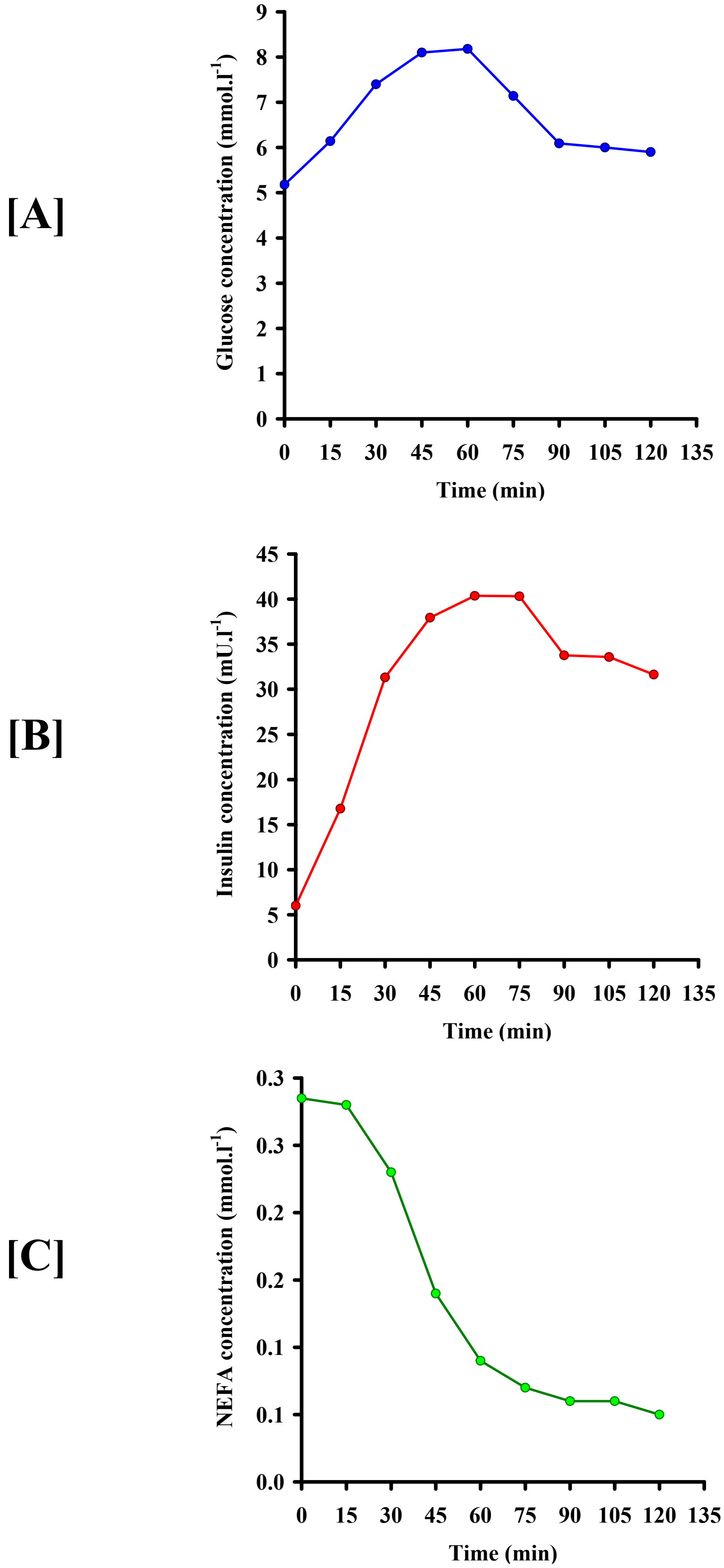


Figure 2.6: [A] glucose, [B] insulin and [C] NEFA response (n = 1) for the glucose pilot - Protocol II: a bolus drink of 20% (w/v) of glucose (30 g in 150 ml) followed by a 20% glucose drink (5 g in 25 ml) every 10 min for 2 h.

[A]

[B]

[C]

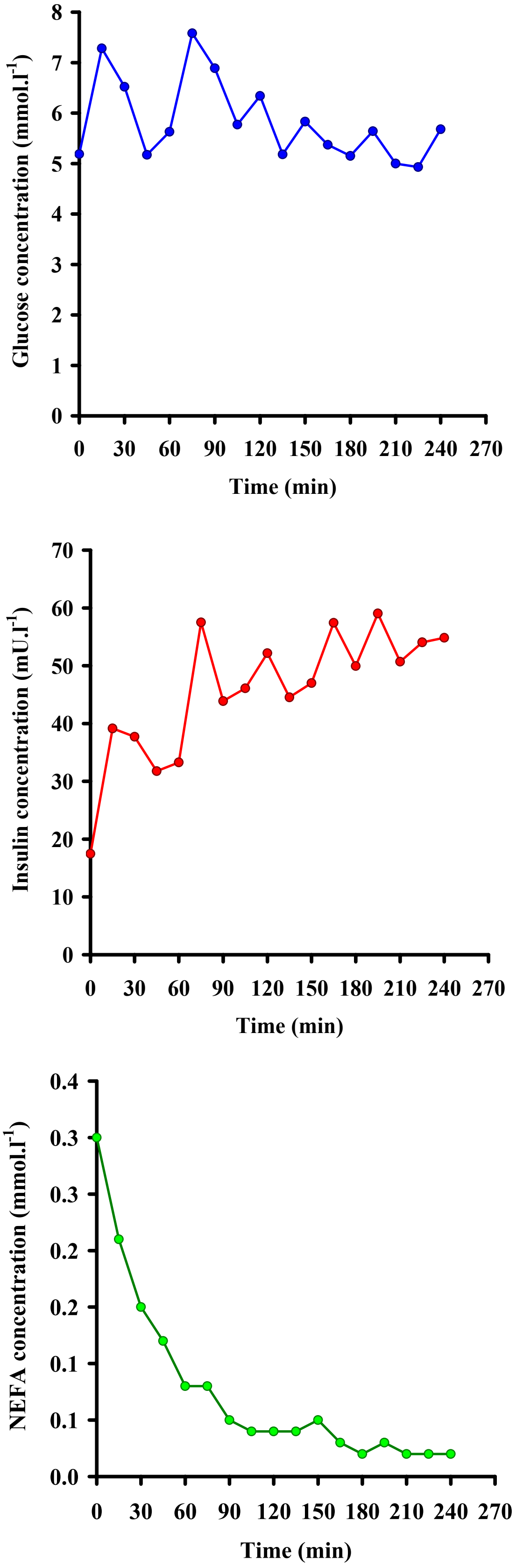


Figure 2.7: [A] glucose, [B] insulin and [C] NEFA response (n = 1) for the glucose pilot - Protocol III: a bolus glucose drink of 30 g (in 100 ml) followed by 15 g of glucose (in 25 ml) every 15 min for 4 hours.

3. Development of a Novel Method to Determine $VLDL_1$ Kinetics

3.1 Introduction

A large body of evidence suggests that increased circulating concentrations of TRLs increase the risk of atherosclerosis (Malloy & Kane, 2001; Tanaka *et al.*, 2001). This is particularly evident in the postprandial state (Karpe, 1999; Schneeman *et al.*, 1993). However, the measurement of a high TRL concentration provides no information regarding the mechanisms responsible for this elevation; i.e. increased rate of synthesis and/or reduced rate of catabolism. As it is important to understand the mechanisms responsible for increased TRL concentrations in different metabolic states, both to advance basic scientific understanding and to help guide therapeutic treatments, studies investigating kinetics of TRL can yield useful data. Such an approach has, for example, revealed that the dyslipidaemia associated with insulin resistance and type 2 diabetes is largely due to an overproduction of hepatically derived large $VLDL_1$ (S_f 60-400) (Watson *et al.*, 2003; Adiels *et al.*, 2005a). These studies typically use precursors labelled with stable or radioactive isotope tracers to measure the synthesis of lipids and apolipoproteins directly (Adiels *et al.*, 2005a; Gill *et al.*, 2004a; Packard *et al.*, 2000; Packard, 1995). Although these techniques yield detailed kinetic data, they are costly, time consuming, labour-intensive and require the use of specialised equipment and techniques in research laboratories.

The aim of the present study was, therefore, to develop a relatively straightforward method of obtaining TRL kinetic data. The method relies on the fact that chylomicrons compete with $VLDL_1$ particles for the same catalytic pathway; i.e. hydrolysis of their TG content by the action of LPL. Previous studies (Karpe & Hultin, 1995; Bjorkegren *et al.*, 1996) have shown that $VLDL_1$ accumulates in plasma after fat ingestion or intravenous infusion of a lipid emulsion (e.g. Intralipid) due to the presence of the newly secreted chylomicrons or chylomicron-like particles, which are the preferred substrate for LPL because of their larger size and TG content (Fisher *et al.*, 1995). Indeed, using stable isotope methods, Björkegren *et* *al.* demonstrated that infusion of Intralipid prevents over 90% of $VLDL_1$ catabolism (Bjorkegren *et al.*, 1996). It was therefore hypothesized in the present study that it would be possible to calculate the production rates of $VLDL_1$-TG and -apoB from the rate of their accumulation during an infusion of Intralipid. The former would be a measure of lipid production while the latter would represent the rate of $VLDL_1$ particle production, as there is one apoB molecule per $VLDL_1$ particle (Elovson *et al.*, 1988). Furthermore, using this approach, it is possible to calculate the rate of Intralipid-TG clearance (a surrogate measure of TRL-TG clearance) from either the steady-state Intralipid-TG concentration during infusion (Rang *et al.*, 2003) or from the exponential decay in Intralipid-TG concentration post-infusion (Rossner, 1974). Here the author reports the development and validation of this 'Intralipid method' to determine TRL kinetics.

3.2 Material and Methods

3.2.1 Subjects

Ten non-smoking healthy subjects (7 males and 3 females) were included in this study after giving written informed consent. All subjects had normal thyroid, liver and renal function and none had acute illness, a history of known cardiovascular disease and hypertension, nor were under medication known to influence carbohydrate or lipid metabolism. The subject information sheet, consent form and health screen for the present study are shown in Appendices A1 and B1. Two subjects had the E3/2 apoE phenotype, seven had the E3/3 phenotype and one had the E4/3 phenotype. The subjects' characteristics are shown in **Table 3.1**. Subjects were requested not to exercise for three days before their study days as this is known to affect TRL metabolism (Gill & Hardman, 2003). In addition, they were asked to weigh and record their dietary intake for two days prior to the Intralipid test and this diet was replicated in those subjects who underwent a second Intralipid test. The study protocol was approved by the Research Ethics Committee of the North Glasgow University Hospitals NHS Trust.

Table 3.1: Subjects' physical and metabolic characteristics (n=10).

	Mean	Range
Age (years)	33.5	(20.0 – 55.0)
Body mass index ($kg.m^{-2}$)	25.9	(20.8 – 34.7)
Waist circumference (cm)	85.1	(65.0 – 113.5)
Waist/hip ratio	0.84	(0.71 – 1.04)
Triglycerides ($mmol.l^{-1}$)	1.36	(0.40 – 4.43)
Total cholesterol ($mmol.l^{-1}$)	4.15	(2.85 – 5.90)
HDL cholesterol ($mmol.l^{-1}$)	1.30	(0.75 – 1.85)
LDL cholesterol ($mmol.l^{-1}$)	2.20	(1.22 – 4.00)
Glucose ($mmol.l^{-1}$)	5.5	(4.3 – 8.0)
Insulin ($mU.l^{-1}$)	8.92	(2.78 – 24.81)
$HOMA_{IR}$	2.41	(0.56 - 8.82)
NEFA ($mmol.l^{-1}$)	0.51	(0.34 – 0.70)
ALT ($U.l^{-1}$)	23	(16-38)

3.2.2 Intravenous Intralipid Test

Each subject reported to the Clinical Investigation Suite in the Department of Vascular Biochemistry in Glasgow Royal Infirmary after an overnight fast of 12 h. Transportation to the hospital was provided for the subjects, when needed, to ensure that they arrived in a rested state. A cannula was introduced into an antecubital vein in both arms; one for administration of Intralipid (purified soybean oil emulsion, Fresenius Kabi Ltd., Warrington, UK) and the other for blood sampling. The cannulae were kept patent by flushing with non-heparinised saline solution (0.9% NaCl). Ten min after cannulation a first baseline blood sample was obtained. A second baseline blood sample was obtained 10 min later.

The intravenous Intralipid test used was a modification of that described by Björkegren and collegues (Bjorkegren *et al.*, 1996). A bolus dose of 20% Intralipid (0.1 $g.kg^{-1}$ body mass) was injected within one min. This was followed immediately by a constant continuous infusion of 10% Intralipid (0.1 $g.kg^{-1}.h^{-1}$). This dose was chosen as Björkegren *et al.* reported that the rate of rise of $VLDL_1$-apoB during Intralipid infusion was no greater for a 0.2 $g.kg^{-1}.h^{-1}$ infusion dose compared with 0.1

$g.kg^{-1}.h^{-1}$, suggesting that the lower dose was sufficient to saturate LPL and prevent measurable $VLDL_1$ catabolism (Bjorkegren *et al.*, 1996). However, experiments with the 0.2 $g.kg^{-1}.h^{-1}$ dose were also performed in the present study to confirm that this was the case in the author's hands (see below). Initially, the infusion period was 120 min, however, during development of the technique this was subsequently shortened to 75 min after it became clear that a 75-min infusion was long enough to induce a sufficient measurable rise in $VLDL_1$-TG and -apoB. **Figure 3.1** shows an example of a subject during an Intralipid trial.

Blood samples were obtained at 15-min intervals during the infusion. Further blood samples were drawn 2.5, 5, 10, 15, 20, 30, 45, 60 and 75 min post-infusion. Initially, the post-infusion period was 3.25 h. However, this was subsequently shortened to 75 min when it became clear that this was sufficient to calculate the Intralipid-TG clearance rate using the exponential decay. All samples were obtained directly into 10-ml potassium EDTA tubes (BD Vacutainer Systems, Plymouth, UK) and placed immediately in ice before centrifuging for 15 min at 3000 rpm and 4^o C.

Aliquots of plasma were frozen immediately at -70^o C, as described in section 2.3, for subsequent analysis of insulin, NEFA, glucose, TG, and total and HDL-cholesterol. The remaining plasma was stored overnight at 4^oC prior to separation of Intralipid and lipoproteins.

3.2.3 Increasing the Intralipid Infusion Rate

Five subjects (2 females and 3 males, age 23-47 y [range], BMI 20.8-28.7 $kg.m^{-2}$, fasting TG 0.47-2.45 $mmol.l^{-1}$) underwent a second test using a higher infusion dose (0.2 $g.kg^{-1}.h^{-1}$) of 10% Intralipid with the same 0.1 $g.kg^{-1}$ bolus dose. This was carried out to determine whether an Intralipid infusion dose of 0.1 $g.kg^{-1}.h^{-1}$ was sufficient to completely prevent measurable lipolysis of $VLDL_1$ by LPL, and therefore enable determination of $VLDL_1$-TG and -apoB production rates from their rises in concentration. If the infusion rate of 0.1 $g.kg^{-1}.h^{-1}$ was sufficient to saturate LPL and block lipolysis of $VLDL_1$, the 0.2 $g.kg^{-1}.h^{-1}$ dose would not result in higher calculated production rates of $VLDL_1$-TG or -apoB compared to the 0.1 $g.kg^{-1}.h^{-1}$ dose. The order of testing was randomized. Other than the higher infusion dose, all conditions of the tests were the same.

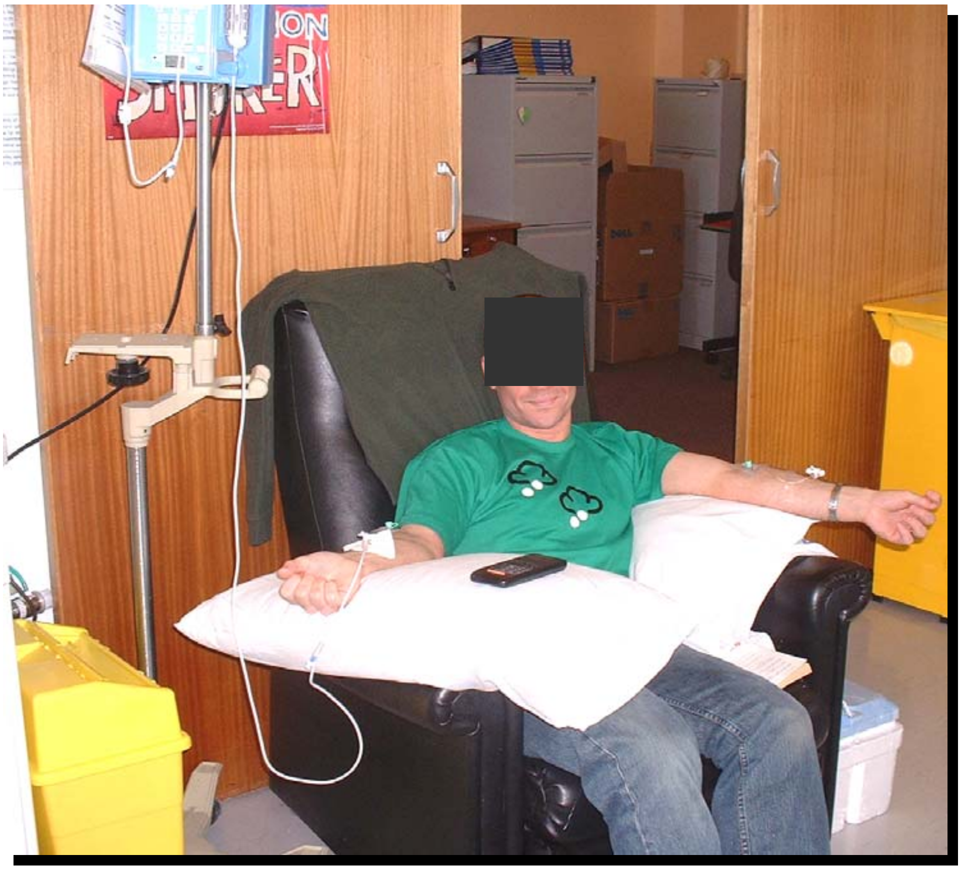


Figure 3.1: A picture of a subject during an Intralipid trial. A cannula is placed in an anticubital vein in each arm; one for the Intralipid infusion of 0.1 g.kg^{-1}.h^{-1} (right arm) using an IVAC Signature Edition Gold pump (ALARIS Medical Systems, Inc.) and one for blood sampling (left arm). An intial bolus Intralipid dose (0.1 g.kg^{-1} body mass) was given in the right arm via a three-way stopcock immediately before starting the infusion. (Published with permission from the subject).

3.2.4 Intralipid (Sf > 400) Separation from Whole Plasma

Two ml of plasma were overlayered with 4 ml of 1.006 $g.ml^{-1}$ density solution in ultra-clear centrifuge tubes and spun at 10,000 rpm and 4^o C for 30 min (Lindgren *et al.*, 1972) using Beckman L8-60M Ultracentrifuge and Beckman 50.4 rotor (Beckman Instruments Inc., UK). Intralipid (d <1.006 $g.ml^{-1}$) was removed in the top 2 ml (IL-1) for subsequent measurements of TG using commercially available kits as described in section 2.4.2. TG concentration was also measured in the middle 1.5 ml fraction (IL-2) to verify complete separation of Intralipid. The final Intralipid-TG concentration was calculated as the addition of these two fractions [IL-1 + (IL-2 × 1.5/2)]. In addition, glycerol was measured in these IL-1 and IL-2 fractions (section 2.4.2) to determine the amount of free glycerol. The final 0.5 ml of the density solution was discarded and the remaining 2 ml Intralipid-free plasma was used for separation of $VLDL_1$ and $VLDL_2$. The CV for the Intralipid-TG separation was 6.9%.

3.2.5 $VLDL_1$ and $VLDL_2$ Separation

$VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) were isolated from plasma using a modification of the cumulative ultracentrifugation density gradient technique as previously described in section 2.4.1. TG concentrations were then measured in the $VLDL_1$ and $VLDL_2$ fractions in all time points and apoB concentrations were also measured directly by immunoturbidimetry as described in section 2.9.2.3. The CVs for separation of $VLDL_1$-TG and -apoB were 5.0% and 3.4%, respectively and for $VLDL_2$-TG and -apoB were 5.8% and 1.4%, respectively.

3.2.6 Fasting Plasma Analysis

Plasma glucose, insulin, total and HDL cholesterol concentrations were analysed in the fasted state and TG and NEFA concentrations were analysed at all time points. LDL cholesterol was calculated in the fasted state using the Friedewald equation (Friedewald *et al.*, 1972). Serum ALT concentrations were measured at screening. All analyses were done as previously described in section 2.4.2.

3.2.7 Correction for Glycerol

Enzymatic kits for TG analysis measure the glycerol that is hydrolyzed from TG by LPL. As Intralipid contains free glycerol as an excipient, it has been reported that it overestimates the true TG concentrations of Intralipid (Howdieshell *et al.*, 1995). Therefore, all Intralipid-TG measurements were corrected for free glycerol and are reported as 'true' TG concentrations [true TG concentration ($mmol.l^{-1}$) = measured TG ($mmol.l^{-1}$) - glycerol ($mmol.l^{-1}$)]. Glycerol concentrations were also measured in 5 subjects in the $VLDL_1$ fraction during infusion and were found to be negligible (influencing $VLDL_1$-TG concentrations by less than 1%).

3.2.8 Kinetic Data Calculations

The clearance rates of Intralipid-TG and production rates of $VLDL_1$-TG and -apoB were calculated as described below using examples from individual subjects.

3.2.8.1 Calculating $VLDL_1$-TG and $VLDL_1$-ApoB Production Rates

The production rates ($mg.h^{-1}$) of $VLDL_1$-TG and -apoB were calculated from the gradient of the linear rise in their pools (total mg in plasma) over time (min) multiplied by 60 min. Pool sizes (mg) were calculated as concentrations in $mg.dl^{-1}$ multiplied by plasma volume [4% of body mass (Packard *et al.*, 1984)] in decilitres.

Figure 3.2A represents the linear increase in TG pool (mg) in the $VLDL_1$ fraction of subject No.3 (female, 55 y, 84.5 kg) with R^2 (goodness-of-fit) value of 0.97 and a gradient of 35.8. Thus, the $VLDL_1$-TG production rate of this subject would be (35.8 × 60) 2147.4 $mg.h^{-1}$ ($609.9\ mg.kg^{-1}.d^{-1}$). Similarly, from **Figure 3.2B**, the $VLDL_1$-apoB production rate of the same subject was (0.85 × 60) 50.9 $mg.h^{-1}$ ($14.5\ mg.kg^{-1}.d^{-1}$).

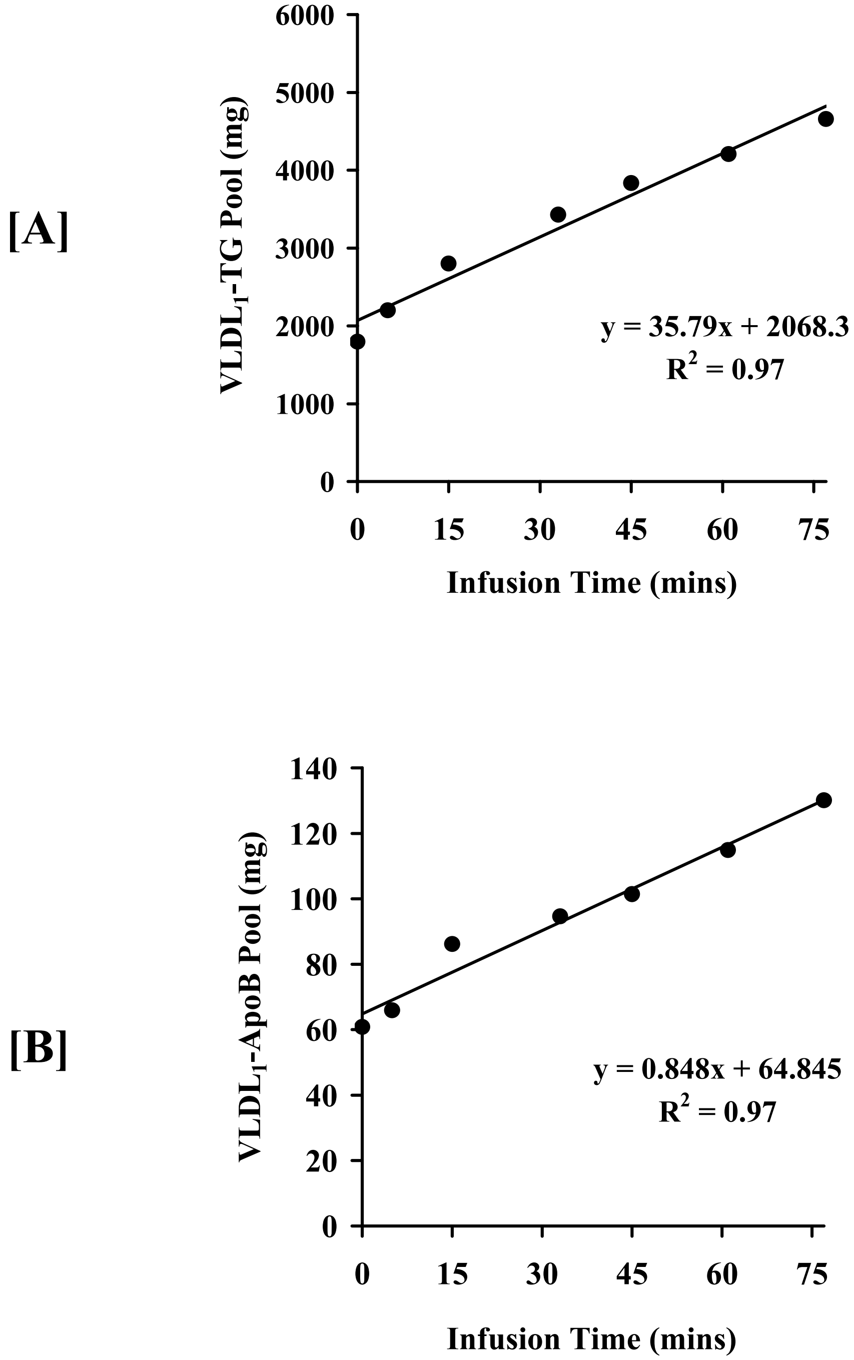


Figure 3.2: Changes in [A] $VLDL_1$-TG and [B] $VLDL_1$-apoB pools (mg) over time in a female subject No. 3 (55 yr, 84.5 kg) during infusion of 10% Intralipid (0.1 $g.kg^{-1}.h^{-1}$). An initial bolus dose of 20% Intralipid (0.1 $g.kg^{-1}$ body mass) was given at 0 min.

3.2.8.2 Calculating VLDL$_1$-TG and VLDL$_1$-ApoB Fractional Synthetic and Catabolic Rates

The fractional synthetic rate (FSR) is defined as the rate of incorporation of a precursor into a product per unit of product mass (Foster *et al.*, 1993), which can be calculated as:

$$\text{FSR} = \frac{\text{initial rate of change in product}}{\text{initial precursor concentration}} \qquad (Eq.\ 1)$$

In this method, the FSR of VLDL$_1$-TG and -apoB (pools.d^{-1}) can be calculated from the gradient of the linear rise in their pool (mg) over time (min) divided by fasting pool size (mg) then multiplied by 60 and 24 (to account for number of minutes in 1 day). However, since the system is in a steady state in fasting conditions, where synthesis of VLDL$_1$ equals its clearance, FSR will also be equivalent to the fractional clearance rate (FCR) in the fasted state (Foster *et al.*, 1993).

Subject No. 3 had a VLDL$_1$-TG and -apoB fasting pool sizes of 1669.5 and 61.7 mg, respectively. According to the equation of the line in **Figure 3.2A**, the subject's VLDL$_1$-TG FSR and FCR were (35.8 ÷ 1669.5 × 60 × 24) 30.9 pools.d^{-1}. Similarly, from **Figure 3.2B**, VLDL$_1$-apoB FSR and FCR were 19.8 pools.d^{-1}.

3.2.8.3 Calculating Intralipid-TG Clearance Rate

Assuming that all TG clearance is Intralipid-TG clearance, it is possible to determine Intralipid-TG clearance rate in two ways:

3.2.8.3.1 The Steady-State (SS) Method

The clearance rate of Intralipid-TG can be calculated from the steady-state concentration during infusion using the following equation (Rang *et al.*, 2003):

$$\text{Clearance rate (ml.min}^{-1}\text{)} = \frac{\text{infusion rate (mg.min}^{-1}\text{)}}{\text{steady state concentration (mg.ml}^{-1}\text{)}} \qquad (Eq.\ 2)$$

In this method, steady state is defined as being achieved when the final 3 values of the Intralipid-TG concentrations differed by less than 13.8% (i.e. two times the CV for the separation of the Intralipid fraction and measurement of the TG. This represents the 95% confidence interval for the measured value). **Figure 3.3A** shows the concentration of Intralipid-TG ± 13.8% during a 75-min infusion for subject No.8 (female, 23 y, 52.0 kg, BMI 20.8 $kg.m^{-2}$). The steady state concentration, i.e. the mean of the last 3 points of infusion, is 0.86 $mmol.l^{-1}$ (0.76 $mg.ml^{-1}$). Since this subject's body mass is 52.0 kg, the infusion rate of 0.1 g of 10% Intralipid per kg of body mass per h equals:

$$\frac{0.1\ g.h^{-1}}{kg} \times 52.0\ kg = 5.2\ g.h^{-1} = 5200\ mg.h^{-1} = 86.67\ mg.min^{-1}$$

From equation (2):

$$\text{Clearance rate} = \frac{86.67\ mg.min^{-1}}{0.76\ mg.ml^{-1}} = 113.7\ ml.min^{-1}$$

Assuming plasma volume [4% of body mass, (Packard *et al.*, 1984)] for this subject was 2080 ml, the clearance rate can also be expressed in $pools.d^{-1}$ as follows:

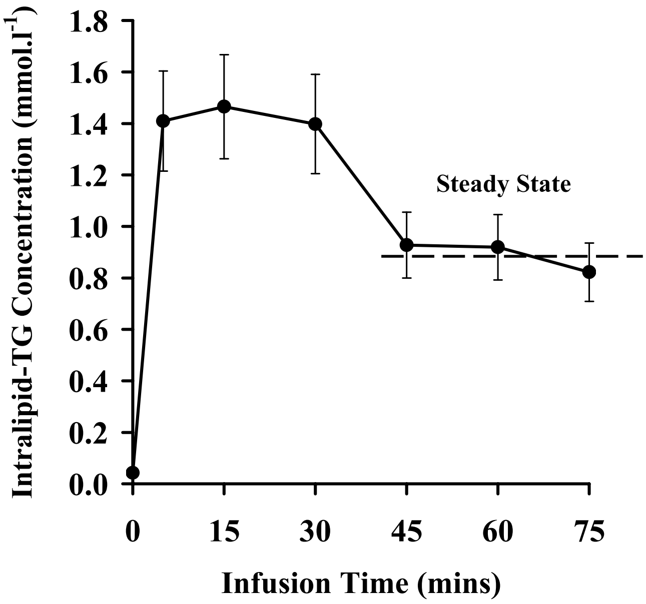
$$\text{Intralipid-TG clearance rate} = \frac{113.7\ ml.min^{-1}}{2080\ ml} \times 60\ min \times 24\ h = 78.9\ pools.d^{-1}$$

3.2.8.3.2 The Exponential (Exp) Method

After stopping the intravenous infusion, Intralipid-TG declines exponentially following first-order kinetics as described by Rössner (Rossner, 1974). **Figure 3.3B** shows the Intralipid-TG concentrations ($mmol.l^{-1}$) post-infusion over time (min) curve for the same female subject (No.8) on a semi-log scale. (In this case, data for the first 45 min post-infusion were used for calculation, as beyond this Intralipid-TG concentrations approached zero increasing the error associated with an exponential curve fit). The equation of the fitted line is:

$$y = k\, e^{-bt} \qquad (\textit{Eq}.\ 3)$$

[A]



[B]

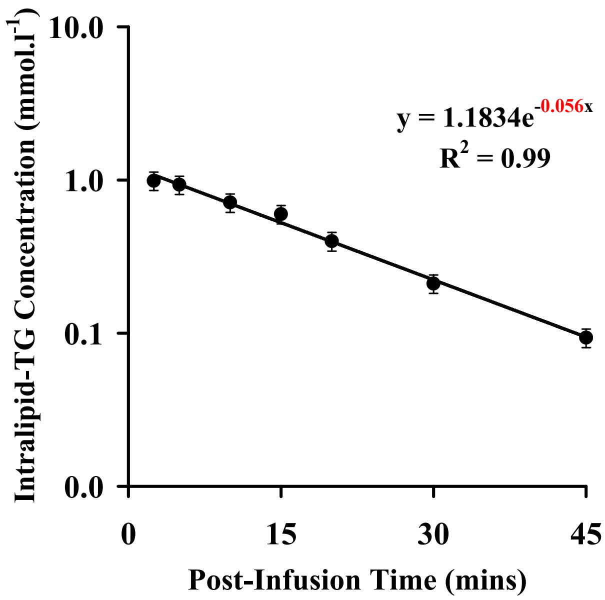


Figure 3.3: Intralipid-TG concentrations (mmol.l^{-1}) [A] during infusion and [B] post-infusion of 0.1 g.kg^{-1}.h^{-1} of 10% Intralipid for a female subject (23 y, 52.0 kg). An initial bolus dose of 20% Intralipid (0.1 g.kg^{-1} body mass) was given at 0 min. The Y-axis error bars are ± 13.8% (i.e. the 95% confidence interval for the measured Intralipid-TG concentration).

where k is the proportionality constant, t is the time, and b is the exponential decay constant, which in turn is defined as:

$$b = \frac{\text{clearance rate (ml.min}^{-1})}{\text{plasma distribution volume (ml)}} \qquad (Eq.\ 4)$$

Hence,

$$\text{Clearance rate (ml.min}^{-1}) = b \times \text{plasma volume (ml)} \qquad (Eq.\ 5)$$

or $\text{in pools.d}^{-1} = b \times 60 \text{ min} \times 24 \text{ h}$

Since the b value from **Figure 3.3B** is 0.056 and the subject's plasma volume was 2080 ml, then from equation (5):

$$\text{Intralipid-TG clearance rate} = 0.056 \times 2080 \text{ ml} = 116.5 \text{ ml.min}^{-1}$$

$$\textit{or} \quad = b \times 60 \text{ min} \times 24 \text{ h} = 80.6 \text{ pools.d}^{-1}$$

Figure 3.4 shows a schematic diagram of the Intralipid method protocol. (For comparison purposes with the stable-isotope method see **Figure 1.7**).

3.2.9 Intralipid Recovery

In order to assess the recovery of plasma-Intralipid in the Intralipid fraction (S_f >400), EDTA plasma was spiked with Intralipid to produce an Intralipid-TG concentration in plasma of ~1.5 $mmol.l^{-1}$ and ~4 $mmol.l^{-1}$. These reflect approximate Intralipid-TG concentrations at the 0.1 $g.kg^{-1}.h^{-1}$ and 0.2 $g.kg^{-1}.h^{-1}$ infusion doses. For each Intralipid concentration, samples of spiked plasma were divided into 10 aliquots and the Intralipid fractions were separated as described in section 3.2.4. TG and glycerol concentrations were measured in plasma before and after addition of the Intralipid (to calculate the actual Intralipid-TG concentration), as well as in the separated Intralipid fractions. The Intralipid recovery was calculated as follows:

$$\%\ \text{recovery} = \frac{\text{separated Intralipid-TG}}{\text{actual Intralipid-TG}} \times 100 \qquad (Eq.\ 6)$$

where actual Intralipid-TG = total TG (plasma with Intralipid) – TG (Intralipid-free plasma).

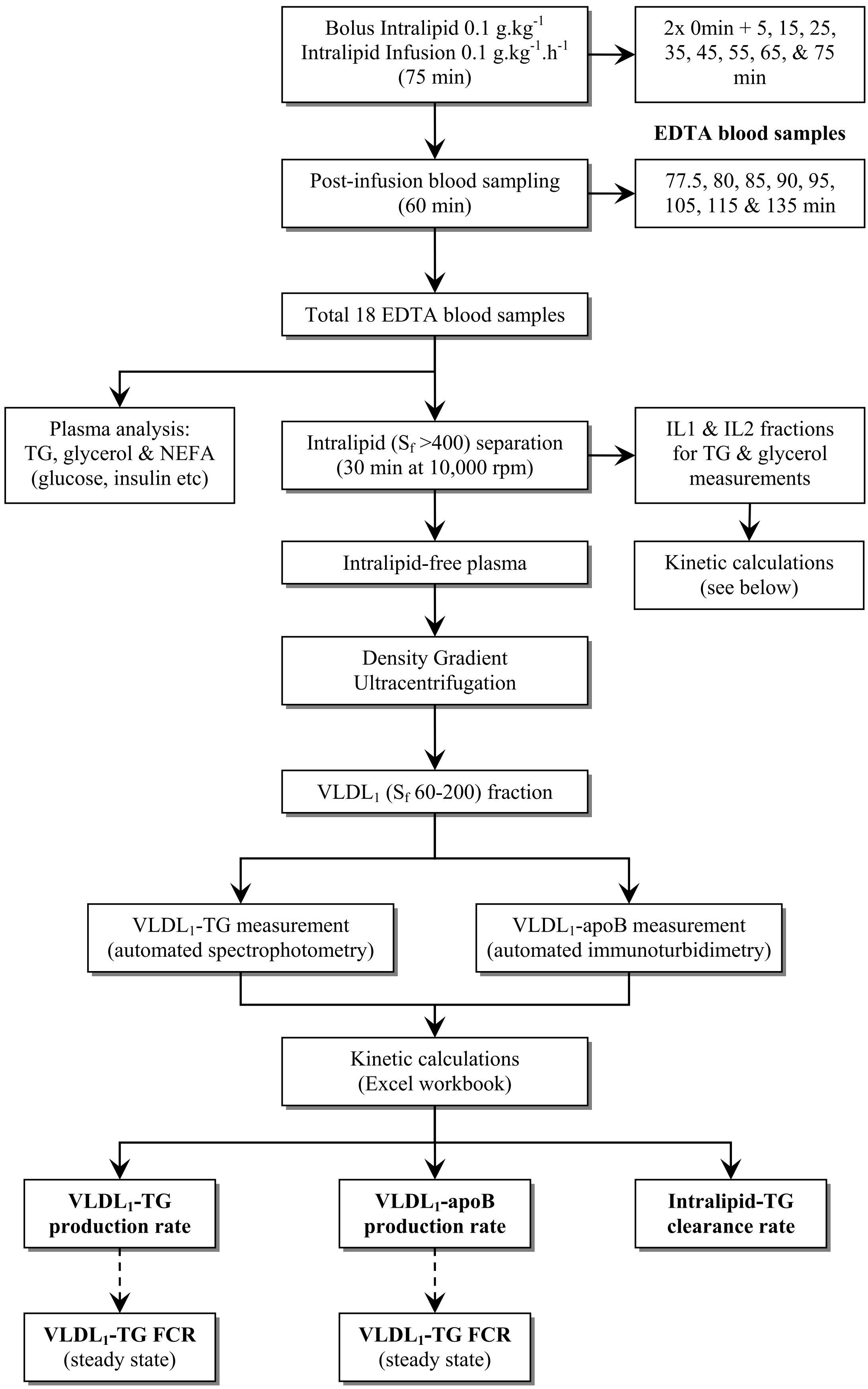


Figure 3.4: Schematic diagram of the 'Intralipid Method' protocol

3.2.10 Statistical Analyses

Statistical analyses were performed using MINITAB for Windows (Version 13.1, MINITAB Inc., State College, PA) and STATISTICA (Release 6.0, StatSoft, Inc, USA). Normality was checked for all the data using the Anderson-Darling test. When data did not approximate a normal distribution, these were log-transformed, specifically TG, glucose, insulin, $HOMA_{IR}$, production rates of $VLDL_1$-TG (expressed in both $mg.h^{-1}$ and $mg.kg^{-1}.d^{-1}$) and $VLDL_1$-apoB (expressed in $mg.h^{-1}$), Intralipid-TG clearance rate and $VLDL_1$-apoB FSR required transformation. Time trends were tested using one-way ANOVA with repeated measures. Paired *t*-tests were used to compare between the Intralipid-TG clearance rates calculated from the steady state and the exponential decay and between the kinetic data obtained from the low and high Intralipid doses. HOMA [calculated as insulin ($mU.l^{-1}$) × glucose ($mmol.l^{-1}$)/22.5] was used as a validated surrogate measure of insulin resistance (Matthews *et al.*, 1985). Relationships between HOMA-estimated insulin resistance ($HOMA_{IR}$), NEFA, BMI, waist circumference and kinetic parameters were assessed using Pearson product-moment correlations. Significance was accepted at the $P < 0.05$ level. Data are presented as mean ± SEM unless otherwise stated. It was not possible to perform a power calculation for this study as the method was still in the developmental stage and thus no data on the reliability of the method were available at this stage.

3.3 Results

3.3.1 Plasma, Intralipid, $VLDL_1$- and $VLDL_2$-TG Concentrations During and Post-Infusion

Figure 3.5A shows the mean plasma-, Intralipid-, $VLDL_1$- and $VLDL_2$-TG responses in 10 subjects during 75-min Intralipid infusion (0.1 $g.h^{-1}.kg^{-1}$ body mass). Plasma-TG concentrations were increased to ~2-3 times the fasting value during the infusion. Similarly, mean Intralipid-TG concentrations increased in response to the bolus dose ($P < 0.001$). $VLDL_1$-TG concentrations rose linearly during the infusion ($P < 0.001$), but $VLDL_2$-TG did not change significantly during the course of the infusion ($P = 0.14$).

After stopping the infusion, the plasma- and Intralipid-TG concentrations decreased exponentially (both *P* <0.001, **Figure 3.5B**). VLDL$_1$-TG continued to rise for ~20 min (*P* <0.001) before plateauing and subsequently decreasing. In subjects where the post-infusion period was extended, VLDL$_1$-TG returned to baseline concentrations within 105-135 min (an example of an earlier Intralipid trial is shown in **Figure 3.6**). The mean VLDL$_2$-TG concentrations remained unchanged for the 75 min post-infusion observation period.

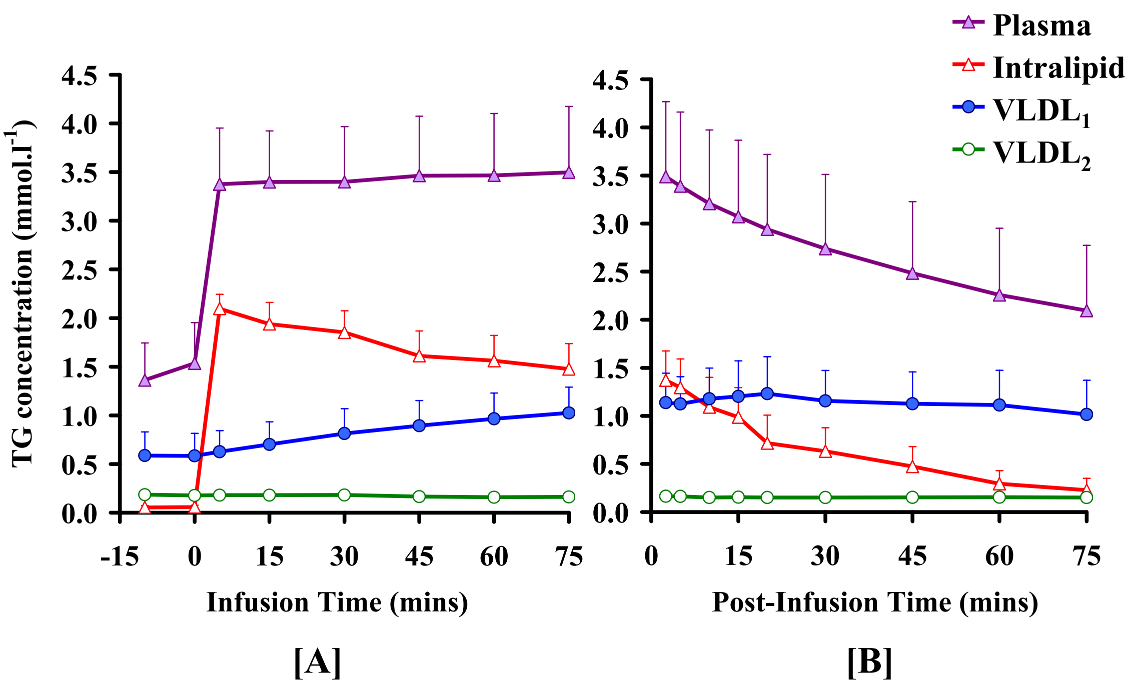


Figure 3.5: Plasma-, Intralipid-, VLDL$_1$- and VLDL$_2$-TG concentrations (mmol.l^{-1}) [A] during infusion of 0.1 g.kg^{-1}.h^{-1} of 10% Intralipid and [B] for 75 min post-infusion. An initial bolus dose of 20% Intralipid (0.1 g.kg^{-1} body mass) was given at 0 min. N = 10, values are mean ± SEM.

3.3.2 VLDL$_1$- and VLDL$_2$-ApoB Concentrations During Infusion

The mean apoB concentration in the VLDL$_1$ (S$_f$ 60-400) fraction increased steadily from fasting levels throughout the infusion and was significantly higher than baseline within 15 min (*P* <0.001). On the other hand, mean VLDL$_2$-apoB concentrations declined significantly (*P* <0.001) during 75 min of infusion. Total (VLDL$_1$ + VLDL$_2$) VLDL-apoB concentrations rose slightly but significantly (*P* <0.05) during infusion (**Figure 3.7**). There was no significant change in the VLDL$_1$ or VLDL$_2$ TG/apoB ratio (expressed in mol:mol) over the 75 min of infusion (*P* = 0.21 and *P* = 0.16, respectively) (**Figure 3.8**).

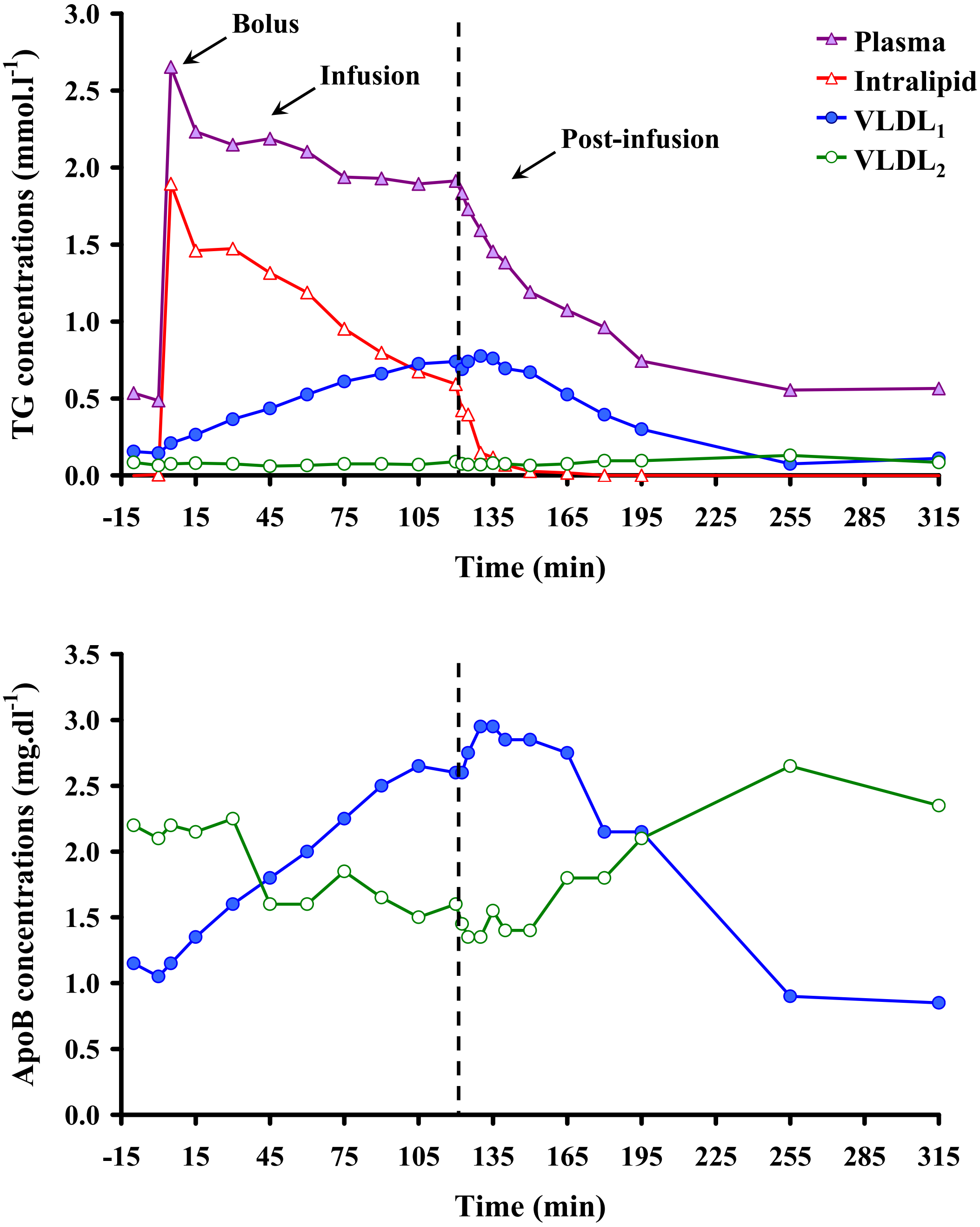


Figure 3.6: An example of a full 135min-Intralipid trial during development of the method. [Top] Plasma-, Intralipid-, VLDL$_1$- and VLDL$_2$-TG and **[Bottom]** VLDL$_1$- and VLDL$_2$-apoB concentrations during and post-infusion for a female subject (34 y, 55.2 kg). A bolus dose of 20% Intralipid (0.1 g.kg^{-1} body mass) was injected at zero min, followed immediately by a constant infusion of 10% Intralipid (0.1 g.kg^{-1}.h^{-1}) for 120 min. The dotted line represents the end of infusion. VLDL$_1$ kinetics for this subject were calculated from the first 75 min of infusion: VLDL$_1$-TG and VLDL$_1$-apoB production rates were 711.2 and 20.7 mg.h^{-1} and fractional catabolic rates were 56.3 and 20.0 pools.d^{-1}, respectively. Intralipid-TG clearance rate was calculated only from its exponential decay post-infusion as 146.9 pools.d^{-1}, as no steady state was achieved during infusion.

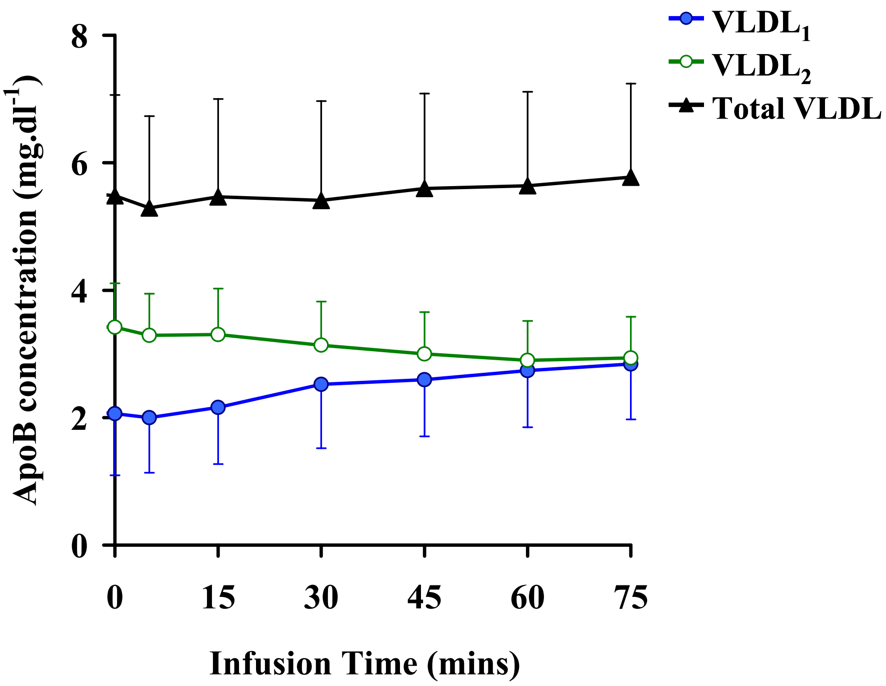


Figure 3.7: $VLDL_1$-, $VLDL_2$- and total VLDL-apoB concentrations ($mg.dl^{-1}$) during infusion of 10% Intralipid ($0.1\ g.kg^{-1}.h^{-1}$). An initial bolus dose of 20% Intralipid ($0.1\ g.kg^{-1}$ body mass) was given at 0 min. N = 10, values are mean ± SEM.

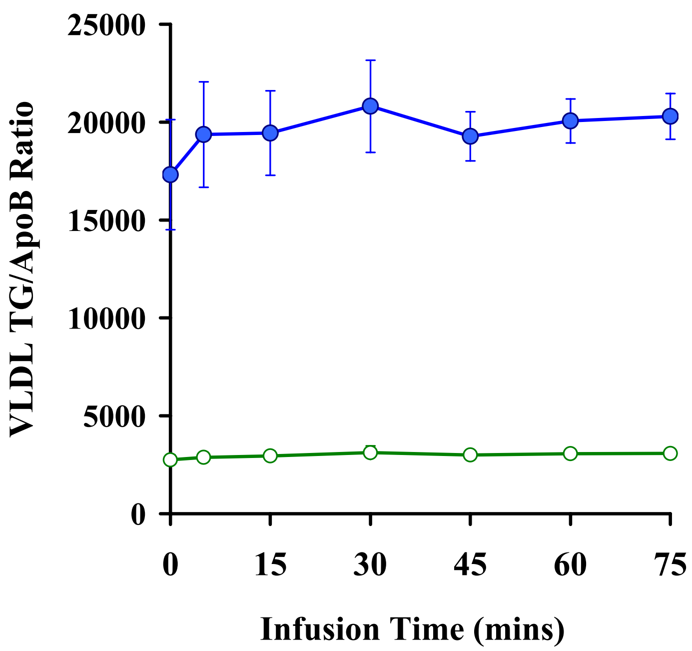


Figure 3.8: $VLDL_1$- and $VLDL_2$-TG/apoB ratios during infusion of 10% Intralipid ($0.1\ g.kg^{1}.h^{-1}$). An initial bolus dose of 20% Intralipid ($0.1\ g.kg^{-1}$ body mass) was given at 0 min. N = 10, values are mean ± SEM.

3.3.3 Kinetic Data

Table 3.2 shows the production rates and FSR of $VLDL_1$-TG and $VLDL_1$-apoB as well as the clearance rates of Intralipid-TG calculated for each subject (n = 10) as previously described. Fasting $VLDL_1$-TG and $VLDL_1$- and $VLDL_2$-apoB concentrations are also presented.

3.3.3.1 $VLDL_1$-TG and $VLDL_1$-ApoB Production Rates

The mean ± SEM (range) production rates for $VLDL_1$-TG and -apoB were 1076.7 ± 224.7 (446.0 – 2563.2) $mg.h^{-1}$ and 25.4 ± 3.9 (12.0 – 50.9) $mg.h^{-1}$, respectively. These corresponded to 333.6 ± 49.1 (198.5 – 609.9) $mg.kg^{-1}.d^{-1}$ and 8.1 ± 0.9 (4.4 – 14.5) $mg.kg^{-1}.d^{-1}$, respectively.

3.3.3.2 $VLDL_1$-TG and $VLDL_1$-ApoB Fractional Synthetic and Catabolic Rates

The mean ± SEM $VLDL_1$-TG and -apoB FSRs, which are equal to the $VLDL_1$-TG and -apoB FCRs in the fasted state (Foster *et al.*, 1993), were 30.2 ± 5.7 (6.5 – 57.8) $pools.d^{-1}$ and 21.1 ± 5.1 (2.2 – 56.2) $pools.d^{-1}$, respectively.

3.3.3.3 Intralipid-TG Clearance Rate

The Intralipid-TG clearance rates calculated for individual subjects by two methods as described above (i.e. SS and Exp) are shown in **Table 3.2**. Eight out of the 10 subjects reached the defined steady state during infusion. The mean ± SEM Intralipid-TG clearance rates in these 8 subjects did not differ significantly between the two calculation methods (52.4 ± 8.6 $pools.d^{-1}$ for SS *vs* 55.3 ± 9.2 $pools.d^{-1}$ for Exp, $P = 0.45$) and the values obtained were strongly correlated ($r = 0.96$, $P < 0.001$). However, since not all subjects reached a steady state, the Intralipid-TG clearance rates mentioned hereafter will refer to that calculated using the exponential method.

Table 3.2: Fasting concentrations, apoE phenotypes and individual lipoprotein kinetic parameters calculated using the 'Intralipid method' (0.1 $g.kg^{-1}.h^{-1}$ infusion dose) (n=10).

Subjects	**Fasting Concentrations**			**Production Rates**				**Intralipid-TG Clearance Rate**		**FSR**[§]	
(apoE phenotype)	**$VLDL_1$-TG**	**$VLDL_1$-ApoB**	**$VLDL_2$-ApoB**	**$VLDL_1$-TG**		**$VLDL_1$-ApoB**		**SS**	**Exp**	**$VLDL_1$-TG**	**$VLDL_1$-ApoB**
	$mmol.l^{-1}$	*$mg.dl^{-1}$*	*$mg.dl^{-1}$*	*$mg.h^{-1}$*	*$mg.kg^{-1}.d^{-1}$*	*$mg.h^{-1}$*	*$mg.kg^{-1}.d^{-1}$*	*$pools.d^{-1}$*	*$pools.d^{-1}$*	*$pools.d^{-1}$*	*$pools.d^{-1}$*
1 (3/3)	0.76	0.8	1.6	625.0	241.1	17.1	6.6	**	79.8	30.2	22.0
2 (3/2)	0.83	1.1	2.9	719.9	214.6	14.8	4.4	82.6	66.1	25.8	10.3
3 (3/2)	1.81	1.8	6.8	2147.4	609.9	50.9	14.5	56.9	56.4	30.9	19.8
4 (4/3)	0.40	0.8	0.9	642.3	235.3	12.0	4.4	72.8	96.5	57.8	13.7
5 (3/3)	0.54	1.1	2.2	711.2	309.2	20.7	9.0	**	146.9	56.3	20.0
6 (3/3)	4.43	10.6	7.7	2563.2	593.2	40.2	9.3	17.3	18.1	6.5	2.2
7 (3/3)	2.15	3.8	3.7	1228.1	406.5	24.9	8.2	39.8	40.5	9.3	5.4
8 (3/3)	0.65	0.3	3.0	446.0	205.8	16.1	7.4	78.7	80.6	44.7	56.2
9 (3/3)	1.04	1.0	3.0	885.5	322.0	24.1	8.8	43.4	54.3	22.6	21.9
10 (3/3)	1.03	0.5	2.9	798.2	198.5	33.0	8.2	27.2	29.5	17.4	39.1

Exp, exponential decay; **FCR**, fractional catabolic rate; **FSR**, fractional synthetic rate; **SS**, steady state.
Subjects 3, 5 and 8 were female subjects.
** Subject did not reach a steady state.
§ As FSR equals FCR in under steady state conditions, the $VLDL_1$-TG and -apoB FSR values are equal to the FCR values in the fasted state.

3.3.4 Effect of Increasing the Intralipid Infusion Rate

For the 5 subjects who underwent Intralipid infusion at the Low (0.1 $g.kg^{-1}.h^{-1}$) and the High (0.2 $g.kg^{-1}.h^{-1}$) doses, there were no significant differences in the mean $VLDL_1$-apoB production rates (Low dose: 23.8 ± 2.8 *vs* High dose: 22.0 ± 1.9 $mg.h^{-1}$, $P = 0.21$) or $VLDL_1$-TG production rates (Low dose: 813.8 ± 127.0 *vs* High dose: 960.9 ± 136.8 $mg.h^{-1}$, $P = 0.10$) between both doses, although there was a tendency for the $VLDL_1$-TG production rate to be higher at the High Intralipid dose. However, it was observed that separation of the large amount of Intralipid from plasma at the higher 0.2 $g.kg^{-1}.h^{-1}$ dose was technically quite difficult and it was suspected that the $VLDL_1$ fraction in some samples may have become slightly contaminated with Intralipid at this dose (**Figure 3.9**). This suggestion is supported by the substantially lower recovery of Intralipid in the Intralipid fraction at high Intralipid concentrations (see below). Calculated FSRs for $VLDL_1$-TG (Low dose: 30.1 ± 8.8 *vs* High dose: 30.4 ± 8.1 $pools.d^{-1}$, $P = 0.94$) and $VLDL_1$-apoB (Low dose: 28.5 ± 8.7 *vs* High dose: 33.4 ± 14.6 $pools.d^{-1}$, $P = 0.64$) did not differ between the two doses. In addition, FSRs for $VLDL_1$-TG ($r = 0.88$, $P = 0.05$) and $VLDL_1$-apoB ($r = 0.95$, $P = 0.01$) between the two doses correlated highly with each other and, when plotted, followed the line-of-equality (**Figure 3.10**).

Furthermore, although a formal reproducibility test was not performed, the repeated two doses provided an opportunity to estimate the test/re-test reproducibility of $VLDL_1$-TG and -apoB production rates. Based on data from these 5 subjects, the within-subject CV for $VLDL_1$-TG production was 20.1% and for $VLDL_1$-apoB production was 12.7%.

3.3.5 Intralipid Recovery

Recovery of the Intralipid-TG in the Intralipid (S_f >400) fraction was 95 ± 7% (mean ± SD) for the 1.5 $mmol.l^{-1}$ Intralipid-TG concentrations and 71 ± 4% for the ~4.0 $mmol.l^{-1}$ Intralipid-TG concentrations.

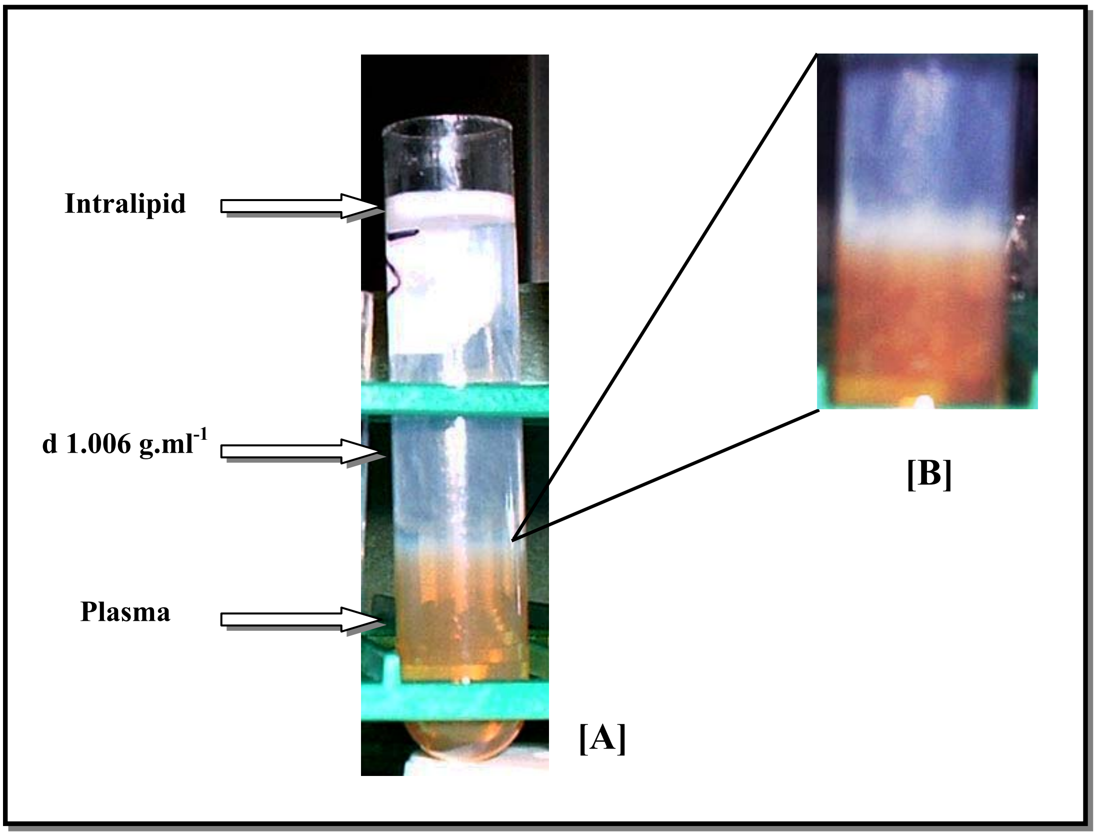


Figure 3.9: [A] An example of Intralipid separation in the High dose of Intralipid infusion (0.2 $g.kg^{-1}.h^{-1}$). Intralipid separation is done by overlayering 2 ml of plasma with 4 ml of density solution 1.006 $g.ml^{-1}$ and centrifuged at 10,000 rpm for 30 min. [B] A magnification of the bottom of the tube showing a thin layer of possible contamination of Intralipid at the top of plasma due to the high concentration of Intralipid.

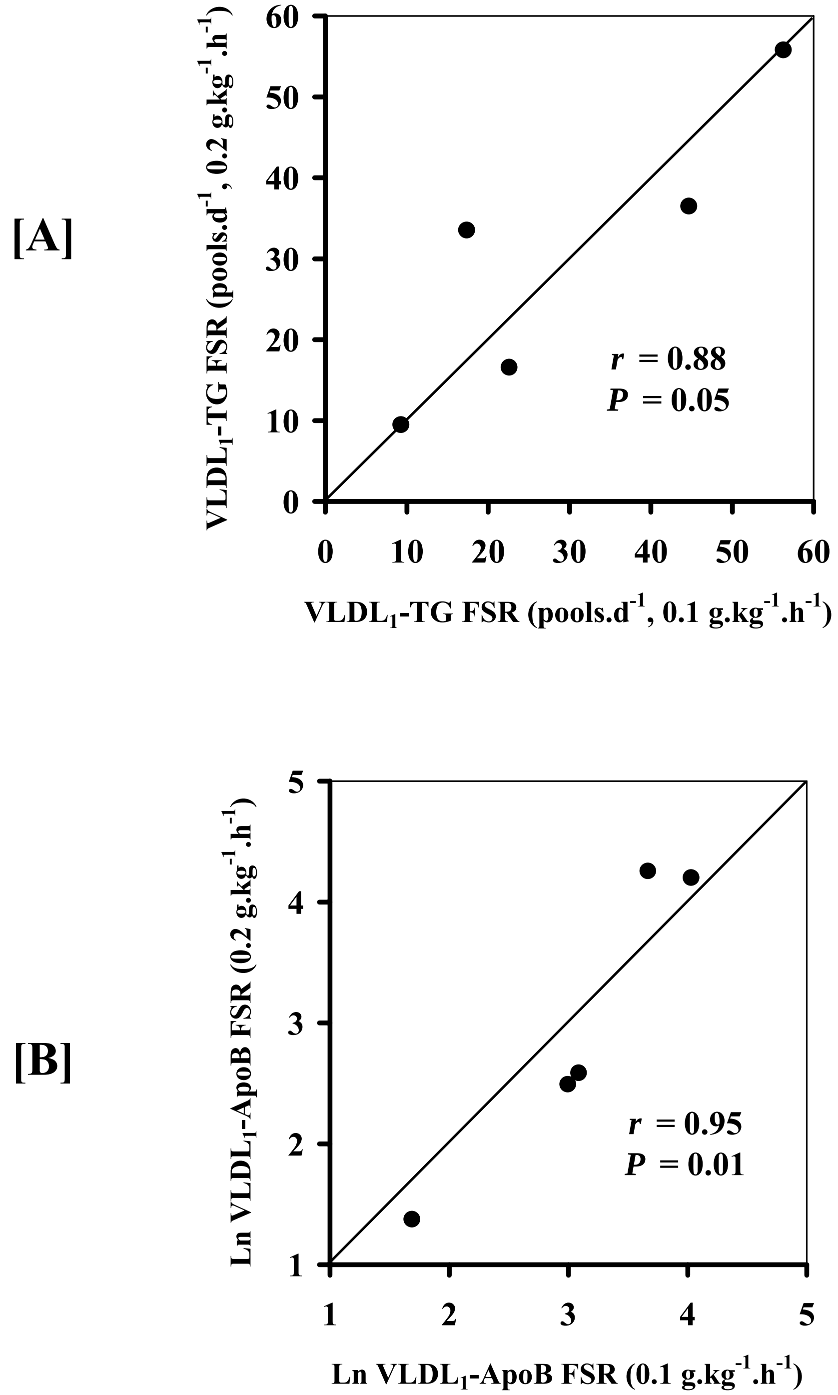


Figure 3.10: Scatterplots (with the line of equality) illustrating the agreement between [A] $VLDL_1$-TG FSR (pools.d^{-1}) and [B] Ln $VLDL_1$-apoB FSR between the Low (0.1 g.kg^{-1}.h^{-1}) and High (0.2 g.kg^{-1}.h^{-1}) doses. FSR is equivalent to FCR in the fasted state. N = 5, *r* and *P* values are for Pearson product-moment correlations between variables. $VLDL_1$-apoB FSR was expressed in pools.d^{-1} prior to log-transformation.

3.3.6 Interrelationships between $VLDL_1$-TG and $VLDL_1$-apoB Production and TG Clearance

Intralipid-TG clearance rate and $VLDL_1$-TG and -apoB production rates (expressed in $mg.h^{-1}$) were significantly inter-related (**Figure 3.11**) with the expected negative correlation between Intralipid-TG clearance and $VLDL_1$-TG (r = -0.67, P = 0.04) and $VLDL_1$-apoB (r = -0.69, P = 0.03) production rates and a positive correlation between $VLDL_1$-TG and -apoB production rates (r = 0.85, P = 0.002). There was also a very strong relationship between $VLDL_1$-TG FSR (which equals the $VLDL_1$-TG FCR in the fasted state) and Intralipid-TG clearance rate (r = 0.90, P < 0.005). The positive correlation between $VLDL_1$-TG and -apoB production rates remained significant between production rates when values were expressed in $mg.kg^{-1}.d^{-1}$ (r = 0.73, P = 0.02). However, the relationships between $VLDL_1$-TG production rate expressed in $mg.kg^{-1}.d^{-1}$ and Intralipid-TG clearance (r = -0.46, P = 0.18) and between $VLDL_1$-apoB production rate expressed in $mg.kg^{-1}.d^{-1}$ and Intralipid-TG clearance (r = -0.28, P = 0.44) were not statistically significant.

3.3.7 Relationships between Kinetic Variables and Subjects Characteristics

Figure 3.12 shows the relationships between the calculated kinetic variables and subjects characteristics, with $VLDL_1$-TG and -apoB production rates expressed in $mg.h^{-1}$. $VLDL_1$-TG and -apoB production rates ($mg.h^{-1}$) correlated strongly and significantly with waist circumference and fasting TG concentration. $VLDL_1$-TG production rate also correlated significantly with $HOMA_{IR}$. Similarly, Intralipid-TG clearance rate was significantly and inversely correlated with waist circumference, fasting TG concentrations and $HOMA_{IR}$. In addition, BMI correlated significantly and positively with $VLDL_1$-TG (r = 0.83, P = 0.003) and $VLDL_1$-apoB (r = 0.81, P = 0.004) production rates and inversely with Intralipid-TG clearance rate (r = -0.60, P = 0.07). Fasting NEFA concentrations were not significantly correlated with any of the kinetic variables. The relationships between $VLDL_1$-TG and -apoB production rates expressed in $mg.kg^{-1}.d^{-1}$ units with BMI, waist circumference, fasting TG concentration and $HOMA_{IR}$ are shown in **Table 3.3**. The correlations between $VLDL_1$-TG production and all of these variables remained strong and statistically significant, however the correlations between $VLDL_1$-apoB production rate and waist circumference and fasting TG were not statistically significant when the production rates were normalized for body mass.

3.3.8 Relationships between Kinetic Variables and ALT Concentrations

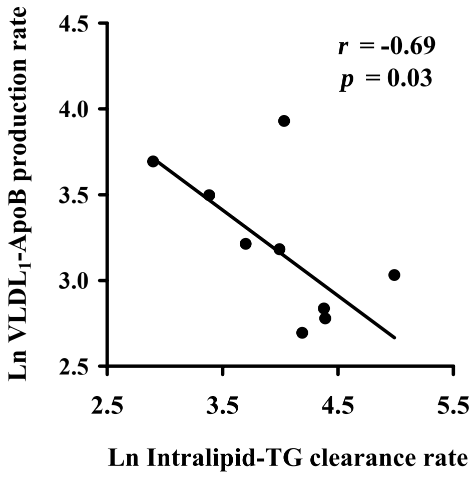
Figure 3.13 shows the relationships between serum ALT concentrations and kinetic variables. Although all subjects had ALT concentrations within the normal range, one subject was on antibiotics shortly prior to participating in the study and was excluded from the following correlations. ALT concentrations correlated significantly with $VLDL_1$-apoB production rate ($r = 0.73$, $P = 0.03$) and Intralipid-TG clearance rate ($r = -0.79$, $P = 0.01$). This indicates that, within normal range, ALT concentrations explained 53% (0.73^2) and 62% (0.79^2) of the variance in $VLDL_1$-apoB production rate and Intralipid-TG clearance rate, respectively. There was a tendency toward a positive correlation with $VLDL_1$-TG production rate, but it did not reach statistical significance ($r = 0.59$, $P = 0.09$).

Table 3.3: Correlations between $VLDL_1$-apoB and -TG production rates (expressed in $mg.kg^{-1}.d^{-1}$) and subjects' characteristics.

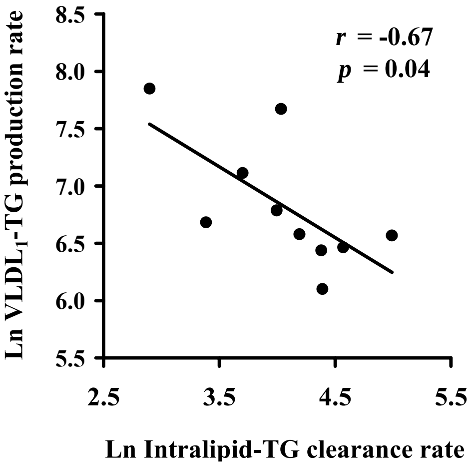
	$VLDL_1$-ApoB Production Rate	**Ln $VLDL_1$-TG Production Rate**
BMI	$r = 0.63$ $P = 0.05$	$r = 0.65$ $P = 0.04$
WC	$r = 0.45$ $P = 0.19$	$r = 0.70$ $P = 0.02$
Ln Fasting TG	$r = 0.54$ $P = 0.11$	$r = 0.79$ $P = 0.01$
Ln $HOMA_{IR}$	$r = 0.40$ $P = 0.25$	$r = 0.79$ $P = 0.01$

BMI, body mass index; **$HOMA_{IR}$**, HOMA-estimated insulin resistance; **WC**, waist circumference. N = 10, *r* and *p* values are for Pearson product-moment correlations between values.

[A]



[B]



[C]

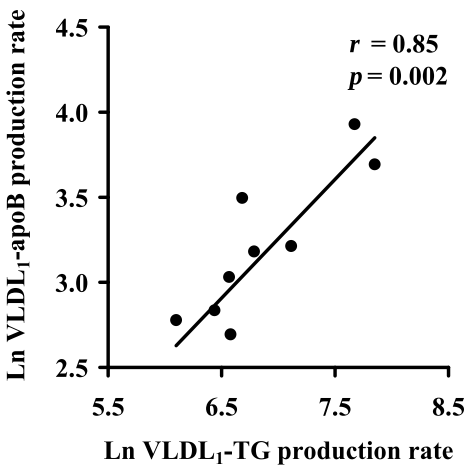


Figure 3.11: Scatterplots (with linear-regression lines of 'best-fit') illustrating the interrelationships between [A] Ln Intralipid-TG clearance rate and Ln $VLDL_1$-apoB production rate, [B] Ln Intralipid-TG clearance rate and Ln $VLDL_1$-TG production rate, and [C] Ln $VLDL_1$-apoB and Ln $VLDL_1$-TG production rates. N = 10, *r* and *P* values are for Pearson product–moment correlations between variables. Production rates and Intralipid-TG clearance rate were expressed in $mg.h^{-1}$ and $pools.d^{-1}$ prior to log-transformation, respectively.

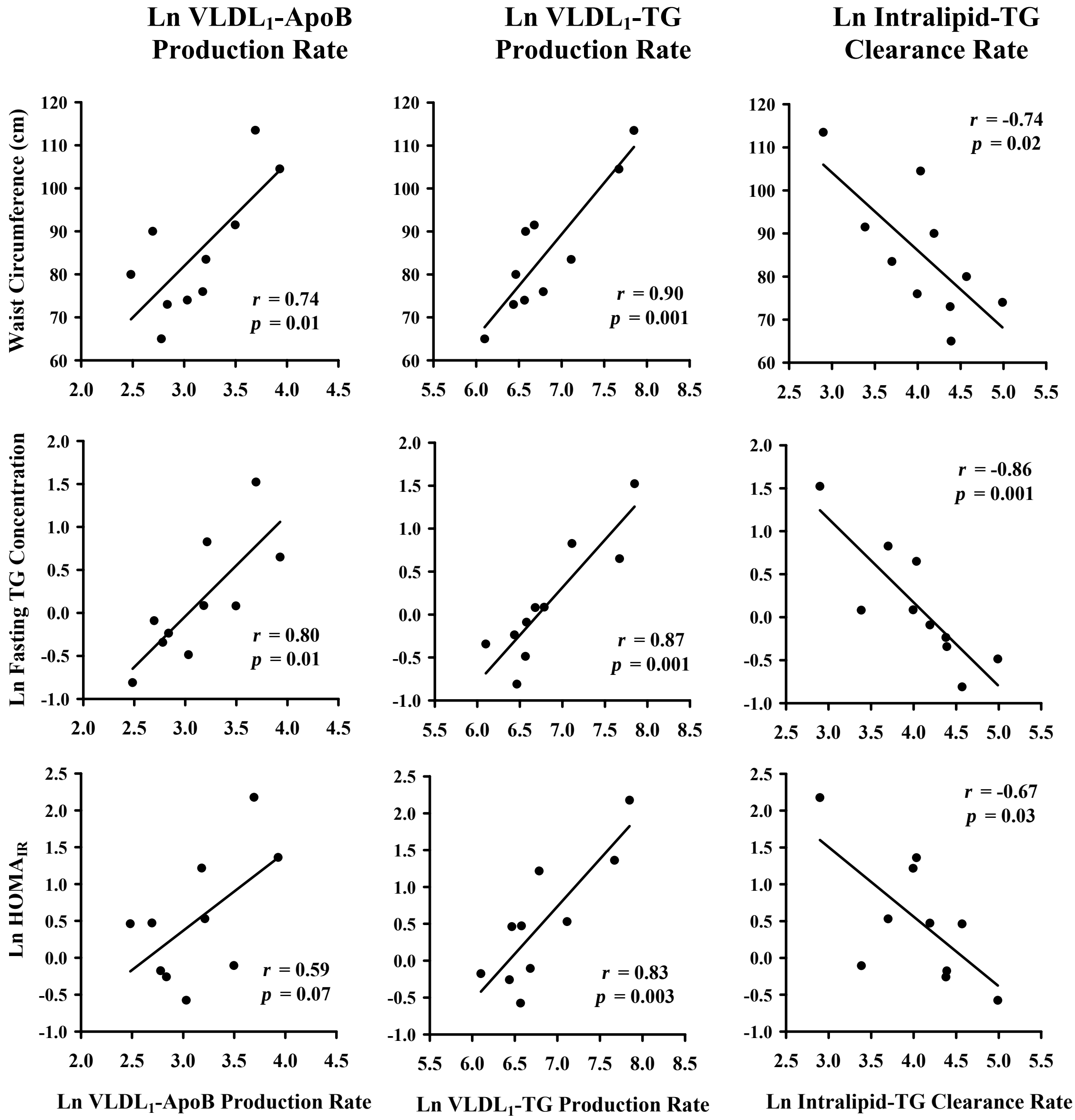
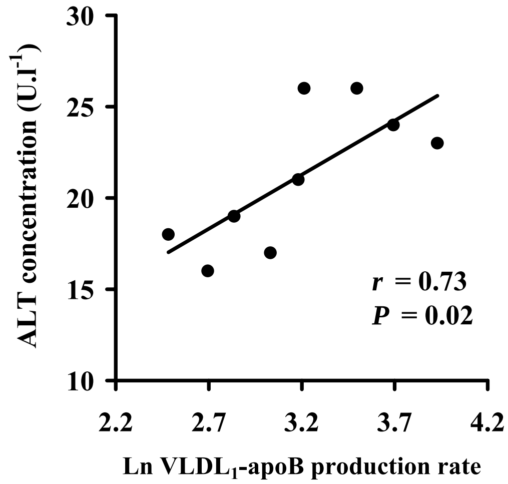
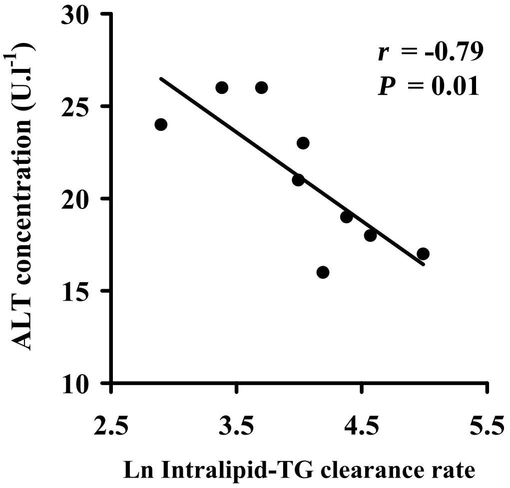


Figure 3.12: Scatterplots (with linear-regression lines of 'best-fit') illustrating the relationships between the kinetic variables [Ln $VLDL_1$-TG production rate (left), Ln $VLDL_1$-apoB production rate (middle) and Ln Intralipid-TG clearance rate (right)] and subjects' characteristics: waist circumference (top), Ln fasting TG concentrations (middle) and Ln HOMA-estimated insulin resistance ($HOMA_{IR}$) (bottom). N = 10, *r* and *p* values are for Pearson product-moment correlations between variables.

[A]



[B]



[C]

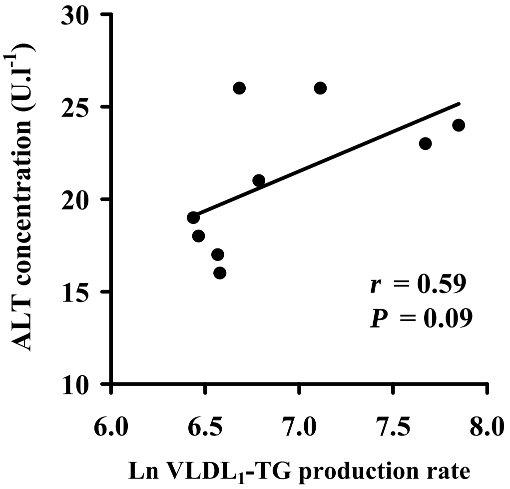


Figure 3.13: Scatterplots (with linear-regression lines of 'best-fit') illustrating the relationships between serum ALT concentration (mU.l^{-1}) and [A] Ln VLDL$_1$-apoB production rate, [B] Ln Intralipid-TG clearance rate and [C] Ln VLDL$_1$-apoB production rate. N = 9, *r* and *P* values are for Pearson product-moment correlations between variables.

3.4 Discussion

In the present study, a relatively straightforward method of determining TRL kinetics was developed. The method relies on the fact that chylomicrons or chylomicron-like particles, such as Intralipid, compete with hepatically-derived large $VLDL_1$ particles for clearance by a common saturable pathway – i.e. hydrolysis of their TG content by LPL (Bjorkegren *et al.*, 1996; Karpe & Hultin, 1995) – and that chylomicrons or chylomicron-like particles are the preferred substrate for LPL (Fisher *et al.*, 1995). Thus, the presence of a sufficient concentration of chylomicrons or chylomicron-like particles in the circulation will almost entirely prevent clearance of $VLDL_1$ by LPL (Bjorkegren *et al.*, 1996) and the rates of $VLDL_1$-TG and -apoB production can therefore be calculated from their rates of rise in concentration. The present work builds on the findings of Björkgren *et al.* who, in studies designed to evaluate the effects of Intralipid infusion on $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) kinetics, found that individual rates of $VLDL_1$-apoB production calculated from the rate of rise of $VLDL_1$-apoB during infusion were virtually identical to those calculated from the 'gold-standard' stable isotope method (see below) (Bjorkegren *et al.*, 1996). The 'Intralipid method' described in the present study enables the determination of the rate of $VLDL_1$-TG (i.e. $VLDL_1$ lipid) and $VLDL_1$-apoB (i.e. $VLDL_1$ particle) production as well as the clearance rate of chylomicron-like particles.

The Intralipid method specifically measures the production rate of large $VLDL_1$ (S_f 60-400) rather than total VLDL (i.e. S_f 20-400). VLDL is a metabolically heterogeneous class of lipoproteins, and it is the larger $VLDL_1$ subclass which competes with chylomicrons/chylomicron-like particles for LPL-mediated clearance and would have its clearance blocked by the presence of Intralipid (Bjorkegren *et al.*, 1996). In contrast, catabolism of the smaller $VLDL_2$ subclass would not be completely blocked by Intralipid as its clearance can occur via the action of hepatic lipase as well as LPL (Packard & Shepherd, 1997). Indeed, as one source of $VLDL_2$ is from catabolism of $VLDL_1$ (the other being direct hepatic production), and this process was blocked by Intralipid infusion, mean $VLDL_2$-apoB concentrations decreased slightly during the infusion although individual responses were more heterogeneous than that observed in $VLDL_1$-apoB; a finding also reported by Björkegren *et al.* (Bjorkegren *et al.*, 1996). This heterogeneity in individual $VLDL_2$-

apoB responses meant that it was not possible to perform any kinetic analyses using the $VLDL_2$ data.

To validate the calculation of $VLDL_1$-TG and -apoB production rates using the 'Intralipid method' it was necessary to consider a number of issues. The first was to determine whether infusing a higher Intralipid dose would influence the calculated $VLDL_1$-TG and -apoB production rates. This was necessary to establish whether the proposed Intralipid infusion dose of 0.1 $g.kg^{-1}.h^{-1}$, was sufficient to saturate LPL and block clearance of $VLDL_1$: if the 0.1 $g.kg^{-1}.h^{-1}$ dose was sufficient, infusing a higher Intralipid dose should not affect calculated production rates. In agreement with the findings of Björkegren *et al.* (Bjorkegren *et al.*, 1996), the calculated $VLDL_1$-apoB production rate in the present study was not changed when a higher, 0.2 $g.kg^{-1}.h^{-1}$ Intralipid infusion dose was used. Similarly, FSRs, which correspond to the FCRs in the fasted state, for $VLDL_1$-TG and -apoB did not differ between the two doses (**Figure 3.10**). Although there was tendency for the calculated $VLDL_1$-TG production rate to be higher with the 0.2 $g.kg^{-1}.h^{-1}$ dose, this was not statistically significant. However, the author feels that the slightly higher apparent $VLDL_1$-TG production rate at the High dose was a methodological, rather than a physiological, issue caused by the difficulty in separating Intralipid at the High dose, leading to the potential contamination of the $VLDL_1$ fraction with Intralipid. This is supported by the fact that Intralipid recoveries at high Intralipid doses were relatively low (71% at an Intralipid concentration of 4 $mmol.l^{-1}$). This contrasts with the near complete recovery of Intralipid at lower Intralipid doses (95% at an Intralipid concentration of 1.5 $mmol.l^{-1}$). This, of course, would not influence the $VLDL_1$-apoB production rate calculations, as Intralipid particles do not contain apoB.

A further issue to consider is whether, following lipolysis by LPL, Intralipid 'remnant' particles may have appeared in the $VLDL_1$ fraction, thereby increasing the measured $VLDL_1$-TG concentration and the apparent $VLDL_1$-TG production rate. However, the author does not believe that this would have had a substantial effect on calculated $VLDL_1$-TG production rates for a number of reasons. Firstly, evidence from the literature suggests that for large TG-rich particles, particularly chylomicron-like particles, lipolysis and particle removal from the plasma is likely to occur simultaneously, rather than by sequential mechanisms (Olivecrona & Olivecrona, 1998; Hultin *et al.*, 1996), with the majority of particles removed from the plasma before conversion to smaller VLDL-sized remnant particles (Karpe *et al.*, 1997). Secondly, as Intralipid contains TG but not apoB, appearance of Intralipid remnants in the $VLDL_1$ fraction would lead to a disproportionate rise in $VLDL_1$-TG compared with $VLDL_1$-apoB, leading to an increase in the $VLDL_1$ TG/apoB ratio. There was no significant increase in this ratio during the infusion (P = 0.21). Thirdly, if the rise in $VLDL_1$-TG was influenced by the appearance of Intralipid remnant particles, then a positive correlation between Intralipid clearance and $VLDL_1$-TG production would be evident (i.e. increased Intralipid clearance would lead to increased $VLDL_1$-TG production). Instead, a negative relationship between Intralipid clearance and $VLDL_1$-TG production (expressed in $mg.h^{-1}$) was observed (i.e. subjects with slow Intralipid clearance also had high $VLDL_1$-TG production) (r = -0.67, P = 0.04). Furthermore, the relationship between $VLDL_1$-apoB production, which would be unaffected by the presence of Intralipid remnant particles, and $VLDL_1$-TG production was very strong, with 71% of the variance in the $VLDL_1$-TG production rate explained by the $VLDL_1$-apoB production rate (r = 0.85, P = 0.002).

Furthermore, it is important to ascertain whether the results obtained are comparable with data obtained using the 'gold standard' stable isotope tracer method. An internal validation of this method was previously undertaken by Björkegren and colleagues (Bjorkegren *et al.*, 1996) in 3 subjects. They reported $VLDL_1$-apoB production rates of 20.0, 25.6 and 7.2 $mg.h^{-1}$ calculated from the Intralipid infusion method with corresponding rates calculated from a stable isotope method of 23.8, 21.6 and 8.0 $mg.h^{-1}$, respectively, indicating that data obtained from the two methods were comparable. In addition, from **Table 3.4**, it is clear that the values for $VLDL_1$-apoB production in the present study are of the same order as those obtained from a number of studies which determined $VLDL_1$-apoB production using staple isotope techniques. Determination of $VLDL_1$-TG production rates using stable isotope tracer methods is technically more difficult than determination of $VLDL_1$-apoB production, and the author is only aware of one group of workers who have evaluated this (Adiels *et al.*, 2005a; Adiels *et al.*, 2006b). The values obtained for $VLDL_1$-TG production in the present study are of the same order as those published by Adiels *et al.* (Adiels *et al.*, 2005a; Adiels *et al.*, 2006b).

This Intralipid method enabled the Intralipid-TG clearance rate to be calculated in two different ways: from the steady state concentration of Intralipid-TG during the infusion, which was defined in the present study as the mean of the final 3 values if these differed by less than 13.8% (i.e. two times the CV for the separation of the Intralipid fraction and measurement of the TG), and from the exponential decrease in Intralipid-TG post-infusion (Rossner, 1974). In subjects where a steady-state Intralipid-TG concentration was achieved, the Intralipid-TG clearance rates calculated from the steady state concentration and from the post-infusion exponential decrease agreed closely (see **Table 3.2**). However, not all subjects achieved a steady-state Intralipid-TG concentration in 75 min of infusion and it is not possible to determine whether a steady-state was achieved for a given subject until sample analysis was completed. Therefore, in practice, it may be easier to use the post-infusion values to determine Intralipid-TG clearance rates, as this ensures that the Intralipid infusion can be kept as short as is necessary to enable calculation of $VLDL_1$-TG and -apoB production rates.

Moreover, the author sought to determine whether this Intralipid method revealed the physiologically expected differences in TRL kinetics between subjects with differing physical and metabolic profiles. As expected, there were strong positive correlations between fasting TG concentrations and $VLDL_1$-TG production rates expressed in either absolute terms or normalized according to body mass and between fasting TG and $VLDL_1$-apoB, production expressed in $mg.h^{-1}$ units, with a strong negative correlation between fasting TG and the Intralipid-TG clearance rates, indicating that those with high TG concentrations exhibited a combination of enhanced $VLDL_1$ production and diminished TG clearance. $VLDL_1$-TG FCR in the fasted state (i.e. with no Intralipid present) was ~45% of the Intralipid-TG clearance rate (30.2 ± 5.7 $pools.d^{-1}$ *vs*. 66.2 ± 11.7 $pools.d^{-1}$, see **Table 3.2**) and there was a very strong correlation between the two variables ($r = 0.90$, $P < 0.0005$), indicating that clearance rates for $VLDL_1$ and chylomicron-like particles within an individual are very tightly linked, consistent with the fact that these particles are cleared by the same pathway. The expected positive correlations between indices of body fatness (waist circumference and BMI) and insulin resistance ($HOMA_{IR}$) and $VLDL_1$-TG and $VLDL_1$-apoB production rates were also observed, in agreement with findings previously reported using stable isotope tracer methods (Gill *et al.*, 2004a). In addition, significant negative relationships were observed between Intralipid-TG clearance and $HOMA_{IR}$ and waist circumference. Thus, the Intralipid method appears to be sensitive enough to detect physiologically relevant differences in TRL kinetics between individuals across the normal and moderately hypertriglyceridaemic range.

Finally, liver fat has been reported to be independently associated with insulin (Westerbacka *et al.*, 2004) and predicted $VLDL_1$ production (Adiels *et al.*, 2006b). It was proposed that insulin resistance induces an increase in the fatty acid flux from adipose tissue to the liver, which leads to the accumulation of fat in the liver, resulting in overproduction of $VLDL_1$ particles (Adiels *et al.*, 2006b). As serum ALT concentrations, within normal range, have been shown to be a marker for liver fat (Westerbacka *et al.*, 2004) and has been shown to predict the onsets of type 2 diabetes (Sattar *et al.*, 2004; Sattar *et al.*, 2007), the metabolic syndrome (Schindhelm *et al.*, 2007b) and CVD events (Schindhelm *et al.*, 2007a), the author investigated whether serum ALT concentrations, would predict hepatic $VLDL_1$ production rate in the present study. Indeed, ALT concentrations correlated significantly with $VLDL_1$-apoB production rate and Intralipid-TG clearance rate, explaining 53% and 62% of their variance; respectively. This suggests that ALT concentrations are significant predictors of hepatic $VLDL_1$ production and further validates the sensitivity of the 'Intralipid Method'.

In conclusion, this chapter describes the development of a novel method to determine TRL kinetics. The 'Intralipid method' provides a relatively straightforward and cost-effective way of determining $VLDL_1$-TG and $VLDL_1$-apoB production rates, and the clearance rate of chylomicron-like particles, which does not require specialised equipment or skill in multicompartmental modelling. The author believes that this method will increase the scope for the study of TRL kinetics, particularly in circumstances where issues related to funding or equipment availability preclude the use of more traditional isotopic tracer methods.

Table 3.4: Comparison of values for $VLDL_1$-TG and $VLDL_1$-apoB production rates (range) calculated in the present study (Intralipid Method) and in previously published studies using the stable-isotope method.

Study	Subjects			Production rates			
	N (m/f)	BMI	TG	$VLDL_1$-ApoB		$VLDL_1$-TG	
		kg.m^{-2}	*mmol.l^{-1}*	*mg.h^{-1}*	*mg.kg^{-1}.d^{-1}*	*mg.h^{-1}*	*mg.kg^{-1}.d^{-1}*
(Bjorkegren *et al.*, 1996)	16 (m)	20.0 - 25.8	0.56 – 1.85	8.0 - 23.8			-
(Demant *et al.*, 1996)	6 (m)	-	1.00 – 2.40	21.2 - 51.8			-
(Pietzsch *et al.*, 1996)	6 (3/3)	20.5 - 25.0	0.70 – 1.46		22.9 - 50.7		-
(Gill *et al.*, 2004a)	16 (8/8)	19.6 - 32.9	1.00 – 3.15	8.5 - 67.8			-
(Adiels *et al.*, 2005a)	17	22.4 - 30.1	0.99 – 2.59		2.9 - 12.5		107 - 347
(Zheng *et al.*, 2006)	5 (f)	22.0 – 27.0	0.89 – 1.58		8.4 ± 5.6*		-
(Adiels *et al.*, 2006b)	18	22.0 – 30.0	0.67 – 3.14		2.9 - 12.5		107 - 352
Intralipid Method	**10 (7/3)**	**20.8 - 34.7**	**0.40 – 4.43**	**12.0 - 50.9**	**4.4 - 14.5**	**446.0 - 2563.2**	**199 - 610**

*Data are mean ± SD.

4. Effects of Acute Hyperinsulinaemia and Hyperglycaemia on $VLDL_1$-TG and $VLDL_1$-ApoB Production and Intralipid-TG Clearance in Normoglycaemic Subjects

4.1 Introduction

Diabetic dyslipidaemia is believed to be initiated by high concentrations of large $VLDL_1$ particles, which, consequently, cause the accumulation of remnant atherogenic particles and the generation of small dense LDL and small dense HDL (Taskinen, 2003). Interestingly, this profile of atherogenic lipoprotein abnormalities along with insulin resistance and hyperinsulinaemia is detectable in both the fasting (Tilly-Kiesi *et al.*, 1996) and postprandial (Johanson *et al.*, 2004) states years before the diagnosis of diabetes and appears to be related more to insulin resistance than hyperglycaemia. Therefore, it is important to understand the role of insulin in the regulation of hepatic $VLDL_1$ production.

Insulin plays a pivotal role in the regulation of hepatic VLDL production and acute hyperinsulinaemia has been shown to suppress VLDL production in normal subjects (Lewis *et al.*, 1993; Malmstrom *et al.*, 1997b). Insulin directly inhibits VLDL assembly and secretion in the liver through a number of mechanisms; e.g. it enhances intracellular apoB degradation (Sparks & Sparks, 1990) and inhibits the maturation process of VLDL assembly (Brown & Gibbons, 2001). In addition, it suppresses the NEFA flux to the liver, thereby reducing substrate availability for VLDL formation (Coppack *et al.*, 1994). However, these suppressive effects may be attenuated or lost in insulin resistant conditions. It has been reported that type 2 diabetic (Malmstrom *et al.*, 1997a; Bioletto *et al.*, 2000) and obese individuals (Lewis *et al.*, 1993; Bioletto *et al.*, 2000) fail to suppress hepatic VLDL production and concentrations (Bioletto *et al.*, 2000) to the same extent as their normal-weight, non-diabetic counterparts.

It is important to recognise that VLDL is a heterogeneous particle consisting of at least two major subclasses - large TG-rich $VLDL_1$ (S_f 60-400) and small, denser $VLDL_2$ (S_f 20-40) (Packard & Shepherd, 1997) - which have been shown to be independently regulated (Packard & Shepherd, 1997; Malmstrom *et al.*, 1998; Gill *et al.*, 2004a). Thus, the apparent effects of regulators on $VLDL_1$ metabolism may be masked or attenuated in studies examining the kinetics of total VLDL without subdivision into $VLDL_1$ and $VLDL_2$ subclasses. Indeed, $VLDL_1$, but not $VLDL_2$, particles have been shown to be the major determinant of plasma TG (Tan *et al.*, 1995) and the target for insulin-mediated VLDL suppression (Gill *et al.*, 2004a; Malmstrom *et al.*, 1998).

Furthermore, it is important to differentiate between VLDL-apoB and VLDL-TG production rates. As there is only one apoB molecule per VLDL particle (Elovson *et al.*, 1988), VLDL-apoB reflects particle number, whereas VLDL-TG is a measure of the major lipid component of VLDL particle. VLDL-apoB production rate may be dissociated from that of VLDL-TG (Kissebah *et al.*, 1981), giving rise to variable TG-to-apoB ratios and, hence, VLDL particle size.

To the best of the author's knowledge, studies examining the effects of acute hyperinsulinaemia on VLDL have either (1) addressed VLDL heterogeneity but considered only the production of $VLDL_1$-apoB, but not $VLDL_1$-TG (Malmstrom *et al.*, 1997b; Malmstrom *et al.*, 1998; Bioletto *et al.*, 2000; Annuzzi *et al.*, 2001); (2) investigated both apoB and TG production rates, but in total VLDL (Lewis *et al.*, 1993; Lewis *et al.*, 1994; Lewis & Steiner, 1996; Mittendorfer *et al.*, 2003b; Lewis *et al.*, 1995), rather than $VLDL_1$ specifically; or (3) studied the composition of VLDL subfractions rather than production rates (Annuzzi *et al.*, 2001; Bioletto *et al.*, 2000). However, at the start of the present study, no information existed on the acute effects of insulin on both $VLDL_1$-TG and -apoB production rates in normoglycaemic subjects. Recently, a non-steady-state stable isotope study by Adiels *et al.* (Adiels *et al.*, 2007) was published, which investigated the acute effect of insulin on the production rates and pool sizes of $VLDL_1$-TG and -apoB in relation to liver fat content in normal and diabetic subjects.

Therefore, the aims of the present study initially were to investigate (1) whether acute hyperinsulinaemia inhibits $VLDL_1$-TG and $VLDL_1$-apoB production to the same extent and (2) factors that might influence the extent to which $VLDL_1$ is suppressed in a group of normoglycaemic individuals with a wide range of adiposity, fasting TG and insulin resistance. This was conducted using a novel, non-stable isotope method to determine $VLDL_1$-TG and -apoB kinetics (Al-Shayji *et al.*, 2007), the development of which was previously described in Chapter 3.

4.2 Subjects and Methods

4.2.1 Subjects

Eight non-smoking healthy subjects (6 men and 2 women) were included in this study after giving written informed consent. All subjects had normal thyroid, liver and renal function and none had acute illness, a history of known cardiovascular disease or hypertension, nor were under medication known to influence carbohydrate or lipid metabolism. The subject information sheet, consent form and health questionnaire were the same as those used for the 'Intralipid Method' study (Appendices A1 & B1). Two subjects had the E3/2 phenotype, five had the E3/3 phenotype and one had the E4/4 phenotype. The subjects' characteristics are shown in **Table 4.1**. The study protocol was approved by the Research Ethics Committee of the North Glasgow University Hospitals NHS Trust.

4.2.2 Study Design

Each subject participated in 2 Intralipid trials (Control and Glucose) in random order at an interval of 2-3 weeks for men and 4 weeks (to control for menstrual cycle) for women. In the Glucose trial, subjects were given an oral bolus of a glucose drink (30 g in 120 ml) an hour before an Intralipid infusion (see below) followed by 10 g of glucose (in 40 ml) at 15-min intervals through out the duration of the trial. This was substituted with equivalent volumes of water in the Control trial (**Figure 4.1**). The dose and frequency of the glucose drink were previously determined, as explained in section 2.10, to elucidate sufficient suppression in NEFA concentrations, before administering the Intralipid infusion, and maintain, as far as possible, a steady state of hyperinsulinaemia and hyperglycaemia throughout the trial.

Subjects were requested not to exercise for three days before their study days as this is known to affect TRL metabolism (Gill & Hardman, 2003). In addition, they were asked to weigh and record their dietary intake for two days prior to the first Intralipid test and this diet was replicated for the second trial.

Table 4.1: Subjects' physical characteristics and mean fasting concentrations (n=8).

	Mean	Range
Age (years)	34	(22-55)
Body Mass (kg)	73.9	(56.0-96.5)
Body mass index ($kg.m^{-2}$)	25.8	(21.0-34.7)
Waist circumference (cm)	83.8	(73.0-104.5)
Waist/hip ratio	0.82	(0.77-0.88)
ALT ($U.l^{-1}$)	20	(15-26)
Triglycerides ($mmol.l^{-1}$)*	1.17	(0.52-2.36)
Total cholesterol ($mmol.l^{-1}$)*	4.21	(3.37-5.65)
HDL cholesterol ($mmol.l^{-1}$)*	1.37	(0.81-1.81)
LDL cholesterol ($mmol.l^{-1}$)*	2.29	(1.47-3.63)
Glucose ($mmol.l^{-1}$)*	5.32	(4.50-5.91)
Insulin ($mU.l^{-1}$)*	7.15	(2.48-15.84)
$HOMA_{IR}$*	1.71	(0.50-3.59)
NEFA ($mmol.l^{-1}$)*	0.50	(0.28-0.77)
Fasting $VLDL_1$-TG ($mmol.l^{-1}$)*	0.47	(0.12-1.31)
Fasting $VLDL_1$-apoB ($mg.dl^{-1}$)*	1.51	(0.38-4.04)

*Values are the mean of Control and Glucose trials

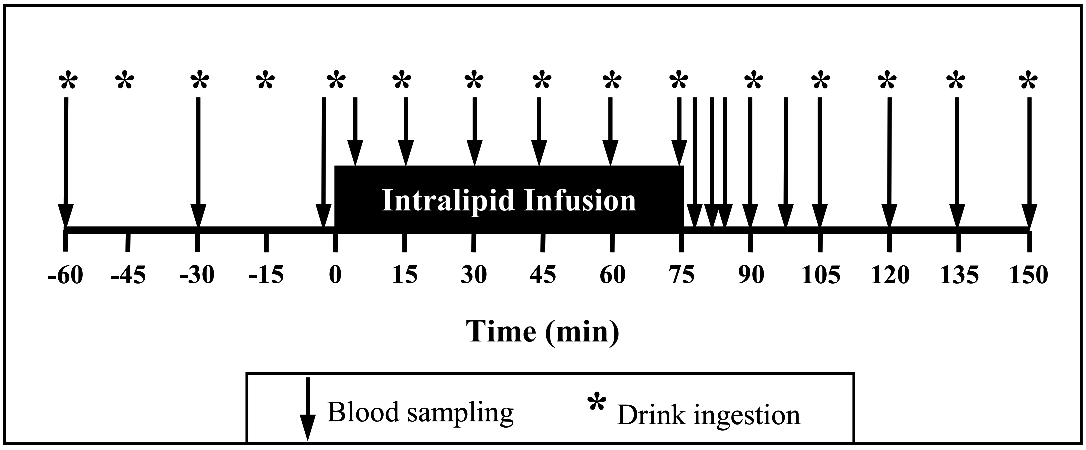


Figure 4.1: Study Protocol. In the Glucose trial a bolus drink of 30 g of glucose in 120 ml water was given at -60 mins, followed by 10 g of glucose in 40 ml of water at 15-min intervals thereafter. In the Control trial, equivalent volumes of water were given at the same intervals.

4.2.3 Intralipid Infusion

TRL kinetics were determined using the 'Intralipid Method' (Al-Shayji *et al.*, 2007) as previously described in Chapter 3. Briefly, subjects reported to the Clinical Investigation Suite in the Department of Vascular Biochemistry in Glasgow Royal Infirmary after an overnight fast of 12 h where they were given a bolus dose of 0.1 $g.kg^{-1}$ of 20% Intralipid followed immediately by an intravenous infusion of 0.1 $g.kg^{-1}.h^{-1}$ of 10% Intralipid for 75 min. Multiple EDTA blood samples were drawn at baseline, before and at 15-min intervals during infusion as well as at 2.5, 5, 10, 15, 20, 30, 45, 60 and 75 min post-infusion.

4.2.4 Analytical Methods

Plasma total and HDL cholesterol concentrations were measured in the fasted state and glucose, TG and NEFA concentrations were measured in all time points. LDL cholesterol was calculated in the fasted state using the Friedewald equation (Friedewald *et al.*, 1972). Plasma insulin concentrations were analysed in the fasted state and every 30 min throughout the trial. Serum ALT concentrations were measured at screening and the apoE phenotype was determined for each subject. All analyses were performed as previously described in sections 2.4.2 to 2.4.4.

Intralipid (S_f >400) and $VLDL_1$ (S_f 60-400) fractions were separated from whole plasma as previously described in sections 3.2.4 and 2.4.1, respectively (Al-Shayji *et al.*, 2007). TG concentrations (corrected for glycerol, section 3.2.7) were measured in the Intralipid and $VLDL_1$ fractions in all time points and apoB concentrations were measured directly in the $VLDL_1$ fraction by immunoturbidimetry as previously described in section 2.9.2.3.

4.2.5 Calculations and Statistical Analyses

$VLDL_1$-TG and $VLDL_1$-apoB productions rates were calculated from their linear rises in the $VLDL_1$ fraction during infusion. Intralipid-TG clearance rate was calculated from its exponential decay post-infusion as described in Chapter 3 (Al-Shayji *et al.*, 2007). The production ratio of TG/apoB (mol:mol) was calculated as an index of the size of the $VLDL_1$ particle being produced.

Time-averaged concentrations for glucose, insulin and NEFA were calculated from their respective areas under the curve from the start of the Intralipid infusion to 75 min post-infusion.

Statistical analyses were performed using MINITAB for Windows (Version 14.0, MINITAB Inc., State College, PA) and STATISTICA (Release 6.0, StatSoft, Inc, USA). Normality was checked for all the data using the Anderson-Darling test. When data did not approximate a normal distribution, these were log-transformed, specifically fasting TG, kinetic data and the time-averaged concentrations of glucose and insulin. Time-trends were tested using two-way ANOVA (trial × time) with repeated measures on both factors. *Post hoc* Fisher LSD tests were used to indentify exactly where any differences lay. Paired *t*-tests were used to compare fasting concentrations and kinetic variables between the Glucose and Control trials. $HOMA_{IR}$ was used as a validated surrogate measure of insulin resistance (Matthews *et al.*, 1985) and was calculated as insulin ($mU.l^{-1}$) × glucose ($mmol.l^{-1}$) / 22.5. To determine factors which predicted the change in $VLDL_1$-TG and -apoB production and Intralipid-TG clearance rates between Control and Glucose trials, Pearson product-moment correlations were performed between the changes (Control minus Glucose) in kinetic variables on one hand and $HOMA_{IR}$, ALT, NEFA and the differences in the time-averaged concentrations of glucose and insulin on the other hand. Significance was accepted at the $P < 0.05$ level. Data are presented as mean ± SEM unless otherwise stated. There were no data available to base a formal power calculation on for the present study. However, in a previous study investigating the effects of hyperinsulinaemia on $VLDL_1$-apoB production using stable isotope methods, a significant effect was seen with five subjects (Malmstrom *et al.*, 1998), suggesting that studying eight subjects in the present study would be sufficient to detect a significant effect of hyperinsulinaemia on $VLDL_1$ metabolism, should such an effect exist.

4.3 Results

4.3.1 Fasting Plasma Concentrations

There was no significant difference in fasting concentrations between the Glucose and Control trials. **Table 4.1** therefore shows the mean of fasting plasma lipid, glucose, insulin, $HOMA_{IR}$ and NEFA concentrations in both trials.

4.3.2 Glucose, Insulin and NEFA Concentrations during and Post-Infusion

Mean plasma glucose concentrations were significantly higher throughout the Glucose trial compared with the Control trial (*P* = 0.005, **Figure 4.2A**). In the Glucose trial, glucose concentrations increased significantly within 30 min of the initial glucose ingestion (i.e. 30 min prior to the Intralipid infusion) (from 5.35 ± 0.18 to 7.92 ± 0.59 mmol.l^{-1}, *P* <0.001) and averaged 7.80 ± 0.12 mmol.l^{-1} during infusion. In the Control trial, glucose concentrations decreased slightly from baseline during the first hour before infusion (from 5.28 ± 0.15 to 5.06 ± 0.11 mmol.l^{-1}, *P* = 0.01), but remained unchanged at an average of 5.08 ± 0.08 mmol.l^{-1} (*P* = 0.44) during infusion.

As expected, insulin concentrations were significantly higher in the Glucose trial compared to the Control trial (*P* = 0.03, **Figure 4.2B**). Like glucose concentrations, insulin concentrations increased significantly 30 min after the start of the glucose drinks in the Glucose trial (from 7.25 ± 1.80 mU.l^{-1} to 47.61 ± 8.78 mU.l^{-1}, *P* = 0.02) and remained elevated (*P* = 0.47) throughout the trial with an average concentration of 64.91 ± 9.7 mU.l^{-1}. This is in contrast to the Control trial, where plasma insulin concentrations did not change significantly from baseline (*P* = 0.91) with an average of 6.87 ± 0.17 mU.l^{-1}.

In the hour prior to the start of the Intralipid infusion, mean plasma NEFA concentrations declined rapidly in the Glucose trial from 0.46 ± 0.07 at baseline to 0.16 ± 0.04 mmol.l^{-1} immediately before infusion (*P* <0.001, **Figure 4.2C**), but did not change significantly from baseline in the Control trial (0.55 ± 0.01 mmol.l^{-1}, *P* = 0.27). When the Intralipid infusion started in both trials, NEFA concentrations

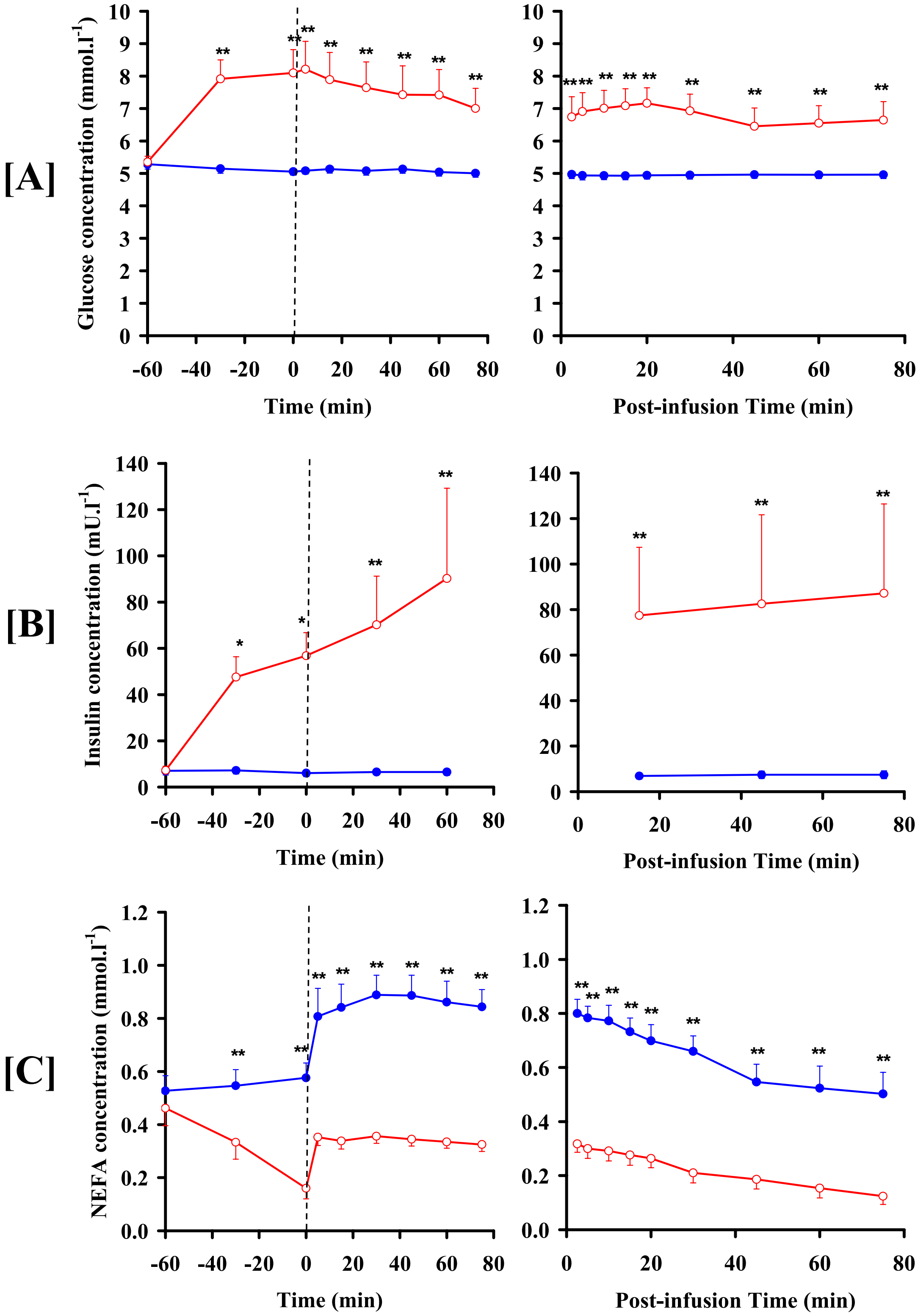


Figure 4.2: Mean [A] glucose, [B] insulin and [C] NEFA concentrations before and during Intralipid infusion (left panel) and post-infusion (right panel) between the Control (●) and Glucose (○) trials. The dotted line represents the beginning of a bolus Intralipid dose of 0.1 g.kg^{-1} followed immediately by a constant Intralipid infusion of 0.1 g.kg^{-1}.h^{-1}. Significant differences between the two trials were tested using two-way ANOVA with (*) representing *P* <0.05 and (**) *P* <0.001.

increased about 2.5-fold and leveled during infusion at an average concentration of 0.85 ± 0.04 $mmol.l^{-1}$ in the Control trial and 0.34 ± 0.02 $mmol.l^{-1}$ in the Glucose trial. Overall, NEFA concentrations were significantly lower throughout the Glucose trial compared to the Control trial (P <0.001).

4.3.3 The Change in Production and Clearance Rates

Individual and mean $VLDL_1$-TG and -apoB production and Intralipid-TG clearance rates in the Control and Glucose trials according to apoE phenotypes are shown in **Figure 4.3**. The $VLDL_1$-TG production rate was lower in the Glucose trial in 6 out of 8 subjects with an overall mean production rate of 805.7 ± 264.0 $mg.h^{-1}$ compared to 969.3 ± 181.6 $mg.h^{-1}$ in the Control trial (P = 0.05, **Figure 4.3A**). Similarly, $VLDL_1$-apoB production rate was lower in the Glucose trial in the same 6 subjects with an overall mean production rate of 21.8 ± 5.3 $mg.h^{-1}$ in the Glucose trial versus 25.8 ± 4.1 $mg.h^{-1}$ in the Control trial (P = 0.04, **Figure 4.3B**). Overall, there was a 25.4% and 20.9% reduction in $VLDL_1$-TG and -apoB production rates, respectively, in the Glucose trials. It is unclear why these two subjects responded differently. However, of note, one of them had the highest $HOMA_{IR}$ value (3.59) among all subjects.

On the other hand, the Intralipid-TG clearance response differed widely amongst subjects (**Figure 4.3C**). While four subjects showed a slight increase in Intralipid-TG clearance rate in the glucose trial, one subject showed a dramatic increase and the remaining three subjects showed a decreased Intralipid-TG clearance rate. The reason for these different responses is unclear. However, it is possibly due to differences in insulin resistance: the subject who had the highest increase in Intralipid-TG clearance rate, had the lowest $HOMA_{IR}$ of 0.5 (mean between the Control and Glucose trials), while the three subjects who had decreased clearance rates in the glucose trial, exhibited the highest $HOMA_{IR}$ concentrations amongst the 8 subjects (1.97, 3.47 and 3.59). This suggestion is supported by a strong correlation between $HOMA_{IR}$ and the change in Intralipid-TG clearance rate (see below). There was no significant difference in Intralipid-TG clearance between the Control and Glucose trials (69.4 ± 12.7 and 103.5 ± 49.8 $pools.d^{-1}$ respectively, P = 0.95), although clearance was 16.9% higher in the Glucose trial. However, this numerical increase in Intralipid-TG clearance was entirely due to one subject who showed a dramatic increase in Intralipid-TG clearance following glucose ingestion. If this subject was excluded from analysis, the Intralipid-TG clearance rates between the Control and Glucose trials were almost identical (58.4 ± 7.3 and 54.8 ± 11.9 pools.d^{-1}, respectively, $P = 0.600$).

4.3.4 $VLDL_1$ Particle Size

The size of the $VLDL_1$ particle being produced (estimated as TG/apoB molar ratio) in response to hyperinsulinaemia and hyperglycaemia varied widely among subjects: five subjects exhibited a decrease in $VLDL_1$ particle size (range 7.4 - 50.6%), whereas 3 subjects showed an increased $VLDL_1$ particle size (range 10.9 - 38.2%). Unlike the Intralipid-TG clearance rate, the reason for this variable response in $VLDL_1$ particles size is unclear and could not be explained by differences in $HOMA_{IR}$. In total, there was a mean reduction of 7.6 ± 10.2% in $VLDL_1$ particle size with glucose ingestion. However, this change was not statistically significant ($P = 0.29$).

4.3.5 Predictors of the Change in Production and Clearance Rates

The change (Control minus Glucose) in NEFA concentrations at zero min (i.e. after the first hour of the start of the drink, and just before the Intralipid infusion) correlated significantly with the change in $VLDL_1$-TG production rate ($r = 0.79$, $P = 0.02$), but not with the change in $VLDL_1$-apoB production rate ($r = 0.59$, $P = 0.13$, **Figure 4.4A**). Overall, the change in NEFA concentrations explained 62% (0.79^2) of the variance in the change in $VLDL_1$-TG production rate.

In addition, the author investigated whether ALT concentrations within normal range would predict the change in hepatic $VLDL_1$-TG and -apoB production in response to hyperinsulinaemia and hyperglycaemia. It was noted that serum ALT concentrations correlated inversely and significantly with the change in $VLDL_1$-TG ($r = -0.83$, $P = 0.01$) and $VLDL_1$-apoB ($r = -0.74$, $P = 0.04$) production rates, explaining 69% and 55% of the variance in change in these responses, respectively (**Figure 4.4B**).

$HOMA_{IR}$ did not correlate significantly with the change in $VLDL_1$-TG and -apoB production rates. However it correlated significantly with the change in Intralipid-

TG clearance rates (*r* = 0.72, *P* = 0.04, **Figure 4.4C**), explaining 52% of the variance of the change in response. This indicates that subjects who had the lowest $HOMA_{IR}$ (i.e. the more insulin sensitive individuals) had the biggest increase in Intralipid-TG clearance rate in response to hyperinsulinaemia.

There was no apparent effect of apoE phenotype on the change in $VLDL_1$-TG and $VLDL_1$-apoB production rates or Intralipid-TG clearance rate (**Figure 4.3**).

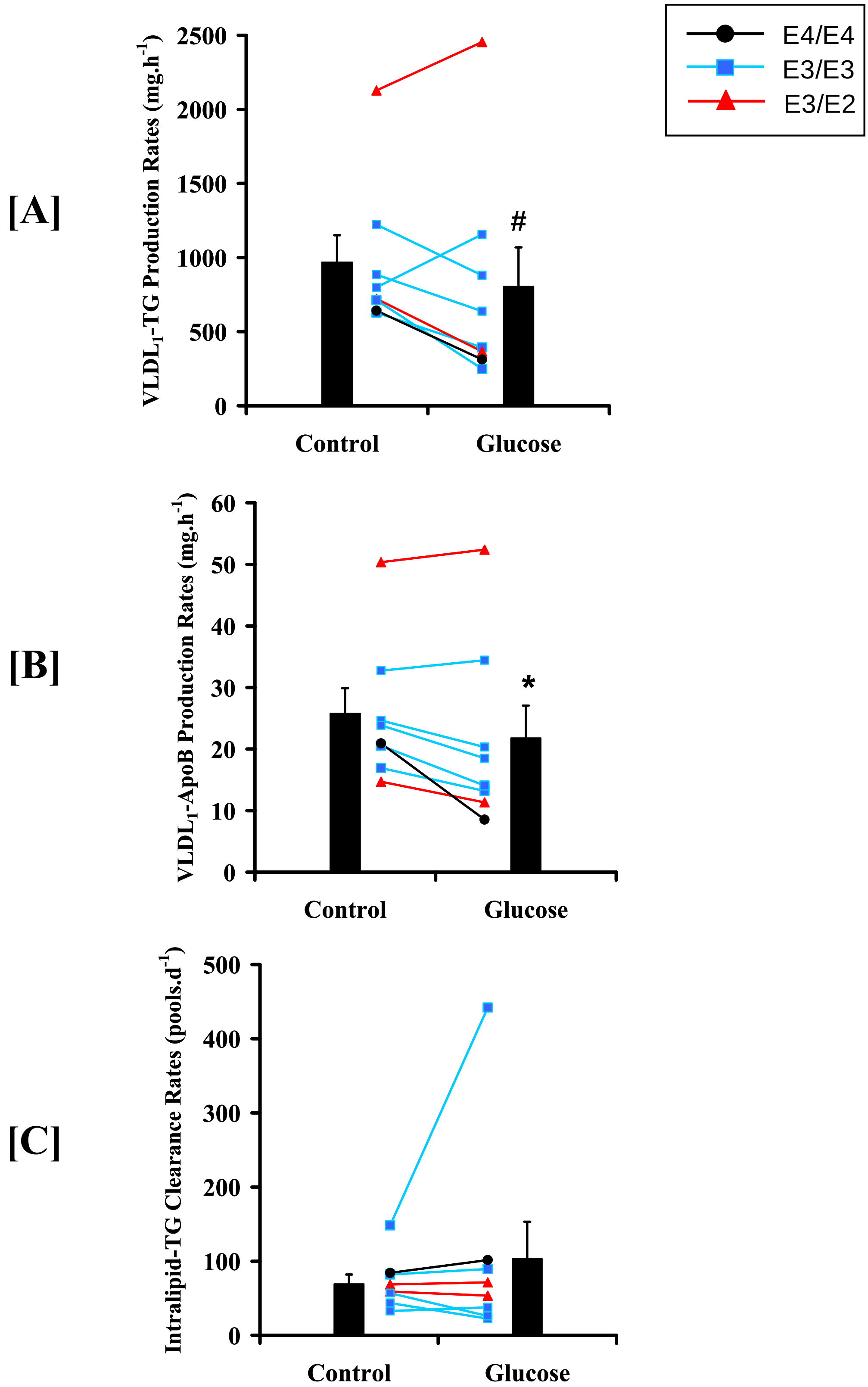


Figure 4.3: Individual (according to apoE phenotype) and mean (bars) [A] VLDL$_1$-TG and [B] VLDL$_1$-apoB production rates and [C] Intralipid-TG clearance rate in the Control and Glucose trials (n = 8). (#) *P* = 0.05, (*) *P* <0.05.

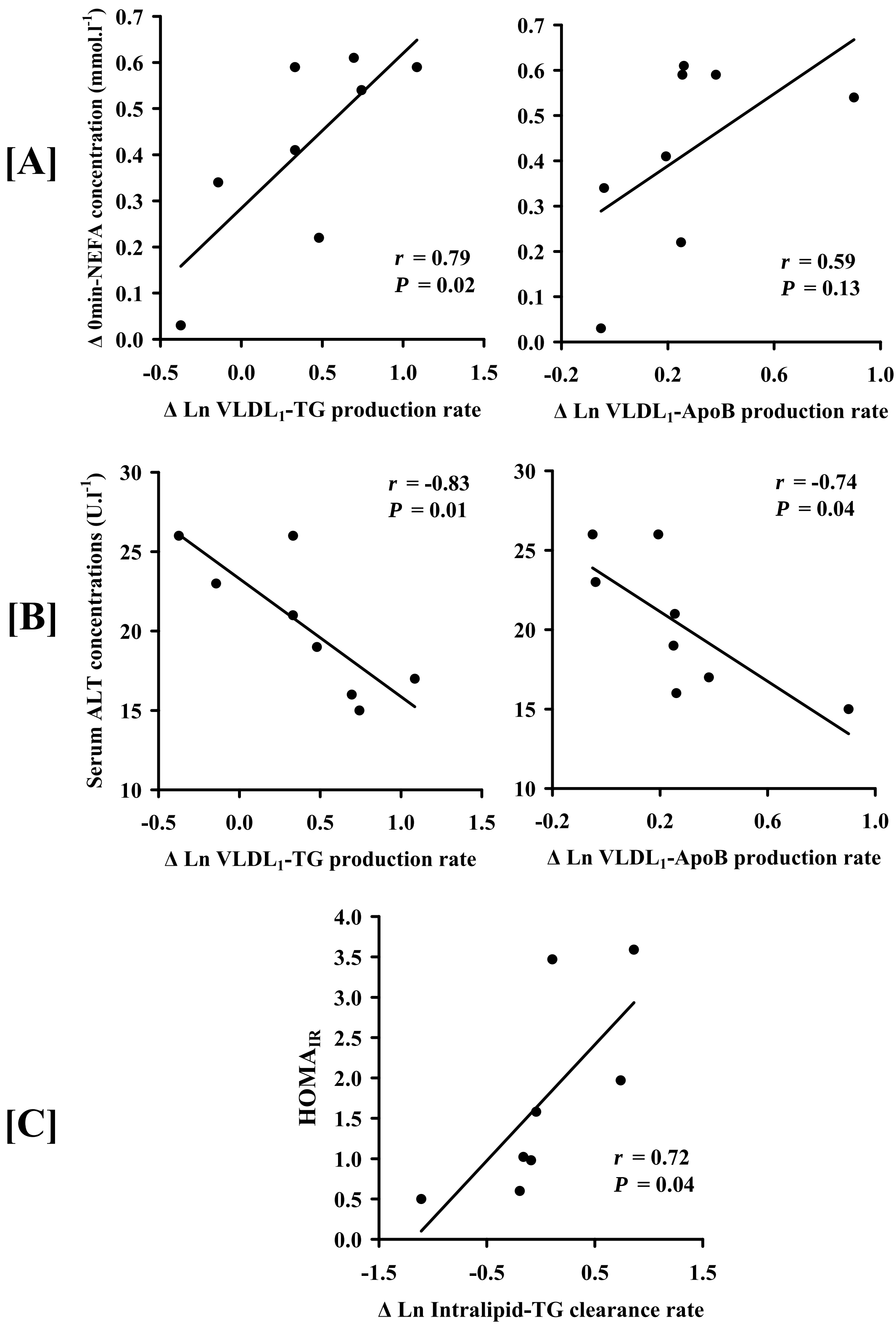


Figure 4.4: Scatterplots (with linear-regression lines of 'best-fit') illustrating the relationships between the change (Control minus Glucose) in [A] NEFA concentrations at 0 min (just before the start of the Intralipid infusion) and the change in $VLDL_I$-TG (left) and $VLDL_I$-apoB production rates (right); between [B] serum ALT concentrations and the change in $VLDL_I$-TG (left) and $VLDL_I$-apoB production rates (right); and [C] $HOMA_{IR}$ and the change in Intralipid-TG clearance rate. N = 8, *r* and *P* values are for Pearson product–moment correlations between variables.

4.4 Discussion

This study aimed to investigate the acute effects of hyperinsulinaemia and hyperglycaemia on the production rates of both $VLDL_1$-TG and -apoB and Intralipid-TG clearance rate in healthy normoglycaemic subjects. Firstly, the present results indicate that insulin acutely suppressed $VLDL_1$-TG and -apoB production rates by 25% and 21%, respectively. However, as there was no significant difference in the size of the $VLDL_1$ particle being produced (i.e. TG/apoB ratio), this suggests equal suppression of $VLDL_1$-TG and -apoB production. This is consistent with the recent findings of Adiels *et al.* (Adiels *et al.*, 2007), who showed that insulin acutely induced a 60% suppression in both $VLDL_1$-TG and $VLDL_1$-apoB production rates in normal, non-diabetic subjects. The work of Adiels *et al.* was concomitant with the present study and, to-date, these are the only two studies that specifically investigated the effect of hyperinsulinaemia on $VLDL_1$-TG and $VLDL_1$-apoB production rates.

The suppression in $VLDL_1$ production in response to insulin in the present study (at 21-25%) was less than the 50-60% reduction reported in $VLDL_1$-TG (Adiels *et al.*, 2007) and $VLDL_1$-apoB (Malmstrom *et al.*, 1997b; Malmstrom *et al.*, 1998) production rates. This could be due to the time of exposure to acute hyperinsulinaemia: in these studies, exogenous insulin was administered for 5-8.5 h, whereas in the present study, $VLDL_1$ production rates were determined during the first 135 min of exposure to hyperinsulinaemia. This is supported by Malmstrom *et al.* (Malmstrom *et al.*, 1997b), who reported a significant detectable reduction in $VLDL_1$-apoB synthesis after 0.5 h of insulin infusion at an average of ~9% per h (range 0% – 24%).

Hepatic assembly of VLDL is a complex process (see section 1.3.2). Briefly, it starts in the rough ER with the co-translational lipidation of apoB-100 forming a 'lipid pocket' and the subsequent interaction with MTP, which catalyses the transfer of TG to the continually synthesised apoB-100 and allowing apoB to fold on a core of neutral lipids. This gives rise to a partially lipidated primordial particle (pre-VLDL), which is retained in the cell by interaction with chaperones and other ER proteins until it is further lipidated to form $VLDL_2$ or sorted to degradation. If apoB fails to be lipidated and misfolds, it is unfolded, retracted to the cytosol, ubiquinated, and sorted to proteasomal degradation (Adiels *et al.*, 2006a). $VLDL_2$ is converted to $VLDL_1$ in the Golgi apparatus by the uptake of a defined lipid load from a fairly rapidly mobilised cytosolic TG pool (Gibbons *et al.*, 2000). Insulin controls a number of enzymatic steps in the assembly and secretion of VLDL by the hepatocyte (Gibbons *et al.*, 2002; Sparks & Sparks, 1994). It downregulates MTP gene expression (Lin *et al.*, 1995), enhances apoB degradation (Sparks & Sparks, 1990), inhibits the secretion of newly synthesised apoB as well as inhibiting VLDL maturation by suppressing transfer of cystosolic TG to pre-VLDL particles (Brown & Gibbons, 2001). This is likely to explain the reduction in $VLDL_1$-TG and - apoB production rates observed in the present study. In addition, insulin decreases NEFA flux to the liver, likely due to inhibition of hormone-sensitive lipase (Lewis *et al.*, 1993) and adipose tissue triglyceride lipase (Kershaw *et al.*, 2006). This consequently decreases substrate availability for VLDL assembly (Coppack *et al.*, 1994), as the influx of NEFA from adipose tissue to the liver normally accounts for 60-80% of hepatic TG (Donnelly *et al.*, 2005). Indeed, in this study, a rapid decrease in plasma NEFA concentrations occurred in the first hour, shortly after induction of hyperinsulinaemia in response to glucose ingestion, which correlated significantly and positively with the suppression in $VLDL_1$-TG and -apoB production in response to insulin: subjects who had the biggest reduction in NEFA concentrations, had the biggest suppression in $VLDL_1$-TG and -apoB production. It should be noted that although NEFA concentrations were significantly lower throughout the Glucose trial compared to the Control trial in response to insulin, NEFA concentrations increased during Intralipid infusion in both trials due to the ‘spill-over’ from Intralipid produced by LPL-mediated TG hydrolysis taking place directly into the circulation (Evans *et al.*, 1999). Therefore, NEFA concentrations during the infusion were not included in the correlations with changes in $VLDL_1$ production rates as they do not reflect the ‘true’ insulin-mediated suppression of NEFA.

Secondly, in the present study, hyperinsulinaemia did not significantly influence Intralipid-TG clearance rate. This is in contrast to the findings of Preiss-Landl *et al.* who reported that insulin upregulates LPL activity in adipose tissue (Preiss-Landl *et al.*, 2002). One likely explanation could be due to the heterogeneity of subjects’ insulin resistance in the present study as there was a significant correlation between $HOMA_{IR}$ and the change in Intralipid-TG clearance rate. While glucose ingestion caused an upregulation in clearance in the more insulin sensitive subjects, it induced a paradoxical downregulation of TG clearance in the more insulin resistant individuals. Indeed, it was previously reported that the more resistant an individual is to insulin-mediated glucose uptake, the lower will be the plasma post-heparin LPL activity and adipose tissue LPL mRNA levels (Maheux *et al.*, 1997).

Finally, the author investigated whether serum ALT concentrations, a marker for liver function and hepatic fat accumulation (Westerbacka *et al.*, 2004), would also predict the change in $VLDL_1$ production in response to hyperinsulinaemia. The present results demonstrate that serum ALT is a strong predictor of the suppression in $VLDL_1$-TG and -apoB production, accounting for 69% and 55% of the variance in the change in production rates, respectively. This finding was recently supported by Adiels *et al.* (Adiels *et al.*, 2007) who showed that individuals with high liver fat failed to suppress $VLDL_1$ production in response to insulin. Whether this reduced suppression of $VLDL_1$ production by insulin is a result of liver fat, a consequence of hepatic insulin resistance or both remains to be uncertain (Adiels *et al.*, 2007). Similarly, it is unclear, in the present study, whether ALT (i.e. liver function) per se is the mediator of the change in $VLDL_1$ production or whether ALT is just a marker for liver fat.

In summary, this study demonstrated that, in normoglycaemic apparently healthy individuals, (1) insulin suppressed $VLDL_1$-TG and -apoB production; and (2) the more insulin sensitive the subject is, the bigger the increase in Intralipid-TG clearance rates due to hyperinsulinaemia and hyperglycaemia. In addition, ALT concentration – a marker of liver function and associated with liver fat content – strongly predicted individuals' ability to suppress $VLDL_1$-TG and -apoB in response to hyperinsulinaemia. Not only do these findings help the basic scientific understanding of the effect of insulin on $VLDL_1$ production, they may also help identifying individuals at higher risk of developing atherogenic dyslipidaemia which is believed to be initiated by insulin resistance, liver fat and hepatic overproduction of $VLDL_1$ particles.

5. Effects of Moderate Exercise on $VLDL_1$ Kinetics in Overweight/Obese Middle-Aged Men

5.1 Introduction

CVD is the main cause of death in the UK, with CHD, being the most common form, accounting for about 101,000 deaths each year (Allender *et al.*, 2007). Elevated plasma concentrations of TG are considered an independent risk factor for CHD (Cullen, 2000). High concentrations of large very low density lipoprotein ($VLDL_1$, S_f 60-400) are the major determinant of plasma TG levels (Tan *et al.*, 1995; Hiukka *et al.*, 2005) and believed to initiate a chain of reactions that generate the atherogenic lipoprotein phenotype associated with insulin resistance conditions (e.g. obesity, type 2 diabetes and the metabolic syndrome) (Bloomgarden, 2007; Taskinen, 2003; Ginsberg *et al.*, 2005). Thus, reducing $VLDL_1$ concentration is likely to induce clinically important changes to the atherosclerotic risk profile.

While patient groups often undergo pharmacological treatments for these lipoprotein disorders, obesity and insulin resistance are growing problems in the population: over half of UK adults are overweight (BMI > 25 $kg.m^{-2}$) and over 20% are obese (BMI > 30 $kg.m^{-2}$) (Allender *et al.*, 2006) and estimated prevalence of the metabolic syndrome in Scottish middle-aged men is ~25% (Sattar *et al.*, 2003). Therefore, it is unfeasible and probably undesirable to subject large sections of the population to long-term pharmacological therapies. Moderate exercise is one potential non-pharmacological therapy for elevated $VLDL_1$ concentrations. There is clear evidence that exercise of this nature can lower fasting and postprandial TG concentrations by 20-25%, mostly in the $VLDL_1$ fraction (Gill *et al.*, 2006), in population groups at increased risk of cardiovascular disease, such as centrally obese middle-aged men (Gill *et al.*, 2004b) and postmenopausal women (Gill & Hardman, 2000). This effect is seen following a single exercise session, so is not mediated by weight loss (Gill *et al.*, 2004b; Gill & Hardman, 2000; Gill *et al.*, 2006). However the mechanism(s) responsible for this TG-reduction are unclear and require further elucidation as it could reflect reduced hepatic $VLDL_1$ production and/or increased LPL-mediated $VLDL_1$ clearance. Interestingly, a prior session of moderate exercise induces a larger TG-reduction in the $VLDL_1$ fraction than the chylomicrons fraction (Gill *et al.*, 2001a; Gill *et al.*, 2006). Because chylomicrons are preferred substrates to LPL above $VLDL_1$ (Karpe *et al.*, 1993a; Fisher *et al.*, 1995), this reduction in $VLDL_1$ was hypothesised to be the result of an exercise-induced suppression of hepatic $VLDL_1$ production rather than increased clearance. However, two recent stable-isotope kinetic studies have demonstrated that, in a group of lean recreationally active young men, a single session of a 90-120 min of moderate intensity exercise (at 60% of $\dot{V}O_{2max}$) resulted in an increased clearance of total VLDL-TG (Magkos *et al.*, 2006; Tsekouras *et al.*, 2007) and a significant decrease in hepatic VLDL-apoB production (Magkos *et al.*, 2006) with no significant change in hepatic VLDL-TG production (Magkos *et al.*, 2006; Tsekouras *et al.*, 2007). However, studies investigating the effects of moderate exercise on large $VLDL_1$ kinetics, especially in overweight/obese middle-aged men, a typical population at which exercise-for-health guidelines are targeted, are lacking. This is important as moderate exercise may have a considerable potential to be used as a first-line therapeutic option for preventing and treating the primary defect in obesity/insulin resistance-related dyslipidaemia.

Kinetics of VLDL are usually determined using stable-, or radio-, isotopic tracer methods, but these techniques are expensive and time-consuming which limits the scope of studies to which these methods can be applied. Earlier in this thesis (Chapter 3) the development of a relatively easy method to determine $VLDL_1$-TG and -apoB production and clearance rates, as well as Intralipid-TG clearance rate (as a surrogate measure of chylomicron-TG clearance) was described (Al-Shayji *et al.*, 2007), which is used in the present study to elucidate the mechanisms by which moderate exercise induces TG lowering.

The aim of the present study was to investigate the effects of a 120-min session of prior moderate exercise on $VLDL_1$-TG and -apoB kinetics and Intralipid-TG clearance rate in a group of overweight/obese middle-aged adults using the Intralipid method.

5.2 Materials and Methods

5.2.1 Subjects

Twelve overweight/obese, middle-aged men participated in this study; their characteristics are shown in **Table 5.1**. All subjects were apparently healthy, normotensive, normoglycaemic, non-smokers who displayed no symptoms of coronary heart disease during a clinical exercise stress test (section 2.5). None was taking any drugs known to affect lipid or carbohydrate metabolism. The subject information sheet, consent form and health questionnaire are shown in Appendices A2 & B2. One subject had E3/2 apoE phenotype, 4 had E3/3 phenotype, 5 had E4/3 phenotype and 2 had E4/4 phenotype. The study was conducted with the approval of North Glasgow University Hospitals NHS Trust Ethics Committee and subjects gave written informed consent prior to participation.

Table 5.1: Characteristics of the participants (n=12).

	Mean ± SD
Age (years)	44 ± 8.4
Body Mass (kg)	95.0 ± 17.1
Body mass index ($kg.m^{-2}$)	31.3 ± 5.1
Waist circumference (cm)	106.9 ± 13.2
Waist/hip ratio	0.98 ± 0.05
Maximal oxygen uptake ($ml.kg^{-1}.min^{-1}$)	37.9 ± 7.2
ALT ($U.l^{-1}$)	39 ± 10

5.2.2 Study Design

Each subject underwent two Intralipid infusions (see below) within an interval of 2 weeks. Preconditions (i.e. control and exercise) were different and administered in random order. On the afternoon prior to one Intralipid infusion subjects walked 120 min on a treadmill at an intensity of ~50% $\dot{V}O_{2max}$ (Exercise trial). In the other trial, subjects performed no exercise on the day preceding the other Intralipid infusion (Control trial). They were asked to weigh and record their dietary intake for two days prior to their first Intralipid infusion and replicate this prior to the second test and were instructed to refrain from alcohol consumption on those two days. No exercise other than the treadmill walk in the Exercise trial was performed during the three days prior to each Intralipid infusion.

5.2.3 *Treadmill Walk*

A preliminary submaximal incremental treadmill test was performed as previously described in section 2.6 at least 1 week prior to the first Intralipid test to estimate $\dot{V}O_{2max}$ and determine the speed and gradient required to elicit 50% $\dot{V}O_{2max}$. The 120-min walk in the Exercise trial was completed ~16-18 h prior to the Intralipid test. Heart rate, O_2 uptake and CO_2 production were measured as previously described in section 2.8. Ratings of perceived exertion (Borg, 1973) were obtained at 15-min intervals during the walk.

5.2.4 *Intralipid Infusion*

$VLDL_1$ kinetics were determined using the 'Intralipid Method' as previously described in Chapter 3 (Al-Shayji *et al.*, 2007) with a slight modification in the frequency of blood sampling during infusion. Briefly, subjects reported to the Clinical Investigation Suite in the Department of Vascular Biochemistry at Glasgow Royal Infirmary in the morning after an overnight fast of 12 h and they were given a bolus dose of 0.1 $g.kg^{-1}$ of 20% Intralipid followed immediately by an intravenous infusion of 0.1 $g.kg^{-1}.h^{-1}$ of 10% Intralipid for 75 min. Multiple EDTA blood samples were drawn at baseline, before and at 10-min intervals during infusion as well as at 2.5, 5, 10, 15, 20, 30, 40 and 60 min post-infusion. The post-infusion period was reduced from 75 to 60 min when it became clear that 60 min was sufficient to calculate the Intralipid-TG clearance rate.

5.2.5 *Analytical Methods*

Plasma glucose, insulin, total and HDL cholesterol and apos C-II, C-III and E concentrations were analysed in the fasted state. TG and NEFA concentrations were analysed in all time points. LDL cholesterol was calculated using the Friedewald equation (Friedewald *et al.*, 1972). Serum ALT concentrations were measured at screening. ApoE phenotype was determined for each subject by isoelectric focusing

using Western blot techniques. All analytical methods were performed as previously described in sections 2.4.2 to 2.4.4.

Intralipid (S_f >400) was separated from whole plasma as previously described in section 3.2.4 with a minor modification. In the present subjects (typical candidates for hyperlipidaemia), it was found that the Intralipid fraction was technically easier to separate at 23° C instead of 4° C. The $VLDL_1$ (S_f 60-400) fraction was separated as previously described in section 2.4.1. TG concentrations were measured in the Intralipid and $VLDL_1$ fractions in all time points and were corrected for glycerol as described in section 3.2.7 (Al-Shayji *et al.*, 2007).

The composition of the $VLDL_1$ fraction (i.e. TG, cholesterol, FC and PL) was measured as described in section 2.4.2. Total protein content was measured manually using a modified Lowry assay (section 2.4.5). CE concentration was determined by multiplying the difference between total cholesterol and free cholesterol concentrations (in $mg.dl^{-1}$) by 1.68 to account for the difference in mass between cholesterol and CE. $VLDL_1$ concentration was calculated by summing the concentrations of these components in $mg.dl^{-1}$ (i.e. TG, FC, CE, PL and protein). ApoB concentrations were measured directly in the $VLDL_1$ fraction by automated immunoturbidimetry (see section 2.9.2.3). In four subjects, where $VLDL_1$-apoB was not detectable by the immunoturbidimetry method, apoB concentrations were measured manually, as described in section 2.9.2.2 by precipitation with Isopropanol and subsequent protein measurement by Lowry method (section 2.4.5).

5.2.6 Calculations and Statistics

$VLDL_1$-TG and -apoB production rates and FCRs, as well as Intralipid-TG clearance rates were determined as previously described in Chapter 3 (Al-Shayji *et al.*, 2007).

Net energy expenditure during the 120-min walk was calculated using indirect calorimetry assuming no protein oxidation (Frayn, 1983). HOMA was used as a validated surrogate measure of insulin resistance (IR) (Matthews *et al.*, 1985).

The number of apoC-II, apoC-III and apoE molecules per $VLDL_1$ particle were calculated, in the fasted state, by dividing the apolipoprotein concentrations in mmol.l^{-1} by the apoB concentration in mmol.l^{-1}. In addition, $VLDL_1$ TG/apoB ratio (mol:mol), representing the size of the particle in the circulation; CE/TG ratio (mol:mol), representing the composition of its core; CE/apoB ratio (mol:mol), representing the CE content per $VLDL_1$ particle; and FC/PL ratio (mg:mg), representing the composition of its surface layer, were also calculated in the fasted state.

Statistical analyses were performed using MINITAB for Windows (Version 14.0, MINITAB Inc., State College, PA) and STATISTICA (Release 6.0, StatSoft, Inc, USA). Normality was checked for all the data using the Anderson-Darling test. When data did not approximate a normal distribution, these were log-transformed, namely insulin, $HOMA_{IR}$, $VLDL_1$-apoB fasting concentrations, TG/apoB ratios, FC/PL ratio, Intralipid-TG clearance rate, $VLDL_1$-apoB FCR and production rate, $VLDL_1$-TG production rate, and the exercise-induced change in $VLDL_1$-apoB FCR and production rate required transformation. Comparisons of fasting values, summary responses and kinetic data between Control and Exercise trials were made using paired *t*-tests and changes over the trial period were assessed by two-way ANOVA (trial × time) with repeated measures on both factors. *Post hoc* Fisher LSD tests were used to indentify exactly where any differences lay. Significance was accepted at the $P < 0.05$ level. Data are presented as mean ± SEM unless otherwise stated.

To determine factors which predicted the change in $VLDL_1$-TG and -apoB production and clearance rates as well as Intralipid-TG clearance rates between Control and Exercise trials, Pearson product-moment correlations were performed between the exercise-induced changes in kinetic variables on one hand and ALT, the exercise-induced changes in glucose, insulin, $HOMA_{IR}$ and NEFA on the other hand.

5.2.7 Power Calculations

The pilot data from 5 subjects who underwent two Intralipid infusions at the 0.1g.kg^{-1}.h^{-1} Intralipid and 0.2 g.kg^{-1}.h^{-1} Intralipid doses (described in Chapter 3), indicate that the standard deviation for the within-subject variability in $VLDL_1$-apoB and $VLDL_1$-TG production are 12.7% and 20.1%, respectively, when preceding diet, exercise and alcohol intake are well-controlled. Based on these data, 12 subjects would enable detection of an 11% difference in $VLDL_1$-apoB production and an 18% difference in $VLDL_1$-TG production, with 80% power at the 0.05 level. Groups of 8-12 subjects clearly show significant effects of a single moderate exercise session on postprandial TG metabolism where a 20-25% reduction in plasma TG concentration is typically observed (Gill & Hardman, 2003).

5.3 Results

5.3.1 Treadmill Walk

Subjects walked at a speed of 4.5 ± 0.2 $km.h^{-1}$ up a gradient of 6.0 ± 0.6%. All subjects completed the 120-min walk without difficulty, with an average perceived exertion of 12.2 ± 0.4 (between 'fairly light' and 'somewhat hard') on the Borg scale of 6-20 (Borg, 1973). Mean $\dot{V}O_2$ was 18.6 ± 0.8 $ml.kg^{-1}.min^{-1}$ (49.2 ± 0.7% $\dot{V}O_{2max}$), mean heart rate was 123 ± 3 $beat.min^{-1}$ and net energy expenditure for the walk was 3.5 ± 0.1 MJ (837 ± 35 kcal).

5.3.2 Fasting Concentrations in the Control and Exercise Trials

Mean plasma and $VLDL_1$ composition and concentrations in the fasted state are shown in **Table 5.2**. In four subjects, $VLDL_1$-apoB was not detectable directly using the immunoturbidimetry method in at least one trial, and, therefore, was measured manually for both the Control and Exercise trials. In addition, fasting apoE concentrations were below limits of detection in 2 subjects in one or two trials, and, therefore, these two subjects were omitted from all apoE calculations. Exercise significantly (P <0.05) reduced fasting plasma TG and glucose concentrations by 21.9 ± 6.0% and 3.0 ± 1.0%, respectively, and increased NEFA by 23.8 ± 12.1%. There was a reduction of 12.6 ± 6.7% in insulin concentrations and of 14.8 ± 6.9% in $HOMA_{IR}$ post-exercise, but these did not reach statistical significance (P = 0.080 and 0.059, respectively). Fasting $VLDL_1$ concentrations were significantly lower by 32.6 ± 9.7% after exercise (P = 0.006), as were fasting concentrations of $VLDL_1$-TG (29.6 ± 9.3%), $VLDL_1$-Cholesterol (43.7 ± 11.4%), $VLDL_1$-apoB (38.2 ± 9.8%), $VLDL_1$-apoC-II (28.0 ± 10.7%), $VLDL_1$-apoC-III (25.0 ± 12.1%) and $VLDL_1$-apoE (27.6 ± 11.4%, n = 10), all P <0.05. There were no significant differences in fasting concentrations of total, HDL- and LDL-cholesterol (**Table 5.2**).

5.3.3 TRL Kinetics in Responses to Exercise

Figure 5.1 shows the mean percentage changes in $VLDL_1$-TG and -apoB kinetics and Intralipid-TG clearance rate and **Figure 5.2** shows the absolute mean and individual production and clearance rates in response to exercise according to apoE phenotypes. There was a significant increase after exercise in Intralipid-TG clearance rate (Control: 47.6 ± 6.8, Exercise: 68.1 ± 9.7 pools.d^{-1}, P <0.001), $VLDL_1$-TG FCR (Control: 16.0 ± 2.1, Exercise: 29.1 ± 4.4 pools.d^{-1}, P = 0.002) and $VLDL_1$-apoB FCR (Control: 10.4 ± 2.0, Exercise: 25.6 ± 5.1 pools.d^{-1}, P = 0.015). However, there was no significant difference between the Control and Exercise trials in the production rates of $VLDL_1$-TG (Control: 1271.5 ± 155.7, Exercise: 1431.7 ± 148.3 mg.h^{-1}, P = 0.104) and $VLDL_1$-apoB (Control: 37.2 ± 7.4, Exercise: 41.5 ± 5.4 mg.h^{-1}, P = 0.335).

5.3.4 $VLDL_1$ Compositional Responses to Exercise

Table 5.3 shows the exercise-induced compositional changes in $VLDL_1$ in the fasted state – reflecting the composition of circulating $VLDL_1$ particles. There was a tendency for a bigger $VLDL_1$ particle (TG/apoB ratio) in the circulation in the Exercise trial (Control: 15392 ± 2178, Exercise: 19449 ± 3150). However, this did not reach statistical significance (P = 0.059). On the other hand, the CE/TG ratio, reflecting the neutral lipid composition of the $VLDL_1$ core, was significantly lower in the Exercise trial (Control: 0.17 ± 0.02, Exercise: 0.12 ± 0.02, P = 0.007). Consequently, the CE/apoB ratio, reflecting CE content per $VLDL_1$ particle, was not significantly different between both trials (Control: 2487 ± 353, Exercise: 2276 ± 564, P = 0.537). PL was not detectable for one subject and, consequently, the FC/PL ratio in both trials for this subject were not included. There was no significant difference in the FC/PL ratio, which reflects the composition of the $VLDL_1$ surface layer, between the Control (0.32 ± 0.01) and Exercise (0.32 ± 0.02) trials (P = 0.606, n = 11).

Moreover, there was no significant difference in the fasting ratios of apoC-II/apoB (Control: 32.6 ± 5.0, Exercise: 42.8 ± 8.6, P = 0.23), apoC-III/apoB (Control: 64.1 ± 7.9, Exercise: 83.8 ± 13.9, P = 0.10) or apoE/apoB (Control: 0.83 ± 0.20, Exercise: 1.12 ± 0.33, P = 0.409, n = 10).

Table 5.2: Plasma and $VLDL_1$ concentrations in the fasted state

	Control	Exercise	*P*-Value
Plasma TG ($mmol.l^{-1}$)	1.54 ± 0.16	1.21 ± 0.15	0.004
Total Cholesterol ($mmol.l^{-1}$)	5.17 ± 0.25	5.14 ± 0.24	0.843
HDL Cholesterol ($mmol.l^{-1}$)	1.05 ± 0.06	1.08 ± 0.07	0.233
LDL Cholesterol ($mmol.l^{-1}$)	3.38 ± 0.31	3.48 ± 0.29	0.409
Glucose ($mmol.l^{-1}$)	5.76 ± 0.09	5.58 ± 0.09	0.010
Insulin ($mU.l^{-1}$)	6.99 ± 1.30	6.29 ± 1.46	0.080
$HOMA_{IR}$	1.81 ± 0.35	1.58 ± 0.37	0.059
NEFA ($mmol.l^{-1}$)	0.55 ± 0.04	0.64 ± 0.03	0.026
$VLDL_1$ concentration ($mg.dl^{-1}$)	94.9 ± 14.1	62.9 ± 11.4	0.006
$VLDL_1$-TG ($mmol.l^{-1}$)	0.67 ± 0.10	0.46 ± 0.08	0.007
$VLDL_1$-C ($mmol.l^{-1}$)	0.47 ± 0.07	0.28 ± 0.06	0.006
$VLDL_1$-apo B ($mg.dl^{-1}$)	2.77 ± 0.44	1.55 ± 0.30	0.004
$VLDL_1$-apo CII ($mg.dl^{-1}$)	1.22 ± 0.18	0.88 ± 0.16	0.017
$VLDL_1$-apo CIII ($mg.dl^{-1}$)	2.52 ± 0.40	1.84 ± 0.37	0.031
$VLDL_1$-apo E ($mg.dl^{-1}$)*	0.16 ± 0.06	0.11 ± 0.05	0.049

* n = 10. All values are mean ± SEM. *P* values are for the difference between the Control and Exercise trials

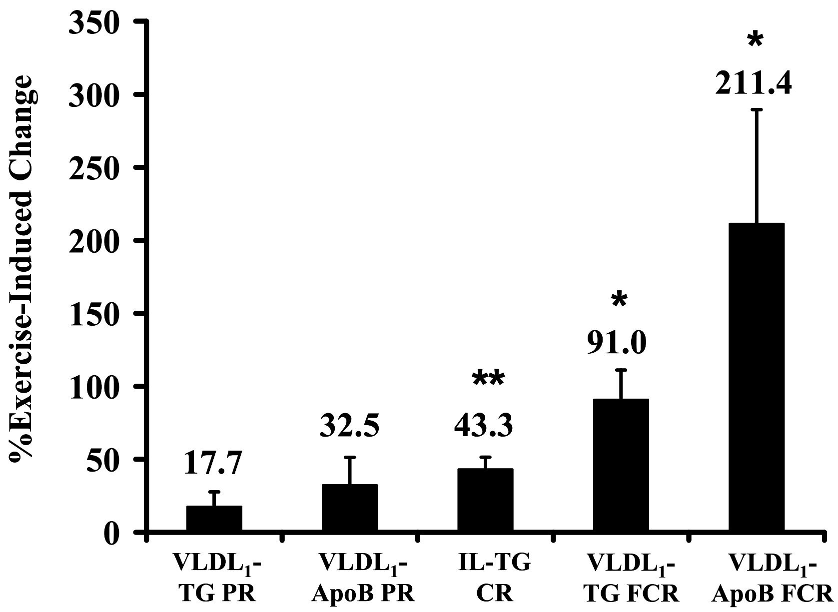


Figure 5.1: The mean percentage change in $VLDL_1$-TG and $VLDL_1$-apoB production rates (PR) and fractional catabolic rates (FCR) and Intralipid (IL)-TG clearance rate (CR) in response to a single session of 120-min brisk walking at ~50% VO_{2max}. (* P <0.05, ** P <0.001).

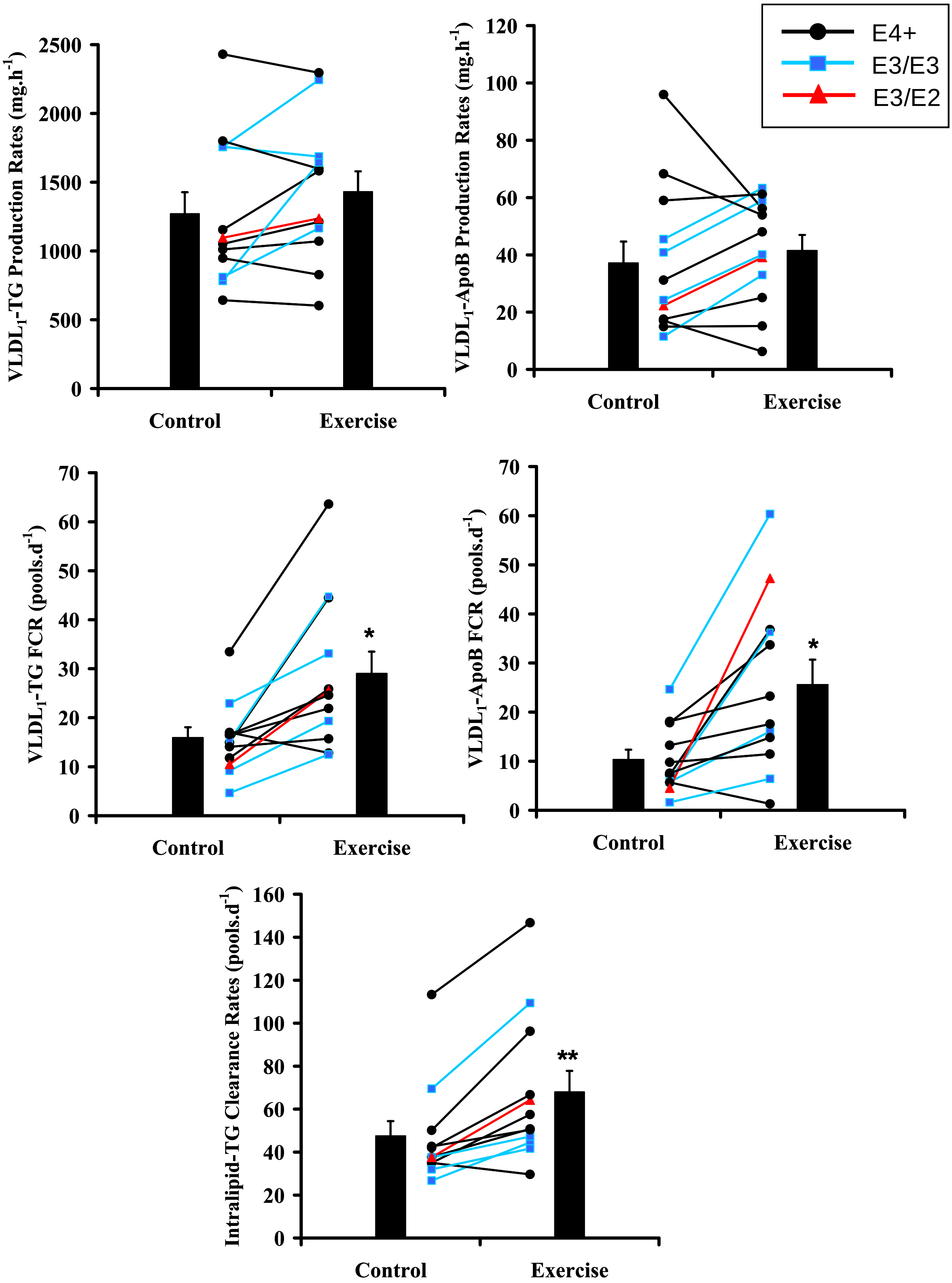


Figure 5.2: Individual (according to apoE phenotype) and mean (bars) VLDL$_1$-TG and VLDL$_1$-apoB production rates (top panel) and fractional catabolic rates (FCR, middle panel) and Intralipid-TG clearance rate (bottom) in response to a single session of 120-min brisk walking at ~50% VO_{2max} (* *P* <0.05, ** *P* <0.001 for the difference in mean response between the Control and Exercise trials).

Table 5.3: Exercise-induced compositional changes to circulating $VLDL_1$ particles

	Control	Exercise	*P*-Value
TG/apoB ratio (mol:mol)	15392 ± 2178	19449 ± 3150	0.059
CE/TG ratio (mol:mol)	0.17 ± 0.02	0.12 ± 0.02	0.007
CE/apoB ratio (mol:mol)	2487 ± 353	2276 ± 564	0.537
FC/PL ratio (mg:mg)*	0.32 ± 0.01	0.32 ± 0.02	0.606

* n = 11. All values are mean ± SEM.

5.3.5 Predictors of the Exercise-Induced Changes in TRL Kinetics

There was a significant correlation (r = 0.61, P = 0.035) between the exercise-induced change in Intralipid-TG clearance rate and the exercise-induced change in $VLDL_1$-TG FCR (**Figure 5.3A**), which suggests that a single mechanism (likely LPL- mediated clearance) was likely to be responsible for both effects.

Although there was no statistically significant effect of exercise on fasting insulin concentrations and $HOMA_{IR}$, a significant inverse relationship was found between the exercise-induced change in $VLDL_1$-apoB FCR and the exercise-induced change in insulin concentrations (r = -0.65, P = 0.022), the exercise-induced change in glucose (r = -0.58, P = 0.049), and, consequently, with the exercise-induced change in $HOMA_{IR}$ (r = -0.68, P = 0.016, **Figure 5.3B**). This shows that those who had the biggest reduction in $HOMA_{IR}$ had the biggest increase in $VLDL_1$-apoB FCR.

No significant correlations were found between ALT or the exercise-induced change in NEFA and any of the exercise-induced changes in $VLDL_1$-TG and -apoB FCR or production rates or Intralipid-TG clearance rate (all P > 0.05). In addition, there was no significant effect of apoE phenotype on the response of the above kinetics to moderate exercise.

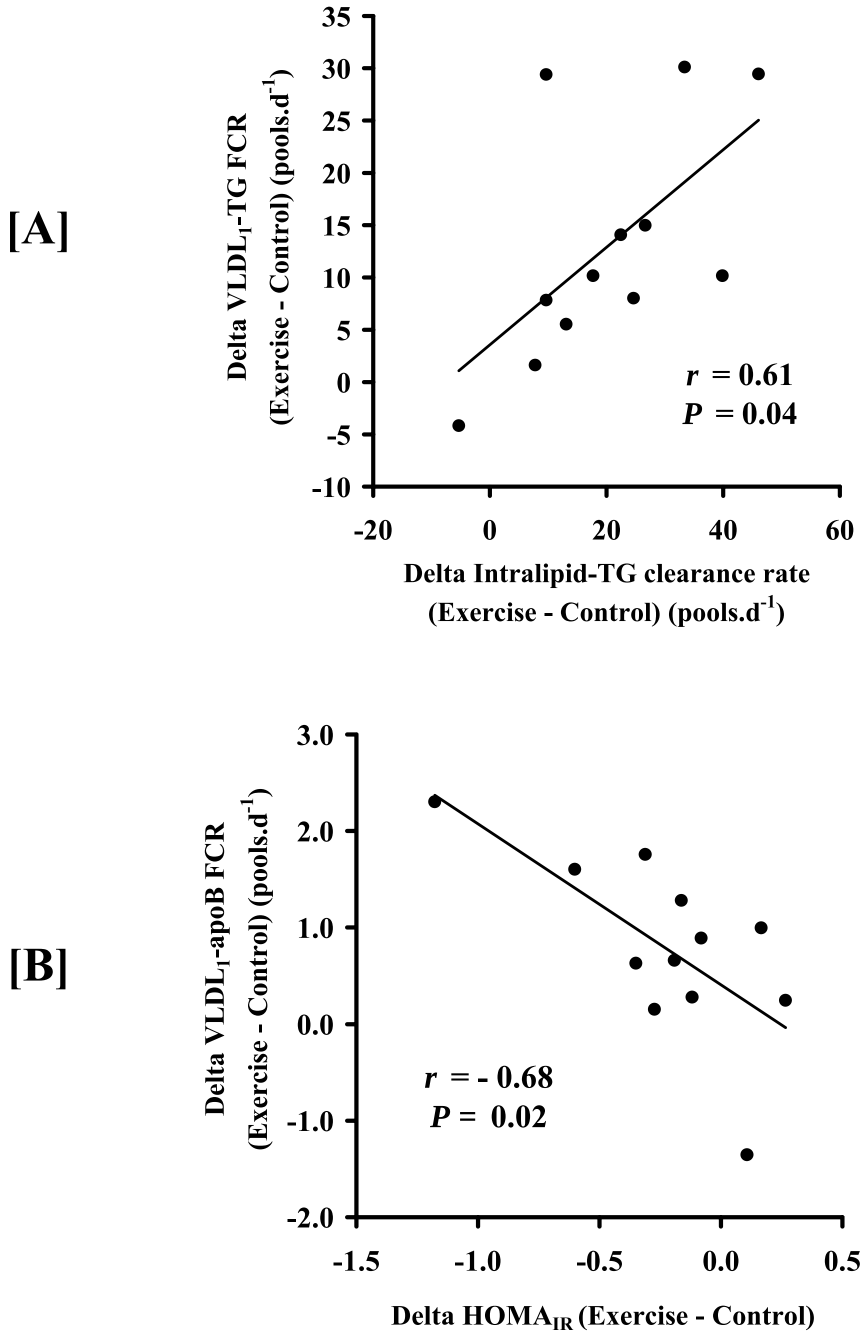


Figure 5.3: Scattergrams indicating the relationships between the exercise-induced changes (Exercise minus Control) in [A] Intralipid-TG and $VLDL_1$-TG FCR (pools.d^{-1}) and [B] $VLDL_1$-apoB FCR (pools.d^{-1}) and $HOMA_{IR}$.

5.4 Discussion

To the best of the author's knowledge, this is the first study to investigate the possible mechanism(s) underlying the hypotriglyceridaemic effect of a prior session of moderate exercise (120-min brisk walk at ~50% $\dot{V}O_{2max}$) in overweight/obese middle-aged men, a group typically targeted in exercise-for-health guidelines.

The results of the present study confirm and expand on earlier observations that a prior session of moderate exercise induces a significant reduction in plasma TG and influences $VLDL_1$ to a greater extent than chylomicrons (Gill *et al.*, 2006). Indeed, in the present study, the change in $VLDL_1$-TG clearance was greater than the change in Intralipid change (91.0% *vs.* 43.3%, respectively). Because chylomicrons are preferred substrates for LPL (Fisher *et al.*, 1995) and, therefore, compete with $VLDL_1$ particles for a LPL-mediated clearance (Karpe *et al.*, 1993b), it was hypothesised earlier that the exercise-induced TG reduction was likely to be the result of a reduced hepatic $VLDL_1$ production rather than increased clearance (Malkova & Gill, 2006). However, this does not seem to be the case. The present results demonstrate that this exercise-induced reduction in TG is due to a significant 91% increase in $VLDL_1$-TG FCR (i.e. clearance), with no significant effect on $VLDL_1$-TG production rate. This is consistent with two recent stable-isotope studies by Magkos *et al.* (Magkos *et al.*, 2006) and Tsekouras *et al.* (Tsekouras *et al.*, 2007) who reported an increased total VLDL-TG clearance, rather than decreased VLDL-TG production following a session of 120-min cycling and 90-min walking (at 60% $\dot{V}O_{2max}$), respectively.

The novelty of this study, however, is that it allowed the simultaneous measurements of $VLDL_1$-TG FCR and Intralipid-TG clearance rate in response to a prior session of moderate exercise. This is important in providing a better understanding of the major possible mechanism(s) involved in the exercise-induced hypotriglyceridaemia as it was difficult to measure the clearance of $VLDL_1$-TG in the presence of chylomicrons/Intralipid because of their competition for the same catabolic pathway (Bjorkegren *et al.*, 1996; Karpe & Hultin, 1995). Importantly, the present results show a significant correlation (r = 0.61, P = 0.035) between the exercise-induced changes in Intralipid-TG and in $VLDL_1$-TG indicating that exercise is upregulating their clearance by the same mechanism/pathway (i.e. LPL). However, it is unclear why $VLDL_1$ appears to develop a higher affinity following exercise for clearance by LPL relative to Intralipid, but this could be due to the compositional changes in the $VLDL_1$ particle post-exercise: the particles in the circulation tended to be ~26% bigger following exercise and more TG-enriched, which may render them more favourable substrates for LPL-mediated lipolysis (Fisher *et al.*, 1995). There are two possible explanations as to why this might be the case. Firstly, the liver might be producing a bigger, more TG-enriched $VLDL_1$ particle following exercise. Consistant with this, circulating NEFA concentrations were 16% higher in the exercise trial than the Control trial, increasing fatty acid availability to the liver, which could lead to larger secreted $VLDL_1$ particle. Secondly, under normal conditions, once in the circulation, the TG content of the newly secreted $VLDL_1$ particle, and consequently its size, are being reduced rapidly by hydrolysis by LPL (Karpe *et al.*, 2007) and the action of CETP, which promotes the transfer of TG from apoB-containing particles (VLDL and LDL) in exchange for CE from HDL (Barter, 2002). The present results show a significant ~29% reduction in $VLDL_1$ CE/TG ratio in the Exercise trial, which is supported by a similar 18% post-exercise reduction in CE/TG ratio reported by Gill *et al* (Gill *et al.*, 2006). This may be suggestive of a reduced *in vivo* CETP activity following moderate exercise, rendering the $VLDL_1$ particle more TG-enriched. However, this explanation is less likely to occur as the CE/apoB ratio, representing the CE content per $VLDL_1$ particle, in the present study was not significantly different between the two trials (Control: 2487 ± 353, Exercise: 2276 ± 564, $P = 0.537$). Unfortunately, conclusive results about the effect of moderate exercise on CETP activity or mass are currently lacking in the literature. While some studies found an increase in CETP mass following exercise (Thomas *et al.*, 2001), others reported an exercise-induced decrease in CETP mass (Magkos *et al.*, 2006). Two other studies found no significant difference in CETP activity post-moderate exercise (Thomas *et al.*, 2004) even after 24 h (Zhang *et al.*, 2002).

Furthermore, although there was no significant difference in the number of apos C-II, C-III and E per $VLDL_1$ between the Exercise and Control trials in this study, a reduced apoC-III and apoE enrichment of $VLDL_1$ was reported in a similar group of participants following moderate exercise (Gill *et al.*, 2006). These apolipoproteins are acquired by VLDL from circulating HDL and apoC-III (Wang *et al.*, 1985) and apo E (Jong *et al.*, 1997) both act to inhibit lipolysis by LPL. This could conceivably have increased the potential of post-exercise $VLDL_1$ particle as an LPL substrate. It is unclear why the present findings differ from the earlier report.

The present results also show a significant 43% increase in Intralipid-TG clearance rate post-exercise, which is likely to reflect increased LPL activity and/or mass. Although early studies of prolonged and vigorous exercise, such as marathon running, clearly demonstrated that this type of exercise increased LPL-mediated TG-clearance (Sady *et al.*, 1986), recent studies of a more moderate exercise are conflicting. While some studies show no significant difference in muscle (Herd *et al.*, 2001) or post-heparin plasma LPL activity (reflecting overall LPL activity from all body tissues) ~16h after a moderate session of exercise (Gill *et al.*, 2003), others reported a significant increase in plasma (Zhang *et al.*, 2002), muscle and adipose tissue LPL activity post-exercise (Perreault *et al.*, 2004). In addition, although Magkos *et al.* (Magkos *et al.*, 2006) found no significant difference in muscle LPL mass, the group reported a significant ~20% increase in plasma LPL concentrations in response to acute moderate exercise. It should be noted, though, that even in the absence of a significant increase in post-heparin LPL activity, the exercise-induced changes in LPL activity was reported to correlate significantly with the exercise-induced changes in fasting and postprandial TG (Gill *et al.*, 2003). Furthermore, if exercise increased the affinity of $VLDL_1$ for clearance, this could have occurred without an increase in measured LPL action using a standard substrate. This further highlights the importance of LPL as a mediator of the TG-lowering effect of moderate exercise.

In addition to increased $VLDL_1$-TG FCR, exercise significantly increased $VLDL_1$-apoB FCR by 211% with no significant influence on $VLDL_1$-apoB production rate. This is in contrast with Magkos *et al.* (Magkos *et al.*, 2006) who reported a significant reduction in total VLDL-apoB production rate with no significant change in VLDL-apoB clearance. A likely explanation for these different findings could be due to the different characteristics of participants. Unlike the normal weight subjects who participated in Magkos *et al.* study (Magkos *et al.*, 2006), the participants in the present study were overweight/obese men with increased abdominal obesity, which makes them likely candidates for insulin resistance and high liver fat content (Karpe & Tan, 2005; Haffner, 2007; Després, 2007; Adiels *et al.*, 2006b). Similar group of subjects with increased liver fat failed to suppress $VLDL_1$ production in response to insulin (Adiels *et al.*, 2007). Indeed, the participants in the present study had higher insulin concentrations compared with the study subjects of Magkos *et al.* (Magkos *et al.*, 2006) (7.0 *vs.* 5.4 $mU.l^{-1}$, respectively) and no significant reductions in insulin or $HOMA_{IR}$ concentrations were observed following exercise in the present study. Interestingly, however, there was a significant correlation in the present study between the exercise-induced change in $HOMA_{IR}$ and the exercise-induced change in $VLDL_1$-apoB FCR, indicating that subjects who had a bigger decrease in $HOMA_{IR}$ following exercise had a bigger increase in $VLDL_1$-apoB FCR (clearance). These exercise-induced changes in insulin sensitivity may augment changes in $VLDL_1$-apoB clearance, but this relationship was not observed for $VLDL_1$-TG.

In summary, a single session of moderate intensity exercise significantly reduced fasting plasma TG, mainly in the $VLDL_1$ fraction, by increasing the $VLDL_1$-TG and $VLDL_1$-apoB FCR (i.e. clearance). The increase in $VLDL_1$ was greater than the increase in Intralipid clearance, suggesting that exercise may have increased the affinity of $VLDL_1$ for LPL-mediated lipolysis (**Figure 5.4**). In contrast, exercise did not decrease hepatic $VLDL_1$-TG or $VLDL_1$-apoB production. However, further studies are needed elucidate the mechanism(s) underlying this increased affinity for clearance of $VLDL_1$, such as *in vitro* studies investigating the affinity of $VLDL_1$ for LPL as well as the change in CETP activity both under control and exercise conditions. This may help improve understanding of the nature of these exercise-induced changes.

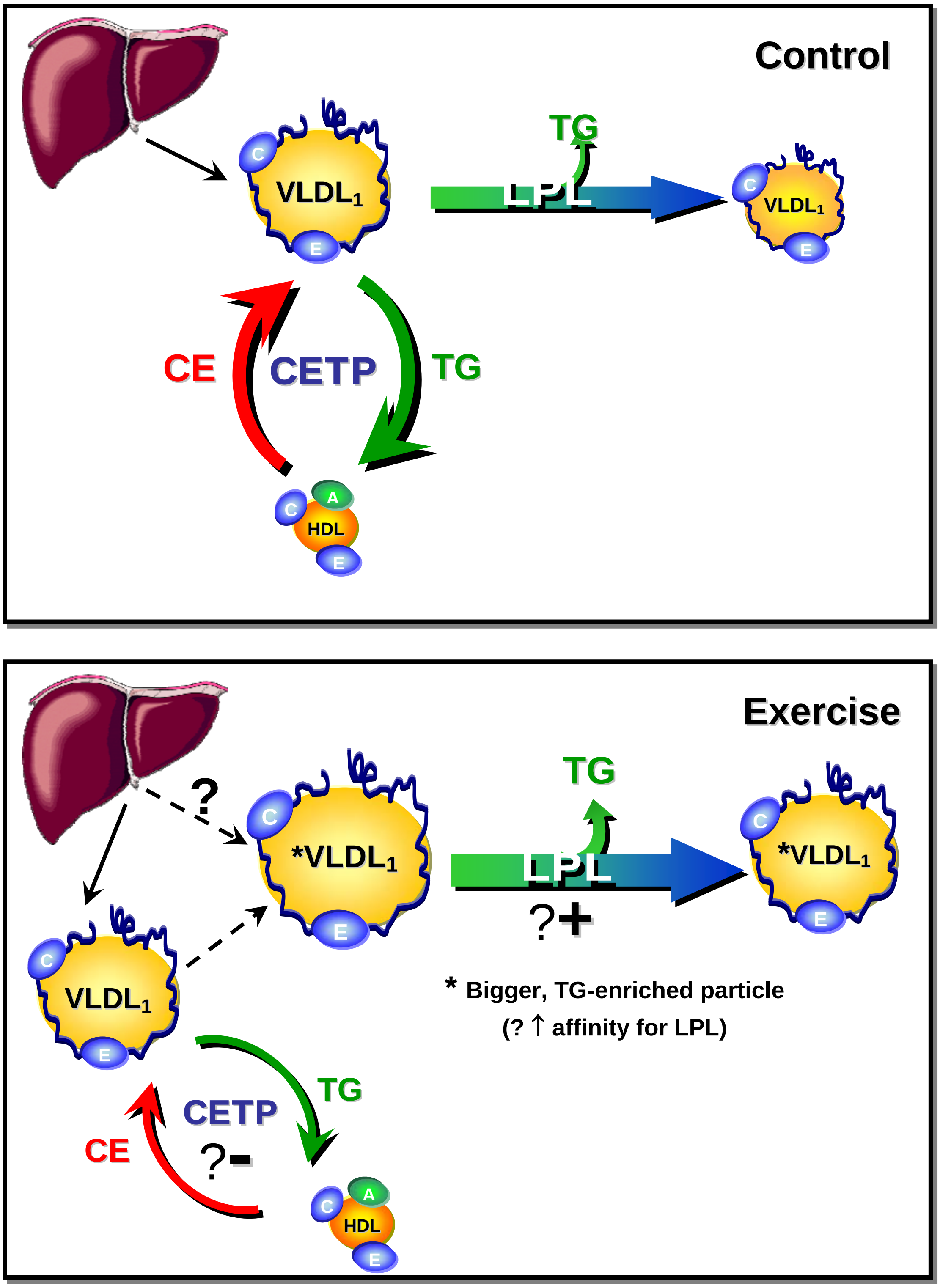


Figure 5.4: Possible mechanisms involved in moderate exercise-induced reduction in VLDL$_1$. Control: once in the circulation, the TG content of the newly secreted VLDL$_1$ particle from the liver, and consequently its size, are being reduced rapidly by hydrolysis by lipoprotein lipase (LPL) and the action of cholesteryl ester transfer protein (CETP), which promotes the transfer of TG from VLDL (and LDL) in exchange for cholestoryl ester (CE) from HDL. **Exercise:** A prior session of moderate exercise significantly reduces VLDL$_1$ concentration by increasing its clearance from plasma, possibly by compositional changes to the VLDL$_1$ particle which render it bigger in size and more TG-enriched, thereby increase its affinity for LPL-mediated lipolysis. The bigger particles are either produced directly by the liver and/or reduced CETP activity. Also, increased LPL activity is likely to play a role.

6. General Discussion

6.1 Summary

There is a growing body of evidence implicating elevated VLDL concentrations, particularly the larger $VLDL_1$ (S_f 60-200) particles, as the primary cause of the atherogenic lipoprotein phenotype in insulin resistance conditions; such as type 2 diabetes (Taskinen, 2003), metabolic syndrome (Grundy, 2006), obesity (Aguilera *et al.*, 2008) and, eventually, coronary heart disease (Abbasi *et al.*, 2002; Tanaka *et al.*, 2001). The fact that this atherogenic dyslipidaemia is detectable years before the onset of the disease (Tilly-Kiesi *et al.*, 1996; Johanson *et al.*, 2004) makes the measurement and regulation of hepatic $VLDL_1$ production of great importance. However, mere measurements of $VLDL_1$ concentrations provide limited information and do not reflect the dynamics of the continual synthesis and clearance of $VLDL_1$ particles. Radioactive and stable-isotope tracers have been successfully used to measure the kinetics of different lipoproteins, including $VLDL_1$. Although such methods yield a large amount of useful information, they are expensive, labour-intensive, time consuming and require the use of specialised laboratories, equipment and skills in multicompartmental modelling. Of note, these methods measure generally either total VLDL-TG and -apoB, or $VLDL_1$- and $VLDL_2$-apoB, but not $VLDL_1$- or $VLDL_2$-TG. It is only recently that Adiels and colleagues (Adiels *et al.*, 2005a) have developed a multicompartmental stable-isotope method to determine both $VLDL_1$- and $VLDL_2$-TG and -apoB kinetics. Thus, this thesis was designed to develop and validate a simpler and cost-effective method – the 'Intralipid Method' – to determine $VLDL_1$ kinetics and use this method to study the effects of insulin and moderate exercise on the regulation of hepatic $VLDL_1$ kinetics.

The method builds on the work of Björkegren *et al.* (Bjorkegren *et al.*, 1996) and relies on the fact that Intralipid (chylomicron-like particles) compete with $VLDL_1$ for the same catalytic pathway (Bjorkegren *et al.*, 1996; Karpe & Hultin, 1995), which allows the accumulation of $VLDL_1$ in the circulation (Bjorkegren *et al.*, 1996) during a constant Intralipid infusion. By measuring the linear rise in $VLDL_1$-TG and -apoB concentrations, it is possible to determine their production rates. In addition, because the method is employed in the fasted state in which there is a steady state where production equals clearance, it is also possible to determine the fractional catabolic rate (FCR, i.e. clearance) of $VLDL_1$-TG and -apoB by dividing their linear rises during the Intralipid infusion by their fasting concentrations. Furthermore, the Intralipid method combines the findings of Rössner (Rossner, 1974), who showed that Intralipid is cleared following first-order kinetics, which allows the determination of Intralipid-TG clearance rate as a surrogate measure of chylomicron-TG clearance.

In addition to the fact that the kinetic data obtained using the Intralipid method are similar to that obtained by the 'gold standard' stable-isotope method (Bjorkegren *et al.*, 1996), this newly developed method has a number of strengths. First, it provides valuable information about TRL kinetics: both $VLDL_1$-TG and $VLDL_1$-apoB production rates *and* Intralipid-TG clearance rate. In addition, under steady state conditions, it allows the simultaneous determination of large $VLDL_1$-TG (rather than total VLDL-TG) and Intralipid-TG clearance rates. This is important for two reasons: (1) it is the high concentrations of large $VLDL_1$ particles that are implicated in atherogenic dyslipidaemia and the progression of atherosclerosis (Taskinen, 2003; Packard, 2003) and, unlike $VLDL_2$, $VLDL_1$ are the target for insulin-induced hepatic suppression (Gill *et al.*, 2004a); (2) due to the fact that Intralipid/chylomicrons and $VLDL_1$ compete for the same LPL-mediated catalytic pathway, it has previously been difficult to determine the clearance rate of $VLDL_1$ in the presence of Intralipid or chylomicrons. As a result, the Intralipid method helped to elucidate the hypotryglyceridaemic effects of exercise on TRL kinetics (Chapter 5). Second, the Intralipid method is sensitive enough to detect physiological changes in hepatic $VLDL_1$ production and Intralipid-TG clearance, such as the effect of insulin on TRL kinetics (Chapter 4). Third, the method is relatively easy and simple enough to be used in any laboratory equipped with only one ultracentrifuge without the need for any other specialised equipment (such as a mass spectrometer). Fourth, estimation of production and clearance rates using the Intralipid method is relatively easy to carry out and can be automated using Excel. Fifth, the protocol for the Intralipid method takes less time to complete than the stable-isotope method, which is beneficial both for the subject and the researcher. While a standard stable-isotope method to measure $VLDL_1$ kinetics requires ~48 h of the subject's time, the Intralipid infusion and post-infusion period of the present method lasts only for about 3.5 h. In addition, unlike the time required (at least 2 weeks) for laboratory techniques and sample and kinetic analyses using the stable-isotope method, the Intralipid method protocol, including lipoprotein separation, sample analysis and kinetic calculations, takes approximately 2-3 days. Sixth, the Intralipid method is cheaper than the stable-isotope method and suitable for laboratories with limited funding or resources as each trial (including investigator/technician time at £20 per hour) costs approximately £750 and 2-3 days to complete compared to the ~£2500 and about two weeks using the stable-isotope method. Finally, because the Intralipid method is safe as well as both time- and cost-effective, it can be used for a large number of subjects, with even multiple trials for each subject.

As a first application, the Intralipid method was used to study the effect of hyperinsulinaemia and hyperglycaemia on $VLDL_1$-TG and -apoB production rates in 8 normoglycaemic subjects (Chapter 4). This was done using a simple frequent oral administration of glucose to provide a pseudo-steady hyperinsulinaemic state as the use of a conventional hyperinsulinaemic normoglycaemic clamp was not possible due to lack of constant medical cover. The result demonstrated that insulin acutely suppressed $VLDL_1$-TG and -apoB production, a finding recently supported by Adiels *et al.* (Adiels *et al.*, 2007). Although hyperinsulinaemia had no apparent effect on Intralipid-TG clearance rate, the change seemed to be related to insulin sensitivity, i.e. the more insulin sensitive the subject was, the biggest the increase in Intralipid-TG clearance rate. One possible limitation of this study is the number of subjects, especially with such a wide range of insulin resistance, which may have diluted the effect of insulin on Intralipid-TG clearance rate. However, the technique would make it very easy to increase the numbers in future studies.

Because moderate exercise has been shown to decrease plasma TG by 20-25%, mostly in the $VLDL_1$ fraction (Gill *et al.*, 2006), the second application of the Intralipid method was to investigate whether this decrease is due to decreased hepatic $VLDL_1$ production, increased clearance or a combination of both (Chapter 5). The present results showed that a session of moderate exercise significantly increased the clearance of $VLDL_1$-TG and -apoB rather than suppress their hepatic production. Although there is no information in the literature about the effect of exercise on

$VLDL_1$ kinetics specifically, these results are consistent with recent findings using stable-isotopes which investigated the effect of moderate exercise on total VLDL-TG (Magkos *et al.*, 2006; Tsekouras *et al.*, 2007) and VLDL-apoB (Magkos *et al.*, 2006) using the stable-isotope method. In addition, because of the nature of the Intralipid method, it provided the opportunity to simultaneously study both Intralipid and $VLDL_1$ clearance, which was previously unfeasible. As a result, a novel finding is observed that this increased exercise-induced clearance of $VLDL_1$ is likely to be the result of increased affinity of $VLDL_1$ to LPL-mediated lipolysis. Furthermore, this study is the first to investigate the kinetics of such effects of exercise in overweight/obese middle-aged men, a group typically targeted in exercise-for-health guidelines.

Adequate liver function is important for $VLDL_1$ homeostasis. Serum ALT concentrations are used clinically as an indication of liver function and, within normal range, have been shown to be a marker for liver fat (Westerbacka *et al.*, 2004). It was recently shown that hepatic overproduction of $VLDL_1$ particles is driven by liver fat accumulation (Adiels *et al.*, 2006b). Results from this thesis show a significant relationship between serum ALT concentrations and hepatic $VLDL_1$ production and regulation. Even within normal range, ALT concentrations correlated significantly with $VLDL_1$ production (Chapter 3) and inversely with the extent to which $VLDL_1$ is suppressed in response to hyperinsulinaemia and hyperglycaemia (Chapter 4): subjects who had higher ALT concentrations, produced more $VLDL_1$ particles and failed to suppress $VLDL_1$ secretion in response to insulin. It is unclear, however, whether this correlation with ALT concentrations reflects liver function *per se* or hepatic fat content, as ALT is a marker for both.

In all studies described in this thesis, 3 subjects were E2/3 phenotype, 11 subjects were E3/3 phenotype, 6 subjects were E3/4 phenotype and 3 subjects were E4/4 phenotype. Previous studies showed that apoE phenotypes influence plasma lipids (Eto *et al.*, 1986) and particularly large $VLDL_1$ concentrations (Bioletto *et al.*, 1998) as well as VLDL-apoB kinetics (Watts *et al.*, 2000; Welty *et al.*, 2000; Demant *et al.*, 1991). However, no apparent effect of apoE phenotype on $VLDL_1$-TG and -apoB production rates or Intralipid-TG clearance rate was observed in this thesis (Appendix C). It is uncertain, however, whether this is a true lack of influence of apoE on $VLDL_1$-TG and -apoB production rates and Intralipid-TG clearance rate or whether it may be due to lack of sufficient numbers for each phenotype. Fortunately, because of the relative ease and cost-effectiveness of the Intralipid method, a bigger study can be conducted which may have not been feasible otherwise using the stable-isotope method.

6.2 Limitations of the Intralipid Method

There are two main limitations to the Intralipid method. The first is that it only allows the determination of $VLDL_1$ kinetics but not $VLDL_2$. This is because $VLDL_2$ is hydrolysed by HL; i.e. Intralipid does not compete with $VLDL_2$ for LPL-mediated lipolysis and, consequently, $VLDL_2$ will not accumulate in the circulation like $VLDL_1$ particles. Although $VLDL_1$ and $VLDL_2$ are regulated independently (Packard & Shepherd, 1997; Gill *et al.*, 2004a), it has been recently reported that while insulin acutely reduced $VLDL_1$ secretion, it increased $VLDL_2$ secretion (Adiels *et al.*, 2007). Although this acute suppression of $VLDL_1$ secretion by insulin was detected using the Intralipid method, it was not possible to investigate this effect on $VLDL_2$. Thus, the addition of $VLDL_2$ kinetics might have helped to complete the picture of the effect of insulin on total VLDL hepatic regulation. The second limitation of the Intralipid method is that the FCR can only be calculated if the subject is in a steady state. For example, it was not possible to determine $VLDL_1$-TG and $VLDL_1$-apoB FCRs in Chapter 4 where $VLDL_1$ production was investigated in response to hyperinsulinemia and hyperglycemia. This is because the steady state value used for FCR calculation is made in the fasted state before the glucose ingestion, which does not represent the steady state value for $VLDL_1$ concentration during glucose ingestion.

Furthermore, the lack of direct comparison with either the stable-isotope or radioactive tracer methods within the same subjects represents a limitation to the validation of the method. Although Björkegren *et al.* (1996) reported a close agreement between the two methods, ideally, further studies on a larger scale should be performed in the future to validate the Intralipid method against the currently used 'gold-standard' methods.

6.3 Future Experiments and Applications of the Method

Results from Chapter 5 suggest that moderate exercise is likely to increase $VLDL_1$ clearance by increasing its size and TG-enrichment, thereby increasing its affinity for LPL-mediated lipolysis. Reports from the literature with respect to the effect of moderate exercise on LPL and CETP activities, which are responsible for $VLDL_1$ TG-enrichment, are conflicting. Therefore, it would be helpful to conduct the following two studies. The first is an *in vitro* study investigating the suggested higher affinity of $VLDL_1$ to LPL post-exercise. This could possibly be done by incubating LPL (obtained commercially or post heparin from a donor) with different concentrations (and possibly at different durations) of the subject's native $VLDL_1$ particles separated from the Control and Exercise trials. $VLDL_1$ lipolysis is then determined by measuring the released NEFA from $VLDL_1$ by LPL over time. In addition, Intralipid could also be incubated with native $VLDL_1$ and LPL to investigate the relative competition between Intralipid and $VLDL_1$ particles for LPL-mediated lipolysis in both the Control and Exercise trials.

Secondly, it is important to compare the activity of CETP in control and post-exercise as data from Chapter 5 suggest an increased TG-enrichment of $VLDL_1$ particles post-exercise. However, current commercial methods that measure CETP activity use the same concentration for donor and accepter particles in both exercise and control trials, which does not necessarily reflect physiological conditions as *in vivo* concentrations of VLDL particles differ between both conditions. Therefore, it would be better to measure CETP activity in an assay in which native $VLDL_1$ from the Exercise and Control trials are used as donor and accepter particles.

These two experiments would aid a better understanding of the effect of exercise on $VLDL_1$ and test the hypothesis that the increased clearance of $VLDL_1$ post-exercise is due to the increased size and TG-enrichment of the $VLDL_1$ particle.

Finally, previous studies have shown that the relationships between obesity, insulin resistance, metabolic syndrome and risk for cardiovascular disease are subject to ethnic variations (Joshi *et al.*, 2007; Lear *et al.*, 2003; Whincup *et al.*, 2002). For example, compared with Caucasian men, South Asian men have excess subcutaneous abdominal adiposity, in spite of similar BMI, which renders them more susceptible to insulin resistance than white men (Chandalia *et al.*, 2007). In addition, although Afro-Caribbean men in the United Kingdom are reported to be as insulin-resistant as South Asian men, they have been found to be less susceptible to the dyslipidaemia that accompanies insulin resistance (Zoratti *et al.*, 2000). Interestingly, Zoratti *et al.* (2000) reported that this favourable lipoprotein profile in Afro-Caribbean may relate to an effective VLDL metabolism. However, there is currently no information available on the effect of ethnicity on $VLDL_1$ kinetics. Therefore, an important application of the Intralipid method would be to investigate $VLDL_1$ kinetics in a different ethnic population. In addition, the method could be used to study $VLDL_1$ kinetics in response to moderate exercise in Kuwaiti subjects and compare that with results from those obtained in Scottish men. Kuwait (the author's homeland) has a high prevalence of obesity (Al-Kandari, 2006), insulin resistance (Al-Shaibani *et al.*, 2004) and type 2 diabetes (Al-Adsani *et al.*, 2004) and ischemic heart diseases are a major cause of death in the country (El-Shazly *et al.*, 2004).

6.4 Conclusion

In conclusion, this thesis describes the development and validation a novel method to determine the kinetics of large $VLDL_1$, and the application of this method to investigate the effects of hyperinsulinaemia/hyperglycaemia and moderate exercise on $VLDL_1$ kinetics. The method is relatively straightforward and cost-effective which means it has potential for future widespread use in situations where isotopic tracer methods are not feasible because of technical or cost limitations.

7. References

Abbasi, F., Brown, B. W., Jr., Lamendola, C., McLaughlin, T. & Reaven, G. M. (2002). Relationship between obesity, insulin resistance, and coronary heart disease risk. *Journal of the American College of Cardiology* **40**: 937-943.

ACSM (2006). American College of Sports and Medicine. Guidelines for exercise testing and prescription, 7th ed. Lippincott Williams & Wilkins, Philadelphia.

Adiels, M., Olofsson, S. O., Taskinen, M. R. & Borén, J. (2006a). Diabetic dyslipidaemia. *Current Opinion in Lipidology* **17**: 238-246.

Adiels, M., Packard, C., Caslake, M. J., Stewart, P., Soro, A., Westerbacka, J., Wennberg, B., Olofsson, S. O., Taskinen, M. R. & Borén, J. (2005a). A new combined multicompartmental model for apolipoprotein B-100 and triglyceride metabolism in VLDL subfractions. *Journal of Lipid Research* **46**: 58-67.

Adiels, M., Taskinen, M. R., Packard, C., Caslake, M. J., Soro-Paavonen, A., Westerbacka, J., Vehkavaara, S., Hakkinen, A., Olofsson, S. O., Yki-Jarvinen, H. & Borén, J. (2006b). Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* **49**: 755-765.

Adiels, M., Westerbacka, J., Soro-Paavonen, A., Hakkinen, A., Vehkavaara, S., Caslake, M., Packard, C., Olofsson, S., Yki-Jarvinen, H., Taskinen, M. & Borén, J. (2007). Acute suppression of VLDL1 secretion rate by insulin is associated with hepatic fat content and insulin resistance. *Diabetologia* **50**: 2356-2365.

Adiels, M., Borén, J., Caslake, M. J., Stewart, P., Soro, A., Westerbacka, J., Wennberg, B., Olofsson, S. O., Packard, C. & Taskinen, M. R. (2005b). Overproduction of VLDL1 Driven by Hyperglycemia Is a Dominant Feature of Diabetic Dyslipidemia. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**: 1697-1703.

Aguilera, C. M., Gil-Campos, M., Canete, R. & Gil, A. (2008). Alterations in plasma and tissue lipids associated with obesity and metabolic syndrome. *Clinical Science* **114**: 183-193.

Al-Adsani, A., Memon, A. & Suresh, A. (2004). Pattern and determinants of dyslipidaemia in type 2 diabetes mellitus patients in Kuwait. *Acta Diabetologica* **41**: 129-135.

Al-Kandari, Y. Y. (2006). Prevalence of obesity in Kuwait and its relation to sociocultural variables. *Obesity Reviews* **7**: 147-154.

Al-Shaibani, H., El Batish, M., Sorkhou, I., Al-Shamali, N., Al-Namash, H., Habiba, S. & Al-Qallaf, B. (2004). Prevalence of insulin resistance syndrome in a primary health care center in Kuwait. *Family Medicine* **36**: 540.

Al-Shayji, I. A. R., Gill, J. M. R., Cooney, J., Siddiqui, S. & Caslake, M. J. (2007). Development of a novel method to determine very low density lipoprotein kinetics. *Journal of Lipid Research* **48**: 2086-2095.

Alam, S., Stolinski, M., Pentecost, C., Boroujerdi, M. A., Jones, R. H., Sonksen, P. H. & Umpleby, A. M. (2004). The effect of a six-month exercise program on very low-density lipoprotein apolipoprotein B secretion in type 2 diabetes. *Journal of Clinical Endocrinology Metabolism* **89**: 688-694.

Alaupovic, P. (1991). Apolipoprotein composition as the basis for classifying plasma lipoproteins. Characterization of ApoA- and ApoB-containing lipoprotein families. *Progress in Lipid Research* **30**: 105-138.

Allender, S., Peto, V., Scarborough, P., Boxer, A. & Rayner, M. (2006). Coronary heart disease statistics British Heart Foundation, London.

Allender, S., Peto, V., Scarborough, P., Boxer, A. & Rayner, M. (2007). Coronary heart disease statistics British Heart Foundation, London.

Altena, T. S., Michaelson, J. L., Ball, S. D., Guilford, B. L. & Thomas, T. R. (2006). Lipoprotein subfraction changes after continuous or intermittent exercise training. *Medicine and Science in Sports and Exercise* **38**: 367-372.

Altena, T. S., Michaelson, J. L., Ball, S. D. & Thomas, T. R. (2004). Single sessions of intermittent and continuous exercise and postprandial lipemia. *Medicine and Science in Sports and Exercise* **36**: 1364-1371.

Annuzzi, G., Iovine, C., Mandarino, B., Patti, L., Di Marino, L., Riccardi, G. & Rivellese, A. A. (2001). Effect of acute exogenous hyperinsulinaemia on very low density lipoprotein subfraction composition in normal subjects. *European Journal of Clinical Investigation* **31**: 118-124.

Arner, P. (1998). Not all fat is alike. *Lancet* **351**: 1301-1302.

Asp, L., Claesson, C., Borén, J. & Olofsson, S. O. (2000). ADP-ribosylation factor 1 and its activation of phospholipase D are important for the assembly of very low density lipoproteins. *The Journal of Biological Chemistry* **275**: 26285-26292.

Asp, L., Magnusson, B., Rutberg, M., Li, L., Borén, J. & Olofsson, S. O. (2005). Role of ADP Ribosylation Factor 1 in the Assembly and Secretion of ApoB-100-Containing Lipoproteins. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**: 566-570.

Avramoglu, R. K., Basciano, H. & Adeli, K. (2006). Lipid and lipoprotein dysregulation in insulin resistant states. *Clinica Chimica Acta* **368**: 1-19.

Bamba, V. & Rader, D. J. (2007). Obesity and atherogenic dyslipidemia. *Gastroenterology* **132**: 2181-2190.

Barrett, P. H. (1998). Kinetics of triglyceride rich lipoproteins: chylomicrons and very low density lipoproteins. *Atherosclerosis* **141**: 35-40.

Barrett, P. H., Chan, D. C. & Watts, G. F. (2006). Thematic review series: Patient-Oriented Research. Design and analysis of lipoprotein tracer kinetics studies in humans. *Journal of Lipid Research* **47**: 1607-1619.

Barrows, B. R. & Parks, E. J. (2006). Contributions of Different Fatty Acid Sources to Very Low-Density Lipoprotein-Triacylglycerol in the Fasted and Fed States. *Journal of Clinical Endocrinology Metabolism* **91**: 1446-1452.

Barrows, B. R., Timlin, M. T. & Parks, E. J. (2005). Spillover of Dietary Fatty Acids and Use of Serum Nonesterified Fatty Acids for the Synthesis of VLDL-Triacylglycerol Under Two Different Feeding Regimens. *Diabetes* **54**: 2668-2673.

Barter, P. J. (2002). Hugh Sinclair Lecture: The regulation and remodelling of HDL by plasma factors. *Atherosclerosis Supplements* **3**: 39-47.

Beylot, M., Martin, C., Beaufrere, B., Riou, J. P. & Mornex, R. (1987). Determination of steady state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer. *Journal of Lipid Research* **28**: 414-422.

Bickerton, A. S., Roberts, R., Fielding, B. A., Hodson, L., Blaak, E. E., Wagenmakers, A. J., Gilbert, M., Karpe, F. & Frayn, K. N. (2007). Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes* **56**: 168-176.

Bioletto, S., Fontana, P., Darioli, R. & James, R. W. (1998). Apolipoprotein E polymorphism and the distribution profile of very low density lipoproteins; an influence of the E4 allele on large (Sf > 60) particles. *Atherosclerosis* **138**: 207-215.

Bioletto, S., Golay, A., Munger, R., Kalix, B. & James, R. W. (2000). Acute hyperinsulinemia and very-low-density and low-density lipoprotein subfractions in obese subjects. *American Journal of Clinical Nutrition* **71**: 443-449.

Bjorkegren, J., Hamsten, A., Milne, R. W. & Karpe, F. (1997). Alterations of VLDL composition during alimentary lipemia. *Journal of Lipid Research* **38**: 301-314.

Bjorkegren, J., Karpe, F., Milne, R. W. & Hamsten, A. (1998). Differences in apolipoprotein and lipid composition between human chylomicron remnants and very low density lipoproteins isolated from fasting and postprandial plasma. *Journal of Lipid Research* **39**: 1412-1420.

Bjorkegren, J., Packard, C. J., Hamsten, A., Bedford, D., Caslake, M., Foster, L., Shepherd, J., Stewart, P. & Karpe, F. (1996). Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *Journal of Lipid Research* **37**: 76-86.

Bjorntorp, P. (1990). "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* **10**: 493-496.

Bland, J. M. & Altman, D. G. (1999). Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* **8**: 135-160.

Blasiole, D. A., Davis, R. A. & Attie, A. D. (2007). The physiological and molecular regulation of lipoprotein assembly and secretion. *Molecular BioSystems* **3**: 608-619.

Bloomgarden, Z. T. (2007). Insulin resistance, dyslipidemia, and cardiovascular disease. *Diabetes Care* **30**: 2164-2170.

Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. *Medicine and Science in Sports* **5**: 90-93.

Borsheim, E., Knardahl, S. & Hostmark, A. T. (1999). Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. *Medicine and Science in Sports and Exercise* **31**: 522-530.

Brown, A. M. & Gibbons, G. F. (2001). Insulin Inhibits the Maturation Phase of VLDL Assembly via a Phosphoinositide 3-Kinase--Mediated Event. *Arteriosclerosis, Thrombosis, and Vascular Biology* **21**: 1656-1661.

Bruce, R. A., Kusumi, F. & Hosmer, D. (1973). Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *American Heart Journal* **85**: 546-562.

Brunzell, J. D., Hazzard, W. R., Porte, D., Jr. & Bierman, E. L. (1973). Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *The Journal of Clinical Investigation* **52**: 1578-1585.

Burton, F. L., Malkova, D., Caslake, M. J. & Gill, J. M. (2008). Energy replacement attenuates the effects of prior moderate exercise on postprandial metabolism in overweight/obese men. *International Journal of Obesity* **32**: 481-489.

Byrne, W. J. (1982). Intralipid or Liposyn--comparable products? *Journal of Pediatric Gastroenterology and Nutrition* **1**: 7-8.

Carlson, L. A. & Hallberg, D. (1963). Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion and of chylomicrones in the dog after single injection. *Acta Physiologica Scandinavica* **59**: 52-61.

Carlson, L. A. & Rossner, S. (1972). A methodological study of an intravenous fat tolerance test with Intralipid R emulsion. *Scandinavian Journal of Clinical and Laboratory Investigation* **29**: 271-280.

Carpentier, Y. A. & Dupont, I. E. (2000). Advances in intravenous lipid emulsions. *World Journal of Surgery* **24**: 1493-1497.

Casillas, J. M., Gremeaux, V., Damak, S., Feki, A. & Perennou, D. (2007). Exercise training for patients with cardiovascular disease. *Annales de Readaptation et de Medecine Physique* **50**: 403-418.

Chan, D. C., Barrett, H. P. & Watts, G. F. (2004a). Dyslipidemia in visceral obesity: mechanisms, implications, and therapy. *American Journal of Cardiovascular Drugs* **4**: 227-246.

Chan, D. C., Chen, M. M., Ooi, E. M. & Watts, G. F. (2008). An ABC of apolipoprotein C-III: a clinically useful new cardiovascular risk factor? *International Journal of Clinical Practice* **62**: 799-809.

Chan, D. C. & Watts, G. F. (2006). Recent advances in the investigation of lipoprotein metabolism using tracer methodology. *Clinical Laboratory* **52**: 353-361.

Chan, D. C., Barrett, P. H. & Watts, G. F. (2004b). Lipoprotein transport in the metabolic syndrome: methodological aspects of stable isotope kinetic studies. *Clinical Science* **107**: 221-232.

Chandalia, M., Lin, P., Seenivasan, T., Livingston, E. H., Snell, P. G., Grundy, S. M. & Abate, N. (2007). Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PLoS ONE* **2**: e812.

Chirieac, D. V., Collins, H. L., Cianci, J., Sparks, J. D. & Sparks, C. E. (2004). Altered triglyceride-rich lipoprotein production in Zucker diabetic fatty rats. *AJP - Endocrinology and Metabolism* **287**: E42-E49.

Chung, B. H., Liang, P., Doran, S., Cho, B. H. S. & Franklin, F. (2004). Postprandial chylomicrons: potent vehicles for transporting cholesterol from endogenous LDL+HDL and cell membranes to the liver via LCAT and CETP. *Journal of Lipid Research* **45**: 1242-1255.

Cohen, J. C., Noakes, T. D. & Benade, A. J. (1989). Postprandial lipemia and chylomicron clearance in athletes and in sedentary men. *American Journal of Clinical Nutrition* **49**: 443-447.

Cohn, J. S., Johnson, E. J., Millar, J. S., Cohn, S. D., Milne, R. W., Marcel, Y. L., Russell, R. M. & Schaefer, E. J. (1993). Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *Journal of Lipid Research* **34**: 2033-2040.

Consolazio, C. F., Johnson, R. E. & Pecora, L. J. (1963). Physiological Measurements of Metabolic Functions in Man McGraw-Hill, New York.

Cooper, A. D. (1997). Hepatic uptake of chylomicron remnants. *Journal of Lipid Research* **38**: 2173-2192.

Coppack, S. W., Jensen, M. D. & Miles, J. M. (1994). In vivo regulation of lipolysis in humans. *Journal of Lipid Research* **35**: 177-193.

Cullen, P. (2000). Evidence that triglycerides are an independent coronary heart disease risk factor. *The American Journal of Cardiology* **86**: 943-949.

Cullen, P. (2003). Triacylglycerol-rich lipoproteins and atherosclerosis--where is the link? *Biochemical Society Transactions* **31**: 1080-1084.

Dashti, N., Manchekar, M., Liu, Y., Sun, Z. & Segrest, J. P. (2007). Microsomal Triglyceride Transfer Protein Activity Is Not Required for the Initiation of Apolipoprotein B-containing Lipoprotein Assembly in McA-RH7777 Cells. *The Journal of Biological Chemistry* **282**: 28597-28608.

Davidson, N. O. & Shelness, G. S. (2000). APOLIPOPROTEIN B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annual Review of Nutrition* **20**: 169-193.

Davignon, J., Gregg, R. E. & Sing, C. F. (1988). Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* **8**: 1-21.

Deeb, S. S., Zambon, A., Carr, M. C., Ayyobi, A. F. & Brunzell, J. D. (2003). Hepatic lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. *Journal of Lipid Research* **44**: 1279-1286.

Demant, T., Bedford, D., Packard, C. J. & Shepherd, J. (1991). Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. *The Journal of Clinical Investigation* **88**: 1490-1501.

Demant, T. & Packard, C. J. (1997). Studies of apolipoprotein B-100 metabolism using radiotracers and stable isotopes. *European Journal of Pediatrics* **156 Suppl 1**: S75-S77.

Demant, T., Packard, C. J., Demmelmair, H., Stewart, P., Bedynek, A., Bedford, D., Seidel, D. & Shepherd, J. (1996). Sensitive methods to study human apolipoprotein

B metabolism using stable isotope-labeled amino acids. *AJP - Endocrinology and Metabolism* **270**: E1022-E1036.

Demant, T., Packard, C. J., Stewart, P., Bedynek, A., Calder, A. G., Shepherd, J. & Seidel, D. (1994). Sensitive mass spectrometry techniques for measuring metabolism of human apolipoprotein B in vivo. *Clinical Chemistry* **40**: 1825-1827.

Després, J. P. (2007). Cardiovascular disease under the influence of excess visceral fat. *Critical Pathways in Cardiology* **6**: 51-59.

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D. & Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *The Journal of Clinical Investigation* **115**: 1343-1351.

Egusa, G., Brady, D. W., Grundy, S. M. & Howard, B. V. (1983). Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *Journal of Lipid Research* **24**: 1261-1267.

El-Shazly, M., Makboul, G. & El Sayed, A. (2004). Life expectancy and cause of death in the Kuwaiti population 1987-2000. *Eastern Mediterranean Health Journal* **10**: 45-55.

Elovson, J., Chatterton, J. E., Bell, G. T., Schumaker, V. N., Reuben, M. A., Puppione, D. L., Reeve, J. R., Jr. & Young, N. L. (1988). Plasma very low density lipoproteins contain a single molecule of apolipoprotein B. *Journal of Lipid Research* **29**: 1461-1473.

Eto, M., Watanabe, K., Iwashima, Y., Morikawa, A., Oshima, E., Sekiguchi, M. & Ishii, K. (1986). Apolipoprotein E phenotypes and plasma lipids in young and middle-aged subjects. *The Tohoku Journal of Experimental Medicine* **148**: 25-34.

Evans, K., Clark, M. L. & Frayn, K. N. (1999). Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *The American Journal of Physiology* **276**: E241-E248.

Ferguson, M. A., Alderson, N. L., Trost, S. G., Essig, D. A., Burke, J. R. & Durstine, J. L. (1998). Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *Journal of Applied Physiology* **85**: 1169-1174.

Fielding, B. A. & Frayn, K. N. (1998). Lipoprotein lipase and the disposition of dietary fatty acids. *The British Journal of Nutrition* **80**: 495-502.

Fisher, E. A. & Ginsberg, H. N. (2002). Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. *The Journal of Biological Chemistry* **277**: 17377-17380.

Fisher, R. M., Coppack, S. W., Humphreys, S. M., Gibbons, G. F. & Frayn, K. N. (1995). Human triacylglycerol-rich lipoprotein subfractions as substrates for lipoprotein lipase. *Clinica Chimica Acta* **236**: 7-17.

Florez, H., Mendez, A., Casanova-Romero, P., Larreal-Urdaneta, C., Castillo-Florez, S., Lee, D. & Goldberg, R. (2006). Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. *Atherosclerosis* **188**: 134-141.

Foster, D. M., Barrett, P. H., Toffolo, G., Beltz, W. F. & Cobelli, C. (1993). Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *Journal of Lipid Research* **34**: 2193-2205.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology* **55**: 628-634.

Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**: 499-502.

Gaw, A., Packard, C. J., Lindsay, G. M., Griffin, B. A., Caslake, M. J., Lorimer, A. R. & Shepherd, J. (1995). Overproduction of small very low density lipoproteins (Sf 20-60) in moderate hypercholesterolemia: relationships between apolipoprotein B kinetics and plasma lipoproteins. *Journal of Lipid Research* **36**: 158-171.

Gibbons, G. F., Brown, A. M., Wiggins, D. & Pease, R. (2002). The roles of insulin and fatty acids in the regulation of hepatic very-low-density lipoprotein assembly. *Journal of the Royal Society of Medicine* **95 Suppl 42**: 23-32.

Gibbons, G. F., Islam, K. & Pease, R. J. (2000). Mobilisation of triacylglycerol stores. *Biochimica et Biophysica Acta* **1483**: 37-57.

Gill, J. M., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Sattar, N., Packard, C. J., Petrie, J. R. & Caslake, M. J. (2006). Effects of a moderate exercise session on postprandial lipoproteins, apolipoproteins and lipoprotein remnants in middle-aged men. *Atherosclerosis* **185**: 87-96.

Gill, J. M., Brown, J. C., Bedford, D., Wright, D. M., Cooney, J., Hughes, D. A., Packard, C. J. & Caslake, M. J. (2004a). Hepatic production of VLDL1 but not VLDL2 is related to insulin resistance in normoglycaemic middle-aged subjects. *Atherosclerosis* **176**: 49-56.

Gill, J. M., Frayn, K. N., Wootton, S. A., Miller, G. J. & Hardman, A. E. (2001a). Effects of prior moderate exercise on exogenous and endogenous lipid metabolism and plasma factor VII activity. *Clinical Science (London, England: 1979)* **100**: 517-527.

Gill, J. M. & Hardman, A. E. (2000). Postprandial lipemia: effects of exercise and restriction of energy intake compared. *American Journal of Clinical Nutrition* **71**: 465-471.

Gill, J. M. & Hardman, A. E. (2003). Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). *The Journal of Nutritional Biochemistry* **14**: 122-132.

Gill, J. M., Herd, S. L. & Hardman, A. E. (2002a). Moderate exercise and post-prandial metabolism: issues of dose-response. *Journal of Sports Sciences* **20**: 961-967.

Gill, J. M., Herd, S. L., Tsetsonis, N. V. & Hardman, A. E. (2002b). Are the reductions in triacylglycerol and insulin levels after exercise related? *Clinical Science (London, England: 1979)* **102**: 223-231.

Gill, J. M., Herd, S. L., Vora, V. & Hardman, A. E. (2003). Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and postprandial states. *European Journal of Applied Physiology* **89**: 184-190.

Gill, J. M., Murphy, M. H. & Hardman, A. E. (1998). Postprandial lipemia: effects of intermittent versus continuous exercise. *Medicine and Science in Sports and Exercise* **30**: 1515-1520.

Gill, J. M. R., Mees, G. P., Frayn, K. N. & Hardman, A. E. (2001b). Moderate exercise, postprandial lipaemia and triacylglycerol clearance. *European Journal of Clinical Investigation* **31**: 201-207.

Gill, J. M. R., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Packard, C. J., Sattar, N., Petrie, J. R. & Caslake, M. J. (2004b). Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *Journal of the American College of Cardiology* **44**: 2375-2382.

Gill, J. M. R., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Perry, C. G., Sattar, N., Packard, C. J., Caslake, M. J. & Petrie, J. R. (2007). Effect of prior moderate exercise on postprandial metabolism in men with type 2 diabetes: Heterogeneity of responses. *Atherosclerosis* **194**: 134-143.

Ginsberg, H. N., Zhang, Y. L. & Hernandez-Ono, A. (2005). Regulation of plasma triglycerides in insulin resistance and diabetes. *Archives of Medical Research* **36**: 232-240.

Goldberg, I. J. (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *Journal of Lipid Research* **37**: 693-707.

Gormsen, L. C., Jensen, M. D., Schmitz, O., Moller, N., Christiansen, J. S. & Nielsen, S. (2006). Energy expenditure, insulin, and VLDL-triglyceride production in humans. *Journal of Lipid Research* **47**: 2325-2332.

Goudriaan, J. R., Espirito Santo, S. M., Voshol, P. J., Teusink, B., van Dijk, K. W., van Vlijmen, B. J., Romijn, J. A., Havekes, L. M. & Rensen, P. C. (2004). The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis. *Journal of Lipid Research* **45**: 1475-1481.

Griffin, B. A. (1997). Low-density lipoprotein subclasses: mechanisms of formation and modulation. *The Proceedings of the Nutrition Society* **56**: 693-702.

Grundy, S. M. (2006). Atherogenic dyslipidemia associated with metabolic syndrome and insulin resistance. *Clinical Cornerstone* **8 Suppl 1**: S21-S27.

Guerin, M., Le Goff, W., Lassel, T. S., Van Tol, A., Steiner, G. & Chapman, M. J. (2001). Proatherogenic Role of Elevated CE Transfer From HDL to VLDL1 and Dense LDL in Type 2 Diabetes : Impact of the Degree of Triglyceridemia. *Arteriosclerosis, Thrombosis, and Vascular Biology* **21**: 282-288.

Gurr, M. I., Harwood, J. L. & Frayn, K. N. (2002). Lipid Biochemistry, 5th ed. Blackwell Science Ltd, Oxford, United Kingdom.

Gusarova, V., Brodsky, J. L. & Fisher, E. A. (2003). Apolipoprotein B100 Exit from the Endoplasmic Reticulum (ER) Is COPII-dependent, and Its Lipidation to Very Low Density Lipoprotein Occurs Post-ER. *The Journal of Biological Chemistry* **278**: 48051-48058.

Haffner, S. M. (2007). Abdominal adiposity and cardiometabolic risk: do we have all the answers? *The American Journal of Medicine* **120**: S10-S16.

Hagberg, J. M., Ferrell, R. E., Katzel, L. I., Dengel, D. R., Sorkin, J. D. & Goldberg, A. P. (1999). Apolipoprotein E genotype and exercise training--induced increases in plasma high-density lipoprotein (HDL)- and HDL2-cholesterol levels in overweight men. *Metabolism* **48**: 943-945.

Hagberg, J. M., Wilund, K. R. & Ferrell, R. E. (2000). APO E gene and gene-environment effects on plasma lipoprotein-lipid levels. *Physiological Genomics* **4**: 101-108.

Hallberg, D. (1965). Studies on the elimination of exogenous lipids from the blood stream. The kinetics for the elimination of chylomicrons studied by single intravenous injections in man. *Acta Physiologica Scandinavica* **65**: 279-284.

Halverstadt, A., Phares, D. A., Wilund, K. R., Goldberg, A. P. & Hagberg, J. M. (2007). Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. *Metabolism* **56**: 444-450.

Hardman, A. E. & Aldred, H. E. (1995). Walking during the postprandial period decreases alimentary lipaemia. *Journal of Cardiovascular Risk* **2**: 71-78.

Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., Macera, C. A., Heath, G. W., Thompson, P. D. & Bauman, A. (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation* **116**: 1081-1093.

Hatters, D. M., Peters-Libeu, C. A. & Weisgraber, K. H. (2006). Apolipoprotein E structure: insights into function. *Trends in Biochemical Sciences* **31**: 445-454.

Havekes, L. M., de Knijff, P., Beisiegel, U., Havinga, J., Smit, M. & Klasen, E. (1987). A rapid micromethod for apolipoprotein E phenotyping directly in serum. *Journal of Lipid Research* **28**: 455-463.

Havel, R. J., Chao, Y., Windler, E. E., Kotite, L. & Guo, L. S. (1980). Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proceedings of the National Academy of Sciences of the United States of America* **77**: 4349-4353.

Havel, R. J., Fielding, C. J., Olivecrona, T., Shore, V. G., Fielding, P. E. & Egelrud, T. (1973). Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. *Biochemistry* **12**: 1828-1833.

Havel, R. J. & Hamilton, R. L. (2004). Hepatic Catabolism of Remnant Lipoproteins: Where the Action Is. *Arteriosclerosis, Thrombosis, and Vascular Biology* **24**: 213-215.

Heath, R. B., Karpe, F., Milne, R. W., Burdge, G. C., Wootton, S. A. & Frayn, K. N. (2003). Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *Journal of Lipid Research* **44**: 2065-2072.

Herd, S. L., Hardman, A. E., Boobis, L. H. & Cairns, C. J. (1998). The effect of 13 weeks of running training followed by 9 d of detraining on postprandial lipaemia. *The British Journal of Nutrition* **80**: 57-66.

Herd, S. L., Kiens, B., Boobis, L. H. & Hardman, A. E. (2001). Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. *Metabolism* **50**: 756-762.

Herd, S. L., Lawrence, J. E. M., Malkova, D., Murphy, M. H., Mastana, S. & Hardman, A. E. (2000). Postprandial lipemia in young men and women of contrasting training status. *Journal of Applied Physiology* **89**: 2049-2056.

Hiukka, A., Fruchart-Najib, J., Leinonen, E., Hilden, H., Fruchart, J. C. & Taskinen, M. R. (2005). Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in type 2 diabetes. *Diabetologia* **48**: 1207-1215.

Hogue, J. C., Lamarche, B., Tremblay, A. J., Bergeron, J., Gagne, C. & Couture, P. (2007). Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. *Journal of Lipid Research* **48**: 1336-1342.

Holmquist, L. (1982). Surface modification of Beckman Ultra-Clear centrifuge tubes for density gradient centrifugation of lipoproteins. *Journal of Lipid Research* **23**: 1249-1250.

Houmard, J. A., Tanner, C. J., Slentz, C. A., Duscha, B. D., McCartney, J. S. & Kraus, W. E. (2004). Effect of the volume and intensity of exercise training on insulin sensitivity. *Journal of Applied Physiology* **96**: 101-106.

Howard, B. V. (1999). Insulin resistance and lipid metabolism. *The American Journal of Cardiology* **84**: 28J-32J.

Howdieshell, T. R., Bhalla, N., DiPiro, J. T., Kuske, T. & Baisden, R. (1995). Effects of free glycerol contained in intravenous fat emulsion on plasma triglyceride determination. *JPEN - Journal of Parenteral and Enteral Nutrition* **19**: 125-126.

Hultin, M., Savonen, R. & Olivecrona, T. (1996). Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling. *Journal of Lipid Research* **37**: 1022-1036.

Hussain, M. M., Shi, J. & Dreizen, P. (2003). Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *Journal of Lipid Research* **44**: 22-32.

Iriyama, K. & Carpentier, Y. A. (1994). Clinical significance of transfer of apolipoproteins between triacylglycerol-rich particles in lipid emulsions and plasma lipoproteins. *Nutrition* **10**: 252-254.

James, A. P., Slivkoff-Clark, K. & Mamo, J. C. (2007). Prior exercise does not affect chylomicron particle number following a mixed meal of moderate fat content. *Lipids in Health and Disease* **6**: 8.

Johanson, E. H., Jansson, P. A., Gustafson, B., Lonn, L., Smith, U., Taskinen, M. R. & Axelsen, M. (2004). Early alterations in the postprandial VLDL1 apoB-100 and apoB-48 metabolism in men with strong heredity for type 2 diabetes. *Journal of Internal Medicine* **255**: 273-279.

Jong, M. C., Dahlmans, V. E., Hofker, M. H. & Havekes, L. M. (1997). Nascent very-low-density lipoprotein triacylglycerol hydrolysis by lipoprotein lipase is inhibited by apolipoprotein E in a dose-dependent manner. *The Biochemical Journal* **328 (Pt 3)**: 745-750.

Joshi, P., Islam, S., Pais, P., Reddy, S., Dorairaj, P., Kazmi, K., Pandey, M. R., Haque, S., Mendis, S., Rangarajan, S. & Yusuf, S. (2007). Risk Factors for Early Myocardial Infarction in South Asians Compared With Individuals in Other Countries. *JAMA: The Journal of the American Medical Association* **297**: 286-294.

Jung, H. R., Turner, S. M., Neese, R. A., Young, S. G. & Hellerstein, M. K. (1999). Metabolic adaptations to dietary fat malabsorption in chylomicron-deficient mice. *The Biochemical Journal* **343 Pt 2**: 473-478.

Kane, J. P. (1983). Apolipoprotein B: structural and metabolic heterogeneity. *Annual Review of Physiology* **45**: 637-650.

Kantor, M. A., Cullinane, E. M., Herbert, P. N. & Thompson, P. D. (1984). Acute increase in lipoprotein lipase following prolonged exercise. *Metabolism* **33**: 454-457.

Karpe, F. (1999). Postprandial lipoprotein metabolism and atherosclerosis. *Journal of Internal Medicine* **246**: 341-355.

Karpe, F., Bickerton, A. S., Hodson, L., Fielding, B. A., Tan, G. D. & Frayn, K. N. (2007). Removal of triacylglycerols from chylomicrons and VLDL by capillary beds: the basis of lipoprotein remnant formation. *Biochemical Society Transactions* **35**: 472-476.

Karpe, F. & Hultin, M. (1995). Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *Journal of Lipid Research* **36**: 1557-1566.

Karpe, F., Olivecrona, T., Hamsten, A. & Hultin, M. (1997). Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. *Journal of Lipid Research* **38**: 949-961.

Karpe, F., Steiner, G., Olivecrona, T., Carlson, L. A. & Hamsten, A. (1993a). Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *The Journal of Clinical Investigation* **91**: 748-758.

Karpe, F. & Tan, G. D. (2005). Adipose tissue function in the insulin-resistance syndrome. *Biochemical Society Transactions* **33**: 1045-1048.

Karpe, F., Tornvall, P., Olivecrona, T., Steiner, G., Carlson, L. A. & Hamsten, A. (1993b). Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis* **98**: 33-49.

Katsanos, C. S. & Moffatt, R. J. (2004). Acute effects of premeal versus postmeal exercise on postprandial hypertriglyceridemia. *Clinical Journal of Sport Medicine* **14**: 33-39.

Kershaw, E. E., Hamm, J. K., Verhagen, L. A., Peroni, O., Katic, M. & Flier, J. S. (2006). Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. *Diabetes* **55**: 148-157.

Kissebah, A. H., Alfarsi, S., Adams, P. W., Seed, M., Folkard, J. & Wynn, V. (1976). Transport kinetics of plasma free fatty acid, very low density lipoprotein triglycerides and apoprotein in patients with endogenous hypertriglyceridaemia: effects of 2,2-dimethyl, 5(2, 5-xylyoxy) valeric acid therapy. *Atherosclerosis* **24**: 199-218.

Kissebah, A. H., Alfarsi, S. & Adams, P. W. (1981). Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in man: Normolipemic subjects, familial hypertriglyceridemia and familial combined hyperlipidemia. *Metabolism* **30**: 856-868.

Ko, K. W., Ohnishi, T. & Yokoyama, S. (1994). Triglyceride transfer is required for net cholesteryl ester transfer between lipoproteins in plasma by lipid transfer protein. Evidence for a hetero-exchange transfer mechanism demonstrated by using novel monoclonal antibodies. *The Journal of Biological Chemistry* **269**: 28206-28213.

Kodama, S., Tanaka, S., Saito, K., Shu, M., Sone, Y., Onitake, F., Suzuki, E., Shimano, H., Yamamoto, S., Kondo, K., Ohashi, Y., Yamada, N. & Sone, H. (2007). Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: a meta-analysis. *Archives of Internal Medicine* **167**: 999-1008.

Kolovou, G. D., Anagnostopoulou, K. K. & Cokkinos, D. V. (2005). Pathophysiology of dyslipidaemia in the metabolic syndrome. *Postgraduate Medical Journal* **81**: 358-366.

Kolovou, G. D. & Anagnostopoulou, K. K. (2007). Apolipoprotein E polymorphism, age and coronary heart disease. *Ageing Research Reviews* **6**: 94-108.

Kraus, W. E., Houmard, J. A., Duscha, B. D., Knetzger, K. J., Wharton, M. B., McCartney, J. S., Bales, C. W., Henes, S., Samsa, G. P., Otvos, J. D., Kulkarni, K. R. & Slentz, C. A. (2002). Effects of the amount and intensity of exercise on plasma lipoproteins. *The New England Journal of Medicine* **347**: 1483-1492.

Lear, S. A., Toma, M., Birmingham, C. L. & Frohlich, J. J. (2003). Modification of the relationship between simple anthropometric indices and risk factors by ethnic background. *Metabolism* **52**: 1295-1301.

Leon, A. S., Togashi, K., Rankinen, T., Després, J. P., Rao, D. C., Skinner, J. S., Wilmore, J. H. & Bouchard, C. (2004). Association of apolipoprotein E polymorphism with blood lipids and maximal oxygen uptake in the sedentary state and after exercise training in the HERITAGE family study. *Metabolism* **53**: 108-116.

Lewis, G. F. & Steiner, G. (1996). Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care* **19**: 390-393.

Lewis, G. F., Uffelman, K. D., Szeto, L. W. & Steiner, G. (1993). Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes* **42**: 833-842.

Lewis, G. F., Uffelman, K. D., Szeto, L. W., Weller, B. & Steiner, G. (1995). Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *The Journal of Clinical Investigation* **95**: 158-166.

Lewis, G. F., Zinman, B., Uffelman, K. D., Szeto, L., Weller, B. & Steiner, G. (1994). VLDL production is decreased to a similar extent by acute portal vs. peripheral venous insulin. *AJP - Endocrinology and Metabolism* **267**: E566-E572.

Lichtenstein, A. H., Hachey, D. L., Millar, J. S., Jenner, J. L., Booth, L., Ordovas, J. & Schaefer, E. J. (1992). Measurement of human apolipoprotein B-48 and B-100 kinetics in triglyceride-rich lipoproteins using [5,5,5-2H3]leucine. *Journal of Lipid Research* **33**: 907-914.

Lin, M. C., Gordon, D. & Wetterau, J. R. (1995). Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. *Journal of Lipid Research* **36**: 1073-1081.

Lindgren, F. T., Jensen, L. C. & Hatch, F. T. (1972). The isolation and quantitative analysis of serum lipoproteins. In Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism., ed. G.J.Nelson, pp. 181-274. John Wiley and Sons, New York.

Lithell, H., Schele, R., Vessby, B. & Jacobs, I. (1984). Lipoproteins, lipoprotein lipase, and glycogen after prolonged physical activity. *Journal of Applied Physiology* **57**: 698-702.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* **193**: 265-275.

MacArthur, J. M., Bishop, J. R., Stanford, K. I., Wang, L., Bensadoun, A., Witztum, J. L. & Esko, J. D. (2007). Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *The Journal of Clinical Investigation* **117**: 153-164.

Magkos, F., Patterson, B. W., Mohammed, B. S. & Mittendorfer, B. (2007). A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men. *AJP - Endocrinology and Metabolism* **292**: E1568-E1574.

Magkos, F., Tsekouras, Y., Kavouras, S. A., Mittendorfer, B. & Sidossis, L. S. (2008). Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure. *Clinical Science* **114**: 59-64.

Magkos, F., Wright, D. C., Patterson, B. W., Mohammed, B. S. & Mittendorfer, B. (2006). Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *AJP - Endocrinology and Metabolism* **290**: E355-E362.

Maheux, P., Azhar, S., Kern, P. A., Chen, Y.-D. I. & Reaven, G. M. (1997). Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. *Diabetologia* **40**: 850-858.

Mahley, R. W. & Ji, Z. S. (1999). Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *Journal of Lipid Research* **40**: 1-16.

Malkova, D., Evans, R. D., Frayn, K. N., Humphreys, S. M., Jones, P. R. M. & Hardman, A. E. (2000). Prior exercise and postprandial substrate extraction across the human leg. *AJP - Endocrinology and Metabolism* **279**: E1020-E1028.

Malkova, D. & Gill, J. M. R. (2006). Effects of exercise on postprandial lipoprotein metabolism. *Future Lipidology* **1**: 743-755.

Malloy, M. J. & Kane, J. P. (2001). A risk factor for atherosclerosis: triglyceride-rich lipoproteins. *Advances in Internal Medicine* **47**: 111-136.

Malmstrom, R., Packard, C. J., Caslake, M., Bedford, D., Stewart, P., Yki-Jarvinen, H., Shepherd, J. & Taskinen, M. R. (1997a). Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia* **40**: 454-462.

Malmstrom, R., Packard, C. J., Caslake, M., Bedford, D., Stewart, P., Yki-Jarvinen, H., Shepherd, J. & Taskinen, M. R. (1998). Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes* **47**: 779-787.

Malmstrom, R., Packard, C. J., Caslake, M., Bedford, D., Stewart, P., Shepherd, J. & Taskinen, M. R. (1999). Effect of heparin-stimulated plasma lipolytic activity on

VLDL APO B subclass metabolism in normal subjects. *Atherosclerosis* **146**: 381-390.

Malmstrom, R., Packard, C. J., Watson, T. D. G., Rannikko, S., Caslake, M., Bedford, D., Stewart, P., Yki-Jarvinen, H., Shepherd, J. & Taskinen, M. R. (1997b). Metabolic Basis of Hypotriglyceridemic Effects of Insulin in Normal Men. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**: 1454-1464.

Mamo, J. C. L., Watts, G. F., Barrett, P. H., Smith, D., James, A. P. & Pal, S. (2001). Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? *AJP - Endocrinology and Metabolism* **281**: E626-E632.

Manchekar, M., Richardson, P. E., Forte, T. M., Datta, G., Segrest, J. P. & Dashti, N. (2004). Apolipoprotein B-containing Lipoprotein Particle Assembly: Lipid capacity of the nascent lipoprotein particle. *The Journal of Biological Chemistry* **279**: 39757-39766.

Marsh, J. B. (2003). Lipoprotein metabolism in obesity and diabetes: insights from stable isotope kinetic studies in humans. *Nutrition Reviews* **61**: 363-375.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**: 412-419.

Mauriege, P., Galitzky, J., Berlan, M. & Lafontan, M. (1987). Heterogeneous distribution of beta and alpha-2 adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *European Journal of Clinical Investigation* **17**: 156-165.

Mead, J. R., Irvine, S. A. & Ramji, D. P. (2002). Lipoprotein lipase: structure, function, regulation, and role in disease. *Journal of Molecular Medicine* **80**: 753-769.

Menzel, H.-J. & Utermann, G. (1986). Apolipoprotein E phenotyping from serum by Western blotting. *Electrophoresis* **7**: 492-495.

Merrill, J. R., Holly, R. G., Anderson, R. L., Rifai, N., King, M. E. & DeMeersman, R. (1989). Hyperlipemic response of young trained and untrained men after a high fat meal. *Arteriosclerosis* **9**: 217-223.

Mittendorfer, B., Patterson, B. W. & Klein, S. (2003a). Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. *American Journal of Clinical Nutrition* **77**: 573-579.

Mittendorfer, B., Patterson, B. W., Klein, S. & Sidossis, L. S. (2003b). VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity. *AJP - Endocrinology and Metabolism* **284**: E708-E715.

Miyashita, M., Burns, S. F. & Stensel, D. J. (2006). Exercise and postprandial lipemia: effect of continuous compared with intermittent activity patterns. *American Journal of Clinical Nutrition* **83**: 24-29.

Morio, B., Holmback, U., Gore, D. & Wolfe, R. R. (2004). Increased VLDL-TAG turnover during and after acute moderate-intensity exercise. *Medicine and Science in Sports and Exercise* **36**: 801-806.

Murphy, M. H., Nevill, A. M. & Hardman, A. E. (2000). Different patterns of brisk walking are equally effective in decreasing postprandial lipaemia. *International Journal of Obesity and Related Metabolic Disorders* **24**: 1303-1309.

Nakano, T., Nakajima, K., Niimi, M., Fujita, M. Q., Nakajima, Y., Takeichi, S., Kinoshita, M., Matsushima, T., Teramoto, T. & Tanaka, A. (2008). Detection of apolipoproteins B-48 and B-100 carrying particles in lipoprotein fractions extracted from human aortic atherosclerotic plaques in sudden cardiac death cases. *Clinica Chimica Acta* **390**: 38-43.

Nielsen, S., Guo, Z., Johnson, C. M., Hensrud, D. D. & Jensen, M. D. (2004). Splanchnic lipolysis in human obesity. *The Journal of Clinical Investigation* **113**: 1582-1588.

Olivecrona, G. & Beisiegel, U. (1997). Lipid binding of apolipoprotein CII is required for stimulation of lipoprotein lipase activity against apolipoprotein CII-deficient chylomicrons. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**: 1545-1549.

Olivecrona, G. & Olivecrona, T. (1998). Clearance of artificial triacylglycerol particles. *Current Opinion in Clinical Nutrition and Metabolic Care* **1**: 143-151.

Olivecrona, T., Hultin, M., Bergo, M. & Olivecrona, G. (1997). Lipoprotein lipase: regulation and role in lipoprotein metabolism. *The Proceedings of the Nutrition Society* **56**: 723-729.

Oliveira, M. R. & Maranhao, R. C. (2002). Plasma kinetics of a chylomicron-like emulsion in normolipidemic obese women after a short-period weight loss by energy-restricted diet. *Metabolism* **51**: 1097-1103.

Olofsson, S. O. & Borén, J. (2005). Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *Journal of Internal Medicine* **258**: 395-410.

Packard, C. & Shepherd, J. (1997). Lipoprotein Heterogeneity and Apolipoprotein B Metabolism. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**: 3542-3556.

Packard, C. J. (1995). The role of stable isotopes in the investigation of plasma lipoprotein metabolism. *Baillière's Clinical Endocrinology and Metabolism* **9**: 755-772.

Packard, C. J. (2003). Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochemical Society Transactions* **31**: 1066-1069.

Packard, C. J., Demant, T., Stewart, J. P., Bedford, D., Caslake, M. J., Schwertfeger, G., Bedynek, A., Shepherd, J. & Seidel, D. (2000). Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *Journal of Lipid Research* **41**: 305-318.

Packard, C. J., Gaw, A., Demant, T. & Shepherd, J. (1995). Development and application of a multicompartmental model to study very low density lipoprotein subfraction metabolism. *Journal of Lipid Research* **36**: 172-187.

Packard, C. J., Munro, A., Lorimer, A. R., Gotto, A. M. & Shepherd, J. (1984). Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *The Journal of Clinical Investigation* **74**: 2178-2192.

Park, Y., Damron, B. D., Miles, J. M. & Harris, W. S. (2001). Measurement of human chylomicron triglyceride clearance with a labeled commercial lipid emulsion. *Lipids* **36**: 115-120.

Park, Y., Grellner, W. J., Harris, W. S. & Miles, J. M. (2000). A new method for the study of chylomicron kinetics in vivo. *AJP - Endocrinology and Metabolism* **279**: E1258-E1263.

Perreault, L., Lavely, J. M., Kittelson, J. M. & Horton, T. J. (2004). Gender Differences in Lipoprotein Lipase Activity after Acute Exercise. *Obesity* **12**: 241-249.

Petitt, D. S. & Cureton, K. J. (2003). Effects of prior exercise on postprandial lipemia: A quantitative review. *Metabolism* **52**: 418-424.

Pietzsch, J., Wiedemann, B., Julius, U., Nitzsche, S., Gehrisch, S., Bergmann, S., Leonhardt, W., Jaross, W. & Hanefeld, M. (1996). Increased clearance of low density lipoprotein precursors in patients with heterozygous familial defective apolipoprotein B-100: a stable isotope approach. *Journal of Lipid Research* **37**: 2074-2087.

Podl, T. R., Zmuda, J. M., Yurgalevitch, S. M., Fahrenbach, M. C., Bausserman, L. L., Terry, R. B. & Thompson, P. D. (1994). Lipoprotein lipase activity and plasma triglyceride clearance are elevated in endurance-trained women. *Metabolism* **43**: 808-813.

Potts, J. L., Fisher, R. M., Humphreys, S. M., Coppack, S. W., Gibbons, G. F. & Frayn, K. N. (1991). Chylomicrons are the preferred substrate for lipoprotein lipase in vivo. *Biochemical Society Transactions* **19**: 314S.

Preiss-Landl, K., Zimmermann, R., Hammerle, G. & Zechner, R. (2002). Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Current Opinion in Lipidology* **13**: 471-481.

Rang, H. P., Dale, M. M., Ritter, J. M. & Moore, P. K. (2003). Pharmacology, 5th ed., pp. 115. Churchill Livingstone, London.

Rashid, S., Watanabe, T., Sakaue, T. & Lewis, G. F. (2003). Mechanisms of HDL lowering in insulin resistant, hypertriglyceridemic states: the combined effect of HDL triglyceride enrichment and elevated hepatic lipase activity. *Clinical Biochemistry* **36**: 421-429.

Rava, P., Athar, H., Johnson, C. & Hussain, M. M. (2005). Transfer of cholesteryl esters and phospholipids as well as net deposition by microsomal triglyceride transfer protein. *Journal of Lipid Research* **46**: 1779-1785.

Rebuffe-Scrive, M., Andersson, B., Olbe, L. & Bjorntorp, P. (1989). Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metabolism* **38**: 453-458.

Redgrave, T. G. (2004). Chylomicron metabolism. *Biochemical Society Transactions* **32**: 79-82.

Redgrave, T. G. & Maranhao, R. C. (1985). Metabolism of protein-free lipid emulsion models of chylomicrons in rats. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **835**: 104-112.

Riches, F. M., Watts, G. F., Hua, J., Stewart, G. R., Naoumova, R. P. & Barrett, P. H. R. (1999). Reduction in Visceral Adipose Tissue Is Associated with Improvement in Apolipoprotein B-100 Metabolism in Obese Men. *Journal of Clinical Endocrinology Metabolism* **84**: 2854-2861.

Rossner, S. (1974). Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experiences with Intralipid. *Acta medica Scandinavica. Supplementum* **564**: 1-24.

Rossner, S. (1982). Long-term reproducibility of the intravenous fat tolerance test. *Scandinavian Journal of Clinical and Laboratory Investigation* **42**: 153-157.

Ruge, T., Svensson, M., Eriksson, J. W. & Olivecrona, G. (2005). Tissue-specific regulation of lipoprotein lipase in humans: effects of fasting. *European Journal of Clinical Investigation* **35**: 194-200.

Rustaeus, S., Stillemark, P., Lindberg, K., Gordon, D. & Olofsson, S. O. (1998). The microsomal triglyceride transfer protein catalyzes the post-translational assembly of apolipoprotein B-100 very low density lipoprotein in McA-RH7777 cells. *The Journal of Biological Chemistry* **273**: 5196-5203.

Sady, S. P., Thompson, P. D., Cullinane, E. M., Kantor, M. A., Domagala, E. & Herbert, P. N. (1986). Prolonged exercise augments plasma triglyceride clearance. *JAMA: The Journal of the American Medical Association* **256**: 2552-2555.

Sattar, N., Gaw, A., Scherbakova, O., Ford, I., O'Reilly, D. S., Haffner, S. M., Isles, C., Macfarlane, P. W., Packard, C. J., Cobbe, S. M. & Shepherd, J. (2003). Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation* **108**: 414-419.

Sattar, N., McConnachie, A., Ford, I., Gaw, A., Cleland, S. J., Forouhi, N. G., McFarlane, P., Shepherd, J., Cobbe, S. & Packard, C. (2007). Serial Metabolic Measurements and Conversion to Type 2 Diabetes in the West of Scotland Coronary Prevention Study: Specific Elevations in Alanine Aminotransferase and Triglycerides Suggest Hepatic Fat Accumulation as a Potential Contributing Factor. *Diabetes* **56**: 984-991.

Sattar, N., Scherbakova, O., Ford, I., O'Reilly, D. S., Stanley, A., Forrest, E., MacFarlane, P. W., Packard, C. J., Cobbe, S. M. & Shepherd, J. (2004). Elevated Alanine Aminotransferase Predicts New-Onset Type 2 Diabetes Independently of Classical Risk Factors, Metabolic Syndrome, and C-Reactive Protein in the West of Scotland Coronary Prevention Study. *Diabetes* **53**: 2855-2860.

Saxena, U., Witte, L. D. & Goldberg, I. J. (1989). Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *The Journal of Biological Chemistry* **264**: 4349-4355.

Schindhelm, R. K., Dekker, J. M., Nijpels, G., Bouter, L. M., Stehouwer, C. D., Heine, R. J. & Diamant, M. (2007a). Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. *Atherosclerosis* **191**: 391-396.

Schindhelm, R. K., Dekker, J. M., Nijpels, G., Stehouwer, C. D., Bouter, L. M., Heine, R. J. & Diamant, M. (2007b). Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the Hoorn Study. *Diabetic Medicine* **24**: 430-435.

Schneeman, B. O., Kotite, L., Todd, K. M. & Havel, R. J. (1993). Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proceedings of the National Academy of Sciences of the United States of America* **90**: 2069-2073.

Scow, R. O. & Olivecrona, T. (1977). Effect of albumin on products formed from chylomicron triacylclycerol by lipoprotein lipase in vitro. *Biochimica et Biophysica Acta* **487**: 472-486.

Segrest, J. P., Jones, M. K., De Loof, H. & Dashti, N. (2001). Structure of apolipoprotein B-100 in low density lipoproteins. *Journal of Lipid Research* **42**: 1346-1367.

Shachter, N. S. (2001). Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Current Opinion in Lipidology* **12**: 297-304.

Shelness, G. S. & Ledford, A. S. (2005). Evolution and mechanism of apolipoprotein B-containing lipoprotein assembly. *Current Opinion in Lipidology* **16**: 325-332.

Shelness, G. S. & Sellers, J. A. (2001). Very-low-density lipoprotein assembly and secretion. *Current Opinion in Lipidology* **12**: 151-157.

Slentz, C. A., Houmard, J. A. & Kraus, W. E. (2007). Modest exercise prevents the progressive disease associated with physical inactivity. *Exercise and Sport Sciences Reviews* **35**: 18-23.

Sniderman, A. D., Bhopal, R., Prabhakaran, D., Sarrafzadegan, N. & Tchernof, A. (2007). Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *International Journal of Epidemiology* **36**: 220-225.

Sparks, J. D. & Sparks, C. E. (1990). Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *The Journal of Biological Chemistry* **265**: 8854-8862.

Sparks, J. D. & Sparks, C. E. (1994). Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **1215**: 9-32.

Stein, O. & Stein, Y. (2005). Lipid transfer proteins (LTP) and atherosclerosis. *Atherosclerosis* **178**: 217-230.

Taghibiglou, C., Carpentier, A., Van Iderstine, S. C., Chen, B., Rudy, D., Aiton, A., Lewis, G. F. & Adeli, K. (2000). Mechanisms of Hepatic Very Low Density Lipoprotein Overproduction in Insulin Resistance: Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *The Journal of Biological Chemistry* **275**: 8416-8425.

Takahashi, S., Sakai, J., Fujino, T., Hattori, H., Zenimaru, Y., Suzuki, J., Miyamori, I. & Yamamoto, T. T. (2004). The very low-density lipoprotein (VLDL) receptor:

characterization and functions as a peripheral lipoprotein receptor. *Journal of Atherosclerosis and Thrombosis* **11**: 200-208.

Tan, C. E., Forster, L., Caslake, M. J., Bedford, D., Watson, T. D. G., McConnell, M., Packard, C. J. & Shepherd, J. (1995). Relations Between Plasma Lipids and Postheparin Plasma Lipases and VLDL and LDL Subfraction Patterns in Normolipemic Men and Women. *Arteriosclerosis, Thrombosis, and Vascular Biology* **15**: 1839-1848.

Tanaka, A., AI, M., Kobayashi, Y., Tamura, M., Shimokado, K. & Numano, F. (2001). Metabolism of triglyceride-rich lipoproteins and their role in atherosclerosis. *Annals of the New York Academy of Sciences* **947**: 207-212.

Taskinen, M. R. (2003). Diabetic dyslipidaemia: from basic research to clinical practice. *Diabetologia* **46**: 733-749.

Thomas, T. R., Horner, K. E., Langdon, M. M., Zhang, J. Q., Krul, E. S., Sun, G. Y. & Cox, R. H. (2001). Effect of exercise and medium-chain fatty acids on postprandial lipemia. *Journal of Applied Physiology* **90**: 1239-1246.

Thomas, T. R., Smith, B. K., Donahue, O. M., Altena, T. S., James-Kracke, M. & Sun, G. Y. (2004). Effects of omega-3 fatty acid supplementation and exercise on low-density lipoprotein and high-density lipoprotein subfractions. *Metabolism* **53**: 749-754.

Thompson, P. D., Tsongalis, G. J., Seip, R. L., Bilbie, C., Miles, M., Zoeller, R., Visich, P., Gordon, P., Angelopoulos, T. J., Pescatello, L., Bausserman, L. & Moyna, N. (2004). Apolipoprotein e genotype and changes in serum lipids and maximal oxygen uptake with exercise training. *Metabolism* **53**: 193-202.

Tilly-Kiesi, M., Knudsen, P., Groop, L. & Taskinen, M. R. (1996). Hyperinsulinemia and insulin resistance are associated with multiple abnormalities of lipoprotein subclasses in glucose-tolerant relatives of NIDDM patients. Botnia Study Group. *Journal of Lipid Research* **37**: 1569-1578.

Tobin, L., Kiens, B. & Galbo, H. (2008). The effect of exercise on postprandial lipidemia in type 2 diabetic patients. *European Journal of Applied Physiology* **102**: 361-370.

Tribble, D. L., Holl, L. G., Wood, P. D. & Krauss, R. M. (1992). Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* **93**: 189-199.

Tsekouras, Y. E., Yanni, A. E., Bougatsas, D., Kavouras, S. A. & Sidossis, L. S. (2007). A single bout of brisk walking increases basal very low-density lipoprotein triacylglycerol clearance in young men. *Metabolism* **56**: 1037-1043.

Tsetsonis, N. V. & Hardman, A. E. (1996a). Effects of low and moderate intensity treadmill walking on postprandial lipaemia in healthy young adults. *European Journal of Applied Physiology* **73**: 419-426.

Tsetsonis, N. V. & Hardman, A. E. (1996b). Reduction in postprandial lipemia after walking: influence of exercise intensity. *Medicine and Science in Sports and Exercise* **28**: 1235-1242.

Tsetsonis, N. V., Hardman, A. E. & Mastana, S. S. (1997). Acute effects of exercise on postprandial lipemia: a comparative study in trained and untrained middle-aged women. *American Journal of Clinical Nutrition* **65**: 525-533.

Tulenko, T. N. & Sumner, A. E. (2002). The physiology of lipoproteins. *Journal of Nuclear Cardiology* **9**: 638-649.

van Tol, A. (2002). Phospholipid transfer protein. *Current Opinion in Lipidology* **13**: 135-139.

Verges, B. (2005). New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. *Diabetes & Metabolism* **31**: 429-439.

Wang, C. S., McConathy, W. J., Kloer, H. U. & Alaupovic, P. (1985). Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *The Journal of Clinical Investigation* **75**: 384-390.

Wang, M. & Briggs, M. R. (2004). HDL: the metabolism, function, and therapeutic importance. *Chemical Reviews* **104**: 119-137.

Watson, K. E., Horowitz, B. N. & Matson, G. (2003). Lipid abnormalities in insulin resistant states. *Reviews in Cardiovascular Medicine* **4**: 228-236.

Watts, G. F., Moroz, P. & Barrett, P. H. (2000). Kinetics of very-low-density lipoprotein apolipoprotein B-100 in normolipidemic subjects: pooled analysis of stable-isotope studies. *Metabolism* **49**: 1204-1210.

Weintraub, M. S., Eisenberg, S. & Breslow, J. L. (1987). Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *The Journal of Clinical Investigation* **80**: 1571-1577.

Weintraub, M. S., Rosen, Y., Otto, R., Eisenberg, S. & Breslow, J. L. (1989). Physical exercise conditioning in the absence of weight loss reduces fasting and postprandial triglyceride-rich lipoprotein levels. *Circulation* **79**: 1007-1014.

Welty, F. K., Lichtenstein, A. H., Barrett, P. H., Dolnikowski, G. G. & Schaefer, E. J. (1999). Human Apolipoprotein (Apo) B-48 and ApoB-100 Kinetics With Stable Isotopes. *Arteriosclerosis, Thrombosis, and Vascular Biology* **19**: 2966-2974.

Welty, F. K., Lichtenstein, A. H., Barrett, P. H., Dolnikowski, G. G. & Schaefer, E. J. (2004). Interrelationships Between Human Apolipoprotein A-I and Apolipoproteins B-48 and B-100 Kinetics Using Stable Isotopes. *Arteriosclerosis, Thrombosis, and Vascular Biology* **24**: 1703-1707.

Welty, F. K., Lichtenstein, A. H., Barrett, P. H., Jenner, J. L., Dolnikowski, G. G. & Schaefer, E. J. (2000). Effects of ApoE Genotype on ApoB-48 and ApoB-100 Kinetics With Stable Isotopes in Humans. *Arteriosclerosis, Thrombosis, and Vascular Biology* **20**: 1807-1810.

Westerbacka, J., Corner, A., Tiikkainen, M., Tamminen, M., Vehkavaara, S., Hakkinen, A. M., Fredriksson, J. & Yki-Jarvinen, H. (2004). Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* **47**: 1360-1369.

Whincup, P. H., Gilg, J. A., Papacosta, O., Seymour, C., Miller, G. J., Alberti, K. G. & Cook, D. G. (2002). Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. *BMJ* **324**: 635.

Williamson, D. H. & Whitelaw, E. (1978). Physiological aspects of the regulation of ketogenesis. *Biochemical Society Symposium* (43) 137-161.

Witter, R. F. & Whitner, V. S. (1972). Determination of serum triglycerides. In Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism., ed. G.J.Nelson, pp. 75-105. John Wiley and Sons, New York.

Wolfrum, C. & Stoffel, M. (2006). Coactivation of Foxa2 through Pgc-1[beta] promotes liver fatty acid oxidation and triglyceride/VLDL secretion. *Cell Metabolism* **3**: 99-110.

Wong, H. & Schotz, M. C. (2002). The lipase gene family. *Journal of Lipid Research* **43**: 993-999.

Xiang, S. Q., Cianflone, K., Kalant, D. & Sniderman, A. D. (1999). Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase. *Journal of Lipid Research* **40**: 1655-1663.

Zhang, J. Q., Ji, L. L., Nunez, G., Feathers, S., Hart, C. L. & Yao, W. X. (2004a). Effect of exercise timing on postprandial lipemia in hypertriglyceridemic men. *Canadian Journal of Applied Physiology* **29**: 590-603.

Zhang, J., Ji, L., Fretwell, V. & Nunez, G. (2006). Effect of exercise on postprandial lipemia in men with hypertriglyceridemia. *European Journal of Applied Physiology* **98**: 575-582.

Zhang, J. Q., Ji, L. L., Fogt, D. L. & Fretwell, V. S. (2007). Effect of exercise duration on postprandial hypertriglyceridemia in men with metabolic syndrome. *Journal of Applied Physiology* **103**: 1339-1345.

Zhang, J. Q., Smith, B., Langdon, M. M., Messimer, H. L., Sun, G. Y., Cox, R. H., James-Kracke, M. & Thomas, T. R. (2002). Changes in LPLa and reverse cholesterol transport variables during 24-h postexercise period. *AJP - Endocrinology and Metabolism* **283**: E267-E274.

Zhang, J. Q., Thomas, T. R. & Ball, S. D. (1998). Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. *Journal of Applied Physiology* **85**: 1516-1522.

Zhang, Y. L., Hernandez-Ono, A., Ko, C., Yasunaga, K., Huang, L. S. & Ginsberg, H. N. (2004b). Regulation of Hepatic Apolipoprotein B-lipoprotein Assembly and Secretion by the Availability of Fatty Acids: I. Differential response to the delivery of fatty acids via albumin or remnant-like emulsion particles. *The Journal of Biological Chemistry* **279**: 19362-19374.

Zheng, C., Ikewaki, K., Walsh, B. W. & Sacks, F. M. (2006). Metabolism of ApoB lipoproteins of intestinal and hepatic origin during constant feeding of small amounts of fat. *Journal of Lipid Research* **47**: 1771-1779.

Zilversmit, D. B. (1979). Atherogenesis: a postprandial phenomenon. *Circulation* **60**: 473-485.

Ziogas, G. G., Thomas, T. R. & Harris, W. S. (1997). Exercise training, postprandial hypertriglyceridemia, and LDL subfraction distribution. *Medicine and Science in Sports and Exercise* **29**: 986-991.

Zoratti, R., Godsland, I. F., Chaturvedi, N., Crook, D., Crook, D., Stevenson, J. C. & McKeigue, P. M. (2000). Relation of plasma lipids to insulin resistance, nonesterified fatty acid levels, and body fat in men from three ethnic groups: relevance to variation in risk of diabetes and coronary disease. *Metabolism* **49**: 245-252.

8. Appendices

Appendix A1: Subject information sheet and consent form – Chapters 3 and 4

Appendix A2: Subject information sheet and consent form – Chapter 5

Appendix B1: Health screen questionnaire – Chapters 3 & 4

Appendix B2: Health screen questionnaire – Chapter 5

Appendix C: Kinetic results for all subjects according to apoE phenotypes

Appendix A1: Subject Information Sheet and Consent Form – Chapters 3 & 4

Version 3, 18th March 2004

UNIVERSITY of GLASGOW

VOLUNTEER INFORMATION SHEET

Title: Development of novel method to determine very low density lipoprotein kinetics

Lay title: A new method of measuring the metabolism of blood fats

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Institute of Biomedical and Life Sciences

Please reply to : Division of Neuroscience and Biomedical Systems,
Institute of Biomedical and Life Sciences,
University of Glasgow, West Medical Building,
University Avenue, Glasgow G12 8QQ
Tel: 0141-330-2916 *Fax*: 0141-330-2915 *E-mail*: j.gill@bio.gla.ac.uk

Frequently Asked Questions

What is the purpose of the study?

The aim of this study is to develop a new method of measuring how fast your liver produces fatty particles (called lipoproteins) and how fast these particles are cleared from your bloodstream. This will help us to determine how effective different treatments are at influencing fat metabolism in future studies.

Why have I been chosen?

You have been chosen because you are a healthy adult aged between 18 and 65 years.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

In the first instance you will be asked to attend for a screening visit in which we will discuss with you and complete confidential questionnaires regarding your health and family history, measure your blood pressure and provide an opportunity for you to ask questions about the study. We will then ask you to help us in one of two different ways:

To provide a fasting blood sample: We will ask you to come to the Clinical Investigation Suite after an overnight fast and provide a simple blood sample. We will take up to 60 ml (4 tablespoons) of blood from you. Your blood will be used to help us to develop laboratory methods for analysing the fat in your bloodstream.

OR

To help us to develop a new method for assessing the metabolism of blood fats: We will ask you to perform either one, two or three experimental trials to help us to develop a new method for assessing the metabolism of lipoproteins. Each trial will involve coming to our Clinical Investigation Suite after an overnight fast. We will then introduce two small plastic tubes called cannulas into a vein in your arms (one in each arm). This is no more painful than giving a normal blood sample. We will take blood from one cannula and use the other to pump small amounts of a fatty substance called Intralipid® into your blood stream. Intralipid® is used in hospitals to feed people through a 'drip' when they are unable to eat normally. The Intralipid® we give you will cause the level of fat in your blood to rise about the same amount as it does after you eat a fatty meal and it will be pumped into your bloodstream for between one and three hours. We will take two blood samples from the cannula before giving you the Intralipid® and then take blood samples at intervals during the Intralipid® infusion and for a maximum of two hours after the Intralipid® pump is stopped. We may ask you to sip small amounts of a glucose drink throughout the trial. We will take a maximum of 250 ml of blood during each trial – this is half the amount of blood that you give when you donate a ‘pint’ of blood. During the experimental trial you will only be able to drink water (or the glucose drink), but we will provide you with some food to eat when the trial is finished. The total duration of each trial will vary between $2^1/_2$ and 6 hours but we will tell you specifically how long each of your own trials will take. You will be able to watch TV or videos, listen to music or read to keep yourself occupied during the experiment.

What do I have to do?

We will ask you to weigh and record everything that you eat and drink and to avoid alcohol and planned exercise for the two days before each Intralipid® test. We will provide you with scales and record sheets to enable you to record your diet easily. If you do more than one test, we will ask you to eat the same diet on the two days leading up to each test. (You will not need to do this if you are just giving us a fasting blood sample).

What are the possible disadvantages and risks of taking part?

Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood. There is a small risk that you could have an allergic reaction to Intralipid® (less than one chance in a million). This usually only occurs in people are allergic to soya protein or eggs, so if you have these allergies you will not be able to participate in this study. In the unlikely event that you do have an allergic reaction to Intralipid® , the appropriate medicines and equipment are in place for you to be treated immediately.

There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol or high blood pressure. If such a problem is revealed, we will inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?

The purpose of this study is to develop a new method of determining how fast lipoprotein particles are produced by the liver and how fast they are broken down and cleared from the bloodstream. We will be able to use this method in future research to help to determine how effective different treatments are at improving the way in which the body handles fat.

What if something goes wrong?

The chances of something going wrong are extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University or hospital will have your name and address removed so that you cannot be recognised from it.

Who has reviewed the study?

This study has been reviewed and approved by the North Glasgow NHS Trust Research Ethics Committee.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting either:

Iqbal AlShayji
Mobile: 07799353689 *E-mail*: ialshayji@yahoo.com
Tel: 0141 2114595

Dr Jason Gill
Tel: 0141 3302916 *E-mail*: j.gill@bio.gla.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.

Volunteer Identification Number for this trial: __________

UNIVERSITY of GLASGOW

CONSENT FORM

Title of Project: Development of novel method to determine very low density lipoprotein kinetics

Lay title: A new method of measuring the metabolism of blood fats

Name of Researcher: ____

Please initial box

1. I confirm that I have read and understand the information sheet dated 18 March 2004 (version 3) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

________________________	____________	____________________
Name of Patient	Date	Signature
________________________	____________	____________________
Name of Person taking consent (if different from researcher)	Date	Signature
________________________	____________	____________________
Researcher	Date	Signature

Blue for patient
Pink for researcher

Appendix A2: Subject Information Sheet and Consent Form - Chapter 5

VOLUNTEER INFORMATION SHEET

Title: Effects of Moderate Exercise on Very Low Density Lipoprotein Kinetics

Lay title: How does exercise affect the metabolism of the liver's fatty particles?

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

Heart disease is the leading cause of death in Scotland and being even slightly overweight, particularly when fat is stored in the tummy region, increases your risk. Many factors contribute to a person's risk of heart disease, but it is thought that the ability to cope with fat contained in food plays a role. A single session of moderate exercise decreases blood fat concentrations following a meal, but is unclear whether this is due to an increase of the clearance of fatty particles by the blood, or their reduced production from the liver. This study will help to investigate the effects of a single brisk walking session lasting from 90 to 120 minutes on the production and clearance of fatty particles. This is important as exercise could be used as a treatment option, rather than drugs, to prevent and treat disturbances in blood fat metabolism.

Why have I been chosen?

You have been chosen because you are a man or a postmenopausal woman aged between 30-60 years who is heavier than the ideal weight for your height.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide, you will be given this information sheet to keep and be asked to sign a consent form. If you do this you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

In the first instance you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health, family history and physical activity level
- measure your blood pressure
- take your height, weight and waist measurements
- take two small blood samples (20 ml or 4 teaspoons) to check the fat and sugar levels in your blood. We will also check if you are anaemic or have any abnormalities in the function of your liver, kidneys and thyroid gland
- provide an opportunity for you to ask questions

These preliminary procedures will enable us to determine whether you fall into the group of people we wish to study and will also ensure that it is perfectly safe for you to take part. In addition, we will inform your GP of your participation in the study and send them a copy of your screening results.

Experimental procedures

A. Preliminary Exercise Test

At the beginning of the study, an exercise test will be undertaken. This will involve walking on a motorised treadmill. If you are not used to walking on a treadmill, we will familiarise you with this before any 'real' sessions are performed. The test is designed to estimate your body's ability to use oxygen and enables us to find the correct speed and gradient for you to walk at during your treadmill walks. This will **not** require a maximal effort and the test will last for about 20 minutes. Heart rate will be monitored and recorded throughout using a heart rate monitor and expired air will be collected at intervals using a mouthpiece and respiratory valve. For safety reasons, the test will be stopped if your heart rate exceeds 85% of your predicted maximum.

B. Body Composition

The amount and distribution of your body fat will be determined by measuring body girths and by using callipers to measure skin fold thickness at four different sites (a sophisticated version of "pinch an inch"). Your height, weight and waist and hip circumferences will also be recorded. You will need to wear only underclothing for these measurements which will be made in private. These measurements only take a few minutes and can be made on the same day as other tests.

C. Main Trials

We will ask you to undertake 2 Intralipid tests, approximately 1-2 weeks apart, in random order. On the day prior to one Intralipid test, a controlled exercise session of 90- to 120-minutes walk will be undertaken (Exercise test). Other than this, conditions (such as alcohol consumption, food eaten, etc) in the days leading up to each trial will be EXACTLY the same. This is explained in detail in the "What do I have to do?" section.

- **Control Test** – We will ask you to come to our Clinical Investigation Suite at Glasgow Royal Infirmary after an overnight fast (i.e. having eaten nothing and with only water to drink for 12 hours). We will then introduce two small plastic tubes called cannulas into a vein in your arms (one in each arm). This is no more painful than giving a normal blood sample. We will take blood from one cannula and use the other to pump small amounts of a fatty substance called Intralipid into your blood stream. Intralipid is used in hospitals to feed people through a 'drip' when they are unable to eat normally. This is a quick way for us to give you a fatty meal. The Intralipid we give you will cause the level of fat in your blood to rise about the same amount as it does after you eat a fatty meal and it will be pumped into your bloodstream for 60 to 75 minutes. We will take a maximum of 250 ml of blood during each trial – this is half the amount of blood that you give when you donate a 'pint' of blood. During the test you will not be able to eat or drink anything except water, but we will provide you with some food to eat when the trial is finished. The total duration of each test will be about 3 hours. You will be able to watch TV or videos, listen to music or read to keep yourself occupied during the experiment.

- **Exercise Test –** This will be identical to the control test, except that we will ask you to come to the University to walk on the treadmill for 90 to 120 minutes on the day before the test.

What do I have to do?

We also ask you to maintain your usual lifestyle (i.e. don't change your diet or exercise habits) for the duration of this study. However, before each Intralipid test, we ask you to do the following:

1. For 3 days before each Intralipid test, refrain from planned or strenuous exercise, other than for personal transportation and the 90- to 120-minute treadmill walk in our laboratory..
2. Weigh and record everything you eat and drink for 2 days before each test. We will provide you with weighing scales and diet sheets to do this.
3. Refrain from alcohol consumption on the day before each test.
4. For the 2 days leading up to the second test, we will ask you to eat the same diet as you did on the two days leading up to the first test. Therefore, we would advise you to eat meals that you will be able to easily repeat during the days preceding both tests.

What are the possible disadvantages and risks of taking part?

- Exercise testing will not be at a maximal level but the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.
- Blood sampling via the cannula may cause minor bruising or an inflammation of the vein. Good practice, however, minimises this risk. Some people may feel faint when they give blood.
- There is a small risk that you could have an allergic reaction to Intralipid (less than one chance in a million). This usually only occurs in people who are allergic to soya protein or eggs, so if you have these allergies you will not be able to participate in this study. In the unlikely event that you do have an allergic reaction to Intralipid, the appropriate medicines and equipment are in place for you to be treated immediately.
- There is a small possibility that taking part in this study will reveal a health problem that you already have such as high cholesterol or high blood pressure. If such a problem is revealed, we will inform your GP to ensure that you receive appropriate treatment.

What are the possible benefits of taking part?

There may be no benefits to you but as a result of being involved in this study you will receive health and fitness information about yourself including fitness tests, dietary assessment, body fat measurement and your cholesterol and blood sugar levels. This study will help us to determine how exercise can improve risk factors for heart disease and diabetes. The findings will be published in scientific journals so that understanding of the way in which exercise decreases the risk of heart disease and diabetes can be increased. This information may help make up better exercise guidelines, particularly for people who are overweight or obese.

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain results and discuss the implications with you.

What if something goes wrong?

The chances of something going wrong are extremely small. We have recently conducted a similar project and there were no problems. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the University or hospital will have your name and address removed so that you cannot be recognised from it. In addition, your records, samples and results will be identified by a number and not your name.

What will happen to the results of the research study?

The results from this study will be presented at scientific meetings and published in scientific journals. A copy of the published results will be sent to you upon request. You will be informed which part of the study you were in, as this information will be confidential and no one else will know your name and which part you participated in.

What will happen to my samples after the study has finished?

The blood samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and heart disease; this may involve investigating new biochemical markers that are not yet identified. Samples will be analysed anonymously and will require a new ethics application before they would be used for future research. If you do not wish your samples to be used for future research, please indicate this on the consent form.

Who has reviewed the study?

This study has been reviewed and approved by the North Glasgow NHS Trust Research Ethics Committee.

Who is organising and funding the research?

This study is being funded by the British Heart Foundation and the Glasgow Royal Infirmary R&D Endowment Fund (Chest, Heart and Stroke).

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting either:

Iqbal AlShayji
Mobile: 07799353689 *E-mail*: i.alshayji.1@research.gla.ac.uk
Tel: 0141 211 4595 or 4596

Dr Jason Gill
Tel: 0141 3302916 *E-mail*: j.gill@bio.gla.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.

Thank You for Your Time and Participation

Volunteer Identification Number: ___________

UNIVERSITY of GLASGOW

CONSENT FORM

Title of Project: Effects of moderate exercise on very low density lipoprotein kinetics

Lay title: How does exercise affect the metabolism of the liver's fatty particles?

Name of Researcher: ____

Please initial box

1. I confirm that I have read and understand the information sheet dated 21/02/2006 (Version 3) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

4. I agree that my GP is to be informed of my participation in this study. ☐

5. I agree for my samples to be used for future research into the prevention and treatment of diabetes and heart disease. This may involve analysis of new biochemical markers not yet identified. Yes ☐ No ☐

Name of Subject	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

Copy for subject
Copy for researcher

Appendix B1: Health Screen Questionnaire – Chapters 3 & 4

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name: ____

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (*i*) their own continuing well-being and (*ii*) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

(a) on medication, prescribed or otherwise	yes [ ]	no [ ]
(b) attending your general practitioner	yes [ ]	no [ ]
(c) on a hospital waiting list	yes [ ]	no [ ]

2. In the past two years, have you had any illness which required you to:

(a) (a) consult your GP	yes [ ]	no [ ]
(b) attend a hospital outpatient department	yes [ ]	no [ ]
(c) be admitted to hospital	yes [ ]	no [ ]

3. Have you ever had any of the following:

(a) Convulsions/epilepsy	yes [ ]	no [ ]
(b) Asthma	yes [ ]	no [ ]
(c) Eczema	yes [ ]	no [ ]
(d) Diabetes	yes [ ]	no [ ]
(e) A blood disorder	yes [ ]	no [ ]
(f) Digestive problems	yes [ ]	no [ ]
(g) Hearing problems	yes [ ]	no [ ]
(h) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(i) Numbness in hands or feet	yes [ ]	no [ ]
(j) Disturbance of vision	yes [ ]	no [ ]
(k) Thyroid problems	yes [ ]	no [ ]
(l) Kidney or liver problems	yes [ ]	no [ ]
(m) Chest pain or heart problems	yes [ ]	no [ ]
(n) Any other health problems	yes [ ]	no [ ]
(o) An allergy to soya protein or eggs	yes [ ]	no [ ]

4. For female volunteers only

(a) Are you pregnant or think that you might be pregnant yes [ ] no [ ]

(b) Do you take the contraceptive pill or other hormone-based contraceptives

yes [ ] no [ ]

(c) Are you postmenopausal yes [ ] no [ ]

(d) Are you receiving Hormone Replacement Therapy (HRT)

yes [ ] no [ ]

5. Have any of your family (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins) ever had any of the following: (if yes please give details including age of first diagnosis)

(a) Any heart problems yes [ ] no [ ]

(b) Diabetes yes [ ] no [ ]

(c) Stroke yes [ ] no [ ]

(d) Any other family illnesses yes [ ] no [ ]

6. Do you currently **smoke** yes [ ] no [ ]

Have you ever smoked yes [ ] no [ ]

If so, for how long did you smoke and when did you stop? ……………………

7. How many units of alcohol do you typically drink in a week? ………………….

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

……………………………………………………………………………………………

Name and address of GP

………………………………………………………………………………

………………………………………………………………………………

Blood pressure measured at screening…………………..mm Hg

Appendix B2: Health Screen Questionnaire – Chapter 5

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name: ____

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (*i*) their own continuing well-being and (*ii*) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

(a) on medication, prescribed or otherwise	yes [ ]	no [ ]
(b) attending your general practitioner	yes [ ]	no [ ]
(c) on a hospital waiting list	yes [ ]	no [ ]

2. In the past two years, have you had any illness which required you to:

(a) consult your GP	yes [ ]	no [ ]
(b) attend a hospital outpatient department	yes [ ]	no [ ]
(c) be admitted to hospital	yes [ ]	no [ ]

3. Have you ever had any of the following:

(a) Convulsions/epilepsy	yes [ ]	no [ ]
(b) Asthma	yes [ ]	no [ ]
(c) Diabetes	yes [ ]	no [ ]
(d) A blood disorder	yes [ ]	no [ ]
(e) Digestive problems	yes [ ]	no [ ]
(f) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(g) Numbness in hands or feet	yes [ ]	no [ ]
(h) Disturbance of vision	yes [ ]	no [ ]
(i) Thyroid problems	yes [ ]	no [ ]
(j) Kidney or liver problems	yes [ ]	no [ ]
(k) Chest pain or heart problems	yes [ ]	no [ ]
(l) Any other health problems	yes [ ]	no [ ]
(m) An allergy to soya protein or eggs	yes [ ]	no [ ]
(n) An allergy to nuts		

4. For female volunteers only

(a) Are you pregnant or think that you might be pregnant yes [ ] no [ ]

(b) Do you take the contraceptive pill or other hormone-based contraceptives

yes [ ] no [ ]

(c) Are you postmenopausal yes [ ] no [ ]

(d) Are you receiving Hormone Replacement Therapy (HRT)

yes [ ] no [ ]

5. Have any of your family (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins) ever had any of the following: (if yes please give details including age of first diagnosis)

(a) Any heart problems yes [ ] no [ ]

(b) Diabetes yes [ ] no [ ]

(c) Stroke yes [ ] no [ ]

(d) Any other family illnesses yes [ ] no [ ]

6. Do you currently **smoke** yes [ ] no [ ]

Have you ever smoked yes [ ] no [ ]

If so, for how long did you smoke and when did you stop? ……………………

7. How many units of alcohol do you typically drink in a week? ………………….

8. Have you taken part in a research study in the last 3 months? yes [ ] no [ ]

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

……………………………………………………………………………………………
……………………………………………………………………………………………
……………………..…………………………………………………………...………………
…………………………………………………………………………….………………

Name and address of GP

……………………………………………………………………………………

……………………………………………………………………………………

Blood pressure measured at screening…………………..mm Hg

Appendix C: Kinetic Results For All Subjects According To ApoE Phenotypes

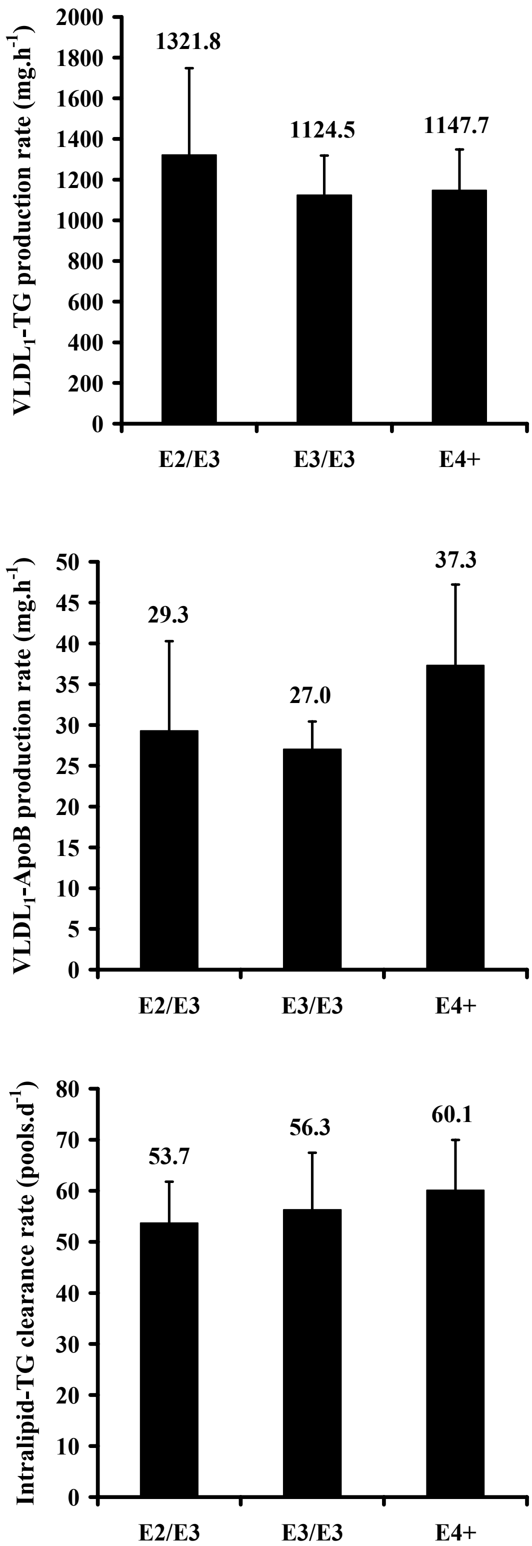


Figure 8.1: Production rates of [A] $VLDL_1$-TG and [B] $VLDL_1$-apoB and [C] Intralipid-TG clearance rate for all subjects (n = 23) according to apoE phenotypes: E2/E3 (n = 3), E3/E3 (n = 11) and E4+ (n = 9). $P > 0.05$ for differences between phenotypes.