Dysbetalipoproteinaemia - Clinical and Laboratory Aspects, Changes in Lipoprotein Composition and Remnant Metabolism Associated with Lipid Lowering Drugs

Dirk Jacobus Blom

MBChB (UCT) MMed (UCT) FCP (SA)

Dissertation presented for the Degree of

Doctor of Philosophy in the Department of

Medicine of the Faculty of Health

Sciences

University of Cape Town

April 2007

Supervisor: Professor A. David Marais

MBChB (UCT) FCP (SA)

Declaration

Dysbetalipoproteinaemia- Clinical and Laboratory Aspects, Changes in Lipoprotein Composition and Remnant Metabolism Associated with Lipid Lowering Drugs

I, Dirk Jacobus Blom, hereby

1. grant the University of Cape Town free licence to reproduce the above thesis in whole or in part for the purpose of research
2. declare that
    a. the above thesis is my unaided work, both in concept and execution, and that apart from the normal guidance received from my supervisor, I have received no assistance except as stated below
    b. neither the substance nor any part of the above thesis has been submitted in the past, or is being submitted for a degree in the University of Cape Town or any other university.

_____ _____

(Dirk Blom) (Date)

Acknowledgements

Professor David Marais first introduced me to the world of lipids, lipoproteins, receptors and familial dyslipidaemias. With his guidance and assistance metabolic pathways, mutations and diseases that initially seemed complex and illogical soon became easy to understand and made perfect sense. Without his enthusiasm and guidance this thesis would never have seen the light of day. I thank him for this.

The lipid clinic at Groote Schuur Hospital has a long history, but the present clinic has largely been shaped by Professor D. Marais and his predecessor, Professor Mike Berger. It would not have been possible to compile the data presented in this thesis without a standardized system of record-keeping, clinical and laboratory databases and routine banking of DNA for consenting patients. Professor D. Marais must take the major credit for establishing a clinic committed to excellence both in patient care and research.

I thank all my colleagues who have either worked at the lipid clinic in the past or are currently still working at the lipid clinic. This thesis would not have been possible without their contributions. Their dedication to patient care and meticulous record keeping is particularly reflected in the clinical sections of this thesis. It is impossible to mention everybody that has made a contribution to the lipid clinic but special thanks go to Dr. Jean Firth who has been a stalwart of the clinic for over twenty years. Dr Karen Wolmarans and Dr Mary Bateman have also worked at the lipid clinic for many years and have made important contributions to patient care.

This thesis could not have been completed without the expert and generous assistance of the technical staff of the University of Cape Town lipid laboratory. Mrs. Pam Byrnes was responsible for the non-denaturing polyacrylamide gradient gel electrophoresis and VLDL-compositional analyses reported in this thesis. Mrs. Mary Moodie, and in later years Miss Bharati Ratanjee, was responsible for extraction and banking of DNA from all patients seen at the lipid clinic. Miss Bharati Ratanjee also assisted with many assays in the laboratory and was always at hand to help develop new methods. Mrs. Sheena Jones performed all the DNA amplifications and restriction enzyme digests necessary to identify the common apolipoproteinE isoforms. She also screened patients for other locally prevalent mutations and amplified DNA for sequencing the binding region of apolipoproteinE. Mrs. Marietjie Levey patiently performed the many sequential ultracentrifugation studies reported in this thesis. The high calibre of work and meticulous attention to detail of all the staff in the laboratory is gratefully acknowledged.

I thank Professors Trevor Redgrave and Hugh Barrett of the University of Western Australia (Perth, Australia) for their contributions to the study of chylomicron remnant metabolism using stable isotope methodology. This test was initially conceived and developed by Professor T. Redgrave. The breath test has in the past been evaluated in patients with familial dyslipidaemia in collaboration with the lipid laboratory at the University of Cape Town. Professor T. Redgrave supplied all the emulsions used in the breath test and measured the enrichment of the ^{13}C-tracer in the breath samples collected in Cape Town. Professor Hugh Barrett developed the kinetic model used to analyze the breath test data. He travelled to Cape Town to introduce the

lipid laboratory to the intricacies of metabolic modelling. He taught me how to model the breath test data using the SAAM II software.

DNA sequencing was outsourced to a commercial entity- Inqaba Biotechnological Industries Pty. Ltd.; Pretoria, South Africa. They delivered high quality sequences and were always willing to repeat sequences if necessary.

Sister Jenny Ross was responsible for the day-to-day administration and running of the lipid clinic at Groote Schuur Hospital. She excelled in her multiple roles as phlebotomist, retriever of results, filing clerk, data capturer, telephonist and patient confidante. This thesis would not have been possible without her efforts.

I thank Bayer for sponsoring the clinical trial reported in this thesis. Their patience and understanding during the difficult phase of protocol development is acknowledged. I am particular grateful to them for allowing further analysis, use and publication of the data generated during the clinical study despite the withdrawal of cerivastatin from the international market.

Sister Rose Jooste was the clinical research co-ordinator responsible for the Bayer W6228/10176 study. Her friendly nature and excellent relationship with the patients contributed substantially to the success of the study. She also made excellent tuna sandwiches for all the patients participating in the breath test. Sister Sue Delport ably assisted her whenever necessary.

The contributions of Mrs. Elizabeth Phillips, secretary to the lipid clinic, and Mr Jeremy Philander, laboratory assistant, also need to be acknowledged. Without their efforts the lipid clinic and laboratory would not be able to run smoothly and efficiently.

I thank all the patients who have faithfully attended the lipid clinic over many years, despite often adverse and difficult circumstances at Groote Schuur Hospital. A special thank you goes to all the patients that participated in the clinical trial described in this thesis. The patients are the foundation of our clinic and I hope this thesis will contribute to improved standards of care for all patients with dysbetalipoproteinaemia.

My final thank you goes to my family without whom none of this would ever have been possible.

Abstract

This thesis examines and reviews multiple aspects of dysbetalipoproteinaemia. Dysbetalipoproteinaemia is a severe, highly atherogenic mixed hyperlipidaemia characterized by the accumulation of remnants of triglyceride-rich lipoproteins. Genetic susceptibility is conferred by mutations in apolipoproteinE, but manifest hyperlipidaemia often only occurs in the presence of metabolic abnormalities that influence lipoprotein production or clearance.

Normal lipid metabolism is briefly reviewed to enable a better understanding of the pathophysiology of dysbetalipoproteinaemia. Hepatic remnant clearance is reviewed in detail with particular emphasis on the roles of all hepatic lipoprotein receptors. The importance of differential hepatic lipoprotein receptor binding affinities according to apoE mutation is emphasized.

Clinical and laboratory records of all patients diagnosed with dysbetalipoproteinaemia at the lipid clinic of Groote Schuur Hospital, Cape Town (South Africa) were retrieved and reviewed. The diagnosis of dysbetalipoproteinaemia was made if cholesterol-enriched VLDL and/or mutations in apoE known to be associated with dysbetalipoproteinaemia were identified in patients with mixed hyperlipidaemia.

Dysbetalipoproteinaemia was diagnosed in 136 patients. Seventy patients were apoE2 homozygotes, the apoE (R145C) mutation was found in 27 patients (23 heterozygotes,

4 homozygotes) and there were 2 apoE (K146Q) heterozygotes. No mutation was found in 37 patients despite sequencing of the apoE ligand binding domain.

The mean total cholesterol and triglyceride at presentation were 11.79 mmol/L and 8.28 mmol/L respectively. Men presented a mean of 6.25 years earlier than women. Characteristic palmar crease xanthomata were found in 25% of patients. Atherosclerotic complications were frequent and peripheral vascular disease was highly prevalent.

The majority of patients had identifiable precipitating disorders or metabolic stressors. The BMI was more than 25 kg/m^2 in almost 80% of patients. At first presentation 27% of patients were diabetic. Other identified precipitants included renal disease, hypothyroidism, alcohol abuse and antiretroviral therapy.

Agarose gel electrophoresis was found to be a poor diagnostic test for dysbetalipoproteinaemia, while non-denaturing polyacrylamide gradient gel electrophoresis is a useful screening test. A ratio of apoB/total cholesterol of less than 0.15 g/mmol predicts dysbetalipoproteinaemia in patients with mixed hyperlipidaemia. The diagnostic utility of VLDL-compositional analysis was evaluated at various diagnostic thresholds.

The effects of lipid lowering therapy in dysbetalipoproteinaemia were evaluated both in an observational fashion and in a double-blind randomized clinical trial. The mean observed reductions in triglycerides and total cholesterol at the clinic were 62% and 52% respectively. In the setting of a clinical trial cerivastatin and fenofibrate lowered

total cholesterol to the same extent, but fenofibrate reduced triglycerides more effectively. The major effect of lipid lowering therapy was to reduce lipid masses in the VLDL1 and VLDL2 fractions. VLDL1 core composition was altered significantly with a decrease in cholesterylester content and a relative increase in triglyceride content.

Chylomicron remnant metabolism was studied in a subset of trial participants. The fatty acid of the cholesterylester in an artificial chylomicron remnant-like emulsion was labelled with ^{13}C. The emulsion was injected intravenously and the fractional catabolic rate was determined by measurement of $^{13}CO_2$ in the breath and compartmental modelling. Treatment with fenofibrate was associated with a significant increase from baseline in the fractional catabolic rate.

Abbreviations

4S	Scandinavian Simvastatin Survival Study
ACAT	acylCoA: cholesterol acyltransferase
ADA	American Diabetes Association
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
apoB	apolipoprotein B
apoE	apolipoprotein E
ARMS	amplification refractory mutation system
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUC	area under the curve
BMI	body mass index
CABG	coronary artery bypass grafting
CE	cholesterol ester
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CHO	chinese hamster ovary
CK	creatinine kinase
CV	cholesterol in VLDL
CVD	cardiovascular disease
CerVD	cerebrovascular disease
C_{VLDL}	cholesterol in VLDL
FC	free cholesterol
FCH	familial combined hyperlipidaemia
FCR	fractional catabolic rate
FFA	free fatty acids
FH	familial hypercholesterolaemia
FH HTZ	FH heterozygotes
FHdysß	FH heterozygotes with apoE2/E2 genotype
geno-	genonegative
geno+	genopositive
GSH	Groote Schuur Hospital
HbA_{1C}	glycated haemoglobin
HDLC	high density lipoprotein cholesterol
HIV	human immunodeficiency virus
HL	hepatic lipase
HMGCoA	3-hydroxy-3-methylglutaryl coenzyme A
HSPG	heparan sulfate proteoglycan
HRT	Hormone replacement therapy
IDL	intermediate density lipoproteins
IEF	isoelectric focusing
IHD	ischaemic heart disease
IMT	intima-media thickness

kb	kilobases
LCAT	lecithin-cholesterol acyltransferase
LDL	low density lipoproteins
LDLC	low density lipoprotein-cholesterol
LPL	lipoprotein lipase
LpX	lipoprotein X
LRP	LDL-receptor related protein
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
MRL	Medical Research Laboratories
mRNA	messenger RNA
NAFLD	non-alcoholic fatty liver disease
NCEP	National Cholesterol Education Program
nm	nanometers
OMIM	Online Mendelian Inheritance in Man
PCI	percutaneous coronary intervention
PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
PGGE	polyacrylamide gradient gel electrophoresis
PI	protease inhibitor
PL	phospholipid(s)
PR	production rates
PS	pool size
PVD	peripheral vascular disease
RAP	receptor associated protein
Rf	retardation factor
RLP-C	remnant-like particles cholesterol
ROC	receiver-operator characteristic curve
rpm	revolutions per minute
SAE	serious adverse events
SCAP	SREBP-cleavage activating protein
SNP	single nucleotide polymorphism
SREBP	sterol regulatory element binding protein
TC	total cholesterol
TEMED	tetramethlylethylenediamine
TG	triglycerides
TGRL	triglyceride-rich lipoproteins
TG_{VLDL}	triglycerides in VLDL
TIA	transient ischaemic attack
TNT	Treat to New Targets Study
TP	triglycerides in plasma
TV	triglycerides in VLDL
UCT	University of Cape Town
ULN	upper limit of normal
VLDL	very low density lipoprotein(s)
WHR	waist-hip ratio

Table of Contents

Table of Contents: Tables

Table of Contents: Figures

1 Chapter One: Introduction to dysbetalipoproteinaemia

1.1 Introduction

Dysbetalipoproteinaemia is an uncommon (approximate incidence in a Western European population of 0.2-1 per 1000 (1)) genetic disorder of lipoprotein metabolism. The molecular defect in dysbetalipoproteinaemia is in apolipoproteinE (apoE) (2), but isolated cases of complete hepatic lipase (HL) deficiency (3;4) with a lipoprotein phenotype similar to that seen in dysbetalipoproteinaemia have been described. The dysbetalipoproteinaemic phenotype is characterized by excessive accumulation of remnants of triglyceride-rich lipoproteins (TGRL), resulting in a variable but often severe mixed hyperlipidaemia in which the mass ratio of plasma triglycerides (TG) to plasma total cholesterol (TC) often approaches unity (5). Lipid infiltration of the skin and/or tendons may occur; palmar crease infiltration leads to the characteristic physical sign of palmar crease xanthomata (or *xanthomata striata palmaris*) seen in a minority of patients. Atherosclerotic complications, with a particular emphasis on peripheral vascular disease (PVD), are frequent and premature and often occur after relatively short periods of hyperlipidaemia (6-10).

1.2 Terminology

Over the years many terms have been used to describe the disorder that I shall refer to as dysbetalipoproteinaemia in this thesis. In 1952 Gofman *et al* (11) described a lipid disorder characterized clinically by tuberous xanthomata over extensor surfaces and planar xanthomata of the palms. Analytical ultracentrifugation revealed increased concentrations of lipoproteins with flotation rates indicating small very low density

lipoproteins (VLDL) and intermediate density lipoproteins (IDL) (S_f= 12-20). Gofman named this disorder *xanthoma tuberosum*. In 1967 Fredrickson published his classification of hyperlipidemias based on paper electrophoretic patterns (12). He identified a lipoprotein pattern with broad intense staining starting in the β zone, where low density lipoproteins (LDL) stain, and extending into the pre- β zone to which VLDL usually migrates (13). This broad β-band pattern was designated as Type III hyperlipidaemia. Fredrickson numbered lipoprotein patterns beginning at the origin, or cathodal end, of the paper electrophoretic strip and allocated numbers as successive bands were unduly prominent (12). Thus Type I hyperlipidaemia describes increased chylomicrons that remain at the origin during paper electrophoresis. Type II hyperlipidaemia is characterized by a prominent β-band, which is the first electrophoretic band in the absence of chylomicronaemia. Type IV hyperlipidaemia refers to an increase in the pre- β zone and as the broad β –band straddles the β and pre- β zone it was allocated the number three. Fredrickson speculated that the patients with *xanthoma tuberosum* described by Gofman might in fact have had Type III hyperlipidaemia, as the lipoprotein patterns on analytical ultracentrifugation were remarkably similar and both cohorts included patients with unusual xanthomata of the hands. Fredrickson also described the unusual migratory behaviour of the ultracentrifugally isolated lipoprotein fraction with density < 1.006 g/ml in patients with dysbetalipoproteinaemia. This VLDL fraction migrated to the β-position instead of the pre- β position and was referred to as β-VLDL (14).

Two of the other terms used to name this disorder also refer to its unusual electrophoretic pattern. Broad- β disease describes the abnormal electrophoretic band seen in patients with this disease. As many as half of all patients with this disorder do

not manifest this characteristic band as subsequent studies have shown (8;15), and using the term broad-β disease may result in clinicians who have little experience with the disorder falsely dismissing the diagnosis if a broad-β band is not seen on electrophoresis. Lipoprotein electrophoresis, which is nowadays generally performed in agarose instead of on paper, is often no longer available in routine chemical pathology laboratories and many clinicians are not familiar with electrophoretic terminology anymore. The term dysbetalipoproteinaemia is not entirely unambiguous either, as it refers to the unusual physical and chemical properties of VLDL (β-VLDL) in this disorder, causing it to migrate to the β-position during electrophoresis. The main lipoprotein disturbance in this disorder is of course not found in the LDL or β-fraction but in larger and less dense lipoproteins such as VLDL and IDL. Nevertheless, the term dysbetalipoproteinaemia is commonly used in the literature. Searching PubMed at *www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed* (accessed on the 13th January 2006) with the search term "type III hyperlipidaemia" retrieved 471 references while the term "dysbetalipoproteinaemia " retrieved 522 references. PubMed does cross reference search terms but the term "dysbetalipoproteinaemia" still retrieved more references, indicating more common usage. The designation familial dysbetalipoproteinaemia is also commonly used in the literature to indicate the genetic nature of the disorder (16-18).

Most apoE variants associated with dysbetalipoproteinaemia are incompletely penetrant and many patients with abnormal apoE will either be normolipidaemic or even hypolipidaemic. These individuals may have detectable β-VLDL and it has been suggested that they be labelled as dysbetalipoproteinaemic while patients that develop overt hyperlipidaemia are classified as having type III hyperlipidaemia (5). This

suggestion has not found general acceptance in the literature and the terms dysbetalipoproteinaemia and type III hyperlipidaemia are often used interchangeably.

Dysbetalipoproteinaemia has also been referred to as remnant removal disease (19). Although this descriptive term describes the main pathophysiological abnormality in dysbetalipoproteinaemia well, it has not been generally adopted and is infrequently found in the literature.

In this thesis I shall only use the term dysbetalipoproteinaemia for the sake of uniformity. Unless specifically stated otherwise, the diagnosis of dysbetalipoproteinaemia in this thesis defines a group of patients with a lipid phenotype characterized by elevation of both plasma triglycerides and total cholesterol secondary to accumulation of cholesterol-rich remnant lipoproteins. Patients were included in this study based on phenotypic criteria and apoE mutations have not been identified in all patients.

1.3 Historical aspects

1.3.1 Overview of the history of atherosclerosis

Evidence of atherosclerosis has been found in some Egyptian mummies (5;20), indicating that this disease is certainly not new to mankind. Galen, the most influential physician of ancient Greece whose teachings dominated the European medical tradition until the Renaissance, described vascular aneurysms but did not describe other forms of atherosclerotic cardiovascular disease. During the Renaissance the resurgent interest in anatomy led to the realization that arteries degenerated with

advancing age and Leonardo da Vinci was one of the first to make this observation. As aneurysms are much more visible than atherosclerosis and often cause dramatic clinical presentations, vascular disease research focussed on them for many centuries. The term arteriosclerosis was first used in the early 19th century to describe the gruel-like atheroma and hardening (sclerosis) of vessels associated with atherosclerosis. Virchow and Rokitansky, two of the leading pathologists of the 19th century, provided detailed pathological descriptions of atherosclerotic lesions and speculated about the causation of the lesion they studied under the microscope. Our modern understanding of atherosclerosis still incorporates elements of their theories.

Experimental research on atherosclerosis first started in Russia in the early 20th century. Ignatowski, a young Russian pathologist, showed that feeding rabbits a protein-rich diet consisting of eggs, meat and milk was associated with the development of atherosclerosis in later life (21). This finding was initially interpreted as confirmation of an earlier hypothesis proposed by Metschnikow that high protein diets accelerate the ageing process. At that time atherosclerosis was seen as a disease of ageing, a chronic process that developed slowly over many years and was an inevitable consequence of senescence. In 1910 Windaus published a paper showing that atherosclerotic aortas had a much higher cholesterol content than unaffected aortas (22). Inspired by Ignatowski's earlier work Anitschkow, an experimental pathologist in St. Petersburg, fed rabbits cholesterol dissolved in sunflower oil and rapidly induced lesions resembling human atherosclerosis. Rabbits fed only sunflower oil did not develop atherosclerotic lesions (23;24). Although Anitschkow published in widely read and well-respected German journals, his work was not taken seriously for many years. Some of the laboratories that did try to replicate Anitschkow's findings

were unable to do so as they used rats and dogs as experimental animals; species in which cholesterol feeding does not provoke atherosclerosis. The plasma cholesterol in Anitschkow's rabbits was also very high (around 12-25 mmol/L) and as human levels are seldom as high as this, the experiments were thought not to be relevant to humans. Possibly the most significant reason for Anitschkow's findings being ignored for so long was that they simply did not fit in with the prevailing view of atherosclerosis as a slowly progressive degenerative disorder (25).

Anitschkow first published his lipid hypothesis of atherogenesis in 1913, but the role of lipids and potential benefits of lipid-lowering therapy in human atherosclerotic disease remained controversial for many years (26;27). Epidemiological and early intervention studies (28;29) provided strong support for the lipid hypothesis but the opposition remained vociferous (30). Only following the discovery of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors, which are now generally known as statins, by Endo was it possible to substantially lower cholesterol levels without major side effects. The Scandinavian Simvastatin Survival Study (4S) (31) was published in 1994 and conclusively showed that lowering cholesterol with simvastatin reduced death and cardiovascular events in a high-risk group of patients with hypercholesterolaemia and established ischaemic heart disease. The 4S persuaded some of the most strident critics of the lipid hypothesis to become enthusiastic proponents of statin therapy (32). Steinberg has recently reviewed the history of the "cholesterol controversy" in considerable detail (25;33-35).

1.3.2 The history of dysbetalipoproteinaemia

Artists depicted tendon xanthomata, which are most commonly associated with familial hypercholesterolaemia (FH), well before their clinical significance was known (36). I am, however, unaware of any artistic depiction of palmar crease xanthomata, the characteristic physical sign of dysbetalipoproteinaemia. This is perhaps not surprising as dysbetalipoproteinaemia is less prevalent than FH and palmar crease xanthomata may be quite subtle and go unnoticed by patients and many physicians.

The history of dysbetalipoproteinaemia starts with Gofman and the first successful characterization of human lipoproteins (37). In 1952 Gofman described 23 patients with cutaneous xanthomata (*xanthoma tuberosum*) and a unique lipoprotein pattern (11). Gofman's work was based on analytical ultracentrifugation, a very costly and labour intensive method restricted to a few laboratories only. Using paper electrophoresis and preparative ultracentrifugation, techniques with greater availability and the capacity to process large amounts of specimens, Fredrickson further described the lipoprotein phenotype of dysbetalipoproteinaemia (14). Cholesterol enriched β-migrating VLDL was the characteristic and main diagnostic feature of dysbetalipoproteinaemia for many years.

Further studies of the lipoprotein phenotype in dysbetalipoproteinaemia demonstrated the presence of lipoprotein particles larger than VLDL and enriched in cholesteryl esters in the post-absorptive state (38;39). Chylomicrons studied during active fat absorption were of normal composition. This finding led to the suggestion that these large lipoprotein particles represent partially degraded chylomicrons or chylomicron-

remnants. Beta-VLDL would similarly contain partially degraded normal VLDL or VLDL-remnants.

The first step to understanding dysbetalipoproteinaemia on a molecular basis was taken when Havel *et al* found that the VLDL fraction of patients with dysbetalipoproteinaemia contained unusually large amounts of a protein called the *arginine-rich* protein (40). This protein was, of course, apoE. Utermann and his co-workers analyzed apoE in more detail using isoelectric focusing (IEF) and found that a specific isoform of apoE was invariably absent in patients with dysbetalipoproteinaemia (41). Much work was subsequently done to understand the genetics of the apoE system and arrive at a uniform system of classification (42-46).

Mahley and co-workers determined the amino-acid sequence of apoE and the molecular differences between the various isoforms of apoE (47-49). Once the causative molecular defect in the vast number of dysbetalipoproteinaemic patients had been found, the pathophysiology of this disorder was soon unravelled. The process by which the pathophysiology of dysbetalipoproteinaemia came to be understood will not be described here, but will be addressed when the pathophysiology of the disorder is reviewed. The topic has also recently been reviewed in detail (50).

1.4 Lipids and lipoproteins

1.4.1 Lipids

Lipids are organic compounds containing aliphatic hydrocarbons. They are generally not soluble in water but will dissolve in nonpolar organic solvents such as chloroform

or ether. Life as we currently know it would not be possible without lipids as they fulfill essential roles in maintaining the structural integrity and biological functioning of all cells. Biological systems contain a multitude of lipids and only those lipids critical to the understanding of lipoprotein metabolism will be briefly introduced here.

Cholesterol is a 27-carbon sterol molecule ($C_{27}H_{46}O$) that is absolutely essential to human life. Its evolutionary importance can be gauged by the fact that more than 100 genes are involved in the regulation of cholesterol synthesis, concentration, transport and metabolism. Cholesterol, its precursors and metabolites are involved in processes as diverse as membrane physiology, nutrient absorption, reproduction, stress responses, calcium metabolism and regulation of salt and water homeostasis (51). It is therefore understandable that there was strong evolutionary pressure to ensure an adequate supply of cholesterol to all cells. All human cells are able to synthesize cholesterol and low levels of intracellular cholesterol activate a feedback system: SREBP-SCAP [sterol regulatory element binding protein (SREBP); SREBP-cleavage activating protein (SCAP)] complexes translocate to the Golgi apparatus where two proteolytic cleavages generate a protein capable of activating multiple genes involved in lipid synthesis and uptake (52).

Cholesterol is also transported in lipoproteins and may be made available to cells with a high demand in this way. Cholesterol may be found either as free cholesterol (FC) or cholesteryl esters (CE). Esterification occurs at the alcohol moiety at position 3 of the cyclopentane phenanthrene. When cholesterol is esterified a long-chain fatty acid is linked to its hydroxyl group. The resulting molecule is less polar than cholesterol and CE are mainly transported in the core of lipoproteins. In plasma lecithin-

cholesterol acyltransferase (LCAT) catalyses the transfer of fatty acids from phosphatidylcholine to cholesterol while acylCoA: cholesterol acyltransferase (ACAT) is responsible for the synthesis of CE in other tissues. Cholesterol is therefore clearly essential for human survival and metabolic errors in cholesterol synthesis result in severe malformation syndromes (53). Yet excess cholesterol is clearly a major factor in the pathogenesis of atherosclerosis, the major cause of mortality in the industrialized world. From an evolutionary perspective, man has evolved elaborate mechanisms to guard against cholesterol deprivation, but deals with cholesterol excess brought about by a diet rich in animal products much less efficiently. Guarding against cholesterol excess might not have been subject to evolutionary pressure as life expectancy was generally shorter than the time needed for atherosclerosis to become overt. Plants have evolved cell membranes that differ from those found in the animal kingdom in several critical properties. It is therefore not surprising that the sterol molecules that have such profound influences on membrane properties differ. Plants do not synthesize cholesterol but a group of closely related sterols known collectively as phytosterols (e.g. sitosterol and campesterol).

Triglycerides (or triacylglycerols) (TG) are esters of glycerol in which all three hydroxyl groups are esterified with fatty acids. The three fatty acids found in triglycerides may be identical or can differ. In biological systems unsaturated fatty acids usually occur at the *sn2* position. Triglycerides are non-polar and are transported in the core of lipoproteins. Triglycerides are energy dense and provide about double the amount of energy (44 kilojoules/gram) found in carbohydrates or proteins. Because triglycerides are so energy dense they are the major storage medium for

excess energy in the body. Most triglycerides in the body are stored in adipocytes. The ability to store energy and survive limited periods of famine was of great evolutionary importance, yet an excess of triglycerides (or their fatty acid constituents) in cells not designed for fat storage may have multiple deleterious consequences, collectively termed lipotoxicity (54). Adipocyte disorders may be associated with profound metabolic consequences (55).

Phospholipids (PL) are chemically much more diverse and complex than the two other lipid classes discussed above. Phospholipids are formed from four components: fatty acids, a phosphate group with a negative charge, an alcohol and a backbone. The backbone may be glycerol in glycerophospholipids or sphingosine in sphingomyelin. Diacylglycerol 3-phosphate (or phosphatidate) is the simplest glycerophospholipid. Two hydroxyl groups of glycerol are esterified to fatty acids while the *sn3* carbon is esterified to phosphoric acid. The major glycerophospholipids derive from diacylglycerol 3-phosphate. The phosphate group of diacylglycerol 3-phosphate becomes esterified to the hydroxyl group of one of several alcohols. Common alcohol moieties are serine, ethanolamine, choline, glycerol and inositol. In animal cells glycolipids are derived from sphingosine. The amino group of the sphingosine backbone is acylated by a fatty acid while the primary hydroxyl group is connected to a sugar. The sugar may be simple as in cerebroside or more complex and branched in gangliosides. Biologically the most important property of phospholipids is their amphipathic nature with both hydrophilic and hydrophobic domains. Phospholipids readily self-assemble in lipid bilayers and are the main constituents of cell membranes. The amphipathic nature of phospholipids means that they are found mainly in the shell of lipoproteins. The unsaturated fatty acids in phospholipids are

prone to free radical or enzymatic oxidation and may then exert significant pro-atherogenic influences (56). Catabolism of many phospholipids requires specific lysosomal enzymes and catalytically deficient mutations of these enzymes may give rise to a lysosomal storage disorder (57).

1.4.2 Apolipoproteins

Human plasma contains a considerable amount of lipid which, according to its biochemical definition, should not be soluble in the aqueous milieu of plasma. Plasma lipids therefore need to be solubilized by associating with proteins. Additionally, such proteins allow for binding to lipoprotein receptors and enzymatic actions. Research on plasma lipoproteins started in the early 20th century with studies on horse lipoproteins (58). During World War II extensive efforts were made to isolate human serum proteins for therapeutic purposes and two major fractions with α1- and β-mobility respectively were recognized (59). Studies of the major protein components of these fractions successively led to the identification and characterization of many apolipoproteins.

The best-known role of apolipoproteins is probably that of "structural backbone" in lipoproteins. Many apolipoproteins have amphipathic regions that aid in the formation and physical organization of lipoproteins. However, not all apolipoproteins have structural roles and many apolipoproteins have multiple biological functions. Thus apolipoproteins also bind to cellular lipoprotein-receptors, regulate enzymes involved in lipid metabolism (such as lipoprotein lipase (LPL)) and facilitate transfer of lipids between lipoproteins.

A full description of all apolipoproteins is beyond the scope of this thesis and only apolipoprotein B (apoB) and apoE will be discussed in detail. Table 1.1 summarizes important features of selected other apolipoproteins.

Table 1-1 Characteristics of Selected Apolipoproteins in Normolipidaemic Fasting Humans

Name	Plasma concentration (g/L)	Molecular mass, Da	Major tissue source	Distribution in lipoproteins				Functional role
				HDL	LDL	IDL	VLDL	
ApoAI	1.3	28016	Liver and intestine	100%				Structural component of HDL, activates LCAT
ApoAII	0.4	17414	Liver and intestine	100%				Activate hepatic lipase
ApoAIV		46465	Liver and intestine					Unknown, may facilitate transfer of apolipoproteins between HDL and chylomicrons, endogenous antioxidant
ApoAV	0.00015	40000	Liver	+	-	?	+	Enhance binding of lipoproteins to endothelium, activates LPL
ApoCI	0.06	6630	Liver	97%		1%	2%	Unknown, may inhibit lipoprotein lipase
ApoCII	0.03	8900	Liver	60%		10%	30%	Activates lipoprotein lipase
ApoCIII	0.12	8800	Liver	60%	10%	10%	20%	Inhibits lipoprotein lipase
ApoD	0.1	29000	Spleen, brain, testes, adrenals	100%				? Role in regulation of arachidonic acid signaling in brain
ApoM	Very low	26000	Liver, kidney	+++	+	+	+	Formation of preβ HDL

Table compiled with the aid of references: (60-67)

1.4.2.1 Apolipoprotein B

ApoB plays a central role in human lipoprotein metabolism. The gene for apoB is located on chromosome 2 and gives rise to two forms of apoB: a truncated protein known as apoB48 and the full-length protein known as apoB100.

ApoB48 is 48% of the amino-terminal portion of apoB100. ApoB48 is synthesized in the intestines while apoB100 is synthesized in the liver. In the intestine apoB messenger RNA (mRNA) undergoes organ-specific editing - a stop-codon is introduced at nucleotide 6666 by a mRNA editase (68)- resulting in the synthesis of a truncated protein. ApoB is a very large protein with a molecular mass of around 550 kDa for apoB100 and 265 kDa for apoB48. It is an amphipathic glycoprotein that tends to form insoluble aggregates in aqueous solutions. In plasma apoB is only found in associations with lipoproteins. ApoB cannot exchange between lipoprotein particles and remains with each lipoprotein particle for its entire "lifespan". Each lipoprotein particle only contains one apoB molecule. ApoB concentrations directly reflect the number of circulating apoB-containing lipoproteins. ApoB-containing lipoproteins are atherogenic and apoB100 can be measured to predict atherosclerotic risk (69;70).

Chylomicrons are synthesized in the intestines with apoB48 serving as the structural template. VLDL is synthesized in the liver with apoB100 serving as the essential structural protein. A critical step in lipoprotein synthesis is the assimilation of adequate lipid so that apoB escapes ubiquitinylation and subsequent degradation. ApoB100 remains with VLDL during its metabolism to IDL and LDL. Apart from its structural role apoB100 also has a receptor-binding domain in its carboxyl-terminal portion. ApoB100 binds to the LDL-receptor in the liver leading to hepatic uptake of

VLDL, IDL and LDL. LDL uptake is almost entirely dependant on apoB mediated LDL-receptor binding, while alternative mechanisms exist for the uptake of TGRL.

ApoB100 mutations may result in hypolipidaemic or hyperlipidaemic phenotypes depending on the change in the apoB100 molecule. Truncating mutations cause hypocholesterolaemia while mutations in the LDL-receptor binding domain cause hypercholesterolaemia (71).

1.4.2.2 Apolipoprotein E

ApoE is a critical factor in remnant lipoprotein metabolism, exerting its influence through multiple mechanisms. ApoE binds to the LDL-receptor, the LDL-receptor related protein (LRP) and heparan sulfate proteoglycans (HSPG) (72) facilitating hepatic uptake of remnant lipoproteins. Increased levels of apoE stimulate VLDL synthesis and inhibit triglyceride hydrolysis (73). All these properties of apoE are important in understanding the pathophysiology of dysbetalipoproteinaemia and will be discussed in detail later.

Shore *et al* first identified ApoE in 1973 (74) as a protein constituent of VLDL. Initial research on apoE focussed on understanding the polymorphic nature of apoE and the role of apoE in lipoprotein metabolism. Clues indicating that apoE may play a role in neurological diseases subsequently began emerging and the role that apoE plays in neurobiology has been researched intensively since. ApoE is one of the primary apolipoproteins in the brain and has roles in neurite growth, neuronal repair and remodelling. The apoE4 isoform is a major susceptibility factor for Alzheimer's disease and has also been linked to poor outcome following traumatic head injuries,

intracerebral haemorrhage and ischaemic stroke. The role of apoE in the central nervous system and neurological disorders will not be discussed in further detail but more information can be found in references (75-78).

1.4.2.2.1 ApolipoproteinE cell biology

The **apoE gene** is located on the long arm of chromosome 19 (19q13.2 MIM 107741) in close association with apoCI, an apoCI pseudogene, apoCII and apoCIV. The gene of 3.7 kilobases (kb) contains four exons separated by three introns. ApoE mRNA is translated to a 317 amino acid nascent polypeptide. This polypeptide is directed to the endoplasmic reticulum by the 18 amino-terminal amino acids that act as a signal sequence (79). In the Golgi apparatus the protein undergoes *O*-linked glycosylation with the addition of carbohydrate moieties, with variable sialization at threonine residue 194 (80) prior to the protein being secreted (81). A significant amount of newly synthesized apoE is degraded prior to secretion (82;83).Secreted apoE may be tightly bound to cell surface proteoglycans (84). Surface-bound apoE may be released and circulate freely or be internalized by the cell, where it can either be degraded or recycled to the cell surface (85-87).

ApoE mRNA is found in many tissues, with the highest level of expression in the liver followed by the brain. ApoE mRNA is found in tissues as diverse as macrophages, spleen, kidney, adrenals and muscles (88). In the brain apoE is secreted by astrocytes (89). In plasma the majority of apoE is of hepatic origin but extrahepatic secretion of apoE, mainly by macrophages, makes up 20-40% of circulating apoE in certain primates (90).

1.4.2.2.2 ApolipoproteinE structure

The mature secreted apoE protein consists of 299 amino acids and has a relative molecular mass of 34 kDa. The apoE molecule has two distinct structural domains, the amino-terminal two thirds of the molecule and the carboxy-terminal one third. A hinge region connects these two domains.

The amino-terminal domain of apoE consists of four amphipathic α-helix bundles (residues 24-42, 54-81, 87-122, 130-164) arranged in an anti-parallel fashion. The hydrophobic faces are orientated towards the interior of the bundle (91). The heparin and receptor-binding sites of apoE are found within the amino-terminal domain. The basic amino acids at positions 136,140,142,143,154,146,147 and 150 are essential for heparin binding (92). This arginine- and lysine-rich region of helix 4 (specifically residues 136-150) also binds to the acidic residues found in the binding regions of the LDL-receptor and LRP. (93). Residues 171-183 contains elements essential for the stabilization and proper alignment of the receptor-binding region (94). For apoE to bind to the LDL-receptor with high affinity it needs to be associated with lipids. When apoE associates with lipids, lysine at position 143 and 146 becomes more exposed to the aqueous phase while amino acids 165-169 form a helix. This change results in enhanced positive electrostatic potential in the receptor-binding region, allowing for high-affinity binding to the LDL-receptor (95;96). The amino-terminal portion of apoE also contains a binding site for scavenger receptor class B type I (97). The physiological importance of this finding still needs to be determined. The interaction of apoE with HSPG, LRP and the LDL-receptor is a highly complex physicochemical process that requires exact conformational alignment of many amino acids. Mutations

in and around the apoE receptor-binding region decrease receptor-binding affinity and depending on the nature of the mutation, binding to the various receptor types may be differentially affected. Mutations that lie outside of the receptor-binding region may still affect receptor binding by secondarily altering the conformation of this region. This principle is illustrated by the altered receptor binding of apoE (R158C) or apoE Leiden.

The structure of the carboxy-terminus (residues 210-299) of apoE is less well understood than the amino-terminus. The carboxy-terminus contains the major lipid binding and self-association regions of apoE. It also contains a heparin binding site (92). The carboxy-terminus of apoE is believed to adopt an amphipathic α-helical conformation with a segment that adopts a coiled-coil helix formation at the lipoprotein binding site (98). Residues 267-299 are responsible for apoE self-association (99), while residues 245-266 determine lipoprotein binding preferences (100;101). The various isoforms of apoE preferentially associate with different lipoprotein classes. ApoE4, for instance, is preferentially found in VLDL lipoproteins. The arginine at position 112 in apoE4 forms a salt bridge with a glutamic acid at position 109 resulting in the side arm of arginine 61 being displaced. This likely alters the interaction of arginine 61 with the carboxy-terminal of apoE (probably with a glutamic acid residue at position 255) and results in altered lipoprotein binding affinity (102).

1.4.2.2.3 ApolipoproteinE polymorphism

ApoE is a highly polymorphic protein. Primary polymorphism is due to genetic variations at the apoE gene locus, while secondary polymorphism is brought about by variable degrees of sialylation at position 194. There are three common primary isoforms of apoE, known as apoE2, apoE3 and apoE4 according to their IEF migration. The common isoforms are the product of three alleles (ε2, ε3 and ε4) at a single gene locus. The three common isoforms differ at amino acid positions 112 and 158. The amino acid substitutions that differentiate the various isoforms also account for the charge difference seen on IEF of apoE. ApoE4 has two positive charges and apoE3 one positive charge relative to apoE2.

Table 1-2 Amino acid differences between the three common apoE isoforms

Isoform	**Amino acid**	
	Position 112	**Position 158**
ApoE2	Cysteine	Cysteine
ApoE3	Cysteine	Arginine
ApoE4	Arginine	Arginine

1.4.2.2.4 ApolipoproteinE population genetics

The ε3 allele is the commonest allele and is often considered the human "wild-type", although the ε4 allele may be the true "ancestral" allele. Almost all animals, including higher primates (103), have the equivalent of a ε4 allele. One could speculate that evolutionary pressure may have favoured the ε3 allele because of its desirable neurobiological properties.

Infectious diseases may also have exerted evolutionary pressure on apoE. There is evidence that apoE influences outcome in several infectious diseases rather than susceptibility to infection. In hepatitis C virus infection the presence of the ε4 allele protects against severe liver disease (104), while patients with the ε2 allele are more severely affected by herpes simplex encephalitis (105). The strongest evidence for infectious diseases exerting evolutionary pressure on apoE comes from the study of apoE genotypes in malaria. Gambian infants homozygous for apoE2 were infected with malaria at a younger age (106;107), while the apoE3/E4 phenotype was enriched amongst infants with severe cerebral malaria and malarial anaemia (108). As apoE2 homozygosity is highly prevalent in Gambia (14%) the authors of one report (107) speculate that early infection with malaria when maternal antibodies are still present may protect against more severe infection later in life. As the studies examined relatively small numbers of patients and have as yet not been replicated in other populations their findings must be treated with caution.

There are six possible apoE phenotypes when the three different genotypes are combined. They are in descending order of frequency for most populations E3/E3, E4/E3, E3/E2, E4/E4, E4/E2, and E2/E2. Certain alleles are more frequent in some populations than others. For instance the ε4 allele is more common in Northern European (14-19%) populations compared to Southern European populations (7-12%) (109). Many African populations, e.g. from Nigeria or Sudan, also have a high prevalence of the ε4 allele. Table 1.3 illustrates gene and phenotype frequencies found in large cohort studies of nondiseased populations.

Table 1-3 Frequencies of common alleles at the apoE gene locus and resultant apoE phenotypes

Population studied	Selection criteria	Sample size	Sex M: F	Genotype: Relative frequency			Phenotype frequencies %						Reference
				ε2	ε3	εe4	E2/E2	E3/E2	E3/E3	E4/E2	E4/E3	E4/E4	
African Nigerians	Unknown	176		0.028	0.662	0.310	0	3	46	3	37	11	(110)
African Americans	Population based	1612		0.131	0.668	0.201	1	18	44	5	27	5	(111)
American Indians	Community based	4541	2:3	0.016	0.855	0.129	0	2.5	72.5	0.5	23.5	1	(112)
C[1]: Framingham, USA	Community based	2258	1:1	0.08	0.787	0.133	0.5	13.1	62.7	1.7	18.8	3.2	(113)
C[1]: Münster, Germany	Factory workers	1557		0.082	0.782	0.136	0.9	11.7	62.2	2.9	19.9	2.2	(114)
C[1]: Finland	Youths	1577		0.039	0.767	0.194	0.3	5.4	58.7	1.8	30.6	3.2	(115)
C[1]: France	Random selection	504		0.081	0.802	0.117	0.8	13.09	64.28	1.6	18.65	1.58	(116)
C[1]: Italy	Random selection	260		0.073	0.827	0.1	0.4	12	68.4	16.5	1.5	1.2	(117)
Chinese	Labourers	141	1:0	0.074	0.844	0.082	1.4	12.1	70.9	0	14.9	0.7	(118)
Japanese	Population based	576		0.037	0.846	0.117	0.3	6.1	71.9	0.7	19.3	1.7	(119)
Mexican Americans	Community based	963		0.039	0.859	0.102	0.21	6.74	73.8	0.73	17.32	1.1	(120)
Shona	Pregnant females	678	0:1	0.159	0.616	0.225	3.7	17.4	40.0	7.1	25.8	6.1	(121)

[1] C=Caucasian population

Table adapted and expanded from reference (109)

1.4.2.2.5 Apolipoprotein E polymorphisms and cardiovascular disease

The apoE polymorphism is one of the most thoroughly studied genetic polymorphisms. The impact of apoE polymorphisms on lipids and cardiovascular disease risk has only been studied at the population level for the three common polymorphisms (ε2, ε3 and ε4) and only these will be considered in this section. The homozygous ε3 genotype, as the most common genotype, is used as the referent against which the influence of other alleles is evaluated. The ε2 allele is generally associated with lower total cholesterol while the ε4 allele elevates total cholesterol (122). The apoE polymorphisms have similar qualitative effects in most populations studied, despite significant differences in mean cholesterol concentrations, diet and exercise, although the magnitude of impact is variable (122). The effect is also observable when studying children (123). The ε2 allele is reported to decrease total cholesterol on average by 0.36 mmol/L while the ε4 allele is associated with an increase of 0.21 mmol/L in the total cholesterol (122). The apoE gene accounts for 8.3% (124) to 1% (113) of the variance in population LDL-cholesterol (LDLC) levels and contributes more to normal cholesterol variability than any other gene identified thus far.

The impact of apoE polymorphisms has also been studied directly at the level of the vessel, either by imaging studies such as angiography or measurement of carotid intima-media thickness (IMT), or at autopsy. In a study of healthy non-diabetic Italians the ε4 allele was associated with significantly increased IMT thickness after statistical adjustment for other atherosclerosis risk factors (117). In an autopsy study of young white males who died traumatically, the apoE genotype was found to

account for 5.7% of the variation in observed atherosclerotic lesions in the thoracic aorta after adjustment for lipid levels (125). In a more recent study the coronary arteries of 700 Finnish men were examined at postmortem. The ε4 allele was found to be a significant risk factor for atherosclerosis in younger and middle-aged men but was not significant anymore in older age (126).

Epidemiological studies have also addressed the role of apoE polymorphisms in cardiovascular disease (CVD). In the MONICA project (Monitoring of Trends and Determinants in Cardiovascular Disease) an increase of 0.01 in the relative frequency of the ε4 allele was associated with an increase of 0.114 mmol/L in the average total cholesterol and an increase in coronary heart disease (CHD) mortality of 24.5/100000. In a subset analysis of the 4S those patients in the placebo group who carried a ε4 allele had a mortality risk ratio of 1.8, independently of other risk factors for atherosclerosis. In patients treated with simvastatin this difference was not observed (127). It is important to note that although apoE polymorphisms have important effects at the epidemiological level, there is as yet no evidence to suggest that apoE genotypes should be determined routinely when screening for CVD risk. In fact, most studies addressing this issue have found areas under the receiver-operating characteristic curve (ROC) of around 0.5 (128-130), indicating that determining apoE genotypes adds no useful information in CVD risk screening for individuals.

1.4.3 Lipoproteins

As discussed previously lipoproteins are complexes of lipids and proteins. They are generally classified according to their ultracentrifugal density, although there are

alternative classification systems based on identifying lipoproteins by the apoproteins present. As a general rule, the density of lipoproteins decreases as their size increases, as larger lipoproteins contain proportionally more lipid, of lower density, and less protein. Lipoproteins are generally not found as a sharp band at any given ultracentrifugal density, but distributed broadly around one or several peaks of maximal concentration along the density gradient.

Lipoproteins are spherical particles and vary in size from more than 1000 nanometers (nm) to around 5-12 nm. Lipoproteins can be visualized by electron microscopy, but accumulation of larger TGRL results in plasma having a turbid to milky appearance (lipaemia) that is visible to the naked eye. This effect is due to light being scattered by lipoprotein particles. The lipid constituents of the outer lipoprotein shell are mainly phospholipids with some free cholesterol. Triglycerides and cholesteryl esters make up the core lipids. Apolipoproteins perform the structural and metabolic roles previously discussed. The physical and chemical characteristics of the main lipoprotein classes are illustrated in the following table.

Table 1-4 **Physico-chemical characteristics of the major lipoprotein classes**

Lipoprotein	Density g/mL [1]	Molecular mass kDa	Electrophoresis [2]	Diameter nm [3]	Lipid Constituents %			Main apolipoproteins
					Triglycerides	Cholesterol	Phospholipid	
Chylomicrons	<0.95	400 x10^3	Origin	75-1200	80-95	2-7	3-9	ApoB48, ApoCI-III, ApoE, ApoAI+II
VLDL	0.95-1.006	10-80 x 10^3	Pre-beta	30-80	55-80	5-15	10-20	ApoB100, ApoCI-III, ApoE
IDL	1.006-1.019	5-10 x 10^3	Broad-beta	25-35	20-50	20-40	15-25	ApoB100, ApoCI-III, ApoE
LDL	1.019-1.063	2.3 x 10^3	Beta	18-25	5-15	40-50	20-25	ApoB100
HDL	1.063-1.210	1.7-3.6 x 10^2	Alpha	5-12	5-10	15-25	20-30	ApoAI, ApoAII, ApoE

[1] g/ml= grams per milliliter

[2] Electrophoretic mobility, bands labelled according to Fredrickson's nomenclature

[3] nm= nanometer

Lp(a) consists of a LDL particle covalently linked to a protein called apo(a). Apo(a) is an extremely polymorphic protein that has close homology to plasminogen. Lp(a) occurs in only a few species. Lp(a) levels in humans vary markedly. The physiological significance of Lp(a) remains unclear and there are still many unanswered questions regarding this enigmatic lipoprotein (131).

1.4.4 Lipoprotein metabolism

This section will briefly review lipoprotein metabolism with particular emphasis on remnant metabolism and clearance. Reverse cholesterol transport in HDL will only be considered where it is relevant to the understanding of the metabolism of apoB containing lipoproteins.

Dietary triglycerides are hydrolyzed by luminal lipases in the intestines and the resulting glycerol, free fatty acids, mono- and acylglycerols are absorbed by enterocytes. In the enterocyte re-esterification of these components yields intact triglycerides again. Chylomicrons are assembled in the endoplasmic reticulum of the enterocyte. ApoB48 is the structural apolipoprotein and apoAI and apoAIV are also associated with nascent chylomicrons. Chylomicrons are exocytosed into lymphatic spaces following translocation to the Golgi apparatus. Chylomicrons enter the plasma compartment via the thoracic duct. In plasma chylomicrons rapidly exchange apolipoproteins with other lipoproteins, especially HDL, and acquire apoE and apoCI-III. Circulating chylomicrons are depleted of triglycerides by the action of LPL. LPL is bound to glycosaminoglycans (heparan sulphate) on the luminal side of capillary endothelial cells and hydrolyzes a proportion of chylomicron triglycerides. The expression of LPL is most pronounced in adipose and muscle tissue. As the

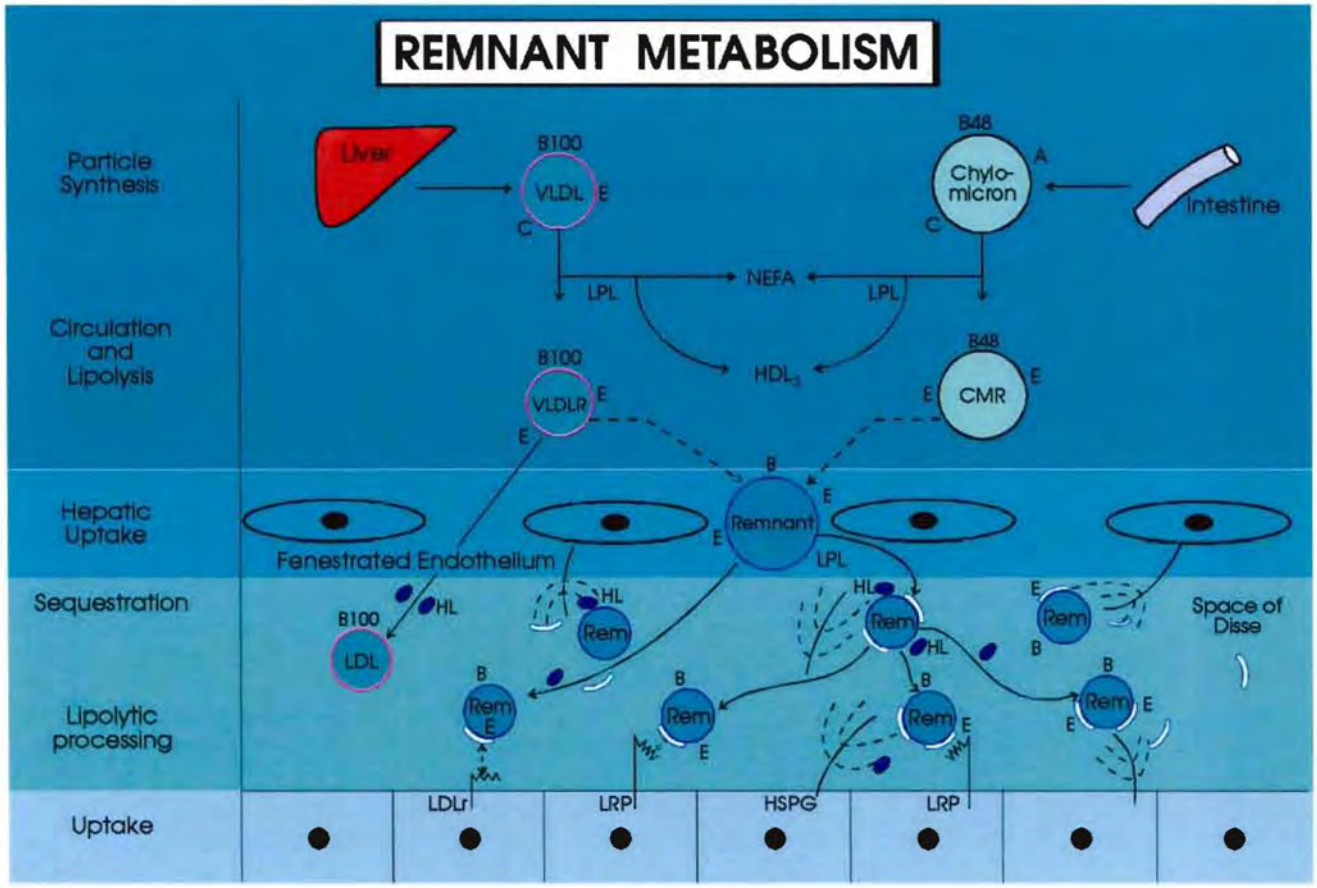
chylomicron undergoes triglyceride hydrolysis some phospholipids and A-apolipoproteins are transferred to HDL. The conformational change in chylomicrons also decreases the binding affinity of the C-apolipoproteins, which then transfer to HDL. LPL reduces the triglyceride content of chylomicrons by about 80-90% before the particle circulates again. The particle is now a chylomicron remnant and is markedly enriched in cholesterol compared to nascent chylomicrons. Chylomicrons undergo rapid metabolism with an average residence time of 5-10 minutes for chylomicron triglycerides in the circulation. The further metabolism of chylomicron remnants will be discussed together with that of VLDL remnants.

VLDL are synthesized in hepatocytes and are quantitatively the most important pathway for triglyceride and cholesterol export from the liver to the circulation. ApoB100 is the structural apolipoprotein of VLDL, but small amounts of C-apolipoproteins and apoE are found on nascent VLDL. The triglycerides in VLDL derive either from uptake of free fatty acids (FFA) bound to albumin and re-esterified in hepatocytes, endogenous *de novo* synthesis or delivery to the liver by remnant lipoproteins. The VLDL secreted by the liver is not uniform in size and lipid composition, but covers a range of species. For practical purposes two main types of VLDL are generally recognized. VLDL may be large and triglyceride rich (VLDL1) or smaller and denser with less triglyceride (VLDL2). In normolipidaemic individuals VLDL1 particles are the major determinant of plasma triglycerides (132;133). In situations where triglycerides are in excess such as diabetes, alcohol abuse or obesity the liver preferentially secretes an excess of VLDL1 (134). Circulating VLDL bind to endothelial LPL where they undergo triglyceride hydrolysis. Because VLDL particles are considerably smaller than chylomicrons they bind fewer molecules of LPL. The

residence time in the blood for VLDL triglycerides is accordingly substantially longer at 15-60 minutes than that of chylomicrons. The action of LPL results in the formation of VLDL remnants. VLDL remnants may be taken up directly by the liver or undergo further metabolism and eventually be converted to LDL. In humans about half of circulating VLDL remnants will undergo conversion to LDL. Small VLDL particles give rise to relatively small remnants that bind apoE less efficiently than larger remnants. Smaller remnants therefore have delayed clearance and are more likely to undergo conversion to LDL than larger remnants. Small remnants often have "intermediate" density between VLDL and LDL and are isolated as IDL.

VLDL remnants are converted to LDL by further lipoprotein remodeling in the circulation. Further triglyceride hydrolysis, mainly mediated by HL, occurs and a small triglyceride-poor and cholesterol-rich particle is formed. Due to its different conformation, LDL loses its binding affinity for apoE and apoB100 remains as the only apolipoprotein. LDL is cleared slowly from the circulation (approximate residence time is three days) and therefore makes up the bulk of circulating lipoproteins. Specific LDL clearance is mediated exclusively by the LDL-receptor. Non-specific clearance by mechanisms such as fluid endocytosis is quantitatively much less important than receptor mediated clearance. LDL has a lower affinity for the LDL-receptor than VLDL remnants and therefore is cleared slowly. The liver takes up the bulk of the circulating LDL, although extrahepatic tissues, such as the adrenals, also contribute to LDL clearance. Figure 1.1 illustrates remnant metabolism.

Figure 1-1 Illustration of remnant metabolism



Legend:

The letters C, E, B48 and B100 indicate the presence of apolipoproteinC, apolipoproteinE, apolipoproteinB48 and apolipoprotein B100 on the respective lipoprotein

VLDL: very low density lipoproteins

LDL: low density lipoproteins

VLDLR: very low density lipoprotein remnant

CMR: chylomicron remnant

Rem: remnant lipoprotein

LPL: lipoprotein lipase

HL: hepatic lipase

LDLr: low density lipoprotein receptor

LRP: low density lipoprotein receptor related protein

HSPG: heparan sulfate proteoglycans

1.4.4.1 Remnant metabolism

Remnant clearance is almost exclusively mediated by the liver. For remnant lipoproteins to be cleared by hepatocytes they need to come in direct contact with hepatocytes first. In the liver the capillary endothelium differs from the endothelium found in most other organs of the body in that it is fenestrated. This fenestrated sinusoidal endothelium is in effect a biofilter that separates the hepatic blood from the plasma found in the space of Disse (135). Nascent chylomicrons are too large to enter the space of Disse, while chylomicron remnants are small enough to pass through the fenestrations. VLDL and its remnants are small enough to freely enter and leave the space of Disse. Pseudocapillarisation of the endothelium occurs in ageing and results in loss of the normal fenestrations. This results in delayed chylomicron remnant clearance and postprandial hypertriglyceridaemia. This mechanism has been suggested as an important factor in the pathogenesis of age-related atherosclerosis (136).

The space of Disse is rich in HSPG (137), apoE (138) and HL (139). Remnants entering the space of Disse may also carry some LPL, but the role of this protein in remnant clearance under physiological conditions is not well defined as yet (72).

Mahley and coworkers (72) suggested that hepatic remnant clearance is a three step process. Rapid sequestration of remnants in the space of Disse is followed by further metabolic processing and finally by receptor mediated uptake.

Remnant lipoproteins entering the space of Disse are rapidly sequestered there. **HSPG** play a very important role in this regard as they are able to bind HL (as well as LPL) and apoE, the other important proteins participating in remnant clearance (140). Heparinase removes the sulfated glycosaminoglycan side chain from proteoglycans. Cells treated with heparinase have significantly reduced remnant binding and uptake compared to untreated cells (141). Experiments during which heparinase is injected into the portal vein of rats followed by injection of labelled β-VLDL show marked reductions in both plasma clearance and hepatic accumulation of the labelled substrate. The affinity and activity of the LRP and LDL-receptor for other ligands was not altered (141). **ApoE** in the space of Disse may be bound to HSPG and by binding to remnants acts as a "bridge" between HSPG and remnants. This results in sequestration of additional remnants. In mice expressing rat apoE, apoE was mainly localized in the sinusoids in the fasting state. Infusion of remnant lipoproteins changed the distribution of the apoE, it was now mainly found over the hepatic parenchymal cells and less was found in the sinusoids (142). The distribution of **HL** is similar to that of the extracellular matrix and HSPG in and around the space of Disse (143). HL is not only important because of its catalytic activity but also because of its role in enhancing remnant binding. Experimental evidence for this comes from experiments in which rat hepatoma cell lines were transfected with various forms of human HL. Wild type HL increased remnant binding and clearance. Catalytically inactive HL with preserved cellular binding affinity also increased remnant binding, although to a lesser extent than wild type HL. Transfection with catalytically active but binding defective HL reduced remnant binding when compared to wild type HL (144). In human HL deficiency β-VLDL is often found in addition to increased triglycerides in all lipoprotein fractions, but especially LDL (145-147). In a study of

three patients with catalytically deficient HL significant differences in lipoprotein composition were found between the two patients who expressed no HL protein and the one patient that expressed a small amount of HL protein. All patients had triglyceride-enriched lipoproteins, but the patient with measurable HL protein did not have marked cholesterol-enrichment of VLDL and IDL, indicating that even catalytically defective HL plays a role in remnant clearance (148).

Following capture and sequestration of remnants further lipolytic processing may occur in the space of Disse. There is some disagreement as to the relative contributions made by HL (144;149) and LPL (150;151) to this process. Further studies examining the amount of LPL transported on remnants and the amount of LPL in the space of Disse under physiological conditions are needed to resolve this issue.

The three receptors that mediate remnant uptake by hepatocytes are the LDL-receptor, LRP and HSPG (140). Both apoB and apoE are ligands for the **LDL-receptor**. ApoE in remnants binds to LDL-receptors with high affinity, leading to rapid endocytosis of remnants. Experiments with fluorescently labeled chylomicron remnants in LDL-receptor deficient mice showed normal sequestration of remnants in sinusoidal spaces but delayed uptake into hepatocytes. This suggests that the LDLR-receptor mediates fast hepatic uptake of remnants but that there are alternative pathways of remnant uptake (152). In another series of experiments with LDL-receptor deficient mice marked accumulation of cholesterol in LDL was found but there was no remnant accumulation (153). This finding is also supportive of the existence of additional alternative pathways to the LDL-receptor for clearing remnants. The **LRP** is a multifunctional receptor and its ligands include HL, LPL and α2-macroglobulin.

The LRP molecule is escorted to the cell surface following intracellular synthesis by a chaperone protein called the receptor associated protein (RAP) (154). Intravenously injected RAP blocks all ligands from binding to the LRP (155), while binding itself to HSPG. HSPG is important to the functioning of the LRP. In Chinese Hamster Ovary (CHO) cell experiments β-VLDL binding is enhanced if the medium is enriched in apoE to mimic the conditions found in the space of Disse. This enhanced binding activity is due to the LRP/HSPG pathway, as LDL-receptor antibodies do not block it substantially. LDL-receptor antibodies block basal (non apoE enriched) binding substantially. Binding of β-VLDL not enriched in apoE was quantitatively comparable in normal cells and cells with mutant HSPG. Enrichment of β-VLDL with apoE increased binding 4-7 fold in normal cells but produced no measurable increase in the cells with mutant HSPG. Heparinase treatment of normal cells eliminated the enhanced binding seen with apoE, while α2-macroglobulin binding was normal in the mutant cells and not influenced by heparinase treatment (144). Functional HSPG is therefore essential on the cell surface to mediate the enhanced binding observed in an apoE-enriched environment. Experiments with LRP-null fibroblasts showed enhanced binding and uptake of apoE-enriched β-VLDL, although the enhancement was not as marked as that observed in wild-type cells. Heparinase treatment eliminated the enhanced binding, proving that **HSPG** alone can make a significant contribution to remnant uptake (156).

A great deal of work has been done examining the relative contribution that each of these three receptors make to remnant metabolism. Much of this work has been done in transgenic animals where a particular gene is either silenced (knocked-out) or

overexpressed. In summary, these studies (152;153;156;157) show that inhibition of either LDL-receptors or HSPG/LRP does not significantly influence remnant clearance, as there is excess capacity in the system. Inhibition of all three receptors results in severe remnant accumulation, as does the deficiency of apoE, which is the critical ligand for all three receptors.

1.5 Pathophysiology of dysbetalipoproteinaemia

The pathogenesis of dysbetalipoproteinaemia is complex and only the last decade has brought a fuller understanding of the molecular mechanisms that result in the dysbetalipoproteinaemic phenotype. In all cases of dysbetalipoproteinaemia considered here dysfunctional apoE is the initial pathophysiological abnormality. The majority of patients with dysbetalipoproteinaemia are homozygous for the ε2 allele of apoE (5), but apoE mutants that are inherited as autosomal dominants and apoE deficiency also need to be considered as precipitating causes.

1.5.1 Precipitating factors

Homozygosity for apoE2 is the most common genetic substrate for dysbetalipoproteinaemia, but less than 10% of apoE2 homozygotes ever develop hyperlipidaemia. Most apoE2 homozygotes are normolipidaemic or even hypolipidaemic (50). Hyperlipidaemia is also not present at birth but develops in adulthood. ApoE2 homozygotes therefore require additional "metabolic stressors" or "second hits" such as diabetes, obesity or hypothyroidism to precipitate hyperlipidaemia.

Transgenic mice expressing human apoE2 at levels of 10-30 mg/dL were hypolipidaemic (158), as are most humans with this genetic makeup. Transgenic apoE2 mice that additionally overexpressed human apoB developed hyperlipidaemia characterized by low levels of LDL-cholesterol and remnant accumulation. This situation may perhaps be analogous to that seen in subjects who co-inherit apoE2 homozygosity and another condition, such as familial combined hyperlipidaemia (FCH), that is characterized by the overproduction of apoB containing lipoproteins (159). Disorders such as diabetes or alcoholism are also often complicated by overproduction of apoB-containing lipoproteins.

Changes in LDL-receptor availability may also precipitate hyperlipidaemia in genetically predisposed subjects. ApoE2 transgenic mice crossed with LDL-receptor null mice developed a dysbetalipoproteinaemic phenotype. In humans FH may be due to mutations in the LDL-receptor. Several studies have addressed the issue of co-inheritance of apoE2 homozygosity and LDL-receptor mutations (160;161). Co-expression of both defects in humans does not have a simple additive effect but results in complex modulation of individual gene expression. This issue will be addressed in more detail later in this thesis. Hypothyroidism affects human lipid metabolism in many ways and decreased availability of LDL-receptors may be a mechanism whereby hyperlipidaemia is precipitated. Ageing is also associated with a decrease in hepatic LDL-receptor numbers. Diets high in cholesterol may also downregulate hepatic LDL-receptor expression.

It is most unusual for women homozygous for apoE2 to develop hyperlipidaemia before menopause, yet in men hyperlipidaemia is occasionally seen in adolescence.

Oestrogen, in physiological amounts, therefore seems to be a protective factor. ApoE2 transgenic rabbits show marked gender differences despite expressing comparable levels of apoE. Male rabbits developed hyperlipidaemia that was reversed by treatment with oestrogens, while female rabbits became hyperlipidaemic following ovariectomy. Oestrogen treatment was associated with increased lipolytic activity and increased LDL-receptor expression (162). Other animal studies have also shown that pharmacological doses of oestrogen can increase LDL-receptor expression. In a human turnover study a postmenopausal female with dysbetalipoproteinaemia was treated with ethinyloestradiol 1μg/kg/day. Turnover studies were performed before and after hormone supplementation. Hyperlipidaemia improved markedly, as seen in other cases of hormone supplementation (163;164), and VLDL catabolism was accelerated significantly. VLDL-production was also increased by a factor of 60% but increased remnant clearance more than compensated for this increase (19).

Renal disease can influence lipoprotein metabolism profoundly through multiple mechanisms. Nephrotic range proteinuria is often associated with overproduction of lipoproteins while patients with chronic renal failure often have impaired lipolytic activity (159;165). Both these mechanisms may contribute to remnant accumulation.

Several classes of drugs are well known to influence lipid metabolism. The molecular mechanisms of drug induced hyperlipidaemia are varied (166;167), but mechanistically increased lipoprotein production or decreased lipoprotein clearance may be operative either alone or in combination. Drugs such as steroids, HIV protease inhibitors (PI), retinoids or beta-blockers may therefore precipitate dysbetalipoproteinaemia in susceptible individuals.

1.5.2 Defective receptor binding

ApoE variants that are associated with the development of dysbetalipoproteinaemia are characterized by defective binding to hepatic lipoprotein receptors. There are, however, important differences between the various apoE mutants in their binding affinity to the hepatic receptors involved in remnant clearance (LDL-receptor, LRP/HSPG). ApoE2 has less than 2% of normal LDL-receptor binding. Although the amino acid change at position 158 from arginine to cysteine, that distinguishes apoE2 from apoE3, lies outside of the direct LDL-receptor binding region it causes secondary structural changes that alter the binding region's conformation significantly. In apoE3 a salt bridge forms between Arg-158 and Asp-154. This does not occur in apoE2 and instead Asp-154 interacts with Arg-150 to form a new salt bridge. This dramatically alters the charge surface presented by apoE2 to its receptor, resulting in decreased receptor binding (168).

The dominantly transmitted mutations in apoE also have reduced LDL-receptor binding (often around 20-50% of normal), but their binding activities are higher than that of apoE2 (50). Conceptually, it not intuitively obvious why these mutants with better LDL-receptor binding than apoE2 are able to cause hyperlipidaemia in a dominant fashion, while two copies of apoE2 and precipitating factors are necessary to cause disease. This phenomenon is partially explained if one considers not only LDL-receptor binding affinity but also binding to HSPG/LRP.

ApoE2 has 50-90% of normal HSPG binding affinity while many of the autosomal dominant variants have very poor binding to HSPG (169). The importance of this receptor pathway is demonstrated by experiments with transgenic mice lacking both murine apoE and functional LDL-receptors. In mice expressing human apoE2 cholesterol levels were approximately double normal, while mice expressing the dominant mutant apoE (Arg142Cys) had cholesterol levels eight times higher than expected. The apoE2 mice were still able to use the HSPG/LRP pathway, but in the absence of LDL-receptors the apoE (Arg142Cys) mice were unable to clear remnants because of their inability to use the alternative HSPG/LRP pathway (50). The HSPG/LRP binding affinity of apoE variants is therefore an important determinant of dominant versus recessive expression of dysbetalipoproteinaemia. The HSPG/LRP pathway is so important because defective function of this pathway impairs both the initial sequestration of remnants in the space of Disse and subsequent receptor-mediated uptake.

1.5.3 ApoE and lipolysis

ApoE2 homozygotes characteristically have low levels of LDL-cholesterol. These low levels of LDL-cholesterol can be explained by several hypotheses. Poor uptake of remnants may lead to cholesterol depleted hepatocytes and upregulation of hepatic LDL-receptors. Remnants may also compete poorly with apoB100 containing LDL for clearance at the LDL-receptor leading to enhanced LDL clearance. Most evidence, however, supports the hypothesis that impaired formation of LDL, due to decreased lipolytic conversion of VLDL to LDL, accounts for the low LDL levels. In transgenic apoE2 + LDL-receptor null mice the LDL-cholesterol levels are low, implying that LDL-receptors are not critical for the LDL-cholesterol lowering effect of apoE2

(170). However, apoE2 enriched VLDL and IDL were poor substrates in an assay of lipolysis. Increasing concentrations of apoE2 resulted in progressively decreasing release of free fatty acids in the lipolytic assay (170). ApoE2 inhibits lipolysis by displacing apoCII, an activator of LPL, from the lipoprotein and adding additional apoCII to the medium partially corrects impaired lipolysis secondary to apoE2 accumulation (170). ApoE also activates HL, but apoE2 is a less effective activator than apoE3 (171;172). HL mediated lipolysis of IDL to LDL is therefore also impaired, leading to decreased LDL production.

1.5.4 ApoE distribution in lipoproteins

ApoE variants do not only differ in their binding affinity towards hepatic lipoprotein receptors but also preferentially associate with certain lipoprotein classes. ApoE2 preferentially associates with HDL (173;174), while certain dominant mutants preferentially associate with VLDL. The presence of Arg-112 determines preferential VLDL association (173). Preferential distribution into VLDL has been documented for apoE-Leiden (175) and apoE (Arg142Cys) (176). In β-VLDL from patients carrying the apoE-Leiden mutation the ratio of mutant to normal apoE approaches 7:1 (175). Enriching VLDL in mutant apoE displaces more apoCII from the particle, impairing its suitability as a lipolytic substrate. The VLDL of transgenic mice expressing similar plasma levels of apoE was a much worse lipolytic substrate if the apoE (Arg142Cys) mutation was introduced rather than apoE2 (50).

1.5.5 ApoE and VLDL production

ApoE plays an important role in the secretion of VLDL triglycerides. Hepatocytes from apoE-deficient mice secrete about 50% less triglycerides than hepatocytes from wild-type mice (177). Conversely overexpression of human apoE in rat hepatocytes was associated with increased VLDL triglyceride secretion that paralleled apoE levels (73). A similar, but more pronounced, effect has also been demonstrated in human apoE3 transgenic rabbits (178). Elevated levels of apoE characterize dysbetalipoproteinaemia and increased VLDL production therefore is an important component in the genesis of hyperlipidaemia (179).

1.5.6 Pathophysiology of apoE deficiency

Dysbetalipoproteinaemia is usually due to the presence of excessive levels of dysfunctional apoE, but occasionally apoE mutations may result in complete or partial apoE deficiency. Several kindreds with apoE deficiency have been described (180-183). Human apoE deficiency is associated with severe hyperlipidaemia, but the phenotype differs from that seen in other forms of dysbetalipoproteinaemia. There is hypercholesterolaemia but hypertriglyceridaemia is not a prominent feature. Remnants are markedly cholesterol-enriched and the ratio of VLDL cholesterol to plasma triglycerides is higher than in dysbetalipoproteinaemia due to binding-defective apoE mutants (183). Triglycerides do not accumulate significantly in apoE deficiency as VLDL-lipolysis is not inhibited and VLDL secretion is not stimulated.

1.5.7 ApoE variants associated with dysbetalipoproteinaemia

Table 1.5 lists some apoE variants that have been reported to cause dysbetalipoproteinaemia.

Table 1-5 ApoE variants associated with dysbetalipoproteinaemia

Mutation	Receptor-binding affinity		Comments	References
	LDL-receptor	HSPG/LRP binding		
Arg158→Cys	<2%	~50%	ApoE2, homozygosity and precipitating factor required for phenotypic expression	
Arg145→Cys			Common in South Africa, homozygotes described	(184)
Arg145→Cys Glu13→Lys	Moderate	Low	ApoE Philadelphia	(185;186)
Arg145→His			ApoE Kochi	(187)
Arg136→Ser	Moderate		Mutant apoE predominates in plasma, found in Spain	(188;189)
Arg136→Cys			ApoE Heidelberg, late onset, dominant inheritance	(190)

Mutation	Receptor-binding affinity		Comments	References
	LDL-receptor	HSPG/LRP binding		
Arg142→Cys	Low	Very low	Very severe hyperlipidaemia, early expression, very rare, used as model in transgenic animal work	(176;191;192)
Arg142→Leu			Identified in single family	(193)
Lys146→Gln	Moderate (~40%)		Large pedigrees studied (Dutch), variable phenotype,	(194-197)
Lys146→Glu	Low	Very low	ApoE-1 Harrisburg, highly penetrant	(198-200)
Lys146→Asn Arg147→Trp			ApoE1-Hammersmith, onset in childhood, phenotype is severe	(201)
Duplication residues 121-127	Low	Very Low	ApoE-Leiden, large pedigrees analyzed, highly penetrant, Dutch population	(175;202)

Mutation	Receptor-binding affinity		Comments	References
	LDL-receptor	HSPG/LRP binding		
Glu244,245→Lys			Apo E Suita, remnant accumulation demonstrated	(203)

Notes:

1. Table based on references (50;204) and Online Mendelian Inheritance in Man (OMIM)
2. The table does not aim to list all apoE mutants or variants
3. ApoE variants that are associated mainly with hypertriglyceridaemia have not been included in this table
4. The table does not list variants associated with lipoprotein glomerulopathy
5. Blank spaces indicate that receptor binding has not been studied as yet

1.5.8 ApoE and lipoprotein glomerulopathy

Lipoprotein glomerulopathy is a very rare disorder (approximately 65 cases reported so far worldwide (205)) that was only officially recognized in 1995. Almost all cases have been reported from Japan with a few cases seen in China and Europe. Lipoprotein glomerulopathy is characterized histologically by lipoprotein thrombi in markedly dilated glomeruli. The histology of lipoprotein glomerulopathy is distinct from that seen in LCAT deficiency which is also a primary lipid disorder that causes renal failure secondarily. The renal histology of LCAT deficiency is characterized primarily by focal segmental glomerulosclerosis, mesangial expansion and "ballooning" of glomerular loops filled with foamy lipid-like material (206).

The renal lesions of lipoprotein glomerulopathy cause mild to severe proteinuria and about half of the patients reported so far have progressed to renal failure. On biochemical evaluation serum levels of apoE are markedly increased (mean 17.1 mg/dL; SEM 1.9 (205) in 40 cases) and hyperlipidaemia is common but not invariable. Mean (± SEM) TG are 3.53 ± 0.32 mmol/L with TC 7.04 ± 0.41 mmol/L (205). These lipid values are somewhat lower than those reported in most cases series of dysbetalipoproteinaemic patients (7;9;10;207). The VLDL is cholesterol enriched, meeting diagnostic criteria proposed for dysbetalipoproteinaemia (208).

Lipoprotein glomerulopathy by definition requires a mutation of apoE to be present. Thus far four mutations have been described in association with this disorder.

Table 1-6 ApoE mutations associated with lipoprotein glomerulopathy

Mutation	Common name	Comments	References
Arg145→Pro	ApoE Sendai	Commonest mutation	(209)
Cys25→Arg	ApoE Kyoto		(210)
Del Leu 141 to Lys 143	ApoE.Tokyo		(211)
Del Gln 156 to Gly 173		Single patient reported	(212)

ApoE mutations are likely the causal factor in this disorder as murine apoE knock-out mice that express apoE Sendai develop an illness very similar to lipoprotein glomerulopathy (213). Recurrences of the disease following renal transplantation are also common. However, not all mice develop renal disease and human gene carriers without renal disease have also been identified (213-215), suggesting that other factors (intrinsic glomerular factors, environmental factors) are important in the pathogenesis of the disorder. Hyperlipidaemia seems to at least play an aggravating role in some cases as histological improvement following intensive lipid-lowering therapy has been described in a few patients (216).

The pathogenesis of lipoprotein glomerulopathy is as yet incompletely understood. The receptor binding affinities of apoE Sendai have been investigated and the LDL-receptor binding is less than 5% of normal but HSPG binding is well preserved at around 66% of that of apoE3 (217). It is speculated that lipoprotein particles carrying abnormal apoE may deposit in predisposed glomeruli where they induce multiple deleterious inflammatory responses in mesangial and glomerular cells (205).

Interestingly histological findings characteristic of lipoprotein glomerulopathy have been reported in a few isolated cases of dysbetalipoproteinaemia due to homozygosity for apoE2 (218;219). Further study of the link between lipids, apoE and renal disease is required.

1.6 The dysbetalipoproteinaemic phenotype

The following section provides a very brief overview of the clinical phenotype of dysbetalipoproteinaemia. Clinical, diagnostic and therapeutic aspects of dysbetalipoproteinaemia will be discussed in greater depth in the relevant chapters of this thesis. This section only intends to provide a brief introduction to the topic.

1.6.1 Clinical features

Palmar crease xanthomata are the distinguishing clinical feature of dysbetalipoproteinaemia and have been regarded as pathognomonic. They are, however, only found in a minority of patients. The reported incidence varies widely amongst case series and is generally lower in more recent reports, where the diagnosis of dysbetalipoproteinaemia is more actively sought in a wider cohort of patients with mixed hyperlipidaemia (6-10;220). Tendon xanthomata, tuberous, tuboeruptive and eruptive xanthomata may also occur but are not unique to dysbetalipoproteinaemia. Dysbetalipoproteinaemia is a highly atherogenic disorder and premature atherosclerosis is common. The distribution of vascular disease differs from that seen in other genetic hyperlipidemias with peripheral vascular disease being highly

prevalent (7-10;220). On occasions hypertriglyceridaemia may be severe enough to cause pancreatitis.

1.6.2 Diagnosis of dysbetalipoproteinaemia

There are no universally accepted laboratory criteria for the diagnosis of dysbetalipoproteinaemia. The diagnosis may be based either on analysis of the lipoprotein phenotype demonstrating remnant accumulation or on analysis of apoE. Dysbetalipoproteinaemia is usually suspected if both triglycerides and total cholesterol are raised and the mass ratio is 1:1 or the molar ratio 1:2.

1.6.2.1 Analysis of lipoprotein phenotype

The lipoprotein phenotype can be determined either by electrophoretic, chromatographic or chemical techniques. Traditionally the diagnosis of dysbetalipoproteinaemia was based on the finding of a broad-beta band on agarose gel (initially paper was used) electrophoresis of plasma with β-mobility of ultracentrifugally isolated VLDL (13). Other suggested electrophoretic methods include immunoelectrophoresis (221), electrophoresis with selective precipitation of VLDL (222;223), isoelectric focusing of plasma (224), modified agarose gel electrophoresis (225), polyacrylamide electrophoresis (15) and polyacrylamide gradient gel electrophoresis (226). Recently the lipid laboratory at the University of Cape Town (UCT) has shown that non-denaturing polyacrylamide gradient gel electrophoresis is probably the best electrophoretic screening technique for dysbetalipoproteinaemia (227). A chromatographic method for the diagnosis of

dysbetalipoproteinaemia has been described (228), but due to its labour-intensive nature, chromatography has not been used as a routine diagnostic tool.

As electrophoretic techniques often require subjective interpretation, attempts were made to develop quantitative diagnostic techniques. These ultracentrifugal techniques all rely on demonstrating cholesterol-enrichment of the VLDL fraction. Using the presence of β-migrating VLDL as a diagnostic gold standard multiple ratios of cholesterol in VLDL to VLDL-TG or plasma-TG were suggested as being diagnostic of dysbetalipoproteinaemia (207;229-232). An alternative method that isolates remnant-like particles by differential antibody binding and then calculates the ratio of cholesterol in these particles to plasma-TG has also been described (233). The other described phenotypic approaches either rely on measurement of apolipoproteins (234;235) or selective lipoprotein precipitation (236).

1.6.2.2 Analysis of apoE

Utermann (41) was the first to report the specific deficiency of the apoE3 isoform in the VLDL of dysbetalipoproteinaemic patients using IEF. IEF techniques subsequently underwent multiple modifications, including neuraminidase treatment to remove sialic acid residues and so remove "minor isoforms" of apoE from the gel. IEF is still used in some laboratories to phenotype apoE, but in general it has been replaced by polymerase chain reaction (PCR) based techniques that can distinguish between the major isoforms of apoE in a single assay. The method described by Hixson and Vernier (237) remains popular. Routine genotyping using this method does not detect the less common dominant variants of apoE and the failure to

demonstrate homozygosity for the ε2 allele therefore does not exclude the diagnosis of dysbetalipoproteinaemia entirely. Dominant apoE mutants may be suspected when the phenotype as determined by IEF and the genotype determined by PCR are discrepant.

1.6.3 Treatment of dysbetalipoproteinaemia

The treatment of dysbetalipoproteinaemia is generally very rewarding with most patients achieving good control of their hyperlipidaemia. The principles of treatment are to identify and control possible precipitating factors, e.g. diabetes, hypothyroidism, obesity, as best as possible. A diet low in total fat with particular emphasis on the reduction of saturated fat is advised. Depending on the body mass index (BMI) additional caloric restriction may be necessary. In the lipid clinic experience most patients require additional lipid-lowering medication. Fibrates, statins and nicotinic acid are all effective (238-240), but cholestyramine should be avoided as it may increase VLDL production (241) and result in worsening hyperlipidaemia.

2 Chapter Two: Methods

This chapter describes the methods used in the retrospective review of the lipid clinic case series of dysbetalipoproteinaemia. Specialized methods such as sequential ultracentrifugation of lipoproteins or studies of remnant metabolism by stable isotope breath test are described in the appropriate chapters.

2.1 Clinical methods

The patients described in this thesis were all seen at the lipid clinic of Groote Schuur Hospital (GSH) in Cape Town, South Africa. GSH is the tertiary teaching hospital affiliated to the UCT. The hospital mainly serves the indigent local population but patients may also be referred from the private medical sector for superspecialist advice. Most patients treated with lipid-lowering therapy continue to attend the clinic in the long-term as until very recently (2005) lipid-lowering therapy could only be dispensed to public sector patients on a script from the lipid clinic that required six-monthly renewal.

The Division of Lipidology of the Department of Medicine comprises the lipid clinic and the associated laboratory at UCT. Currently the lipid clinic at GSH is the only facility in South Africa that has access to the full range of diagnostic services necessary to diagnose dysbetalipoproteinaemia. The clinic has a particular interest in dysbetalipoproteinaemia and there is a low threshold for investigating patients with mixed hyperlipidaemia.

2.1.1 Clinical documentation

Almost all patients referred to the lipid clinic are initially seen by a consultant. Patients not personally seen by a consultant at their first visit are presented to a consultant by junior physicians. From 1990 onwards patient data has been captured in standardized admission forms, to ensure completeness of data collection. All data are prospectively captured in a Paradox (Corel ®) database. Clinical data are generally complete for patients seen after 1990. Before 1990 a standardized clerking sheet was also used but less information was collected and there was not a strong emphasis on ensuring completeness of data collection. Follow-up visits are also recorded using a standardized format. The lipid clinic notes are kept in duplicate; the original remains in the lipid clinic file (held at the lipid laboratory) while a carbon copy is filed in the patient's folder held by the hospital. This system facilitates rapid access to notes for research and enquiries and guards against data loss due to mislaid or lost hospital folders.

2.1.2 Collection of clinical data

History taking and physical examination generally follow well-established traditions. When taking the history special attention is paid to the following points, which are often neglected in standard medical histories:

- Clinical manifestations of atherosclerosis
- Dietary history by non-quantitative food recall and description
- Detailed family history covering 4 generations if possible

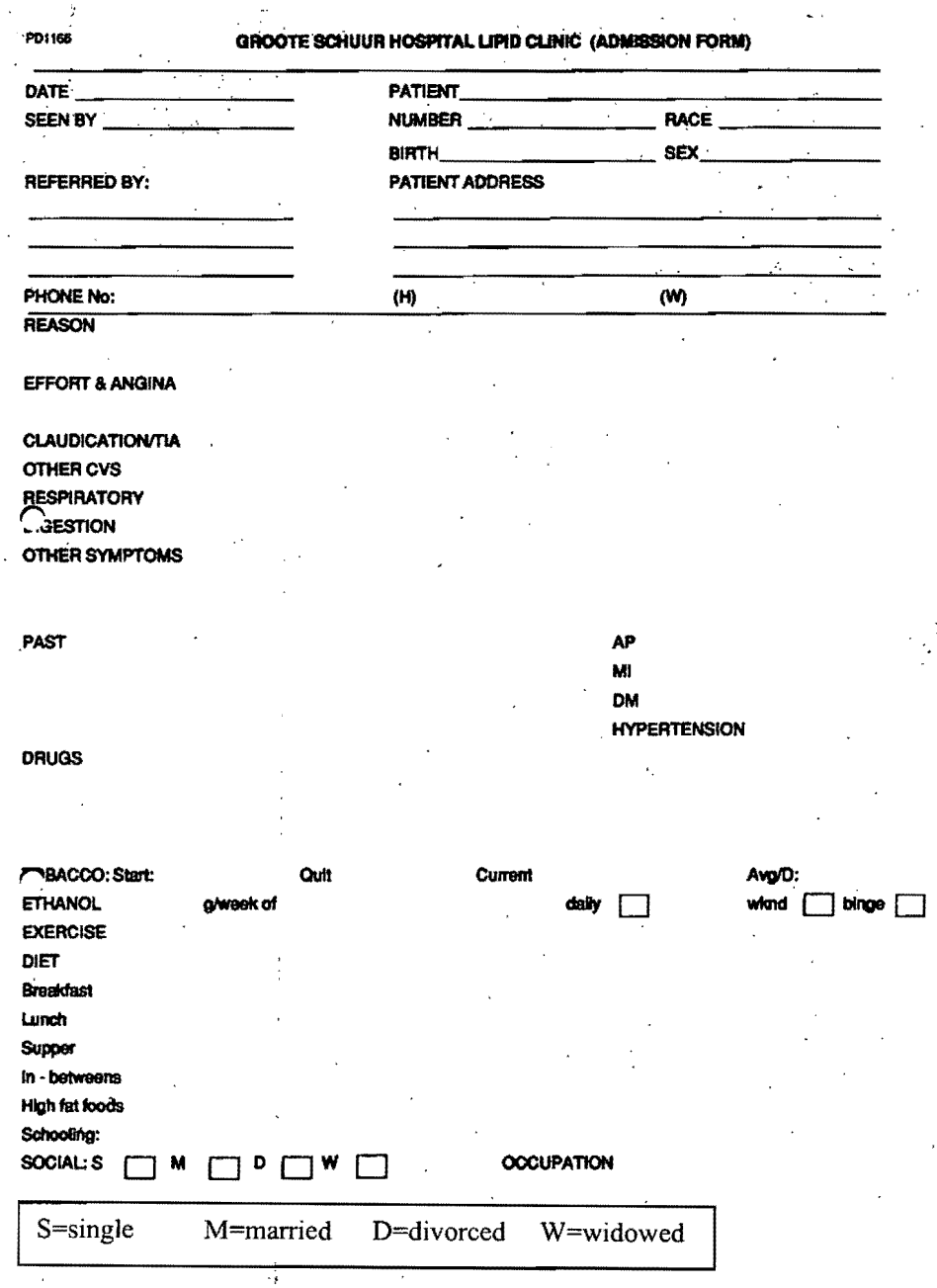
- Evaluation of physical activity

The physical examination particularly emphasizes the following points:

- Physical stigmata of dyslipidaemia
- Evidence of vascular disease

Following the initial consultation a letter detailing the patient's history, physical findings, diagnosis and the recommended treatment is sent to the referring doctor. Figures 2.1-5 are reproductions of the stationery in use at the clinic.

Figure 2-1 Page 1 of initial visit data sheet



PD1165 GROOTE SCHUUR HOSPITAL LIPID CLINIC (ADMISSION FORM)

DATE _____ PATIENT _____

SEEN BY _____ NUMBER _____ RACE _____

BIRTH _____ SEX _____

REFERRED BY: PATIENT ADDRESS

_____ _____

_____ _____

_____ _____

PHONE No: (H) (W)

REASON

EFFORT & ANGINA

CLAUDICATION/TIA

OTHER CVS

RESPIRATORY

DIGESTION

OTHER SYMPTOMS

PAST AP

MI

DM

HYPERTENSION

DRUGS

TOBACCO: Start: Quit Current Avg/D:

ETHANOL g/week of daily ☐ wknd ☐ binge ☐

EXERCISE

DIET

Breakfast

Lunch

Supper

In - betweens

High fat foods

Schooling:

SOCIAL: S ☐ M ☐ D ☐ W ☐ OCCUPATION

S=single M=married D=divorced W=widowed

Note: The pedigree of the patient is drawn on the reverse (blank) side of this form

Figure 2-2 Page 2 of initial visit data sheet

PD 1141

GSH LIPID CLINIC ADMISSION FORM (2)

PATIENT:

HEIGHT ___,m ___MASS _____,kg ___ BMI ____

Waist_____ Hip _____ WHR_____

Arcus cornealis

Xanthelasma

Tendon xanthoma

Cutaneous xanthoma

GENERAL EXAMINATION

PULSE BP Erect Supine

Site	Radial	brachial	femoral	poplit	dors. ped	post tib	cartoid
R							
L							

N=Normal
R=Reduced
A=Absent
B=Bruit
E=Ectatic

AORTA RENAL

JVP APEX AUSCULT

ECG (DATE _____)

RESPIRATORY

ABDOMINAL

NEUROLOGIC

URINALYSIS

›id tests 1 1 = mM/L 2 = m 3 = %	DATE		2	DATE		3	DATE	
	TG			Apo A			Origin	
	T Chol			Apo B			Beta	
	HDL - C			Apo (a)			pre - B	
	LDL - C						Alpha	

APPEARANCE

CHEMISTRY	Glucose			Alk Ph			Urate	
	Protein			GGT			Urea	
	Albumin			ALT			Creat	
	Bili			AST				

Previous investigation:

DATE								

Figure 2-3 Page 3 of initial visit data sheet

GSH LIPID CLINIC ADMISSION FORM (3)

PATIENT

RISKS ASSESSMENT

TRIGLYCERIDE		FAMILY HIST	HYPERTENSION		
TOTAL CHOL		SMOKING	DIABETES MELL		
LDL-CHOL		EXERCISE	RENAL DISEASE		
HDL - CHOL		DIET	HYPERURICAEMIA		
App (a)		OBESITY			

CLINICAL ASSESSMENT

LIPID DISORDER

FREDRICKSON TYPE:

OTHER CLINICAL PROBLEMS

1

2

3

4

5

6

7

8

MANAGEMENT

FAMILY STUDIES

DIET

OTHER

DRUGS

FOLLOW - UP

COMPUTERISED ☐ DICTATED ☐

Figure 2-4 Page 1 of follow-up visit data sheet

PD 1140

GSH LIPID CLINIC FOLLOW -Up (1)	PATIENT . . . . . SEX . . . . FOLDER . . . . . RACE . . . . BIRTH		
	Lp(a)	GGE	TYPE

LIPID CHEMISTRY					
DATE/ASSAY					
TRIGLYCERIDE					
TOTAL CHOL.					
HDL - CHOL.					
LDL -CHOL.					

CLINICAL & DRUGS (PTO FOR NOTES)					
DATE OF VISIT					
MASS/HEIGHT					
BP AND PULSE					

OTHER CHEMISTRY					
Glucose/URATE					
UREA/CREAT.					
AST/ALT.					
GGT/ALK.P.					
CPK/LDH					

Figure 2-5 Page 2 of follow-up visit data sheet

PD 1143

GSH LIPID CLINIC FOLLOW - UP (2) BRIEF CLINICAL NOTES TO INCLUDE COMMENT ON EXERCISE, DIET, AND SIDE EFFECTS.	PATIENT . . . . . . FOLDER . . . . . SEX . . . . . . BIRTH . . . . . RACE . . . . .

DATE _____ SEEN BY _____

DATE _____ SEEN BY _____

DATE _____ SEEN BY _____

DATE _____ SEEN BY _____

DATE _____ SEEN BY _____

2.1.3 Consent for DNA extraction and storage

The lipid clinic at GSH not only aims to provide expert clinical care to patients but actively engages in research. All patients are therefore given the opportunity to consent to storage and analysis of their DNA. The consent covers specific genetic tests that may be necessary diagnostically, e.g. genotyping of apoE in patients with mixed hyperlipidaemia and further research investigations. The University Research Ethics Committee has approved the "DNA consent form". More than 98 % of patients consent to storage and further use of their DNA.

Figure 2-6 DNA consent form

THE LIPID CLINIC AND THE LIPID LABORATORY AT THE UNIVERSITY OF CAPE TOWN & GROOTE SCHUUR HOSPITAL
CONSENT FOR DIAGNOSTIC & RESEARCH INVESTIGATION

Please read carefully and feel free to ask any questions you may have. If you agree, place a check (T) in the relevant block; if you disagree, delete the relevant clause.

I, ... hereby consent to the removal of blood 9, skin 9, or other tissue (specify) ..... from myself and/or ..... to whom I am the (relationship) .....

The purpose of the investigation is to perform
9 diagnostic tests for dyslipidaemia or other metabolic disease that may predispose to atherosclerosis
9 research investigations currently and in future, but subject to the conditions of the institutions above and the ethics review that govern the research at these institutions. This may mean the determination of alternative inherited defects to explain my condition, or other factors which may have a bearing on the outcome of my condition, or for testing of any other gene that may be under research.

The result(s) of the analysis carried out on the sample(s) will be made known to me, via my doctor, in accordance with the relevant protocol if and when available. The doctor(s) to be informed:

.....

Additionally, I authorise that the results be made known to the following family member(s):

.....

After due explanation by ....., I understand that

(1) Conventional procedures and techniques are employed by trained staff to obtain and process the samples and that consequently the risk to my health is minimal.
(2) Confidentiality and anonymity of the sample(s) is(are) respected: the samples are coded by numbers. My written permission is required for release of identifiable information to any other party.
(3) Precise diagnoses may not always be possible because of limited knowledge about the cause(s) of the condition(s). Even under the best conditions, current understanding and teehnology are imperfect. The research may thus be of no direct benefit to me but the samples may provide general insight into disease and health.
(4) The cost involved for me will be determined according to scheduled tariffs, or borne by research budgets, and that no tests for which payment is required will be undertaken without prior consultation.
(5) No commercial claims can be made on developments from these investigations by myself.
(6) Permission to investigate may be withdrawn at any time by me, without prejudice to my future medical care.

Signed ..... Date.....

Witnessed by (Signatures) ....

Printed Names & Dates ....

Admin/formA5.wpd

2.2 Special investigations

2.2.1 Routine biochemical investigations

Following a twelve-hour fast all patients routinely attend for venesection approximately a week before their initial consultation. All investigations listed below were performed in the chemical pathology laboratory of GSH using automated analyzers. Standard laboratory quality control procedures were followed to ensure reliability and reproducibility of the results. Cholesterol and TG were measured by standard enzymatic methods. High density lipoprotein cholesterol (HDLC) was initially measured after precipitation with phosphotungstic acid but in 2004 the laboratory switched to a homogenous method using polyethylene glycol modified enzymes (Roche Diagnostics). ApoB was measured by a commercially available immunoturbidimetric assay (Roche Diagnostics). The following investigations were done in all patients:

Lipid investigations:

- Triglyceride
- Total cholesterol
- HDLC
- LDLC is calculated using Friedewald's equation (242) provided TG is less than 4.5 mmol/L

Note: The Friedewald equation should not be used in patients with dysbetalipoproteinaemia and no values for LDLC are given in this study unless LDLC was determined ultracentrifugally.

- ApoAI
- ApoB
- Lipoprotein (a)

Other investigations:

- TSH, T_4 requested if TSH is abnormal
- Glucose
- Renal function: urea, creatinine
- Liver function tests: albumin, bilirubin, AST, ALT, GGT, ALP
- Urate
- Creatinine kinase

2.2.2 Electrophoresis

2.2.2.1 Agarose gel electrophoresis

Agarose gel electrophoresis was performed utilizing the Beckman Paragon Lipo ® system according to the manufacturer's instructions. Briefly, 5 µl of plasma (or serum on occasions) was applied to the agarose gel using the template supplied by the manufacturer. Gels were electrophoresed at 100V for 30 min in a barbital buffer (pH 8.6). Following electrophoresis gels were fixed in an acetic acid solution, dried and then stained with Sudan Black followed by destaining in ethanol solutions.

All gels were reviewed by a single observer (ADM) at the time of the patient's initial presentation and assigned a classification according to the WHO criteria (243). A TG

of 2.3 mmol/L was used to differentiate between IIa and IIb patterns. Gels that showed uniform staining from the β-area to the pre-β area were classified as Type III. Routine agarose gel electrophoresis was performed at the chemical pathology laboratory. The agarose gels for the Bayer W6228/10176 (hereafter referred to as Bayer Study) were done at the lipid laboratory. These gels were scanned with the GelDoc system (Bio-Rad Corporation) and densitometric analysis was undertaken.

2.2.2.2 Polyacrylamide gradient gel electrophoresis (PGGE)

Non-denaturing polyacrylamide gradient gel electrophoresis was undertaken in 2-8% polyacrylamide gels with a 3% acrylamide concentration in the stacking gel. The higher acrylamide concentration in the stacking gel is necessary to allow safe handling and loading of the gels. Lipoproteins are prestained with Sudan Black, so that gels can be inspected immediately after electrophoresis and require no further processing.

Technique:

Polyacrylamide mini-gels (6 cm long) were freshly cast prior to each electrophoretic run. A 30% stock solution of acrylamide with 2.7% bisacrylamide was used to prepare an 8% solution of acrylamide in a buffer containing 13.6 g% Tris and 30% (v/v) glycerol at pH 8.8. A 2% acrylamide solution was prepared in Tris 13.6 g%. Polymerization was activated in 4 ml of each solution by adding 30 µl of ammonium persulphate (100 mg/ml) and 15 µl of tetramethlylethylenediamine (TEMED). The activated solutions were placed in the two chambers of a gradient maker with a small magnet in each chamber to permit mixing as the gel was poured from the chamber

containing the 8% acrylamide solution. The stacking gel contains 3% acrylamide stock in 1.68 g% Tris at a pH of 6.8. Once the separation gel has set, the stacking gel solution is polymerized by adding 100 µl of ammonium persulphate and 4.0 µl of TEMED. Using this solution and a comb 15 lanes are prepared for electrophoresis.

Fasted EDTA- plasma samples were prestained for lipids with Sudan Black using a similar method to Gambert et al (244). Briefly, 100µl of plasma was incubated with 50µl of lipid stain (1 % w/v Sudan Black in ethylene glycol) for an hour at 4 °C and then spun for 20 min at 10000G. Equal volumes of supernatant and saturated sucrose were mixed and 12 µl of the resultant solution was loaded per well.

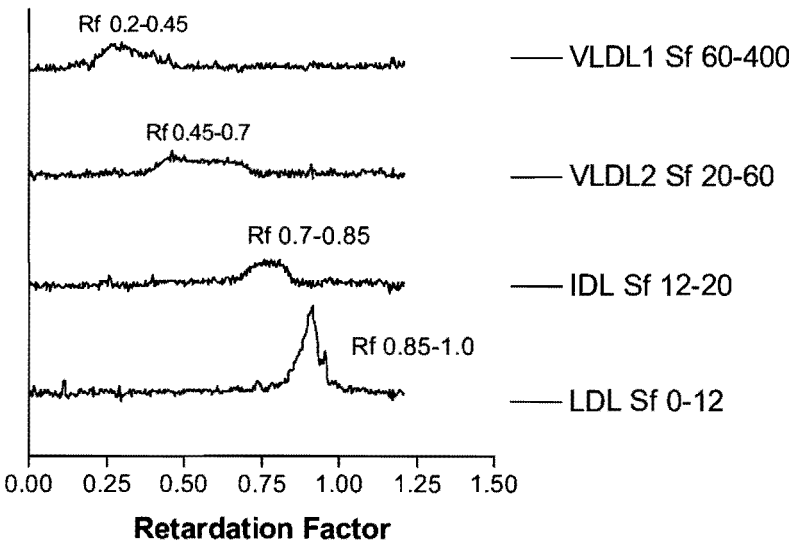
Gels were run for 12-18 hours at 130 V at 4° C in a Tris Glycine (pH 8.3) buffer utilizing the Bio-Rad Minigel apparatus. The gels were subsequently analyzed without any further processing.

The gels were visually inspected and reports used a simple terminology that avoids inferring lipoprotein separation by density. The bands corresponding to LDL species usually appeared sharp and well defined and migrated to the distal fifth of the gel (anodal end). Migration of lipoproteins on successive gels was assessed relative to two control lanes, in which previously selected plasma with a known large (A) and small (B) LDL species were run.

2.2.2.2.1 PGGE analysis

Gels were calibrated with lipoprotein fractions (VLDL1, VLDL 2, IDL and LDL) isolated by sequential density-gradient ultracentrifugation, according to a previously described method (245). These fractions were prestained for lipids as described above and then electrophoresed on the same gel. Optical density profiles were obtained by video densitometry and the migration range of each lipoprotein fraction was expressed in terms of the retardation factor (Rf), for which the beginning of the separation gel was taken as zero and the peak of small dense LDL (B species) as 1.0, as this is the apo-B-containing lipoprotein that migrates furthest in this system. VLDL1 (Sf 60-400) has a Rf of 0.2-0.45, VLDL2 (Sf 20-60) has a Rf of 0.45-0.7, IDL (Sf 12-20) has a Rf of 0.7-0.85 and LDL (Sf 0-12) has a Rf of 0.85 to 1.0. Chylomicrons are found at Rf < 0.2.

Figure 2-7 Calibration of PGGE



Gels run after June 1998 were electronically captured with the Bio-Rad GelDoc 1000 system (Bio-Rad, USA). Gels are placed in a photographic chamber and images are captured by a mounted video camera. The image is digitalized and transferred to a personal computer for further analysis with the Bio-Rad MultiAnalyst software (Bio-Rad, USA) or alternatively raw optical density and migration distance data can be exported for analysis with other software. Baseline optical density was measured for each gel at the beginning of the separation gel between lanes. The value obtained was subtracted from the optical density readings for the lanes.

2.2.2.2.2 PGGE terminology

Gels are described using an "in-house" terminology that allows for rapid and reproducible description of gels. The following section explains this terminology.

As PGGE separates lipoproteins according to size, with size being inversely proportional to migration distance, the largest lipoproteins are found at the origin. Chylomicron sized particles do not migrate into the gel and remain at the origin. Such staining is referred to as O. Particles migrating further than the origin but not into the "LDL area" stain in the midzone of the gel and are termed M. The M region is quite large, making up about two thirds of the gel. Therefore lipoproteins migrating to the beginning of the M zone are termed M-early (M-e) while those migrating further are termed M-late (M-l). Remnant particles are found in the M-l zone. Particles that migrate to the lower third of the gel are termed to be in the "LDL" area. The largest particle is assigned the letter A while the smallest particle is termed B. Faintly staining bands are indicated by lowercase. The assignment of lowercase or upper-case

letters in the reporting is subjective by nature, but as all reports have been given by a single observer the problem of inter-observer variability is minimized. I is found in an intermediate position between A and B and further subdivisions are made- termed Aearly (Ae), A/I and I/B. Particles that migrate further than the B marker are termed B-post. Unusually an extraordinary large LDL-sized particle may be seen above A, and is termed pre-A. It represents Lp (a). The figures illustrate the naming convention.

Figure 2-8 Naming convention for PGGE

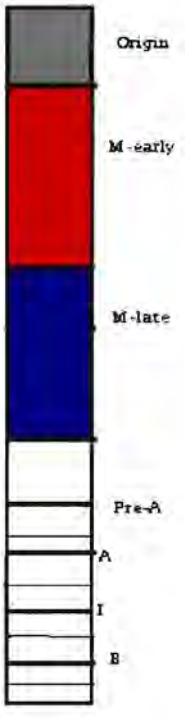
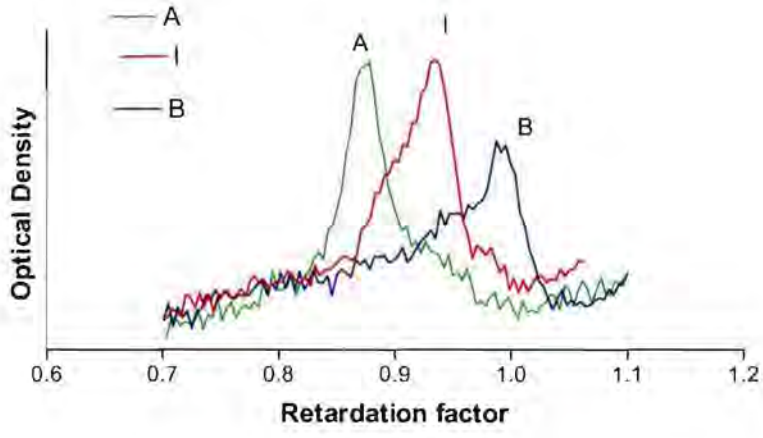


Figure 2-9 LDL species size on PGGE



2.2.3 VLDL compositional analysis

For VLDL compositional analysis 5.5 ml of fasted plasma was adjusted to a density of 1.3 g/ml by addition of 2.74 g of KBr. A saline solution of density 1.0063 g/ml is prepared. Five ml of the density-adjusted plasma is loaded in a Beckman SW40 rotor tube. Using a peristaltic pump saline is layered over the plasma to 2 mm below the rim of the tube. The tubes are balanced in the SW40 rotor and then spun at 33000 revolutions per minute (rpm) for 17 hours at a temperature of 15° Celsius.

Following ultracentrifugation the VLDL supernatant is aspirated quantitatively and assayed for cholesterol and triglycerides together with the unused density-adjusted plasma. The ratio of cholesterol to triglyceride in VLDL is calculated (C_{VLDL}/TG_{VLDL}). The ratio of cholesterol in VLDL to plasma TG is also calculated C_{VLDL}/TG_{Plasma}). These two ratios were initially reported as mass ratios and this tradition has been maintained in the lipid laboratory to allow for easier comparison

with previously published work. All ratios describing cholesterol-enrichment of VLDL in this thesis are therefore mass ratios.

2.2.4 Genotyping

2.2.4.1 DNA extraction and storage

Genomic DNA was extracted from buffy coats using the method described by Parzer (246). This method yields good quantities (about 250 μg of DNA from 5ml of whole blood) of relatively pure DNA. Extracted DNA is labelled with a unique identifying number and stored frozen at -20° C. The "DNA number" allocated to each patient is used in all laboratory databases and allows for easy searching across multiple databases.

2.2.4.2 Determination of common apoE isoforms

The common apoE isoforms were determined according to the method first described by Hixson and Vernier (237) with some minor modifications. The PCR is performed in a final volume of 50 μl. The PCR mix used is listed below.

	(*Boehringer)	(*Promega)
Water	32.8μl	29.8μl
10x Buffer*	5.0μl	5.0μl
dNTPs (2mM)	2.5μl (100uM)	2.5μl
F4 (20uM)	2.5μl (1 uM)	2.5μl
F6 (20uM)	2.5μl (1 uM)	2.5μl
DMSO	2.5μl (5%)	0.2μl
Taq polymerase (5 units/ul)*	0.2μl (1unit)	0.2μl
DNA (denatured)	2.0μl	2.0μl
$MgCl_2$ 25mM/L	0	3.0μl

Primer F4 (5'-ACAGAATTCGCCCCGGCCTGGTACAC-3')
Primer F6 (5'-TAAGCTTGGCACGGCTGTCCAAGGA-3')

PCR conditions: 1 cycle at 95° C for 5min

35 cycles of 94.5° C for 45 seconds and finally 60 ° C for 1 minute

Post PCR 10 µl of product is run on an agarose gel to confirm that amplification was successful. Thirty µl of PCR product is digested with 10 units of HhaI at 37° C overnight. Digested product is loaded and run on a 12% polyacrylamide gel utilizing a sucrose loading buffer. The gel is stained with ethidium bromide and photographed under UV light in the GelDoc apparatus.

The various apoE isoforms have differing numbers of HhaI cleavage sites, resulting in unique band patterns for each isoform. This is illustrated in figure 2-10 and 2-11.

Figure 2-10 HhaI cleavage sites

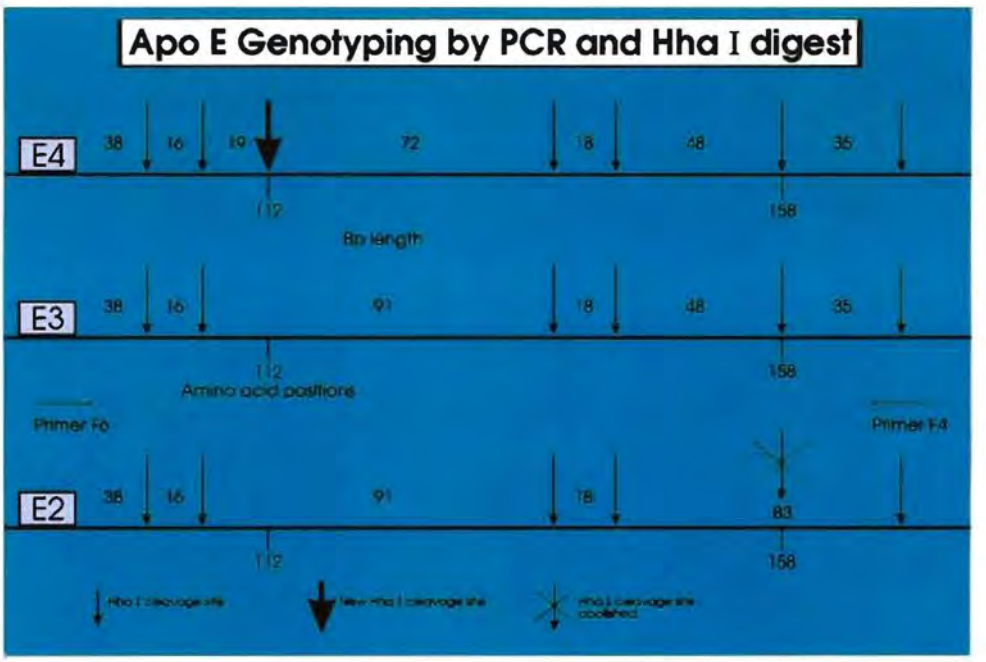
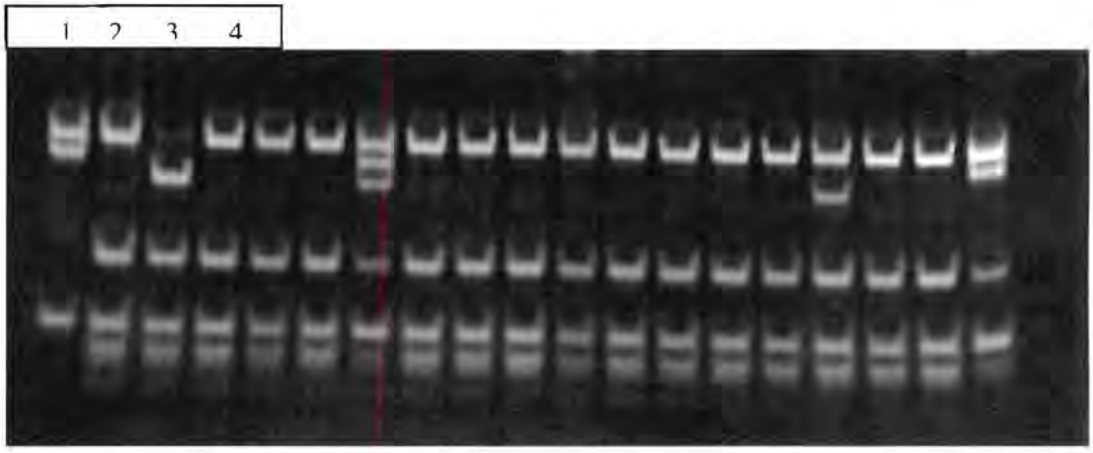


Figure 2-11 Band patterns after HhaI cleavage

	2/2	3/3	4/4	2/3	2/4	3/4
91	—	—		—	—	—
83	—			—	—	
72			—		—	—
48		—	—	—	—	—
38	—	—	—	—	—	—
35		—	—	—	—	—

Figure 2-12 Example of gel used to determine apoE isoforms



Lanes 1, 2 and 3 are loaded with samples of known genotypes: ε2/ε2, ε3/ε3 and ε4/ε 4

2.2.4.3 Determination of apoE (Arg145→Cys) mutation

Two methods are in use at the lipid laboratory to test for this mutation. The methods are used interchangeably and show excellent agreement with each other.

2.2.4.3.1 Method 1

The restriction enzyme BbvI cuts at the sequence: GCAGC. In the wildtype the following fragments are generated (from 5' to 3'): 25, 108, 33 and 78 bp. The corresponding fragments in the mutant are 25, 108 and 111 bp.

The same DNA fragment that was amplified for determination of apoE isoforms is used for this assay. The fragment is cut for two hours at 65° C using 2 U of BbvI (Fermentas). Cut products are separated by electrophoresis on a 12% polyacrylamide gel (4 hours at 110 V) and stained with ethidium bromide. Visualization is under UV lighting.

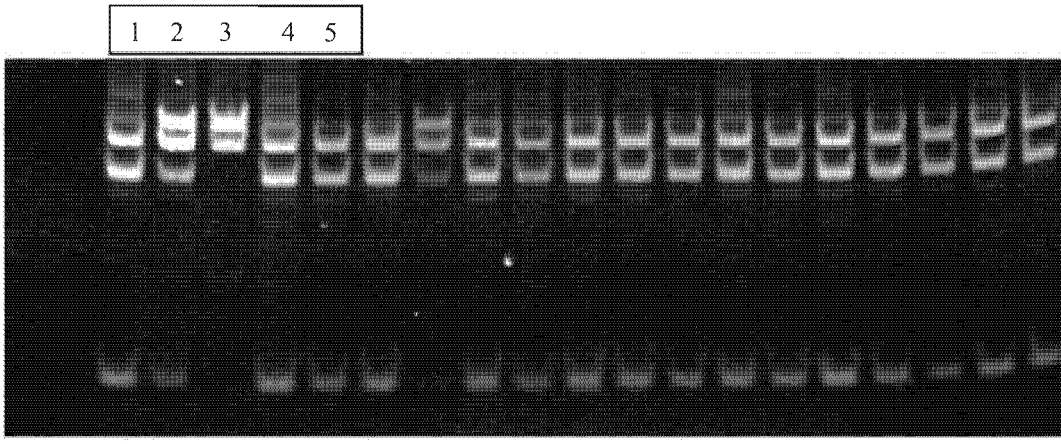
Bands seen following BbvI digest:

Homozygote for ApoE (R145C): 111, 108 and 25

Heterozygote for ApoE (R145C): 111,108, 78, 33 and 25

Wildtype: 108, 78, 33 and 25

Figure 2-13 BbvI digest for ApoE (R145C)



Lane 1: Wildtype

Lane 2: Heterozygote

Lane 3: Homozygote

Lane 4: Wildtype

Lane 7: Heterozygote

Note: The 25 bp fragment is not seen on this gel

2.2.4.3.2 Method 2

The ApoE (R145C) mutation can also be detected by using a mismatch assay (Amplification Refractory Mutation System –ARMS) developed in the lipid laboratory with the help of Professor Howard Henderson of the Department of Chemical Pathology at UCT.

Two sets of PCR are performed- one set with a primer that will amplify wildtype DNA and one set with a "mutant" primer that will yield product only if the apoE (R145C) mutation is present.

Normal: (5'- ATCGCTGAGGAGCCGCTTTCG-3')

Mutant: (5'-ATCGCTGAGGAGCCGCTTTC*A*-3')

Opposite Primer F6: (5'-TAAGCTTGGCACGGCTGTCCAAGGA-3')

DNA denatured in 10% DMSO is used for this assay. The PCR cycles are:

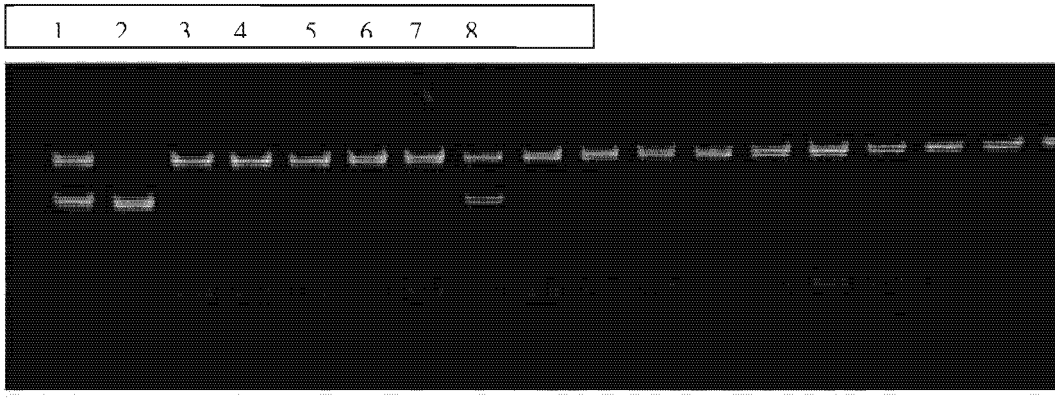
1 cycle at 95° C for 35 seconds

35 cycles of 94.5 °C for 45 seconds, 60° C for 60 seconds, 72° C for 60 seconds

1 cycle at 72° C for 3 minutes

The amplified product is run on a 10% polyacrylamide gel. PCR products from the two PCR reactions per patient are loaded in a single lane separated by a time interval of 30 minutes. The PCR product of the reaction with the mutant primer is generally loaded first.

Figure 2-14 ARMS assay for apoE (R145C)



Lane 1: Heterozygote Both primers have successfully amplified and two bands of product are visible.

Lane 2: Homozygote Only the mutant primer has yielded product and a single band is visible that has migrated further into gel due to earlier loading.

Lane 3: Wildtype Only the wildtype primer has yielded product.

Lane 8: Heterozygote

2.2.4.4 Determination of apoE (Lys146→Gly) mutation

This mutation is also screened for using an ARMS assay. The primers were designed in the lipid laboratory with the assistance of Professor Howard Henderson of the Department of Chemical Pathology at UCT. The PCR conditions are identical to those used for the apoE (R145C) mutation. The PCR products are also run on a 10% polyacrylamide gel with double, time-delayed loading of lanes. The RCR product obtained with the mutant primer is again loaded first.

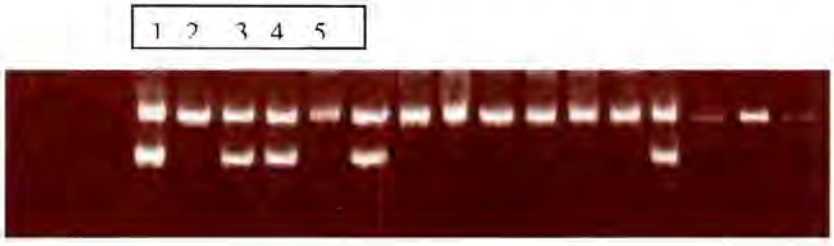
The following primers are used:

Mutant: (5'-GGCATCGCGGAGGAGCCGATG-3')

Wildtype: (5'-GGCATCGCGGAGGAGCCGATT-3')

Opposite Primer F6: (5'-TAAGCTTGGCACGGCTGTCCAAGGA-3')

Figure 2-15 ARMS assay for ApoE (K146Q)



Lane 1: Heterozygote Both primers have been amplified and two bands are visible. The bands are separated not by size but by delayed loading.

Lane 2: Wildtype The mutant primer has not amplified and only one band is visible.

Lane 3: Heterozygote

2.2.4.5 ApoE sequencing

In patients with cholesterol-enriched VLDL meeting the diagnostic criteria set out in this thesis, but with no identified apoE mutation, a portion of the apoE molecule was sequenced in an attempt to identify mutations that were not screened for or are novel.

Due to limitations of funds it was not possible to sequence the entire apoE molecule, but the focus was on the receptor-binding region of apoE as most identified mutations have been found in this region. The PCR fragment obtained when determining the common apoE isoforms by the method of Hixson (237) was sequenced.

Sequencing was not performed in-house but outsourced to a commercial biotechnology company (Inqaba Biotechnological Industries Pty. Ltd.; Pretoria, South Africa).

Genomic DNA was amplified as described in section 2.2.4.2. PCR products were separated in agarose gels; the relevant bands were cut from the gel and submitted to Inqaba Biotechnological Industries. The DNA was purified at Inqaba Biotechnological Industries and the fragment was sequenced. Fragments were sequenced by the chain termination method with fluorescent labels on an automated sequencer. Inqaba Biotechnological Industries e-mailed completed sequences to the laboratory for review. Technically inadequate sequences were repeated. All fragments were sequenced bidirectionally (forward + reverse).

All sequences were reviewed by two independent observers and compared with the previously published sequence.

2.3 Statistical Methods

2.3.1 Data Management

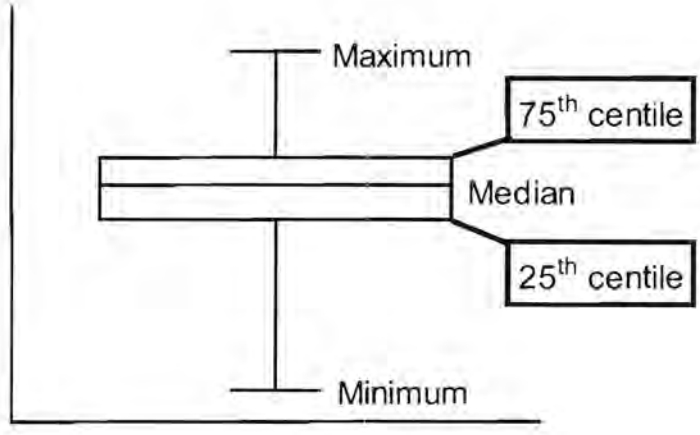
Clinical data were captured from original source documentation held at the lipid laboratory and entered in a Paradox (Corel ® Version 9) database. If clinical notes

had gone missing from the folder held at the lipid laboratory the patient's hospital folder was requested. Results of special investigations performed at the lipid laboratory were captured by linkage of multiple databases held in the laboratory (e.g. genotyping results) to the main database using the unique "DNA number" of each patient. Data preceding the computerization of the laboratory was extracted from archived laboratory records and entered manually.

2.3.2 Data Analysis

Data to be analyzed was transferred either to Quattro Pro (Corel ®) or to Graph Pad Prism (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). All graphs were generated using Prism. Where box and whiskers plots are presented they are all of the following type.

Figure 2-16 Illustrative box and whiskers plot



2.3.3 Statistical analysis

The data were analyzed using standard statistical methods. Normally distributed variables were analyzed using parametric tests. Non-parametric tests were used for skewed data or logarithmic transformation was performed. Categorical variables were analyzed in contingency tables. The statistical methods used for each analysis will be indicated in the individual sections.

2.4 Reviewing the Cape Town experience of dysbetalipoproteinaemia

2.4.1 Dysbetalipoproteinaemia: definition for this study

For the purposes of this study dysbetalipoproteinaemia was defined by both phenotypic and genotypic criteria. Patients were classified as dysbetalipoproteinaemic if they fulfilled one **or** both of the criteria below.

Phenotypic Criteria:

The demonstration of cholesterol-enriched VLDL has been the phenotypic diagnostic test of choice for patients with suspected dysbetalipoproteinaemia for many years. Fredrickson himself suggested it should replace agarose gel electrophoresis of ultracentrifugally isolated VLDL (207). Within the literature there is not universal agreement as to the level of cholesterol-enrichment of VLDL that should be regarded as diagnostic. In this study we defined dysbetalipoproteinaemia using the mass ratios given below. These ratios are currently in use at the lipid laboratory and offer higher

specificity, at the cost of reduced sensitivity, when compared to other diagnostic ratios.

Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$ (CV/TV) ≥ 0.42

or

Cholesterol $_{VLDL}$/ Triglyceride $_{Plasma}$ (CV/TP) ≥ 0.30

VLDL compositional analysis was performed on multiple occasions in some patients. A single positive result was sufficient for inclusion in the study.

Genotypic Criteria:

Patients were also diagnosed with dysbetalipoproteinaemia if one of the following genotypes was documented in the presence of mixed hyperlipidaemia:

- Homozygosity for the ε2 isoform of apoE
- ApoE (R145C) mutation detected
- ApoE (K146Q) mutation detected

Based on these diagnostic criteria the dysbetalipoproteinaemic patients can be divided into several groups.

Table 2-1 Categories of dysbetalipoproteinaemic patients

VLDL-Compositional Analysis	Genotyping	
	Detected	Not Detected
Positive	Geno [1]+ VLDL composition [2]+	Geno [1]- VLDL composition [2]+
Negative	Geno [1]+ VLDL composition [2]-	Not dysbetalipoproteinaemia

[1] Genotype: analysis of apoE for mutations known to be associated with dysbetalipoproteinaemia

[2] VLDL-compositional analysis

Patients with positive VLDL compositional analysis but no detected apoE mutation are referred to as "genotype negative" (Geno-) in this thesis. In this specific context the term genotype negative must be taken to indicate that no mutations in apoE were detected using a limited array of genotypic tests. ApoE mutations may still be present, but the patients are referred to as genotype negative for the purpose of this thesis.

2.4.2 Case finding

As the diagnosis of dysbetalipoproteinaemia was based on laboratory criteria all laboratory records of apoE genotyping and VLDL compositional analysis were reviewed for patients that met the inclusion criteria. Additionally the clinical database was searched for patients that had been labelled as dysbetalipoproteinaemic or where the diagnosis had been considered but no special investigations had been performed.

Historically the policy from about 1985 onwards has been to perform VLDL compositional analysis in all patients with possible dysbetalipoproteinaemia. Patients seen prior to 1985 were often not investigated further. In these patients apoE genotyping was done if a DNA sample was available. VLDL compositional analysis was requested if the patient still attended the lipid clinic.

3 Chapter Three: Clinical and laboratory findings

3.1 Demographics

One hundred and thirty-six patients with dysbetalipoproteinaemia were included in the database. The first patient presented in January 1969 and the database was closed in February 2004.

Table 3-1 Ethnic and sex distribution of dysbetalipoproteinaemic patients

Ethnicity	Males (%) [1]	Females (%)[1]	Sum (%)[1]
Caucasian	27 (19.9)	19 (13.9)	46 (33.8)
Mixed Race	37 (27.2)	40 (29.4)	77 (56.6)
Indian	1 (0.7)	0 (0)	1 (0.7)
African	10 (7.4)	2 (1.4)	12 (8.8)
All	75 (55.1)	61 (44.9)	136

[1] The percentage indicated is relative to all dysbetalipoproteinaemic patients in the series

Percentages have been rounded to the first decimal

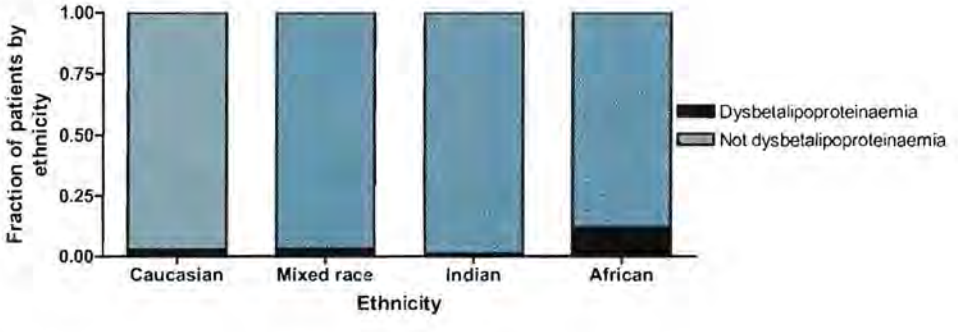
Table 3-2 Ethnic and sex distribution of all other patients seen at the GSH lipid clinic up to February 2004

Ethnicity	Males (%) [1]	Females (%) [1]	Sum (%) [1]
Caucasian	866 (20.1)	814 (18.9)	1680 (39.0)
Mixed Race	1027 (23.9)	1424 (33.1)	2451 (56.9)
Indian	59 (1.4)	26 (0.6)	85 (2.0)
African	54 (1.3)	36 (0.8)	90 (2.1)
All	2006 (46.8)	2300 (53.2)	4306

[1] The percentage indicated is relative to the total number of patients

Percentages have been rounded to the first decimal

Figure 3-1 Relative proportions of dysbetalipoproteinaemia within ethnic groupings



Chi-square test P<0.0001

The proportion of patients with dysbetalipoproteinaemia at the lipid clinic within each ethnic group ranges from 1-3% for all ethnic groups, except for African patients in whom dysbetalipoproteinaemia was diagnosed in 12%. There are several possible

explanations for the high prevalence of dysbetalipoproteinaemia in African patients attending the clinic. African patients historically had worse access to medical services than other population groups and thus would often only be referred to a tertiary center if they had obvious physical signs of a lipid disorder. This referral pattern would preferentially select patients with genetic dyslipidaemias that are associated with obvious cutaneous xanthomata. The high frequency of the apoE (R145C) mutation in the African population also contributes to the high prevalence of the disorder (see below).

3.1.1 Comparison with previously published case series

Most published case series originate from Europe (6;9;10) or the United States of America (5;7;220). It is therefore difficult to compare the ethnic mix in the case series due to the different populations studied. All case series reviewed (5-7;9;10;220) reviewed report a slight excess of male patients with a pooled (6;7;9;10) gender distribution of 3:2 for male: female. We recorded a very similar gender distribution with a moderate excess of male patients. As the genetics of dysbetalipoproteinaemia are not sex-linked, the male excess reflects the influence of environmental and biological factors, of which oestrogen is likely the most important in premenopausal females.

3.2 ApoE genotypes

In the 136 patients with dysbetalipoproteinaemia apoE mutations were identified in 99 (73%). ApoE2 homozygosity was the most commonly identified molecular cause of

dysbetalipoproteinaemia (71 % of identified mutations). Locally the apoE (R145C) mutation was also highly prevalent and accounted for 27% of identified mutations. The apoE (R145C) mutation was found in all population groups studied. However, three of the four apoE (R145C) homozygotes were African. None of the homozygous subjects had a family history suggestive of consanguinity. This suggests that the background frequency of the apoE (R145C) mutation may be relatively high in the black population. The apoE (K146Q) mutation was identified in two patients. Both patients had Dutch/Belgian ancestry. This mutation was also first described in Dutch and Belgian patients (194).

Table 3-3 ApoE genotypes in the dysbetalipoproteinaemia cohort

Genotype		**Sex**		**Ethnicity**				*Total*
		Male	Female	Caucasian	Mixed race	Indian	Black	
ApoE2/E2		39	31	28	36	1	5	*70*
ApoE (R145C)	Heterozygote	15	8	6	14	0	3	*23*
	Homozygote	2	2	0	1	0	3	*4*
ApoE (K146Q)		2	0	2	0	0	0	*2*
Genotype not identified		*17*	*20*	*10*	*26*	*0*	*1*	*37*

3.2.1 ApoE (R145C) mutation

The apoE (R145C) mutation was first described in 1982 by Rall (47). Rall identified this mutation in two African American patients with dysbetalipoproteinaemia whose

apoE competed unusually well with iodinated LDL in human fibroblasts. One patient was homozygous for this mutation while the other was heterozygous (18;47). Emi and Hsia subsequently described further cases of dysbetalipoproteinaemia secondary to this mutation, also in African American patients (18;47;247;248). In 1997 de Villiers described 10 patients, including 4 homozygotes, with this mutation (184). The patients all attended the lipid clinic at Groote Schuur Hospital. The mode of inheritance was shown to be autosomal dominant with incomplete penetrance. There is no gene-dose effect and homozygotes and heterozygotes are affected similarly.

In 1991 Lohse described a 24 year-old woman with severe dysbetalipoproteinaemia whose apoE migrated as apoE4 during IEF. The patient was found to be a homozygous carrier of apoE (E13K; R145C). The allelic variant was named apoE4-Philadelphia (185). When the kindred were subsequently traced, six heterozygous mutation carriers were identified. Heterozygosity for the apoE4-Philadelphia allele was associated with "a moderate form" of dysbetalipoproteinaemia (186). Heterozygotes had various degrees of hyperlipidaemia (less severe than the homozygous patient) in the presence of cholesterol-enriched VLDL. None of the heterozygotes had cutaneous manifestations of hyperlipidaemia. For the apoE4-Philadelphia mutation there is therefore a suggestion that a gene-dose effect may apply and that the inheritance is partially dominant.

The apoE4-Philadelphia mutation was excluded in the original cohort of apoE (R145C) patients reported from Cape Town by *Aval* digestion of PCR products generated using apoE exon 3 specific primers (184;186). Two of the apoE (R145C) homozygotes had also previously been phenotyped as apoE2 homozygotes by IEF.

3.2.1.1 ApoE (R145C) population distribution

The apoE (R145C) mutation has been identified in patients with diverse ethnic and geographic origins. The GSH lipid clinic has identified Caucasians (with ancestry from the United Kingdom), Africans and Mixed Race patients with this mutation. The identification of three unrelated African homozygotes suggests that the background prevalence of the apoE (R145C) allele in the African population may be high. This has not been formally examined as yet, but information is available from two studies examining lipoprotein metabolism in pregnancy.

In the first study apoE genotypes and the presence of the apoE (R145C) mutation were determined in pregnant patients presenting to the GSH obstetric unit with gestational proteinuric hypertension (pre-eclampsia) and in women with uncomplicated pregnancies. All participants in this study were African and were almost entirely of Xhosa extraction. DNA extraction and amplification was successful in 180 of 184 subjects. The apoE (R145C) mutation was found in 11/180 (6%) of patients. All subjects were heterozygous for the mutation. There was no association between apoE genotypes and the occurrence of gestational proteinuric hypertension (249).

Tanyanyiwa studied lipid profiles, PGGE and apoE genotypes in 690 pregnant women attending two large public health care facilities in Harare, Zimbabwe. Almost all patients were of Shona extraction. ApoE2 homozygotes (25/690) were not screened

for the apoE (R145C) mutation and 564 women were finally successfully tested for this mutation (121).

Table 3-4 ApoE (R145C) mutation in Zimbabwean patients

		N	%
ApoE (R145C)	Heterozygotes	29	5.15
	Homozygotes	2	0.35
	No mutation detected	533	94.5

The total prevalence of 5.5% for this mutation is very similar to that observed in the previous study, although the two studies were conducted in geographically and ethnically disparate regions: Cape Town, South Africa and Harare, Zimbabwe. The detection of two homozygotes again supports the contention that there is a high prevalence of this mutation in Africans with Zimbabwean ancestry.

3.2.1.2 ApoE (R145C) and the common apoE isoforms

All carriers of the apoE (R145C) mutation had at least one ε3 allele when genotyped according to Hixson (237). The genotype was ε3/ ε3 in all homozygotes.

Table 3-5 Common isoforms of apoE in apoE (R145C) mutation carriers

ε2/ ε3	ε3/ ε3	ε4/ ε3
3	21	3

Due to the small number of subjects not homozygous for the ε3 allele, the influence of the other apoE allele on clinical and laboratory characteristics was not examined.

3.2.1.3 Haplotype analysis of the apoE (R145C) gene

Haplotype analysis of the apoE (R145C) mutation in patients attending the lipid clinic at GSH has been undertaken previously. This work was done by a student (Aron Abera) for a BSc (Hons) degree in the Department of Genetics under supervision of Professor Howard Henderson of the Department of Chemical Pathology and Professor A. David Marais of the Department of Medicine (250).

DNA from 47 subjects who either carried the apoE (R145C) mutation or were relatives of mutation carriers was examined. DNA from 30 healthy normolipidaemic controls of African ethnic origin was also studied. Two microsatellite markers and one intragenic single nucleotide polymorphism (SNP) were selected for study. The two microsatellite markers were (distance from apoE gene given in brackets):

- D19S417 (5.9 Mb)
- D19S408 (1.3 Mb)

The SNP was located in intron 2 of the apoE gene.

PCR products for the microsatellite markers were analyzed on 9% non-denaturing polyacrylamide gels. The SNP was analyzed in agarose gels following restriction of the PCR fragment with Tsp509I.

Haplotypes were constructed using the common isoforms of apoE and the three markers described above.

The figure below illustrates the haplotypes constructed in the largest kindred with the apoE (R145C) mutation.

Figure 3-2 Haplotypes in a kindred with the apoE (R145C) mutation

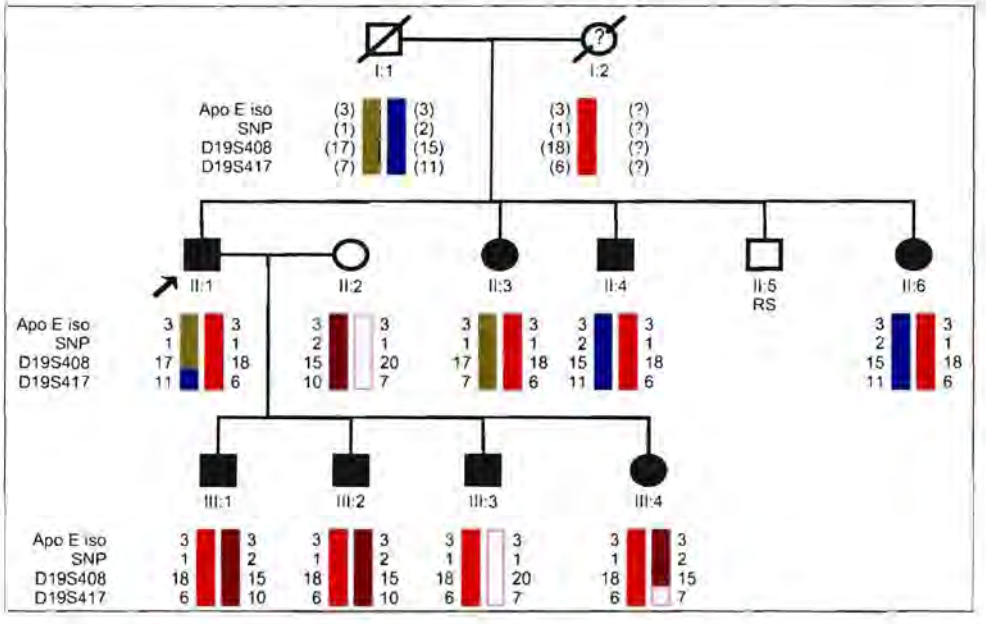


Illustration courtesy of A. Abera

Of the three kindreds studied, two had similar haplotypes for the mutated allele. All three kindreds studied were of Mixed Race. DNA amplification was only successful in two African patients homozygous for the apoE (R145C) mutation. These individuals were both heterozygous at the microsatellite markers and differed in 7 of

the 8 possible repeat lengths (see figure 3.6). There were no differences in the SNP genotypes.

Table 3-6 Microsatellite markers in two African patients homozygous for the apoE (R145C) mutation

	D19S408 Genotype ("CA" repeat length)	*D19S417 Genotype ("CA" repeat length)*
Patient 1	(15,17)	*(8,12)*
Patient 2	*(17,21)*	*(6,16)*

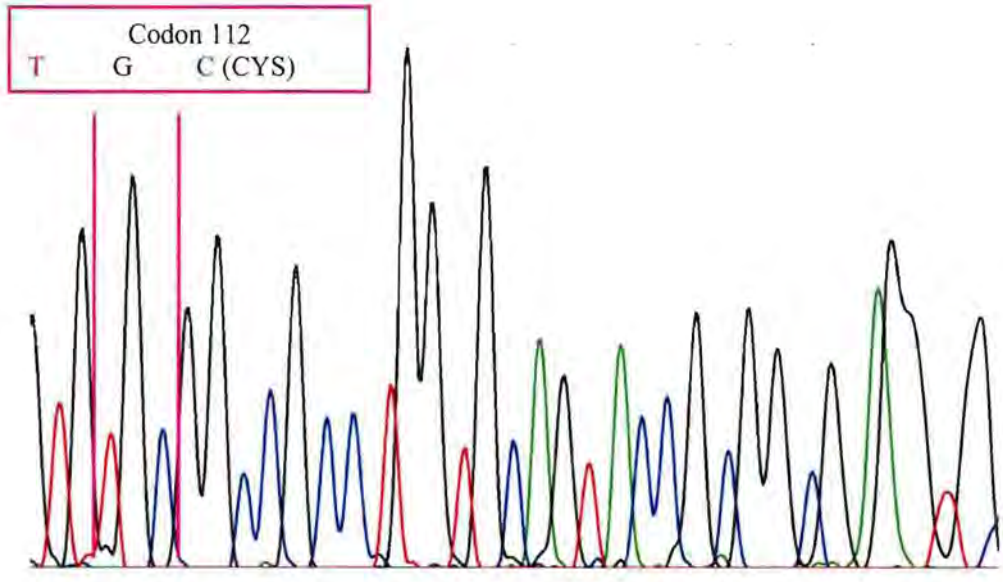
The allele frequency of the two microsatellite markers in the control samples of African origin was also compared to that observed in all African mutation carriers. Significant differentiation was found at both microsatellite markers, but due to the small number of specimens studied and the relative distance of the chosen markers from the apoE gene locus the study was unable to conclusively differentiate between a single origin and multiple origins of the mutation in African patients.

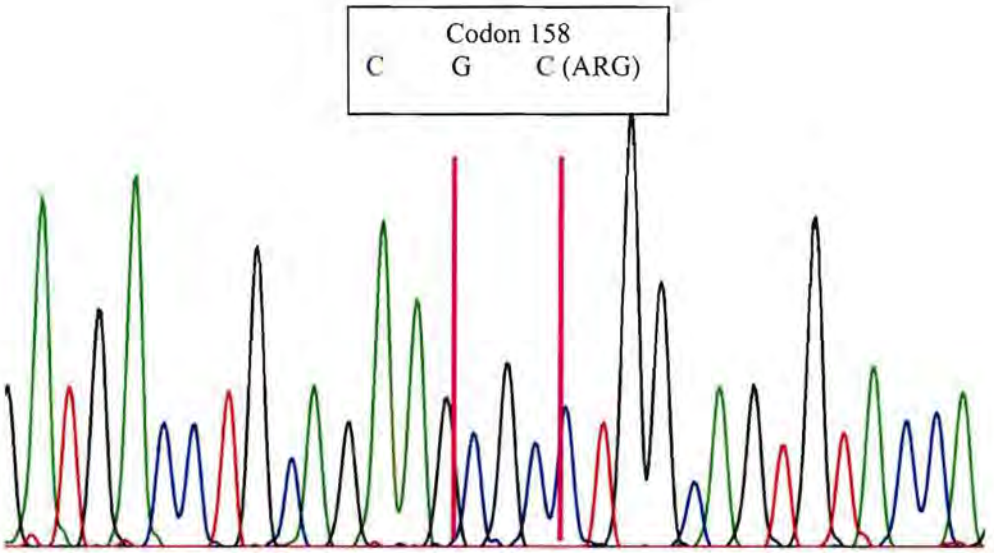
However, given the diverse ethnic origins of individuals carrying the apoE (R145C) mutation it is likely that the mutation is recurrent. The C→T change observed at position 4031 of the apoE gene with the apoE (R145C) mutation is an example of a change occurring in a CpG dinucleotide sequence, a mutational hotspot (251).

3.2.2 ApoE sequences in patients with no identified apoE mutations

The binding region of apoE was sequenced in all patients without an identified apoE genotype as detailed in section 2.2.4.5. Adequate quality forwards and reverse sequences were obtained in all subjects. No mutations were identified in any of the patients. The apoE genotype as determined by the Hixson method (237) was confirmed by sequencing in all patients.

Figure 3-3 ApoE sequence in a genonegative patient (E3/E3)

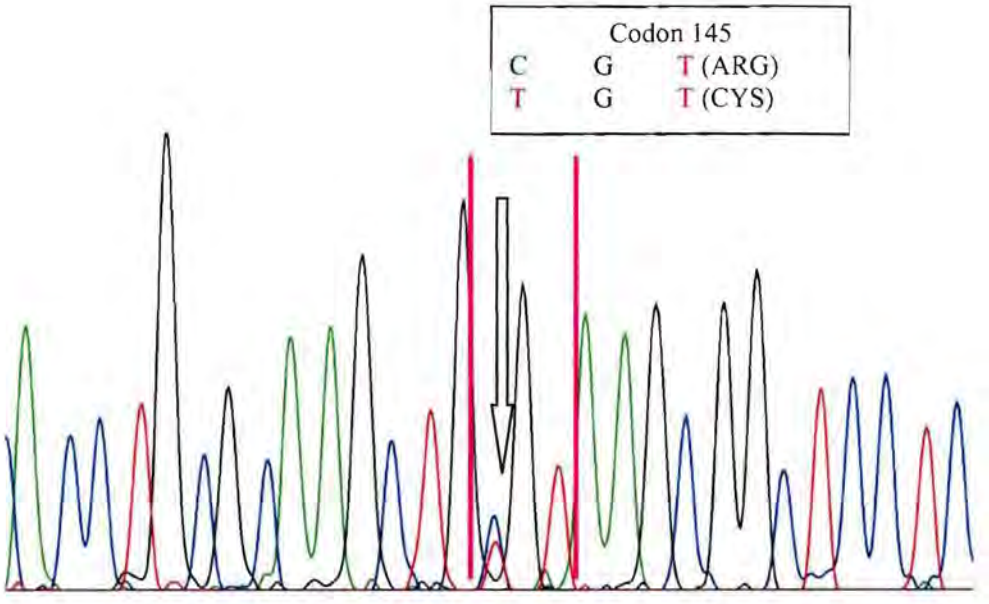




Although this investigation did not find any mutations in apoE, it is of course still possible that subjects may have mutations outside the sequenced fragment. Large deletions or insertions may also have been missed.

Samples from patients with the apoE (R145C) and apoE (K146Q) mutations were also submitted for sequencing and the mutations were confirmed. The apoE (R145C) sequence variation is illustrated in figure 3.3.

Figure 3-4 Heterozygous apoE (R145C) mutation carrier



3.3 Total clinical experience

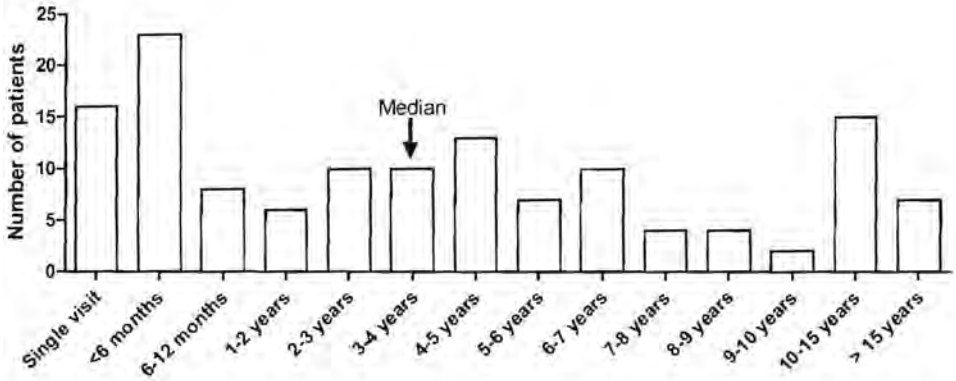
All patient records were reviewed in February 2004 and the date of the last consultation was noted. The duration of follow-up was calculated as the difference between the date at presentation and the date of the last consultation. Where available, information on the final outcome was collected.

3.3.1 Follow-up

The mean duration of follow-up was 4.74 ± 5.16 years (median 3.55) with a range from 0 to 23.14 years. Sixteen patients were either lost to follow-up or referred elsewhere after their initial visit, but for all other patients data from at least one

follow-up visit are available. The mean age at the last documented visit was 55.00 ± 12.47 years with a range of 10.54 to 85.21 years.

Figure 3-5 Duration of follow-up



Note: Data censored February 2004

The total cumulative experience is 640 patient-years.

3.3.2 Final outcomes

In February of 2004 75 patients were known to be alive (as judged by a clinic attendance less than six months previously or personal contact), 15 patients had died and the final outcome could not be determined with certainty in 46 patients. Many of the patients with unknown outcome are presumed to have died, considering their age and morbidity when last seen at the clinic. They may have died at home or at other medical facilities with no formal documentation regarding their death available in their GSH folders. The two commonest causes of death were CVD and renal failure.

Of the 75 patients known to be alive, 67 regularly attend the lipid clinic for treatment and monitoring while 8 receive their treatment in the private sector or at other hospitals.

3.4 Age at presentation

Information regarding the age at initial presentation was available for 135 patients. The interval between the onset of hyperlipidaemia and presentation to the lipid clinic is obviously highly variable and cannot be determined reliably. The age at presentation is therefore the best approximation available and allows for comparisons to be made between different groups of dysbetalipoproteinaemic patients. Table 3.7 illustrates ages at presentation by patient categories.

Table 3-7 Ages at presentation

Patient categories		Age at presentation (years)				
		Number	Mean	Median	Standard deviation	Range
All patients		135	50.19	51.36	11.39	6.93-80.80
Males		74	47.37	47.84	11.30	6.93-67.20
Females		61	53.62	53.76	10.53	22.6-80.80
Geno (+)	All	98	49.95	50.78	10.71	6.93-71.27
	E2/E2	69	49.93	50.00	9.77	23.18-71.27
	E (R145C) All	27	50.00	53.47	13.15	6.93-65.15
	E (R145C) hetero	23	50.56	54.27	13.42	6.93-65.15
	E (R145C) homo	4	46.81	44.83	12.71	33.82-63.76
	E (K146Q)	2	49.87	49.87	12.29	41.18-58.56
Geno (-)		37	50.83	51.67	13.17	22.60-80.80

There was a very wide range in age of presentation. The two youngest patients (6.93 and 22.60 years at presentation) both had severe renal disease with nephrotic range proteinuria, which may cause severe hyperlipidaemia even in the absence of genetic errors in lipid metabolism (252). The oldest patient (80.80 years old at presentation) presented with ischaemic heart disease and the diagnosis of dysbetalipoproteinaemia was made when her severe mixed hyperlipidaemia was investigated.

3.4.1 Factors influencing age at presentation

The following factors were examined for their influence on the age at initial presentation.

3.4.1.1 Gender

Males presented at a younger age than females. When analyzed by subgroup this finding was confirmed in the following groups:

- Cohort as a whole
- Genopositive patients
- apoE2 homozygotes

In the smaller patient categories (apoE (R145C) mutation and genonegative patients) females also presented later than men but the differences did not reach statistical significance. This may be due to the smaller number of patients and the wide scatter of the data. The other subgroups are too small to allow for meaningful analysis by gender.

Table 3-8 Gender differences in age at presentation

Category	Sex	Number	Mean	SD	Difference	P value [1]
All Patients	Males	74	47.37	11.30	6.25	0.0013
	Females	61	53.62	10.63		
Geno (+)	Males	57	47.48	11.65	5.90	0.0065
	Females	41	53.38	8.22		
E2/E2	Males	38	47.01	10.16	6.49	0.0051
	Females	31	53.51	8.06		
E (R145 C) Hetero	Males	15	47.65	15.38	8.35	0.16
	Females	8	56.00	6.12		
Geno (-)	Males	17	46.98	10.43	7.1	0.10
	Females	20	54.09	14.63		

[1] Unpaired t-test

3.4.1.2 ApoE Genotype

There were no differences in age at presentation when patients were compared by genotypic status (mean age 49.95 ± 10.71 vs. 50.83 ± 13.17, P=0.69 for genopositive vs. genonegative patients by unpaired t-test). Amongst patients with identified apoE mutations the nature of the mutation did not influence the age at presentation (mean age 49.93 ± 9.77 vs. 50.56 ± 13.42, P=0.81 by unpaired t-test-for apoE2 homozygotes vs. apoE (R145C) heterozygotes). The number of apoE (R145C) homozygotes is

small and their mean age of 46.81 ± 12.71 years at presentation was not significantly different from heterozygous mutation carriers (P=0.61, unpaired t-test).

3.4.1.3 Ethnicity

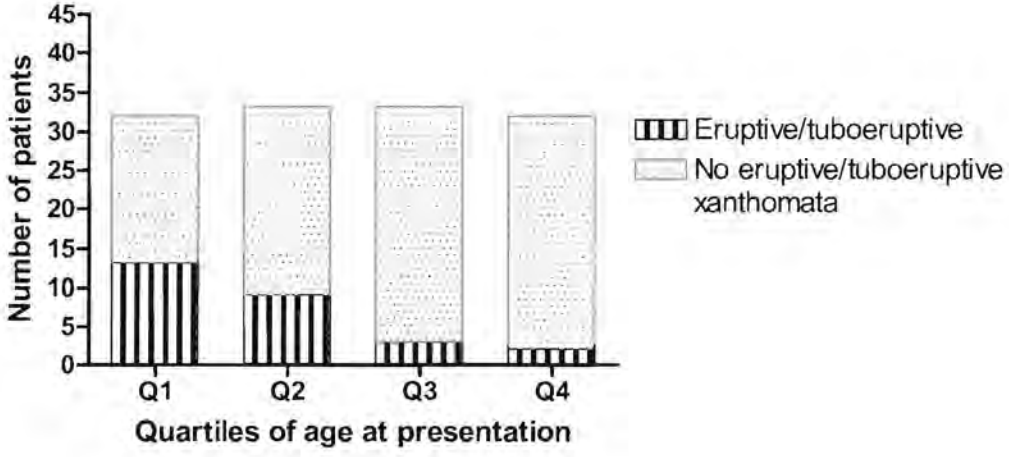
The age at presentation was very similar for Caucasian and mixed race patients (51.73 ± 11.52 years vs. 50.20 ± 10.20 years, P=0.44 by unpaired t-test) but Africans presented on average about 7 years earlier at a mean age of 42.96 ± 16.89 years, although there was a wide range of ages at presentation from 6.93 to 65.10 years. Interestingly, in a study of FH at the lipid clinic Caucasian patients presented the earliest and African patients the latest. African patients were generally only seen following a complication of atherosclerosis (253). This finding probably reflects higher awareness of FH and better access to screening in the Caucasian population together with the often less obvious physical signs of FH.

3.4.1.4 Physical signs of a lipid disorder

Cutaneous xanthomata (tuberous, tuboeruptive and eruptive xanthomata) are probably the most dramatic external signs of dysbetalipoproteinaemia and are readily noticed by the patient and the physician, although in the GSH lipid clinic experience the initial referral is often to a dermatologist rather than a lipidologist. Palmar crease xanthomata and tendinous xanthomata are usually only detected at the initial lipid clinic visit.

Patients with eruptive/tuboeruptive xanthomata were significantly younger at presentation than patients without these xanthomata (42.90 ± 12.06 years vs. 52.23 ± 10.44 years, P<0.0001, unpaired t-test). The high prevalence of eruptive/tuboeruptive xanthomata in younger patients is well illustrated if patients are divided into quartiles by age of presentation.

Figure 3-6 Eruptive/tuboeruptive vs. quartiles of age at presentation



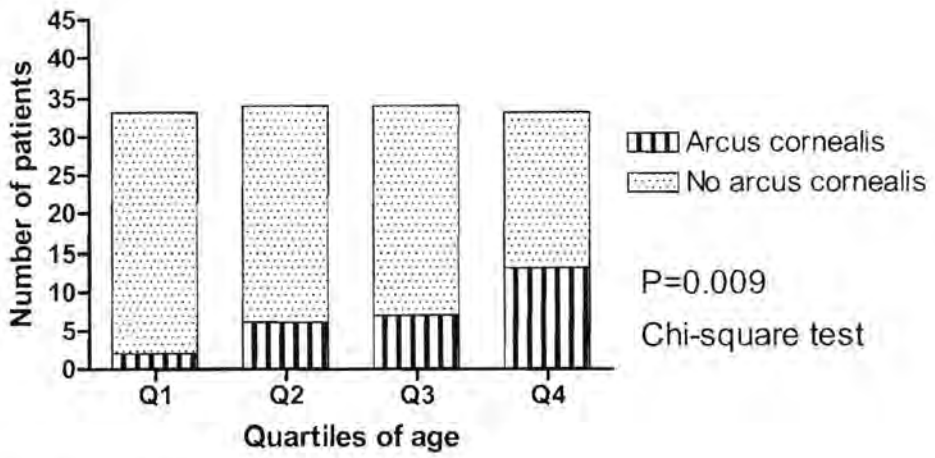
Chi-square test P=0.005

Quartile 1: 6.93-43.71
Quartile 2: 43.72- 51.51
Quartile 3: 51.52- 57.68
Quartile 4: 57.68-80.80

The finding of palmar crease xanthomata did not influence the age of presentation to the lipid clinic (48.15 ± 9.17 years vs. 50.90 ± 12.04 years, P=0.22 by unpaired t-test for palmar crease xanthomata vs. none). The same was true for tendinous xanthomata (50.98 ± 9.44 years vs. 50.05 ± 11.05 years, P=0.72 by unpaired t-test for tendinous xanthomata vs. none). Xanthelasmata also showed no relationship to the age at

presentation (49.89 ± 7.59 years vs. 50.24 ± 11.74 years, P=0.92 by unpaired t-test for xanthelasmata vs. none). As expected the prevalence of arcus cornealis increased with age (56.16 ± 9.01 years vs. 48.64 ± 11.52 years, P=0.0017 by unpaired t-test for arcus vs. none).

Figure 3-7 Arcus cornealis by quartiles of age at presentation



Quartile 1: 6.93-43.71
Quartile 2: 43.72- 51.51
Quartile 3: 51.52- 57.68
Quartile 4: 57.68-80.80

3.4.1.5 Metabolic precipitants of dysbetalipoproteinaemia

Dysbetalipoproteinaemia is often unmasked by clinical conditions such as alcoholism, diabetes or hypothyroidism. We compared the ages at presentation of patients categorized by the clinical disorder thought to be the dominant precipitating factor by the admitting clinician. Alcohol abuse was associated with a significantly younger age at presentation when compared to those patients in whom no secondary cause was identified. Alcohol intake was estimated from the patient's self reported drinking

habits and may not always truly reflect actual alcohol consumption. The fact that patients who abuse alcohol are younger at presentation than other patients may simply be due to alcohol abuse often setting in at a younger age than the other clinical conditions considered.

Table 3-9 Ages at presentation by metabolic precipitants

	Number	Mean	Median	SD	Range	P value [4]
Diabetes [1]	36	52.19	54.24	10.48	27.40-71.27	0.88
Hypothyroidism	6	56.02	54.17	13.71	39.75-80.80	0.20
Alcohol abuse [2]	6	39.00	38.99	8.41	29.49-49.38	0.01
Renal disease [3]	13	48.39	52.62	18.31	6.93-60.39	0.57
None of above	74	50.35	50.00	10.16	23.18-75.97	

[1] Diabetes previously diagnosed or diagnosed at the first consultation

[2] Alcohol abuse was defined as consumption of more than 140 grammes of alcohol per week for females and 210 grammes per week for males

[3] Chronic renal failure, proteinuria (> 1+ on dipstick), nephrotic syndrome

[4] Compared to patients with none of the above conditions (unpaired t-test)

Obesity was highly prevalent. Thirty-three % of patients had a BMI of more than 30 kg/m^2 and the waist circumference exceeded the values recommended (men<102 cm, women <88 cm) in the third report of the National Cholesterol Education Program

(NCEP III) (254) in 50% of patients. Obesity was analyzed without reference to other metabolic precipitants due to its high prevalence.

Table 3-10 Age at presentation and obesity

Measure of obesity		Number [1]	Age at presentation				P value [2]
			Mean	Median	SD	Range	
BMI	≥ 30	43	49.98	51.66	12.33	6.93-80.80	0.69
(kg/m^2)	< 30	84	50.66	52.67	9.77	27.04-66.92	
Waist [3]	> NCEP	54	51.39	52.67	9.57	27.04-68.43	0.04
(cm)	< NCEP	54	47.16	49.06	12.80	6.93-80.80	

[1] Incomplete data for some patients

[2] Data not normally distributed, Mann Whitney test

[3] Men >102 cm, women >88 cm

The finding that an increased waist circumference was associated with a higher age at presentation is somewhat surprising. Abdominal obesity is predictive of insulin resistance and would be expected to accelerate the phenotypic expression of dysbetalipoproteinaemia.

3.4.1.6 Comparison with previous case series

All previous case series report a younger age at presentation in males than females (6;7;9;10), with the mean reported difference ranging from 5 (10) to 17 years (6). Earlier case series (6;7) report younger mean ages at onset than those published later

(9;10). This may well reflects ascertainment bias as Borrie (6) studied patients seen at a dermatology clinic for xanthomata and Morganroth (7) reports on patients referred to the National Institute of Health. It is likely that younger and more severely affected patients would be referred to such centers. Feussner (10) describes 64 hyperlipidaemic apoE2 homozygotes identified at a single tertiary center by systematic screening of patients referred with mixed hyperlipidaemia. The setting of Feussner's study is therefore very similar to the GSH lipid clinic and the mean ages at presentation are very similar to those seen in this study (values in brackets) at 48.9 years (47.37) for males and 53.2 (53.62) years for females. There are no published reports examining the influence of physical stigmata, apoE genotype and comorbidities on the age at presentation.

3.5 Lipid investigations

There was a very wide range of lipid values at initial presentation. Dysbetalipoproteinaemia may present with "moderate" dyslipidaemia in a few patients but on occasions may also causes extremely severe dyslipidaemia. Table 3.11 illustrates the lipid values at presentation. Patients who were referred on treatment and for whom untreated lipid values could not be traced were excluded from this analysis. Triglyceride values were not normally distributed and analytic statistics were performed following logarithmic transformation.

Table 3-11 Untreated lipid values at presentation

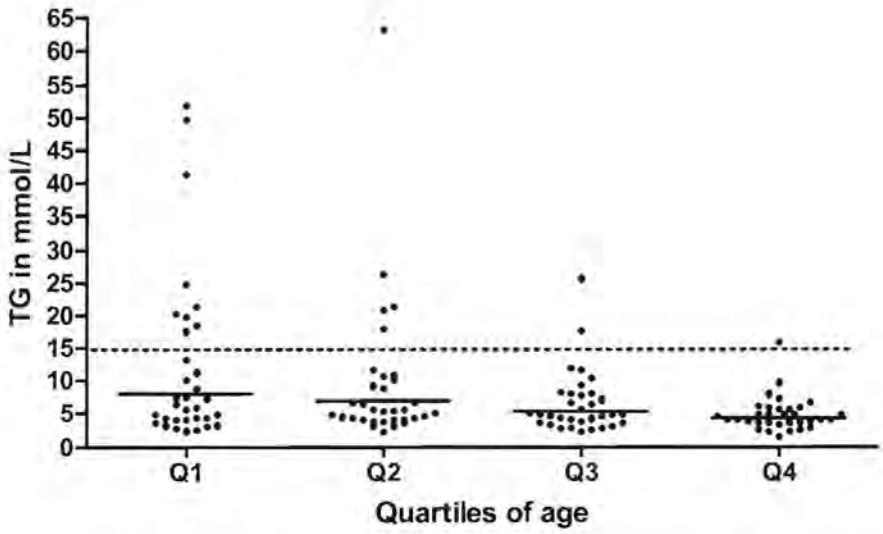
All Patients	Number	Mean	Median	SD	Range
TG	131	8.28[1]	4.90	9.39	1.6-63.20
TC	133	11.79[1]	10.42	5.04	4.8-34.30
HDLC	118	1.07[1]	1.00	0.37	0.5-2.1

[1] All values in mmol/L

3.5.1 Triglycerides

In patients with FH (253) and the general population (255) triglycerides increase with age. In dysbetalipoproteinaemic subjects hypertriglyceridaemia is often associated with cutaneous xanthomata (see below) and may lead to acute pancreatitis. There is therefore a positive bias towards earlier referral of hypertriglyceridaemic patients. This bias is confirmed by analyzing triglyceride levels at presentation by quartiles of age.

Figure 3-8 Triglycerides vs. age at presentation quartiles



ANOVA P=0.02

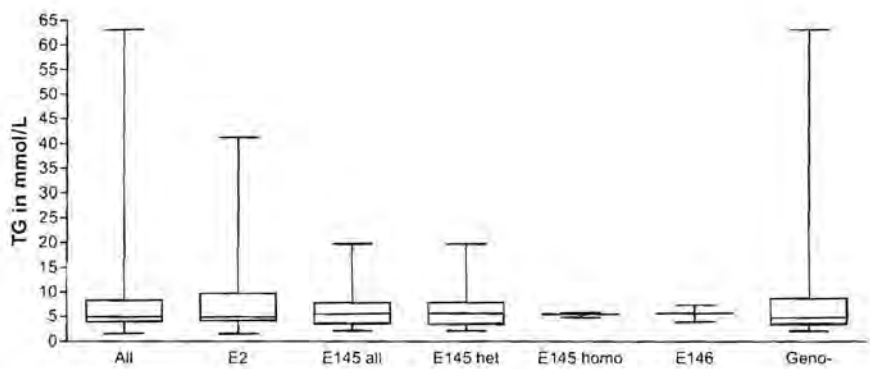
The scatter plot (—— geometric mean) shows that most patients with severe hypertriglyceridaemia (here defined as TG>15 mmol/L and indicated by the broken line) present at a younger age.

The nature of apoE mutations influences remnant metabolism differentially. TG levels may therefore differ between apoE mutants.

Table 3-12 Triglycerides by apoE mutation

Genotype			Number	Triglyceride mmol/L			
				Mean	Median	SD	Range
Geno +	E2/E2		67	4.9	7.8	7.0	1.6-41.3
	E (R145C)	All	26	6.7	5.6	4.3	2.2-19.7
		Hetero	23	6.9	5.6	4.6	2.2-19.7
		Homo	3	5.3	5.5	0.48	4.8-5.7
	E (K146Q)		2	5.7	5.7	2.5	3.9-7.4
Geno-			37	10.3	4.8	14.4	2.2-63.2

Figure 3-9 Triglycerides by apoE mutation



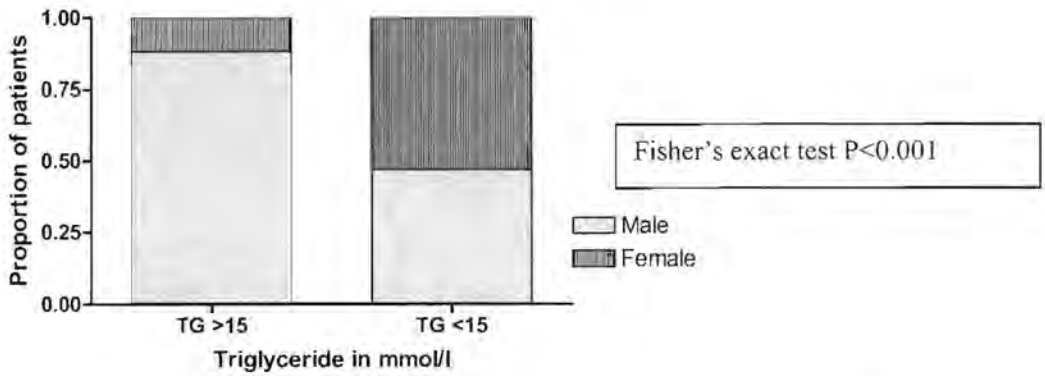
There were no significant differences in TG values at presentation between apoE2 homozygotes and heterozygous carriers of the apoE (R145C) mutation (P=0.81, unpaired t-test). Homozygosity for the apoE (R145C) mutation was not associated with higher TG than the heterozygous state (P=0.78, unpaired t-test). Patients without

an identified apoE mutation had similar TG to those in whom an apoE mutation had been identified (P=0.67, unpaired t-test). There were no significant differences in TG between males and females (geometric mean 6.45 vs. 5.55 for males vs. females; P=0.23 by unpaired t-test).

Patients with severe hypertriglyceridaemia (TG>15 mmol/L) are of particular concern because they are at high risk of acute pancreatitis. Special examination was done to determine whether any factors were particularly associated with severe hypertriglyceridaemia. There were 17 patients with severe hypertriglyceridaemia.

Diabetes (P=0.78, Fisher's exact test), genotypic status (P=0.56, Fisher's exact test), BMI (30.17 ± 5.30 vs. 28.52 ± 4.42, P=0.19 by unpaired t test for TG >15 mmol/L vs. TG <15 mmol/L) and waist circumference > NCEP Criteria (P=0.10, Fisher's exact test) were not associated with severe hypertriglyceridaemia. However, severe hypertriglyceridaemia was significantly associated with alcohol abuse (P=0.02, Fisher's exact test) and gender (P<0.001, Fisher's exact test).

Figure 3-10 Severe hypertriglyceridaemia and gender



In this cohort men were more likely than women to present with severe hypertriglyceridaemia. Although alcohol abuse was more frequent amongst males (data not shown) the absolute number of patients with severe hypertriglyceridaemia that abused alcohol was only three.

3.5.1.1 Comparison with previously published case series

The mean TG of 8.28 mmol/L in our series lies within the range of 5.7 mmol/L (256) to 8.1mmol/L (10) reported in previous studies. The association of plasma triglycerides with physical stigmata of dyslipidaemia and comorbid conditions has not been systematically reported previously.

3.5.2 Total cholesterol

The levels of total cholesterol and triglyceride are correlated (r^2=0.54 by linear regression, P<0.0001) and it is therefore not surprising that these lipids have similar associations with clinical and metabolic features.

Figure 3-11 Correlation of triglycerides and total cholesterol

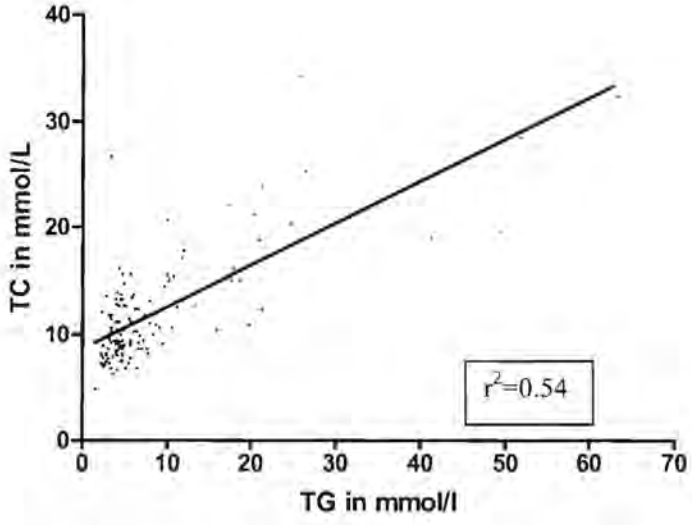


Figure 3-12 Total cholesterol vs. age at presentation quartiles

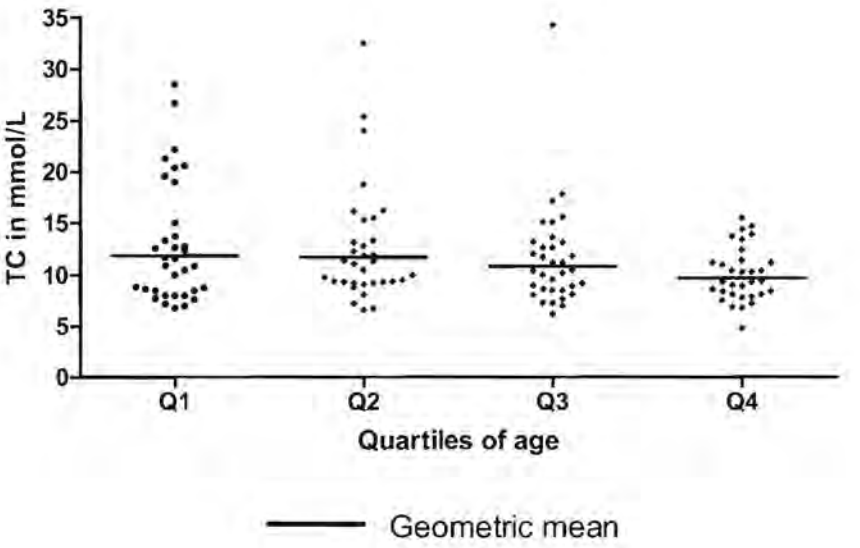
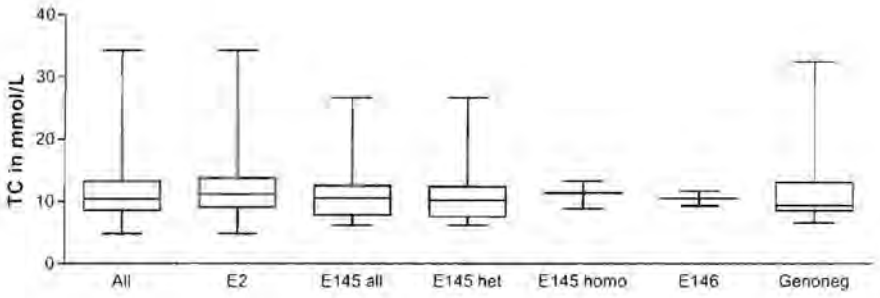


Table 3-13 Total cholesterol by apoE mutation

Genotype			Number	Total cholesterol			
				Mean	Median	SD	Range
Geno +	E2/E2		68	12.02	11.20	4.60	4.8-34.30
	E (R145C)	All	26	10.72	10.55	4.25	6.2-27.20
		Hetero	23	10.66	10.20	4.48	6.2-27.20
		Homo	3	11.18	11.40	2.23	8.84-13.30
	E (K146Q)		2	10.50	10.50	1.69	9.30-11.70
Geno-			37	12.19	9.40	6.34	6.60-32.50

Figure 3-13 Total cholesterol by apoE mutation



There were no significant differences between the total cholesterol at presentation between E2 homozygotes and heterozygous carriers of the apoE (R145C) mutation (P=0.13, unpaired t-test). Homozygosity for the apoE (R145C) mutation was not associated with higher total cholesterol than the heterozygous state (P=0.64, unpaired t-test). Patients without an identified apoE mutation had similar total cholesterol to those in whom an apoE mutation had been identified (P=0.88, unpaired t-test). There was no significant difference in total cholesterol between males and females (geometric mean 10.81 vs. 11.22, P=0.96, unpaired t-test).

3.5.2.1 Comparison with previously published case series

The mean TC of 11.79 mmol/L is similar to that reported in previous case series where the aggregate mean of 4 series (6;7;9;10) is 11.84 mmol/L. Factors influencing TC levels have not been reported on previously.

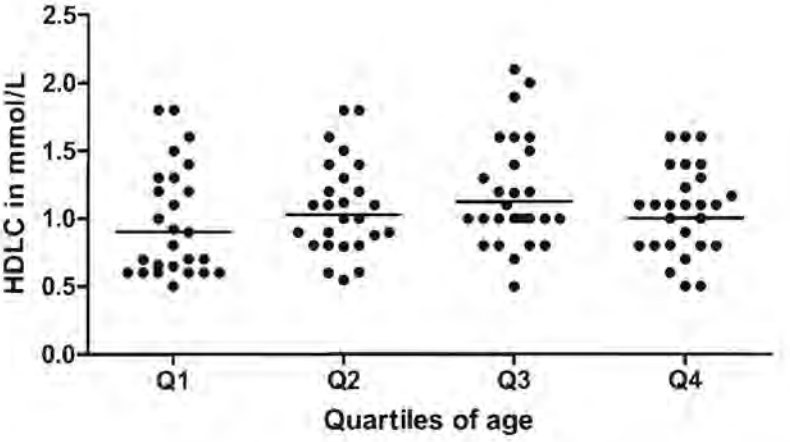
3.5.3 Ratio of total cholesterol to triglyceride

The ratio of total cholesterol to triglycerides often approaches unity when expressing lipids by mass or 2:1 if lipids are expressed in molar terms (5). In our series the mean molar ratio was 2.07 ± 1.03 (Median 1.96, Range 0.39-7.63). There were no significant differences in this ratio between any subgroups of dysbetalipoproteinaemic patients (data not shown). Because the high variability of the ratio of total cholesterol to triglyceride in dysbetalipoproteinaemia a ratio that significantly differs from 2 does not exclude the diagnosis. The mean molar ratio was 4.45 ± 3.21 (Median 3.75, Range 0.11-34.50) in the total database of 5952 lipid clinic patients. Of 38 patients with a ratio of exactly 2:1, only 2 were dysbetalipoproteinaemic.

3.5.4 HDL Cholesterol

HDLC levels are highly variable in patients with dysbetalipoproteinaemia. In our cohort the HDLC range was from 0.5-2.1 mmol/L. HDLC levels did not show a consistent association with age at presentation in our series.

Figure 3-14 HDLC vs. age at presentation quartiles



ANOVA P=0.22

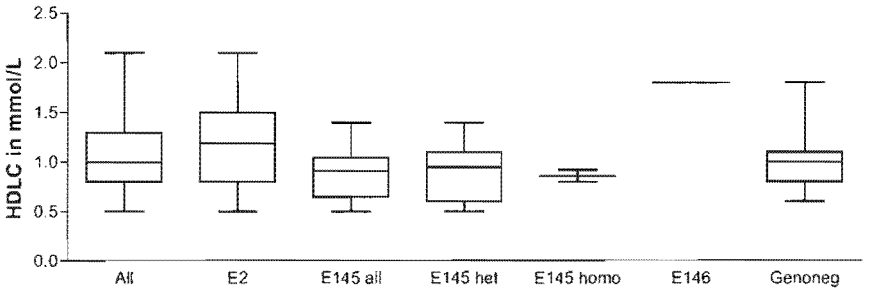
_____ (geometric mean)

The HDLC level varied significantly when analyzed by apoE mutation.

Table 3-14 HDLC by apoE mutation

Genotype			Number	HDL Cholesterol mmol/L			
				Mean	Median	SD	Range
Geno +	E2/E2		63	1.18	1.19	0.41	0.5-2.1
	E (R145C)	All	24	0.88	0.91	0.25	0.5-1.4
		Hetero	22	0.88	0.95	0.26	0.5-1.4
		Homo	2	0.86	0.86	0.08	0.8-0.92
	E (K146Q)		1		1.8		
Geno-			30	0.99	1.0	0.27	0.6-1.8

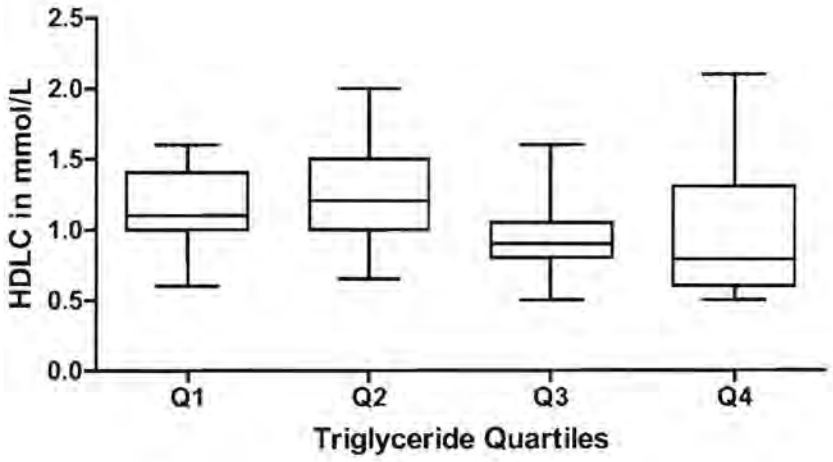
Figure 3-15 HDLC by apoE mutation



There were significant differences between the HDLC at presentation between apoE2 homozygotes and heterozygous carriers of the apoE (R145C) mutation (P=0.002, unpaired t-test). Homozygosity for the apoE (R145C) mutation was not associated with altered HDLC levels compared to the heterozygous state (P=0.91, unpaired t-test). Patients without an identified apoE mutation had similar HDLC to those in whom an apoE mutation had been identified (P=0.16, unpaired t-test). HDLC levels are generally higher in women than men. This was also true in our cohort although the difference was not statistically significant (1.03 ±0.36 mmol/L vs. 1.12 ± 0.38 mmol/L for male vs. female, P=0.15, unpaired t test).

Hypertriglyceridaemia is often associated with low levels of HDLC. In our series mean HDLC levels were highest in those patients in the lower quartiles of triglyceride levels. HDLC levels may have been overestimated in some hypertriglyceridaemic patients due to incomplete precipitation of apoB-containing lipoproteins.

Figure 3-16 HDLC by triglyceride quartiles



ANOVA (Kruskal-Wallis test) P=0.003

3.5.4.1 Comparison with previously reported case series

Not all previous case series report HDLC, but where reported, values are similar to the mean value seen in our series. There have also been significant changes in the methodology used to determine HDLC over the years and this may affect the comparability of results (257).

3.5.5 Apolipoproteins

3.5.5.1 Apolipoprotein B

Apolipoprotein B levels reflect the number of circulating apoB-containing lipoproteins. Larger lipoproteins contain proportionally less apoB and more lipid than smaller lipoproteins. Remnant lipoproteins are larger than LDL and are relatively cholesterol-enriched. In patients with equivalent elevations of TC, assuming no major differences in HDLC, one would thus expect to find lower levels of apoB in patients with remnant accumulation. The mean apoB level in our cohort was 1.24 ±0.34 g/L. Table 3.15 shows the mean apoB levels by apoE genotype.

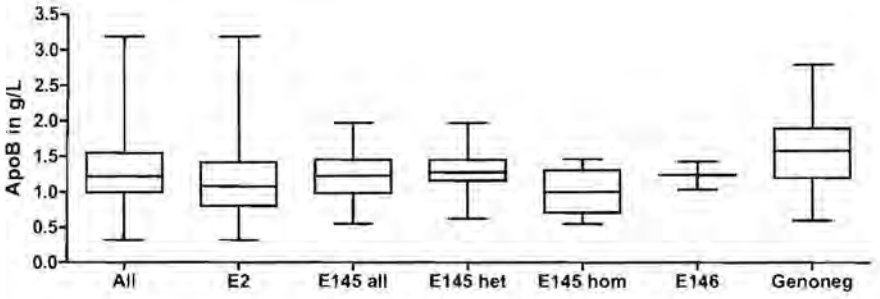
Table 3-15 ApoB (g/L) levels by apoE genotype

Genotype			Number	Apolipoprotein B (g/L)			
				Mean	Median	SD	Range
Geno +	E2/E2		58	1.15	1.08	0.48	0.32-3.19
	E (R145C)	All	23	1.21	1.22	0.36	0.55-1.97
		Hetero	19	1.25	1.27	0.34	0.62-1.97
		Homo	4	1.00	1.00	0.39	0.55-1.46
	E (K146Q)		2	1.24	1.24	0.28	1.04-1.43
Geno-			30	1.60	1.58	0.54	0.6-2.8

ANOVA for data sets apoE2/E2, apoE (R145C) heterozygotes and genonegative P=0.0003

(Tukey's post-test for E2/E2 vs. genonegative P<0.0001, apoE (R145C) vs. genonegative P<0.05)

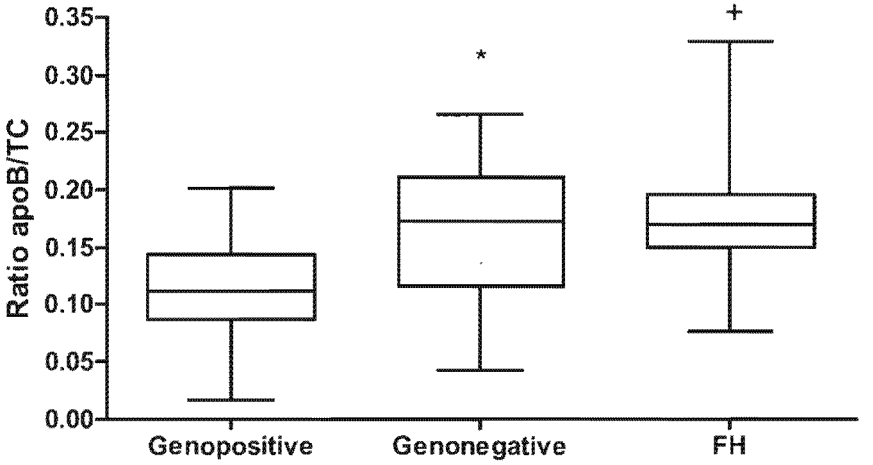
Figure 3-17 ApoB (g/L) by apoE mutation



ApoB levels were significantly higher in patients without a known apoE mutation than in apoE2 homozygotes and apoE (R145C) mutation carriers. The apoB levels did not differ significantly between apoE2 homozygotes and apoE (R145C) mutation carriers. In a cohort of 916 patients with FH seen at the GSH lipid clinic and evaluated in the same laboratory, the mean apoB level was 1.56 ± 0.39 g/L with a mean TC of 9.10 ± 1.68 mmol/L. The TC was 11.0 ± 3.96 mmol/L ($P<0.0001$, Mann-Whitney test) in the dysbetalipoproteinaemic patients for whom apoB levels were available.

The ratio of apoB/TC was significantly lower in patients with dysbetalipoproteinaemia than those with FH (0.13 ± 0.05 vs. 0.17 ± 0.04, $P<0.0001$, Mann-Whitney test for dysbetalipoproteinaemia vs. FH). On subgroup analysis patients with no known apoE mutation have significantly higher apoB/TC ratios than those with identified apoE mutations and more closely resemble patients with FH. This could be due to the concurrence of LDL hypercholesterolaemia and cholesterol-enriched VLDL in genonegative patients. A detailed ultracentrifugal analysis would be necessary to resolve this issue.

Figure 3-18 ApoB/TC ratio in dysbetalipoproteinaemia and FH



Kruskal-Wallis test P<0.0001

Post-test

* P<0.001 vs. genopositive patients

\+ P<0.001 vs. genopositive patients

The difference between genonegative and FH patients was not statistically significant

3.5.5.2 ApolipoproteinAI

ApoAI levels ranged from 0.29-2.49 g/L with a mean of 1.17 ± 0.33 g/L. ApoAI levels did not differ significantly by apoE genotype (data not shown). ApoAI levels did not differ significantly between patients with dysbetalipoproteinaemia and patients with FH (1.17 ± 0.33 g/L vs. 1.15 ± 0.32 g/L, P=0.55 by unpaired t-test for dysbetalipoproteinaemia vs. FH).

3.5.5.3 Comparison with previously published case series

Previously published case series have not reported details on apolipoprotein levels and comparisons are therefore not possible.

3.5.6 VLDL compositional analysis

VLDL compositional analysis was done at least once in 134/136 (99%) of patients. In total 258 VLDL compositional analyses were performed, with a mean number of 1.9 analyses per patient. In this section the data are confined to the results of those compositional analyses performed when patients were not receiving lipid-lowering medication. VLDL compositional analysis was performed in 98 patients in the untreated state.

3.5.6.1 Ratio of cholesterol $_{VLDL}$/triglyceride $_{VLDL}$ (CV/TV)

The ratio of CV/TV is generally elevated in patients with dysbetalipoproteinaemia, but does not always reach the proposed diagnostic threshold of 0.42 (230), particularly in patients with severe hypertriglyceridaemia. Table 3.16 lists the CV/TV ratios by genotype.

Table 3-16 CV/TV ratio by apoE genotype in untreated patients

Genotype			Number	Ratio CV/TV			
				Mean	Median	SD	Range
Geno +	E2/E2		50	0.53	0.51	0.19	0.21-1.34
	E (R145C)	All	17	0.52	0.43	0.35	0.16-1.76
		Hetero	16	0.51	0.43	0.36	0.16-1.76
		Homo	1	0.68			
	E (K146Q)		1	0.37			
Geno-			28	0.49	0.47	0.15	0.20-1.02

Note: For technical reasons this ratio could not be determined in one patient, therefore sum of all patients is only 97

The CV/TV ratio did not differ significantly by apoE genotype (data not shown).

Figure 3-19 Ratio CV/TV by apoE genotype in untreated patients

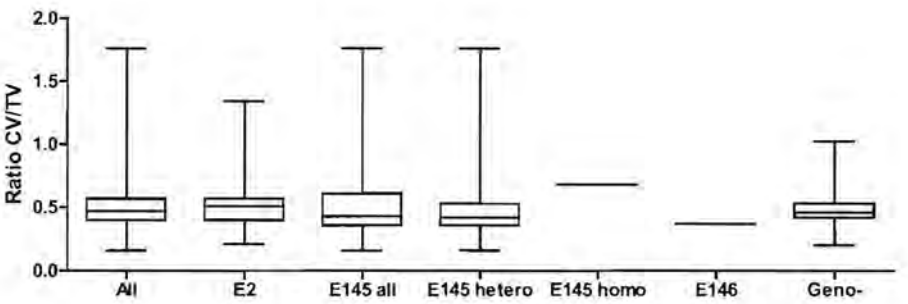


Table 3.17 illustrates in how many patients the diagnosis of dysbetalipoproteinaemia would have been made purely on phenotypic criteria using a diagnostic threshold of either 0.42 (230) or 0.35 (231) for the ratio CV/TV.

Table 3-17 Patients meeting phenotypic diagnostic criteria (CV/TV) in the untreated state

Genotype			Number	Ratio CV/TV	
				≥ 0.42	≥ 0.35
Geno +	E2/E2		50	37	46
	E (R145C)	All	17	10	12
		Hetero	16	9	11
		Homo	1	1	1
	E (K146Q)		1	0	1
Geno-			28	22	25

3.5.6.2 Ratio of cholesterol $_{VLDL}$/triglyceride $_{Plasma}$ (CV/TP)

The ratio of CV/TP ranged widely from 0.10 to 0.71. The ratio was significantly higher in apoE2 homozygotes than in patients with no identified genotype (Kruskal-Wallis test P=0.002, Dunn's multiple comparison test for apoE2 homozygotes vs. genonegative P<0.01; Columns analyzed apoE2 homozygotes, apoE (R145C) heterozygotes and genonegative).

Table 3-18 CV/TP ratio by apoE genotype in untreated patients

Genotype			Number	Ratio CV/TP			
				Mean	Median	SD	Range
Geno +	E2/E2		51	0.36	0.35	0.1	0.14-0.66
	E (R145C)	All	17	0.33	0.30	0.16	0.1-0.71
		Hetero	16	0.33	0.30	0.16	0.1-0.71
		Homo	1	0.37			
	E (K146Q)		1	0.37			
Geno-			28	0.28	0.28	0.08	0.14-0.46

Table 3.19 shows how many untreated patients reached the diagnostic threshold of 0.30 (207) or 0.25 (231) for the CV/TP ratio.

Table 3-19 Patients meeting phenotypic (CV/TP) diagnostic criteria in the untreated state

Genotype			Number	Ratio CV/TP	
				≥ 0.30	≥ 0.25
Geno +	E2/E2		51	41	44
	E (R145C)	All	17	9	10
		Hetero	16	8	10
		Homo	1	1	1
	E (K146Q)		1	1	1
Geno-			28	12	19

3.5.6.3 Comparison with previously published case series

Most of the previously published case series do not report the degree of VLDL-cholesterol enrichment found in their patients, but simply use VLDL-compositional analysis as a diagnostic criterion. Where the results are reported, they are similar to those in this cohort (10).

3.5.7 Agarose gel electrophoresis

The interpretation and classification of agarose gel electrophoresis always involves a measure of judgment and subjectivity and is therefore not fully standardizable. However, all agarose gels in this series were reviewed and classified by a single observer, eliminating intra-observer variability. Reports on agarose gel electrophoresis were available for 113 patients.

Thirty-two % of patients had agarose gel electrophoresis "typical" of dysbetalipoproteinaemia, i.e. broad-beta bands. Amongst genopositive patients 41% of patients had a broad-beta band. In an additional 27% of patients the suspicion of dysbetalipoproteinaemia was raised on review of agarose gel electrophoresis, but the pattern observed was not entirely characteristic. By way of example, patients with severe hypertriglyceridaemia at presentation and staining of chylomicrons at the origin in addition to staining of the ß and pre-ß areas were classified as having a Type V pattern. If lipoproteins stained relatively uniformly in the ß and pre-ß areas without a clear discernible shoulder, underlying dysbetalipoproteinaemia was suspected.

Table 3-20 Fredrickson classification of agarose gel electrophoresis

Fredrickson	N [1]	%
I	0	0
IIa	2	2
IIb	28	20
III	36	32
IV	2	2
V	9	8
III suspected [2]	31	27
Not classified [3]	1	1
Other [4]	4	4

[1] Number of patients

[2] Broad-beta band suspected, but electrophoretogram not entirely characteristic, see table 3.21 below for more details

[3] Gels not classifiable (technically inadequate)

[4] This group includes patients studied on lipid-lowering treatment with normal electrophoresis

Table 3-21 Final Fredrickson classification of agarose gels suggestive of dysbetalipoproteinaemia

Final Fredrickson classification	N
IIb	18
IV	6
V	7

Of the 36 patients with typical broad-beta bands on agarose gel electrophoresis 32 were genopositive (Fisher's exact test P=0.004 comparing the prevalence of typical broad-beta band by genotypic status). In patients without a typical broad-beta band the most common pattern was Type IIb (38/113), followed by Type V (15/113) and Type IV (8/113).

As is to be expected mean TG levels were highest in the patients with Type V patterns and lowest in those with IIa patterns (data not shown). Patients with a typical broad-beta band had significantly higher mean TG levels than those patients ultimately classified as having a Type IIb pattern with the suspicion of an underling broad-beta band (TG for typical Type III pattern 8.54 ± 10.24 mmol/ vs. 4.74 ± 3.59 mmol/L for patients classified as Type IIb pattern with suspicion of underlying broad-beta band; P=0.0007 by Mann Whitney test). TG levels did not differ significantly between patients with Type IIb patterns, whether an underlying broad-beta band was suspected or not (TG for typical Type IIb 4.49 ± 1.78 mmol/L vs. 4.74 ± 3.59 mmol/L for patients with possible underlying broad-beta band; P=0.43 by Mann-Whitney test). TC levels were higher in patients with typical broad-beta bands than those classified as having an IIb pattern with possible underlying dysbetalipoproteinaemia, but the

difference was not statistically significant (TC for typical Type III pattern 12.05 ± 5.75 mmol/L vs. 9.58 ± 2.38 mmol/L for patients classified as Type IIb pattern with suspicion of underlying broad-beta band; P=0.058 by Mann Whitney test). Having more cholesterol-enriched VLDL or physical stigmata of dysbetalipoproteinaemia (palmar crease xanthoma, cutaneous or tendinous xanthomata) was not associated with a higher prevalence of agarose gel electrophoretograms characteristic of dysbetalipoproteinaemia (data not shown).

There were 8477 records of agarose gel electrophoreses in the clinic database at the time that the dysbetalipoproteinaemia database was closed. Of these 78 had been classified as Fredrickson Type III without a diagnosis of dysbetalipoproteinaemia having been made subsequently. Not all patients had continued follow-up at the lipid clinic, and therefore special investigations for dysbetalipoproteinaemia were not completed in all patients. Of the 78 patients apoE genotypes were available for 65.

Table 3-22 ApoE genotypes in patients with Fredrickson Type III pattern who were not diagnosed with dysbetalipoproteinaemia

ApoE genotype	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
N	8	4	31	20	2

54 patients had been evaluated for the apoE (R145C) mutation and all were negative. VLDL compositional analysis had been undertaken on 51 occasions in 34 patients. None of the ratios were considered diagnostic for dysbetalipoproteinaemia. Although not all patients with Type III patterns on agarose gel electrophoresis were extensively

worked-up for dysbetalipoproteinaemia this finding does indicate that agarose gel electrophoresis is not entirely specific for dysbetalipoproteinaemia.

3.5.8 Polyacrylamide gradient gel electrophoresis

Non-denaturing PGGE is performed routinely on all patients attending the lipid clinic for the first time. Currently the total experience is of about 26000 lanes of PGGE run over the last fifteen years. PGGE separates lipoproteins by size and the gradient chosen in the UCT lipid laboratory retains only apoB-containing lipoproteins within the gel. Dysbetalipoproteinaemia is characterized by the accumulation of remnant lipoproteins and low levels of LDL. Separating lipoproteins by size one would therefore expect to see little or no LDL-sized material but an excess of larger lipoproteins of IDL and VLDL2 size. The dysbetalipoproteinaemic phenotype is clearly highly variable and in cases where lipid metabolism is severely deranged - especially when TG levels are markedly elevated - chylomicron and VLDL1-sized lipoproteins may also accumulate. The abnormal distribution of lipoproteins by size in dysbetalipoproteinaemia had already been observed by Shepherd in 1975 using agarose column chromatography (228).

The observation that many patients with dysbetalipoproteinaemia had an unusual PGGE profile led to further investigation. It was found that a pattern characterized by accumulation of IDL-sized lipoproteins and the absence of LDL-sized lipoproteins was highly specific (95%) and moderately sensitive (72%) for the diagnosis of dysbetalipoproteinaemia (227;258). The control group for this study was made up of patients with mixed hyperlipidaemia in whom dysbetalipoproteinaemia had been

excluded by VLDL compositional analysis and genotyping for the common apoE isoforms and locally prevalent dominant apoE mutation. Analysis of the area under the curve (AUC) of densitometric tracings further improved diagnostic accuracy. A ratio of AUC of more than 0.5 for IDL/LDL area was 100% specific and 89% sensitive for the diagnosis of dysbetalipoproteinaemia.

PGGE patterns were divided into four arbitrarily named categories:

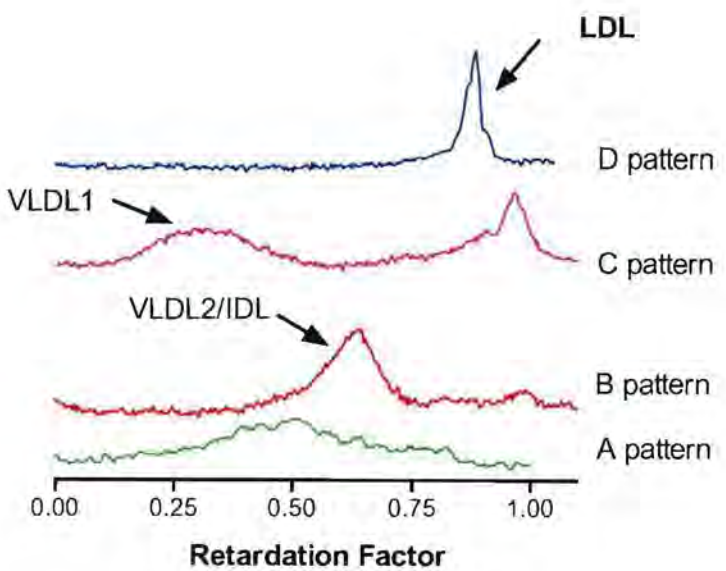
A=VLDL and IDL only (Rf 0.45-0.85)

B=VLDL and IDL, faint staining in LDL region (Rf 0.85-1.0)

C=VLDL and IDL, prominent staining in LDL region (Rf 0.85-1.0)

D=LDL only (Rf 0.85-1.0)

Figure 3-20 Densitometric scans of PGGE patterns



Note: The relative OD scales have been adjusted for the various tracings to emphasize TGRL.

Figure 3-21 PGGE patterns

Note: The photograph above is a composite of several gels. It has been electronically altered to increase contrast and sharpness, but no other changes have been made to the images. The densitometric tracings in Figure 3.20 are not those of the lanes above.

The publication of the above findings described pattern A in 72% of untreated patients with genopositive dysbetalipoproteinaemia. Pattern B was found in 20% of subjects, while 8% had pattern C. None of the patients studied in the untreated state had pattern D (258). If treated patients are included in the cohort as many as 8% may have pattern D. PGGE patterns may therefore undergo substantial change in individual patients as hyperlipidaemia is reversed. The effect of lipid-lowering treatment on PGGE patterns

will be discussed in greater detail in Chapter 4 in the context of the controlled conditions of a clinical trial. The next section reports on the experience with PGGE in the full cohort of dysbetalipoproteinaemic patients.

3.5.8.1 PGGE patterns

PGGE reports were available for 135 of the patients. About half of all patients had a PGGE pattern considered highly characteristic of dysbetalipoproteinaemia (A pattern). In 69% of patients the PGGE pattern was very suggestive of dysbetalipoproteinaemia (A or B pattern).

Table 3-23 PGGE reports in dysbetalipoproteinaemia

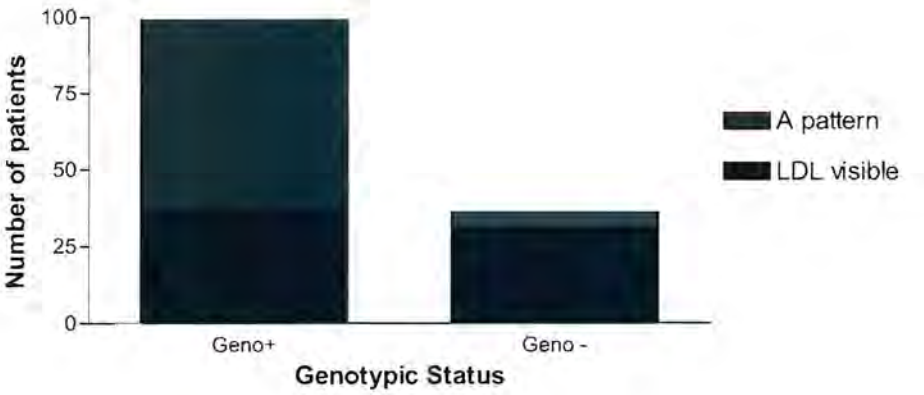
Pattern	A	B	C	D
Number	67	26	31	11
Percentage of total	50	19	23	8

Table 3-24 PGGE patterns by apoE genotype

Genotype			Number	PGGE pattern			
				A	B	C	D
Geno +	E2/E2		69	54	8	1	6
	E (R145C)	All	28	8	9	10	1
		Hetero	24	7	7	9	1
		Homo	4	1	2	1	0
	E (K146Q)		2	0	0	2	0
Geno-			36	5	9	18	4

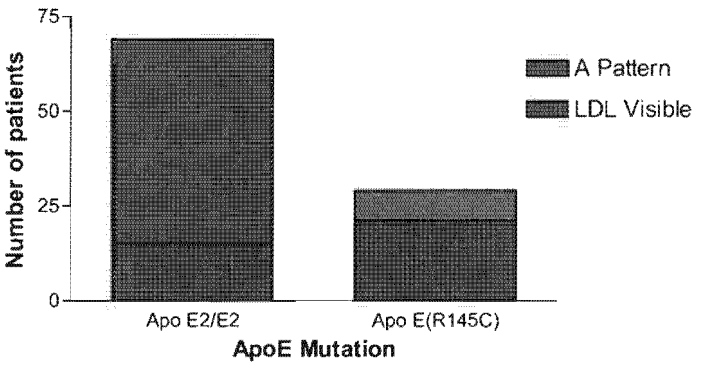
Genopositive patients were significantly more likely than genonegative patients to have an A pattern with no LDL-sized lipoproteins visible on staining ($P<0.0001$ by Fisher's exact test).

Figure 3-22 PGGE with no visible LDL by apoE genotypic status



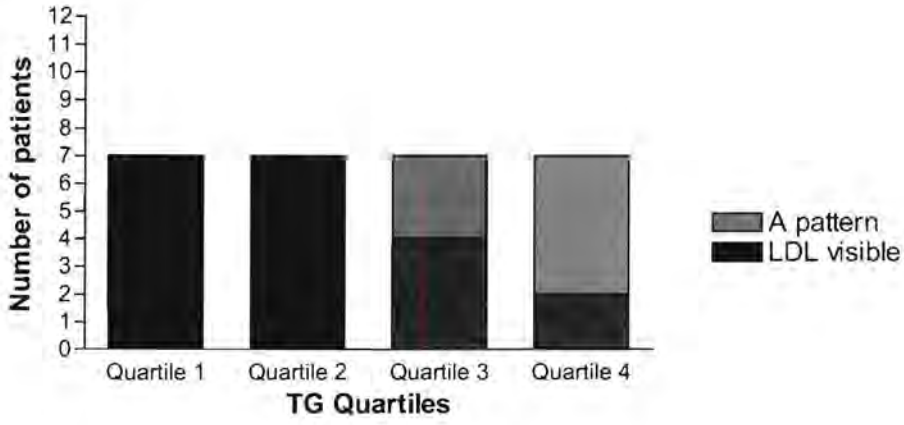
PGGE patterns also differed according to the underlying apoE mutation. Patients with the apoE (R145C) mutation were significantly more likely to have visible LDL-sized lipoprotein particles than apoE2 homozygotes (P<0.0001 by Fisher's exact test).

Figure 3-23 PGGE patterns by apoE mutation



In patients with the apoE (R145C) mutation LDL-sized particles were seen more frequently in patients with TG levels in the lower two quartiles (P=0.005 by Chi-square test). In apoE2 homozygotes TG levels (quartiles) did not have a statistically significant relationship to the presence of visible LDL-sized lipoproteins (P=0.24 by Chi-square test).

Figure 3-24 PGGE patterns by TG quartiles in patients with the apoE (R145C) mutation



All laboratory records of PGGE were reviewed for reports that would be highly suggestive of a diagnosis of dysbetalipoproteinaemia. There were 26026 PGGE reports in total. There were 333 reports of patients with visible staining only in the range of VLDL and IDL-sized particles with no staining of LDL-sized particles. Of these 333 reports, 112 referred to patients that had not been included in the dysbetalipoproteinaemia database.

Table 3-25 Origin of specimens with PGGE reports suggesting dysbetalipoproteinaemia

Source	Details	N
Epidemiological or screening study	ART in children [1]	4
	Coronary care [2]	4
	PCOS [3]	4
	Population screen [4]	5
	NAFLD [5]	6
	PET [6]	15
	Other [7]	11
Referred by other labs		5
Lipid clinic [8]		47
Origin unclear		11

[1] Specimens from a study investigating the effects of antiretroviral therapy (ART) on the lipid profile of HIV-infected children. No clinical data available.

[2] Specimens received from the coronary care unit. No clinical data available.

[3] Specimens from a study investigating lipid abnormalities in patients with polycystic ovarian syndrome (PCOS). No clinical data available.

[4] Specimens received as part of an epidemiological survey (259). No clinical data available.

[5] Specimens received and processed during the course of a study investigating non-alcoholic fatty liver disease (NAFLD). No clinical data available.

[6] Specimens from a study examining lipid abnormalities in patients with preeclampsia. DNA was available for these patients and 5 patients were homozygous for apoE2 and the apoE (R145C) mutation was detected in two. Unfortunately no clinical details are available.

[7] Specimens from various other sources.

[8] Specimens that had banked DNA. Most, but not all, of the patients with banked DNA would have been seen as patients at the lipid clinic.

On the 47 specimens linked to the lipid clinic by DNA numbers the following data were available:

Table 3-26 Genotypic and phenotypic results in patients with PGGE patterns suggestive of dysbetalipoproteinaemia

ApoE Isoform					
E2/E2	E2/E3	E3/3	E3/4	E4/E4	NT [1]
9	7	1	14	5	11

ApoE (R145C) mutation [2]		
POS	NEG	NT
1	21	25

VLDL compositional analysis		
POS	NEG	NT
1	18	28

[1] NT= Not tested

[2] Patients with apoE2 homozygosity were not routinely tested for this mutation

There were 11 patients in who no further specialized investigations to diagnose dysbetalipoproteinaemia had been requested. These patients were mainly seen in the first few years following the establishment of the clinic. The clinic's interest in dysbetalipoproteinaemia was not fully developed at that stage and there was less experience in the interpretation of PGGE. Ten of the 47 (21%) patients were found to have apoE genotypes compatible with the diagnosis of dysbetalipoproteinaemia. These patients were not included in the dysbetalipoproteinaemic cohort studied here because they had been diagnosed after the database had been locked (6), their records could not be retrieved (1) or samples had been sent for PGGE and DNA analysis, but the patients had in fact not been seen at the lipid clinic (3).

Thirteen patients had been investigated extensively for dysbetalipoproteinaemia. These patients had all had apoE genotyping to determine the common apoE isoforms, exclusion of the apoE (R145C) mutation and VLDL compositional analysis. In many patients the VLDL compositional analysis had been repeated on several occasions. The diagnosis of dysbetalipoproteinaemia could not be confirmed in these patients.

It is conceivable that many of the patients with PGGE suggestive of dysbetalipoproteinaemia but without clinical details may well have had this disorder. ART, particularly if PI are used, is known to precipitate dyslipidaemia and dysbetalipoproteinaemia may well occur in genetically susceptible individuals (260;261). Patients in coronary care units have manifest atherosclerosis and this population may therefore also be enriched for dysbetalipoproteinaemia. PCOS is commonly associated with obesity and insulin resistance (262), both factors that may precipitate dysbetalipoproteinaemia. Similar considerations apply to patients with NAFLD (263). In the population survey about 1000 people were screened (259). A rate of dysbetalipoproteinaemia of 5:1000 is higher than previously reported (1), but it must be considered that lipid levels were not known for these patients and that some samples may have originated from individuals with normal or even low lipid values. The high rate of PGGE patterns suggestive of dysbetalipoproteinaemia in patients with preeclampsia is of interest. In a recent antenatal study in Zimbabwe using the same PGGE method as described here, 30 of 660 (4.5%) participants had a pattern highly suggestive of dysbetalipoproteinaemia. Of these 30 subjects 14 had apoE mutations (8 apoE (R145C); 6 apoE2/E2). The dysbetalipoproteinaemia pattern only persisted in two subjects in the postnatal state, strongly suggesting that pregnancy is a sufficient metabolic stressor in genetically predisposed subjects to tilt lipid metabolism towards remnant accumulation (121). Pathology laboratories in the private sector often referred samples with "unusual" lipid values to our laboratory. A common reason for referral was marked discrepancy between the directly measured LDLC and the LDLC expected by Friedewald calculation. This is the case in dysbetalipoproteinaemia.

Although an A pattern on PGGE is not entirely specific for dysbetalipoproteinaemia, PGGE remains a very useful screening investigation. The pattern was uncommon (333/26026 or 1.3%) and was positively associated with dysbetalipoproteinaemia in 238 instances. In the 95 instances where dysbetalipoproteinaemia was suspected but could not be proven clinical information was not available and further investigations could not be done in 61 cases. Many of these patients had clinical backgrounds that are positively associated with the development of dysbetalipoproteinaemia.

3.5.8.1.1 LDL particle sizes by PGGE

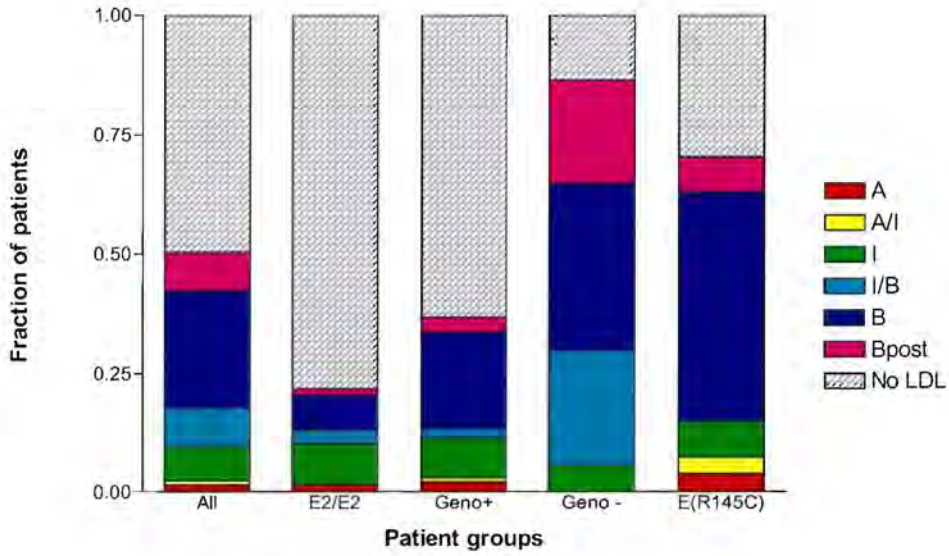
LDL species size, when studied in non-dysbetalipoproteinaemic subjects, is influenced by many variables (264). Hypertriglyceridaemia is strongly associated with small dense LDL particles (265). Other variables such as activity of plasma lipases, cholesterol ester transfer protein, oestrogen levels (266) and activity of plasminogen activator inhibitor-1 (267) also influence LDL particle size. LDL particle size is further influenced by genetic factors that interact in a complex fashion with environmental and hormonal influences (268;269). From an epidemiological point of view age, male sex, obesity, hypertension, diabetes and the metabolic syndrome are associated with an increased prevalence of small dense LDL particles (270).

There is no published data on LDL species size in dysbetalipoproteinaemic patients. This is not surprising as low levels of LDL and accumulation of remnants of TGRL characterize this disorder. The little LDL formed in dysbetalipoproteinaemia is likely to be small and dense as there are high levels of circulating remnant lipoproteins that clear slowly. TGRL may exchange TG with LDL and subsequent delipidation of LDL

leads to the formation of small dense LDL. Conversely apoE2 does not activate HL as well as apoE3 (171;172) and low HL levels are associated with larger LDL species.

In those patients of our cohort with visible LDL-sized particles the particle size, determined relative to the two control LDL species - A (large) and B (small) - loaded on every gel, was predominantly, but not exclusively, small.

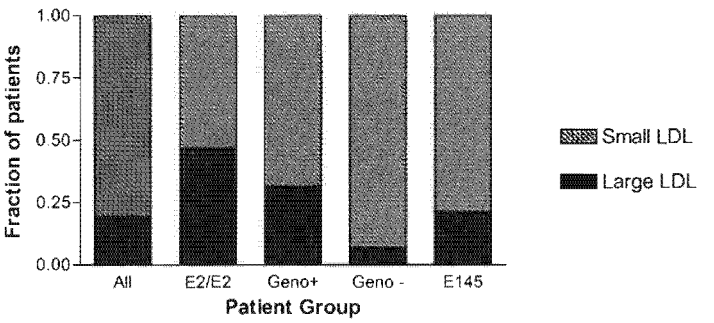
Figure 3-25 LDL particle sizes by PGGE (proportional representation)



Note: Capital letters in this instance only indicate LDL-size and do not refer to the intensity of staining (see PGGE terminology for more details)

Lipoproteins that migrated further than I (i.e. I/B, B and Bpost) were classified as small LDL species. All other LDL species were classified as large.

Figure 3-26 LDL species in dysbetalipoproteinaemic patients with LDL-sized lipoproteins (proportional representation)



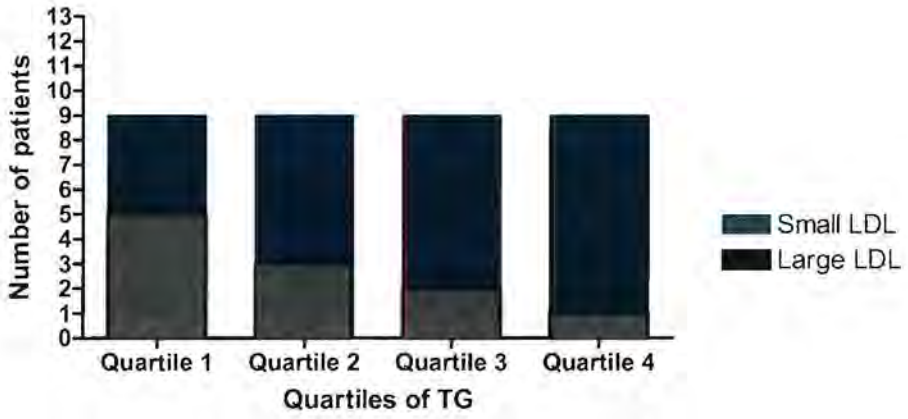
Although the majority of dysbetalipoproteinaemic patients have small dense LDL, the prevalence of small dense LDL is much higher in patients without identified apoE mutations than those with known apoE mutations (P=0.006, Fischer's exact test). Patients with apoE (R145C) mutations also had a higher prevalence of small LDL species than apoE2 homozygotes but the difference was not statistically significant (P=0.28, Fischer's exact test). Patients without identified apoE mutations are very unlikely to have large LDL particles.

Table 3-27 LDL size by apoE genotype

		Large LDL	Small LDL	Fisher's exact test
Genotype	Geno+	12	22	0.006
	Geno-	2	29	
ApoE mutation	E2/E2	7	8	0.28
	E (R145C)	5	15	

As the vast majority of patients without identified apoE mutations had small dense LDL it was not feasible to investigate the effect of hypertriglyceridaemia on LDL size in this subgroup. In genopositive patients the proportion of patients with large LDL species declined progressively in the higher quartiles of TG levels, but the change was not statistically significant (P=0.21 by Chi-square test with 9 patients in each quartile).

Figure 3-27 LDL species size in genopositive patients by quartiles of TG values



Griffin (271) has previously suggested that 1.5 mmol/L is the TG threshold above which increasing levels are strongly positively associated with the presence of small dense LDL. It is therefore not surprising that the majority of patients with visible LDL sized lipoproteins had small dense LDL. Of interest is that large LDL species were found in a few patients despite the presence of multiple factors associated with the formation of small dense LDL. This phenomenon was also observed in a few patients with FH (253) and remains as yet unexplained. Speculatively, differences in HL and CETP activity and altered remodelling of LDL may account for these differences.

3.6 Physical stigmata of dyslipidaemia

3.6.1 Palmar crease xanthomata

Palmar crease xanthomata (sometimes also called *xanthoma striata palmaris*) are characteristic yellow deposits of lipid in the palmar and digital creases. In rare cases the feet may be involved in a similar fashion. Palmar crease xanthomata are usually planar but may on occasions contain small nodules. The lipid clinic experience of palmar crease xanthoma is that they are usually not symptomatic and patients are often unaware of them. They are usually bilateral, but unilateral involvement of the dominant hand has been seen. Typically palmar crease xanthomata resolve completely once hyperlipidaemia has been adequately controlled. The pathophysiology of palmar crease xanthomata is not well understood or researched, but as these lesions are pathognomonic of dysbetalipoproteinaemia (5) it seems reasonable to assume that remnant lipoproteins differ from other lipoproteins in their ability to extravasate in defined anatomical microcirculations and induce xanthoma formation. Lesions similar to palmar crease xanthoma may be seen in patients with high concentrations of lipoprotein X (LpX). With LpX the infiltration of the palmar creases often lacks the characteristic yellow/orange colour seen in dysbetalipoproteinaemia, as carotene is poorly soluble in the aqueous core of LpX.

Figure 3-28 Hand with palmar crease xanthomata

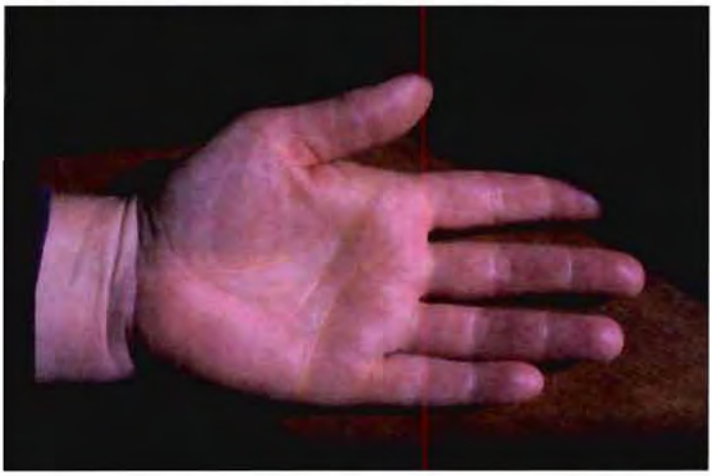


Figure 3-29 Palmar crease infiltration

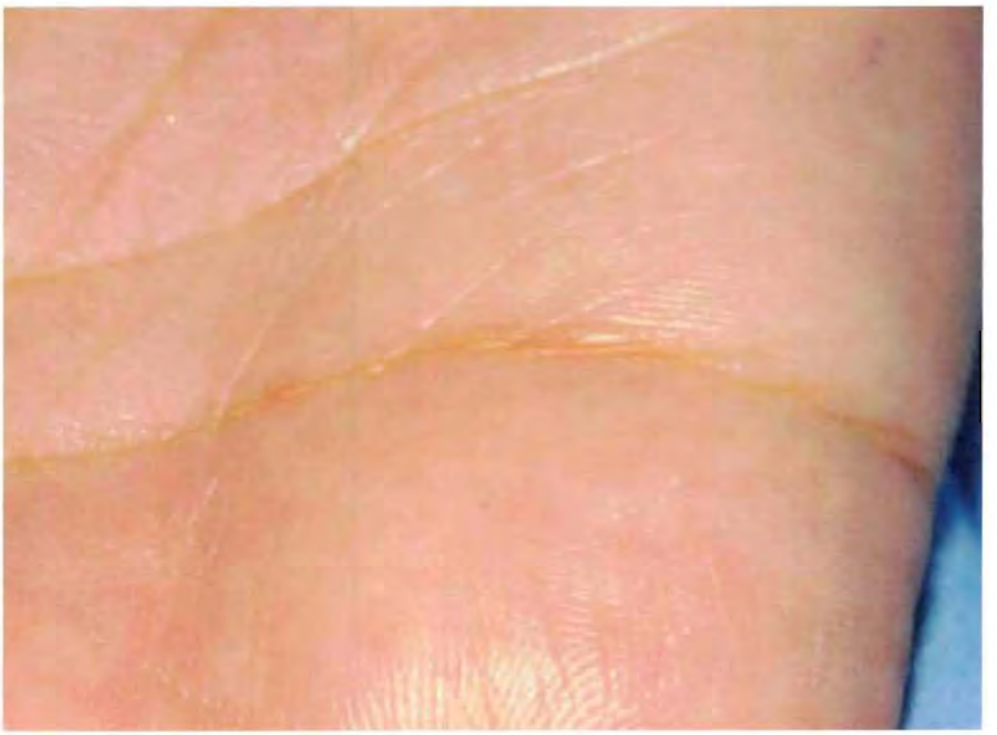


Figure 3-30 Digital crease infiltration

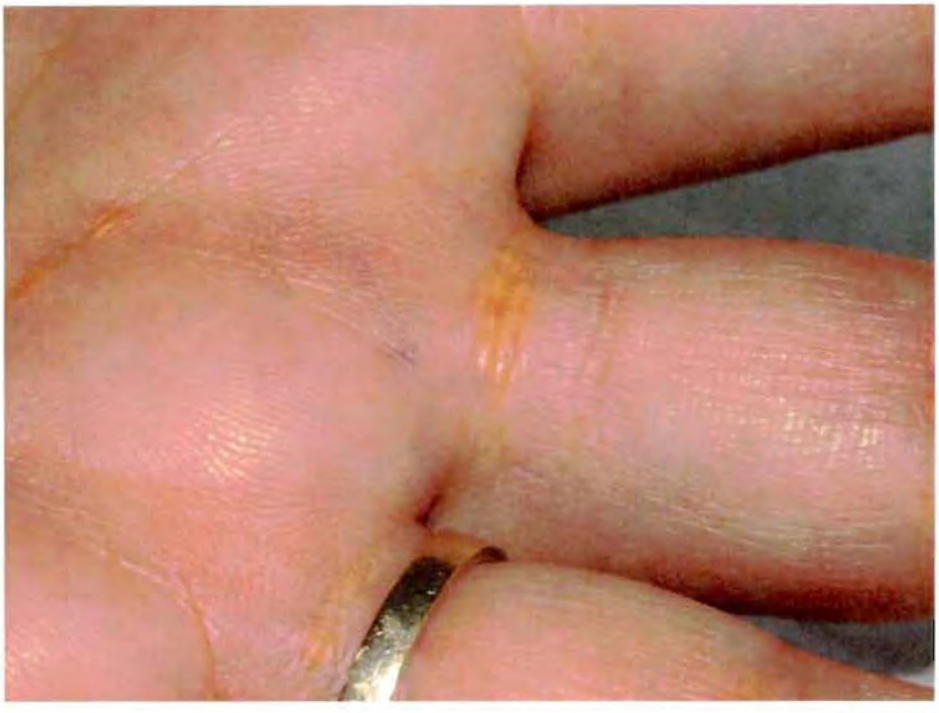


Figure 3-31 Infiltration of toe crease

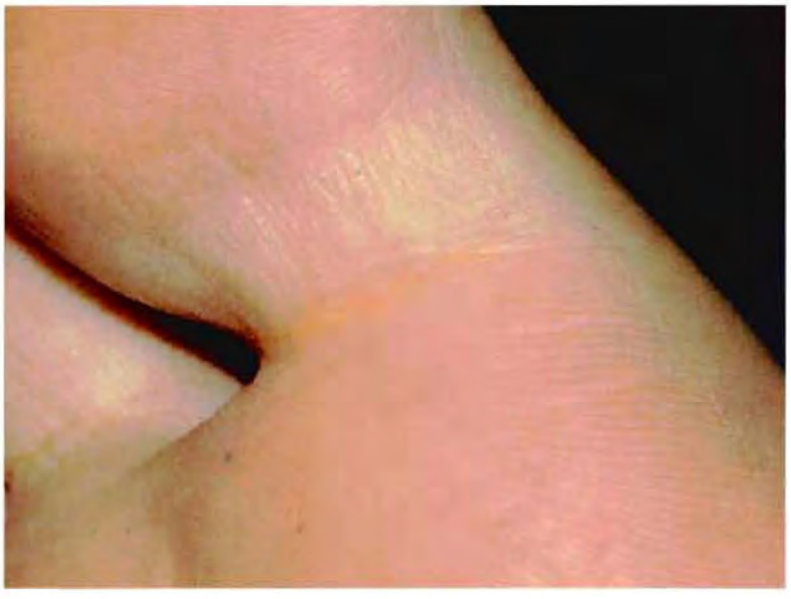


Figure 3-32 Palmar crease infiltration with LpX

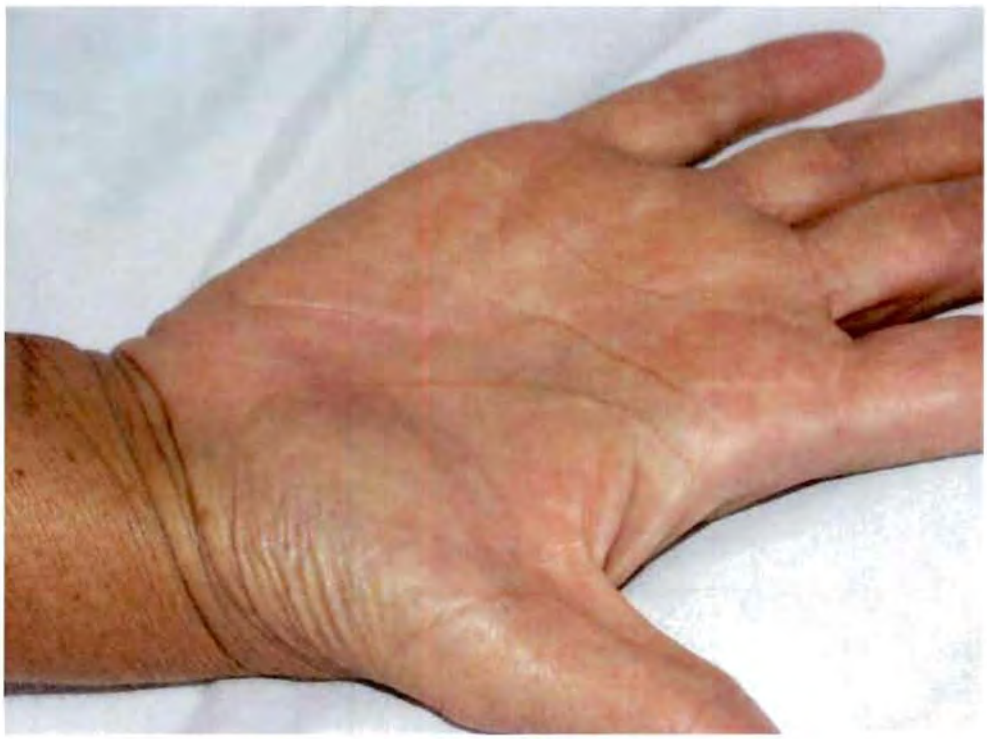
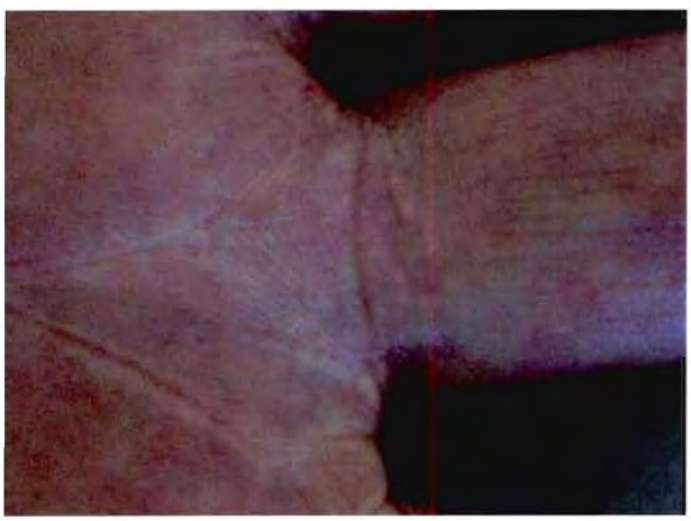


Figure 3-33 Close-up of palmar crease with LpX



Information on palmar crease xanthomata was available in 134 patients and palmar crease xanthomata were found in 34 patients (25%). Palmar crease xanthomata were not associated with a younger age at presentation (see above). Palmar crease xanthomata were significantly more common in patients homozygous for apoE2 than in carries of the apoE (R145C) mutation or patients with no identified apoE mutation (P<0.0001, Chi-square test). Only one of the genonegative patients had palmar crease xanthomata.

Table 3-28 Palmar crease xanthoma by apoE genotype

Genotype			Palmar crease xanthomata	
			Present	Absent
Geno +	E2/E2		28	39
	E (R145C)	All	4	22
		Hetero	3	19
		Homo	1	3
	E (K146Q)		1	1
Geno-			1	35

Palmar crease xanthomata were found in 33% of female patients and 23% of males but this difference was not statistically significant (P=0.07, Fisher's exact test). The presence of palmar crease xanthomata was associated with higher levels of total cholesterol and VLDL that was more cholesterol-enriched.

Table 3-29 Palmar crease xanthomata and lipid values

Lipid value	Palmar crease xanthomata		P value [1]
	Present	Absent	
TG	8.06 ± 10.02	8.49 ± 7.02	0.25
TC	13.55 ± 5.83	11.19 ± 4.63	0.02
HDLC	1.06 ± 0.35	1.15 ± 0.43	0.22
Ratio CV/TV [2]	0.55 ± 0.13	0.48 ± 0.12	0.01
Ratio CV/TP [3]	0.36 ± 0.09	0.31 ± 0.09	0.005

[1] Unpaired t-test, skewed data was log transformed before analysis

[2] Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$

[3] Cholesterol $_{VLDL}$/ Triglyceride $_{Plasma}$

The finding that palmar crease xanthomata are associated with VLDL that is highly cholesterol enriched lends some support to the speculation that the size, composition and nature of remnant particles determine their propensity towards forming palmar crease xanthomata.

3.6.1.1 Comparison with previously published case series

The reported prevalence of palmar crease xanthomata is highly dependant on the initial criteria used to diagnose dysbetalipoproteinaemia. Thus it is not surprising that Borrie (6) reports palmar crease xanthomata in 13/18 (72%) of patients seen at a

dermatology clinic. Similarly Morganroth (7) reports palmar crease xanthomata in 64% of patients seen at the NIH. Feussner (10) found palmar crease xanthomata in 39% of their less highly selected sample. Overall the lipid clinic experience is of palmar crease xanthomata in only 25% of patients, but the occurrence of palmar crease xanthomata was strongly linked to the apoE genotype (see above). The prevalence in apoE2 homozygotes of 28/67 (42%) is very similar to that reported by Feussner.

3.6.2 Eruptive and tuboeruptive xanthomata

Eruptive and tuboeruptive xanthomata are not characteristic of dysbetalipoproteinaemia but may occur whenever there is severe hypertriglyceridaemia. Eruptive xanthomata are usually the first cutaneous manifestation of severe hypertriglyceridaemia, while tuboeruptive xanthomata are generally associated with hyperlipidaemia of longstanding duration. These two types of cutaneous xanthomata are considered together here as the distinction between eruptive and tuboeruptive xanthomata in practice is somewhat subjective and the clinical records do not allow for retrospective classification. Although these xanthomata are usually asymptomatic, they generally are dramatic enough to persuade patients to seek medical advice.

Figure 3-34 Eruptive xanthomata on the trunk and buttocks

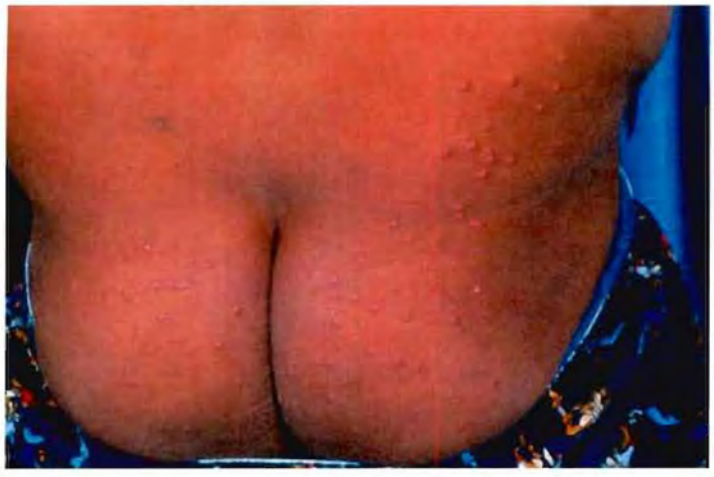


Figure 3-35 Tuboeruptive xanthomata at the elbow

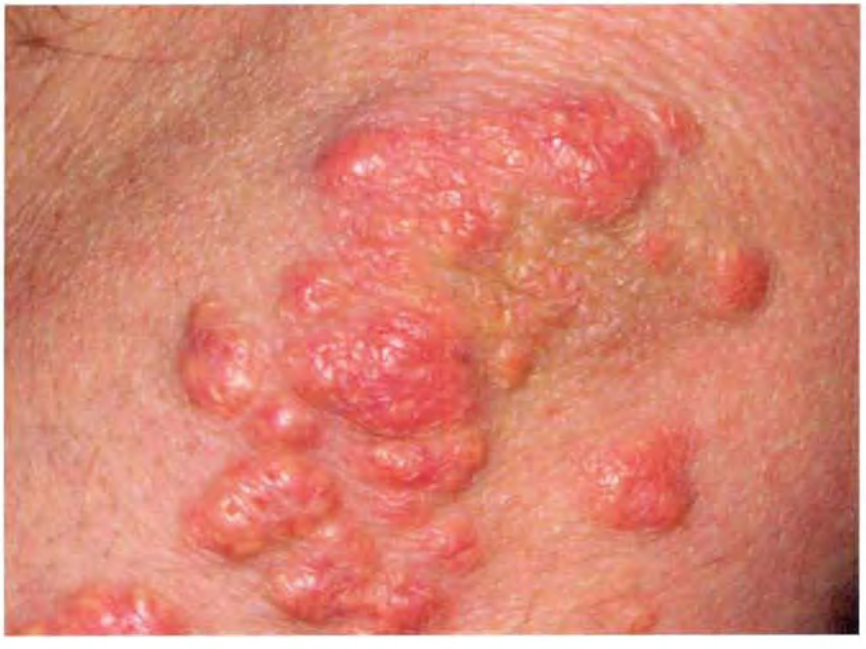
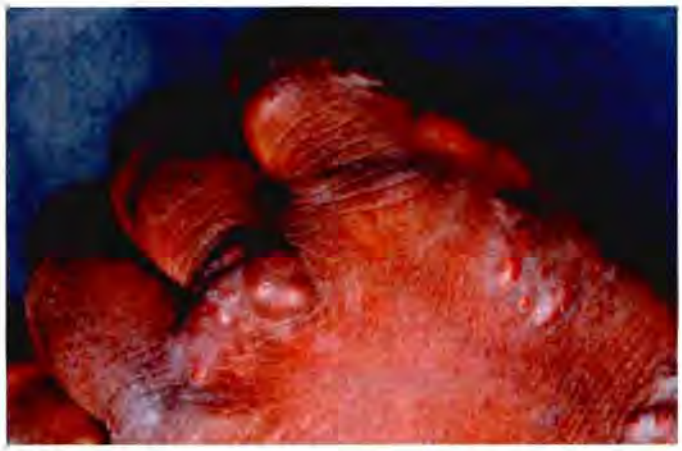


Figure 3-36 Tuboeruptive xanthomata in an African patient



Eruptive or tuboeruptive xanthomata were found in 30 of 133 (23%) of patients. As previously shown the presence of these xanthomata was associated with a significantly younger age at presentation. The highest incidence of eruptive/tuboeruptive xanthomata was found in patients homozygous for apoE2.

Table 3-30 Eruptive/tuboeruptive xanthomata by genotype

Genotype			Eruptive/tuboeruptive xanthomata	
			Present	Absent
Geno +	E2/E2		20	48
	E (R145C)	All	5	22
		Hetero	2	20
		Homo	3	1
	E (K146Q)		1	1
Geno-			4	32

Chi-square P=0.008

Eruptive/tuboeruptive xanthomata were found in 20% of female patients and 24% of males (P=0.54, Fisher's exact test).

Table 3-31 Eruptive/tuboeruptive xanthomata and lipid values

Lipid value	Eruptive/tuboeruptive xanthomata		P value [1]
	Present	Absent	
TG	14.30 ± 11.80	6.58 ± 7.89	<0.0001
TC	16.01 ± 6.54	10.64 ± 3.90	<0.001
HDLC	1.13 ± 0.52	1.07 ± 0.33	0.94
Ratio CV/TV [2]	0.54 ± 0.27	0.50 ± 0.13	0.20
Ratio CV/TP [3]	0.36 ± 0.12	0.32 ± 0.08	0.01

[1] Unpaired t-test, skewed data were log transformed before analysis

[2] Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$

[3] Cholesterol $_{VLDL}$/Triglyceride $_{Plasma}$

In our series the finding of eruptive/tuboeruptive xanthomata was generally associated with severe hyperlipidaemia. VLDL cholesterol-enrichment was also higher in patients with such xanthomata, but the difference was only statistically significant for the ratio CV/TP.

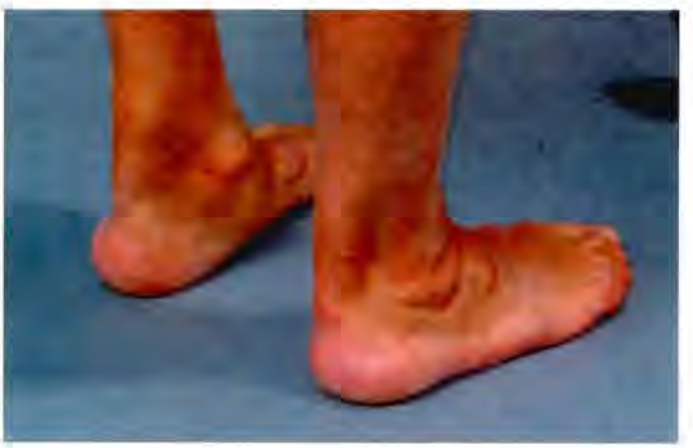
3.6.2.1 Comparison with previously published case series

Similar to palmar crease xanthomata, the reported prevalence of eruptive/tuboeruptive xanthomata is highly variable and higher in the older studies. The prevalence of 29% in apoE2 homozygotes is higher than the 11% recorded by Feussner (10), but lower than that reported in all the other series.

3.6.3 Tendinous xanthomata

Tendinous xanthomata are firm subcutaneous nodules attached to or forming an integral part of the tendon. Tendinous xanthomata always indicate serious errors of lipoprotein metabolism. They are seen most commonly in patients with FH but also occur in patients with cerebrotendinous xanthomatosis, phytosterolaemia and dysbetalipoproteinaemia. Tendinous xanthomata most commonly involve the Achilles tendon followed by the extensor tendons of the hand.

Figure 3-37 Achilles tendon xanthomata in a patient with FH



In our series of dysbetalipoproteinaemic patients all tendinous xanthomata were located in the Achilles tendons. Seventeen % (23/134) of patients had tendinous xanthomata at presentation. Tendinous xanthomata were most prevalent amongst apoE2 homozygotes.

Table 3-32 Tendinous xanthomata by genotype

Genotype			Tendinous xanthomata	
			Present	Absent
Geno +	E2/E2		17	52
	E (R145C)	All	1	25
		Hetero	0	23
		Homo	1	3
	E (K146Q)		1	1
Geno-			4	32

Chi-square test P=0.04

There was no significant difference (P=0.65, Fisher's exact test) in the prevalence of tendinous xanthomata between males (19%) and females (15%). None of the lipid values analyzed was predictive of the presence of tendinous xanthomata, although there may be a trend for a higher TC in those with tendinous xanthomata.

Table 3-33 Tendinous xanthomata and lipid values

Lipid value	Tendinous xanthomata		P value [1]
	Present	Absent	
TG	5.45 ± 3.29	8.87 ± 10.17	0.11
TC	13.31 ± 5.89	11.44 ± 4.82	0.08
HDLC	1.14 ± 0.50	1.07 ± 0.34	0.91
Ratio CV/TV [2]	0.49 ± 0.11	0.51 ± 0.18	0.86
Ratio CV/TP [3]	0.35 ± 0.07	0.32 ± 0.10	0.15

[1] Unpaired t-test, skewed data were log transformed before analysis

[2] Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$

[3] Cholesterol $_{VLDL}$/ Triglyceride $_{Plasma}$

3.6.3.1 Comparison with previously published case series

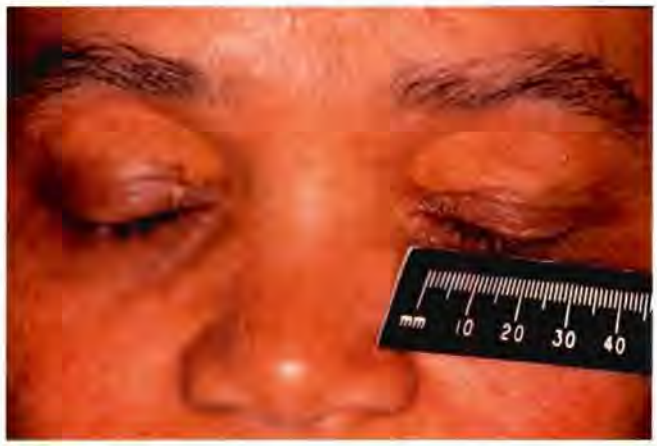
The prevalence of tendinous xanthomata in previously published case series is highly variable. Feussner (10) and Stuyt (9) respectively report tendinous xanthomata in only 1% and 3% of their study populations. Morganroth (7) found tendon xanthomata in 23% of patients in his series. This is similar to the 17% prevalence in the current series and the 25% prevalence in apoE2 homozygotes. The large variability in the prevalence of tendon xanthomata cannot be explained easily, especially the large difference observed between this series and that of Feussner (10). Tendon xanthomata are more difficult to detect than cutaneous xanthomata and when the tendon

abnormalities are subtle clinicians do not always agree on the clinical significance of these abnormalities.

3.6.4 Xanthelasmata

Xanthelasmata are soft yellow-orange papules or plaques found around the eyes and lids. Their prevalence increases with age and is generally higher in women than men. They may occur in normolipidaemic individuals but are often associated with subtle derangements of lipid profiles such as higher VLDL and lower HDL levels (272). Xanthelasmata are not useful in the differential diagnosis of lipid disorders and generally only alert the clinician to the need for performing lipid analysis.

Figure 3-38 Xanthelasmata



Xanthelasmata were found in 8% (11/133) of patients. No difference in the occurrence of xanthelasmata was observed between the various apoE genotypes. Xanthelasmata were significantly (P=0.02, Fisher's exact test) more common in female patients (17%) than in male patients (3%).

Table 3-34 Xanthelasmata by genotype

Genotype			Xanthelasmata	
			Present	Absent
Geno +	E2/E2		7	61
	E (R145C)	All	2	25
		Hetero	2	21
		Homo	0	4
	E (K146Q)		1	1
Geno-			1	35

Chi-square test P=0.14

In this series of patients there were no significant differences in lipid values between patients with and without xanthelasmata. However, there were only 11 patients with xanthelasmata, limiting the statistical power of this analysis.

Table 3-35 Xanthelasmata and lipid values

Lipid value	Xanthelasmata		P value [1]
	Present	Absent	
TG	6.44 ± 2.53	8.33 ± 9.71	0.97
TC	11.82 ± 2.63	11.73 ± 5.20	0.59
HDLC	1.18 ± 0.43	1.07 ± 0.36	0.41
Ratio CV/TV [2]	0.53 ± 0.12	0.51 ± 0.18	0.44
Ratio CV/TP [3]	0.34 ± 0.07	0.32 ± 0.10	0.53

[1] Unpaired t-test, skewed data were log transformed before analysis

[2] Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$

[3] Cholesterol $_{VLDL}$/ Triglyceride $_{Plasma}$

3.6.4.1 Comparison with previously published case series

The prevalence of xanthelasmata is generally low, at 3-6 % (9;10), in previously published series. The 8% prevalence in this series is in accord with this finding.

3.6.5 Arcus cornealis

Arcus cornealis is an opaque (white) arc or circle seen at the periphery of the cornea. In its early stages it may not be circumferential as it generally develops superiorly and inferiorly before becoming circumferential. Age is the dominant influence on the prevalence of arcus cornealis, with the prevalence approaching 100% at the age of 80 years (273). Arcus cornealis is generally not useful in the differential diagnosis of lipid disorders. Its main utility is in alerting the physician to the possible presence of hyperlipidaemia if found in a young (<40 years of age) subject. In this series there was a progressive rise in the prevalence of arcus cornealis with increasing age (see above).

Figure 3-39 Arcus cornealis in a patient with dysbetalipoproteinaemia

Arcus cornealis was found in 21% (28/132) patients. The prevalence of arcus cornealis was not affected by apoE mutations.

Table 3-36 Arcus cornealis by genotype

Genotype			Arcus cornealis	
			Present	Absent
Geno +	E2/E2		15	52
	E (R145C)	All	4	23
		Hetero	4	19
		Homo	0	4
	E (K146Q)		0	2
Geno-			9	27

Chi-square test	P=0.61

The prevalence of arcus cornealis was 22% in males and 24% in females (P=0.83, Fisher's exact test). In this small group of patients no changes in the prevalence of arcus cornealis according to lipid values at initial presentation was demonstrable.

Table 3-37 Arcus cornealis and lipid values

Lipid value	Arcus cornealis		P value [1]
	Present	Absent	
TG	6.33 ± 4.96	8.67 ± 10.16	0.32
TC	10.91 ± 5.41	11.96 ± 4.93	0.18
HDLC	1.09 ± 0.38	1.08 ± 0.37	0.92
Ratio CV/TV [2]	0.51 ± 0.10	0.51 ± 0.19	0.67
Ratio CV/TP [3]	0.32 ± 0.09	0.33 ± 0.09	0.93

[1] Unpaired t-test, skewed data were log transformed before analysis

[2] Cholesterol $_{VLDL}$/Triglyceride $_{VLDL}$

[3] Cholesterol $_{VLDL}$/ Triglyceride $_{Plasma}$

3.6.5.1 Comparison with previously published case series

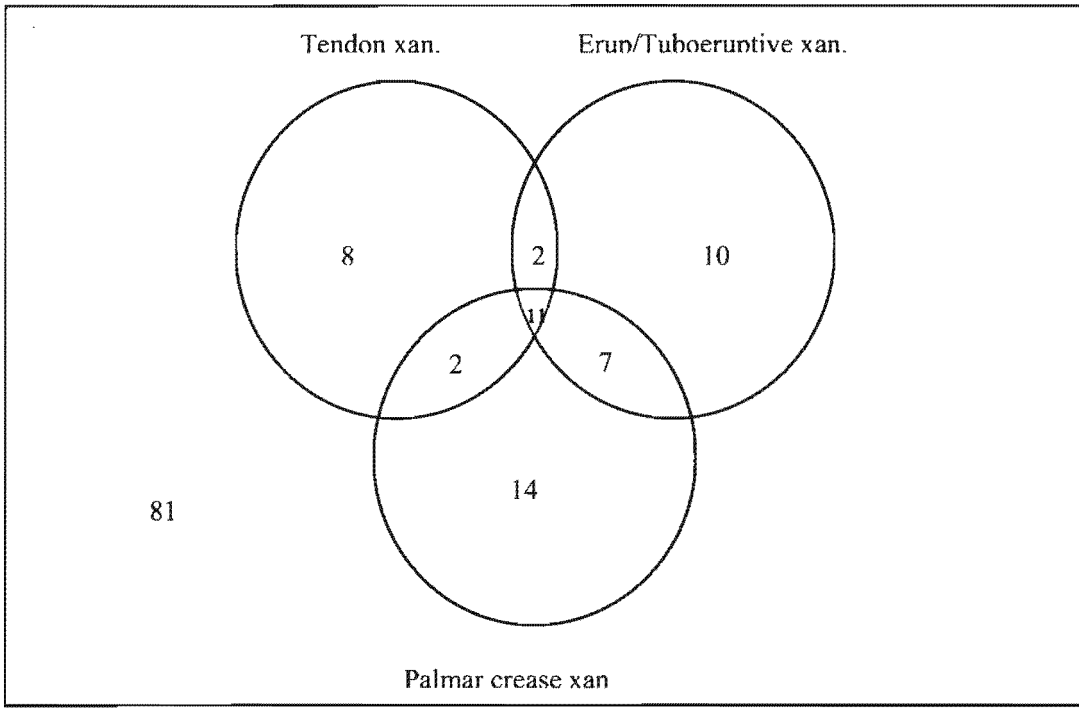
Arcus cornealis is not a useful physical sign in the differential diagnosis of dyslipidaemias and increases in prevalence with age. Information on its prevalence is therefore not included in all cases series. Where reported the prevalence ranges from

1.6-11% (6;7;10). A higher prevalence of arcus cornealis was found in this series (21%), but a higher mean age of our cohort may be partially explanatory

3.6.6 Clustering of physical signs of dyslipidaemia

The physical signs of dyslipidaemia cluster in patients. In this analysis only those physical signs indicative of severe disturbances in lipoprotein metabolism have been considered. There were 54 patients with tendinous, eruptive/tuboeruptive or palmar crease xanthomata and 22 patients had at least two of these signs, while all three signs were found in 11 patients.

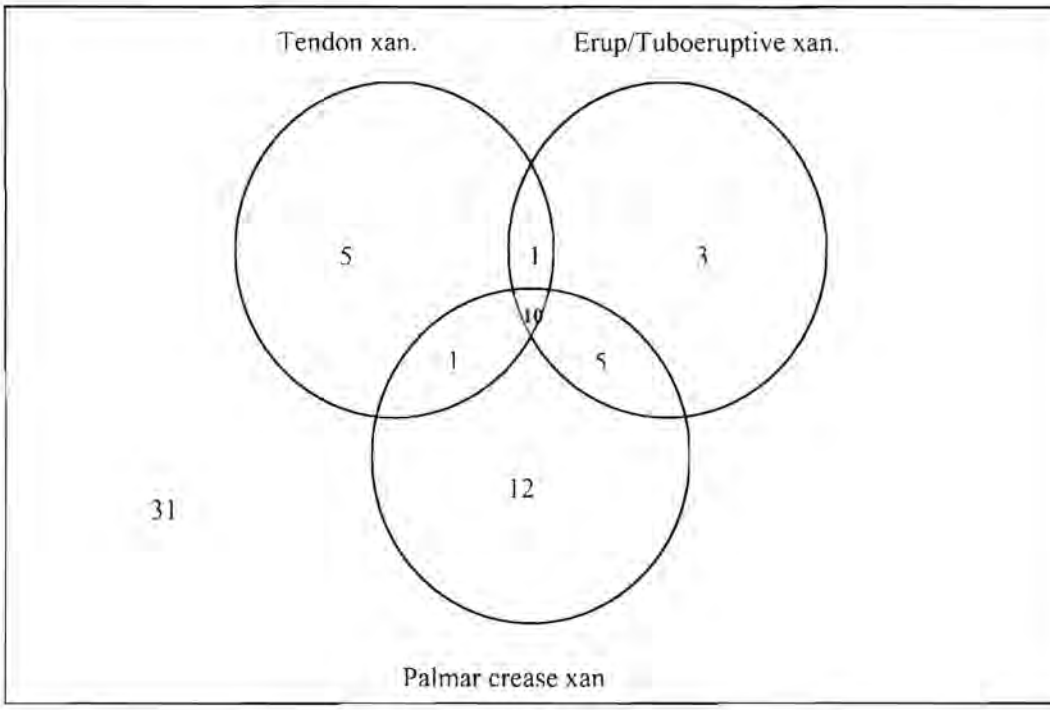
Figure 3-40 Clustering of physical signs in complete cohort



The numbers indicate the number of patients with the physical sign.

Most of the physical signs of dyslipidaemia were found in patients homozygous for apoE2 and in this cohort 37/68 (54%) had at least one physical sign.

Figure 3-41 Clustering of physical signs in apoE2 homozygotes



3.7 Atherosclerotic complications

All clinical records were reviewed to gather data on atherosclerotic complications. Data were collected on atherosclerotic complications present at diagnosis as well as those that set in during follow-up at the clinic. The duration of follow-up at the clinic was very variable and consequently the total atherosclerotic burden in this population cannot be estimated accurately. The approximate age of the patient when the atherosclerotic complication set in was calculated. Comparisons were made with FH patients attending the same clinic (253).

Ischaemic heart disease (IHD) was defined as a history of myocardial infarction, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) or a convincing clinical history of angina pectoris. PVD was defined by a history of surgery for PVD (amputation, bypass surgery), angiographic intervention or by a convincing history of peripheral claudication. Cerebrovascular disease (CerVD) was defined as a history of a previous stroke or transient ischaemic attack (TIA).

3.7.1 Ischaemic Heart Disease

At presentation 48/135 (36%) patients had IHD and during follow-up another 11 developed IHD. Therefore 59/135 (44%) of patients are known to have developed IHD. As previously stated, this figure likely underestimates the true burden of IHD due to incomplete follow-up.

The apoE genotype did not influence IHD status either at presentation or at final follow-up (data not shown). The prevalence of IHD did not differ between males and females (32% vs. 39%, P=0.47; Fisher's exact test for male vs. female).

Table 3.38 illustrates the prevalence of IHD in relationship to other cardiovascular risk factors such as smoking, diabetes and hypertension.

Table 3-38 IHD and other cardiovascular risk factors

		IHD		P Value [1]
		Present	Absent	
Diabetes	Present	24	12	0.0007
	Absent	31	63	
Hypertension	Present	42	32	0.0009
	Absent	17	45	
Smoking [2]	Present	33	40	0.47
	Absent	20	33	

Note: Complete data was not available on all risk factors for all patients; therefore the number of patients with IHD is not always equal

[1] Fisher's exact test

[2] Smoking is defined as any history of smoking

Diabetes and hypertension were strongly predictive of IHD, but in this analysis smoking was not associated with IHD. The total number of pack years smoked at the time of presentation was derived by multiplying the number of years smoked by the average number of cigarettes smoked per day and dividing by twenty. There was no difference in smoking exposure between patients with and without IHD (mean pack years 26.8 ± 11.6 vs. 26.8 ± 17.5, P=0.61 by Mann-Whitney test for IHD vs. no IHD patients). Increased IHD prevalence was also not found in the top quartile of nicotine exposure (data not shown).

The mean age at which IHD became clinically overt was 52.2 ± 9.5 (range 32-75) years. The mean age of onset of IHD was not significantly influenced by apoE genotype (data not shown). IHD was, however, diagnosed at a significantly younger age in males than females (49.4 ± 10.4 vs.56.6 ± 7.6, P=0.004 unpaired t-test for male vs. female). Table 3.39 shows the mean ages at which IHD was diagnosed in the presence of other cardiovascular risk factors.

Table 3-39 Age at diagnosis of IHD in the presence of other cardiovascular risk factors

	Age at diagnosis of IHD		P Value
	Risk Factor		
	Present	Absent	
Diabetes	52.8 ± 8.3	52.7 ± 10.8	0.98
Hypertension	54.3 ± 8.6	48.4 ± 10.6	0.03
Smoking	50.0 ± 9.4	57.3 ± 8.8	0.007

In patients who had never smoked the diagnosis of IHD was made on average seven years later than in smokers. Diabetes increased the prevalence of IHD but did not further accelerate its presentation. Hypertension was somewhat paradoxically associated with more advanced age at the time IHD was diagnosed.

3.7.1.1 Comparison with previously published case series

In a cohort of patients with FH attending the same clinic the prevalence of IHD at presentation was 43%. The mean age at onset of IHD was 43 years for males and 49 years for females (253). Although patients with FH were on average 6 years younger when IHD set in, they would have been hyperlipidaemic from birth while dysbetalipoproteinaemic patients would only have become hyperlipidaemic following a fairly prolonged period of normo- or even hypolipidaemia.

The prevalence of IHD cannot be compared directly across several case series as diagnostic criteria may vary and are not always disclosed. Additionally referral patterns to lipid clinics may introduce significant selection bias. The final prevalence of 44% for IHD in this cohort is the highest reported as yet, but does include prolonged follow-up in many patients. The prevalence of diabetes is also higher in this series than that reported in most other series (see below). This may have contributed to the higher prevalence of IHD together with a positive selection bias towards IHD in referrals to the GSH lipid clinic.

Morganroth (7) reports a difference of 11.6 years in the mean age of onset for IHD between males (38.1) and females (49.7). This series documents earlier onset in males (49.4) than females (56.0) but does not find the same degree of prematurity of IHD. Stuyt (9) analyzed risk factors for IHD in 39 patients and only higher levels of total cholesterol and smoking were predictive of IHD. The small number of hypertensive (21%) and diabetic patients (3%) in Stuyt's study did not allow for adequate assessment of the impact of these risk factors.

3.7.2 Peripheral vascular disease

At their first evaluation 37/134 (28%) of patients had evidence of PVD. During follow-up PVD was diagnosed in one additional patient.

The apoE genotype did not influence the prevalence of PVD either at presentation or at final follow-up (data not shown). The prevalence of PVD did not differ between males and females (23% vs. 30%, P=0.43; Fisher's exact test for male vs. female).

Table 3.40 illustrates the prevalence of PVD in relationship to other cardiovascular risk factors.

Table 3-40 PVD and other cardiovascular risk factors

		PVD		P Value [1]
		Present	Absent	
Diabetes	Present	21	24	0.001
	Absent	17	74	
Hypertension	Present	30	44	0.0005
	Absent	8	54	
Smoking [2]	Present	24	49	0.10
	Absent	10	43	

Note: Complete data was not available on all risk factors for all patients; therefore the number of patients with PVD is not always equal

[1] Fisher's exact test

[2] Smoking is defined here as any history of smoking

As seen with the analysis of IHD prevalence diabetes and hypertension were strongly associated with a diagnosis of PVD. The estimated total smoking exposure did not differ between patients with and without PVD (28.6 vs. 25.9 pack years, P=0.47 by unpaired t-test for PVD vs. no PVD).

The mean age at which PVD was diagnosed was 56.42 ± 7.87 years with a range from 36 to 70 years. The mean age of diagnosis of PVD was not influenced by apoE genotype (data not shown) or gender (56.53 ± 9.3 vs. 55.75, P=0.77 by unpaired t-test for males vs. females).

Table 3-41 Age of diagnosis of PVD in the presence of other cardiovascular risk factors

	Age at diagnosis of PVD		P Value
	Risk Factor		
	Present	Absent	
Diabetes	55.0 ± 8.5	57.9 ± 8.2	0.29
Hypertension	56.8 ± 8.8	56.4 ± 4.7	0.49
Smoking	57.0 ± 6.5	55 ± 12.2	0.54

None of the cardiovascular risk factors examined was associated with an earlier diagnosis of PVD in this cohort, although diabetes and hypertension were strong

predictors of a diagnosis of PVD. The number of patients with diagnosed PVD was smaller than those with IHD. IHD diagnosis generally tended to predate the diagnosis of PVD.

3.7.2.1 Comparison with previously published case series

The incidence of PVD is very high in dysbetalipoproteinaemia when compared to other genetic dyslipidaemias. In patients with FH attending the GSH lipid clinic the prevalence of PVD, using the same definition, was only 4% (253) compared with 28% in dysbetalipoproteinaemic patients. The two most recently published case series (9;10) report a higher prevalence of PVD than IHD with ratios of 1.81:1 (10) and 1.21:1 (9). The two earlier series (6;7) report ratios of 0.78:1 (7) and 0.5:1 (6). The ratio of 0.77:1 in this series is very similar to that reported by Morganroth (7). Both later series also report a high absolute prevalence of PVD (31.1 and 46%). Diagnostic criteria for PVD are not disclosed in all papers and where physical findings such as absent or reduced pulses are used to diagnose PVD significant observer bias may be introduced. Interestingly, this series did not find a significant difference in age of onset of PVD between males and females for PVD, contrary to the findings in IHD and Morganroth's paper (7).

3.7.3 Cerebrovascular disease

There were only 6/134 (4%) patients with clinical evidence of CerVD. Four patients had a history of a cerebrovascular accident and TIA's had been diagnosed in 2. Three patients were apoE2 homozygotes, 2 carried the apoE (R145C) mutation and in one no apoE mutation had been identified. There were 4 males and 2 females with CerVD.

The mean age at diagnosis was 56.9 ± 10.6 with a range of 38-68 years. Two patients were diabetic and all patients were hypertensive. The small number of patients with CerVD precludes formal assessment of the influence of other cardiovascular risk factors.

3.7.3.1 Comparison with previously published case series

In all previous studies CerVD is the least common atherosclerotic complication of dysbetalipoproteinaemia. The reported prevalence ranges from 0-11% but the series with the highest reported prevalence included data on ultrasonographically detected carotid artery stenosis in the definition of CerVD.

3.8 Pancreatitis

Severe hypertriglyceridaemia is a well-known precipitant of acute pancreatitis. The pathogenesis of hypertriglyceridaemic pancreatitis is imperfectly understood. The level of hypertriglyceridaemia required to trigger an episode of acute pancreatitis can vary markedly between patients and even for an individual patient. In the GSH lipid clinic the experience is that hypertriglyceridaemia of less than 15 mmol/L is seldom associated with acute pancreatitis (274;275).

Two of the patients included in this case series presented to the lipid clinic following episodes of acute pancreatitis. Pancreatitis was attributed to hypertriglyceridaemia in both patients as there was no history of alcohol abuse and no gallstones were visible ultrasonographically.

Patient 1: This was a 50 year old man seen following an episode of acute pancreatitis. Unfortunately TG values at the time of pancreatitis were not available, but the TG was still 5.5 mmol/L after being kept nil per mouth for several days in hospital. Original laboratory records could not be traced to see whether lipaemia had been commented upon at any stage. ApoE2 homozygosity was subsequently documented, but no further follow-up information is available.

Patient 2: This 34 year old lady presented following an episode of severe pancreatitis requiring prolonged hospital admission. The TG level was 7.5 mmol/L following several days of fasting. The patient had eruptive and tuboeruptive xanthomata suggesting prolonged severe hypertriglyceridaemia. Type II DM had been diagnosed several months prior to admission. No mutations in apoE could be identified. Several further episodes of acute pancreatitis have occurred subsequently resulting in the formation of a pancreatic pseudocyst. During times of non-compliance with lipid-lowering therapy the TG value has been as high as 30 mmol/L.

Although acute pancreatitis is an unusual complication of dysbetalipoproteinaemia, patients with severe hypertriglyceridaemia must be made aware of this risk. Immediate institution of a very low fat diet (<10g/day of triglycerides) for several days helps to rapidly lower triglycerides. Any factors such as uncontrolled diabetes, alcohol abuse or medications that may be exacerbating the hypertriglyceridaemia should be modified as far as possible. If the clinician is not aware that several days of

fasting may drop TG levels substantially, the diagnosis of hypertriglyceridaemic pancreatitis may be dismissed inappropriately if only moderately elevated TG levels are found.

3.9 Disorders precipitating dysbetalipoproteinaemia

Dysbetalipoproteinaemia is generally not evident at birth and often only becomes clinically overt in the presence of "metabolic stressors". ApoE mutations differ in their requirement for metabolic cofactors. In the GSH lipid clinic series 33% of patients had identifiable medical disorders, excluding obesity, which may have precipitated dyslipidaemia. Including obesity, defined either by BMI>30 kg/m^2 or a waist circumference exceeding NCEP ATP III guidelines, 68% of patients had an identifiable precipitating factor. In the patients with unknown apoE mutations medical disorders were identified more frequently, this was attributable to the higher prevalence of diabetes (see below). In apoE (R145C) mutation carriers metabolic disorders, excluding obesity, were identified in 38 % of patients compared to 17% of apoE2 homozygotes (P=0.06, Fisher's exact test). Obesity was the commonest identifiable precipitating factor and abdominal obesity was particularly prevalent.

3.9.1 Diabetes

Diabetes is a well-known precipitant of dysbetalipoproteinaemia and thus it is not surprising that this disorder was highly prevalent in this series. All diabetics were clinically classified as Type II based on features such as age at onset, association with obesity, presence of other markers of the metabolic syndrome and presentation without ketoacidosis (276). There was no standardized policy for treating diabetes at

the lipid clinic but patients generally received lifestyle and nutritional advice followed by oral hypoglycaemic therapy with either metformin or sulfonylureas or both. Insulin was generally prescribed when combination oral hypoglycaemic therapy failed to achieve a glycated haemoglobin (HbA_{1C}) value of less than 7%. No patients were prescribed thiazolidenediones as these drugs were never available on the GSH formulary.

At initial presentation 36/135 (27%) of patients were diabetic. During follow-up diabetes was diagnosed in a further 9 patients resulting in a prevalence of at least 33% with some diagnoses of diabetes likely not recorded due to incomplete follow-up. Diabetes was significantly more common in patients without an identified mutation in apoE than in those patients with known apoE mutations. Diabetes was more common in apoE (R145C) mutation carriers (30%) than in apoE2 homozygotes (16%), but the difference was not statistically significant (P=0.16, Fisher's exact test). Assessing diabetic status at final follow-up confirmed the excess of diabetes seen in patients with no known apoE mutation (data not shown).

Table 3-42 Diabetes at initial presentation by apoE genotype

Genotype			Diabetes	
			Present	Absent
Geno +	E2/E2		11	58
	E (R145C)	All	8	19
		Hetero	7	16
		Homo	1	3
	E (K146Q)		0	2
Geno-			17	20

Chi-square P=0.004 for apoE2/E2 vs. apoE (R145C) vs. Geno-

At the initial clinic visit 33% of females and 22% of males were diabetic (P=0.17, Fisher's exact test). Diabetes was positively associated with higher BMI and WHR. Analyzing patients according to their final diabetic status showed even more marked differences in the WHR measured at the initial presentation (data not shown).

Table 3-43 Diabetes at initial presentation and measures of obesity

		Diabetic	Non-diabetic	P Value
BMI		30.17 ± 5.13	28.16 ± 4.13	0.03
Waist [1]	Male	100.50 ± 13.92	95.11 ± 11.52	0.18
	Female	100.4 ± 10.31	91.71 ± 11.71	0.01
WHR[2]	Male	1.00 ± 0.04	0.95 ± 0.07	0.05
	Female	1.00 ± 0.08	0.88 ± 0.08	<0.0001

[1] Waist circumference in cm

[2] Waist-hip ratio

The relatively small number of patients (9) that developed diabetes during follow-up did not allow for further comparative analysis, as full anthropometric data were only available for six of these patients.

Hypertension is a common comorbidity of diabetes and in this series 80% of diabetic patients were hypertensive compared to 42% of the non-diabetic population (P<0.0001, Fisher's exact test). There was no difference (P=0.70, Fisher's exact test) in the rates of smoking between diabetic (61%) and non-diabetic patients (56%).

3.9.1.1 Comparison with previously published case series

This study found a diabetes prevalence of 27%. This prevalence is much higher than that reported in all other series. Even when considering only those patients with an identified apoE mutation the prevalence of diabetes at 24% remains very high. ApoE (R145C) carriers had a particularly high prevalence of diabetes (30%) and when considering only apoE2 homozygotes the prevalence of diabetes at 16% was similar to the 14% reported by Feussner (10). Yet Stuyt (9) identified only a single patient (3%) with diabetes. The differing prevalences may possibly be partially explained by differences in the criteria used to diagnose diabetes and the intensity of surveillance for this disorder. Morganroth (7) reports that 55% of patients tested had an abnormal glucose tolerance test. Using the more stringent criteria currently applicable to the diagnosis of Type II DM it is conceivable that diabetes may have been identified in more of these patients. Type II DM is generally asymptomatic in its early phases and several years may pass between the onset of hyperglycaemia and symptoms. All the GSH lipid clinic patients with dysbetalipoproteinaemia are actively screened for diabetes by means of a yearly fasting glucose. Additionally all patients with severe mixed hyperlipidaemia are evaluated for dysbetalipoproteinaemia and the dyslipidaemia is not ascribed to diabetes before dysbetalipoproteinaemia has been excluded.

3.9.2 Hypothyroidism

Hypothyroidism was identified in 6/136 (4%) of patients. In 4 patients thyroid dysfunction was thought to be the main precipitant of hyperlipidaemia, while 2 patients had renal disorders in addition to the thyroid dysfunction. All patients were treated with thyroxine but none of the four patients for whom follow-up data are

available achieved adequate control of hyperlipidaemia without lipid-lowering medication.

3.9.2.1 Comparison with previous case series

The prevalence of hypothyroidism (4%) in this series is very similar to the reported rate of 1-5% (7;9;10).

3.9.3 Renal disease

Clinically significant renal disease was identified in 13/136 (10%) patients. There were 8 patients with diabetic nephropathy, 3 patients with nephrotic syndrome secondary to primary glomerulonephritis, 1 patient with autosomal dominant polycystic kidney disease and 1 patient with hypertensive nephropathy. Complete remission of renal pathology was only achieved in one patient. This was a young boy (7 years old at presentation) with membranous nephropathy secondary to chronic hepatitis B infection and extremely heavy proteinuria. His TC at presentation was 26.7 mmol/L with a TG of 3.5 mmol/L. He had extremely cholesterol-enriched VLDL (CV/TV 1.76, CV/TP 0.71) and was found to be heterozygous for the apoE (R145C) mutation. He was treated successfully with lamivudine for his hepatitis B infection and the membranous nephropathy resolved. Following remission of proteinuria the patient's lipids normalized and lipid-lowering medication was discontinued. Interestingly the typical GGE "A" pattern reverted to a LDL dominated "D" pattern.

It is difficult to quantify the metabolic impact of renal disease in the other patients as co-morbidities such as diabetes were present in many and the renal pathology was irreversible.

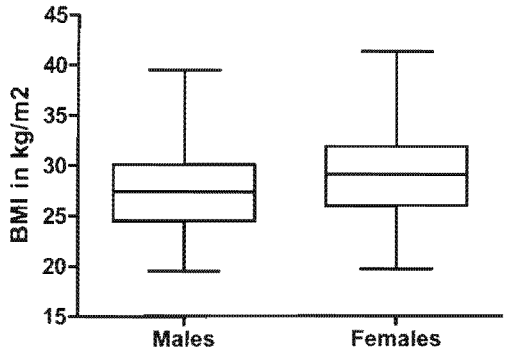
3.9.3.1 Comparison with previously published case series

Feussner (10) documents renal disease in 6% of patients but presents no detailed information on the nature of the renal disease. This series found renal disease in 10% of patients with a high prevalence of diabetic nephropathy. As the prevalence of diabetes was also high in our series the apparently higher prevalence of renal disease in our series may be related to excess cases of diabetic nephropathy. Morganroth and Stuyt (7;9) did not identify renal disease as a significant precipitant of dysbetalipoproteinaemia in any of their patients.

3.9.4 Obesity

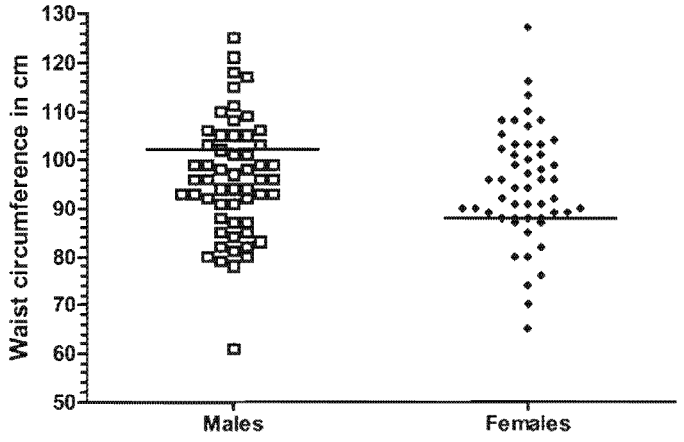
Seventy-nine % of patients with dysbetalipoproteinaemia were overweight (BMI 25-30 kg/m^2) and 33% obese (BMI >30 kg/m^2). The mean BMI for all patients was 28.68 ± 4.54 kg/m^2. The BMI of female patients was significantly higher than that of male patients (29.56 ± 4.61 kg/m^2vs. 27.89 ± 4.37 kg/m^2, P=0.04 unpaired t-test for females vs. males). There were no significant differences in BMI between patients with differing apoE genotypes (data not shown). In 50% of patients the waist circumference exceeded the gender-specific threshold recommended by the NCEP ATP III guidelines (254). Abdominal obesity was highly prevalent in females (73%) while the rate was significantly (P<0.0001, Fisher's exact test) lower in males (29%).

Figure 3-42 BMI in dysbetalipoproteinaemic patients



P<0.04 by unpaired t-test

Figure 3-43 Waist circumference in dysbetalipoproteinaemic patients



The bars indicate the NCEP ATP III recommended maximal waist circumference by gender.

The prevalence of obesity as defined by a BMI of more than 30 kg/m^2 did not increase with quartiles of age at presentation (P=0.79, Chi-square test) and the mean BMI also

did not differ between the quartiles (data not shown). Waist circumference was analyzed separately for males and females. There were no significant changes in waist circumference with age for either sex (data not shown).

3.9.4.1 Comparison with previously published case series

Rates of overweight and obesity are also very high in previously published case series. In Feussner's series (10) 72% of patients were overweight, while Stuyt (9) reports obesity (defined as a body weight higher than 120% of ideal body weight according to insurance tables) in 62% of patients. All studies, except Feussner (10), report higher levels of obesity in female compared to male patients. None of the previous studies report data on waist circumference, as the deleterious metabolic effects of abdominal obesity have only been appreciated more recently.

3.9.5 Alcohol abuse

Alcohol abuse was identified in 6/134 (4%) patients. These patients were all male and relatively young. Unfortunately the lipid response to decreased alcohol intake could not be evaluated accurately, as all patients received dietary and lipid-lowering therapy in addition to advice regarding their alcohol intake.

3.9.5.1 Comparison with previously published case series

None of the previously reported series include data on the prevalence of alcohol abuse, although the link between excessive alcohol intake and hypertriglyceridaemia is well established (10;277;278).

3.9.6 Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART)

In the pre-ART era it was known that HIV infection and AIDS were associated with dyslipidaemia. The dyslipidaemia was characterized by low levels of HDLC, moderately severe hypertriglyceridaemia and the presence of small dense LDLC (279-281). High levels of inflammatory cytokines were thought to account for the dyslipidaemia (281).

Following the introduction of ART a lipodystrophy syndrome was first described in 1998 (282-284). The lipodystrophy syndrome is a complex constellation of altered fat distribution, dyslipidaemia and insulin resistance. Antiretroviral drugs differ markedly in their potential to cause dyslipidaemia. Amongst the protease inhibitors ritonavir causes the most hypertriglyceridaemia, while stavudine (d4T) is the nucleoside reverse transcriptase inhibitor that affects lipid metabolism the most (285;286). The molecular mechanisms of ART-associated dyslipidaemia have not been elucidated conclusively, but may involve alteration in SREBP signalling (287-289), protection of apoB from proteasomal degradation (290), endocrine alterations (291;292) and mitochondrial toxicity (293). Cardiovascular disease and diabetes are increasingly of concern in long-term HIV survivors (294).

In 1999 a case report of severe hyperlipidaemia (TC 29.5 mmol/L and TG 27.3 mmol/L) associated with cutaneous xanthoma in a patient receiving ritonavir based ART was published in the Lancet (260). The apoE phenotype was E2/E2 and the response to a fibrate was excellent. Following this initial case report there has been increased interest in the role that apoE plays in modulating the hyperlipidaemic phenotype induced by ART (261;295).

In the GSH lipid clinic experience there were no patients with dysbetalipoproteinaemia solely due to HIV infection. The series includes one patient with ART associated dysbetalipoproteinaemia. This was a young male with apoE2 homozygosity and no other metabolic risk factors except for ART.

ART, on a meaningful scale, has only been available to patients in the South African public health care sector since late 2004. Owing to the interest in this subject an additional review was done of ART-associated dysbetalipoproteinaemia from the close of the main database up to December 2006.

There were four additional cases of ART-associated dysbetalipoproteinaemia. The patients were all receiving "second-line" PI (Ritonavir boosted Lopinavir) based ART after failing or not tolerating the "first-line" non-nucleoside reverse transcriptase inhibitor based therapy. Three of the patients were apoE2 homozygotes and one had the apoE (R145C) mutation. Dyslipidaemia was discovered incidentally in at least two of the four patients when serum sent for other investigations was noted to be lipaemic.

Only a small fraction of ART-associated dyslipidaemia is likely being seen at the lipid clinic because lipid testing is not included in the routine monitoring of patients receiving "first-line" ART and many physicians also lack awareness of the potential metabolic complications of ART. Treatment guidelines currently recommend testing for dyslipidaemia only after PI-based therapy has been initiated. Currently there are few patients taking PI based therapy, but this number will steadily increase as more patients receive ART for longer periods of time.

3.9.6.1 Comparison with previously published case series

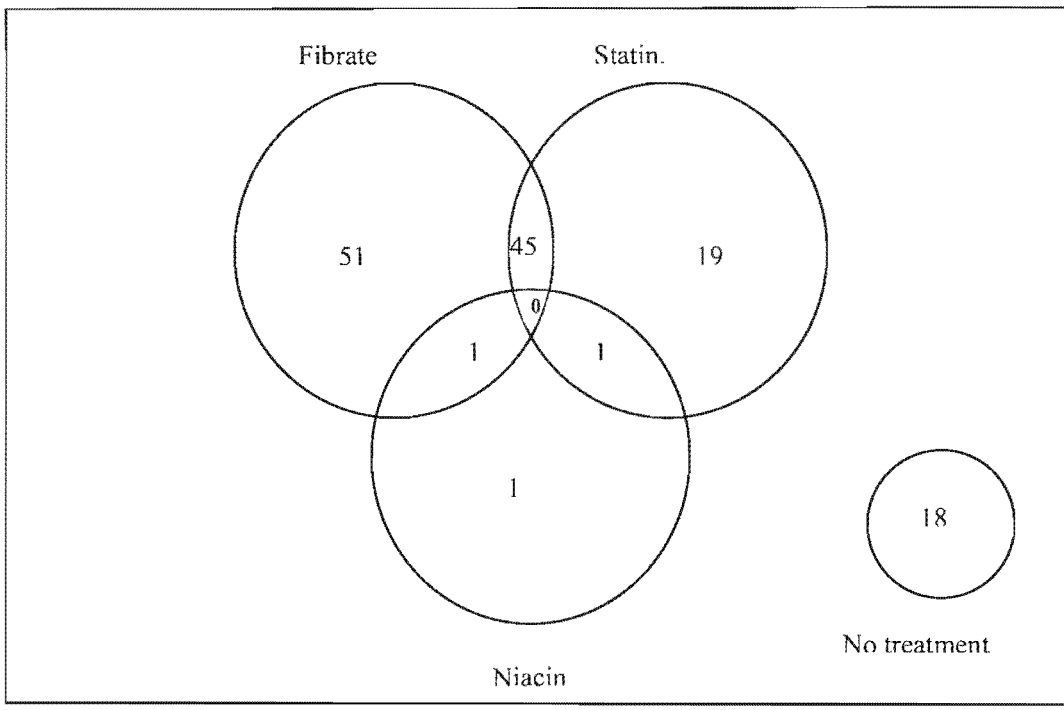
HIV infection has only become prominent in the last two decades and effective ART has been available for an even shorter time. None of the previously published series could therefore include any data on HIV infection and ART.

3.10 Treatment experience

This section presents observational data regarding the treatment of dysbetalipoproteinaemia over a period of more than 20 years. The data should be interpreted cautiously as it is observational in nature and may be subject to unrecognized bias. It does, however, present a "real-world" experience of treating dysbetalipoproteinaemia in a resource limited setting. Lipid-lowering drugs, especially statins, were usually prescribed at submaximal dosages due to budgetary constraints.

Drugs were analyzed by class, as analysis by individual compound would result in the formation of multiple small subgroups. Figure 3.44 illustrates the frequencies with which the various drug classes were prescribed.

Figure 3-44 Drug classes used to treat dysbetalipoproteinaemia at the lipid clinic



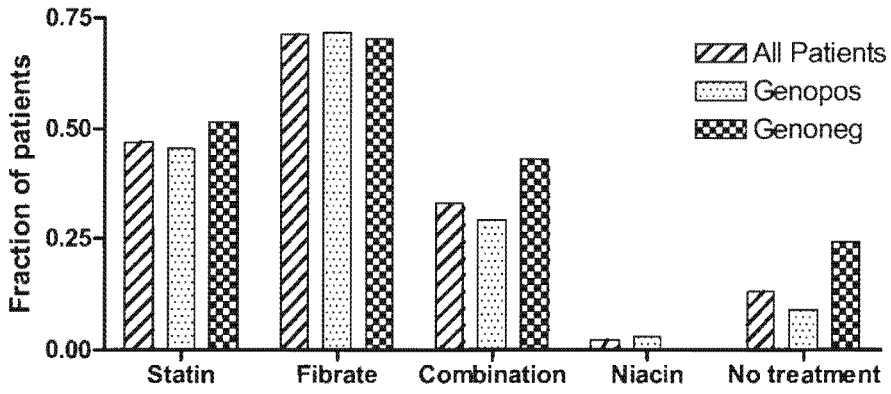
Note: No treatment indicates that no treatment data was available for the patient

The majority of treated patients (97 of 118 patients) were prescribed fibrates, either as monotherapy or in combination with a statin. The very high usage of fibrates reflects the general prescribing practice at the GSH lipid clinic, where patients with mixed hyperlipidaemia suspicious of dysbetalipoproteinaemia are initially started on a fibrate following lifestyle modification and control of precipitating factors. Patients that are adequately controlled on fibrate monotherapy are maintained on this and if control is

poor a low dose statin is co-prescribed. The 18 patients classified as not having received treatment were either not prescribed drug therapy at their initial visit and then lost to follow-up or did not have follow-up data to evaluate the response to lipid-lowering medication.

There were no significant differences (P=0.22 by Chi-square) in the drug classes prescribed to patients according to whether there was a known apoE mutation or not.

Figure 3-45 Relative proportion of drug classes prescribed to patients by apoE genotype



P=0.22 by Chi-square analysis for genopositive vs. genonegative patients

Increasing duration of treatment was associated with increasing use of combination therapy. This may well reflect some loss of control as dietary compliance wanes, patients age and additional medical problems such as diabetes set in or diabetic control deteriorates with time. After five years of follow-up significantly more patients were on combination therapy (P=0.0002 by Fisher's exact test).

Figure 3-46 Treatment of dysbetalipoproteinaemia according to duration of follow-up

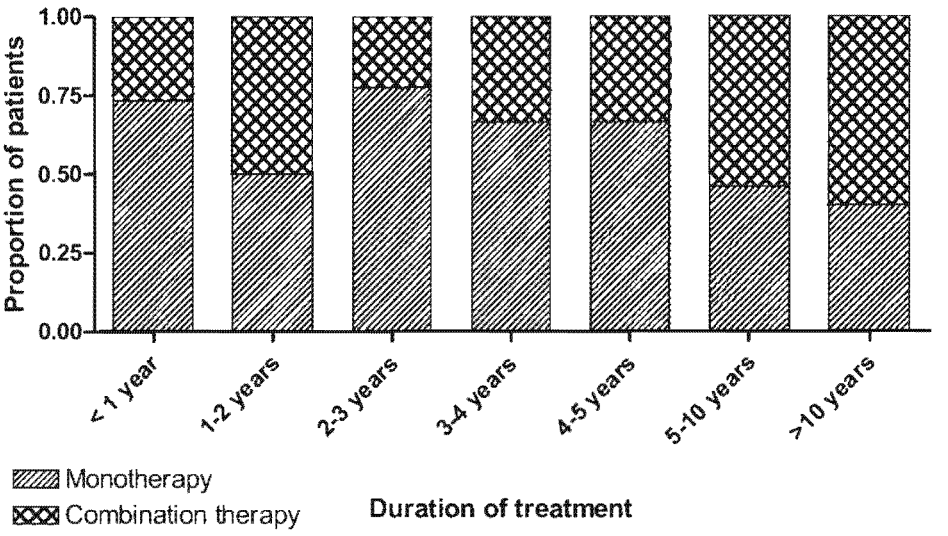
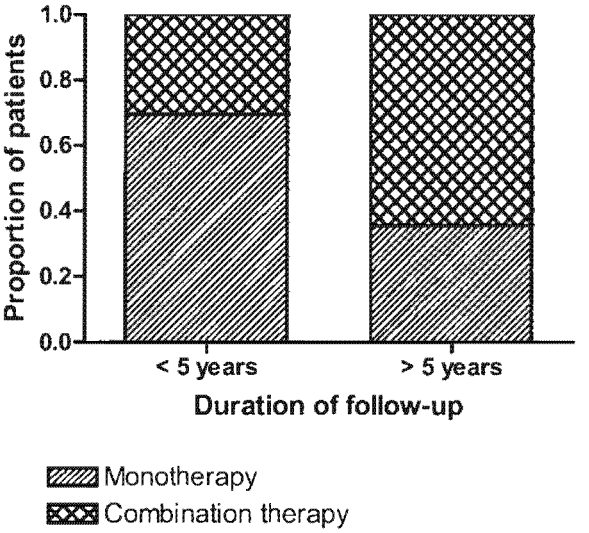


Figure 3-47 Treatment of dysbetalipoproteinaemia after five years of follow-up



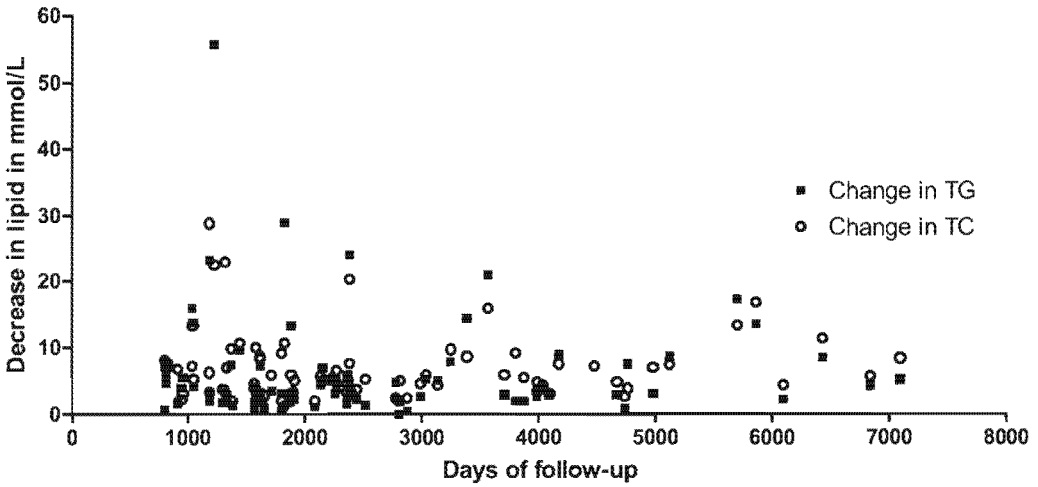
P=0.0002 (Fisher's exact test)

3.10.1 Lipid responses to therapy

The lipoprotein profile that in the view of the author was the most optimal (designated as "best lipid profile") was sought in the records. The lipid response was only assessed in patients that had been followed-up at the GSH lipid clinic for at least two years, so that there was sufficient opportunity to adjust medications. These patients would have been seen a minimum of four times as prescriptions have to be renewed every six months according to South African law. Additionally the prescription of lipid-lowering drugs was restricted to lipid clinics for the majority of the study period.

In patients that had been followed-up for more than two years the absolute decreases seen in TG and TC values scattered widely when analyzed by duration of follow-up. Increasing duration of follow-up beyond 2 years was not associated with further improvement in lipid control. It is therefore reasonable to analyze patients with more than 2 years of follow-up as a group without considering duration of follow-up in the analysis.

Figure 3-48 Response to lipid lowering therapy and duration of follow-up



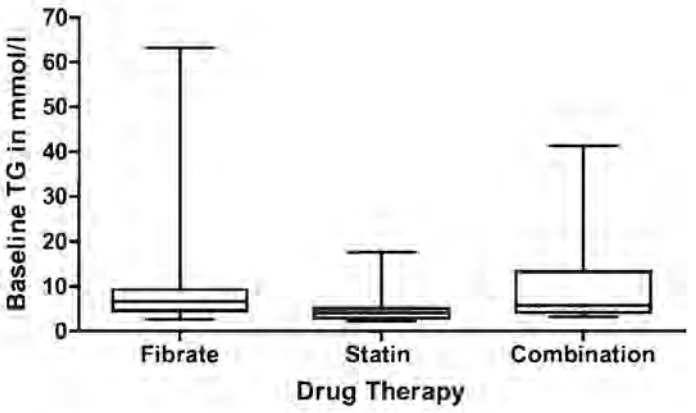
3.10.1.1 Triglycerides

The mean decrease in TG from untreated values to the "best recorded level" was 6.11 mmol/L (median 3.2, range 0-55.6). The absolute decrease in TG was highest for those patients with the highest TG at baseline (r^2=0.98 for linear regression of change in TG vs. baseline TG). The mean percentage reduction in TG from baseline was 62% (median 68%, range 0-92%). On lipid-lowering therapy the mean achieved TG was 2.43 mmol/L (median 2.0 mmol/L, range 0.3-12.4 mmol/L). The baseline TG and "best" TG were highly significantly different (P<0.0001 by Wilcoxon signed rank test). A TG<2.3 mmol/L was achieved in 64% of patients.

Patients treated with fibrates (9.26 ± 10.28 mmol/L) or statin + fibrate combination therapy (9.71 ± 8.90 mmol/L) tended to have higher baseline TG (mean ± SD) values than those treated with statins (5.19 ± 4.44 mmol/L), although the difference did not

reach statistical significance (P=0.06 by Kruskal-Wallis test). This trend likely simply reflects the fact that clinicians are more likely to prescribe fibrates as the degree of hypertriglyceridaemia increases.

Figure 3-49 Baseline TG values and lipid-lowering therapy



ANOVA (Kruskal-Wallis) P=0.06

The "best" TG levels achieved did not differ by which therapy was prescribed (fibrate 2.26 ± 1.50 mmol/L, statin 2.14 ± 1.03 mmol/L, combination therapy 2.71 ± 2.20 mmol/L; P=0.67 by Kruskal-Wallis test); this likely reflects ongoing adjustment of the treatment to achieve optimal lipid control.

The absolute and relative decrease in TG achieved as well as the "best" TG levels did not differ between patients with and without identified mutations in apoE (data not shown). Diabetic patients responded less well to treatment than non-diabetic patients and had significantly higher "best" TG levels than non-diabetic patients (3.13 ± 2.18

mmol/L vs. 2.18 ± 1.29 mmol/L; P=0.04 by Mann-Whitney test for diabetes vs. non-diabetic) although there was no significant difference in baseline levels (8.32± 8.50 mmol/L vs. 8.64 ± 9.49 mmol/L; P=0.59 by Mann-Whitney test for diabetes vs. non-diabetic). Similarly, patients with obesity defined by a BMI>30 kg/m^2 had higher treated levels than non-obese patients (3.09 ± 2.38 mmol/L vs. 2.18 ± 1.32 mmol/L; P=0.03 by Mann-Whitney test for obese vs. non-obese), despite similar baseline levels (8.20± 8.53 mmol/L vs. 8.57 ± 9.62 mmol/L; P=0.88 by Mann-Whitney test for obese vs. non-obese). When obesity was defined by a waist circumference that exceeded the NCEP ATP III recommendations (254) similar TG values were achieved (2.90 ± 2.22 mmol/L vs. 2.18 ± 1.34 mmol/L; P=0.10 by Mann-Whitney test for obese vs. non-obese). Baseline differences were not statistically significant (7.78 ± 7.33mmol/L vs. 10.32 ± 13.32 mmol/L; P=0.85 by Mann-Whitney test for obese vs. non-obese). When abdominal obesity was defined by WHR, patients in the lowest quartile (i.e. those patients with the least abdominal obesity) achieved significantly lower "best" TG levels than those in the highest quartile (1.92 ± 1.80 mmol/L vs. 3.82 ± 2.68 mmol/L, P=0.002 by Mann-Whitney test for quartile 1 vs. quartile 4) despite comparable baseline levels (8.21 ± 7.49 mmol/L vs. 10.05 ± 9.70 mmol/L, P=0.41 by Mann-Whitney test for quartile 1 vs. quartile 4).

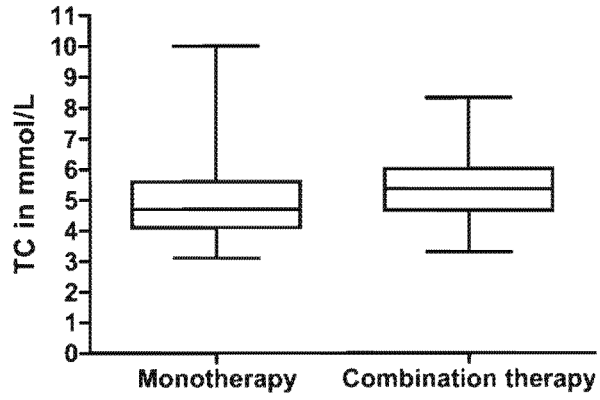
3.10.1.2 Total cholesterol

The mean decrease in TC from baseline was 6.84 mmol/L (median 5.50, range 1.0-28.80 mmol/L). The mean percentage reduction in TC was 52.4% (median 55.6%, range 15.2-85.8%). Patients with the highest cholesterol at baseline had the largest absolute reduction in TC values (r^2=0.94 for linear regression of absolute change in

cholesterol vs. baseline cholesterol). The mean TC on lipid-lowering therapy was 5.15 mmol/L (median 5.05 mmol/L, range 3.10-10.0 mmol/L), which was significantly lower ($P<0.0001$ by Wilcoxon signed rank test) than the baseline TC value of 11.98 ± 5.39 mmol/L in those patients whose response to treatment could be analyzed. The TC was less than or equal to 4.5 mmol/L in a third of patients and 5.0 mmol/L or less in 49% of patients.

The TC at baseline did not differ by the lipid lowering therapy chosen (fibrate therapy 11.88 ± 5.21 mmol/L, statin therapy 12.57 ± 6.93 mmol/L, combination therapy 12.15 ± 5.58 mmol/L; $P=0.90$ by Kruskal-Wallis test). The "best" TC also did not differ significantly when analyzed by lipid-lowering therapy (fibrate therapy 4.96 ± 1.54 mmol/L, statin therapy 4.82 ± 0.86 mmol/L, combination therapy 5.42 ± 1.21 mmol/L; $P=0.08$ by Kruskal-Wallis test). When analyzing treatment response by the use of monotherapy or combination therapy, the patients treated with combination therapy had significantly higher "best" TC levels (4.93 ± 1.40 mmol/L vs. 5.42 ± 1.21 mmol/L, $P=0.02$ by Mann Whitney test for monotherapy vs. combination therapy). This does not imply that combination therapy is inferior to monotherapy, but indicates that patients that fail initial monotherapy are likely to be treated with combination therapy. Patients failing monotherapy may be less responsive to therapy for a variety of reasons and may therefore not achieve excellent lipid values.

Figure 3-50 Best TC achieved for monotherapy vs. combination therapy



P=0.02 by Mann Whitney test

While the presence of an identified apoE mutation did not affect TG response to therapy it did significantly affect the reductions seen in TC. Patients with identified apoE mutations achieved significantly better reduction in TC than those in whom no mutation had been identified, despite having higher initial TC values.

Table 3-44 TC response to therapy by apoE genotype

	Genotypic Status	Mean	Median	SD	P Value [1]
Baseline TC	Geno+	12.42	11.20	5.12	0.02
	Geno-	10.89	9.30	5.97	
Best TC	Geno+	4.99	4.70	1.30	0.03
	Geno-	5.54	5.30	1.31	
Absolute reduction	Geno+	7.44	6.80	4.90	<0.0001
	Geno-	5.34	3.8	5.32	
Relative reduction (%) [2]	Geno+	56	58	15	0.0005
	Geno-	44	42	14	

[1] Mann Whitney test

[2] Percentage reduction

Diabetic patients responded less well to therapy than non-diabetic patients. This finding may, however, be partially explained by the fact that proportionally more patients with diabetes did not have identified apoE mutations (see above).

Table 3-45 TC response to therapy by glycaemic status

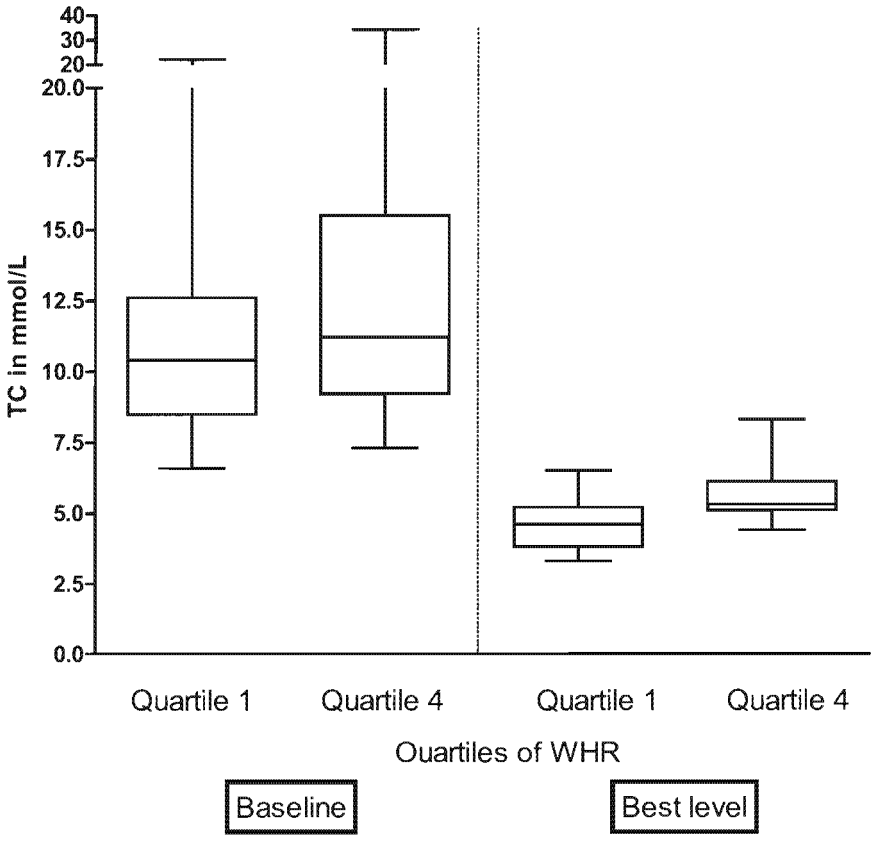
	Glycaemic Status	Mean	Median	SD	P Value [1]
Baseline TC	Diabetes	10.35	9.20	3.56	0.04
	No diabetes	12.56	10.90	5.82	
Best TC	Diabetes	5.68	5.60	1.33	0.01
	No diabetes	4.96	4.7	1.27	
Absolute reduction	Diabetes	4.67	3.80	2.97	0.005
	No diabetes	7.61	6.20	5.43	
Relative reduction	Diabetes	42	43	14	0.005
	No diabetes	56	58	15	

[1] Mann Whitney test

Patients with a BMI>30 kg/m^2 responded similarly to lipid lowering therapy to patients with a BMI<30 kg/m^2 (5.45 ± 1.31 mmol/L vs. 5.05 ± 1.35 mmol/L, P=0.21 by Mann Whitney test for the "best" TC in obese vs. non-obese patients). Patients with a waist circumference exceeding NCEP ATP III recommendations (254) tended to have higher TC levels on treatment (5.36 ± 1.15 mmol/L vs. 4.88 ± 1.35 mmol/L, P=0.07 by Mann Whitney test for excessive waist circumference vs. normal waist circumference). Assessing abdominal obesity with the use of the WHR showed that patients in the lowest quartile of WHR (with the least abdominal obesity) had lower "best" TC levels than those in the highest quartile (4.60 ± 0.89 mmol/L vs. 5.68 ± 1.11 mmol/L, P=0.007 by Mann Whitney test for quartile 1 vs. quartile 4) despite

similar baseline levels of TC (14.00 ± 8.12 mmol/L vs. 11.13 ± 4.38 mmol/L, P=0.36 by Mann Whitney test for quartile 1 vs. quartile 4).

Figure 3-51 Quartiles of WHR and response to therapy for TC



Note: The Y-axis is discontinuous

3.10.1.3 High density lipoprotein cholesterol

Lipid lowering therapy was associated with an increase in HDLC from baseline in most patients. The mean increase in HDLC was 0.28 mmol/L (median 0.3, range –0.7

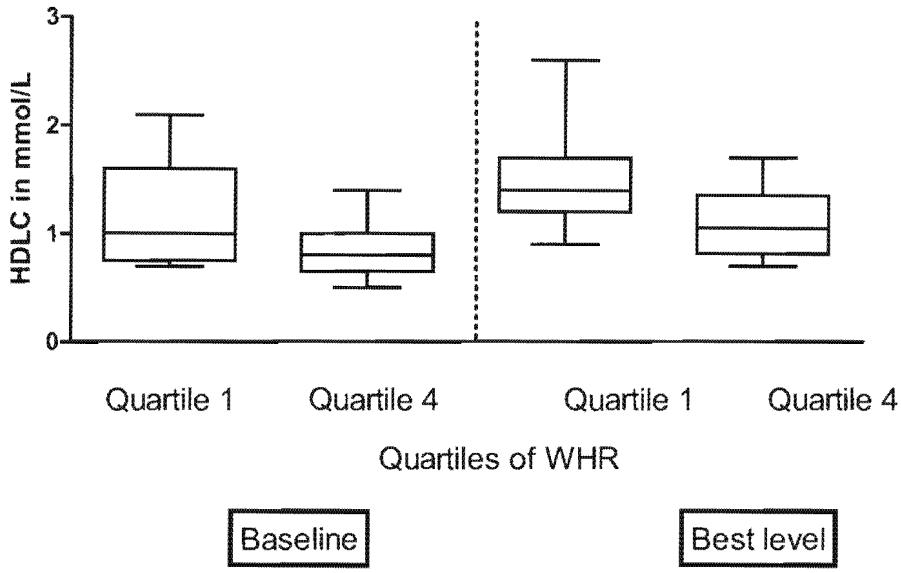
to 1.9 mmol/L). The correlation between baseline HDLC and the observed increase was weak (r^2=0.11 by linear regression) with lower baseline HDLC levels tending to increase more than higher levels of HDLC. The baseline HDLC and "best" HDLC were significantly different (1.01 mmol/L ± 0.35 mmol/L vs. 1.29 ± 0.44 mmol/L, P<0.0001 by Wilcoxon signed rank test for baseline vs. "best" HDLC levels). The mean percentage increase in HDLC was 34% (median 30, range –43 to 271%). On therapy the mean HDLC was 1.29 mmol/L (median 1.20, range 0.7-2.6 mmol/L). On therapy 76% of patients had a HDLC ≥ 1.0 mmol/L and in 43% of patients the HDLC was ≥ 1.3 mmol/L.

HDLC did not differ significantly at baseline according to final treatment allocation (fibrate therapy 1.03 ± 0.36 mmol/L, statin therapy 1.04 ± 0.18 mmol/L, combination therapy 0.99 ± 0.40 mmol/L; P=0.55 by Kruskal-Wallis test). The "best" HDLC did not differ by type of lipid lowering therapy provided (fibrate therapy 1.31 ± 0.48 mmol/L, statin therapy 1.41 ± 0.52 mmol/L, combination therapy 1.22 ± 0.33 mmol/L; P=0.59 by Kruskal-Wallis test).

HDLC showed equivalent increases irrespective of the presence of an identified apoE mutation (data not shown). HDLC also did not differ at baseline or on therapy between diabetics and non-diabetics (1.16 ± 0.31 mmol/L vs. 1.34 ± 0.47 mmol/L, P=0.15 by Mann-Whitney test for "best" HDLC in diabetics vs. non-diabetics). Obesity assessed either by BMI>30 kg/m^2 or waist circumference exceeding NCEP ATP III recommendations (254) did not influence baseline HDLC levels or the change with therapy (data not shown). There were, however, significant differences in HDLC response when patients were stratified by WHR (1.47 ± 0.41 mmol/L vs. 1.09

± 0.30 mmol/L, P=0.01 by Mann-Whitney test for "best" HDLC in quartile 1 of WHR vs. quartile 4).

Figure 3-52 HDLC and response to therapy by quartiles of WHR



The baseline HDLC levels were also higher in the quartile of lowest WHR, although the difference did not meet formal criteria for statistical significance (1.17 ± 0.48 mmol/L vs. 0.84 ± 0.24 mmol/L, P=0.07 by Mann-Whitney test).

3.10.1.4 Apolipoproteins and PGGE

ApoB and apoAI were only measured routinely at presentation and therefore it is not possible to examine changes in these variables with treatment. ApoE was not measured in any of the patients due to budgetary constraints. PGGE was only

performed routinely at presentation, so that it is not possible to discern changes in lipoprotein distribution with treatment in this cohort.

4 Chapter Four: A clinical trial evaluating lipid-lowering therapy for dysbetalipoproteinaemia

Lipid-lowering therapy for dysbetalipoproteinaemia was evaluated in a clinical trial sponsored by Bayer Pharmaceuticals. The trial was approved both by the Medicines Control Council of South Africa and the Humans Research Ethics Committee of the University of Cape Town. The trial enrolled dysbetalipoproteinaemic subjects from the lipid clinic at Groote Schuur Hospital and was conducted on the premises of the University of Cape Town. Permission was obtained from the sponsors to use trial data in this thesis. The results of this trial were never published due to the worldwide withdrawal of cerivastatin when cerivastatin was found to have a higher rate of muscle toxicity than other statins.

4.1 Study Bayer W6228/10176

Title: A single center, randomized, double-blind, cross-over study to compare the efficacy, tolerability and safety of cerivastatin 0.4 mg, fenofibrate 200 mg and combination of both once a day in the treatment of patients with Type III hyperlipoproteinaemia.

4.1.1 Study design and protocol

The Bayer W6228/10176 study (hereafter referred to as the Bayer study) was a randomized, double-blind crossover study of a statin, a fibrate and the combination of

both. The inclusion criteria specified a phenotypic diagnosis (cholesterol-enriched VLDL) of dysbetalipoproteinaemia and patients could be enrolled even if no mutation in apoE had been identified.

Inclusion criteria

- Males or females aged 18-60 years
- Previously ultracentrifugally proven dysbetalipoproteinaemia with
  - CV/TV $\geq$ 0.42 or
  - CV/TP $\geq$ 0.30
- Patients compliant with American Heart Association Step I diet
- Written informed consent provided

Patients were excluded if they had medical conditions that could influence lipid metabolism significantly. These included:

- Type I DM
- Type II DM with a glycated haemoglobin of >10%
- Hypothyroidism unless adequately replaced with thyroxine
- Nephrotic syndrome or creatinine > 176 µmol/L
- Consumption of more than 14 alcoholic drinks per week
- Congestive cardiac failure (NYHA III or IV)
- HIV infection or ART

Other exclusionary criteria were:

- TG >25 mmol/L at any stage of the study or a previous history of pancreatitis
- Severe hypertension (Systolic BP >180 or diastolic BP>110 mmHg)
- Major cardiovascular event (MI, CVA, CABG, PCI) within 6 months of study enrollment
- Significant arrhythmia or conduction disturbance
- Unexplained creatinine kinase (CK) > 3x upper limit of normal (ULN)
- Active liver disease or AST and/or ALT > 3x ULN
- Gastrointestinal disease that may affect drug absorption
- Severe psychiatric disease
- Use of drugs that interfere with cerivastatin or fenofibrate metabolism
- Intolerance of statins or fibrates

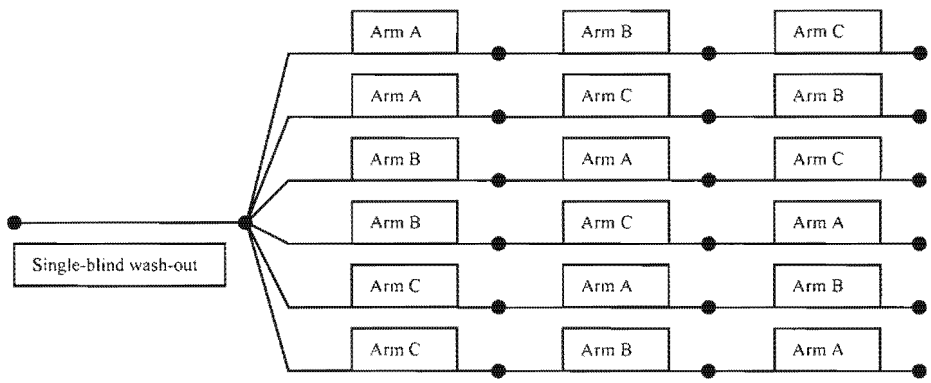
The study design was that of a double-blind randomized crossover trial. The trial consisted of 4 treatment cycles of 6 weeks duration each. The first treatment cycle was identical for all trial participants and was a single-blind placebo washout period to obtain untreated baseline lipid values. Patients were then randomly allocated to one of three possible treatment arms: The enrollment target was 36 patients.

- Arm A: Cerivastatin 0.4 mg/d, Fenofibrate placebo
- Arm B: Cerivastatin placebo, Fenofibrate 200 mg/d
- Arm C: Cerivastatin 0.4 mg/d, Fenofibrate 200 mg/d

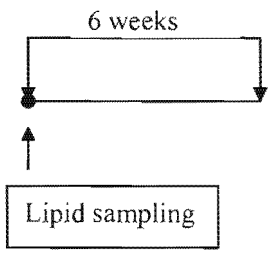
Patients were randomized in blocks of six according to a schedule generated by the study sponsor. Following completion of each treatment arm patients randomly crossed

over to the next treatment arm, till they had been exposed to all three possible treatment options. Patients were evaluated at the completion of each treatment period in the fasting state. There was no wash-out between the treatment periods.

Figure 4-1 Bayer W6228/10176 trial design



Legend:



4.1.2 Laboratory evaluation

At each visit the following laboratory tests were performed.

Lipid evaluation:

- Fasting TG, TC and HDLC
- * VLDL compositional analysis
- * Sequential lipoprotein ultracentrifugation and complete compositional analysis
- * PGGE
- Remnant turnover by means of stable isotope breath test (in selected subset of patients only- samples analyzed at the University of Western Australia, Perth)

Safety monitoring:

- Full blood count
- Urinalysis by dipstick
- Serum sodium, potassium, calcium, phosphate, uric acid, creatinine, urea
- Serum total protein, albumin, bilirubin, AST and ALT
- Serum amylase
- Serum CK
- Serum glucose
- Thyroid stimulating hormone and free T_4 (initial visit only)
- Pregnancy test (initial visit only in women of childbearing potential)

All laboratory tests except those marked with * were performed in the chemical pathology laboratory of Groote Schuur Hospital. Tests marked with * were performed at the lipid laboratory.

4.2 Study results

4.2.1 Bayer Study

4.2.1.1 Study course

The study was terminated prematurely due to the worldwide withdrawal of cerivastatin in 2001 following reports of increased rates of rhabdomyolysis in patients taking cerivastatin and in particular when gemfibrozil was taken concurrently (296;297). None of the enrolled patients completed all three treatment arms of the study. The premature termination of the study obviously limits statistical power, but a limited analysis of the results will be presented. Study data were analyzed by the author by means of a *per protocol* analysis.

All patients that had completed at least one treatment arm while being at least 80% compliant with study medication (assessed by count of returned tablets) were included in the analysis of that arm. The order in which medications had been allocated was not considered, as treatment carryover effects were not expected.

Thirty-five patients were screened and enrolled in the study. None of the patients completed the study. Twenty patients completed at least one six-week period on study medication. Three patients withdrew consent; two of these patients were less than 80% compliant on therapy due to adverse events related to study medication. One patient withdrew consent in the absence of treatment related adverse events.

Table 4-1 Study arms completed and reasons for early discontinuation

	Enrolled	Placebo wash-out	Treatment periods [1]		
			1	2	3
Male	20	16	12	10	0
Female	15	12	8	7	0
Total	**35**	**28**	**20**	**17**	**0**
Attrition (per period)	0	7	8	3	17
Reasons for non-completion					
Study terminated		5	6	1	17
TG too high		1	0	0	0
Prohibited drug		1	0	0	0
Death following MI		0	1	0	0
Consent withdrawn [2]		0	1	2	0

[1] Number of six-week periods on study drug that were completed

[2] Reasons given for withdrawing consent were myalgia, panic attacks and no reason given

Table 4-2 Medication arms completed

	Cerivastatin	Fenofibrate	Cerivastatin + Fenofibrate
Allocated	29	28	26
Completed	13	14	10
Non-compliant [1]	0	1	1
Analyzable	13	13	9

[1] Compliance < 80% as assessed by counting returned medication

4.2.1.2 Patient characteristics

The data presented here is that of the 20 patients that completed at least one treatment arm, as these are the patients that contributed data to the analysis reported below. Of the 20 patients 8 were overweight (BMI > 25 kg/m^2 and < 30 kg/m^2) and 5 were obese (BMI >30 kg/m^2). ApoE mutations were identified in 16/20 (80%) of patients.

Table 4-3 Clinical characteristics of the patients that completed at least one treatment arm

		Male	Female	All patients
n		12	8	20
Age at enrollment		51.84 ± 6.17	53.13 ± 4.15	52.35 ± 5.34
Anthropometry	Height (m)	1.72 ± 0.09	1.59 ± 0.05	1.67 ± 0.10
	Weight (kg)	82.13 ± 8.54	68.19 ± 8.54	76.55 ± 13.59
	BMI (kg/m^2)	27.87 ± 3.85	27.04 ± 3.59	27.54 ± 3.77
	Waist (cm)	93.25 ± 10.18	85.13 ± 9.45	90.00 ± 10.47
	Hip (cm)	99.75 ± 8.20	100.00 ± 9.32	99.85 ± 8.42
	WHR	0.94 ± 0.07	0.85 ± 0.04	0.90 ± 0.07
Concomitant conditions	Diabetes	1	3	4
	Hypertension	4	2	6
	IHD	4	1	5
	PVD	2	2	4
	CerVD [1]	2	0	2

Values are given as mean ± SD

[1] CerVD: Cerebrovascular disease

Table 4-4 ApoE genotypes in patients that completed at least one treatment arm

ApoE2/E2	ApoE (R145C)	No apoE mutation identified	**Total**
12	4	4	**20**

4.2.1.3 Lipid results

The lipid results reported in this section are those of the 20 patients that completed at least one treatment arm of the study. The baseline results of the two patients that were non-compliant with active treatment are included, because there is no reason to suspect that they did not discontinue their usual lipid-lowering therapy as instructed and they were compliant on double placebo in the wash-out phase. Of the patients analyzed in this section 16 had an identified apoE mutation (12 E2/E2 and 4 apoE (R145C)). The group of patients with no identified apoE mutations was too small (3 analyzable patients for treatment effects) to allow an adequately powered statistical analysis of differences in responses to therapy according to the presence or absence of apoE mutations.

4.2.1.3.1 Total cholesterol

Following the single-blind placebo washout period the mean total cholesterol was 10.56 ± 3.46 mmol/L (median 9.50 mmol/L, range 6.90- 20.90 mmol/L). The TC was significantly lowered from baseline in all treatment arms (P<0.0001, ANOVA).

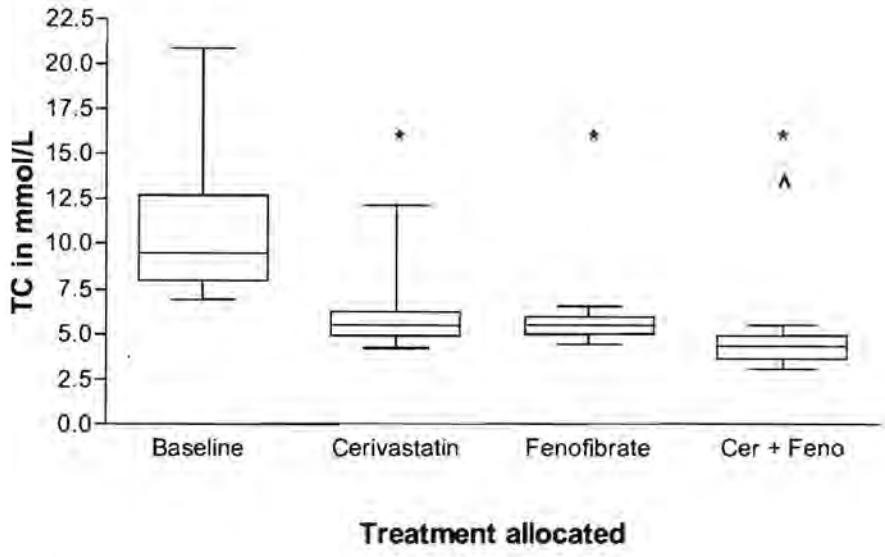
Combination therapy was significantly more effective than cerivastatin alone ($P<0.05$, Tukey's post test) in lowering the total cholesterol.

Table 4-5 TC during lipid-lowering therapy

Total cholesterol						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	10.56	9.5	3.46	20	6.90-20.90	<0.001	<0.001	<0.001
Cerivastatin	5.95	5.4	2.03	13	4.20-12.20		>0.05	<0.05
Fenofibrate	5.46	5.40	0.64	13	4.40-6.50			>0.05
Combination	4.26	4.30	0.79	9	3.00-5.40			

[1] TC log-transformed prior to analysis

Figure 4-2 TC during lipid-lowering therapy

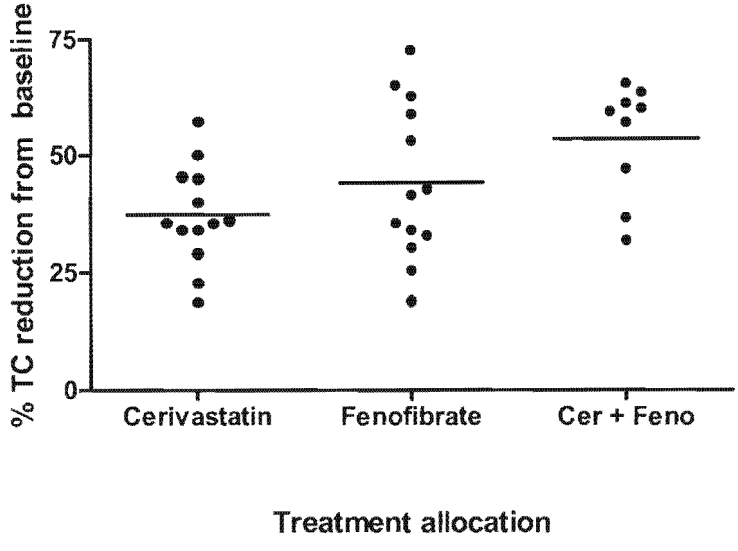


Legend: ** P <0.001 vs. baseline*

^ P <0.05 vs. cerivastatin

The mean reduction in TC from baseline was 37.2 ± 10.6 % for cerivastatin, 44.07 ± 16.68 % for fenofibrate and 53.43 ± 12.11 % for the combination of fenofibrate and cerivastatin (ANOVA P=0.03, P<0.05 by Tukey's post test for cerivastatin vs. combination therapy).

Figure 4-3 TC reduction from baseline



Drug development studies report a 26 % decrease in TC with the 0.4 mg dose of cerivastatin (298;299) in patients with primary hypercholesterolaemia. As dysbetalipoproteinaemia generally responds well to lipid-lowering therapies it is not surprising that a more profound reduction in cholesterol was seen in this study.

4.2.1.3.2 Triglycerides

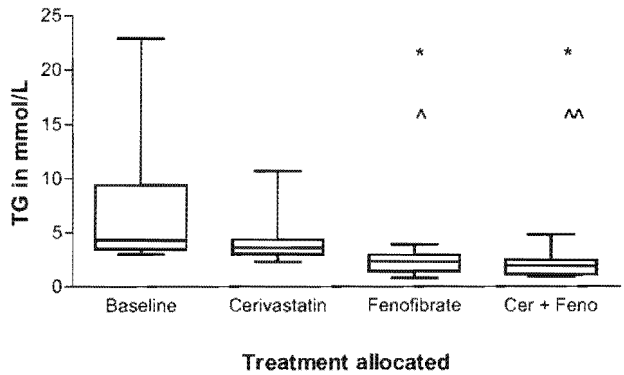
The mean TG in the untreated state following the wash-out period was 7.11 ± 5.48 mmol/L (median 4.3, range 3.00-22.90 mmol/L). Triglycerides decreased in all treatment arms (ANOVA, $P<0.0001$), but the change from baseline was only statistically significant for fenofibrate and combination therapy.

Table 4-6 TG during lipid-lowering therapy

Triglycerides						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	7.11	4.3	5.48	20	3.0-22.9	>0.05	<0.001	<0.001
Cerivastatin	4.15	3.6	2.17	13	2.3-10.7		<0.05	<0.01
Fenofibrate	2.23	2.30	0.93	13	0.8-3.9			>0.05
Combination	2.04	1.90	1.19	9	0.9-4.8			

[1] Analysis conducted on log-transformed values

Figure 4-4 TG during lipid-lowering therapy



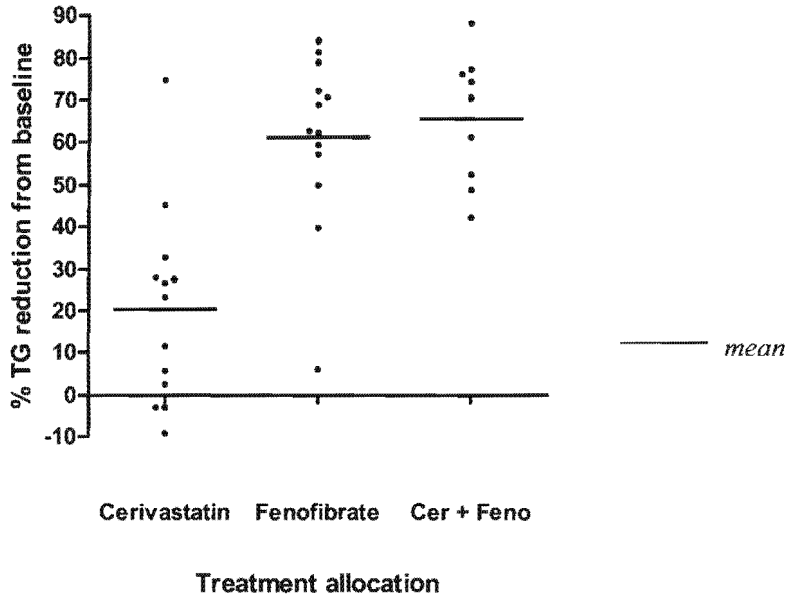
Legend: ** P< 0.001 vs. baseline*

^^ P<0.01 vs. cerivastatin

^ P<0.05 vs. cerivastatin

The mean reduction in TG with cerivastatin was 20.2 ± 23.2 %, 61.1 ± 20.7 % with fenofibrate and 65.8 ± 15.3 % with combination therapy (ANOVA P<0.0001, Tukey's post test P<0.001 for cerivastatin vs. fenofibrate and cerivastatin vs. combination therapy). The TG response to cerivastatin monotherapy was highly variable with 5 patients showing either an increase or a less than 10% decrease in TG values, while one patient reduced his TG by more than 70% from a very high baseline value of 22.90 mmol/L. Fenofibrate therapy and combination therapy consistently reduced triglycerides more than cerivastatin monotherapy.

Figure 4-5 TG reduction from baseline (%)



Note: Negative values indicate an increase in TG values

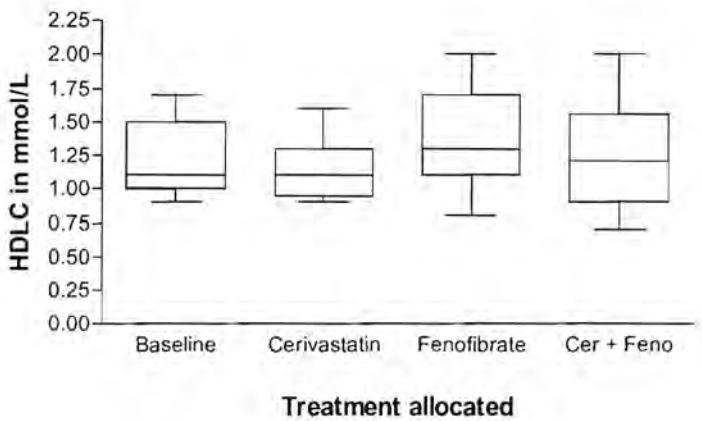
4.2.1.3.3 HDLC

The mean HDLC following discontinuation of lipid-lowering therapy was 1.24 ± 0.28 mmol/L (median 1.10 mmol/L, range 0.90-1.70 mmol/L). Mean HDLC increased when fenofibrate was prescribed, but none of the changes in HDLC reached statistical significance (ANOVA, P=0.36). HDLC values were highly variable.

Table 4-7 HDLC during lipid-lowering therapy

	HDLC					Tukey's multiple comparison test		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.24	1.10	0.28	20	0.90-1.70	>0.05	>0.05	>0.05
Cerivastatin	1.15	1.10	0.23	13	0.90-1.60		>0.05	>0.05
Fenofibrate	1.37	1.30	0.35	13	0.80-2.0			>0.05
Combination	1.24	1.20	0.43	9	0.7-2.0			

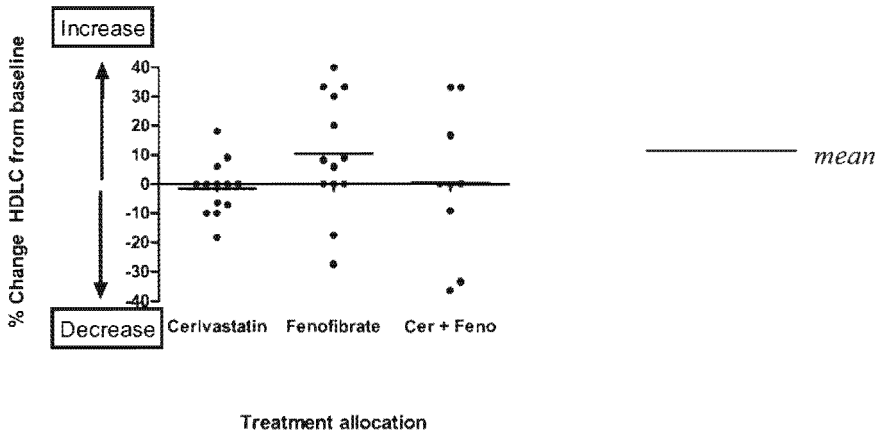
Figure 4-6 HDLC levels during lipid-lowering treatment



No statistically significant differences

The mean change in HDLC with cerivastatin was a decrease of 1.4 ± 9.3 %, fenofibrate increased HDLC by 10.4 ± 20.3 % while combination therapy resulted in a mean decrease of 0.5 ± 25.0 % (ANOVA, P=0.25). The changes in HDLC were highly variable, ranging from increases of about 40% to reductions of 35%.

Figure 4-7 Change in HDLC from baseline (%)



Hypertriglyceridaemia is often associated with low levels of HDLC. The relationship between TG levels and HDLC was explored at baseline and following therapy with lipid lowering medications. At baseline there was no clear-cut relationship between TG levels and HDLC, but in patients on fenofibrate or combination therapy HDLC and TG were inversely correlated.

Figure 4-8 Relationship between TG and HDLC at baseline

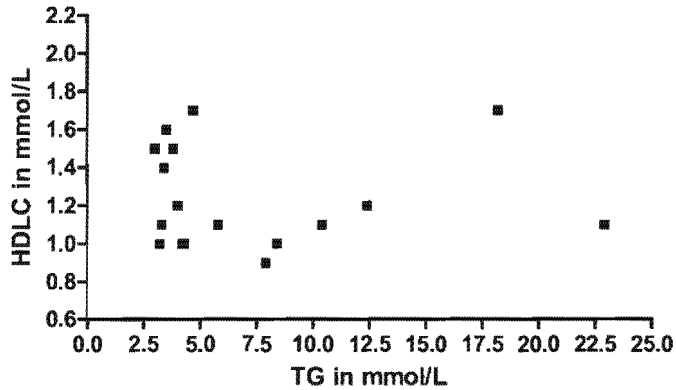
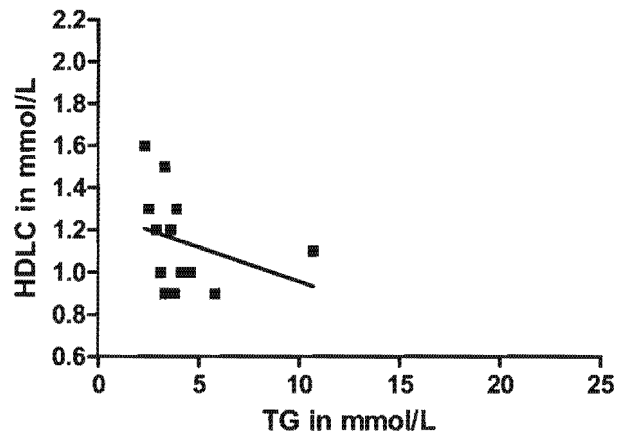
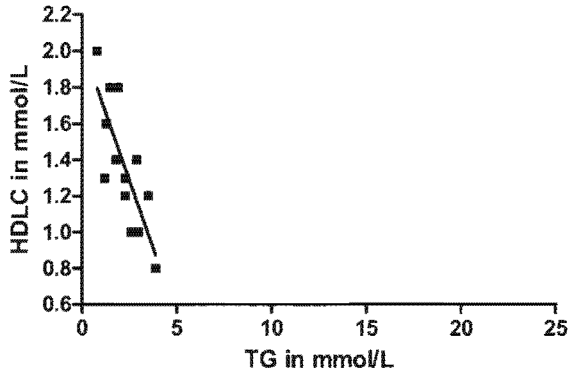


Figure 4-9 Relationship between TG and HDLC on cerivastatin



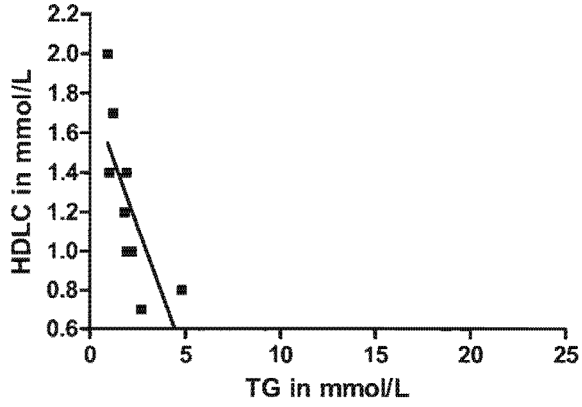
Linear regression r^2 =0.10

Figure 4-10 Relationship between TG and HDLC on fenofibrate



Linear regression r^2 =0.62

Figure 4-11 Relationship between TG and HDLC on combination therapy



Linear regression r^2 =0.56

It was noted that some patients with marked hypertriglyceridaemia had HDLC cholesterol levels that seemed inappropriately high and that the HDLC actually decreased in these patients as TG levels improved. Baseline TG was stratified by

quartiles and HDLC levels in the top and bottom quartiles were compared on and off treatment. Patients in the quartile with the highest TG (mean TG 15.98 ± 5.58 mmol/L) had a mean HDLC of 1.28 ± 0.29 mmol/L in the untreated state, which decreased to 1.00 ± 0.28 mmol/L when lipid-lowering therapy was given. Conversely patients in the lowest quartile of TG (mean TG 3.23 ± 0.17 mmol/L) had untreated HDLC values of 1.25 ± 0.24 mmol/L that increased to 1.28 ± 0.32 mmol/L with treatment. It is therefore likely that the assay used to measure HDLC in this study gives erroneously high readings in some patients with marked hypertriglyceridaemia. This phenomenon obviously also obscures changes in HDLC brought about by medication.

4.2.1.3.4 Previously published data on treating dysbetalipoproteinaemia (plasma lipids)

4.2.1.3.4.1 Historical overview

The atherogenicity of dysbetalipoproteinaemia was recognized soon after the initial descriptions of the disorder. It is therefore not surprising that attempts at treating dysbetalipoproteinaemia beyond dietary modification were made as soon as lipid-lowering medication became available. The first reports of successful treatment of dysbetalipoproteinaemia describe the beneficial lipid response observed with oestrogen therapy in postmenopausal females (163;164). Two subsequent studies further expand the experience with hormone replacement therapy (HRT); in one study oestrogen was prescribed (300) while the other study utilized tibilone (301), a synthetic steroid with weak oestrogenic, progestogenic and androgenic properties. The vast majority of patients included in these studies were postmenopausal females. Of

the two males studied, one responded by developing severe hyperlipidaemia (164), while the other withdrew due to the effects of oestrogen on sexual functioning and body habitus (163).

Subsequent studies describe the use of nicotinic acid (302) (or the closely related acipimox (303)), alone or in combination with clofibrate. The therapeutic effects of statins is described for the first time in a case report from 1986 (304). The use of bile acid sequestrants, colestipol in this case, was soon abandoned when its TG raising effects were recognized (305). Subsequent studies have documented the responses to newer fibrates (306-312) or explored treatment with various statins (240;313-318). There are only few reported direct comparisons of statin and fibrate treatment (238;316;318) and there are no published reports of direct comparison of statin, fibrate and combination therapy.

4.2.1.3.4.2 Overview of drug trials in dysbetalipoproteinaemic patients

Note: Trials may be listed more than once if they included more than one treatment arm.

Table 4-8 First attempts at treating dysbetalipoproteinaemia

Author	Year	N [1]	Diagnosis [2]	ApoE		Drug [3]	Dose [4]	Duration	Baseline Values (mmol/L)				% Change [5]				Comments	Ref
				E2/E2	Other				TC	TG	HDLC	LDLC	TC	TG	HDLC	LDLC		
Kushwaha	1977	6	AG +UC	NA	NA	OES	1µg/kg										1	(163)
Falko	1979	3	UC+IF	3	0	OES	1µg/kg	8w	10.54	5.95	1.04	3.41	53	23	48	53	2	(164)
Stuyt	1981	1	UC+IF	1	0	PLA	500ml	24d	8.01	5.84			16(I)	6(I)			3	(319)
Hoogwerf	1984	5	UC+IF	2	3	NA	3g	12w	9.17	4.90	1.04	3.86	28	43	28	11	4	(302)
Stuyt	1985	10	UC+IF	10	0	ACP	750mg	6w	10.00	7.99	0.91	3.05	30	48	7	10		(303)
Hoogwerf	1985	7	UB+IF	2	5	COL	20g	8w	7.51	3.91	0.96	3.78	4	34(I)	2(D)	32	5	(305)
Hoogwerf	1985	7	UB+IF	2	5	C+C		8w	7.51	3.91	0.96	3.78	21	35	30	15	5	(305)
Dallongeville	1991	9	UB+GE	6	0	OM3	6g	12w	8.57	6.34	1.92	0.78	26	57	14	21(I)		
De Beer	2002	4	IF/GE	4	0	TIB	2.5mg	8w	13.53	6.82	1.37	NA	51	64	16 (D)	NC	6	(301)

[1] Number of patients in study

[2] Indicates the criteria on which the diagnosis of dysbetalipoproteinaemia was based

AG *Agarose gel electrophoresis*

GE *Genetic analysis of apoE*

IF *Isoelectric focusing*

UB *Ultracentrifugation and demonstration of β-migrating VLDL*

UC *Ultracentrifugation with compositional analysis*

[3] Drugs

ACP *Acipimox*

C+C *Colestipol (20g/d)+Clofibrate (2g/d)*

COL *Colestipol*

OES *Ethinyl oestradiol*

OM3 *Omega-3 fatty acid in capsule form*

PLA *Single plasma infusion (pooled from two healthy donors)*

TIB *Tibolone*

[4] Total daily dosage

[5] Indicates the percent change from the baseline untreated value

Except where indicated the change in TG, TC and LDLC is a decrease while changes in HDLC refer to increases (Decrease= (D), Increase= (I))

Comments

1. Patients were studied in a metabolic unit under different dietary conditions and exposed to drug for varying lengths of time. One male patient was included in the study. Lipid parameters improved in all patients, at times dramatically, during drug exposure. The *ad hoc* nature of this study does not allow for calculation of responses.
2. The study included one male patient who had marked exacerbation of his hyperlipidaemia on oestrogen. The baseline data presented is that of all 3 patients, but the response is the change in lipids in the two female patients.
3. Plasma infusion was not effective; the change shown likely reflects the normal variability in lipid levels of dysbetalipoproteinaemic patients.
4. The diagnosis of dysbetalipoproteinaemia seems uncertain in at least two patients on retrospective review of the data presented.
5. The diagnosis of dysbetalipoproteinaemia seems uncertain in some patients.
6. The authors do not report quantitative data on LDLC, but do report that the change in LDLC was not statistically significant.

Table 4-9 Fibrates for dysbetalipoproteinaemia

Author	Year	N [1]	Diagnosis [2]	ApoE		Drug [3]	Dose [4]	Duration	Baseline Values (mmol/L)				% Change [5]				Comments	Ref
				E2/E2	Other				TC	TG	HDLC	LDLC	TC	TG	HDLC	LDLC		
Hoogwerf	1984	5	UC+IF	2	3	CLO	2g	12w	9.17	4.90	1.04	3.86	23	40	22	2	1	(302)
Hoogwerf	1984	4	UC+IF	2	2	GEM	1200mg	12w	9.14	4.98	1.04	3.68	23	56	35	1	2	(302)
Stuyt	1985	10	UC+IF	10	0	CLO	2g	6w	8.88	5.7	0.93	3.14	24	34	10	11		(303)
Hoogwerf	1985	7	UB+IF	2	5	CLO	2g	8w	7.51	3.91	0.96	3.78	11	37	25	6(1)		(305)
Packard	1986	6	UC+IF	6	0	BEZ	600mg	8w	11.13	6.69	1.14	3.46	47	68	28	19		(307)
Fruchart	1987	9	UC+UB+IF	9	0	FEN	100mg	12w	9.29	5.51	0.83	3.11	37	56	25	7		(320)
Houlston	1988	13	UC	NA	NA	GEM	1200mg	16m	12.6	10.2	1.1		40	70	45		3	(310)
Kuo	1988	8	UC+IF	8	0	GEM	1200mg	24+18m	9.84	5.16	0.96	4.95	46	65	54	41	4	(309)
Lussier-Cacan	1989	9	UC+UB+IF	9	0	FEN	300mg	12w	9.30	5.51	0.83	3.11	37	56	25	7	5	(308)
Illingworth	1990	12	UC+IF	10	2	CLO			12.95	8.77	0.91	3.60	40	52	32	1		(316)
Larsen	1994	6	UC+IF	6	0	CLO	2g	8w	11.27	7.18	1.11	3.57	40	48	9	12		(321)
Larsen	1994	6	UC+IF	6	0	GEM	1200mg	8w	11.27	7.18	1.11	3.57	54	70	7	22		(321)

Author	Year	N [1]	Diagnosis [2]	ApoE		Drug [3]	Dose [4]	Duration	Baseline Values (mmol/L)				% Change [5]				Comments	Ref
				E2/E2	Other				TC	TG	HDLC	LDLC	TC	TG	HDLC	LDLC		
Zhao	1994	8	UC+UB+IF	8	0	GEM	1200mg		11.87	6.08	0.95	1.87	45	63	28	0.5		(306)
Feussner	1997	23				GEM							26	27	15			(312)
Feussner	1997	23				FEM							39	39	28			(312)
Civeira	1999	10	UE+UC	1	9	GEM	1200	4w	8.37	3.64	0.99	4.53	17	55	2	5	6	(322)

[1] Number of patients in study

[2] Indicates the criteria on which the diagnosis of dysbetalipoproteinaemia was based

GE *Genetic analysis of apoE*

IF *Isoelectric focusing*

UB *Ultracentrifugation and demonstration of β-migrating VLDL*

UC *Ultracentrifugation with compositional analysis*

[3] Drugs

BEZ *Bezafibrate*

CLO *Clofibrate*

FE *Fenofibrate*

FEM *Fenofibrate (micronized)*

GEM *Gemfibrozil*

[4] Total daily dosage

[5] Indicates the percent change from the baseline untreated value

Except if indicated the change in TG, TC and LDLC is a decrease while changes in HDLC refer to increases (Decreases= (D), Increases+ (I))

Comments

1. The diagnosis of dysbetalipoproteinaemia seems uncertain in some patients.
2. One patient did not complete this arm of the study.
3. LDLC values were not reported.
4. Patients were treated for two prolonged periods interrupted by a drug-free interval. The results reported are those at the conclusion of the first treatment period.
5. This article reports on further analysis of lipoproteins in a cohort of patients initially reported by Fruchart (320).
6. This study included a very high number of patients with autosomal dominant mutations in apoE.

Table 4-10 Statins for dysbetalipoproteinaemia

Author	Year	N [1]	Diagnosis [2]	ApoE		Drug [3]	Dose [4]	Duration	Baseline Values(mmol/L)				% Change [5]				Comments	Ref
				E2/E2	Other				TC	TG	HDLC	LDLC	TC	TG	HDLC	LDLC		
East	1986	1	UC+IF	1	0	MEV	40mg	16	10.88	5.49	0.75	2.9	56	49	13	25	1	(304)
Vega	1988	3	UC+IF	3	0	LOV	40mg	8w	10.37	5.69	0.59	2.31	39	37	30	40		(313)
Illingworth	1990	12	UC+IF	10	2	LOV	40mg	12w	12.95	8.77	0.91	3.60	44	42	26	32		(316)
Stuyt	1990	12	UC+IF			SIM	80mg	6w	12.30	8.77			54	48				(314)
Feussner	1992	19	UB+UC+IF	19	0	SIM	20mg	8w	13.24	13.47	0.72	3.54	39	42	18	36	2	(318)
Feussner	1992	13	UB+UC+IF	13	0	SIM	40mg	8w	9.70[6]	10.72[6]	0.72[6]	2.33[6]	13[7]	24[7]	29[7]	22[7]	2	(318)
Feussner	1992	6	UB+UC+IF	6	0	S+G	40/450	8w	9.18[8]	9.64[8]	0.78[8]	1.45[8]	15[9]	17[9]	7.7(D)[9]	19(I)[9]	2	(318)
Zhao	1994	9	UC+IF	3	6	SIM	20mg	10w	9.08	4.09	0.99	2.94	44	37	5	44	3	(323)
Gylling	1994	5	UC+IF	5	0	LOV	40mg	12w	8.2	6.2	1.0	1.5	43	63	20	20		(324)
Knopp	1996	20	UC+GE	20	0	PRA	40mg	6w					36	22				(325)
Civeira	1999	10	UE+UC	1	9	SIM	20mg	4w	8.37	3.64	0.99	4.53	26	18	6 (D)	30	4	(238)
van Dam	2002	36	GE	34	2	ATR	40mg	40w	9.11	4.64	1.18	1.87	46	40	2 (D)	38		(240)
Ishigami	2003	4	IF	4	0	ATR	20mg	4w	8.21	3.71	1.40	1.30	52	56	16	44		(315)

[1] Number of patients in study

[2] Indicates the criteria on which the diagnosis of dysbetalipoproteinaemia was based

GE *Genetic analysis of apoE*

IF *Isoelectric focusing*

UB *Ultracentrifugation and demonstration of β-migrating VLDL*

UC *Ultracentrifugation with compositional analysis*

[3] Drugs

ATR *Atorvastatin*

LOV *Lovastatin*

MEV *Mevinolin*

PRA *Pravastatin*

S+G *Simvastatin +gemfibrozil*

SIM *Simvastatin*

[4] Total daily dosage

[5] Indicates the percent change from the baseline untreated value

Except if indicated the change in TG, TC and LDLC is a decrease while changes in HDLC refer to increases (Decreases= (D), Increases (I)))

[6] Indicates the lipid levels on 20mg of simvastatin

[7] Indicates additional changes in lipids by increasing simvastatin dose to 40 mg

[8] Indicates lipid levels on 40mg of simvastatin

[9] Indicates additional changes in lipids by adding gemfibrozil 450 mg to simvastatin 40 mg

Comments

1. This is the first reported use of a statin in patients with dysbetalipoproteinaemia.
2. In this trial patients were started on 20 mg simvastatin, which was uptitrated to 40 mg if the dyslipidaemia remained uncontrolled. Gemfibrozil was added to simvastatin 40 mg if necessary. Patients with an initial adequate response were maintained on simvastatin 20 mg.
3. Summary statistics were calculated from the data provided in the paper. The study includes a large number of patients with autosomal dominant apoE mutations.
4. The study included many patients with autosomal dominant apoE mutations.

Table 4-11 Trials comparing statins and fibrates

Note: This table only lists studies that compared statin and fibrate treatment prospectively in the same patient cohort.

Author	Year	N [1]	ApoE		Statin [2]	Fibrate [3]	%Change statin				% Change fibrate				Comments	Ref
			E2/E2	Other			TC	TG	HDLC	LDLC [4]	TC	TG	HDLC	LDLC		
Illingworth	1990	12	10	2	LOV 40mg	CLO 2g	44	42	26	32	40	52	32	1	1	(316)
Civeira	1999	10	1	9	SIM 20mg	GEM 1200mg	26	18	6 (D)	30	17	55	2	5	2	(238)

[1] Number of patients in study

[2] Statin

LOV Lovastatin

SIM Simvastatin

[3] Fibrates

CLO Clofibrate

GEM Gemfibrozil

[4] LDLC was determined by ultracentrifugation

Comments

1. LDLC was significantly lower with lovastatin treatment, but there was no significant difference in outcome by treatment for any of the other lipid measures.
2. This study included many patients with autosomal dominant mutations in apoE. Patients did not respond as well to treatment as in many of the other studies including only apoE2 homozygotes and it is doubtful whether the study is truly representative of the lipid response seen in the vast majority of dysbetalipoproteinaemic patients. The fibrate was more effective at reducing triglycerides, VLDLC and increasing HDLC. The statin reduced LDLC more effectively.

4.2.1.3.4.3 Bayer study versus historical data

In previous studies (see above) statin treatment of dysbetalipoproteinaemic patients was associated with decreases in TC ranging from 26-56%. The 37% reduction observed in TC using cerivastatin 0.4 mg/d correlates well with the changes seen using approximately equipotent (as judged by their LDLC lowering potency in non-dysbetalipoproteinaemic patients) dosages of other statins. With 40 mg/d of lovastatin TC decreases by 39% (313) to 44 % (316). Simvastatin 20mg/d reduces TC by 26 (238) to 44 % (323). The 26% decrease observed with simvastatin in the study by *Civeira et al* (238) is unusually low and likely not representative of the majority of dysbetalipoproteinaemic patients. The study included only one apoE2 homozygote. Studies using fibrates report TC reductions ranging from 11-57%. The response to fenofibrate, given in varying dosages, is 37% (320) to 39% (312). The 44% reduction of TC observed in our study using 200 mg of micronized fenofibrate is well within the range of expected responses.

Although results of statin and fibrate trials are not directly comparable because of variations in patient selection and characteristics, fibrates are in general associated with better reductions in TG than statins. This finding is confirmed in the two studies that directly compare fibrates and statins (238;316). In the Bayer study cerivastatin lowered TG by 20%, which is less than the 37-63% observed in studies using lovastatin 40mg/d (313;316;317) or the 18-42% (238;318) observed using simvastatin 20mg/d. The poor TG-lowering efficacy of cerivastatin markedly contrasts with the 61% reduction in TG seen with fenofibrate. The TG lowering efficacy of fenofibrate

in the Bayer study was similar to the 56% reduction observed by Fruchart (320), but better than the 39% reduction seen by Feussner (312).

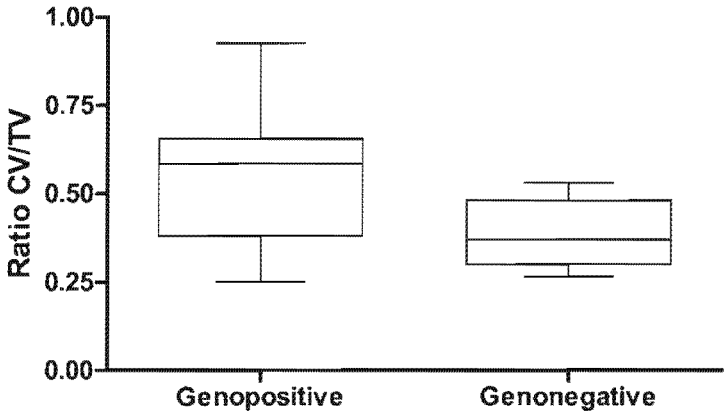
As the HDLC assay used for this study was not reliable (see above), changes in HDLC will be discussed in the section dealing with lipoprotein analysis by sequential ultracentrifugation. Similarly VLDLC and LDLC will be discussed in the context of the detailed analysis of lipoproteins by sequential ultracentrifugation.

4.2.1.4 VLDL compositional analysis

4.2.1.4.1 Ratio CV/TV

Following discontinuation of lipid-lowering medication the mean CV/TV ratio was 0.51 ± 0.17 (median 0.51, range 0.25-0.93) in the 28 patients that completed the placebo washout phase. In the 20 patients that completed at least one treatment arm the mean ratio was 0.52 ± 0.17 (median 0.52, range 0.25-0.93). The CV/TV ratio at baseline in patients that continued in the study beyond the placebo washout phase, did not differ significantly (P=0.30, unpaired t-test) from those that were discontinued early due to study termination. Patients with an identified apoE mutation had VLDL that was more cholesterol-enriched than those with no known apoE mutation (CV/TV 0.54 ± 0.18 for genopositive patients vs. 0.38 ± 0.10 in genonegative patients; unpaired t-test P=0.03).

Figure 4-12 Ratio CV/TV in genopositive vs. genonegative patients at baseline



Unpaired t-test P=0.03

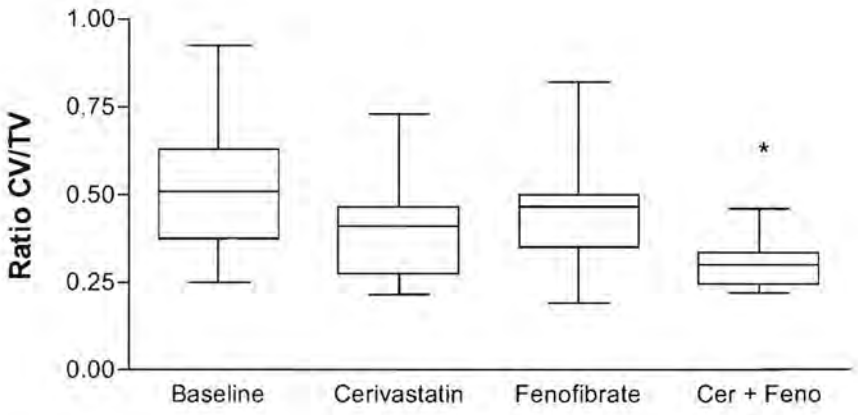
Only patients with a previously reported VLDL compositional analysis considered diagnostic of dysbetalipoproteinaemia (CV/TV ≥ 0.42 and/or CV/TP ≥ 0.30) were enrolled in this study. Amongst the 28 patients that completed the placebo washout phase the CV/TV ratio was ≥ 0.42 in 18. Amongst the genopositive patients 16/21 had a positive ratio while the ratio was only positive in 2/7 genonegative patients (P=0.06, Fisher's exact test).

Lipid-lowering treatment not only decreased plasma lipids (see above), but also changed VLDL composition significantly (ANOVA P=0.005).

Table 4-12 Ratio CV/TV with lipid-lowering therapy

	Ratio CV/TV					Tukey's multiple comparison test		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.52	0.51	0.17	20	0.25-0.93	>0.05	>0.05	<0.01
Cerivastatin	0.40	0.41	0.14	13	0.22-0.73		>0.05	>0.05
Fenofibrate	0.45	0.46	0.15	13	0.19-0.82			>0.05
Combination	0.30	0.30	0.07	9	0.22-0.46			

Figure 4-13 Ratio CV/TV during lipid-lowering treatment

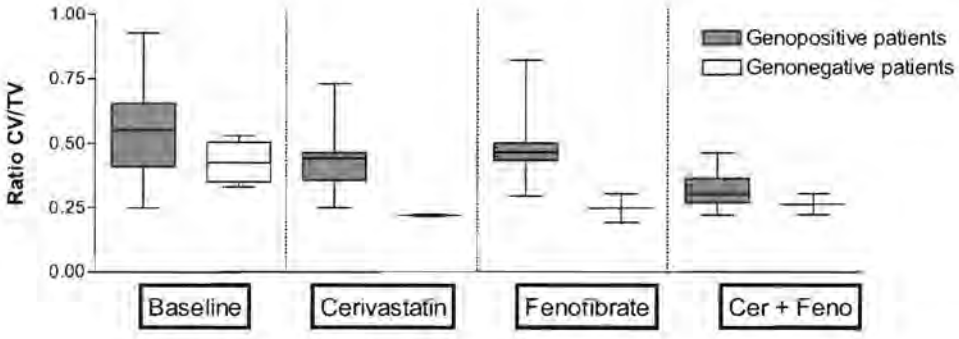


Legend: * *P<0.01 for combination vs. baseline (Tukey's multiple comparison test)*

ANOVA P=0.005

There were only four analyzable patients without identified apoE mutations. These patients had less cholesterol-enriched VLDL at baseline (see above), and the CV/TV ratio also decreased following institution of lipid-lowering therapy; always remaining lower than the ratio observed in genopositive patients.

Figure 4-14 Ratio CV/TV with lipid-lowering therapy in genopositive and genonegative patients

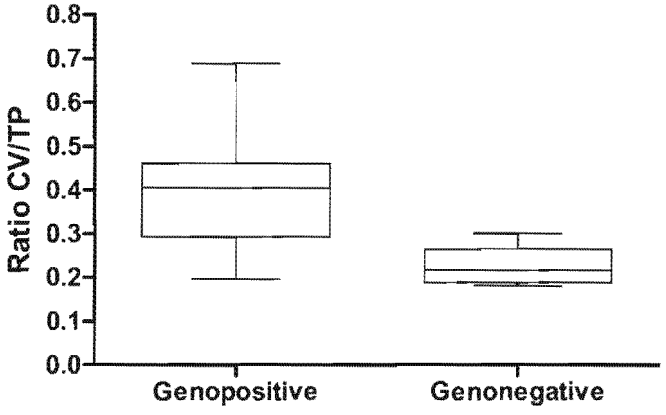


4.2.1.4.2 Ratio CV/TP

At baseline the mean ratio of CV/TP was 0.35 ± 0.12 (median 0.35, range 0.14-0.55) in the 28 patients that completed the placebo washout phase. The ratio in the 20 patients with analyzable results was 0.37 ± 0.11 (median 0.37, range 0.20-0.55) and did not differ significantly (P=0.18 by unpaired t-test) from that seen in patients that were discontinued from the study prior to completion of a treatment arm. Patients

with an identified apoE mutation had significantly higher ratios than patients in whom no apoE mutation had been identified (CV/TP 0.40 ± 0.12 for genopositive vs. 0.23 ± 0.04 for genonegative patients; P=0.0002 by unpaired t-test).

Figure 4-15 Ratio CV/TP in genopositive vs. genonegative patients at baseline



Unpaired t-test P=0.0002

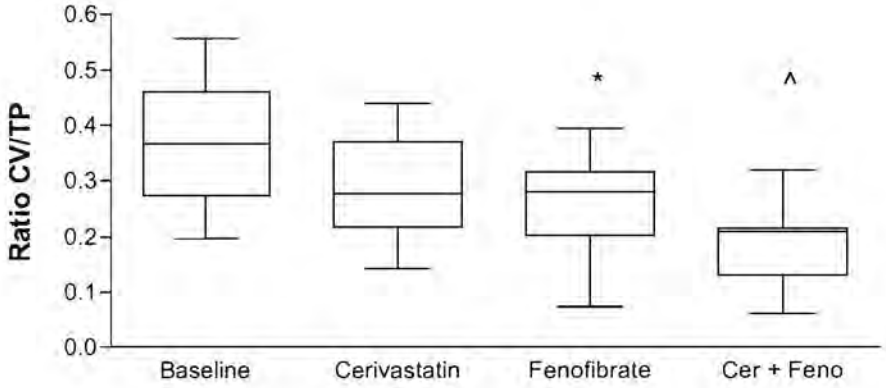
The CV/TP ratio was positive in 17/28 patients at baseline. It was positive in 17/21 genopositive patients and 1/7 genonegative patients (P=0.003, Fischer's exact test). Both the CV/TV and the CV/TP ratio were negative in 9 patients, while both ratios were positive in 16 patients. Three patients had discordant ratios.

The CV/TP ratio decreased from baseline in all treatment arms (ANOVA P<0.0001).

Table 4-13 Ratio CV/TP with lipid-lowering therapy

Ratio CV/TP						Tukey's multiple comparison test		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.37	0.37	0.11	20	0.20-0.55	>0.05	<0.01	<0.001
Cerivastatin	0.28	0.28	0.09	13	0.14-0.44		>0.05	>0.05
Fenofibrate	0.26	0.28	0.09	13	0.07-0.39			>0.05
Combination	0.18	0.20	0.07	9	0.06-0.32			

Figure 4-16 Ratio CV/TP during lipid-lowering therapy



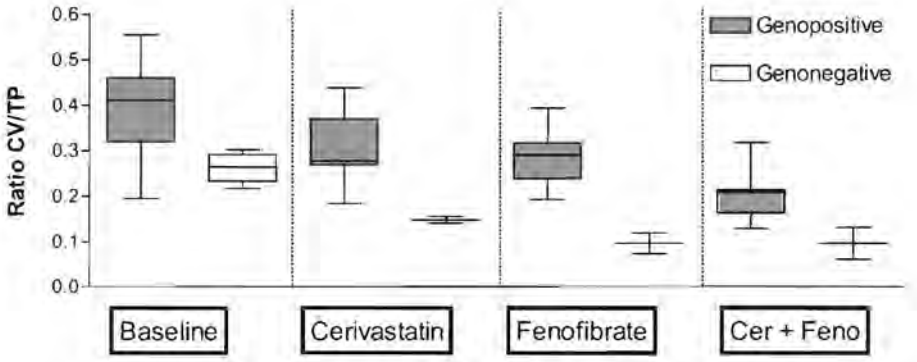
Legend: * *P<0.01 for fenofibrate vs. baseline (Tukey's multiple comparison test)*

^ *P<0.001 for combination therapy vs. baseline (Tukey's multiple comparison test)*

ANOVA P<0.0001

Patients with no identified apoE mutation also responded to lipid-lowering therapy with a reduction in the cholesterol-enrichment of their VLDL, however, they had consistently lower CV/TP ratios than genopositive patients.

Figure 4-17 Ratio CV/TP with lipid-lowering therapy in genopositive and genonegative patients



4.2.1.4.3 Previously published data on changes in the VLDL compositional analysis with dysbetalipoproteinaemia treatment

Table 4.14 lists studies that report changes in VLDL compositional analysis with lipid-lowering treatment. For ease of comparison where molar ratios have been reported they have been converted to mass ratios. Many studies only report VLDL compositional analysis at baseline and do not provide sufficient raw data to calculate changes induced by lipid-lowering therapy.

Table 4-14 Changes in VLDL compositional analysis with lipid-lowering therapy

For more details on the studies listed below, please refer to the previous tables on the treatment of dysbetalipoproteinaemia.

Author	Year	Drug [1]	Baseline Values		Treated Values		% Change [2]		Comments	Ref
			CV/TV	CV/TP	CV/TV	CV/TP	CV/TV	CV/TP		
East	1986	MEV 40mg		0.60		0.29		52	1	(304)
Packard	1986	BEZ 600mg		0.97		0.77		21		(307)
Fruchart	1987	FEN100mg		0.40		0.30		25		(308;320)
Dallongeville	1991	OM3 6g		0.36		0.33		8		(326)
van Dam	2002	ATR 40mg		0.50		0.34		32		(240)
Ishigami	2003	ATR 20mg		0.50		0.29		43		(315)

Notes;

[1] Drugs

ATR *Atorvastatin*

BEZ *Bezafibrate*

FEN *Fenofibrate*

MEV *Mevinolin*

OM3 *Omega-3 fatty acids*

[2] Refers to change from baseline value

[3] Comments

1. Values averaged from two treatment periods

Although few studies specifically report on changes in VLDL composition, and if so only on the ratio of VLDLC to TG in plasma, lipid-lowering treatment is invariably associated with VLDL that is less cholesterol-enriched. A similar response is observed in the Bayer study with both ratios used to assess VLDL composition decreasing with treatment. The decreases observed are of similar magnitude as those observed in the studies reported above.

4.2.1.5 Lipoprotein fractions: absolute masses

Due to technical difficulties in the processing and analysis of samples, data on lipoprotein composition were only available for 18 patients. Of the 18 patients 17 completed two treatment arms and one patient only completed a single arm. Most patients (15/18) had an identified apoE mutation: 11 were homozygous for apoE2 and 4 carried the apoE (R145C) mutation.

4.2.1.5.1 Method

4.2.1.5.1.1 Sequential ultracentrifugation

Lipoprotein composition was analyzed by sequential ultracentrifugation using Lindgren's method (245) with modifications.

Density solutions

Two solutions are made that can be combined in varying proportions to make a solution of the desired density.

1. Saline solution of density 1.006 g/ml. (22.80 g NaCl and 0.2g disodium EDTA is dissolved in distilled water, 2 ml of 1mmol/L NaOH is added and the volume brought to 2 L. Thereafter a further 6 ml of distilled water is added.)
2. Saline-NaBr solution of density 1.182 g/ml. (249.8 g of NaBr is dissolved in 1 L of saline of density 1.006 g/ml)

Sample preparation

Two ml of plasma is adjusted to a density of 1.118 g/ml by addition of 0.341 g NaCl and careful mixing.

Tube preparation

Beckman Ultra-Clear ™ tubes for the SW40 rotor are coated with polyvinyl chloride to make them wettable and assist in gradient creation (327). Briefly, 2 g of polyvinyl alcohol is dissolved in 50 ml of distilled water by gentle stirring and heating. Fifty ml of isopropanol is added to the hot solution with continued gentle stirring and heating. Once the solution is clear it is allowed to cool to room temperature. The Beckman tubes are filled with this solution for 15 minutes and the solution is then aspirated. The tubes are allowed to dry and any residual fluid at the bottom of the tube is aspirated. The tubes are filled with distilled water overnight and then finally rinsed with distilled water again and air dried.

Gradient creation

The gradient is created by careful overlayering of solutions. Initially 0.50 ml of the 1.182 g/ml solution is pipetted into the tubes. Further solutions are added with the use of a peristaltic pump. The following solutions are added to the d=1.182 g/ml solution from the bottom to the top

1. 2.0 ml plasma adjusted to d=1.118 g/ml
2. 1.0 ml of density solution 1.0988 g/ml
3. 1.0 ml of density solution 1.0860 g/ml
4. 1.0 ml of density solution 1.0790 g/ml
5. 1.0 ml of density solution 1.0722 g/ml
6. 2.0 ml of density solution 1.0641 g/ml
7. 2.0 ml of density solution 1.0588 g/ml

Ultracentrifugation

Ultracentrifugation is performed using a Beckman L80 Optima centrifuge and SW 40 rotor. The temperature of the centrifuge is 23 ° Celsius. The centrifuge is set to accelerate normally, but deceleration is set at 0 to avoid mixing of the sample.

Table 4.15 gives the time and speed schedule used for the ultracentrifugation, as well as the volumes removed at each step.

Table 4-15 Sequential ultracentrifugation schedule

Lipoprotein fraction	Speed (rpm)	Time Hours: Minutes	Volume removed (ml)
VLDL1	39000	1:38	1.0
VLDL2	18500	15:41	0.5
IDL	39000	2:35	0.5
LDL	30000	21:10	1.0

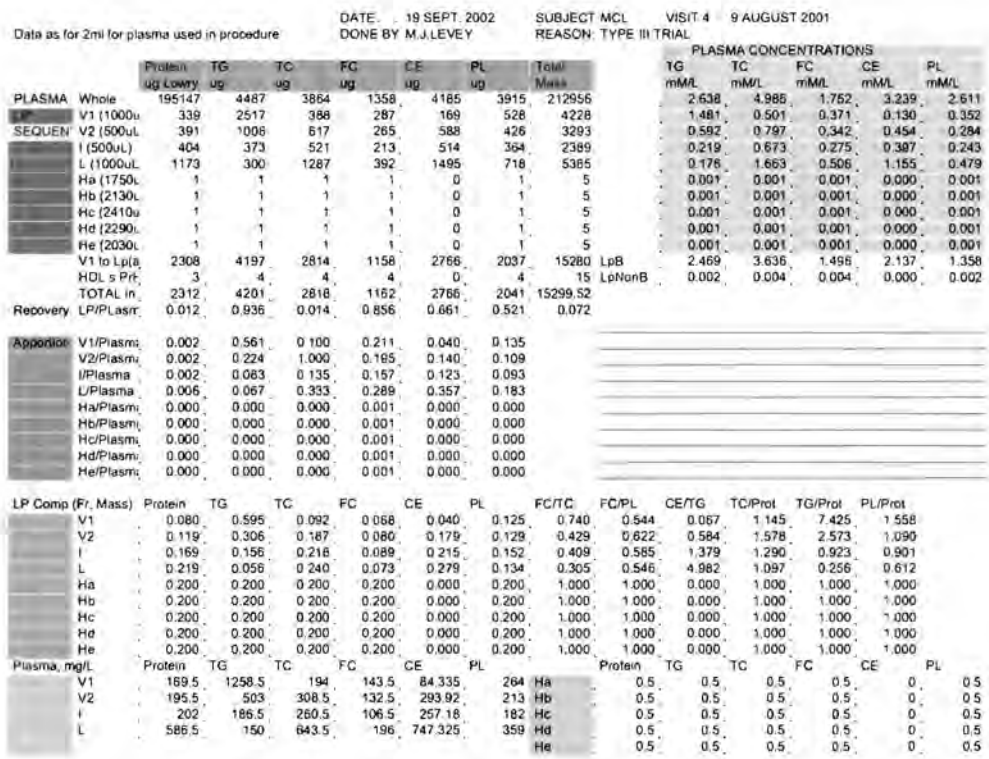
The 1.0 ml supernatant removed following the first centrifugation is replaced with 1.0 ml of solution d=1.0588 g/ml. Subsequently the removed supernatants are no longer replaced.

4.2.1.5.1.2 Lipoprotein fraction analysis

The TC, FC, PL and TG were determined enzymatically in the lipoprotein fractions using commercially available kits (Roche, Wako) according to the manufacturer's instruction. Esterified cholesterol was calculated as the difference between TC and CE. The mass of CE was calculated as 1.67 x esterified cholesterol. Protein was measured using a modification of the Lowry method (328) with bovine serum albumin as a standard.

Total lipoprotein mass was calculated as the sum of FC, CE, TG, PL and protein. All data were entered in a Quattro Pro spreadsheet (Corel) that automatically calculated all derived values.

Figure 4-18 Example of lipoprotein fractional composition spreadsheet



Data as for 2ml for plasma used in procedure | DATE: 19 SEPT. 2002 | DONE BY M.J.LEVEY | SUBJECT MCL | VISIT 4 | 9 AUGUST 2001 | REASON: TYPE III TRIAL

		Protein ug Lowry	TG ug	TC ug	FC ug	CE ug	PL ug	Total Mass
PLASMA	Whole	195147	4487	3864	1358	4185	3915	212956
LP	V1 (1000u	339	2517	388	287	169	528	4228
SEQUEN	V2 (500uL	391	1008	617	265	588	426	3293
	I (500uL)	404	373	521	213	514	364	2389
	L (1000uL	1173	300	1287	392	1495	718	5365
	Ha (1750L	1	1	1	1	0	1	5
	Hb (2130L	1	1	1	1	0	1	5
	Hc (2410u	1	1	1	1	0	1	5
	Hd (2290L	1	1	1	1	0	1	5
	He (2030L	1	1	1	1	0	1	5
	V1 to Lp(a	2308	4197	2814	1158	2766	2037	15280
	HDL s Pr	3	4	4	4	0	4	15
	TOTAL in	2312	4201	2818	1162	2766	2041	15299.52
Recovery	LP/Plasm	0.012	0.936	0.014	0.856	0.661	0.521	0.072

PLASMA CONCENTRATIONS

	TG mM/L	TC mM/L	FC mM/L	CE mM/L	PL mM/L
	2.638	4.986	1.752	3.239	2.611
	1.481	0.501	0.371	0.130	0.352
	0.592	0.797	0.342	0.454	0.284
	0.219	0.673	0.275	0.397	0.243
	0.176	1.663	0.506	1.155	0.479
	0.001	0.001	0.001	0.000	0.001
	0.001	0.001	0.001	0.000	0.001
	0.001	0.001	0.001	0.000	0.001
	0.001	0.001	0.001	0.000	0.001
	0.001	0.001	0.001	0.000	0.001
LpB	2.469	3.636	1.498	2.137	1.358
LpNonB	0.002	0.004	0.004	0.000	0.002

Apportior	V1/Plasm	0.002	0.561	0.100	0.211	0.040	0.135
	V2/Plasm	0.002	0.224	1.000	0.195	0.140	0.109
	I/Plasma	0.002	0.083	0.135	0.157	0.123	0.093
	L/Plasma	0.006	0.067	0.333	0.289	0.357	0.183
	Ha/Plasm	0.000	0.000	0.000	0.001	0.000	0.000
	Hb/Plasm	0.000	0.000	0.000	0.001	0.000	0.000
	Hc/Plasm	0.000	0.000	0.000	0.001	0.000	0.000
	Hd/Plasm	0.000	0.000	0.000	0.001	0.000	0.000
	He/Plasm	0.000	0.000	0.000	0.001	0.000	0.000

LP Comp (Fr. Mass)	Protein	TG	TC	FC	CE	PL	FC/TC	FC/PL	CE/TG	TC/Prot	TG/Prot	PL/Prot
V1	0.080	0.595	0.092	0.068	0.040	0.125	0.740	0.544	0.067	1.145	7.425	1.558
V2	0.119	0.306	0.187	0.080	0.179	0.129	0.429	0.622	0.584	1.578	2.573	1.090
I	0.169	0.156	0.218	0.089	0.215	0.152	0.409	0.585	1.379	1.290	0.923	0.901
L	0.219	0.056	0.240	0.073	0.279	0.134	0.305	0.546	4.982	1.097	0.256	0.612
Ha	0.200	0.200	0.200	0.200	0.000	0.200	1.000	1.000	0.000	1.000	1.000	1.000
Hb	0.200	0.200	0.200	0.200	0.000	0.200	1.000	1.000	0.000	1.000	1.000	1.000
Hc	0.200	0.200	0.200	0.200	0.000	0.200	1.000	1.000	0.000	1.000	1.000	1.000
Hd	0.200	0.200	0.200	0.200	0.000	0.200	1.000	1.000	0.000	1.000	1.000	1.000
He	0.200	0.200	0.200	0.200	0.000	0.200	1.000	1.000	0.000	1.000	1.000	1.000

Plasma, mg/L	Protein	TG	TC	FC	CE	PL
V1	169.5	1258.5	194	143.5	84.335	264
V2	195.5	503	308.5	132.5	293.92	213
I	202	186.5	260.5	106.5	257.18	182
L	586.5	150	643.5	196	747.325	359

	Protein	TG	TC	FC	CE	PL
Ha	0.5	0.5	0.5	0.5	0	0.5
Hb	0.5	0.5	0.5	0.5	0	0.5
Hc	0.5	0.5	0.5	0.5	0	0.5
Hd	0.5	0.5	0.5	0.5	0	0.5
He	0.5	0.5	0.5	0.5	0	0.5

4.2.1.5.2 Plasma lipids

Analysis of lipoprotein composition included determination of plasma lipids as well. Lipids were analyzed independently of the results obtained in the routine chemical pathology laboratory. Results for TC and TG were very similar to those reported above and will not be presented in detail here.

4.2.1.5.2.1 Plasma free cholesterol (FC)

Plasma FC concentration decreased significantly from baseline with all treatments (ANOVA P<0.0001). Free cholesterol was the lowest with combination therapy, followed by fenofibrate and cerivastatin, but none of the differences between treatment arms was statistically significant.

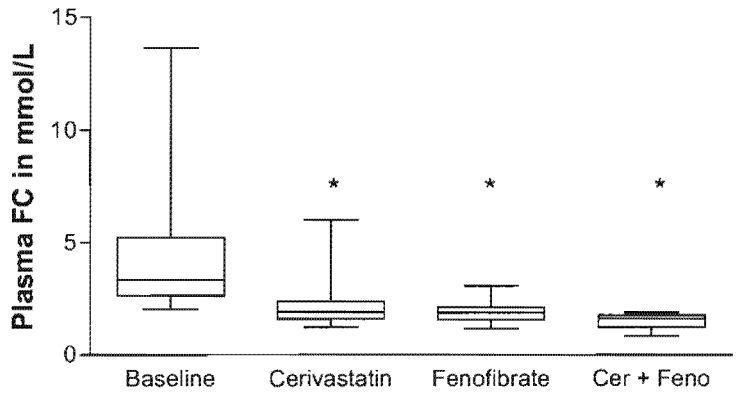
Table 4-16 Plasma free cholesterol concentration and lipid-lowering therapy

ANOVA P<0.0001

Plasma FC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	4.43	3.36	2.97	18	2.03-13.64	P<0.001	P<0.001	P<0.001
Cerivastatin	2.28	1.92	1.26	13	1.24-6.01		P>0.05	P>0.05
Fenofibrate	1.90	1.88	0.47	13	1.18-3.09			P>0.05
Combination	1.48	1.60	0.35	9	0.84-1.88			

[1] Data log transformed prior to analysis

Figure 4-19 Plasma free cholesterol concentration and lipid-lowering therapy



*Legend: * P<0.001 vs. baseline*

There were no differences in free cholesterol and in the response to treatment between apoE2 homozygotes and apoE (R145C) carriers (data not shown).

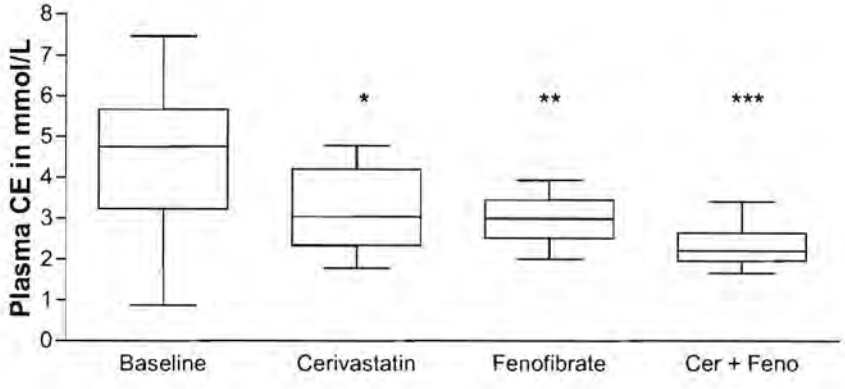
4.2.1.5.2.2 Plasma cholesterol ester (CE)

Plasma CE concentration decreased from baseline values in all treatment arms (ANOVA P<0.0001). Combination therapy was associated with the lowest levels of plasma CE, followed by fenofibrate and cerivastatin. Differences between the various treatment arms were not statistically significant.

Table 4-17 Plasma cholesterol ester concentration and lipid-lowering therapy

Plasma CE (mmol/L)						Tukey's multiple comparison test		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	4.53	4.78	1.70	18	0.88-7.45	<0.05	<0.01	<0.001
Cerivastatin	3.17	3.03	0.96	13	1.78-4.78		>0.05	>0.05
Fenofibrate	3.00	3.00	0.60	13	2.00-3.93			>0.05
Combination	2.35	2.22	0.52	9	1.66-3.41			

Figure 4-20 Plasma CE concentration and lipid-lowering therapy



*Legend: * P<0.05 vs. baseline/ ** P<0.01 vs. baseline/ *** P<0.001 vs. baseline*

Plasma CE was significantly lower at baseline in the apoE (R145C) mutation carriers than apoE2 homozygotes (2.92 ± 0.95 mmol/L for apoE (R145C) vs. 4.94 ± 0.96 for apoE2/E2; P=0.03 by unpaired t-test). Following resumption of lipid-lowering therapy the differences between the two genotypes were no longer significant (data not shown).

4.2.1.5.2.3 Plasma phospholipid (PL)

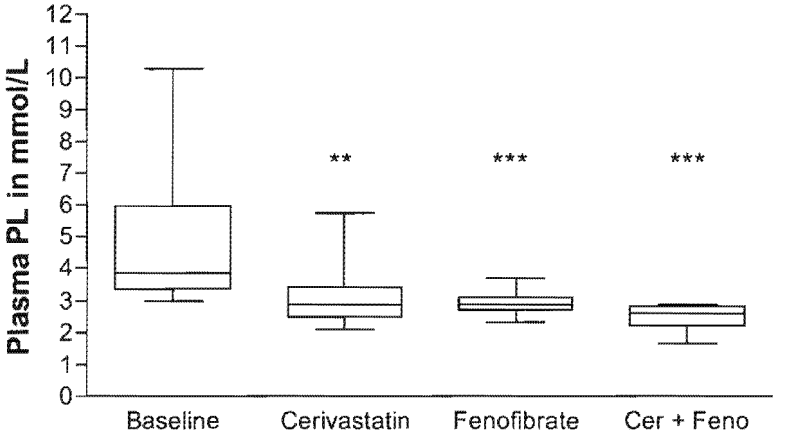
The mean PL concentration at baseline was 4.61 ± 1.95 mmol/L. All treatments significantly decreased baseline PL (ANOVA P<0.0001).

Table 4-18 Plasma PL concentration and lipid-lowering therapy

Plasma PL (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	4.61	3.87	1.95	18	2.95-10.29	<0.01	<0.001	<0.001
Cerivastatin	3.09	2.89	0.91	13	2.11-5.73		>0.05	>0.05
Fenofibrate	2.91	2.87	0.34	13	2.29-3.68			>0.05
Combination	2.46	2.61	0.40	9	1.62-2.84			

[1] Data log transformed for analysis

Figure 4-21 Plasma PL concentration and lipid-lowering therapy



Legend: ** *P<0.01 vs. baseline*

*** *P<0.001 vs. baseline*

There were no differences in PL concentration according to the nature of the apoE mutation (data not shown).

4.2.1.5.3 VLDL1

4.2.1.5.3.1 VLDL1 protein

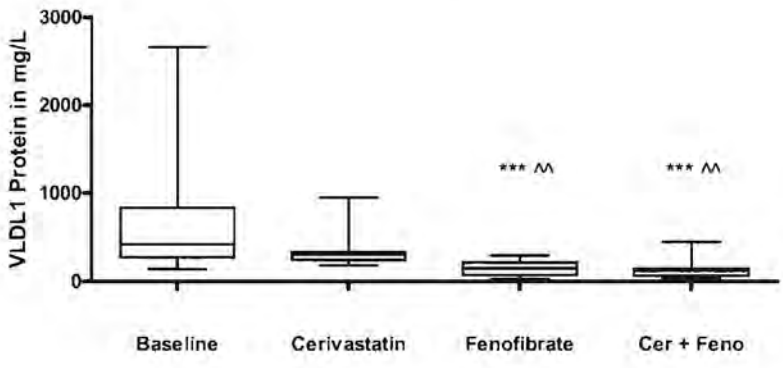
VLDL1 protein decreased significantly from baseline following institution of lipid-lowering therapy (ANOVA, P<0.0001). Fenofibrate and combination therapy were significantly more effective in decreasing VLDL1 protein than cerivastatin monotherapy.

Table 4-19 VLDL1 protein and lipid-lowering therapy

VLDL1 protein (mg/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	681	420	693	18	141-2664	>0.05	<0.001	<0.001
Cerivastatin	345	301	199	13	181-951		<0.01	<0.01
Fenofibrate	149	148	81	13	28-293			>0.05
Combination	140	122	40	9	37-446			

[1] Data log transformed for analysis

Figure 4-22 VLDL1 protein and lipid-lowering therapy



*Legend: *** P<0.001 vs. baseline*

^^ P<0.01 vs. cerivastatin

There were no significant differences in VLDL1 protein between the two apoE genotypes studied, either at baseline or with lipid-lowering therapy (data not shown).

4.2.1.5.3.2 VLDL1 triglycerides

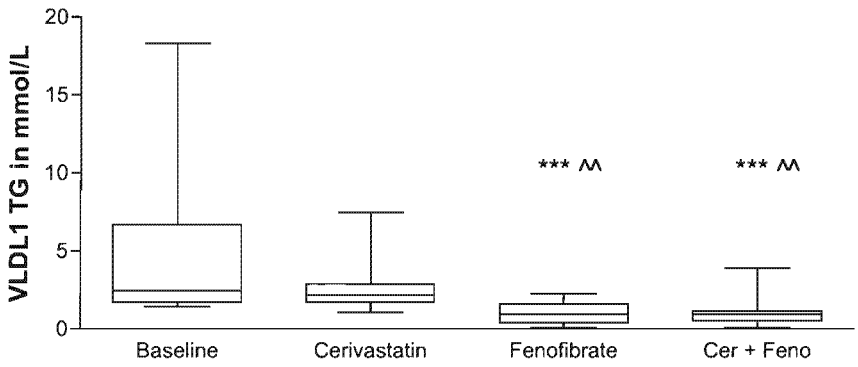
VLDL1 TG concentration decreased from baseline with lipid-lowering therapy (ANOVA P<0.0001), but the change seen with cerivastatin did not reach statistical significance on post-test.

Table 4-20 VLDL1 TG concentration and lipid-lowering therapy

VLDL1 TG (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	4.41	2.46	4.26	18	1.45-18.31	>0.05	<0.001	<0.001
Cerivastatin	2.66	2.17	1.64	13	1.05-7.48		<0.01	<0.01
Fenofibrate	1.04	0.97	0.67	13	0.08-2.27			>0.05
Combination	1.11	0.95	1.11	9	0.07-3.92			

[1] Data log transformed prior to analysis

Figure 4-23 VLDL1 TG and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

There were no statistically significant differences in VLDL1 TG levels between the two-apoE genotypes at any stage during the study (data not shown).

4.2.1.5.3.3 VLDL1 total cholesterol

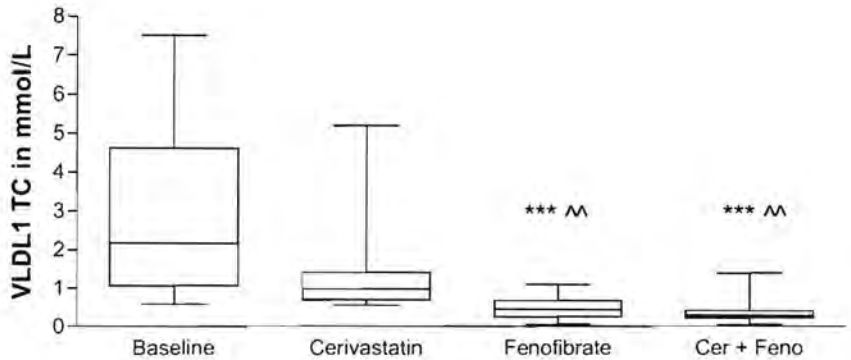
Total cholesterol concentration in VLDL1 decreased from baseline with all lipid-lowering treatments (ANOVA, P<0.0001), but the decrease seen with cerivastatin did not reach significance on post-test. There were no significant differences in VLDL1 TC between the two apoE genotypes studied at any stage (data not shown).

Table 4-21 VLDL1 TC concentration and lipid-lowering therapy

VLDL1 TC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	2.90	2.17	2.32	18	0.58-7.51	>0.05	<0.001	<0.001
Cerivastatin	1.33	0.98	1.24	13	0.56-5.19		<0.01	<0.01
Fenofibrate	0.47	0.43	0.29	13	0.05-1.09			>0.05
Combination	0.39	0.29	0.39	9	0.04-1.38			

[1] Data log transformed prior to analysis

Figure 4-24 VLDL1 TC and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.3.4 VLDL1 free cholesterol

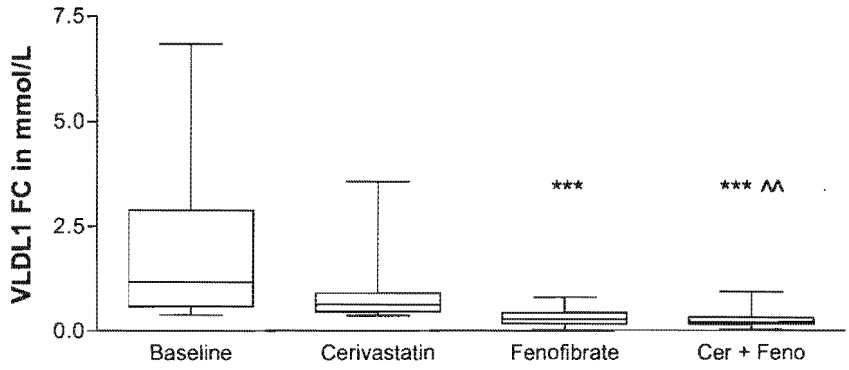
The levels of FC in VLDL1 responded similarly to TC to lipid-lowering therapy. FC concentration decreased with all therapies (ANOVA, P<0.0001), but the change observed with cerivastatin did not reach statistical significance. There were no differences between the two apoE genotypes studied (data not shown).

Table 4-22 VLDL1 FC response to lipid-lowering therapy

VLDL1 FC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.86	1.16	1.82	18	0.38-6.85	>0.05	<0.001	<0.001
Cerivastatin	0.86	0.62	0.85	13	0.37-3.57		>0.05	<0.01
Fenofibrate	0.33	0.28	0.22	13	0.02-0.81			>0.05
Combination	0.28	0.20	0.26	9	0.03-0.94			

[1] Data log transformed prior to analysis

Figure 4-25 VLDL1 FC and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.3.5 VLDL1 cholesterol ester

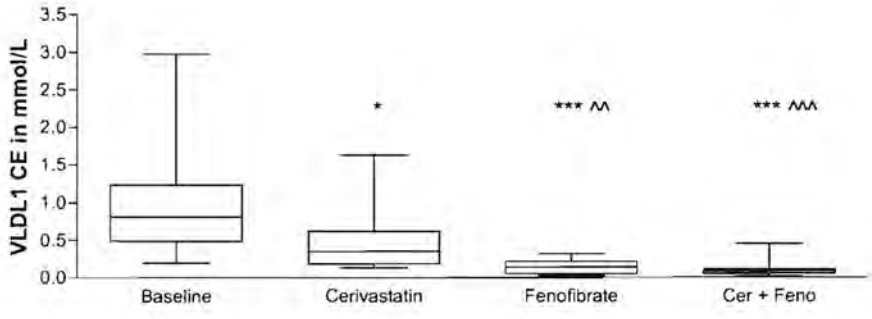
VLDL1 CE decreased significantly from baseline in all treatment arms (ANOVA, P<0.0001). The decrease was greatest for combination therapy followed by fenofibrate and cerivastatin treatment. There were no significant differences at any stage in VLDL1 CE between the two apoE genotypes studied (data not shown).

Table 4-23 VLDL1 CE and lipid-lowering therapy

VLDL1 CE (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.03	0.81	0.77	18	0.20-2.98	<0.05	<0.001	<0.001
Cerivastatin	0.46	0.35	0.40	13	0.13-1.63		<0.01	<0.001
Fenofibrate	0.15	0.14	0.09	9	0.03-0.32			>0.05
Combination	0.12	0.09	0.13	9	0.02-0.45			

[1] Data log transformed prior to analysis

Figure 4-26 VLDL1 CE and lipid-lowering therapy



Legend: * *P<0.05 vs. baseline* *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin* ^^^ *P<0.001 vs. cerivastatin*

4.2.1.5.3.6 VLDL1 phospholipid

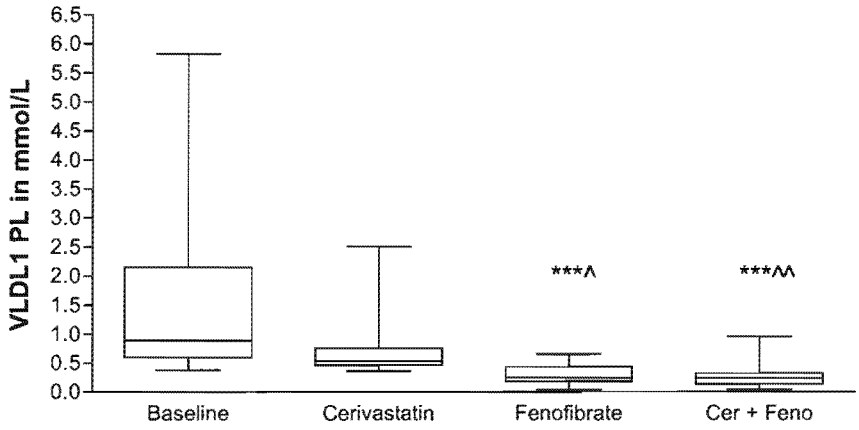
The PL content of the VLDL1 fraction decreased with all lipid-lowering treatments (ANOVA, P<0.0001), but the decrease was not statistically significant with cerivastatin. There were no significant differences in VLDL1 PL at any stage between the two apoE genotypes studied (data not shown).

Table 4-24 VLDL1 phospholipid concentration and lipid-lowering therapy

VLDL1 PL (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.56	0.89	1.55	18	0.38-5.83	>0.05	<0.001	<0.001
Cerivastatin	0.75	0.53	0.58	13	0.36-2.51		<0.05	<0.01
Fenofibrate	0.30	0.25	0.17	13	0.03-0.65			>0.05
Combination	0.29	0.24	0.27	9	0.03-0.96			

[1] Data log transformed prior to analysis

Figure 4-27 VLDL1 PL and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^ *P<0.05 vs. cerivastatin* ^^ *P<0.01 vs. cerivastatin*

4.2.1.5.4 VLDL2

4.2.1.5.4.1 VLDL2 protein

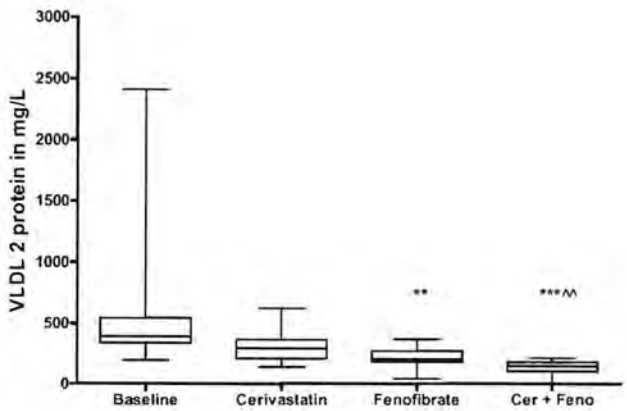
VLDL2 protein concentration decreased from baseline with all therapies (ANOVA, P<0.0001), but the change seen with cerivastatin was not significant on post-test analysis. There were no significant differences at any stage between the two apoE genotypes studied (data not shown).

Table 4-25 VLDL2 protein concentration and lipid-lowering therapy

VLDL2 protein (mg/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	563	389	503	18	199-2410	>0.05	<0.01	<0.001
Cerivastatin	301	294	123	13	139-620		>0.05	<0.01
Fenofibrate	216	205	79	13	40-369			>0.05
Combination	137	148	63	9	6-215			

[1] Data log transformed prior to analysis

Figure 4-28 VLDL2 protein and lipid-lowering therapy



Legend: ** *P<0.01 vs. baseline* *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.4.2 VLDL2 triglycerides

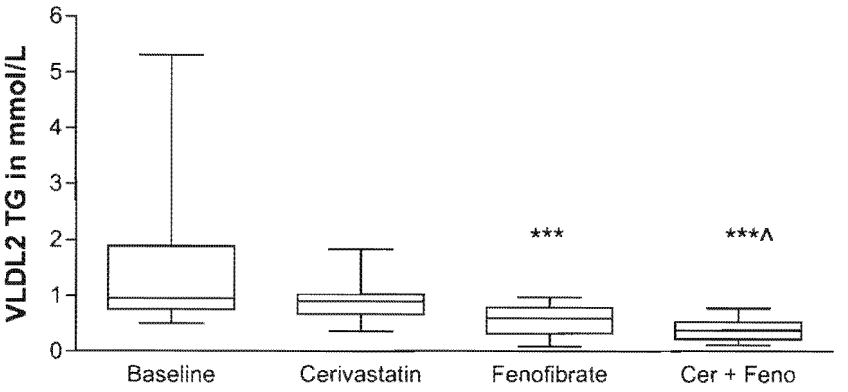
VLDL2 TG concentration decreased from baseline values in all three treatment arms (ANOVA, P<0.0001), but the change with cerivastatin did not reach statistical significance on post test. There were no statistically significant differences between the two apoE genotypes studied (data not shown).

Table 4-26 VLDL2 TG concentration and lipid-lowering therapy

VLDL2 TG (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.42	0.95	1.20	18	0.50-5.31	>0.05	<0.001	<0.001
Cerivastatin	0.86	0.89	0.37	13	0.36-1.83		>0.05	<0.05
Fenofibrate	0.55	0.59	0.27	13	0.09-0.97			>0.05
Combination	0.39	0.38	0.20	9	0.11-0.77			

[1] Data log transformed prior to analysis

Figure 4-29 VLDL2 TG and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^ *P<0.05 vs. cerivastatin*

4.2.1.5.4.3 VLDL2 total cholesterol

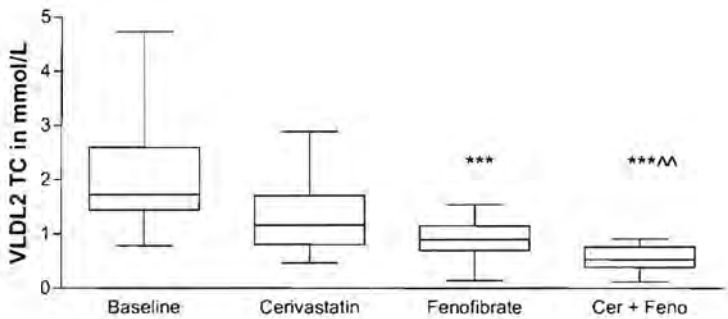
VLDL2 TC concentration decreased from baseline with all lipid-lowering therapies (ANOVA, P<0.0001), but the change seen with cerivastatin did not reach statistical significance. At baseline the VLDL2 TC was significantly lower in patients with the apoE (R145C) than that seen in apoE2 homozygotes (2.59 ± 1.04 vs. 1.39 ± 0.22, P=0.01 by unpaired t-test for apoE2 homozygotes vs. apoE (R145C) mutation carries). With lipid-lowering therapy no statistically significant differences were observed between the two genotypes.

Table 4-27 VLDL2 TC concentration and lipid-lowering therapy

VLDL2 TC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	2.10	1.72	1.03	18	0.78-4.73	>0.05	<0.001	<0.001
Cerivastatin	1.32	1.16	0.68	13	0.472.90		>0.05	<0.01
Fenofibrate	0.89	0.90	0.35	13	0.14-1.55			>0.05
Combination	0.54	0.53	0.26	9	0.12-0.92			

[1] Data log transformed prior to analysis

Figure 4-30 VLDL2 TC and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.4.4 VLDL2 free cholesterol

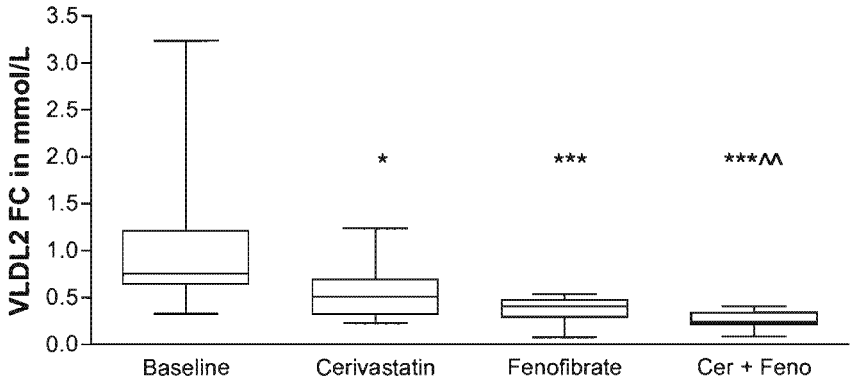
VLDL2 FC concentration decreased significantly from baseline in all treatment arms (ANOVA, P<0.0001). The apoE genotype did not exert any significant influence on VLDL2 FC (data not shown).

Table 4-28 VLDL2 FC and lipid-lowering therapy

VLDL2 FC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.00	0.75	0.65	18	0.33-3.23	<0.05	<0.001	<0.001
Cerivastatin	0.55	0.51	0.27	13	0.23-1.23		>0.05	<0.01
Fenofibrate	0.37	0.40	0.13	13	0.07-0.53			>0.05
Combination	0.25	0.23	0.10	9	0.08-0.40			

[1] Data log transformed prior to analysis

Figure 4-31 VLDL2 FC and lipid-lowering therapy



Legend: * *P<0.05 vs. baseline* *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.4.5 VLDL2 cholesterol ester

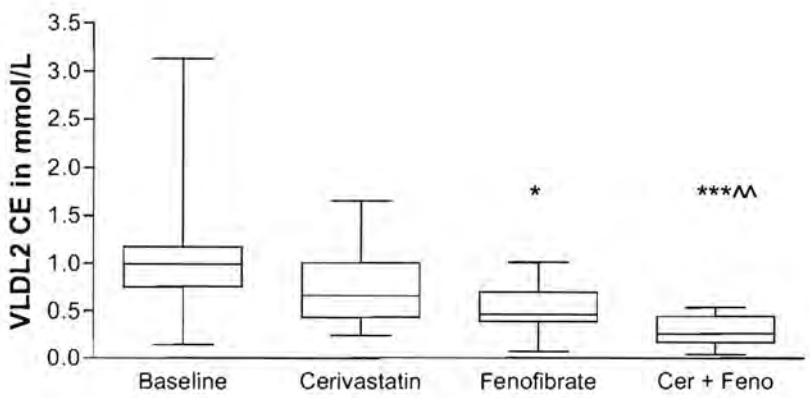
VLDL2 CE concentration decreased from baseline values in all three treatment arms (ANOVA, P<0.0001), but the change with cerivastatin was not statistically significant on post test. There were no significant differences at any stage between the two apoE genotypes studied (data not shown).

Table 4-29 VLDL2 CE concentration and lipid-lowering therapy

VLDL2 CE (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.10	1.00	0.72	18	0.14-3.13	>0.05	<0.05	<0.001
Cerivastatin	0.77	0.66	0.43	13	0.24-1.66		>0.05	<0.01
Fenofibrate	0.52	0.47	0.24	13	0.07-1.01			>0.05
Combination	0.29	0.26	0.17	9	0.04-0.53			

[1] Data log transformed prior to analysis

Figure 4-32 VLDL2 CE and lipid-lowering therapy



Legend: * *P<0.05 vs. baseline* *** *P<0.001 vs. baseline*

^^ *P<0.01 vs. cerivastatin*

4.2.1.5.4.6 VLDL2 phospholipid

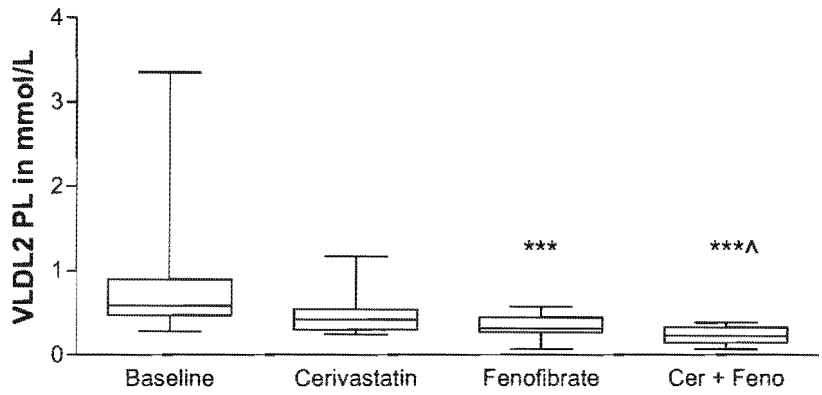
VLDL2 PL concentration decreased with lipid-lowering therapy (ANOVA, P<0.0001), but as with the other components of VLDL2 the change seen with cerivastatin did not reach statistical significance. There were also no differences at any stage between the two apoE genotypes studied in VLDL2 PL levels (data not shown).

Table 4-30 VLDL2 PL concentration and lipid-lowering therapy

VLDL2 PL (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.85	0.59	0.74	18	0.28-3.35	>0.05	<0.001	<0.001
Cerivastatin	0.48	0.42	0.25	13	0.24-1.17		>0.05	<0.05
Fenofibrate	0.34	0.32	0.13	13	0.07-0.58			>0.05
Combination	0.23	0.22	0.10	9	0.07-0.39			

[1] Data log transformed prior to analysis

Figure 4-33 VLDL2 PL and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^ *P<0.05 vs. cerivastatin*

4.2.1.5.5 IDL

4.2.1.5.5.1 IDL protein

The protein content of the IDL fraction decreased from baseline in all treatment arms (ANOVA, P=0.0005), but differing from the results seen in the fractions with larger lipoproteins (VLDL1 + VLDL2) changes with cerivastatin reached statistical significance, while those with fenofibrate were not statistically significant. The apoE

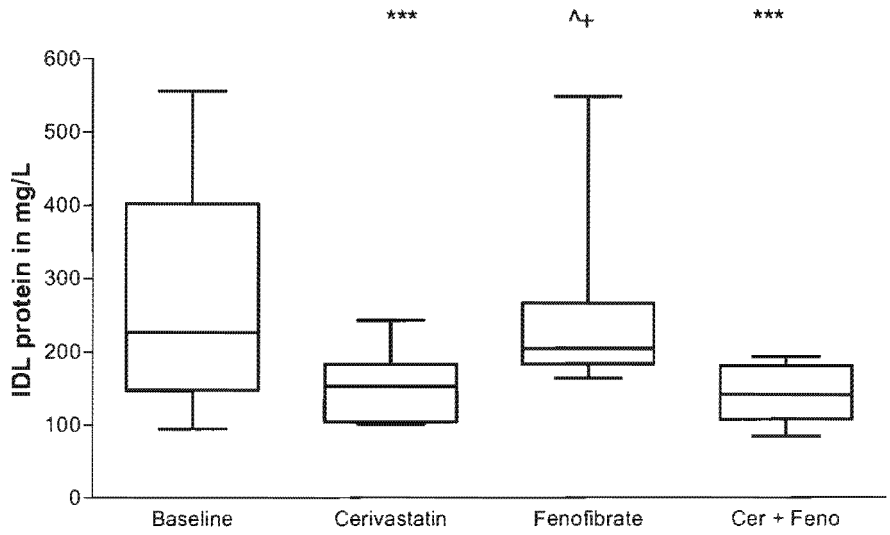
genotype did not exert a statistically significant influence on IDL protein levels at any stage (data not shown).

Table 4-31 IDL protein and lipid-lowering therapy

IDL protein (mg/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	270	226	139	18	95-556	<0.001	>0.05	<0.001
Cerivastatin	153	153	50	13	100-243		<0.05	>0.05
Fenofibrate	242	204	100	13	164-548			<0.05
Combination	143	141	39	9	84-193			

[1] Data log transformed prior to analysis

Figure 4-34 IDL protein and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

^ *P<0.05 vs. cerivastatin*

\+ *P<0.05 vs. combination therapy*

4.2.1.5.5.2 IDL triglycerides

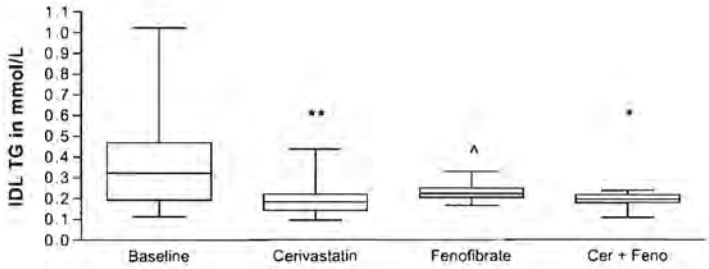
Lipid-lowering therapy was associated with lowering of IDL TG concentration in all three treatment arms (ANOVSA, P=0.0025), but the change was only significant when cerivastatin formed part of the therapy. The two apoE genotypes studied did not show statistically significant differences at any stage (data not shown).

Table 4-32 IDL TG concentration and lipid-lowering therapy

IDL TG (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.35	0.32	0.21	18	0.11-1.02	<0.01	>0.05	<0.05
Cerivastatin	0.19	0.18	0.09	13	0.1-0.44		<0.05	>0.05
Fenofibrate	0.23	0.22	0.04	13	0.17-0.33			>0.05
Combination	0.19	0.20	0.04	9	0.11-0.24			

[1] Data log transformed prior to analysis

Figure 4-35 IDL TG and lipid-lowering therapy



Legend: * *P<0.05 vs. baseline* ** *P<0.01 vs. baseline*

^ *P<0.05 vs. cerivastatin*

4.2.1.5.5.3 IDL total cholesterol

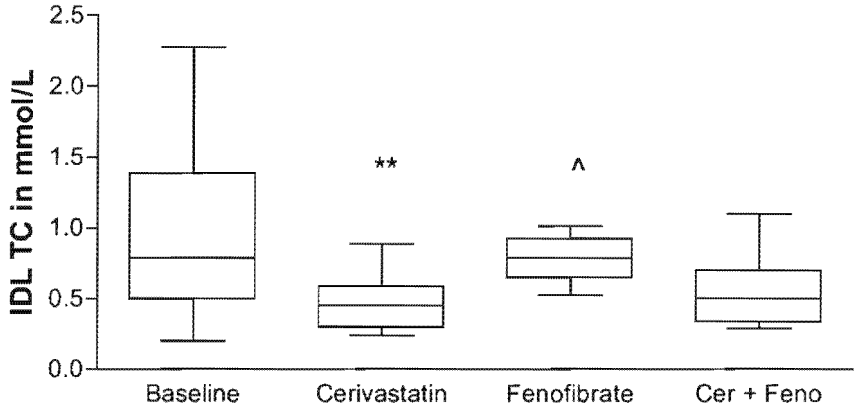
IDL TC concentration fell from baseline once lipid-lowering treatment was provided (ANOVA, P=0.0012), but this change was only statistically significant for cerivastatin monotherapy. At baseline IDL TC was significantly lower in patients with the apoE (R145C) mutation when compared to apoE2 homozygotes (1.11 ± 0.54 mmol/L vs. 0.44 ± 0.19 mmol/L for apoE2/E2 vs. apoE (R145C) IDL TC, P=0.01 by unpaired t-test). Once lipid-lowering treatment had been started the difference in IDL TC was no longer statistically significant (data not shown).

Table 4-33 IDL TC concentration and lipid-lowering therapy

IDL TC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.97	0.79	0.56	18	0.20-2.27	<0.01	>0.05	>0.05
Cerivastatin	0.47	0.46	0.20	13	0.24-0.89		<0.05	>0.05
Fenofibrate	0.79	0.79	0.16	13	0.53-1.01			>0.05
Combination	0.55	0.50	0.26	9	0.29-1.10			

[1] Data log transformed prior to analysis

Figure 4-36 IDL TC and lipid-lowering therapy



Legend: ** *P<0.01 vs. baseline*

^ *P<0.05 vs. cerivastatin*

4.2.1.5.5.4 IDL free cholesterol

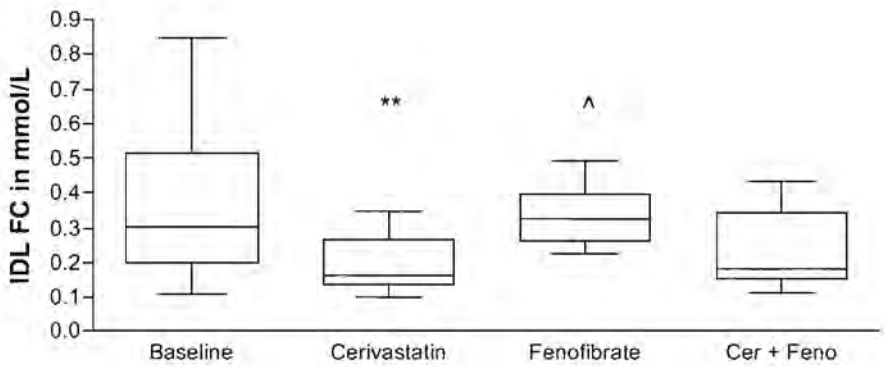
IDL FC concentration responded similarly to TC to lipid-lowering therapy, with a decrease from baseline values in all treatment arms (ANOVA, P=0.0032), but statistical significance on post-testing only in the cerivastatin group. IDL FC was significantly lower (at baseline only) in the apoE (R145C) group compared to apoE2 homozygotes (0.44 ± 0.22 mmol/L vs. 0.18 ± 0.05 mmol/L for apoE2/E2 vs. apoE (R145C) IDL TC, P=0.02 by unpaired t-test).

Table 4-34 IDL FC concentration and lipid-lowering therapy

IDL FC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.38	0.30	0.22	18	0.11-0.85	<0.01	>0.05	>0.05
Cerivastatin	0.20	0.16	0.08	13	0.10-0.35		<0.05	>0.05
Fenofibrate	0.33	0.33	0.08	13	0.23-0.49			>0.05
Combination	0.24	0.18	0.11	9	0.12-0.43			

[1] Data log transformed prior to analysis

Figure 4-37 IDL FC and lipid-lowering therapy



Legend: ** *P<0.01 vs. baseline*

^ *P<0.05 vs. cerivastatin*

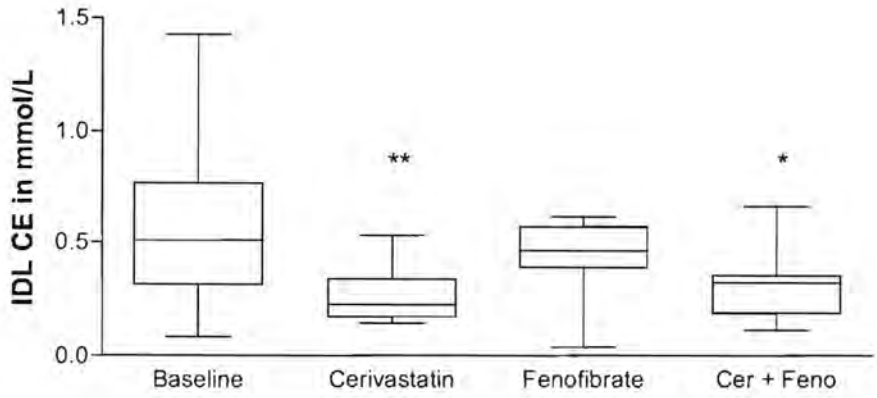
4.2.1.5.5.5 IDL cholesterol ester

IDL CE concentration decreased from baseline in all treatment arms (ANOVA, P=0.0036), but the change with fenofibrate monotherapy did not reach statistical significance on post test. The lower IDL TC level in the apoE (R145C) mutation carriers at baseline is due to both lower FC and CE values (0.66± 0.35 mmol/L vs. 0.25± 0.14 mmol/L for apoE2/E2 vs. apoE (R145C) IDL TC, P=0.009 by unpaired t-test).

Table 4-35 IDL CE concentration and lipid-lowering therapy

	IDL CE					Tukey's multiple comparison test		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.58	0.51	0.35	18	0.09-1.43	<0.01	>0.05	<0.05
Cerivastatin	0.27	0.23	0.12	13	0.14-0.54		>0.05	>0.05
Fenofibrate	0.46	0.47	0.15	13	0.04-0.61			>0.05
Combination	0.31	0.32	0.16	9	0.12-0.66			

Figure 4-38 IDL CE and lipid-lowering therapy



*Legend: * P<0.05 vs. baseline ** P<0.01 vs. baseline*

4.2.1.5.5.6 IDL phospholipid

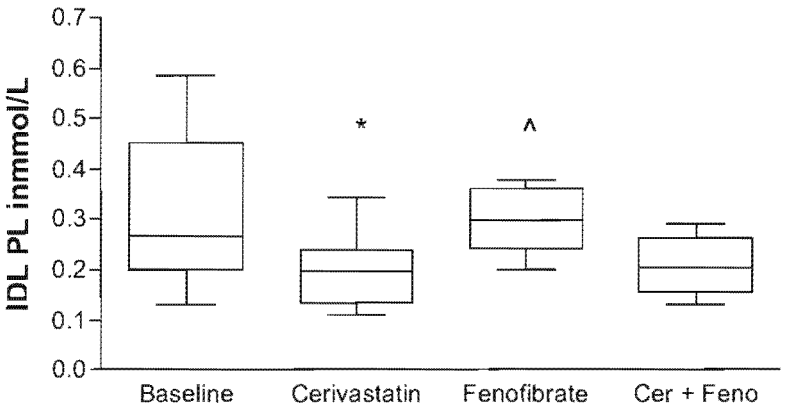
The PL concentration in IDL decreased when cerivastatin was prescribed, either as monotherapy or in combination with fenofibrate (ANOVA, P=0.0023). The final concentrations of PL were similar in both cerivastatin-containing arms, but on post test the change was not significant in the combination therapy arm, likely due to the smaller number of patients in this treatment arm.

Table 4-36 IDL PL concentration and lipid-lowering therapy

IDL PL (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.97	0.79	0.56	18	0.20-2.27	<0.01	>0.05	>0.05
Cerivastatin	0.47	0.46	0.20	13	0.24-0.89		<0.05	>0.05
Fenofibrate	0.79	0.79	0.16	13	0.53-1.01			>0.05
Combination	0.55	0.50	0.26	9	0.29-1.10			

[1] Data log transformed prior to analysis

Figure 4-39 IDL PL and lipid-lowering therapy



Legend: ** *P<0.01 vs. baseline*

^ *P<0.05 vs. cerivastatin*

4.2.1.5.6 LDL fraction

4.2.1.5.6.1 LDL protein

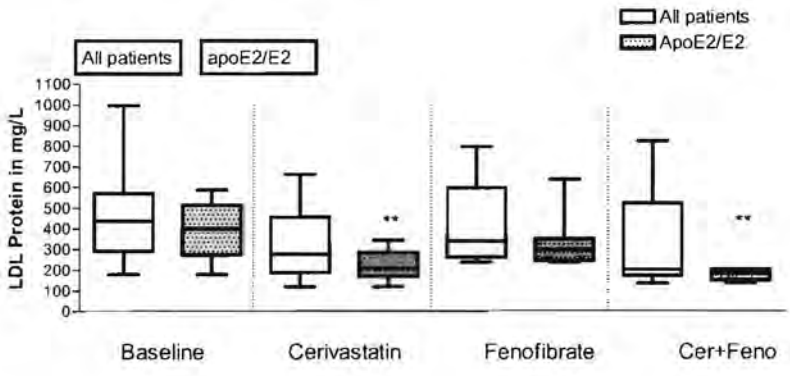
Lipid-lowering therapy was associated with small changes in LDL protein concentration. LDL protein concentration decreased with cerivastatin-containing therapy, but none of the changes reached statistical significance (ANOVA, P=0.07). ApoE2 homozygotes showed a significant decrease in LDL protein with cerivastatin and combination therapy (ANOVA, P=0.0006). Although there was no significant difference between apoE2 homozygotes and apoE (R145C) carriers at baseline, LDL protein was significantly lower in apoE2 homozygotes in each treatment arm (data shown in figure 4.40).

Table 4-37 LDL protein concentration and lipid lowering therapy

	LDL protein (mg/L)					Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	482	437	239	18	180-997	>0.05	>0.05	>0.05
Cerivastatin	326	277	174	13	120-664		>0.05	>0.05
Fenofibrate	425	339	190	13	238-797			>0.05
Combination	337	203	234	9	136-823			

[1] Data log transformed prior to analysis

Figure 4-40 LDL protein and lipid-lowering therapy



*Legend: ** P<0.01 vs. baseline*

All significance levels refer to statistical analyses within the cohort of apoE2 homozygotes.

4.2.1.5.6.2 LDL triglycerides

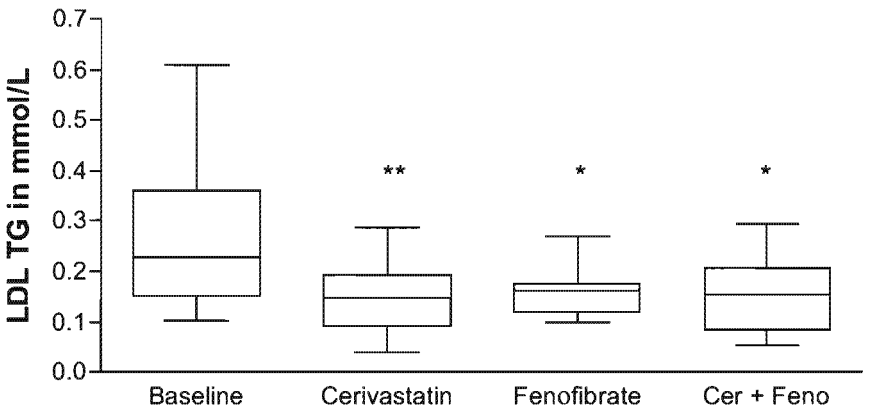
Lipid-lowering treatment reduced the TG concentration within LDL to a similar extent in all treatment arms (ANOVA, P=0.0028). The apoE genotype did not influence LDL TG levels in a significant fashion (data not shown).

Table 4-38 LDL TG concentration and lipid-lowering therapy

LDL TG (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.27	0.23	0.14	18	0.10-0.61	<0.01	<0.05	<0.05
Cerivastatin	0.15	0.15	0.07	13	0.04-0.29		>0.05	>0.05
Fenofibrate	0.16	0.16	0.04	13	0.10-0.27			>0.05
Combination	0.15	0.16	0.07	9	0.05-0.30			

[1] Data log transformed prior to analysis

Figure 4-41 LDL TG and lipid-lowering therapy



Legend: * *P<0.05 vs. baseline* ** *P<0.01 vs. baseline*

4.2.1.5.6.3 LDL total cholesterol

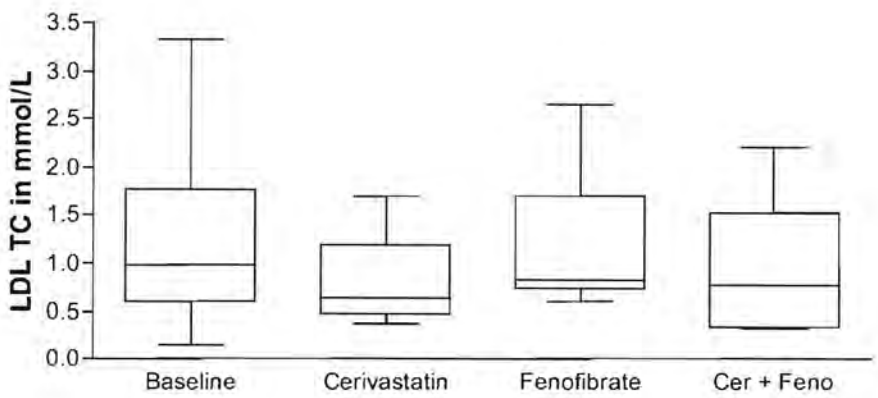
LDL TC levels were generally low at baseline, as is to be expected in patients with dysbetalipoproteinaemia. Levels decreased with lipid-lowering therapy, but none of the changes was statistically significant (ANOVA, P=0.42).

Table 4-39 LDL TC concentration and lipid-lowering therapy

	LDL TC (mmol/L)					Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	1.25	0.98	0.91	18	0.14-3.33	>0.05	>0.05	>0.05
Cerivastatin	0.83	0.64	0.44	13	0.37-1.71		>0.05	>0.05
Fenofibrate	1.18	0.82	0.66	13	0.60-2.65			>0.05
Combination	0.96	0.78	0.67	9	0.31-2.21			

[1] Data log transformed prior to analysis

Figure 4-42 LDL TC and lipid-lowering therapy



4.2.1.5.6.4 LDL free cholesterol

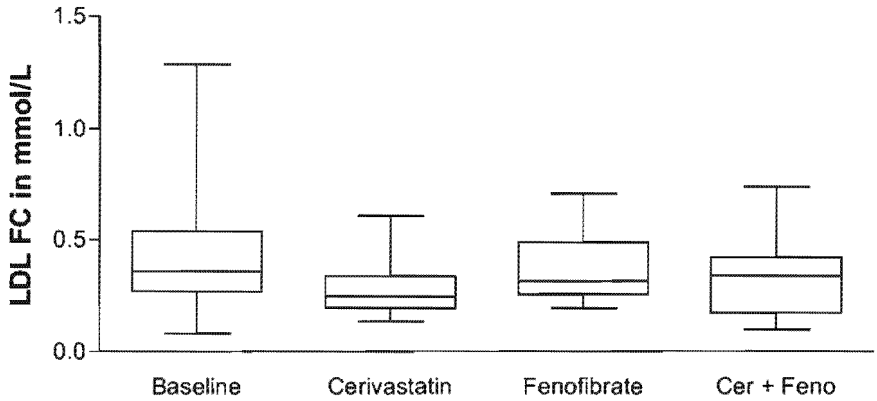
LDL FC concentration did not change significantly with the initiation of lipid-lowering treatment (ANOVA, P=0.27). There were no statistically significant differences in LDL FC between the two apoE genotypes studied (data not shown).

Table 4-40 LDL FC concentration and lipid-lowering treatment

LDL FC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.44	0.36	0.28	18	0.09-1.28	>0.05	>0.05	>0.05
Cerivastatin	0.28	0.25	0.13	13	0.14-0.61		>0.05	>0.05
Fenofibrate	0.37	0.31	0.16	13	0.20-0.71			>0.05
Combination	0.33	0.34	0.20	9	0.10-0.74			

[1] Data log transformed prior to analysis

Figure 4-43 LDL FC and lipid-lowering therapy



4.2.1.5.6.5 LDL CE

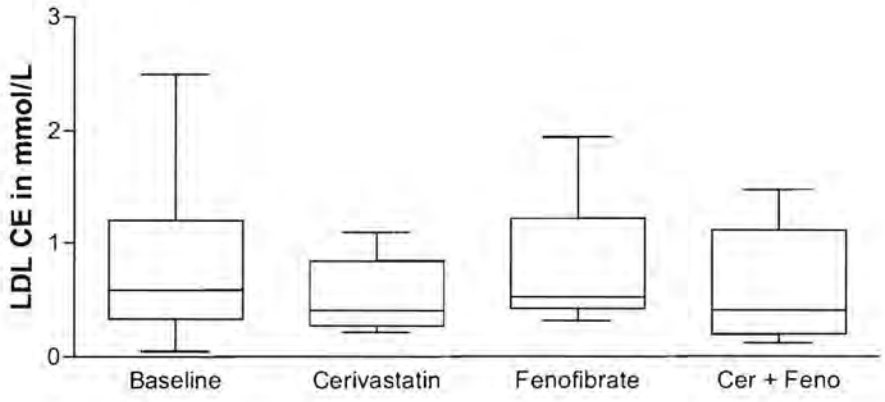
LDL CE concentration was lowest when cerivastatin-containing therapy was given, but none of the differences in LDL CE reached statistical significance (ANOVA, P=0.50). There were no statistically significant differences in LDL CE between the two apoE genotypes studied (data not shown).

Table 4-41 LDL CE concentration and lipid-lowering therapy

LDL CE (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.81	0.58	0.66	18	0.05-2.49	>0.05	>0.05	>0.05
Cerivastatin	0.54	0.40	0.32	13	0.22-1.09		>0.05	>0.05
Fenofibrate	0.81	0.52	0.51	13	0.31-1.94			>0.05
Combination	0.63	0.40	0.49	9	0.13-1.48			

[1] Data log transformed prior to analysis

Figure 4-44 LDL CE and lipid-lowering therapy



4.2.1.5.6.6 LDL phospholipid

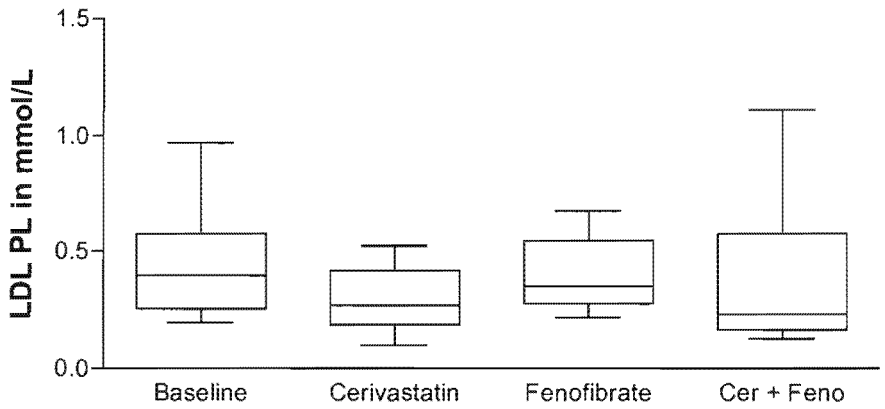
LDL PL concentration was lowest with cerivastatin therapy. None of the changes seen in LDL PL were statistically significant (ANOVA, P=0.16). There were no statistically significant differences in LDL PL between the two apoE genotypes studied (data not shown).

Table 4-42 LDL PL concentration and lipid-lowering therapy

LDL PL (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.45	0.40	0.24	18	0.19-0.97	>0.05	>0.05	>0.05
Cerivastatin	0.30	0.27	0.14	13	0.10-0.53		>0.05	>0.05
Fenofibrate	0.40	0.35	0.16	13	0.22-0.68			>0.05
Combination	0.39	0.24	0.32	9	0.13-1.11			

[1] Data log transformed prior to analysis

Figure 4-45 LDL PL and lipid-lowering therapy



4.2.1.5.7 HDL fraction

HDL was collected in five fractions following sequential ultracentrifugation. The data presented here represents the sum of all the fractions collected.

4.2.1.5.7.1 HDL protein

Denser HDL fractions were contaminated by plasma proteins, resulting in very high levels of measured protein. The protein concentrations are several orders of magnitude higher than those measured in other fractions and therefore clearly do not reflect levels of HDL associated proteins. The protein data will therefore not be presented here.

4.2.1.5.7.2 HDL triglycerides

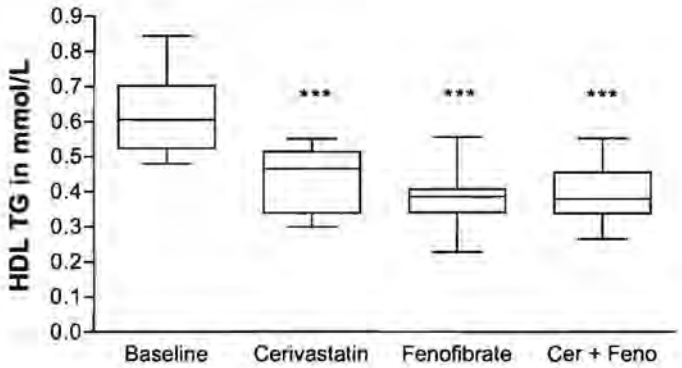
Although HDL is not a TGRL lipid-lowering therapy was associated with a definite decrease in HDL TG mass from baseline (ANOVA, P<0.0001). HDL TG mass decreased in all three treatment arms.

Table 4-43 HDL TG and lipid-lowering therapy

HDL TG (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.62	0.61	0.10	18	0.48-0.84	<0.001	<0.001	<0.001
Cerivastatin	0.44	0.47	0.09	13	0.30-0.55		>0.05	>0.05
Fenofibrate	0.39	0.39	0.08	13	0.23-0.56			>0.05
Combination	0.40	0.38	0.09	9	0.27-0.55			

[1] Data log transformed prior to analysis

Figure 4-46 HDL TG and lipid-lowering therapy



Legend: *** *P<0.001 vs. baseline*

4.2.1.5.7.3 HDL total cholesterol

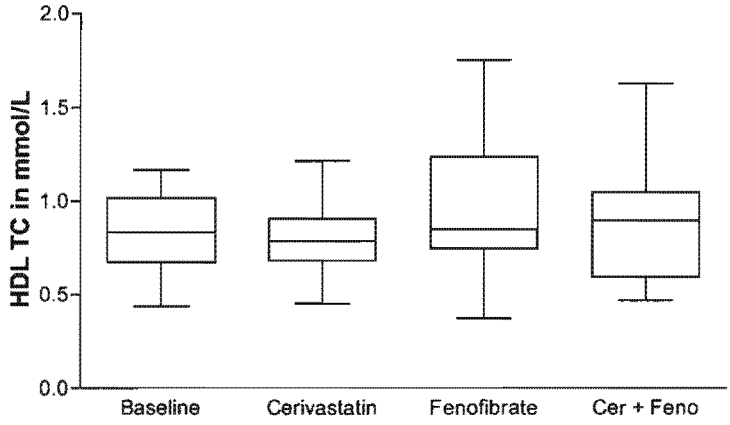
The HDL TC concentration was low and increased when fibrates were prescribed. However, none of the changes in HDL TC reached statistical significance (ANOVA, P=0.59).

Table 4-44 HDL TC and lipid lowering therapy

HDL TC (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.84	0.84	0.21	18	0.44-1.17	>0.05	>0.05	>0.05
Cerivastatin	0.81	0.79	0.23	13	0.45-1.22		>0.05	>0.05
Fenofibrate	0.95	0.85	0.36	13	0.37-1.75			>0.05
Combination	0.89	0.90	0.35	9	0.47-1.63			

[1] Data log transformed prior to analysis

Figure 4-47 HDL TC and lipid-lowering therapy



4.2.1.5.7.4 HDL free cholesterol

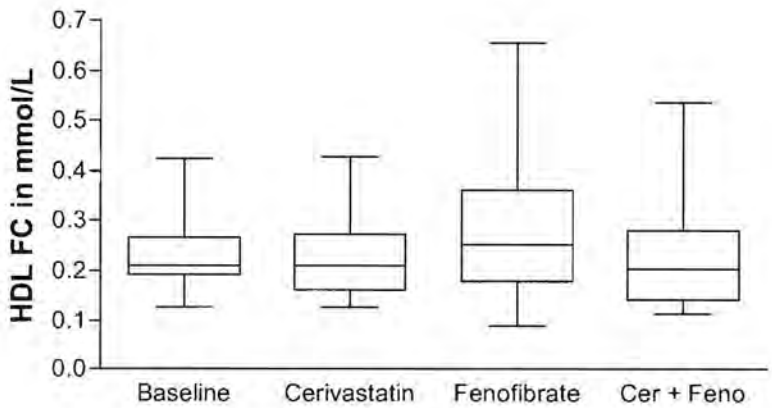
HDL FC concentration levels did not change significantly with lipid-lowering treatment (ANOVA, P=0.52).

Table 4-45 HDL FC concentration and lipid-lowering therapy

	HDL FC (mmol/L)					Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combinatior
Baseline	0.24	0.21	0.08	18	0.13-0.43	>0.05	>0.05	>0.05
Cerivastatin	0.22	0.21	0.08	13	0.13-0.43		>0.05	>0.05
Fenofibrate	0.28	0.25	0.14	13	0.09-0.66			>0.05
Combination	0.23	0.20	0.13	9	0.11-0.54			

[1] Data log transformed prior to analysis

Figure 4-48 HDL FC and lipid-lowering therapy



4.2.1.5.7.5 HDL cholesterol ester

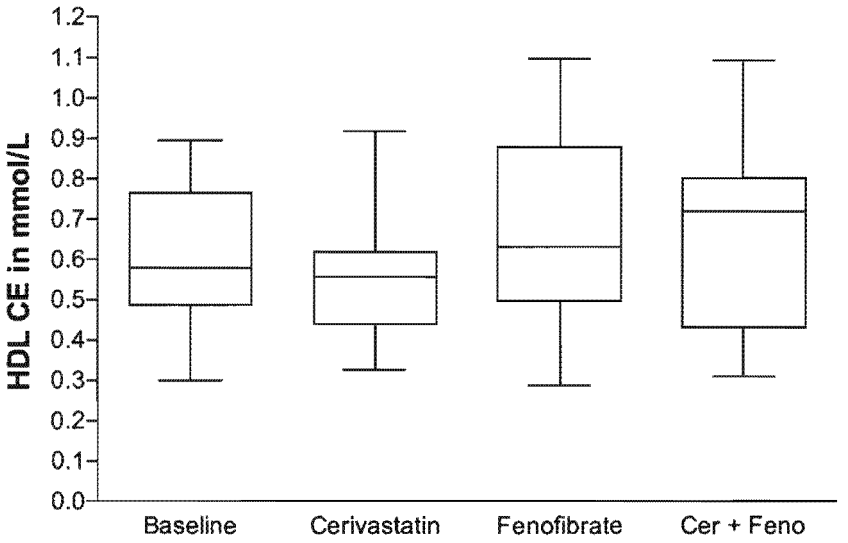
HDL CE concentration increased from baseline when fenofibrate-containing therapy was prescribed, but none of the changes seen were statistically significant (ANOVA, P=0.61).

Table 4-46 HDL CE concentration and lipid-lowering therapy

HDL CE (mmol/L)						Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.61	0.58	0.18	18	0.30-0.90	>0.05	>0.05	>0.05
Cerivastatin	0.56	0.57	0.18	13	0.33-0.92		>0.05	>0.05
Fenofibrate	0.67	0.63	0.24	13	0.29-1.10			>0.05
Combination	0.65	0.72	0.24	9	0.31-1.10			

[1] Data log transformed prior to analysis

Figure 4-49 HDL CE and lipid-lowering therapy



4.2.1.5.7.6 HDL phospholipid

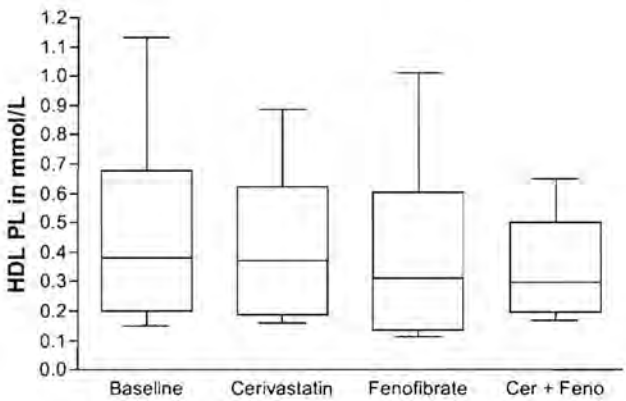
HDL PL concentration levels were lower in patients on fibrate-containing therapy, but none of the changes in HDL PL were significant (ANOVA, P=0.83).

Table 4-47 HDL PL concentration and lipid-lowering therapy

	HDL PL (mmol/L)					Tukey's multiple comparison test [1]		
	Mean	Median	SD	N	Range	P vs. Cerivastatin	P vs. Fenofibrate	P vs. Combination
Baseline	0.44	0.38	0.27	18	0.15-1.13	>0.05	>0.05	>0.05
Cerivastatin	0.41	0.37	0.26	13	0.16-0.88		>0.05	>0.05
Fenofibrate	0.38	0.31	0.29	13	0.11-1.01			>0.05
Combination	0.35	0.30	0.17	9	0.17-0.65			

[1] Data log transformed prior to analysis

Figure 4-50 HDL PL and lipid-lowering therapy



4.2.1.6 Lipoproteins fractions: compositional changes

This section presents a brief overview of the relative compositional changes in the various lipoprotein fractions. Detailed data on compositional changes will only be presented where of relevance.

4.2.1.6.1 VLDL1 fractional composition

Table 4-48 VLDL1 fractional composition

VLDL1		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
Prot/TM[1]	(Mean ± SD)	0.10 ± 0.02	0.10 ± 0.01	0.11 ± 0.03	0.12 ± 0.10	0.54
	P vs. baseline		>0.05	>0.05	>0.05	
TG/TM	(Mean ± SD)	0.53 ± 0.06	0.60 ± 0.05	0.57 ± 0.07	0.60 ± 0.10	0.07
	P vs. baseline		>0.05	>0.05	>0.05	
TC/TM	(Mean ± SD)	0.16 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.10 ± 0.02	0.0002
	P vs. baseline		<0.05	<0.05	<0.001	
FC/TM	(Mean ± SD)	0.10 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.01	0.03
	P vs. baseline		>0.05	>0.05	<0.05	
CE/TM	(Mean ± SD)	0.11 ± 0.05	0.08 ± 0.03	0.08 ± 0.04	0.05 ± 0.02	0.001
	P vs. baseline		>0.05	<0.05	<0.01	
PL/TM	(Mean ± SD)	0.16 ± 0.03	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	0.09
	P vs. baseline		>0.05	>0.05	>0.05	

VLDL1		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
FC/TC	(Mean ± SD)	0.60 ± 0.13	0.65 ± 0.10	0.66 ± 0.12	0.70 ± 0.07	0.16
	P vs. baseline		>0.05	>0.05	>0.05	
FC/PL	(Mean ± SD)	0.61 ± 0.15	0.56 ± 0.11	0.53 ± 0.10	0.50 ± 0.09	0.08
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TG	(Mean ± SD)	0.22 ± 0.10	0.13 ± 0.07	0.14 ± 0.08	0.09 ± 0.04	0.001
	P vs. baseline		<0.05	<0.05	<0.01	
TC/Prot	(Mean ± SD)	1.80 ± 0.57	1.38 ± 0.42	1.25 ± 0.42	1.15 ± 0.60	0.006
	P vs. baseline		>0.05	<0.05	<0.05	
TG/Prot	(Mean ± SD)	5.91 ± 1.81	6.46 ± 0.76	5.58 ± 1.58	6.63 ± 2.57	0.44
	P vs. baseline		>0.05	>0.05	>0.05	
PL/Prot	(Mean ± SD)	1.74 ± 0.41	1.55 ± 0.25	1.51 ± 0.31	1.56 ± 0.56	0.36
	P vs. baseline		>0.05	>0.05	>0.05	

[1] TM=Total mass (sum of TG, TC, PL and protein)

4.2.1.6.2 VLDL2 fractional composition

Table 4-49 VLDL2 fractional composition

VLDL2		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
Prot/TM[1]	(Mean ± SD)	0.15 ± 0.03	0.14 ± 0.01	0.16 ± 0.04	0.14 ±0.05	0.42
	P vs. baseline		>0.05	>0.05	>0.05	
TG/TM	(Mean ± SD)	0.33 ± 0.10	0.35 ± 0.07	0.32 ± 0.08	0.36 ± 0.07	0.64
	P vs. baseline		>0.05	>0.05	>0.05	
TC/TM	(Mean ± SD)	0.25 ± 0.06	0.24 ± 0.05	0.24 ±0.04	0.23 ± 0.06	0.74
	P vs. baseline		>0.05	>0.05	>0.05	
FC/TM	(Mean ± SD)	0.11 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.21
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TM	(Mean ± SD)	0.23 ± 0.09	0.23 ± 0.06	0.24 ± 0.05	0.20 ± 0.08	0.57
	P vs. baseline		>0.05	>0.05	>0.05	
PL/TM	(Mean ± SD)	0.17 ± 0.03	0.16 ± 0.02	0.18 ± 0.02	0.19 ± 0.03	0.08
	P vs. baseline		>0.05	>0.05	>0.05	
FC/TC	(Mean ± SD)	0.47 ± 0.14	0.43 ± 0.04	0.43 ± 0.05	0.50 ± 0.01	0.22
	P vs. baseline		>0.05	>0.05	>0.05	
FC/PL	(Mean ± SD)	0.67 ± 0.16	0.60 ± 0.12	0.57 ± 0.08	0.61 ± 0.18	0.27
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TG	(Mean ± SD)	0.81 ± 0.47	0.70 ± 0.28	0.81 ± 0.36	0.61 ± 0.41	0.53
	P vs. baseline		>0.05	>0.05	>0.05	

VLDL2		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
TC/Prot	(Mean ± SD)	1.64 ± 0.36	1.66 ± 0.37	1.60 ± 0.30	2.32 ± 2.35	0.32
	P vs. baseline		>0.05	>0.05	>0.05	
TG/Prot	(Mean ± SD)	2.22 ± 1.04	2.45 ± 0.41	2.18 ± 0.75	3.96 ± 4.94	0.20
	P vs. baseline		>0.05	>0.05	>0.05	
PL/Prot	(Mean ± SD)	1.13 ± 0.23	1.17 ± 0.20	1.20 ± 0.25	2.10 ± 2.63	0.16
	P vs. baseline		>0.05	>0.05	>0.05	

[l] TM=Total mass (sum of TG, TC, PL and protein)

4.2.1.6.3 IDL fractional composition

Table 4-50 IDL fractional composition

IDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
Prot/TM[l]	(Mean ± SD)	0.20 ± 0.06	0.21 ± 0.02	0.22 ± 0.05	0.19 ± 0.04	0.59
	P vs. baseline		>0.05	>0.05	>0.05	
TG/TM	(Mean ± SD)	0.23 ± 0.09	0.23 ± 0.05	0.18 ± 0.04	0.22 ± 0.05	0.19
	P vs. baseline		>0.05	>0.05	>0.05	
TC/TM	(Mean ± SD)	0.27 ± 0.05	0.25 ± 0.04	0.28 ± 0.02	0.27 ± 0.07	0.22
	P vs. baseline		.0.05	>0.05	>0.05	
FC/TM	(Mean ± SD)	0.11 ± 0.02	0.10 ± 0.02	0.12 ± 0.04	0.12 ± 0.03	0.38

IDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TM	(Mean ± SD)	0.27 ± 0.07	0.24 ± 0.05	0.27 ± 0.07	0.26 ± 0.08	0.49
	P vs. baseline		>0.05	>0.05	>0.05	
PL/TM	(Mean ± SD)	0.18 ± 0.02	0.21 ± 0.03	0.21 ± 0.02	0.21 ± 0.04	0.01
	P vs. baseline		>0.05	<0.05	>0.05	
FC/TC	(Mean ± SD)	0.40 ± 0.07	0.43 ± 0.06	0.43 ± 0.15	0.44 ± 0.10	0.80
	P vs. baseline		>0.05	>0.05	>0.05	
FC/PL	(Mean ± SD)	0.61± 0.13	0.52 ± 0.11	0.59 ± 0.22	0.59 ± 0.22	0.52
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TG	(Mean ± SD)	1.39 ± 0.64	1.13 ± 0.49	1.57 ± 0.55	1.31 ±0.70	0.33
	P vs. baseline		>0.05	>0.05	>0.05	
TC/Prot	(Mean ± SD)	1.42 ± 0.41	1.18 ± 0.24	1.33 ± 0.22	1.57 ± 0.84	0.22
	P vs. baseline		>0.05	>0.05	>0.05	
TG/Prot	(Mean ± SD)	1.27 ± 0.72	1.09 ± 0.30	0.87 ± 0.25	1.20 ± 0.29	0.17
	P vs. baseline		>0.05	>0.05	>0.05	
PL/Prot	(Mean ± SD)	0.94 ± 0.23	0.97 ± 0.10	0.99 ± 0.21	1.13 ± 0.46	0.32
	P vs. baseline		>0.05	>0.05	>0.05	

[1] TM=Total mass (sum of TG, TC, PL and protein)

4.2.1.6.4 LDL fractional composition

Table 4-51 LDL fractional composition

LDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
Prot/TM[1]	(Mean ± SD)	0.29 ± 0.06	0.28 ± 0.03	0.28 ± 0.05	0.27 ± 0.04	0.60
	P vs. baseline		>0.05	>0.05	>0.05	
TG/TM	(Mean ± SD)	0.14 ±0.06	0.11 ± 0.03	0.09 ± 0.03	0.12 ± 0.05	0.04
	P vs. baseline		>0.05	<0.05	>0.05	
TC/TM	(Mean ± SD)	0.26 ± 0.07	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.07	0.42
	P vs. baseline		>0.05	>0.05	>0.05	
FC/TM	(Mean ± SD)	0.10 ± 0.02	0.10 ± 0.03	0.10 ± 0.02	0.11 ± 0.03	0.65
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TM	(Mean ± SD)	0.27 ± 0.10	0.30 ± 0.05	0.32 ± 0.06	0.29 ± 0.09	0.38
	P vs. baseline		>0.05	>0.05	>0.05	
PL/TM	(Mean ± SD)	0.20 ± 0.03	0.20 ± 0.03	0.20 ± 0.03	0.22 ± 0.06	0.46
	P vs. baseline		>0.05	>0.05	>0.05	
FC/TC	(Mean ± SD)	0.39 ± 0.10	0.36 ± 0.08	0.34 ± 0.08	0.39 ± 0.11	0.44
	P vs. baseline		>0.05	>0.05	>0.05	
FC/PL	(Mean ± SD)	0.50 ± 0.14	0.53 ± 0.15	0.48 ± 0.07	0.55 ± 0.27	0.74
	P vs. baseline		>0.05	>0.05	>0.05	
CE/TG	(Mean ± SD)	2.42 ± 1.60	2.96 ± 1.40	3.77 ± 1.56	3.07 ± 1.68	0.14

LDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
	P vs. baseline		>0.05	>0.05	>0.05	
TC/Prot	(Mean ± SD)	0.97 ± 0.45	1.01 ± 0.19	1.07 ± 0.22	1.12 ± 0.42	0.70
	P vs. baseline		>0.05	>0.05	>0.05	
TG/Prot	(Mean ± SD)	0.50 ± 0.21	0.40 ± 0.12	0.35 ± 0.11	0.43 ± 0.13	0.046
	P vs. baseline		>0.05	<0.05	>0.05	
PL/Prot	(Mean ± SD)	0.72 ± 0.27	0.70 ± 0.08	0.74 ± 0.13	0.83 ± 0.28	0.48
	P vs. baseline		>0.05	>0.05	>0.05	

[1] TM=Total mass (sum of TG, TC, PL and protein)

4.2.1.6.5 HDL fractional composition

As plasma proteins contaminated the HDL fractions, only data showing relationships between lipid constituents of HDL will be displayed here.

Table 4-52 HDL fractional composition

HDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
FC/TC	(Mean ± SD)	0.28 ± 0.07	0.27 ± 0.08	0.29 ± 0.06	0.26 ± 0.07	0.79
	P vs. baseline		>0.05	>0.05	>0.05	
FC/PL	(Mean ± SD)	0.76 ± 0.44	0.73 ± 0.41	1.03 ± 0.64	0.86 ± 0.60	0.43
	P vs. baseline		>0.05	>0.05	>0.05	

HDL		Baseline	Cerivastatin	Fenofibrate	Combination	ANOVA
CE/TG	(Mean ± SD)	1.02 ± 0.36	1.39 ± 0.57	1.79 ± 0.68	1.67 ± 0.56	0.001
	P vs. baseline		>0.05	<0.01	<0.05	

4.2.1.7 Lipoprotein fractions: Summary statistics

4.2.1.7.1 Triglycerides

Approximately 80% of the total decrease in TG in all three treatment arms was accounted for by decreased TG content of VLDL1 and VLDL2, the lipoprotein subclasses which carry most of the plasma TG. Fenofibrate (either as monotherapy or as part of combination therapy) was more effective in reducing TG mass in the TGRL with an average reduction of 60-75% in VLDL1+VLDL2 TG compared to the 40% reduction with cerivastatin alone. Combination therapy was not significantly more effective in reducing TGRL TG content than fenofibrate monotherapy. IDL TG content was reduced by 34-45%; the reduction of 34% seen with fenofibrate was not statistically significant. LDL TG mass was reduced to a very similar degree (41-44%) by all therapies. Although the reductions in triglyceride mass of IDL and LDL were mostly statistically significant (except the change in IDL triglyceride mass with fenofibrate) the contribution to overall triglyceride reduction was about 10% for cerivastatin and 5% for fenofibrate and combination therapy. More than 90% of reduction in plasma triglycerides in the cerivastatin arm was accounted for by changes

in apoB-containing lipoproteins with the corresponding figure for fenofibrate and combination therapy being 94%.

Table 4.53 and table 4.54 respectively show the absolute and relative mean reduction in TG in plasma and the apoB-containing lipoprotein fractions in the treatment arms of the study.

Table 4-53 Absolute mean reduction in TG (in mmol/L) from baseline

Fraction	Cerivastatin (mmol/L)	Fenofibrate (mmol/L)	Cer + Feno (mmol/L)
Plasma	2.84	4.73	4.85
VLDL1	1.74	3.36	3.30
VLDL2	0.56	0.87	1.02
IDL	0.15	0.12	0.16
LDL	0.12	0.11	0.11

Table 4-54 Mean percentage reduction in TG from baseline within lipoprotein fractions

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
Plasma	41.46	69.05	70.8
VLDL1	39.55	76.36	75
VLDL2	39.44	61.27	71.83
IDL	42.86	34.29	45.71
LDL	44.44	40.74	40.74

Table 4.55 illustrates the contribution of each lipoprotein fraction to triglyceride reduction in plasma.

Table 4-55 Percentage of reduction in total TG accounted for by each lipoprotein fraction

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
VLDL1	61.27	71.04	68.04
VLDL2	19.72	18.39	21.03
IDL	5.28	2.54	3.30
LDL	4.23	2.33	2.27
Sum of % reduction	90.49	94.29	94.64

4.2.1.7.1.1 Changes in triglyceride according to apoE genotype

Patients with the apoE (R145C) mutation had higher median TG at baseline than apoE2 homozygotes (7.85 vs. 4.06 mmol/L for apoE (R145C) vs. apoE2/E2), although the difference did not reach statistical significance (P=0.21 by unpaired t-test following logarithmic transformation). The small number of subjects in the apoE (R145C) group limits the statistical power of any analysis. In these more hypertriglyceridaemic patients, reductions in VLDL1 TG accounted for most of the observed TG reduction with less TG mass reduction attributable to denser lipoprotein fractions.

Table 4-56 Absolute mean reduction in TG (in mmol/L) from baseline by apoE genotype

Fraction	Cerivastatin (mmol/L)		Fenofibrate (mmol/L)		Cer + Feno (mmol/L)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	2.55	5.66	4.29	8.20	4.25	8.31
VLDL1	1.20	4.91	2.64	7.24	2.36	7.25
VLDL2	0.75	0.42	1.07	0.65	1.22	0.84
IDL	0.18	0.04	0.14	0.05	0.20	0.05
LDL	0.11	0.11	0.10	0.09	0.13	0.07

Table 4-57 Mean percentage reduction in TG from baseline within lipoprotein fractions by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	39.67	54.47	66.71	78.94	66.20	79.93
VLDL1	32.03	59.77	70.79	88.11	63.28	88.16
VLDL2	46.24	31.57	66.22	49.09	75.34	63.48
IDL	46.89	16.04	38.16	18.78	51.91	20.00
LDL	46.60	39.63	41.93	34.56	56.02	24.07

Table 4-58 Percentage of reduction in total TG accounted for by each lipoprotein fraction by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
VLDL1	46.86	86.75	61.56	88.29	55.55	87.24
VLDL2	29.43	7.42	25.05	7.93	28.77	10.11
IDL	6.93	0.71	3.35	0.61	4.60	0.60
LDL	4.19	1.94	2.24	1.10	3.02	0.84
Sum of %	87.41	96.82	92.21	97.93	91.95	98.80

4.2.1.7.2 Total cholesterol

Plasma TC decreased 39%-57% from baseline with treatment. The decrease was greatest with combination therapy, followed by fenofibrate and cerivastatin. Most of the reduction in plasma TC was accounted for by changes in the cholesterol content of VLDL1 and VLDL2. Differences did, however, emerge between the treatment allocations. With fenofibrate monotherapy approximately 90% of the total reduction in plasma TC was explained by changes in VLDL1 and VLDL2. With cerivastatin monotherapy this figure was only 66%, while combination therapy resulted in an 80%

decrease of the cholesterol content of VLDL1 and VLDL2. The most striking difference was observed in the VLDL1 fraction where fenofibrate (as monotherapy or as combination therapy) decreased cholesterol 83-86%, compared to the 54% reduction observed with cerivastatin. Fenofibrate was less effective in reducing IDL and LDL cholesterol than therapies containing cerivastatin. IDL cholesterol decreased by 18% with fenofibrate while therapies containing cerivastatin resulted in a 42-50% decrease. For LDL cholesterol the reduction with fenofibrate was less than 5%, while cerivastatin containing therapies achieved a 22-33% reduction. Overall 93-95% of the reduction in plasma cholesterol was explained by changes in apoB containing lipoproteins.

Table 4-59 Absolute mean reduction in TC (in mmol/L) from baseline

Fraction	Cerivastatin (mmol/L)	Fenofibrate (mmol/L)	Cer + Feno (mmol/L)
Plasma	3.51	4.06	5.11
VLDL1	1.57	2.42	2.50
VLDL2	0.77	1.20	1.56
IDL	0.49	0.18	0.41
LDL	0.42	0.06	0.28

Table 4-60 Mean percentage reduction in TC from baseline within lipoprotein fractions

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
Plasma	39.24	45.34	57.17
VLDL1	54.33	83.74	86.51
VLDL2	36.84	57.42	74.64
IDL	50.52	18.56	42.27
LDL	33.60	4.80	22.40

Table 4-61 Percentage of reduction in total TC accounted for by each lipoprotein fraction

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
VLDL1	44.73	59.66	48.89
VLDL2	21.94	29.59	30.50
IDL	13.96	4.44	8.02
LDL	11.97	1.48	5.48
Sum of %	92.59	95.17	92.88

4.2.1.7.2.1 Changes in total cholesterol according to apoE genotype

In the genotype positive patients plasma cholesterol was reduced 38-64% with therapy. The greatest reduction in plasma cholesterol was achieved in the apoE2/E2 patients with combination therapy (64%), while the lowest reduction was 38% in the apoE (R145C) mutation carriers with fenofibrate monotherapy. Comparing the two genotypes the response to therapy of TC in the denser fractions IDL and LDL differed. In apoE2/E2 patients taking cerivastatin-containing regimes there was a reduction of 33-50% in TC within IDL and LDL TC, while fenofibrate reduced the values by28% and 12% respectively. In the apoE (R145C) mutation carriers with cerivastatin monotherapy there was a reduction of 9-12% in IDL and LDL TC, while combination therapy hardly changed IDL (reduced by 1%) and LDL TC increased by 52%. Fenofibrate monotherapy in the apoE (R145C) mutation carriers increased IDL and LDL TC 77% and 85% respectively. Correspondingly in the apoE (R145C) group the reductions in VLDL1 and VLDL2 total cholesterol were more than 100% of the final reduction in cholesterol, as cholesterol mass in denser fractions increased.

Table 4-62 Absolute mean reduction in TC (in mmol/L) from baseline by apoE genotype

Fraction	Cerivastatin (mmol/L)		Fenofibrate (mmol/L)		Cer + Feno (mmol/L)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	3.85	3.40	4.76	3.10	6.07	4.15
VLDL1	1.57	2.55	2.43	3.65	2.46	3.80
VLDL2	0.98	0.33	1.55	0.51	1.95	0.85
IDL	0.56	0.04	0.31	-0.34 [1]	0.52	0.01
LDL	0.30	0.12	0.12	-0.85 [1]	0.38	-0.52 [1]

[1] Minus sign indicates that the absolute mass increased from baseline with treatment

Table 4-63 Mean percentage reduction in TC from baseline within lipoprotein fractions by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	40.32	41.15	49.85	37.59	63.60	50.29
VLDL1	52.52	62.10	81.68	89.13	82.44	92.77
VLDL2	36.58	23.96	57.58	36.78	72.35	61.33
IDL	50.89	9.70	28.23	-76.98 [1]	47.24	1.14
LDL	33.25	12.13	12.64	-85.10 [1]	41.80	-52.00 [1]

[1] Minus sign indicates that the TC increased from baseline

Table 4-64 Percentage of reduction in TC accounted for by each lipoprotein fraction by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
VLDL1	40.65	74.88	51.14	117.88	40.47	91.65
VLDL2	25.56	9.79	32.54	16.49	32.06	20.54
IDL	14.67	1.26	6.58	-10.93 [1]	8.64	0.12
LDL	7.86	3.57	2.42	-27.45 [1]	6.27	-12.53 [1]
Sum of %	88.74	89.50	92.68	96.00	87.44	99.78

[1] Minus sign indicates that the TC increased from baseline

4.2.1.7.3 Free cholesterol and cholesterol ester

Both FC and CE levels decreased from baseline with treatment. The percentage change of plasma FC was greater than that of CE for all therapies. The percentage reduction in plasma FC was greatest for combination therapy, followed by fenofibrate and cerivastatin.

FC and CE were reduced by approximately equivalent percentages within VLDL1, although the reductions were much higher for fenofibrate and combination therapy. In VLDL2 FC was reduced more than CE, except for combination therapy where the reductions were very similar. In IDL CE was reduced more than free cholesterol. In LDL FC and CE were reduced by similar percentages with cerivastatin containing therapies, while fenofibrate resulted in no reduction of CE.

Table 4-65 Absolute mean reduction in FC and CE (in mmol/L) from baseline within lipoprotein fractions

Fraction	Cerivastatin (mmol/L)		Fenofibrate (mmol/L)		Cer + Feno (mmol/L)	
	FC	CE	FC	CE	FC	CE
Plasma	2.15	1.36	2.53	1.53	2.94	2.18
VLDL1	0.99	0.57	1.54	0.88	1.59	0.91
VLDL2	0.45	0.33	0.63	0.58	0.74	0.81
IDL	0.18	0.31	0.05	0.13	0.15	0.27
LDL	0.15	0.27	0.06	0	0.1	0.18

Table 4-66 Mean percentage reduction in FC and CE from baseline within lipoprotein fractions

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	FC	CE	FC	CE	FC	CE
Plasma	48.53	30.02	57.11	33.77	66.37	48.12
VLDL1	53.23	55.34	82.80	85.44	85.48	88.35
VLDL2	45.45	30.00	63.64	52.73	74.75	73.64
IDL	47.37	53.45	13.16	22.41	39.47	46.55
LDL	34.09	32.93	13.64	0.00	22.73	21.95

Table 4-67 Percentage of reduction in FC and CE accounted for by each lipoprotein fraction

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	FC	CE	FC	CE	FC	CE
VLDL1	28.21	16.24	37.93	21.67	31.12	17.81
VLDL2	12.82	9.40	15.52	14.29	14.48	15.85
IDL	5.13	8.83	1.23	3.20	2.94	5.28
LDL	4.27	7.69	1.48	0.00	1.96	3.52
Sum %	50.43	42.17	56.16	39.16	50.49	42.47

4.2.1.7.3.1 Changes in free cholesterol and cholesterol ester according to apoE genotype

In the apoE (R145C) mutation carriers FC reduction in apoB-containing lipoproteins explained 74-96% of the total reduction observed in plasma cholesterol. In the apoE2/E2 group the corresponding figures for free cholesterol are 43-50%. In the apoE (R145C) mutation carriers increases in IDL and LDL CE were more important in accounting for the changed TC within these fractions than changes in FC.

Table 4-68 Absolute mean reduction in FC and CE (in mmol/L) from baseline within lipoprotein fractions by apoE genotype

Fraction	Cerivastatin (mmol/L)				Fenofibrate (mmol/L)				Cer + Feno (mmol/L)			
	ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R145C)	
	FC	CE	FC	CE	FC	CE	FC	CE	FC	CE	FC	CE
Plasma	2.12	1.74	*3.22*	*0.19*	2.72	2.05	*3.35*	*-0.24*	3.26	2.82	*3.57*	*0.59*
VLDL1	0.81	0.75	*2.21*	*0.34*	1.36	1.08	*2.92*	*0.74*	1.37	1.09	*2.99*	*0.82*
VLDL2	0.58	0.40	*0.23*	*0.10*	0.80	0.75	*0.35*	*0.15*	0.94	1.01	*0.45*	*0.39*
IDL	0.21	0.35	*0.01*	*0.02*	0.13	0.18	*-0.10*[1]	*-0.24*[1]	0.19	0.32	*-0.01*[1]	*0.01*
LDL	0.10	0.20	*0.08*	*0.05*	0.07	0.05	*-0.20*[1]	*-0.6*[1]*5*	0.12	0.26	*-0.08*[1]	*-0.43*[1]

[1] Minus sign indicates that the FC or CE increased from baseline

Table 4-69 Mean percentage reduction in FC and CE from baseline within lipoprotein fractions by apoE genotype

Fraction	Cerivastatin (%)				Fenofibrate (%)				Cer + Feno (%)			
	ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R1	
	FC	CE	FC	CE	FC	CE	FC	CE	FC	CE	FC	C
Plasma	45.84	35.12	*60.21*	*6.34*	58.81	41.44	*62.68*	*-8.29*	70.48	57.15	*66.78*	*2*
VLDL1	47.08	60.11	*68.63*	*38.26*	78.56	86.01	*90.57*	*83.86*	79.23	86.93	*92.83*	*9*
VLDL2	47.29	29.71	*32.29*	*14.41*	65.10	55.11	*50.24*	*22.16*	76.20	74.22	*64.43*	*5*
IDL	47.32	52.53	*7.94*	*7.60*	28.75	26.88	*-57.78*[1]	*-97.48*[1]	44.09	48.82	*-7.50*[1]	*3*
LDL	30.59	34.93	*23.53*	*6.87*	20.65	7.93	*-63.22*[1]	*-95.29*[1]	36.06	45.33	*-26.41*[1]	*-*

[1] Minus sign indicates that the FC or CE increased from baseline

Table 4-70 Percentage of reduction in FC and CE accounted for by each lipoprotein fraction by apoE genotype

Fraction	Cerivastatin (%)				Fenofibrate (%)				Cer + Feno (%)			
	ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R145C)		ApoE2/E2		ApoE (R145C)	
	FC	CE	FC	CE	FC	CE	FC	CE	FC	CE	FC	CE
VLDL1	21.15	19.52	*65.00*	*9.90*	28.55	22.59	*94.07*	*23.81*	22.58	17.90	*72.02*	*19.64*
VLDL2	15.11	10.49	*6.65*	*2.88*	16.82	15.75	*11.35*	*4.86*	15.44	16.63	*10.87*	*9.43*
IDL	5.41	9.01	*0.42*	*0.56*	2.66	3.73	*-3.35*[1]	*-7.86*[1]	3.20	5.31	*-0.33*[1]	*0.22*
LDL	2.70	5.17	*2.21*	*1.37*	1.47	0.95	*-6.53*[1]	*-20.90*[1]	2.02	4.26	*-2.04*	*-10.46*
Sum %	44.37	44.19	*74.28*	*14.72*	49.51	43.01	*95.54*	*-0.10*[1]	43.24	44.10	*80.53*	*18.83*

[1] Minus sign indicates that the FC or CE increased from baseline

4.2.1.7.4 Phospholipid

Plasma phospholipid concentration decreased significantly from baseline in all three treatment arms. The decrease was greatest with combination therapy, followed by fenofibrate and cerivastatin. There were no statistically significant differences between the decreases achieved with the various therapeutic regimens. The decrease in plasma phospholipid was mainly accounted for by the decreased phospholipid mass in TGRL. Reduced phospholipid in TGRL accounted for 77% of the decrease with cerivastatin, 87% with cerivastatin/fenofibrate and 100% with fenofibrate. The proportional reduction in phospholipid was greatest in VLDL1, and fenofibrate-containing therapy was significantly more effective here, achieving a decrease of 81%, compared to the 52 % seen with cerivastatin. In IDL and LDL only cerivastatin significantly decreased phospholipid

Table 4-71 Absolute mean reduction in PL (in mmol/L) from baseline

Fraction	Cerivastatin (mmol/L)	Fenofibrate (mmol/L)	Cer + Feno (mmol/L)
Plasma	1.53	1.70	2.15
VLDL1	0.81	1.26	1.27
VLDL2	0.37	0.51	0.61
IDL	0.11	0.01	0.10
LDL	0.15	0.05	0.06

Table 4-72 Mean percentage reduction in PL from baseline within lipoprotein fractions

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
Plasma	33.19	36.88	46.64
VLDL1	51.92	80.77	81.41
VLDL2	43.53	60.00	71.76
IDL	35.48	3.23	32.26
LDL	33.33	11.11	13.33

Table 4-73 Percentage of reduction in PL accounted for by each lipoprotein fraction

Fraction	Cerivastatin (%)	Fenofibrate (%)	Cer + Feno (%)
VLDL1	52.94	74.12	59.07
VLDL2	24.18	30.00	28.37
IDL	7.19	0.59	4.65
LDL	9.80	2.94	2.79
Sum of %	94.12	107.65	94.88

4.2.1.7.4.1 Changes in phospholipid according to apoE genotype

In the genotype positive patients similar decreases in plasma PL concentration were seen compared to the total patient group. Comparing the two genotypes studied here, differences were noted in the phospholipid mass of the denser lipoproteins IDL and LDL. In the apoE2/E2 patients the PL mass in IDL and LDL decreased 14-15% with

fenofibrate monotherapy and 37-50% with cerivastatin-containing therapy. These decreases accounted for approximately 6% (with fenofibrate) and 14-17% (with cerivastatin containing therapy) of total PL decreases. In the apoE (R145C) mutation carriers IDL and LDL phospholipid increased 34-86% with fenofibrate and combination therapy. Cerivastatin caused a minor increase (5%) in IDL phospholipid, but phospholipid in LDL fell by 13%. The changes in PL paralleled those observed for the behaviour of TC.

Table 4-74 Absolute mean reduction in PL (in mmol/L) from baseline by apoE genotype

Fraction	Cerivastatin (mmol/L)		Fenofibrate (mmol/L)		Cer + Feno (mmol/L)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	1.57	1.89	1.89	2.10	2.49	2.24
VLDL1	0.73	1.63	1.16	2.24	1.14	2.26
VLDL2	0.52	0.16	0.68	0.25	0.81	0.31
IDL	0.13	-0.01 [1]	0.05	-0.11 [1]	0.17	-0.06 [1]
LDL	0.14	0.06	0.06	-0.15 [1]	0.18	-0.38 [1]

[1] Minus sign indicates that the PL mass increased from baseline

Table 4-75 Mean percentage reduction in TC from baseline within lipoprotein fractions by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
Plasma	32.84	38.42	39.42	42.58	51.94	45.35
VLDL1	48.97	64.04	78.13	87.97	76.46	88.67
VLDL2	49.33	26.84	64.48	41.48	77.20	51.31
IDL	37.26	-5.26 [1]	14.14	-60.05 [1]	48.29	-32.11 [1]
LDL	39.69	13.64	15.89	-34.32 [1]	50.33	-86.48 [1]

[1] Minus sign indicates that the PL increased from baseline

Table 4-76 Percentage of reduction in PL accounted for by each lipoprotein fraction by apoE genotype

Fraction	Cerivastatin (%)		Fenofibrate (%)		Cer + Feno (%)	
	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)	ApoE2/E2	ApoE (R145C)
VLDL1	46.47	86.40	61.60	106.82	45.75	100.94
VLDL2	32.99	8.66	35.82	12.05	32.55	13.97
IDL	8.31	-0.53	2.62	-5.43 [1]	6.79	-2.72 [1]
LDL	9.10	3.17	3.03	-7.19 [1]	7.28	-16.99 [1]
Sum of %	96.87	97.71	103.06	106.25	92.37	95.20

[1] Minus sign indicates that the PL increased from baseline

4.2.1.8 Lipoprotein fractions: Graphical representation

4.2.1.8.1 Lipoprotein fractions: absolute masses

These graphs show the absolute mass of the lipid component of each lipoprotein fraction at baseline and during the three treatment arms.

Figure 4-51 VLDL1 lipids

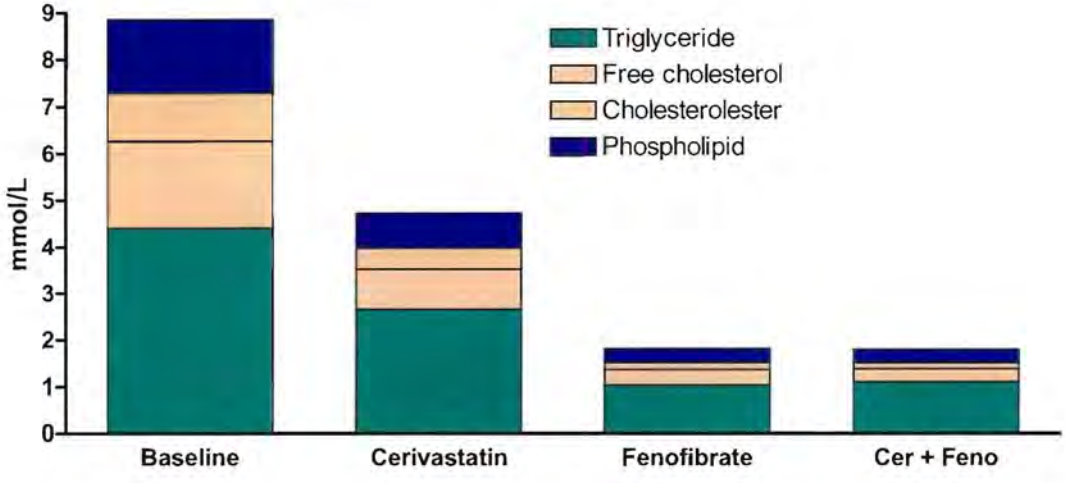


Figure 4-52 VLDL2 lipids

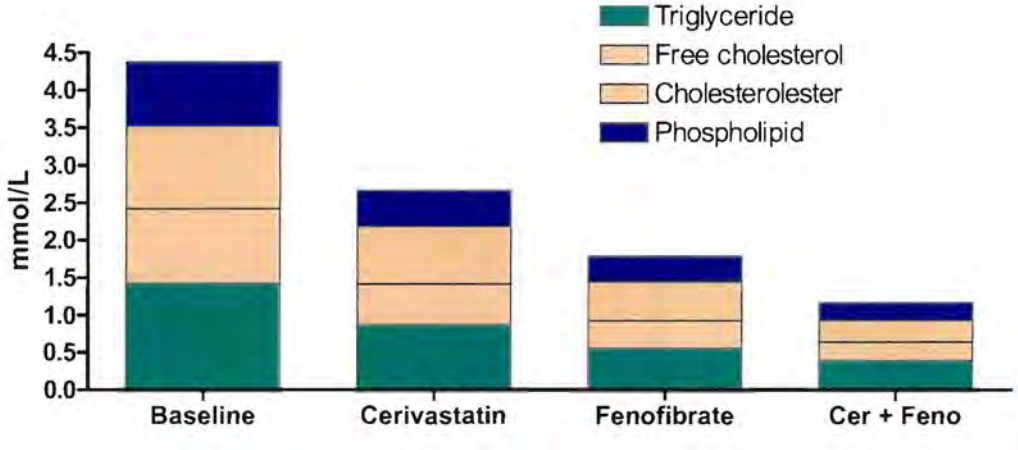


Figure 4-53 IDL lipids

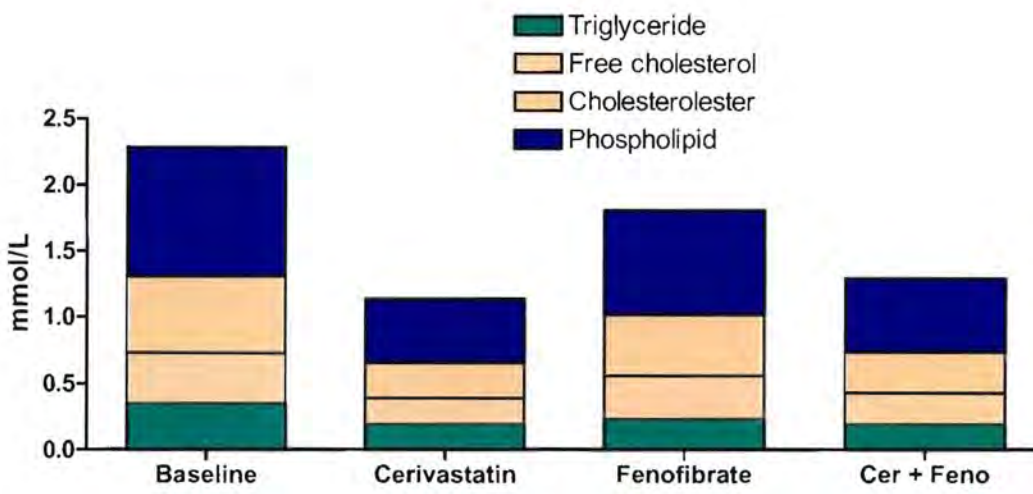


Figure 4-54 LDL lipids

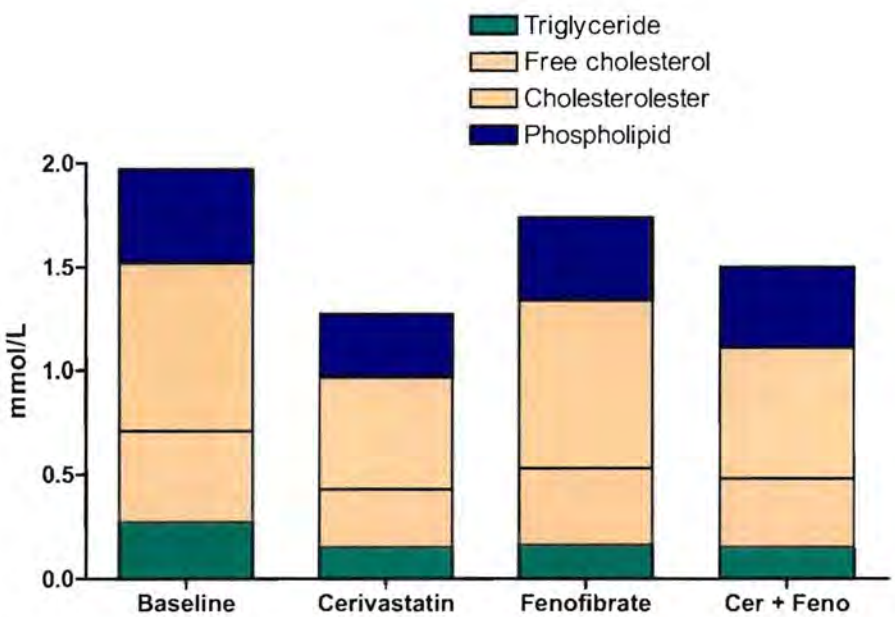
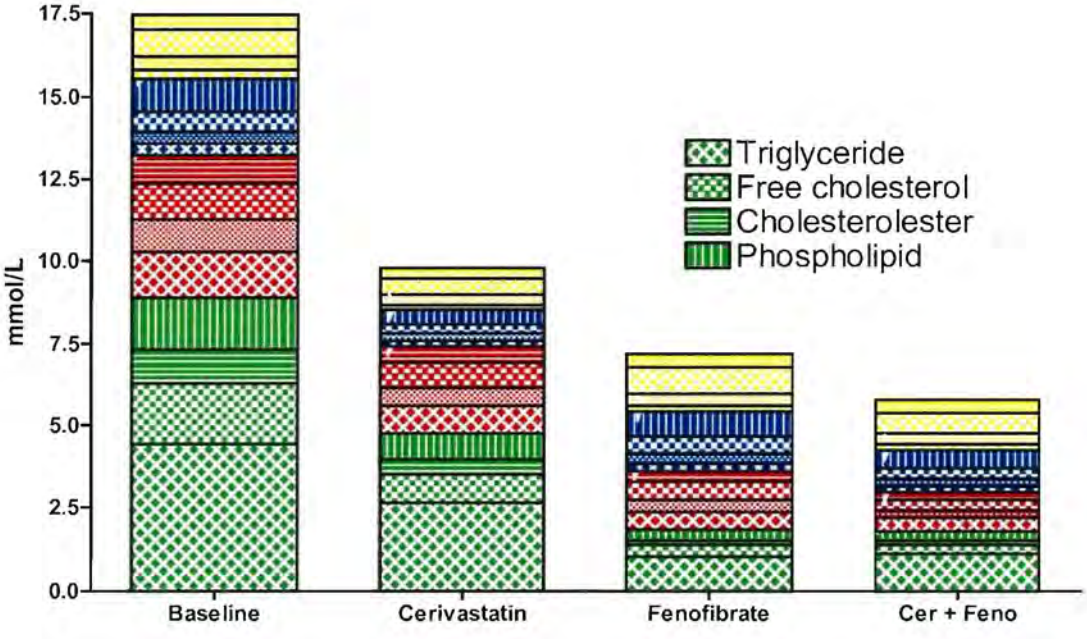


Figure 4-55 All apoB-containing lipoproteins



Legend: Pattern *Lipid constituents*

Colour *Lipoprotein fraction*

Green: VLDL1

Red: VLDL2

Blue: IDL

Yellow: LDL

4.2.1.8.2 Lipoprotein fractions: relative masses

These graphs show the relative contribution of protein and each lipid component to the total analyzed mass within each lipoprotein fraction.

Figure 4-56 VLDL1 relative masses

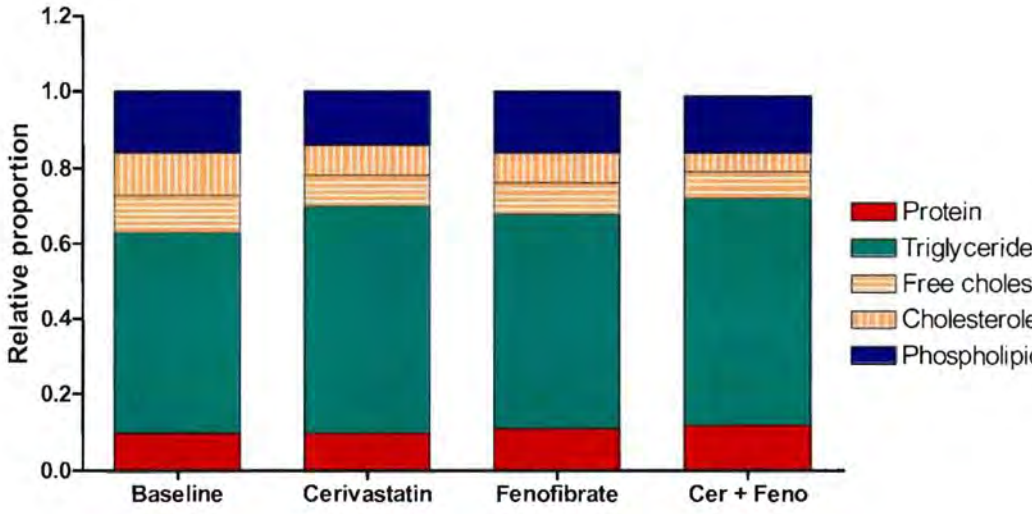


Figure 4-57 VLDL2 relative mass

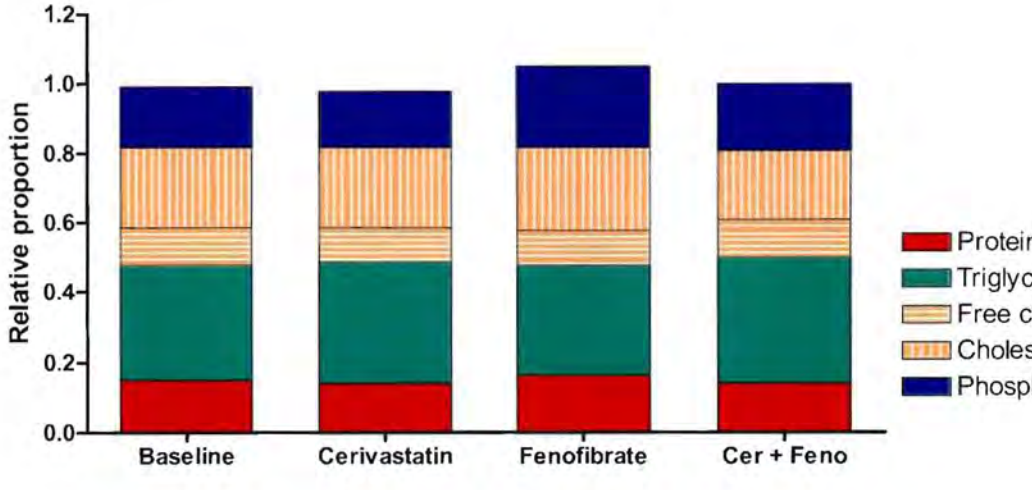


Figure 4-58 IDL relative mass

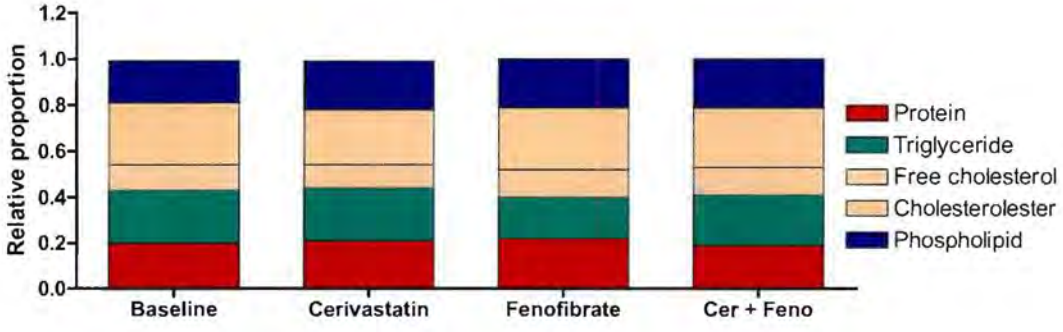


Figure 4-59 LDL relative mass

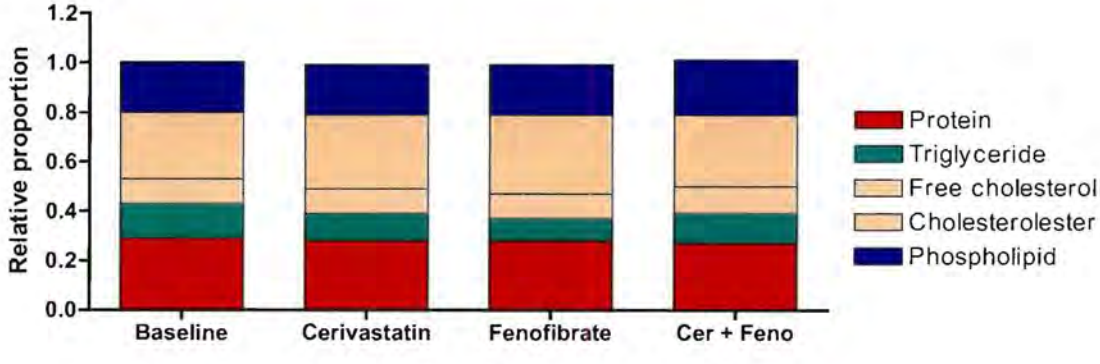
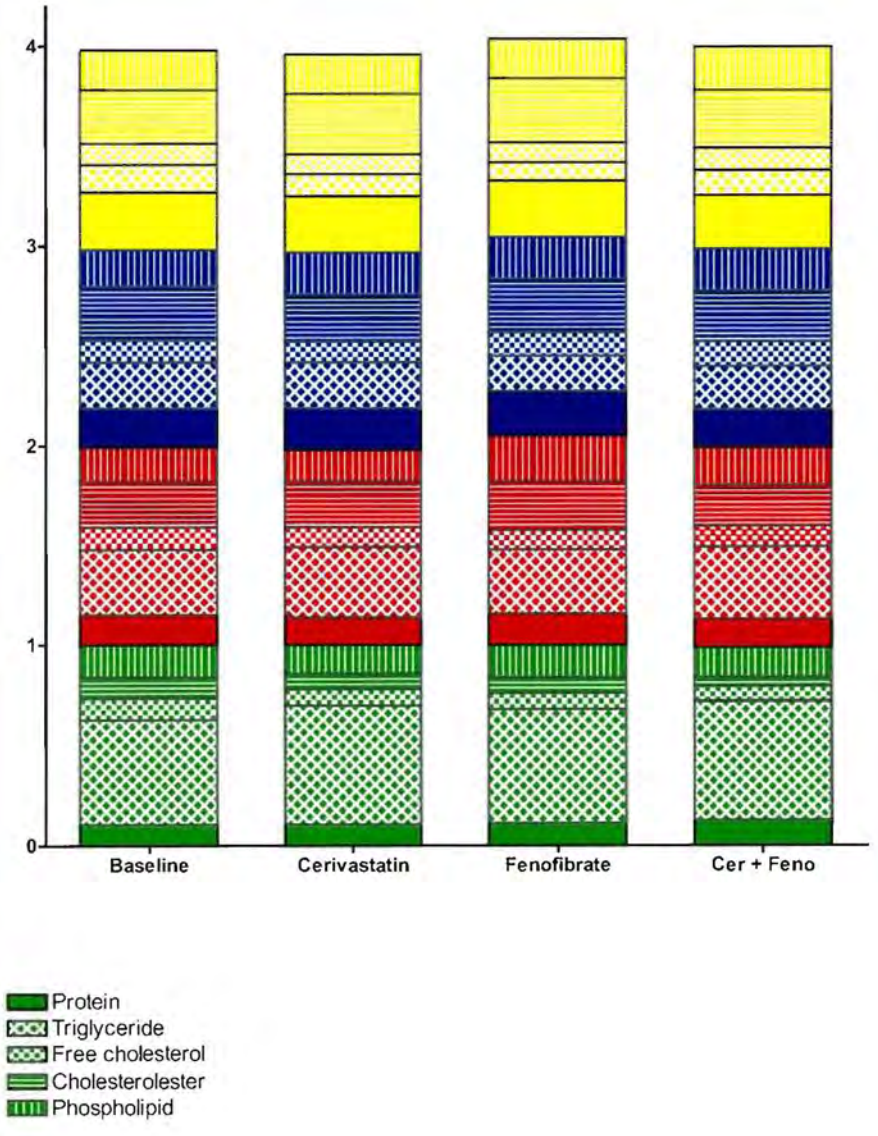


Figure 4-60 All apoB containing lipoproteins- relative masses



Legend: Pattern *Lipid constituents*

Colour *Lipoprotein fraction*

Green: VLDL1

Red: VLDL2

Blue: IDL

Yellow: LDL

4.2.1.9 Previously published data on changes in lipoprotein fractions with lipid-lowering therapy in dysbetalipoproteinaemic patients

Most studies evaluating lipid-lowering therapy for patients with dysbetalipoproteinaemia report limited data on ultracentrifugal analysis of lipoprotein fractions. The most commonly reported data is ultracentrifugally derived VLDLC and LDLC, while only a few studies report detailed data on lipoprotein composition. Ultracentrifugal techniques also vary between studies. In many studies IDLC is included in the ultracentrifugally derived LDLC fraction.

4.2.1.9.1 VLDL

Most studies do not subdivide VLDL into VLDL1 and VLDL2, but report on VLDL as a single fraction. Table 4.77 illustrates changes in VLDL previously reported in response to lipid-lowering therapy. For more details on individual studies please refer to section 4.2.1.3.4.2.

Table 4-77 VLDL response to lipid-lowering therapy in previously published studies

Author	Year	Drug [1]	VLDL isolation [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Hoogwerf	1984	NA 3g	SS D<1.006	4.25	3.60	54	47		(302)
Hoogwerf	1984	CLO 2g	SS D<1.006	4.25	3.60	49	45		(302)
Hoogwerf	1984	COL 10g	SS D<1.006	3.03	2.94	+32	+53		(305)
Hoogwerf	1984	CLO 2g	SS D<1.006	3.03	2.94	51	55		(305)
Hoogwerf	1984	C+C	SS D<1.006	3.03	2.94	52	51		(305)
Stuyt	1985	ACP 750mg	SS D<1.006	4.76	4.18	40	33	1	(303)
Stuyt	1985	CLO 2g	SS D<1.006	6.01	6.34	45	50	1	(303)

Author	Year	Drug [1]	VLDL isolation [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Packard	1986	BEZ 600mg	SUC	6.52		75			(307)
East	1986	MEV 40mg	NS	6.64	3.86	73	56	4	(304)
Kuo	1988	GEM 1.2g	SS D<1.006	3.94	3.98	45	65	2	(309)
Vega	1988	LOV 40mg	SS D<1.006	7.52		45			(313)
Lussier-Cacan	1989	FEN 300mg	SS D<1.006	5.36	4.80	70	65		(308)
Illingworth	1990	LOV 40mg	SS D<1.006	7.79		53			(316)
Illingworth	1990	CLO 2g	SS D<1.006	7.79		61			(316)
Dallongeville	1991	OM3 6g	SUC	4.90	5.77	54	62		(326)
Feussner	1992	SIM 20mg	SS D<1.006	8.95		45			(318)
Larsen	1994	GEM 1.2g	SS D<1.006	6.29		59			(321)

Author	Year	Drug [1]	VLDL isolation [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Larsen	1994	CLO 2g	SS D<1.006	6.29		79			(321)
Zhao	1994	GEM 1.2g	DG	6.57	4.66	72	70	3	(306)
Gylling	1995	LOV 40mg	DG	3.7	3.8	54	50		(317)
Civeira	1999	SIM 20mg	SUC	1.60	2.01	20	9	5	(238)
Civeira	1999	GEM 1.2g	SUC	2.02	2.05	50	53	5	(238)
van Dam	2002	ATR 40mg	DG	4.24	4.08	59	46		(240)
Ishigami	2003	ATR 20mg	SUC	4.30	2.77	76	62		(315)

[1] Drug and daily dosage

ATR *Atorvastatin*

BEZ *Bezafibrate*

C+C *Colestipol (20g) + Clofibrate 2g*

CLO *Clofibrate*

COL *Colestipol*

FEN *Fenofibrate*

GEM *Gemfibrozil*

LOV *Lovastatin*

MEV *Mevinolin*

NA *Nicotinic acid*

OM3 *Omega-3 fatty acids*

SIM *Simvastatin*

[2] Refers to the method by which VLDL was isolated

SS D<1.006 Single step ultracentrifugation with isolation of fraction less dense than 1.006 g/mL

SUC Sequential ultracentrifugation

DG density gradient ultracentrifugation

[3] VLDLC at baseline

[4] VLDL TG at baseline

[5] Reduction (in %) from baseline values (+ indicates an increase from baseline)

Comments

1. The study included a washout period between the two arms, therefore the baselines differ
2. The study consisted of two prolonged periods of drug treatment interrupted by a washout; the data reported refers to the first period
3. VLDLC and VLDL TG calculated as the sum of lipids in VLDL1 and VLDL2
4. Ultracentrifugal method not stated in article.
5. The study protocol specified a washout period between each treatment arm.

The reported reductions in VLDLC with lipid-lowering therapy differ widely, ranging from a low of 20% with simvastatin therapy in a group of patients with autosomal dominantly inherited dysbetalipoproteinaemia and low levels of VLDLC at baseline (322), to reductions of more than 70% with both fibrates (321) and statins (315). The observed reductions in VLDLC (taken as the sum of VLDL1 and VLDL2) of 48%, 73% and 80% for cerivastatin, fenofibrate and combination therapy respectively in the Bayer study fall well within the previously reported range. In the two studies directly comparing statin and fibrate treatment (238;316) fibrates consistently produce greater reductions in VLDLC than statins. In the Bayer study the same discrepancy in VLDLC response was observed when fenofibrate was given.

Lipid-lowering therapy in dysbetalipoproteinaemia is generally associated with marked reductions in VLDL TG mass, except for colestipol which raised VLDL TG markedly (305). In head to head comparisons of statins and fibrates (238;316) fibrates reduce VLDL TG more effectively than statins. In this study a similar response was observed, with VLDL TG reductions of 40%, 73% and 74 % respectively for statin, fibrate and combination therapy.

4.2.1.9.2 IDL

The majority of published studies do not specifically report changes in IDL lipids, but table 4.78 lists studies that do supply data on the IDL fraction.

Table 4-78 IDL response to lipid-lowering therapy in previous studies of dysbetalipoproteinaemia

Author	Year	Drug [1]	IDL isolation [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Lussier-Cacan	1989	FEN 300mg	SUC	1.42	0.33	40	44		(308)
Dallongeville	1991	OM3 6g	SUC	0.98	0.47	8	0		(326)
Zhao	1994	GEM 1.2g	DG	2.47	0.70	34	51		(306)
Gylling	1995	LOV 40mg	DG	0.9	0.2	33	50		(317)
Civeira	1999	SIM 20mg	SUC	0.67	0.19	41	15	1	(238)
Civeira	1999	GEM 1.2g	SUC	0.74	0.21	35	25	1	(238)
van Dam	2002	ATR 40mg	DG	1.80	0.59	51	46		(240)
Ishigami	2003	ATR 20mg	SUC	1.19	0.28	62	56		(315)

[1] Drug and daily dosage

ATR *Atorvastatin*

CLO *Clofibrate*

FEN *Fenofibrate*

GEM *Gemfibrozil*

LOV *Lovastatin*

OM3 *Omega-3 fatty acids*

SIM *Simvastatin*

[2] Refers to the method by which IDL was isolated

SUC Sequential ultracentrifugation

DG density gradient ultracentrifugation

[3] IDL TC at baseline

[4] IDL TG at baseline

[5] Reduction (in %) from baseline values

Comments

1. The study included a washout period between the two arms, therefore the baselines differ

While fibrates were more effective than statins in reducing VLDL lipids this finding does not hold true for denser lipoprotein fractions. Civeira (238) found that simvastatin reduced IDL TC more than gemfibrozil, while gemfibrozil reduced the TG mass within IDL more than simvastatin. In the Bayer study cerivastatin was as effective as other statins in reducing IDL TC and TG. Fenofibrate was less effective at reducing IDL TC than one would have expected reviewing the previously published literature. The reduction in IDL TC was 51%, 18% and 43 % for cerivastatin, fenofibrate and combination therapy respectively. IDL TG was reduced by 44%, 37% and 47 % respectively.

4.2.1.9.3 LDL

Most studies report changes in LDLC, although the methods used to determine LDLC vary. None of the studies uses the Friedewald equation (242) to calculate LDLC, which would clearly have been inappropriate. Most studies used the Lipid Research Clinics protocol to measure LDLC (329). This protocol specifies removal of VLDL by ultracentrifugation ($d<1.006$ g/ml) and precipitation of apo-B containing lipoproteins. HDLC is measured in the supernatant and LDLC calculated by subtraction of VLDLC and HDLC from the plasma TC. As this technique only removes VLDL and not IDL before precipitation of HDL the LDLC derived in this fashion does include IDLC. As can be seen from table 4.78 IDLC may be as high as 1.0-2.0 mmol/L in patients with dysbetalipoproteinaemia.

Table 4.79 shows LDLC responses to lipid-lowering therapy in previously published studies.

Table 4-79 LDL response to lipid-lowering therapy in previously published studies of dysbetalipoproteinaemia

Author	Year	Drug [1]	LDLC determination [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Hoogwerf	1984	NA 3g	LRC	3.86		11			(302)
Hoogwerf	1984	CLO 2g	LRC	3.86		2			(302)
Hoogwerf	1985	COL 20g	LRC	3.78		32			(305)
Hoogwerf	1985	CLO 2g	LRC	3.78		+6			(305)
Hoogwerf	1985	C+C	LRC	3.78		15			(305)
Stuyt	1985	ACP 750mg	LRC	3.05		10		1	(303)
Stuyt	1985	CLO 2g	LRC	3.14		11		1	(303)

Author	Year	Drug [1]	LDLC determination [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Packard	1986	BEZ 600mg	SUC	3.46		19			(307)
East	1986	MEV 40mg	NS	2.9		25			(304)
Kuo	1988	GEM 1.2g	LRC	4.95		41			(309)
Vega	1988	LOV 40	LRC	2.31		40			(313)
Lussier-Cacan	1989	FEN 300mg	SUC	1.66		+25			(308)
Illingworth	1990	LOV 40	LRC	3.6		32			(316)
Illingworth	1990	CLO 2g	LRC	3.6		1			(316)

Author	Year	Drug [1]	LDLC determination [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Dallongeville	1991	OM3 6g	LRC (M)	1.92		+20			(326)
Feussner	1992	SIM 20mg	LRC	3.54		36			(318)
Larsen	1994	GEM 1.2g	LRC	3.57		22			(321)
Larsen	1994	CLO 2g	LRC	3.57		12			(321)
Zhao	1994	GEM 1.2g	DG	1.87	0.41	0	49		(306)
Gylling	1995	LOV 40mg	DG	1.5	0.2	20	0		(317)
Civeira	1999	SIM 20mg	SUC	4.53		30		1	(238)
Civeira	1999	GEM 1.2g	SUC	4.48		2		1	(238)
van Dam	2002	ATR 40mg	DG	1.87		38			(240)

Author	Year	Drug [1]	LDLC determination [2]	Baseline values (mmol/L)		% Reduction [5]		Comments	Ref
				TC [3]	TG [4]	TC	TG		
Ishigami	2003	ATR 20mg	SUC	1.30	0.23	44	63		(315)

[1] Drug and daily dosage

ATR *Atorvastatin*

BEZ *Bezafibrate*

C+C *Colestipol (20g) + Clofibrate 2g*

CLO *Clofibrate*

COL *Colestipol*

FEN *Fenofibrate*

GEM *Gemfibrozil*

LOV *Lovastatin*

MEV *Mevinolin*

NA *Nicotinic acid*

OM3 *Omega-3 fatty acids*

SIM *Simvastatin*

[2] Refers to the method by which LDLC was measured

LRC Lipid research clinic protocol (IDL included with LDL fraction)

LRC (M) A modified protocol including ultracentrifugation at d=1.019g/mL to remove IDL

SUC Sequential ultracentrifugation

DG density gradient ultracentrifugation

[3] LDLC at baseline

[4] LDL TG at baseline

[5] Reduction (in %) from baseline values

Comments

1. Study involved a drug washout between the treatment arms; the baselines are therefore not identical

On review of the reported baseline LDLC levels it is obvious that studies reporting LDLC using the LRC methodology generally report higher LDLC levels than those using ultracentrifugal techniques that exclude IDLC. In the former LDLC levels approximate 3.5 mmol/L, while the latter report LDLC of less than 2.0 mmol/L in most instances. The only notable exception is the paper by Civeira and colleagues (238) that reports a baseline LDLC of almost 4.5 mmol/L. As previously discussed the patients in this study are atypical in many respects.

As seen from the table above LDLC may rise, fall or remain unchanged with lipid-lowering therapy. LDLC generally decreases more with statins than fibrates (238;316), which was also replicated in the Bayer study. In this study cerivastatin, fenofibrate and combination therapy lowered LDLC by 34%, 6% and 23% respectively. The effect of fibrate therapy on LDLC may be very dependant on the baseline characteristics of the patients entered into the study, as patients with high baseline TG levels are more likely to respond to lipid-lowering therapy with LDLC increases.

Very few studies report on changes in LDL TG mass, but all studies except Gylling *et al* (317), find decreased LDL TG with lipid-lowering therapy. The decreases of 44%, 41% and 44% observed with cerivastatin, fenofibrate and combination therapy in the Bayer study fall within the previously documented range.

4.2.1.9.4 HDLC

There have been significant changes in the methodology used to measure HDLC over the last few decades (257). The CDC recommended reference method involves removal of TGRL by ultracentrifugation followed by precipitation of apoB-containing lipoproteins and measurement of cholesterol in the supernatant (330). Early precipitation based assays on whole plasma are subject to significant interference from elevated TG, which often preclude complete precipitation of aggregated apoB-containing lipoproteins and lead to falsely elevated HDLC levels. Newer generation homogenous HDLC assays are said to be free of interference by TG (331), except if present in extremely high concentrations. This, however, does not apply to patients with dysbetalipoproteinaemia where newer methods may give either falsely low (332) or falsely high results (333). Measurement of HDLC in dysbetalipoproteinaemic patients is therefore problematic and this is especially so if they are severely hypertriglyceridaemic.

The HDLC responses to lipid-lowering therapy reported in tables 4.8-4.11 are highly variable, ranging from modest decreases to increases as high as 54% (309). The majority of studies do, however, report more modest changes in HDLC. In the Bayer study the ultracentrifugally derived HDLC decreased 4 % with cerivastatin while increasing 13% with fenofibrate and 6% with combination therapy. These responses are within the lower range of what has been previously reported in the literature.

Only Ishigami *et al* (315) report on changes in HDL TG in a study of apoE2 homozygotes treated with atorvastatin 20mg. HDL TG mass decreased by 26% and 36% in HDL_2 and HDL_3 respectively. This is comparable to the 29% reduction in HDL TG mass observed in the cerivastatin arm of the Bayer study.

4.2.1.9.5 Studies that report changes in compositional analysis of lipoprotein fractions

Only few of the trials listed in tables 4.8-4.11 report detailed information on changes in compositional analysis of lipoproteins following treatment of dysbetalipoproteinaemia. Ishigami *et al* (315) report lipid masses within each lipoprotein fraction, but do not report protein levels within lipoprotein fractions making it impossible to calculate lipoprotein composition. Lussier-Cacan *et al* (308) report complete compositional data for nine dysbetalipoproteinaemic patients treated with fenofibrate. Zhao et al report compositional data in two studies. The first study (306) reports eight patients homozygous for apoE2 treated with gemfibrozil, while the second study (323) reports the effects of simvastatin treatment in nine patients with a variety of apoE mutations.

4.2.1.9.5.1 VLDL composition

Two studies report on VLDL compositional changes in patients taking fibrates. Zhao *et al* (306) report that gemfibrozil significantly decreased PL relative mass in VLDL1 (Baseline: 14.2 ±1.4; Therapy: 11.9 ± 2.7) and CE relative mass in VLDL 2 (Baseline: 32.3 ± 41, Therapy: 27.0 ± 4.0). The relative protein content of VLDL2 increased significantly (Baseline: 9.5 ± 1.0; Therapy: 12.4 ± 1.7). No other significant changes were observed in the composition of either VLDl1 or VLDL2. In contradistinction Lussier-Cacan *et al* (308) analyzed VLDL as a single fraction and found significant changes in almost all compositional parameters.

Table 4-80 VLDL composition as found by Lussier-Cacan *et al*

Parameter	Baseline [1]	Fenofibrate [1]	P [2]
FC/TM [3]	8.2 ± 1.2	6.8 ± 1.0	<0.02
CE/TM	32.7 ± 9.4	19.4 ± 2.5	<0.005
TG/TM	35.1 ± 9.6	47.5 ± 2.2	<0.01
PL/TM	17.5 ± 1.2	16.1 ± 0.8	<0.025
PR [4]/TM	6.5 ± 1.9	10.2 ± 1.5	<0.001
CE/TG	1.09 ±0.66	0.41 ± 0.06	<0.02
CE/PR	5.83 ± 3.34	1.95 ± 0.45	<0.01

[1] Mean ± SD

[2] P by unpaired t-test

[3] Total mass

[4] Protein

Evaluating simvastatin treatment Zhao *et al* found that the relative TG mass in VLDL2 increased significantly in apoE2 homozygotes (Baseline: 29.6 ± 2.3; Therapy: 39.6 ± 4.3). No other significant changes were found in VLDL1 or VLDL2. The statistical power of this study is, however, limited as the authors analyzed patients with different apoE mutations separately, and therefore only had three subjects in each group.

In the Bayer study lipid-lowering therapy was associated with significant changes in the composition of VLDL1, while VLDL2 composition remained virtually unchanged. The changes in VLDL1 composition were for the most part analogous to those observed by Zhao and Lussier-Cacan. In particular we also observed significant reductions in FC and CE relative mass in VLDL1 as well as a reduction in the CE/TG ratio. Although many other of the changes in compositional indices in our study did not reach statistical significance we observed changes similar to those previously reported by Lussier-Cacan, namely an increase in the relative TG mass and a reduction in PL content.

4.2.1.9.5.2 IDL

Zhao (306) found no significant changes in IDL composition with gemfibrozil therapy while Lussier-Cacan (308) found statistically significantly decreased CE (Baseline: 44.1 ± 6.5; Therapy: 33.0 ± 6.3) and increased TG relative mass (Baseline: 14.8 ± 5.4; Therapy: 23.0 ± 4.8). Simvastatin did not change the composition of IDL in any of the apoE genotypes studied by Zhao *et al* (323). In the Bayer study significant changes in IDL composition were not discernible either with fibrate or statin treatment.

4.2.1.9.5.3 LDL

Zhao (306) reports decreased relative TG mass in LDL with gemfibrozil therapy (Baseline 11.1 ± 2.1; Therapy 8.0 ± 1.3), while Lussier-Cacan found increased PL content with fenofibrate (Baseline 17.1 ± 1.3; Therapy: 20.0 ± 1.8). Simvastatin was not associated with any significant changes in LDL composition (323).

In the Bayer study there was a significant decrease in relative TG mass but no change in the relative PL mass.

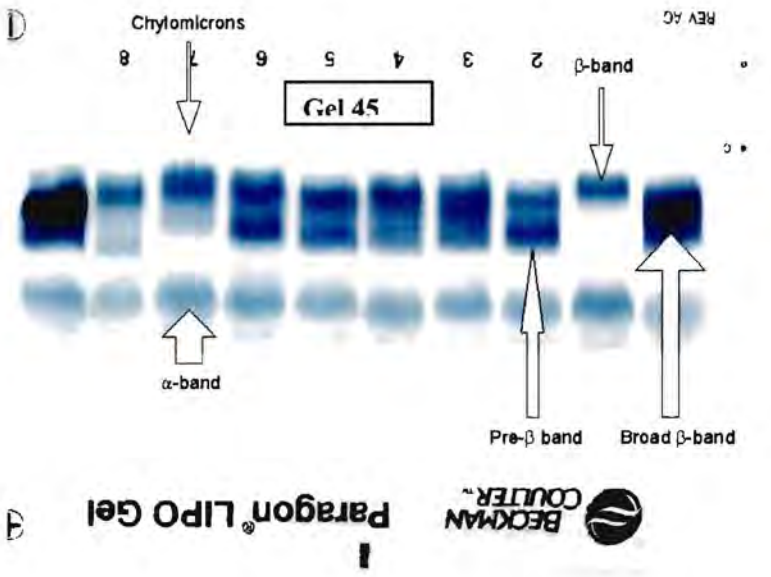
4.2.1.9.5.4 HDL

There are no reports in the published literature of HDL compositional changes with lipid-lowering therapy in dysbetalipoproteinaemic subjects and comparisons with previously published data are therefore not possible.

4.2.1.10 Agarose gel electrophoresis

All patients had agarose gel electrophoresis at baseline and at completion of each treatment arm. Plasma and aliquots of the lipoprotein fractions obtained by sequential ultracentrifugation was electrophoresed. Agarose gel electrophoresis was done within 72 hours of venesection. All plasma and ultracentrifugal samples were kept refrigerated until electrophoresis was performed. Gels were reported on in the conventional fashion and then also scanned for densitometric analysis. Optical density data were exported to GraphPad Prism and analyzed further.

Figure 4-61 Agarose gel (plasma)



Illustrated below are two representative agarose gels of plasma and lipoprotein fractions, showing the different VLDL migration patterns for different patients.

Figure 4-62 Agarose gel of plasma and lipoprotein fractions (Patient JJV)

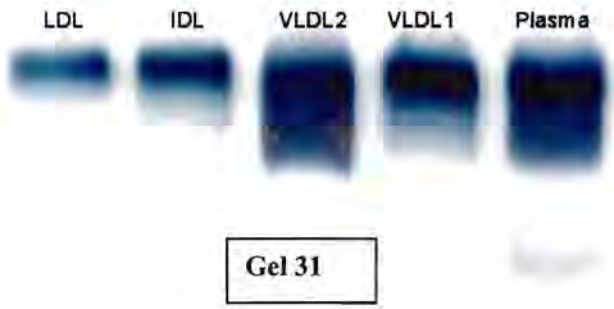
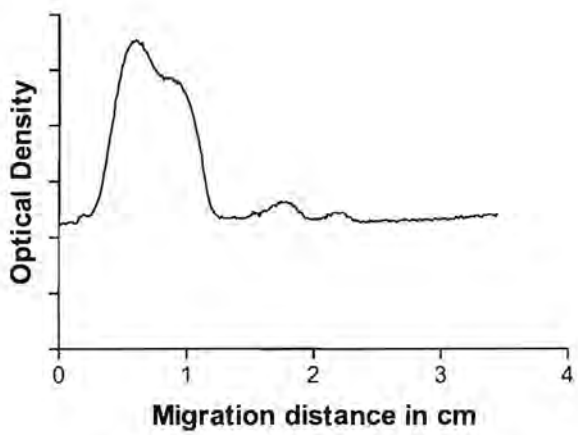


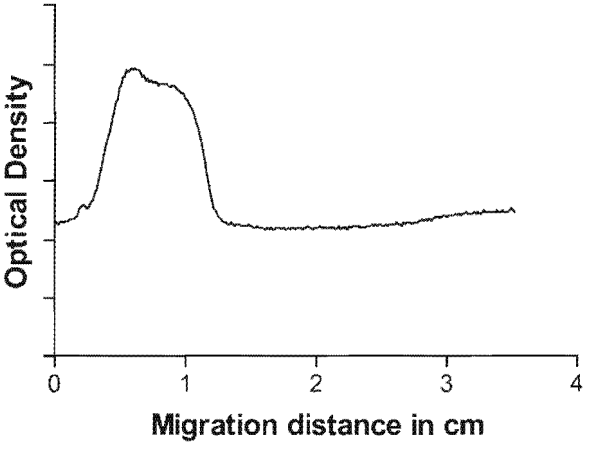
Figure 4-63 Densitometric scans of plasma and lipoprotein fractions (Pat JJV)

The OD scans are those of figure 4.62. The patient is a male apoE2 homozygote. The sample was taken in the untreated state following drug washout.

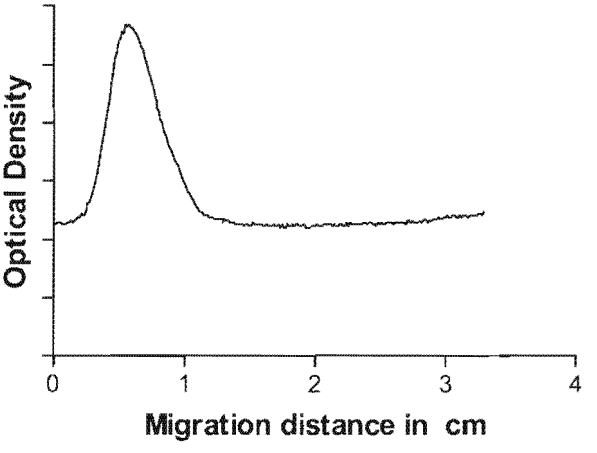
Plasma



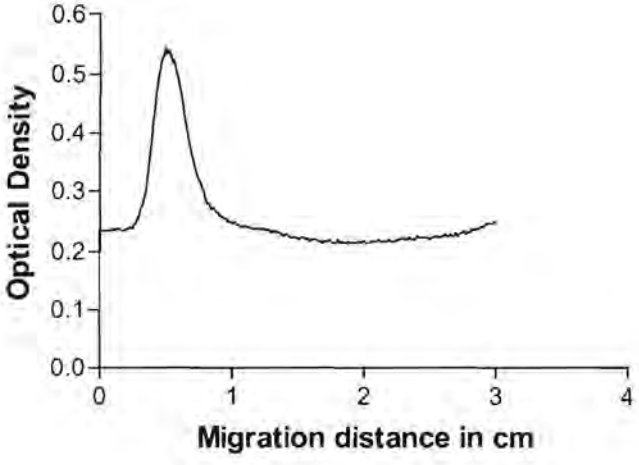
VLDL1


Optical Density
0
1
2
3
4
Migration distance in cm

VLDL2


Optical Density
0
1
2
3
4
Migration distance in cm

IDL


Optical Density
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
Migration distance in cm

LDL

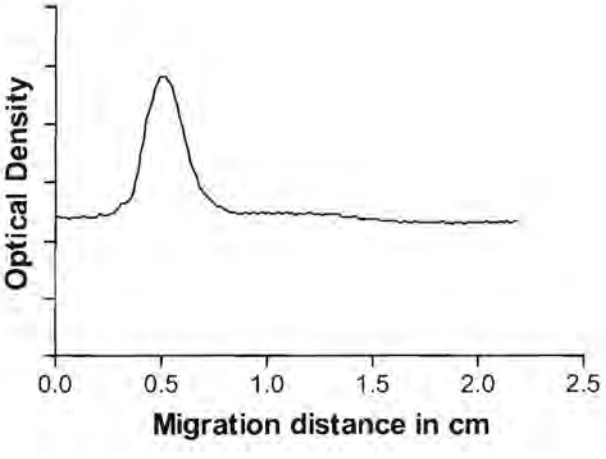

Optical Density
0.0
0.5
1.0
1.5
2.0
2.5
Migration distance in cm

Figure 4-64 VLDL migration relative to plasma (Pat JJV)

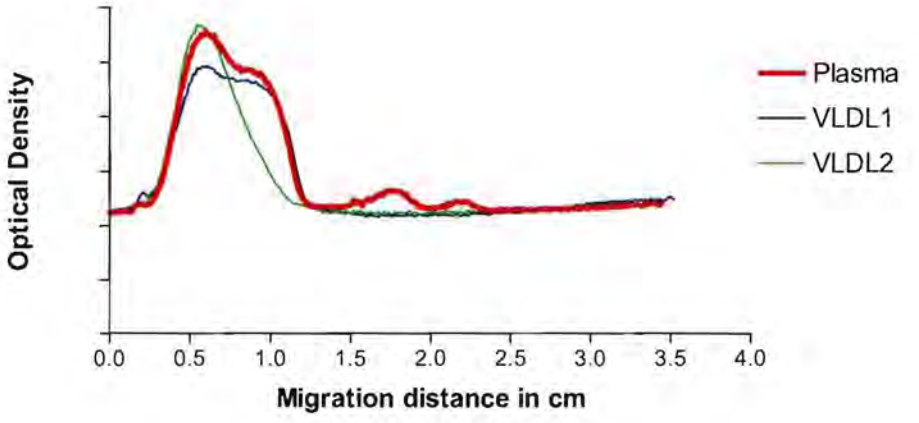


Figure 4-65 IDL and LDL migration relative to plasma (Pat JJV)

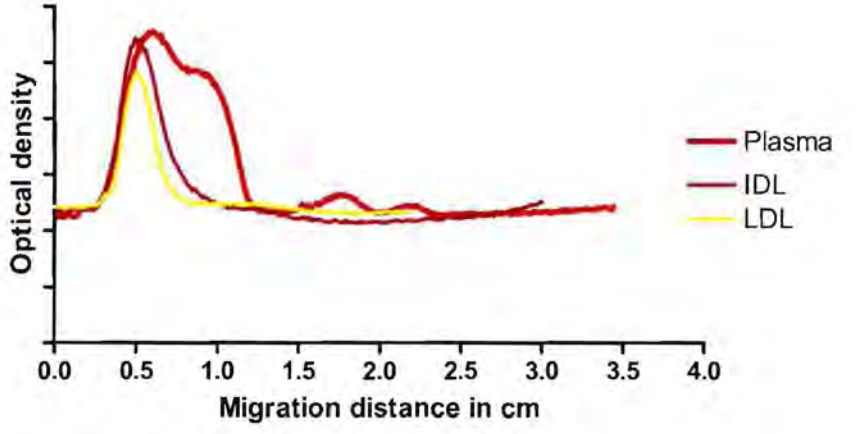


Figure 4-66 VLDL migration relative to LDL (Pat JJV)

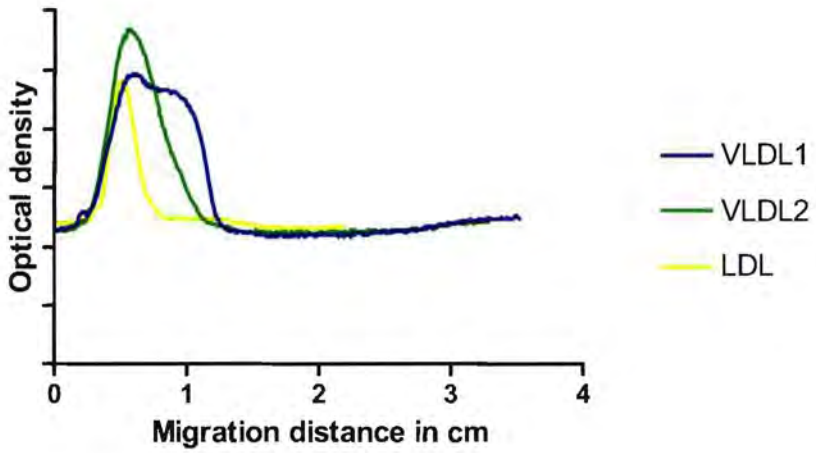


Figure 4-67 Agarose electrophoresis of plasma and lipoprotein fractions (Pat FCC)

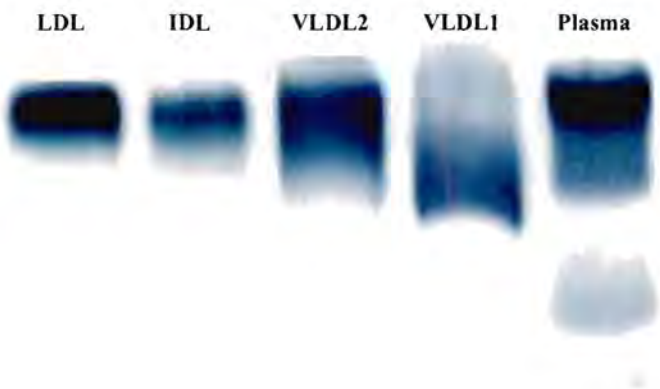
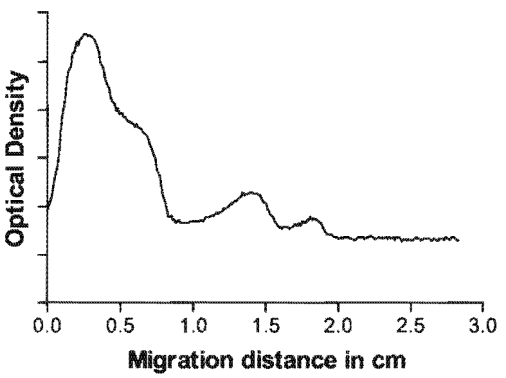


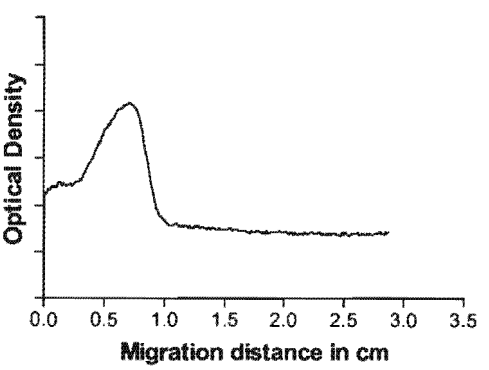
Figure 4-68 Densitometric scans of plasma and lipoprotein fractions (Pat FCC)

The OD scans are those of figure 4.69. The patient is male and has no identified apoE mutation. The sample was taken in the untreated state following drug washout.

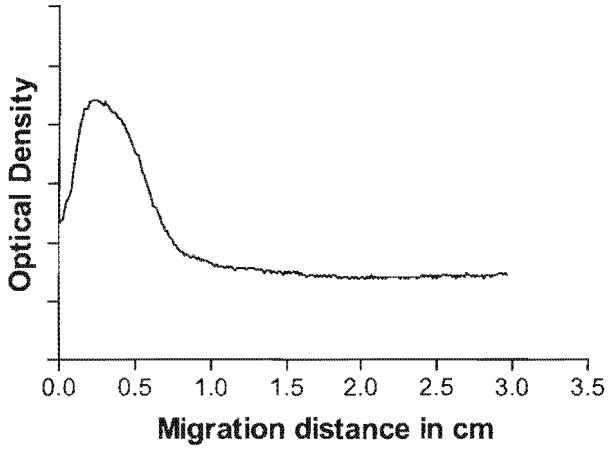
Plasma



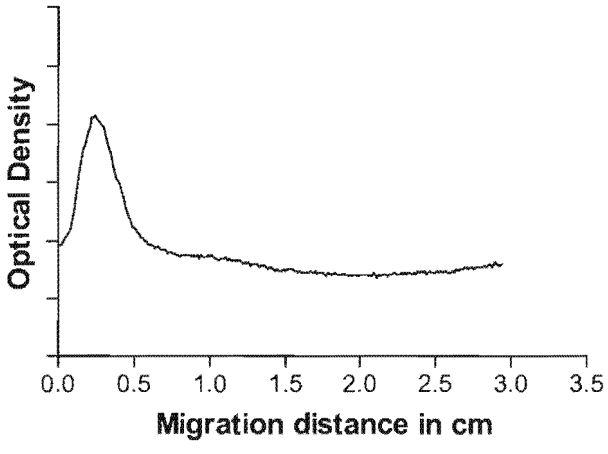
VLDL1



VLDL2


Optical Density
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Migration distance in cm

IDL


Optical Density
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Migration distance in cm

LDL

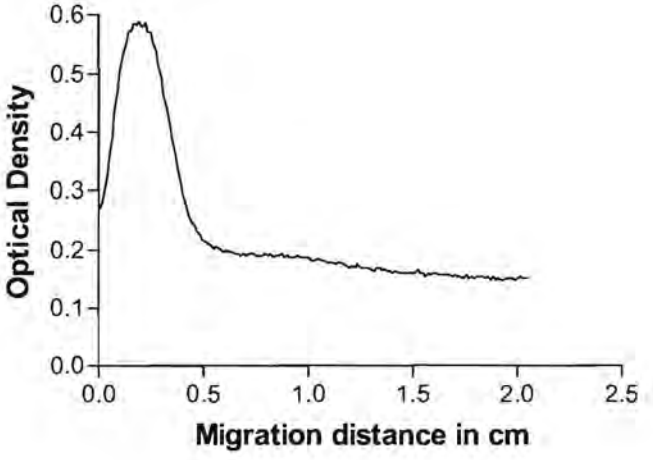


Figure 4-69 VLDL migration relative to plasma (Pat FCC)

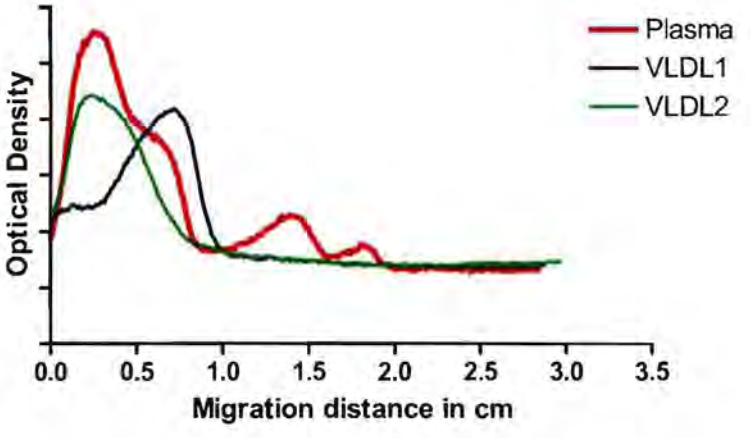


Figure 4-70 IDL and LDL migration relative to plasma (Pat FCC)

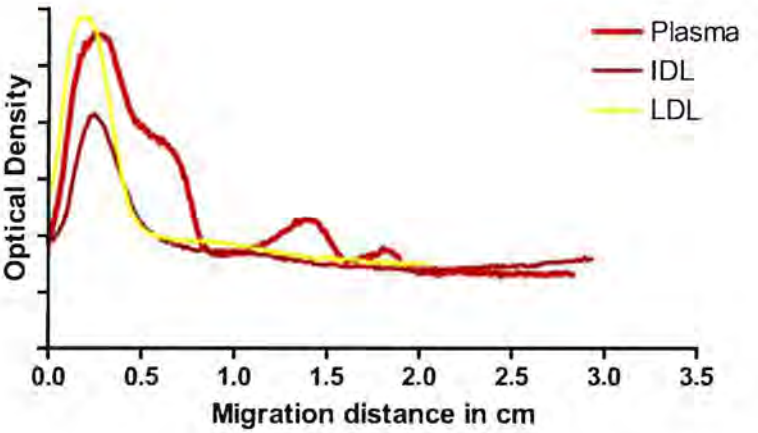
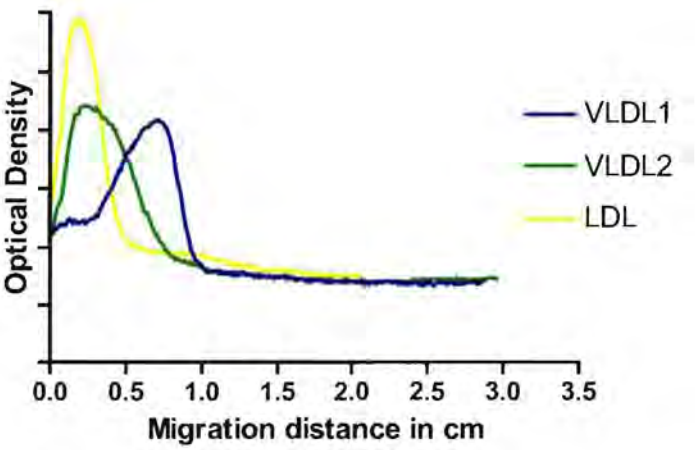


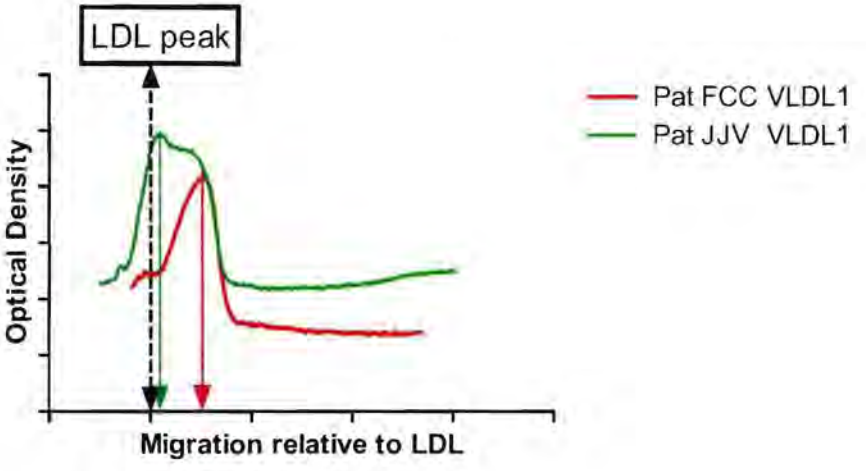
Figure 4-71 VLDL migration relative to LDL (Pat FCC)



From the above graphs it is clear that the VLDL1 fractions of patients show significant differences in the distance migrated. Figure 4.72 compares the migration of

VLDL1 in the two examples listed above. The graph has been normalized with the LDL peak of each patient set at a migration distance of 1 cm.

Figure 4-72 VLDL1 migration: Patients FCC and JJV

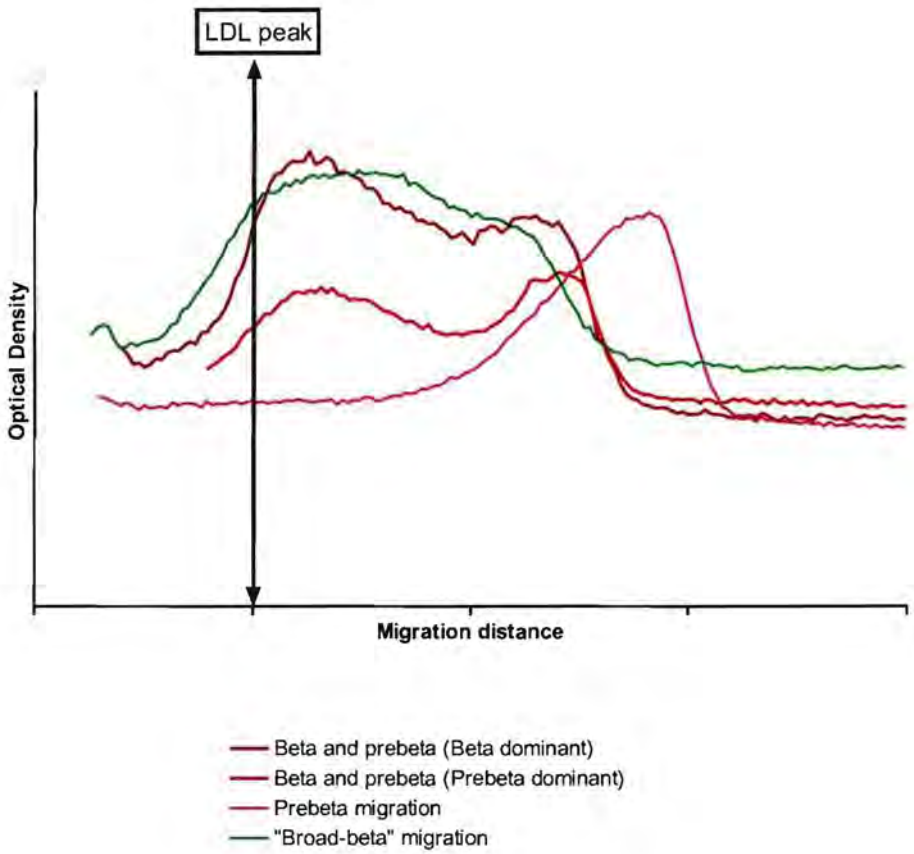


VLDL1 migration did not only differ by migration distance, but four patterns of VLDL1 migration defined by the number, location and morphology of peaks seen on optical density scanning were identified.

VLDL1 migration patterns:

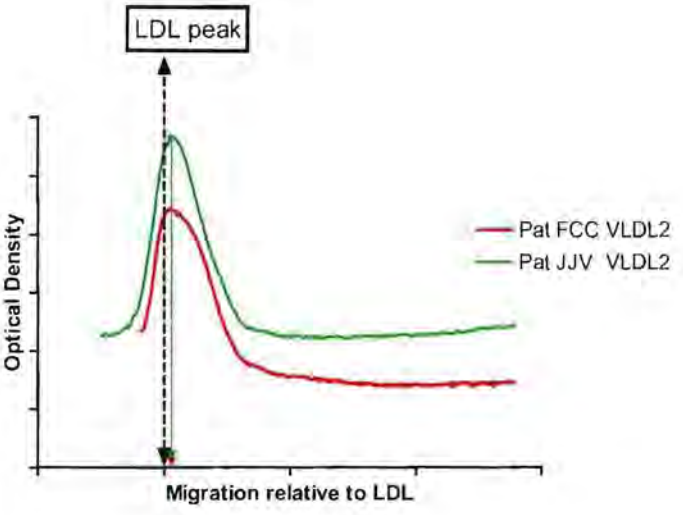
1. Two distinct peaks with beta and prebeta mobility (Beta peak dominant)
2. Two distinct peaks with beta and prebeta mobility (Prebeta dominant)
3. Prebeta peak only
4. Single, broad peak originating in the beta area and extending to the prebeta area, labelled as "broad-beta" migration

Figure 4-73 Categorization of VLDL1 migration patterns



VLDL2 band morphology was much more uniform than VLDL1 band morphology. VLDL2 bands had a single peak that often, but not always, migrated with beta mobility.

Figure 4-74 VLDL2 migration: Patients FCC and JJV



IDL migration was highly uniform as a single, narrow band that migrated with beta mobility (not illustrated).

4.2.1.10.1 Agarose gel electrophoresis of plasma

In the untreated state 40% (8 of 20) patients had a Fredrickson Type III pattern. This pattern was only found in patients with identified apoE mutations. The next most common pattern was Fredrickson Type IIB. The introduction of lipid-lowering therapy was associated with significant changes in electrophoretic patterns. In patients with a Type III pattern at baseline this pattern persisted in 3/5 patients treated with cerivastatin, 1/8 patients treated with fenofibrate and 1/4 of patients taking combination therapy. Two patients had a Type III pattern only when taking cerivastatin, these patients respectively had Type V and IIB patterns at baseline. Fenofibrate and combination therapy were associated with significantly lower TG levels than cerivastatin therapy (see above) and it is therefore not surprising that IIB

patterns were seen most commonly with cerivastatin treatment followed by fenofibrate and combination therapy (P=0.0042 by Chi-square test).

Table 4-81 Agarose gel electrophoresis of plasma

Patient group			Baseline	Cerivastatin	Fenofibrate	Combination
Agarose electrophoresis pattern	All patients	IIA	0	1	7	8
		IIB	11	8	6	1
		III	8	4	1	1
		IV	0	0	0	0
		V	1	0	0	0
	Genopositive	IIA	0	1	6	5
		IIB	7	6	5	1
		III	8	4	1	1
		IV	0	0	0	0
		V	1	0	0	0
	Genonegative	IIA	0	0	1	3
		IIB	4	2	1	0
		III	0	0	0	0
		IV	0	0	0	0
		V	0	0	0	0

4.2.1.10.2 Agarose gel electrophoresis of lipoprotein fractions

VLDL1 patterns changed significantly from baseline with lipid-lowering treatment (P=0.0001 by Chi-square test). At baseline 79% (15/19) of patients had VLDL1 that

included a beta-migrating component, even if this was not dominant. Following institution of lipid-lowering therapy a prebeta band only was clearly the dominant pattern in patients receiving fenofibrate either alone or in combination with cerivastatin. In both the cerivastatin and fenofibrate arms there were two instances each of a "broad-beta" migrating VLDL1. Different patients were affected in each case, but interestingly these patients came from the top TG quartile of their respective treatment arm (detailed data not shown). As previously shown TG levels were higher in the cerivastatin group and this group also had more patients with beta-migrating VLDL1.

Table 4-82 VLDL1 patterns on agarose gel electrophoresis

	Baseline	Cerivastatin	Fenofibrate	Combination
Two peaks Beta dominant	5	0	0	0
Two peaks Prebeta dominant	1	4	0	1
Prebeta only	4	7	12	9
"Broad-beta"	9	2	2	0

Although there were only four patients with no identified apoE mutations in this analysis cohort they all had prebeta migrating VLDL1 and migratory behaviour did not change with lipid-lowering therapy. All patients with identified apoE mutations had a beta-migratory component to their VLDL1 at baseline.

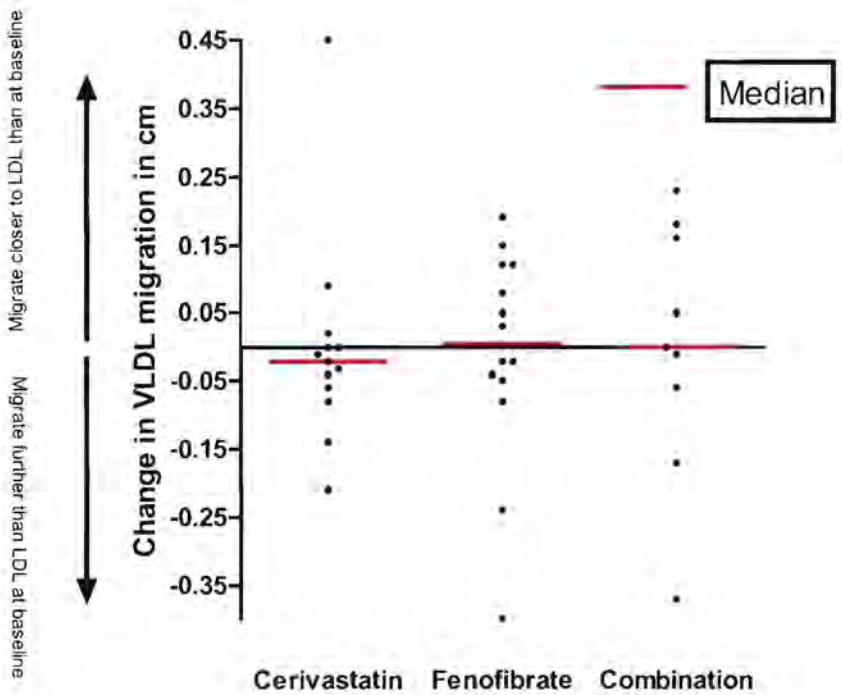
Table 4-83 VLDL1 patterns according to apoE mutation status

	Baseline		Cerivastatin		Fenofibrate		Combination	
	Geno +	**Geno -**	**Geno +**	**Geno -**	**Geno +**	**Geno -**	**Geno +**	**Geno -**
Two peaks Beta dominant	5	0	0	0	0	0	0	0
Two peaks Prebeta dominant	1	0	4	0	0	0	1	0
Prebeta only	0	4	5	2	10	2	6	3
"Broad-beta"	9	0	2	0	2	0	0	0

VLDL2 morphology did not change from baseline with lipid lowering treatment and VLDL2 uniformly migrated as a single peak. The VLDL2 peak was generally found in the beta area of the gel, although it did on average migrate a fraction further than LDL. VLDL2 migration was assessed relative to LDL migration for each sample by determining the distance between the two peaks. The mean ± SD difference was 0.14 ± 0.15 cm at baseline, 0.16 ± 0.15 cm with cerivastatin, 0.14 ± 0.10 cm with fenofibrate and 0.16 ± 0.12 with combination therapy (ANOVA, P=0.93).

The changes in VLDL migration relative to LDL migration with lipid-lowering therapy were assessed individually for each patient. Lipid-lowering therapy was associated with changes in VLDL2 migration relative to LDL migration in both directions.

Figure 4-75 Changes in individual VLDL2 migration from baseline relative to LDL migration



IDL migration was very uniform throughout the study as a single narrow band with beta mobility. The mean ± SD distance between IDL and LDL peaks at baseline was 0.03 ± 0.04 cm, 0.03 ± 0.03 cm with cerivastatin, 0.02 ± 0.03 cm with fenofibrate and 0.01 ± 0.04 cm with combination therapy (ANOVA, P=0.34).

4.2.1.11 Polyacrylamide gradient gel electrophoresis

Samples for PGGE were frozen at -80° Celsius and stored until the study had been completed. Electrophoresis was then performed on batched samples. Previous experience in the lipid laboratory (unpublished) had indicated that electrophoretic patterns are not altered by storage at -80° Celsius.

All samples were assigned a classification according to the scheme illustrated in figure 3.20. OD tracings were also obtained to measure the Rf, and therefore the size, of LDL if particles of this size were seen on the gel.

At baseline the majority of patients (15/21) had the A pattern which is highly characteristic of dysbetalipoproteinaemia.

Table 4-84 PGGE patterns at baseline and with lipid-lowering therapy

Pattern	Baseline	Cerivastatin	Fenofibrate	Combination
A	15	9	8	3
B	1	2	3	3
C	4	2	1	1
D	1	0	2	3

PGGE responded variably to lipid-lowering therapy. In many patients there was no change in PGGE pattern despite significant lowering of serum lipids. When changes in PGGE patterns were observed they commonly were: staining of LDL-sized particle where there previously had been none, increased size of LDL peak or reduction in TGRL on the gel.

The following figures illustrate a few representative responses to lipid-lowering therapy.

Figure 4-76 PGGE pattern showing only minor variations with therapy (pat JCJ)

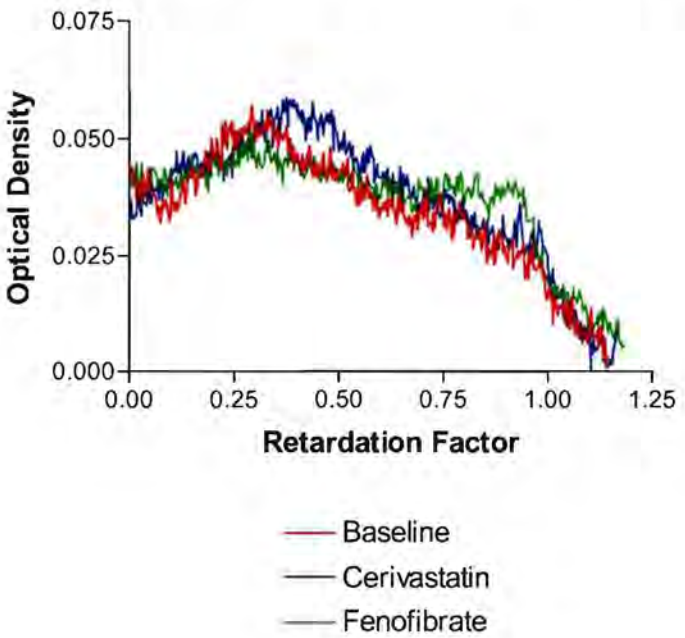


Figure 4-77 PGGE pattern showing increasing amounts of LDL-sized particles (Pat MHR)

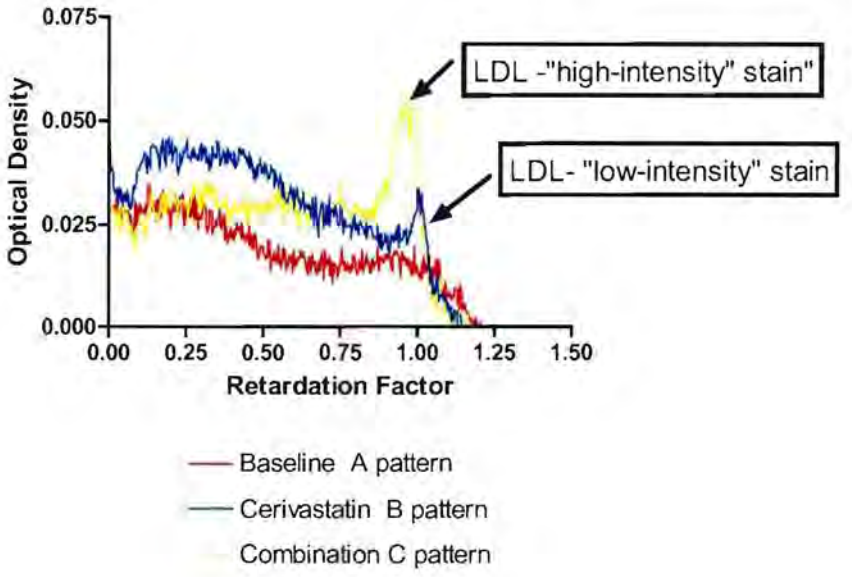
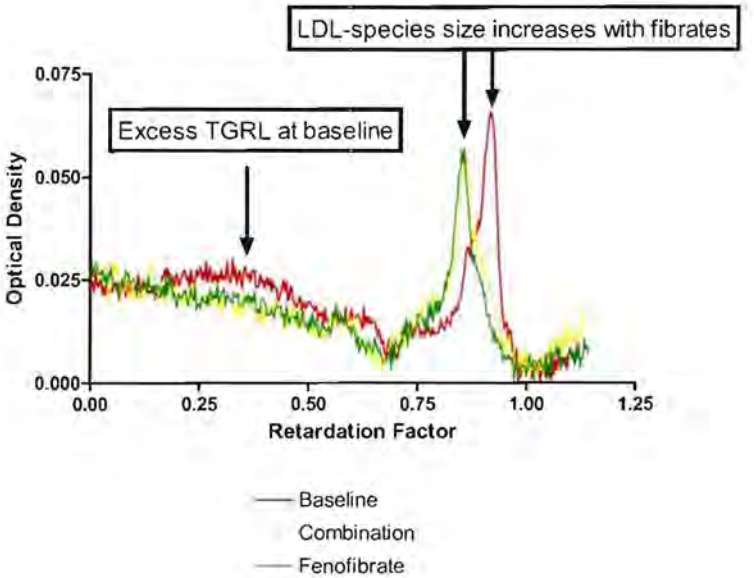


Figure 4-78 PGGE pattern showing reduction in TGRL and change in LDL-species size (Pat KLL)



Fifty-three % (8/15) patients with an A pattern at baseline retained this pattern throughout the study. In the other 7 subjects LDL-sized particles became visible in 73 % (8/11) of gels studied. The staining of LDL-sized particles was interpreted as low intensity in all but one of these patients. None of the patients that had visible LDL-sized particles on the gel at baseline reverted to an A pattern with lipid-lowering therapy.

In those patients with LDL-sized particles visible in the untreated state the most frequent change was a reduction in TGRL (2/5) or no change (2/5). In one patient a marked reduction in the intensity of staining of LDL-sized particles was observed.

4.2.1.11.1 PGGE and apoE genotype

There were marked differences in the distribution of PGGE between patients with identified apoE mutations and those without. At baseline almost all patients with an identified apoE genotype had no visible LDL staining, while all genonegative patients had visible LDL-sized particles (P=0.001 by Chi-square test for presence of LDL-sized particles by genotype at baseline).

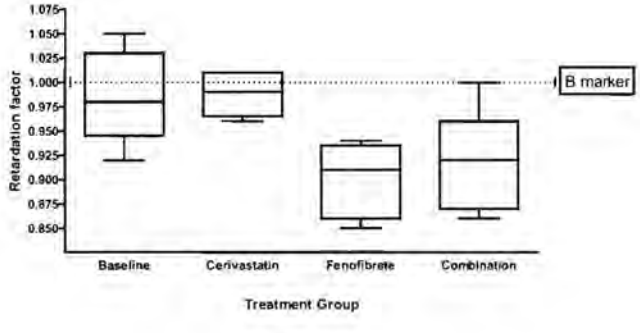
Table 4-85 PGGE patterns and apoE genotype

	Baseline		Cerivastatin		Fenofibrate		*Combination*	
	Geno+	Geno-	Geno+	Geno-	Geno+	Geno-	Geno+	*Geno-*
A	15	0	9	0	8	0	3	*0*
B	0	0	2	0	3	0	3	*0*
C	1	3	0	2	1	0	1	*0*
D	*0*	*1*	*0*	*0*	*0*	*2*	*0*	*3*

4.2.1.11.2 LDL-size and lipid-lowering treatment

In patients with visible LDL-sized particles LDL particle-size was analyzed by comparing retardation factors measured at peak OD. Peak LDL-size was larger in the two treatment arms containing fenofibrate than at baseline or with cerivastatin (P=0.009 by ANOVA; Tukey's multiple comparison test with P<0.05 for baseline and cerivastatin vs. fenofibrate). This is consistent with the described effects of fibrates on LDL-species size.

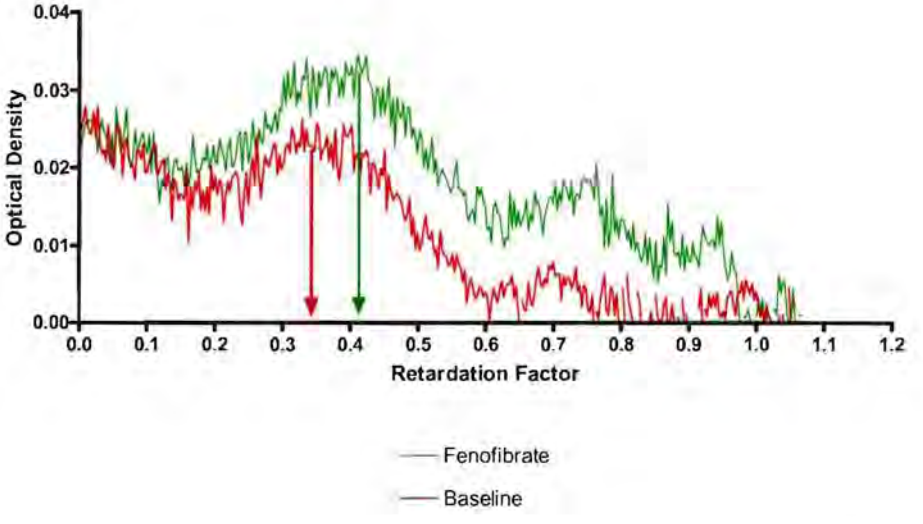
Figure 4-79 LDL-species size with lipid-lowering therapy



4.2.1.11.3 TGRL size and lipid lowering treatment

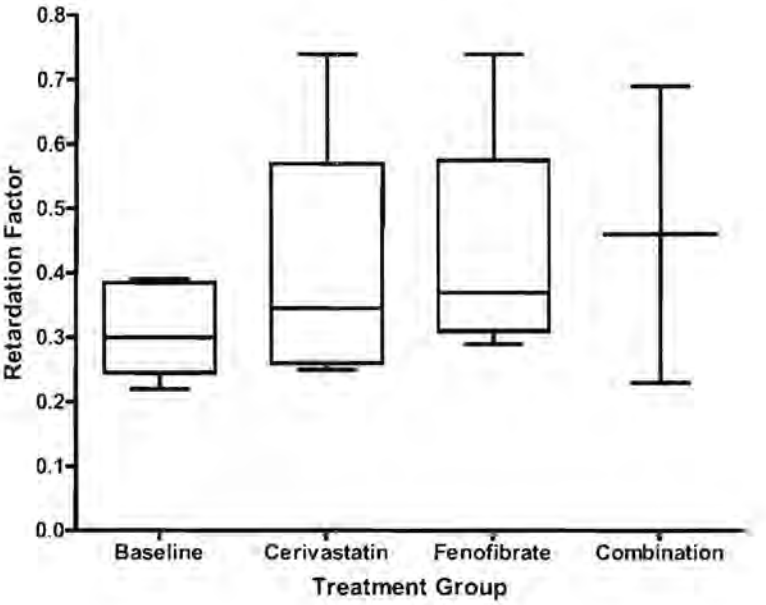
The size of TGRL was examined on and off treatment in the 8 patients that maintained an A pattern throughout the entire study period. The Rf was measured at the peak optical density of TGRL staining.

Figure 4-80 Determination of TGRL size in patients with A pattern



The size of TGRL decreased from baseline with lipid-lowering therapy, but the difference was not statistically significant (ANOVA P=0.57).

Figure 4-81 Change in Rf of TGRL with lipid-lowering therapy in patients maintaining an A pattern throughout study period



4.2.1.12 Safety Results

This section includes data from all patients who received at least one dose of study medication. Patients with compliance less than 80% compliance were included in the safety assessment.

4.2.1.12.1 Serious adverse events (SAEs)

Two SAEs occurred during the course of the study. Both of the SAEs were assessed as probably not related to study medication. A brief description and commentary on the SAEs is provided.

- A 51-year old male with pre-existing IHD (2 previous myocardial infarctions) and frequent but stable angina pectoris had a fatal anterolateral myocardial infarction 8 days after completing the placebo washout and commencing therapy with cerivastatin. The patient died approximately 36 hours after the onset of chest pain of cardiogenic shock complicated by acute renal failure and pulmonary oedema. The patient had undergone coronary angiography 17 years before being enrolled into the study at which stage severe diffuse distal coronary artery disease not amenable to surgical or percutaneous intervention had been demonstrated. Clearly, severe IHD was present at baseline and the occurrence of a MI in this setting is not entirely unexpected. Yet there is concern that withdrawal of lipid-lowering therapy may have increased the risk of an acute cardiac event. In 2002 Heeschen et al (334) reported an approximately 3-fold increase in death and non-fatal MI in patients admitted with an acute coronary syndrome when statins were withdrawn at admission. Much of this effect was subsequently found to have been due to an error in statistical analysis (335). Although not studying dysbetalipoproteinaemia the "Treat to New Targets " (TNT) trial provides the most definite evidence on the effect of statin withdrawal in patients with stable coronary artery disease. In this study 9395 patients with IHD who had been taking a statin entered a six week washout phase followed by an eight week open label phase of atorvastatin 10 mg/d. Cardiac event rates did not differ between the two phases, suggesting that short-term withdrawal of statins is not associated with excess cardiac morbidity (336).

- A 56-year-old male patient underwent laparoscopic cholecystectomy for acute cholecystitis 22 days after starting fenofibrate, following washout of previous lipid-lowering medications. Unfortunately, an adequate description of the gallstones was not provided and they were not preserved for chemical analysis. The patient had previously been exposed to various fibrates and statins over a period of more than 10 years. Fibrates increase the relative concentration of phospholipids and cholesterol in bile, whereas the concentration of bile acids is reduced (337). Increased cholesterol concentration or saturation of bile may promote formation of cholesterol stones (338). Fibrates influence biliary composition mainly by decreasing the activity of cholesterol-7-α-hydroxylase (CYP7A1), the first enzyme in the major pathway of bile acid synthesis (339). The effect of fibrates on CYP7A1 activity is PPARα mediated reduced mRNA transcription. Additionally PPARα stimulation increases mRNA levels for ABCG5, MDR3 and SREBP2 slightly (340). In this particular patient long-term fibrate therapy may have increased the risk of cholesterol gallstones forming (although there is no proof that the patient did in fact have cholesterol stones), but there is no evidence to suggest that fenofibrate precipitates cholecystitis in patients with gallstones.

4.2.1.12.2 Adverse events leading to discontinuation of study medication

Three patients discontinued study medication before the sponsor terminated the study. One patient withdrew consent, but had not experienced any study related adverse

events. Two patients discontinued study medications due to medication related adverse events.

- A 45-year-old female patient experienced myalgia, backache and headache while taking cerivastatin during her second treatment period. The myalgia was not associated with elevation of serum CK. She had previously taken fenofibrate without problems. The myalgia improved following statin withdrawal and subsequently recurred during routine follow-up following trial discontinuation when a statin (atorvastatin) was added to bezafibrate in an attempt to improve lipid control. This patient's myalgia clearly relates to statin exposure. Myalgia is a well known side effect of statins, although the exact mechanism remains to be determined (341).

- A 42-year-old male patient withdrew from the study while taking cerivastatin in the second treatment period. He had been randomized to cerivastatin and fenofibrate in the first treatment period. The patient described symptoms highly suggestive of panic attacks from the beginning of the study, including the single-blind placebo phase, but felt that these symptoms had been aggravated by study medication. He was initially persuaded to continue with study medication, but later decided to withdraw from the study. The patient has had ongoing symptoms of anxiety while taking other lipid-lowering medications. These symptoms are much more likely to relate to an underlying anxiety disorder than drug side effects.

4.2.1.12.3 Other clinical adverse events

Other clinical adverse events included upper respiratory tract infections, dental infections, headache, gastrointestinal disturbances and musculoskeletal (excluding myalgia) problems. None of these adverse events occurred at a higher frequency than would be expected in routine clinical practice. These other clinical adverse events were evenly distributed amongst all trial phases, including the placebo period. None of the other adverse events was judged clinically to be related to study drug. Detailed data on other clinical adverse events is not presented here.

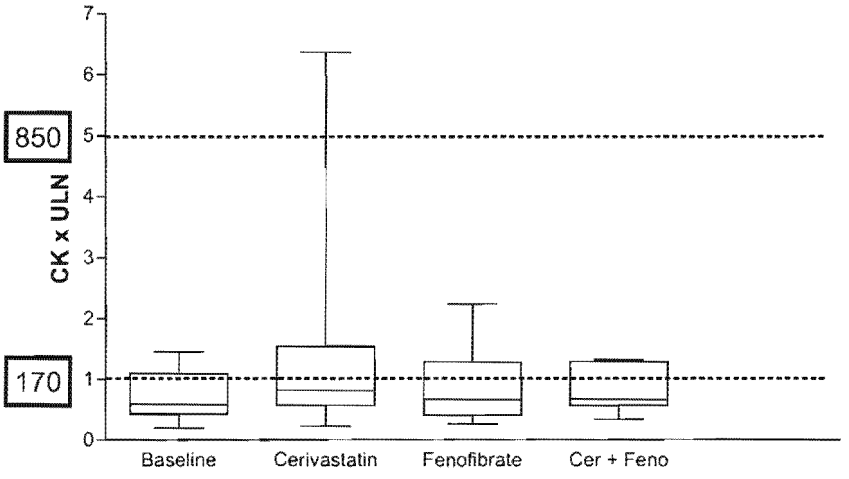
4.2.1.12.4 Laboratory monitoring

4.2.1.12.4.1 Creatine kinase

During the study one patient developed myalgia, without elevation of CK, while taking cerivastatin (see above) and withdrew from the study.

The laboratory ULN for CK for this study was 170 U/L. In this small study rhabdomyolysis did not occur. A single patient had an elevation in CK of more than 5 x ULN. This male patient had a CK of 6.4 x ULN while taking cerivastatin. He reported having an influenza-like syndrome around the time of his study visit with fever, muscle aches and respiratory symptoms. He was not discontinued from the study and when next assessed, while taking combination therapy, his CK was only marginally elevated at 1.3 x ULN and there was no myalgia.

Figure 4-82 CK (x ULN) during the study



Legend: The graph shows the CK values in relationship to the ULN.

There was no significant change in mean CK from baseline during any of the treatment arms (ANOVA; P=0.40).

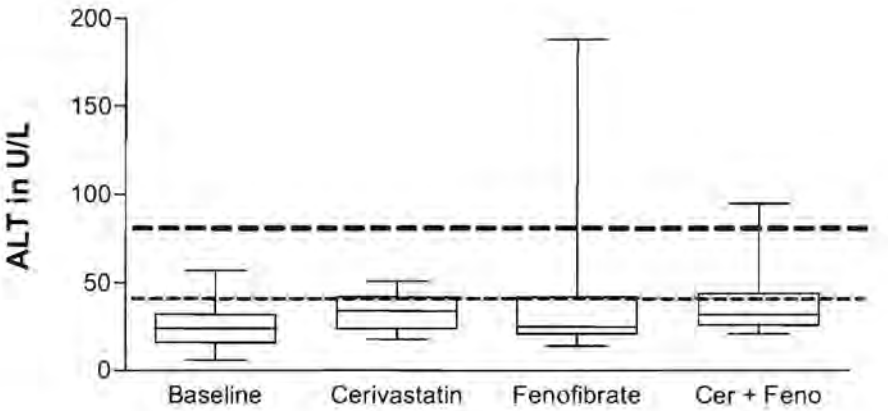
4.2.1.12.4.2 Liver function tests

4.2.1.12.4.2.1 Transaminases

The local laboratory reference ranges for transaminases in this study were 1-38 U/L for aspartate aminotransferase (AST) and 1-41 for alanine aminotransferase (ALT). Following withdrawal of lipid lowering medication the mean ALT was 25.6 ± 13.0 U/L (median 24.0, range 6-57 U/L) and the mean AST 19.4 ± 5.0 U/L (median 19.0, range 10-34 U/L).

The mean ALT increased from baseline in each treatment arm. The mean ALT with cerivastatin, fenofibrate and combination therapy was 33.8 ± 10.8 U/L, 40.4 ± 44.0 U/L and 38.3 ± 21.4 U/L respectively (ANOVA of log transformed data, P=0.05).

Figure 4-83 ALT during lipid lowering therapy



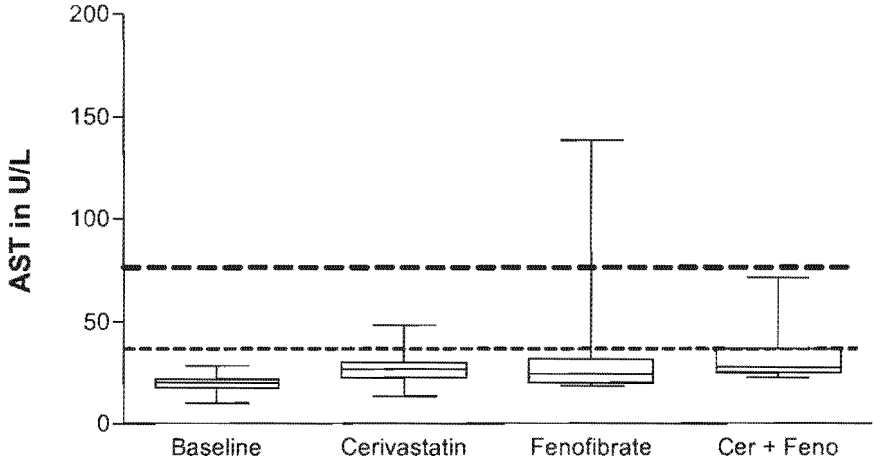
Legend: The thin dashed line indicates the ULN and the thick dashed line 2 x ULN

ANOVA P=0.05

Tukey's multiple comparison tests- all comparisons P>0.05

The mean AST with cerivastatin, fenofibrate and combination therapy was 26.0 ± 8.1 U/L, 33.0 ± 31 U/L and 32.7 ± 14.5 U/L (ANOVA of log transformed data, P=0.004)

Figure 4-84 AST during lipid lowering therapy



Legend: The thin dashed line indicates the ULN and the thick dashed line 2 x ULN

ANOVA P=0.004

Tukey's multiple comparison test: Baseline vs. Fenofibrate P<0.05

Baseline vs. Combination therapy P<0.01

An increase of more than 2 x ULN in the ALT and/or AST was observed in two patients:

- A female patient had an ALT of 188 U/L and an AST of 139 U/L while taking fenofibrate. This patient had taken combination therapy during the previous treatment arm with an ALT of 41 U/L and an AST of 38 U/L. At baseline her

ALT was 14 U/L with an AST of 17 U/L. The study was terminated two days after the patient had completed the fenofibrate arm and bezafibrate has subsequently been prescribed with the transaminases remaining within the normal range. There was no other identifiable reason for this patient's transaminitis and the elevation in transaminases therefore is ascribed to study medication.

- A male patient was noted to have an ALT of 95 U/L and an AST U/L of 71 while taking combination therapy. Previously, while taking cerivastatin monotherapy, his ALT and AST were 36 U/L and 29 U/L respectively. At baseline the ALT was 25 U/L with an AST of 27 U/L. The patient currently takes bezafibrate and his transaminases are normal. No other causes of transaminitis were identified and the transaminitis was ascribed to study medication.

If the two patients with increases of more than 2 x ULN in transaminases are excluded from the analysis as outliers the change in ALT with therapy is no longer significant (ANOVA of log transformed data, P=0.07) but the change in AST remains highly significant (ANOVA of log transformed data, P=0.0006).

Many patients treated with statins show transient increases in transaminase values, but elevations in ALT of >3 x ULN on at least two occasions, in clinical trials of various statins, occurred in only 70 patients per 100000 patient-years of statin exposure (342). Clinical liver disease or acute liver failure is extremely rare with statins and the rate of acute liver failure in patients taking statins is similar to the background rate of idiopathic acute liver failure in the general population (343). In one of the largest

trials of fenofibrate to date (FIELD study) ALT reached 3 x ULN or more on at least one occasion in a similar number of patients on fenofibrate and placebo (11/4895 and 26/4895 patients respectively). Of the patients allocated fenofibrate 944 were taking statins at the end of the trial as well with no reported increase in hepatic side-effects (344). Fenofibrate is there generally safe from a hepatic point of view and combination statin and fibrate therapy is generally not associated with excess hepatic risk.

4.2.1.12.4.2.2 Alkaline phosphatase (ALP)

Fibrates are known to reduce serum ALP levels (345;346). This effect is largest with bezafibrate and smallest with gemfibrozil, with fenofibrate having intermediate activity (347). Monitoring of ALP has been suggested as an indirect method of monitoring compliance in patients taking fibrates (348). Unfortunately ALP measurement was not included in the panel of biochemical tests when the study was conceived and therefore no data on ALP are available.

4.2.1.12.4.2.3 Other measures of hepatic function

Albumin, total protein and bilirubin were all within normal limits at baseline and none of these values changed significantly with therapy (data not shown).

4.2.1.12.4.3 Amylase

None of the patients on the study developed pancreatitis and the amylase remained within normal limits at all visits. Lipid-lowering treatment had no influence on the amylase values (data not shown).

4.2.1.12.4.4 Renal function tests

Increased levels of plasma urea and creatinine have been reported in patients taking fenofibrate. The reported increases in serum creatinine range from 8-18 % (349), and are generally reversible on discontinuation of fenofibrate. In the FIELD study creatinine was 10-12 μmol/L higher in the fenofibrate arm, but when a subset of 661 patients was retested 8 weeks after completion of the study there were no differences in creatinine between the two arms (344). Increased serum creatinine secondary to fenofibrate is not associated with reductions in glomerular filtration rate measured by sensitive techniques (350), but seems to be due to enhanced endogenous creatinine production (351). Many trials do not report changes in serum urea, but when they are reported, they are generally of similar magnitude as the changes in creatinine. Although fenofibrate therapy elevates serum creatinine, it is associated with reduced progression to microalbuminuria (352) in diabetes and with a reduced need for retinal laser therapy for diabetic retinopathy (344). The mechanism of these beneficial effects on the microvasculature is not clear and it is also not known to what extent they are secondary to the lipid effects of fenofibrate.

4.2.1.12.4.4.1 Urea

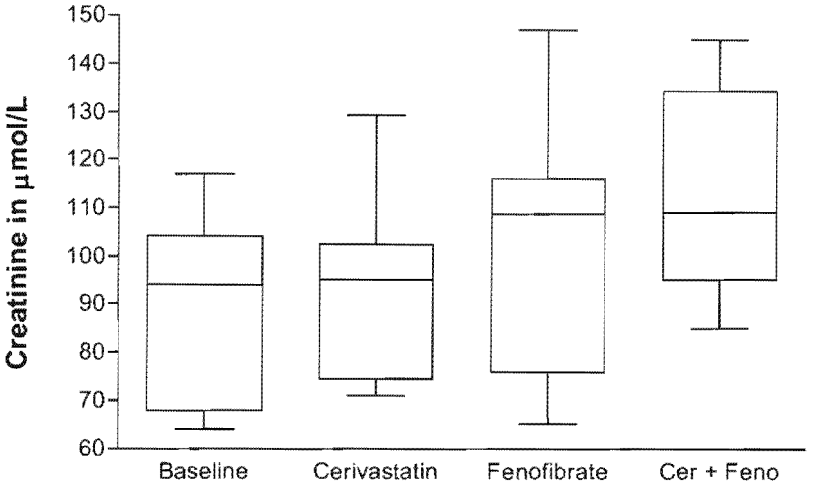
The mean urea at baseline was 5.2 ± 1.2 mmol/L (median 5.1, range 2.7-7.0 mmol/L). When taking cerivastatin, fenofibrate and combination therapy the urea levels respectively were 4.8 ± 1.1 mmol/L, 5.7 ± 1.7 mmol/L and 6.1 ± 1.6 mmol/L

(ANOVA, P=0.09). Although urea levels were increased in patients taking fenofibrate, the changes were not statistically significant in this small cohort.

4.2.1.12.4.4.2 Creatinine

The mean baseline creatinine was 90.6 ± 17.7 μmol/L (median 94, range 64-117 μmol/L). On cerivastatin therapy the creatinine was 93.6 ± 19.0 μmol/L, it was 101 ± 23.0 μmol/L on fenofibrate and 113 ± 19.8 μmol/L on combination therapy (ANOVA, P=0.03). The mean increase in creatinine from baseline with fenofibrate (as mono- or combination therapy) was 15.6 μmol/L. Following discontinuation of fenofibrate the creatinine returned to the patient's baseline level.

Figure 4-85 Creatinine during lipid-lowering therapy



ANOVA P=0.03

4.2.1.12.4.4.3 Electrolytes

Sodium, potassium, calcium and inorganic phosphate were all within normal limits and showed no significant changes with therapy (data not shown).

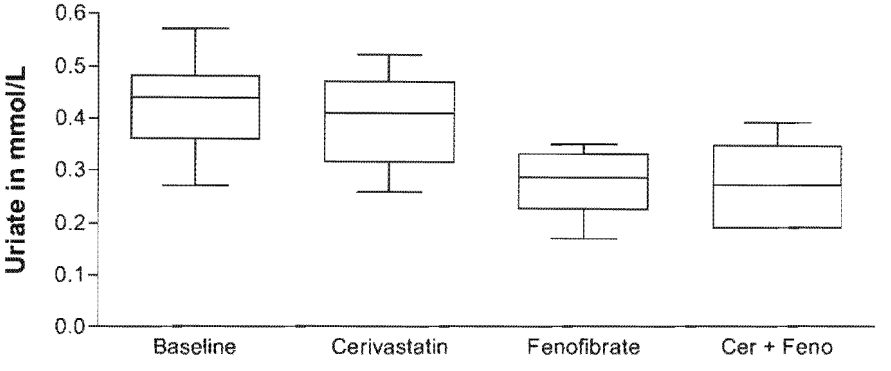
4.2.1.12.4.5 Urate

Fenofibrate is unique amongst the fibric acid derivatives in its ability to reduce serum urate concentrations. This effect is seen in healthy normolipidaemic volunteers (353) and in hyperlipidaemic patients (354). In a study of hyperuricaemic men with gout fenofibrate reduced urate by a mean of 19%. This effect was due to a 36% increase in urate clearance (355). Fenofibrate has been suggested as alternative or add-on therapy for patients poorly responsive or intolerant of allopurinol (355-357).

The mean baseline urate was high in the study cohort at 0.42 ± 0.08 mmol/L (median 0.44, range 0.27-0.57 mmol/L). This is not surprising considering the high prevalence of hypertension (39%) and the frequent use of thiazide diuretics (35%) in the study population. There is also a well-reported association of hyperuricaemia with features of the metabolic syndrome such as hypertriglyceridaemia, hypertension and impaired glucose tolerance (358-360); conditions that are found in many dysbetalipoproteinaemic subjects.

With cerivastatin monotherapy the urate was unchanged at 0.40 ± 0.08 mmol/L but decreased to 0.28 ± 0.06 mmol/L with fenofibrate and to 0.27 ± 0.07 mmol/L with combination therapy (ANOVA, P<0.0001). The mean decrease in urate when fenofibrate was taken was 0.14 mmol/L (34%).

Figure 4-86 Urate during lipid-lowering therapy



ANOVA P<0.0001

Tukey's multiple comparison tests

All comparisons P<0.0001 except for:

Baseline vs. cerivastatin P>0.05

Cerivastatin + fenofibrate vs. fenofibrate P>0.05

None of the trial participants had a clinical history of gout and no acute attacks of gout were observed during the study. Rapid lowering of urate in patients with gout

with allopurinol may occasionally precipitate acute gouty arthritis, but adding fenofibrate to allopurinol as additional hypouricaemic therapy in patients with poorly controlled gout did not precipitate acute gout, despite substantial lowering of serum urate (355).

4.2.1.12.4.6 Glucose

Of the 20 patients that had analyzable results 7 (35%) were diabetic. The mean fasting glucose at baseline in the non-diabetic patients was 5.45 mmol/L (median 5.4, range 4.3-6.8 mmol/L). Of the non-diabetic patients five met the American Diabetes Association (ADA) criteria for the diagnosis of impaired fasting glucose (IFG) or pre-diabetes of a fasting glucose of 5.6-6.9 mmol/L (361). The mean glucose in patients with IFG was 6.08 mmol/L (median 6.05, range 5.6-6.8 mmol/L). In patients with diabetes the mean fasting baseline glucose was 8.02 mmol/L (median 7.5 mmol/L, range 3.5-13.8 mmol/L).

Lipid-lowering therapy did not change fasting glucose significantly in any treatment arm (data not shown). None of the patients with IFG developed overt diabetes during the course of the study.

4.2.1.12.4.7 Haematological parameters

Haemoglobin, erythrocyte indices, white cell count (WCC) and platelets were generally within normal limits and did not change appreciably with lipid-lowering therapy (data not shown). No patient had abnormalities on haematological testing that were of clinical concern.

5 Chapter Five: Breath test to study chylomicron remnant metabolism

A subset of patients participating in the Bayer study was enrolled in a study of chylomicron remnant metabolism using a stable isotope breath test. This test was first developed at the University of Western Australia, Perth. Following initial experience in animal models (362-364) this technique has been applied to human subjects with hereditary and other forms of dyslipidaemia (365;366). It has subsequently been used to examine chylomicron remnant metabolism in a wide variety of clinical and experimental settings (367-374). Dysbetalipoproteinaemic subjects have previously been shown to have a markedly reduced fractional catabolic rate (FCR) when studied with this test (365).

5.1 Material and methods

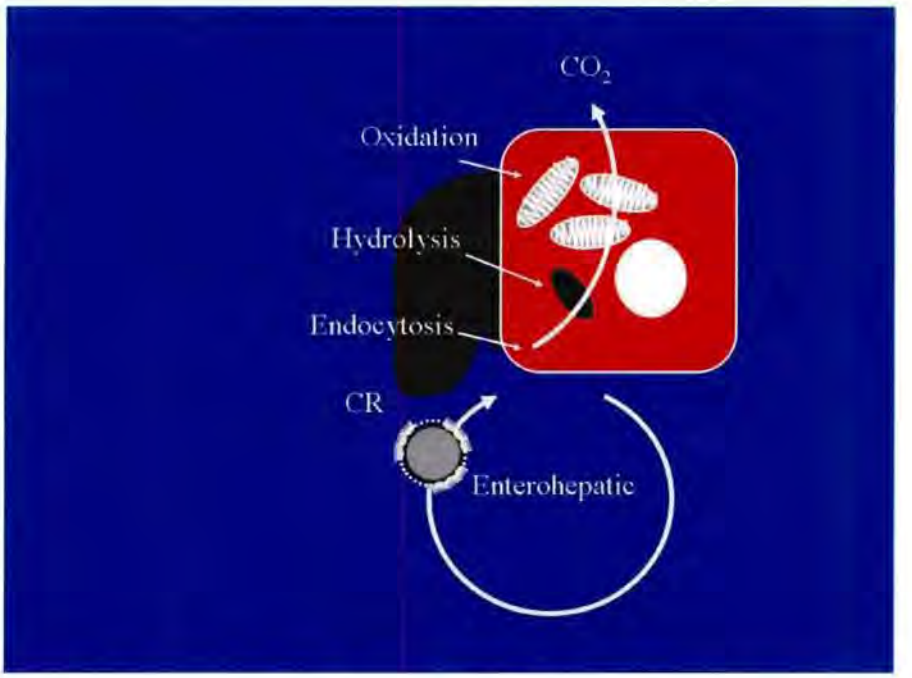
5.1.1 Introduction

Standard methods for assessing the kinetics of chylomicron remnant metabolism involve measurement of plasma triglycerides, retinyl esters or apoB48 following an oral fat load (375;376). Measuring fasting apoB48 levels may also predict the kinetic response of apoB48 and retinyl esters to an oral fat load (377). Unfortunately, none of these tests clearly distinguishes between defective lipolysis and defective hepatic remnant binding. Nascent chylomicrons must undergo partial lipolysis before they can be cleared as chylomicron remnants (378;379) and accumulation of apoE2 is known

to inhibit lipolytic activity (380). Retinyl-esters may also exchange into TGRL of hepatic origin (381), limiting their usefulness for the study of chylomicron remnant metabolism. Kinetic studies also require multiple venesections, decreasing patient acceptability.

The breath test described here circumvents many of the problems associated with previous measurements of chylomicron remnant metabolism. The test uses artificial lipid emulsions mimicking chylomicron remnant particles. These chylomicron remant-like particles associate with apoE in the circulation following intravenous injection, without any requirement for further lipolysis. The chylomicron remnant-like particles are endocytosed by the liver following apoE-mediated binding to hepatic lipoprotein receptors. ^{13}C is a stable (nonradioactive) carbon isotope and serves as the tracer in this experiment. The label is in the fatty acid moiety of the cholesteryl ester. Following endocytosis of chylomicron remnant-like particles and lysosomal hydrolysis of fatty acids, mitochondrial oxidation liberates ^{13}C which is ultimately excreted in the breath as $^{13}CO_2$. Measuring the enrichment of ^{13}C in end-tidal CO_2 therefore allows one to track the catabolism of the injected chylomicron remnant-like particles.

Figure 5-1 Breath test of chylomicron remnant metabolism



Picture: Professor T.Redgrave (University of Western Australia, Perth,)

Following intravenous injection the chylomicron remnant-like particle associates with apoE in the circulation and is endocytosed by the liver. Hydrolysis of the fatty acid moiety of cholesteryl ester and subsequent oxidation liberates ^{13}C which appears in breath as $^{13}CO_2$

CR: Chylomicron remnant-like particle

5.1.2 Preparation of emulsions

All emulsions were prepared at the University of Western Australia, frozen and then shipped to Cape Town. They were stored at -20° C and thawed on the day of use.

The relative composition by mass (%) of the lipid emulsions was as follows:

- Triolein 55.9 ± 1.6 %
- Phosphaditylcholine 28.2 ± 1.8 %
- Cholesteryl oleate 8.2 ± 0.7 %
- Cholesterol 7.7 ± 0.7 %

The lipid particles had a mean diameter of 55 ± 3 nm as measured by negative-stain electron microscopy.

Cholesteryl [^{13}C] oleic acid was synthesized from uniformly labelled [^{13}C] oleic acid (Novachem, Victoria; Australia) and cholesterol. Equimolar amounts of [^{13}C] oleic acid and cholesterol were reacted for 17 hours at room temperature in dry carbon tetrachloride with 107 mol% of dicyclohexylcarboiimide in the presence of 16 mol% N,N-dimethylaminopyridine. A 1% volume of water was added and the mixture was stirred for 4 hours. Subsequently the mixture was extracted with petroleum spirits and then 2% diethyl ether in petroleum. After filtration through a small silica column, thin layer chromatography was performed. Fractions with cholesteryl ester were combined, reduced in volume and purified by flash chromatography on silica gel. The fraction obtained was dried under high vacuum to constant mass. The cholesteryl [^{13}C] oleate thus obtained was a colourless oil and its structure was confirmed by proton nuclear magnetic resonance.

Cholesteryl [^{13}C] oleic acid (70mg), cholesterol (24mg), triolein (135 mg) and phosphaditylcholine (75mg) were emulsified by sonication for 1 hour in 8.5 ml of 2.2% glycerol in water. Following sonication the mixture was centrifuged at 3000 rpm

for 10 min to remove titanium fragments and then filtered through a 0.22 μm filter into sterile vessels. All emulsions were tested for sterility and pyrogen contamination before being frozen at -20° C.

5.1.3 Clinical protocol

Chylomicron remant metabolism was studied on the same day as the patient's scheduled routine trial visit. Following collection of routine blood samples the chylomicron remnant-like emulsion was injected into an antecubital vein utilizing an appropriately sized butterfly needle.

Figure 5-2 Chylomicron remnant-like emulsion prior to injection

End-expiratory breath samples were collected in sterile tubes with the aid of a short drinking straw. Patients were instructed to exhale slowly but fully through the drinking straw placed in the tube. At the end of expiration patients quickly removed the drinking straw from the tube and replaced the cap immediately. Patients were

asked to practice the collection of alveolar air under supervision several times before the actual study commenced.

Figure 5-3 Equipment used to collect end-tidal breath samples

Patients collected breath samples at time zero (before the injection of the lipid emulsion) and at the following intervals:

- Every ten minutes for the first hour
- Every twenty minutes for the next hour
- Every 30 min for the next five hours
- Hourly for the next seven hours
- Once at 24 hours

Patients remained at the clinical trial facility for the first seven hours of the investigation. During their stay at the trial facility patients were asked to remain seated in their chairs except for visits to the toilet. They remained fasting. After seven hours patients consumed a standardized lunch provided for them (tuna sandwiches).

After consuming their lunch patients were allowed to go home with instructions to continue collecting breath samples. They were asked to refrain from any vigorous physical activity and to avoid consuming carbonated beverages. Additional sandwiches were provided for supper, although this meal was not consumed under supervision.

5.1.4 Breath sample analysis

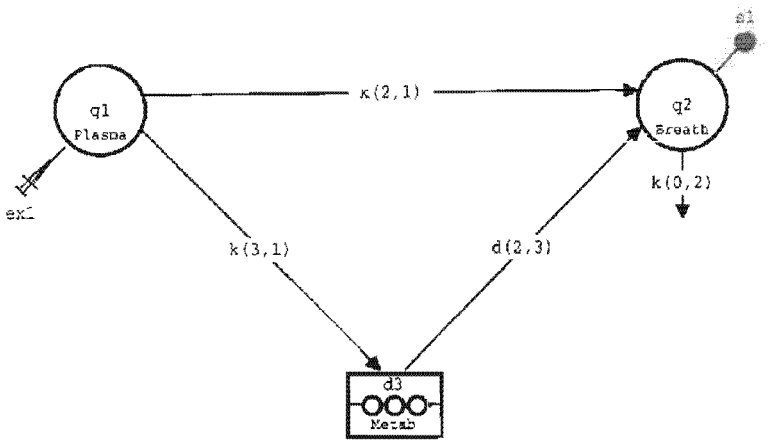
All breath samples were shipped to the University of Western Australia and analyzed there. The CO_2 in the breath samples was analyzed by isotope mass ratio spectroscopy using a Finnigan BreathmatPlus machine (Thermoquest Systems Pty. Ltd, Sydney; Australia). The ratio of $^{13}CO_2/^{12}CO_2$ was referenced to Peedeebelimnite standard values and the delta unit value was calculated using the Breathmat software. The delta unit values reference a sample of limestone, a standard in the ^{13}C isotope field, and basal (non-enriched) values correspond approximately 1% ^{13}C. There may be small variations amongst patients in the basal ^{13}C values, depending on the diet consumed. Endogenous ^{13}C enrichment varies between different food groups (382) and patients on very low fat diets have previously been shown to have starting enrichment values about 2 units higher than normolipidaemic control subjects (365).

5.1.5 Kinetic analysis of ^{13}C-enrichment of CO_2

Kinetic data were analyzed using a previously developed and published compartmental model (365;366;368;373). The model is a simple three-compartment

model. The model assumes that the fractional rate constants are first order and time invariant.

Figure 5-4 Compartmental model used to fit breath test enrichment data



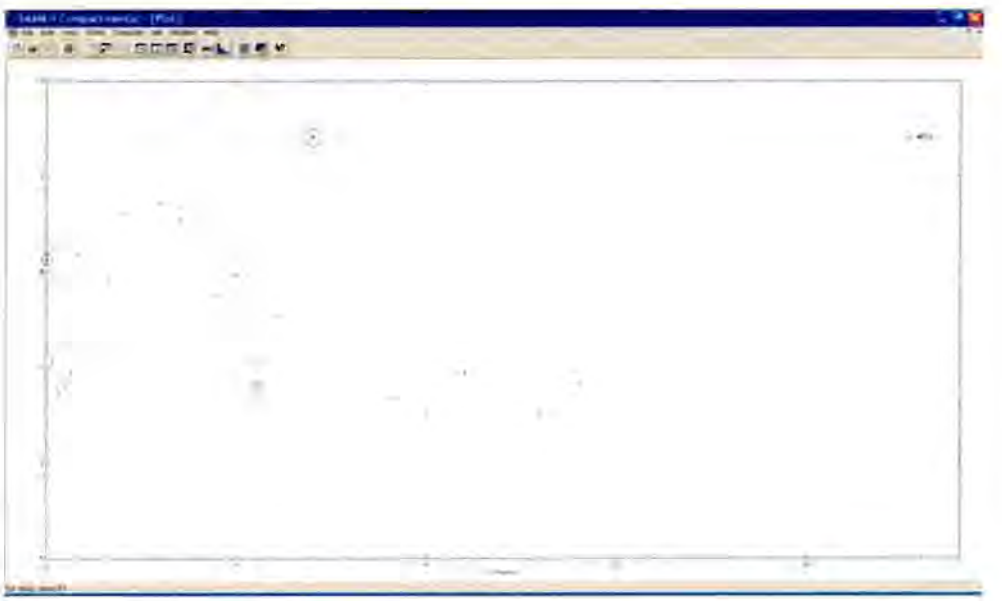
Compartment 1 ("Plasma") is the plasma compartment into which the labelled chylomicron remnant-like emulsion is injected (ex1). Compartment 2 ("Breath") represents labelled CO_2 in breath and is sampled (s1) during the course of the experiment. The third compartment ("Metab") is the primary pathway through which the remnant emulsion is cleared. It represents all the extrahepatic and hepatic processes that take place before the ^{13}C label is released and found in the breath. Compartment 3 therefore accounts for binding of the chylomicron remnant-like emulsion to hepatic lipoprotein receptors, endocytosis, hydrolysis and finally oxidation of the oleate. The metabolic processes associated with movement of label from compartment 1 to 3 and then to 2 are of course not instantaneous and compartment 3 may therefore also be described as a "delay compartment".

K(2,1) represents the fractional transfer rate of tracer from compartment 1 to compartment 2, while k(3,1) describes the fractional transfer rate of tracer from compartment 1 to compartment 3. The main movement of tracer is represented by k(3,1). The fractional catabolic rate (FCR) is calculated as the sum of k(2,1) and k(3,1).

The observed $^{13}CO_2$ data was fitted in Cape Town to the above model using the SAAM II program (SAAM Institute, Seattle; USA). Model rate constants and FCR were estimated after the observed values had been fitted to the model.

The following figures illustrate the fitting of experimental values to the compartmental model.

Figure 5-5 Data plot prior to fitting data to compartmental model



The circled data points were excluded from the data set as outliers.

Figure 5-6 Compartmental model fitted to data points

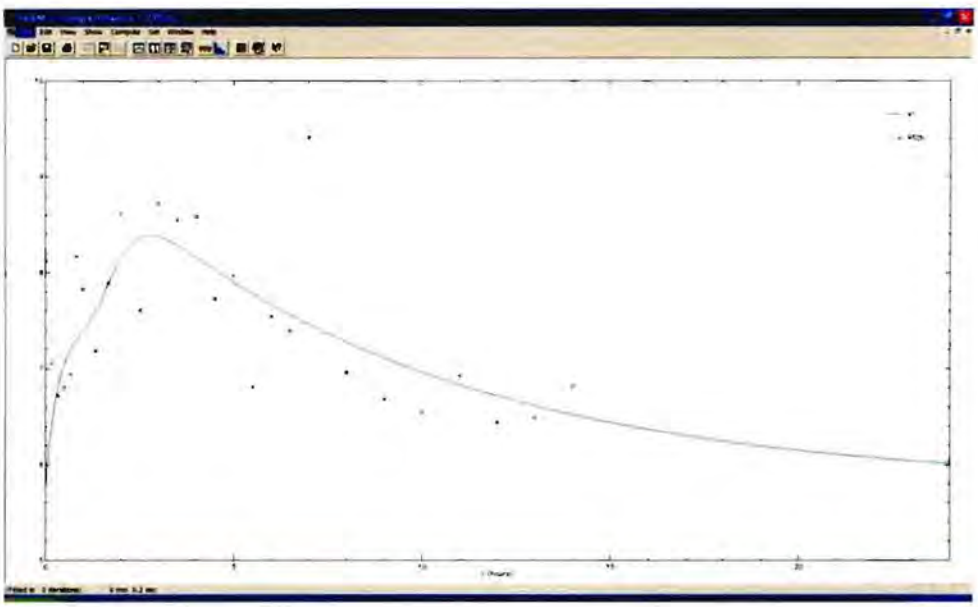
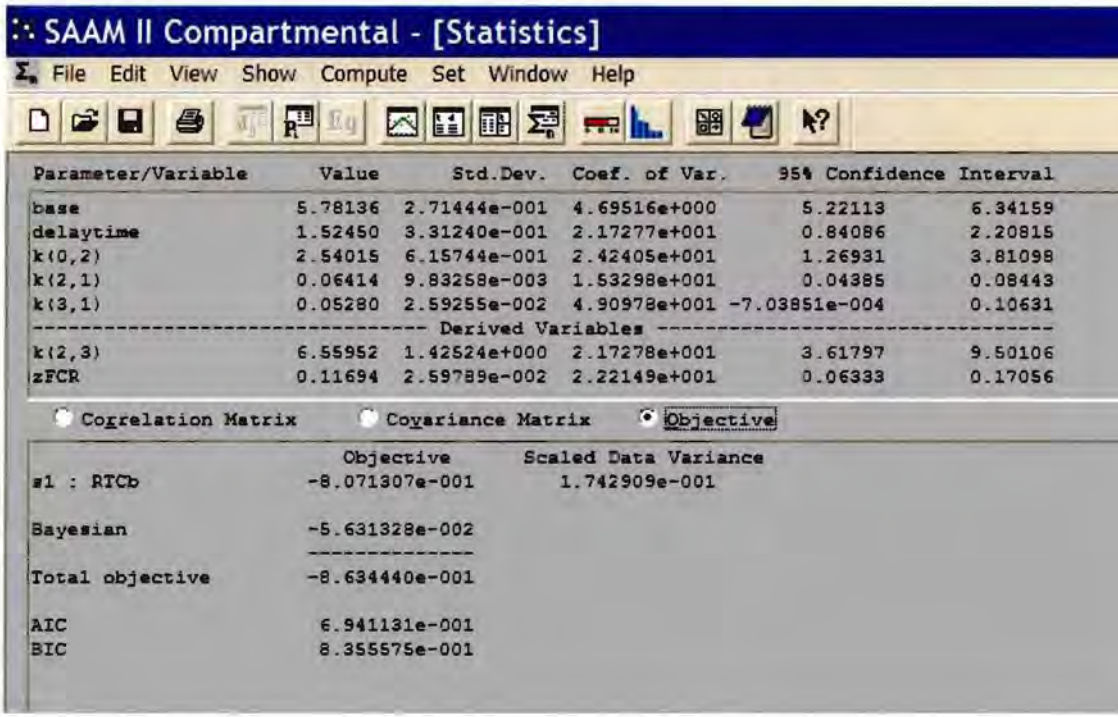


Figure 5-7 Kinetic variables obtained after fitting data to compartmental model



5.1.6 Illustrative Results

The following figures illustrate a few characteristic curves previously obtained in normolipidaemic and hyperlipidaemic populations. The data are all means with standard error of the mean bars and the line represents that fitted by the compartmental model to the averaged data.

Figure 5-8 Breath enrichment with $^{13}CO_2$ in normolipidaemic subjects

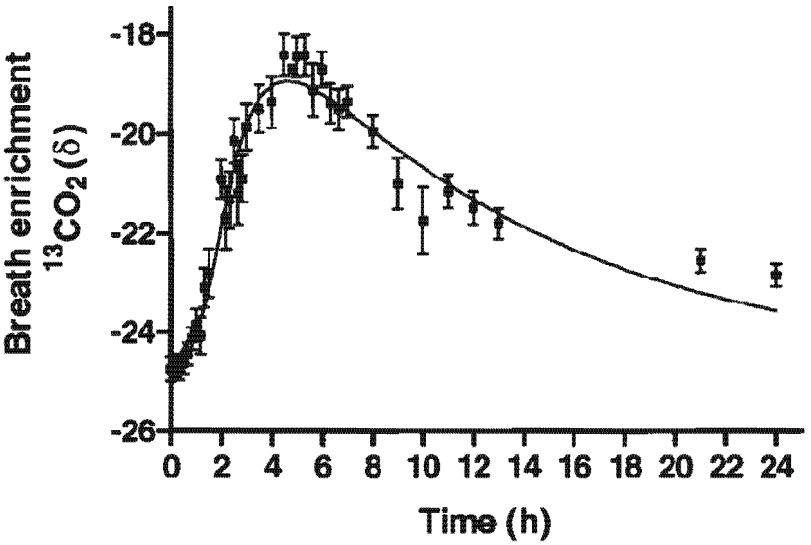

Breath enrichment
$^{13}CO_2$ (δ)
-18
-20
-22
-24
-26
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (h)

Figure 5-9 Breath enrichment with $^{13}CO_2$ in subjects with Type I hyperlipidaemia

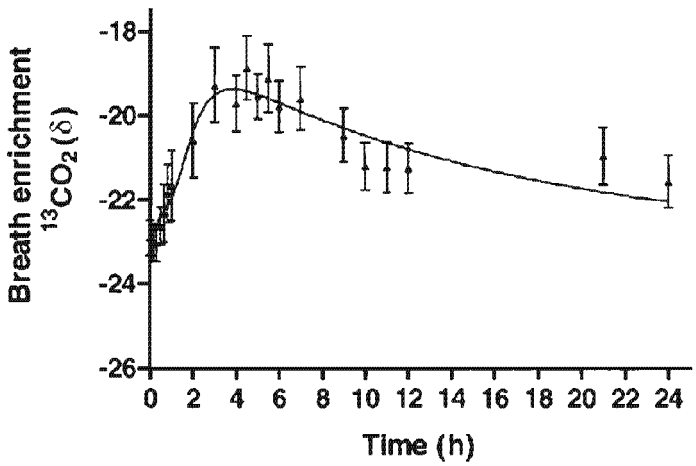
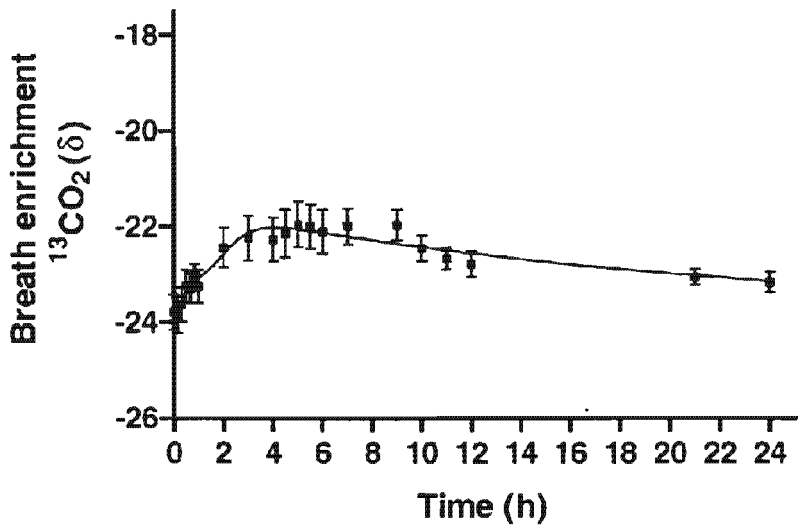


Figure 5-10 Breath enrichment with $^{13}CO_2$ in subjects with dysbetalipoproteinaemia



Figures 5.8-5.10 are taken from reference (365). The patients with Type I hyperlipidaemia and dysbetalipoproteinaemia were studied in Cape Town.

5.1.7 Limitations of breath test

Although the breath test was designed primarily to evaluate chylomicron remnant clearance; the appearance of the $^{13}CO_2$ label in breath does not only reflect the speed at which emulsion particles enter the liver for catabolism. Variations in respiratory quotient, fatty acid uptake and oxidation may also influence the results of the breath test.

In the circulation cholesteryl ester transfer protein (CETP) activity may potentially confound the interpretation of the breath test. CETP may transfer the labelled cholesteryl oleate from the emulsion to other lipoproteins before the cholesteryl oleate can be catabolized. Expansion of the acceptor lipoprotein pool may also be relevant as transfer of cholesteryl esters may be enhanced despite comparable CETP activity. However, in a previous study breath test results in patients with Type I hyperlipidaemia and a very large TGRL pool, were no different from those obtained in control subjects (365). In a study evaluating the effect of atorvastatin in viscerally obese men baseline CETP activity was not correlated with FCR and atorvastatin did not affect CETP activity significantly (373).

Once the chylomicron remnant-like emulsion has been injected it rapidly acquires apoE in the circulation. ApoE binds to hepatic lipoprotein receptors (LDL-receptor,

LRP/HSPG) and mediates the uptake of emulsion particles. Functional alterations in these receptors may alter the observed FCR. In humans the breath test FCR does not differ between normolipidaemic individuals, patients with heterozygous FH and patients with homozygous FH (366). Additionally there were no differences in FCR between homozygous patients with LDL-receptor mutations that differed substantially in their binding affinities for LDL apoB. In humans the LDL-receptor is therefore is not critical for clearance of the chylomicron remnant-like emulsion. However, alterations in expression and function of hepatic lipoprotein receptors may still affect chylomicron remnant clearance. In viscerally obese men atorvastatin increased the breath test FCR and there was a significant inverse relationship between changes in FCR and plasma LDLC, suggesting that decreased competition between LDLC and remnants of TGRL for the LDL-receptor may be a mechanism by which atorvastatin increased the breath test FCR (373). This finding does suggest that LDL-receptors do have a role to play in chylomicron remnant metabolism. Little is known about the differential effect statin therapy may have on the expression and functionality of various hepatic lipoprotein receptors.

In subjects consuming a very low-fat, high carbohydrate diet endogenous production of CO_2 will be higher leading to dilution of the label. Similarly physical activity is associated with excess oxidation of unlabelled substrates and dilution of the label. Consumption of carbonated beverages could potentially lead to additional CO_2 contamination of the alveolar air sample. The influence of the last two factors can be limited by studying patients under controlled conditions.

Alterations of fatty acid pool size and flux may also conceivably affect the breath test. Hepatocytes may either oxidize or re-esterify fatty acids. The degree to which fatty acids will be oxidized depends on hepatic glycogen stores and the relative concentrations of insulin and glucagon. Low hepatic glycogen stores with high levels of glucagon and low levels of insulin would, for instance, be associated with increased fatty acid oxidation. All patients were studied in the fasting state and fed a standardized meal. Additionally no significant differences were found when normolipidaemic volunteers were studies in the fasted and fed state (365).

5.2 Results

The Bayer study protocol stipulated that 12 of the 36 planned patients would be enrolled in the breath test substudy. Due to the early termination of the study fewer patients were enrolled in the substudy and none had breath tests during all treatment arms. Six patients that were recruited for the breath test substudy entered the trial substantially later than the main cohort of patients and could therefore only be studied at baseline.

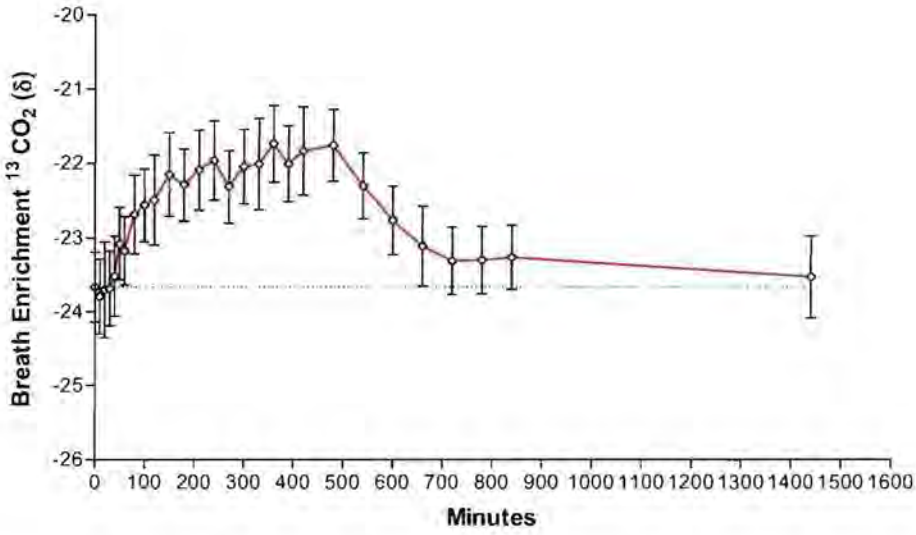
Table 5-1 Number of patients studied with breath test during Bayer study

Treatment	N
Baseline	14
Cerivastatin	5
Fenofibrate	5
Combination	4

5.2.1 $^{13}CO_2$ profiles at baseline (prior to fitting data to compartmental model)

In the untreated state the ^{13}C profile was similar to that previously reported in dysbetalipoproteinaemic subjects (365), with a delayed and smaller rise in tracer excretion when compared to normolipidaemic controls.

Figure 5-11 $^{13}CO_2$ profile at baseline in all patients



The symbols indicate the mean values and the error bars the SEM. The line connects the means and does not represent the fitted data.

Patients with identified apoE mutations had a smaller and slower rise in tracer excretion than those patients in whom no apoE mutation had been identified. Genopositive patients had higher initial starting enrichment values than genonegative

patients (see Fig 5.12) and the difference is therefore best illustrated by showing the absolute change in $^{13}CO_2$ enrichment (Fig 5.13).

Figure 5-12 $^{13}CO_2$ at baseline in genopositive and genonegative patients

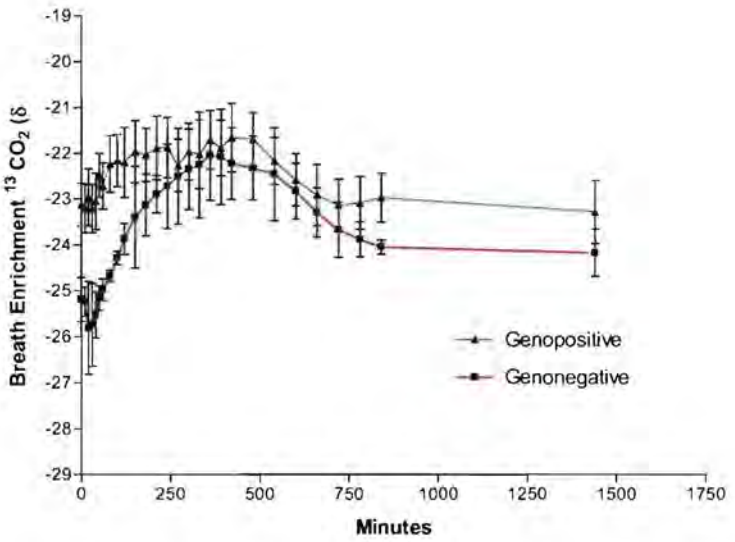


Figure 5-13 $^{13}CO_2$ at baseline in genopositive and genonegative patients (absolute changes)

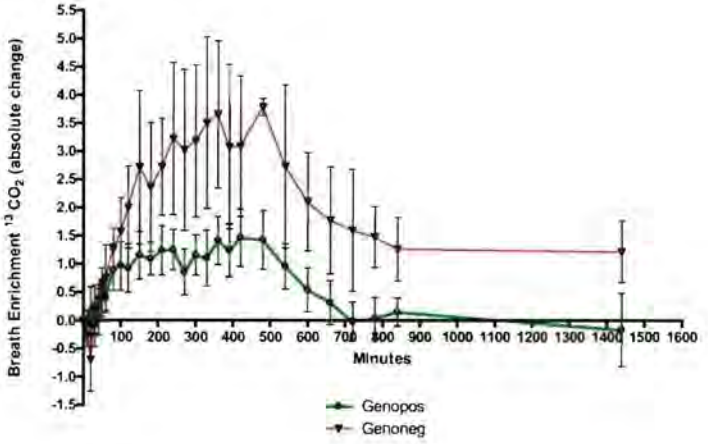
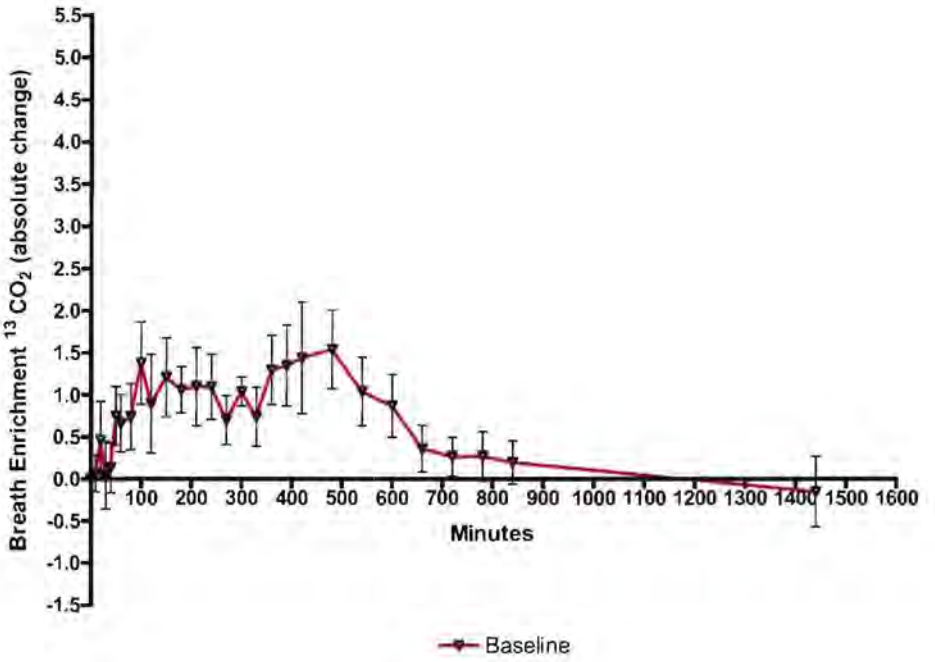


Figure 5.14 shows the $^{13}CO_2$ response at baseline in those 8 patients that had at least one breath test done while taking lipid-lowering therapy.

Figure 5-14 $^{13}CO_2$ at baseline in patients who had breath tests while taking lipid-lowering medication



5.2.2 $^{13}CO_2$ profiles with lipid-lowering therapy (data not fitted to compartmental model)

In patients on lipid-lowering therapy $^{13}CO_2$ was excreted earlier and more rapidly with larger absolute changes than in the untreated state. The $^{13}CO_2$ profiles changed most, by simple visual inspection, in patients taking fenofibrate either as monotherapy or in combination with cerivastatin.

Figure 5-15 $^{13}CO_2$ in patients taking cerivastatin (absolute change)

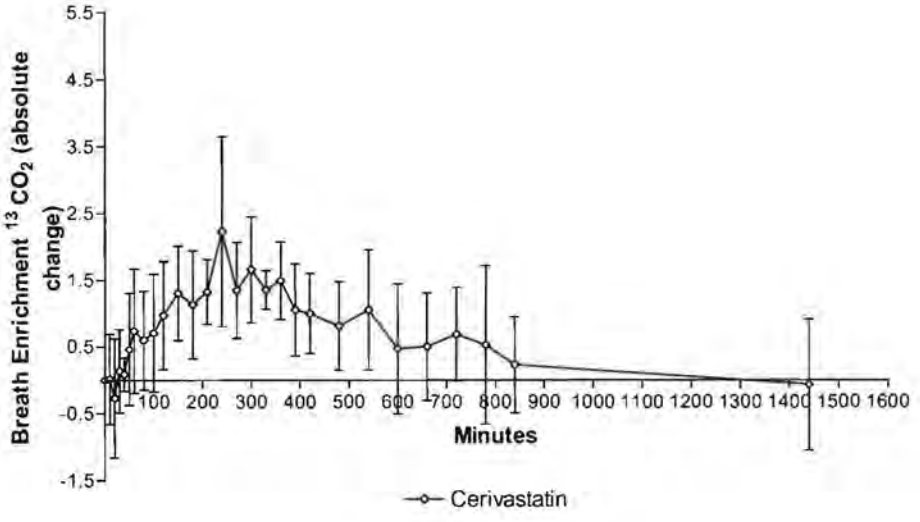


Figure 5-16 $^{13}CO_2$ in patients taking fenofibrate (absolute change)

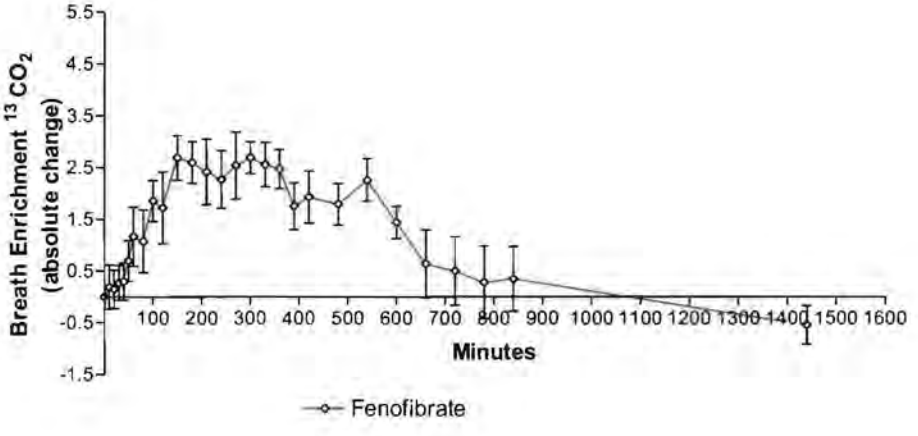


Figure 5-17 $^{13}CO_2$ in patients taking cerivastatin and fenofibrate (absolute change)

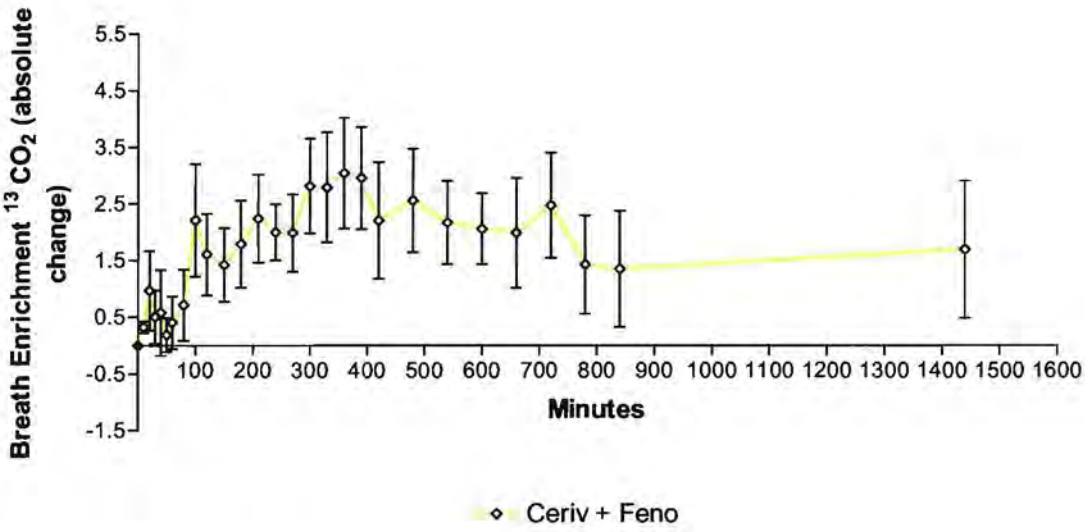
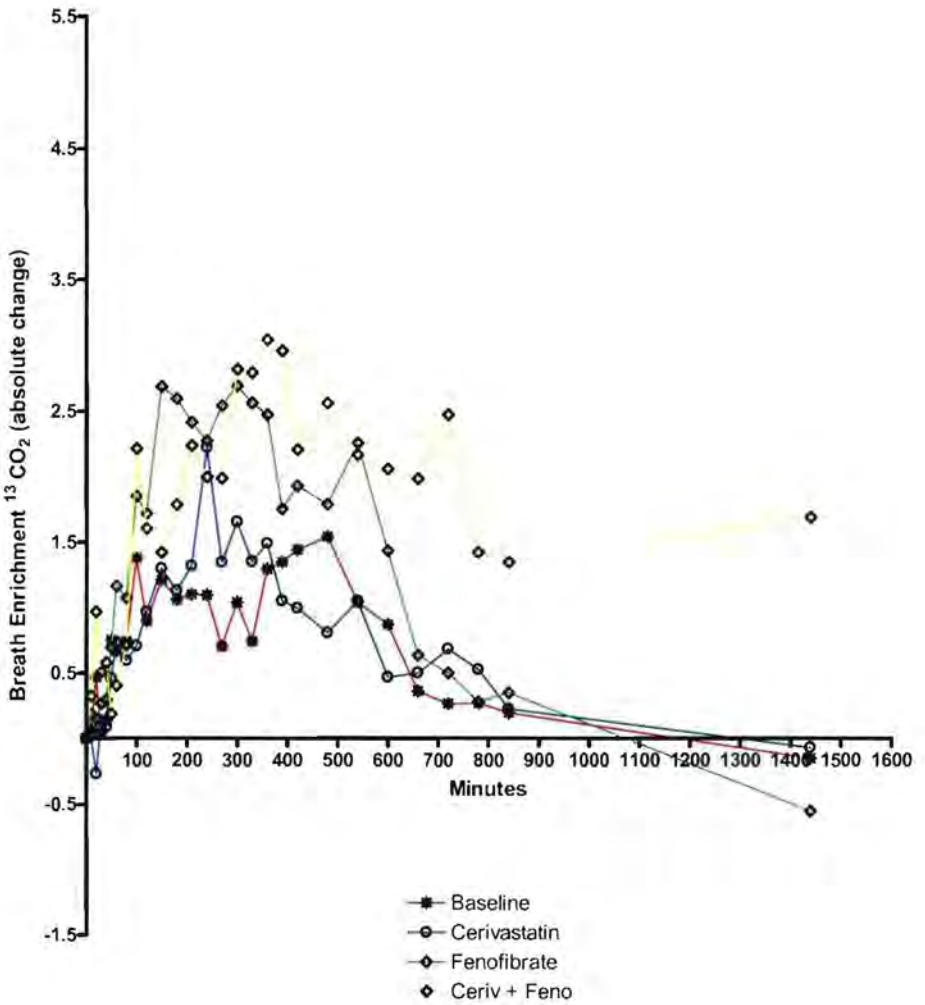


Figure 5-18 $^{13}CO_2$ at baseline and with lipid-lowering therapy (overlay of graphs)



5.2.3 Breath test data fitted to compartmental model

5.2.3.1 Individual patient example

The following figures illustrate the modelling of data for an individual patient and the changes observed in the profile. In this patient lipid-lowering therapy resulted in higher peak enrichment of the breath with $^{13}CO_2$ and earlier attainment of this peak.

Figure 5-19 Patient MHH: Compartmental modeling at baseline

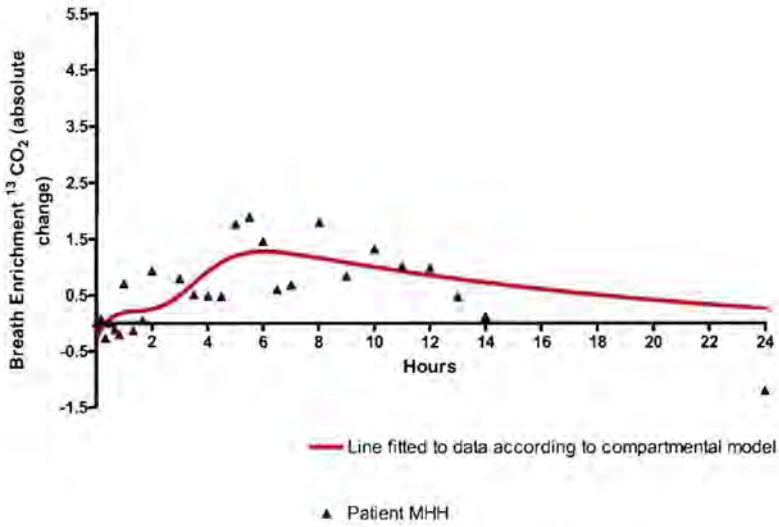


Figure 5-20 Patient MHH: Compartmental modeling on cerivastatin

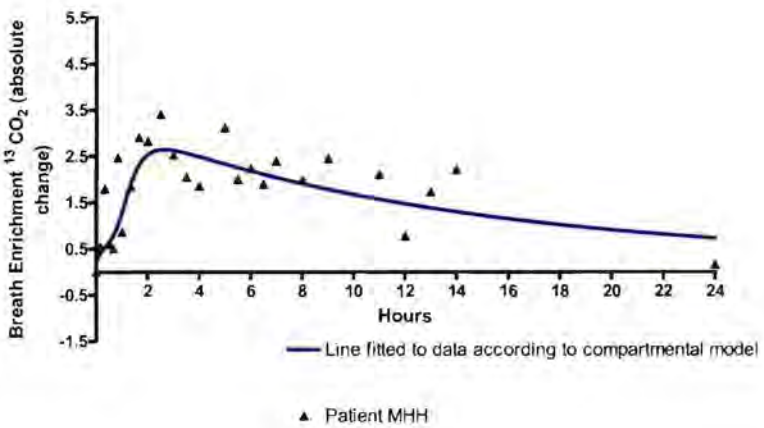


Figure 5-21 Patient MHH: Compartmental modeling on fenofibrate

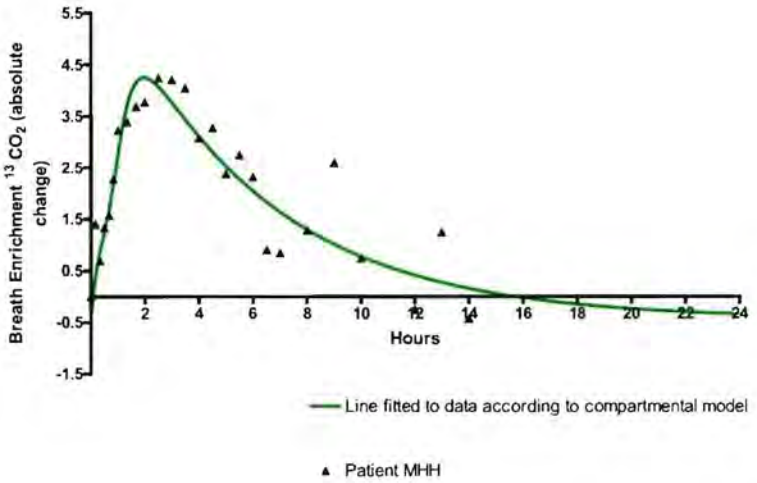
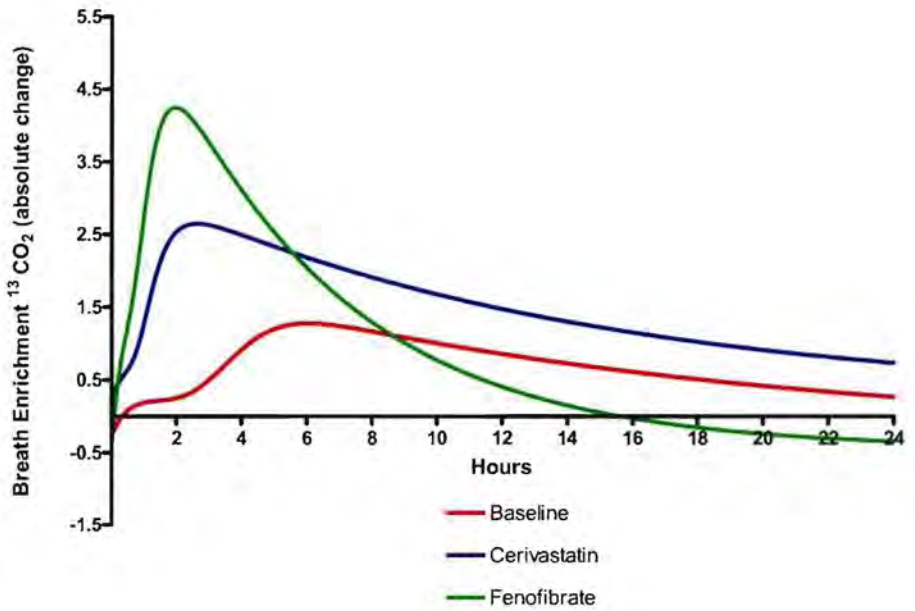


Figure 5-22 Patient MHH: Overlay of fitted data



5.2.3.2 Study cohort

The following figures illustrate the lines derived by fitting the compartmental model to the averaged data for each treatment period. The $^{13}CO_2$ enrichment follows a similar contour during all treatment periods except for the combination therapy group. In the latter group the peak enrichment is substantially delayed compared to the other groups. Lipid-lowering therapy is consistently associated with higher maximum enrichment.

Figure 5-23 Compartmental modeling of averaged data at baseline (all patients)

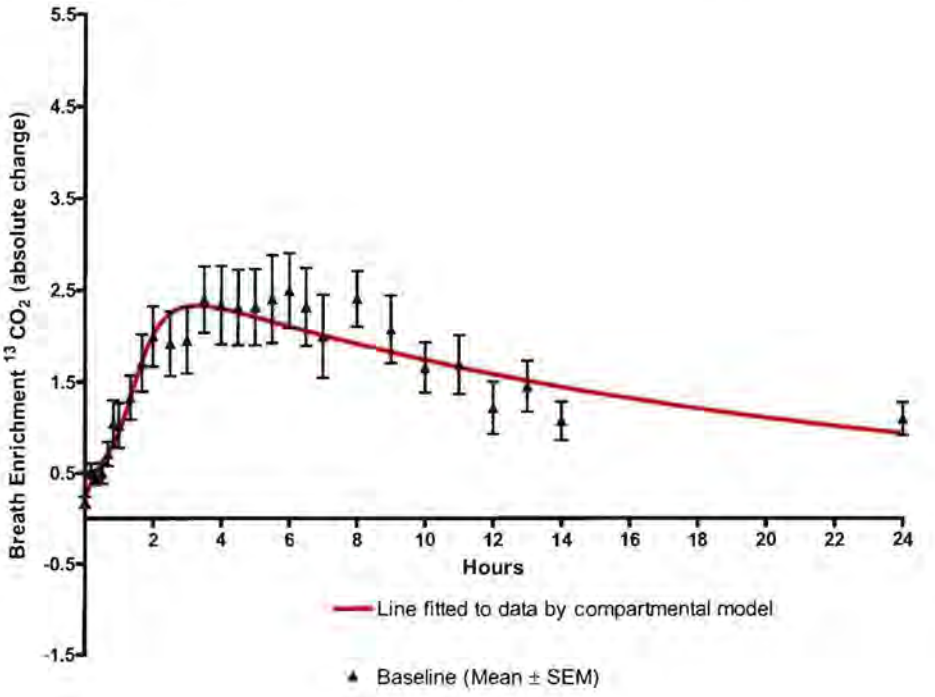


Figure 5-24 Compartmental modeling of averaged data at baseline (patients that completed more than one breath test)

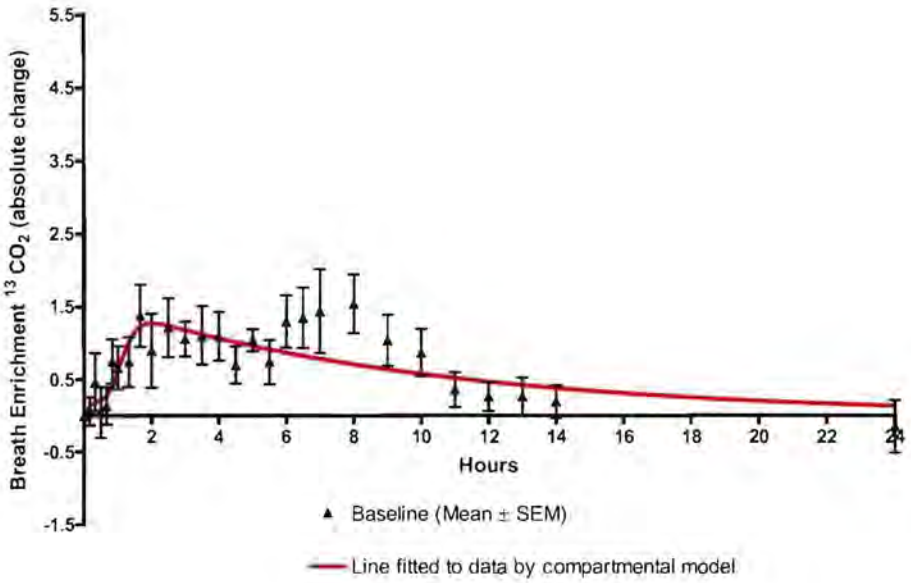


Figure 5-25 Compartmental modeling of averaged data on cerivastatin

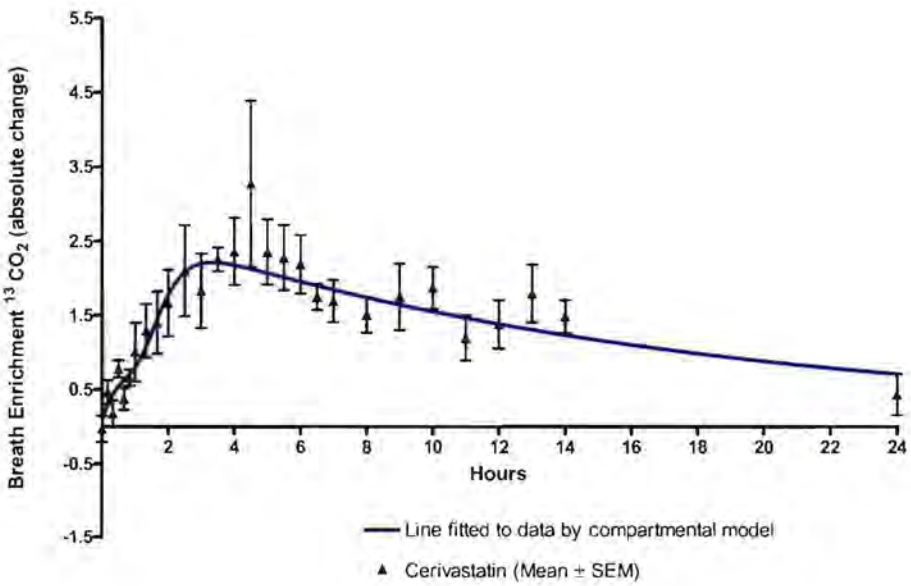


Figure 5-26 Compartmental modeling of averaged data on fenofibrate

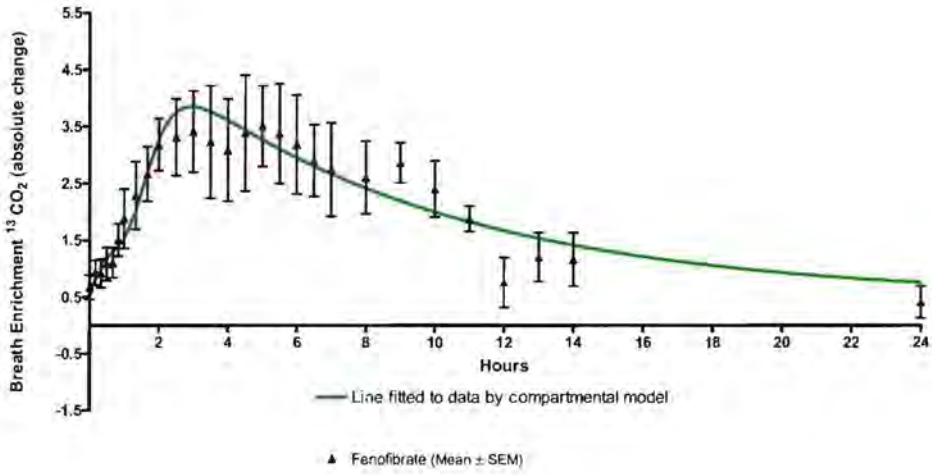


Figure 5-27 Compartmental modeling of averaged data on cerivastatin and fenofibrate

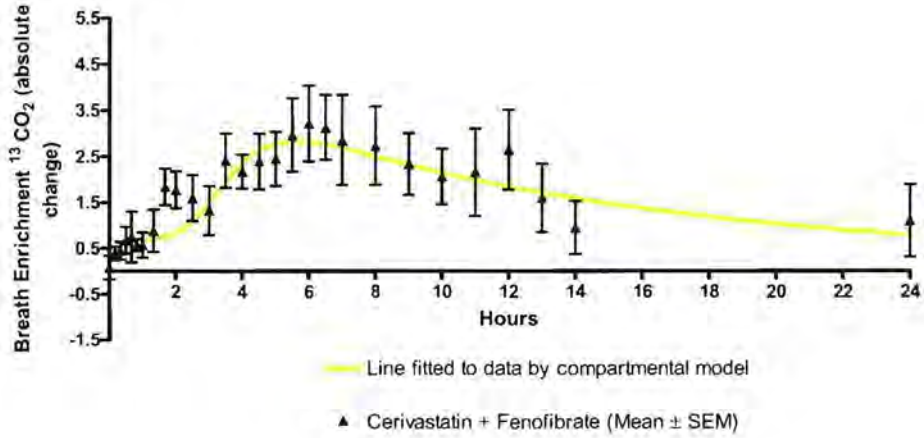
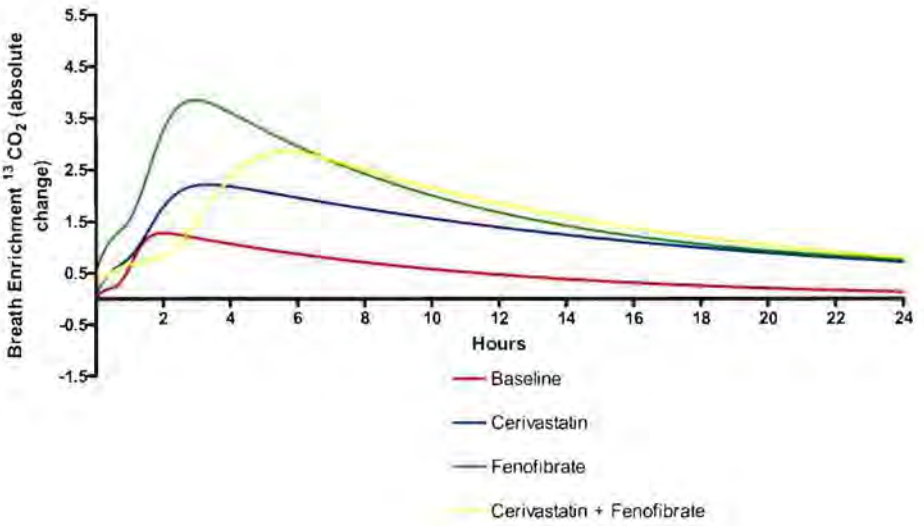


Figure 5-28 Overlay of compartmental models fitted to averaged data



5.2.4 Kinetic variables

Table 5.2 shows the model rate constants and FCR for the labelled chylomicron remnant-like infusion at baseline and with lipid-lowering therapy. Relative to baseline there was a significant increase in FCR and k(3,1) with fenofibrate monotherapy. The breath test results are congruent with the changes in lipids and lipoprotein composition as fenofibrate was associated with the largest reduction in VLDL1 lipids. Chylomicron remnants are found mainly found in this fraction and a reduction in VLDL1 lipids does therefore imply an increase in FCR. Interpretation is, however, complicated by the fact that the VLDL1 fraction does not contain chylomicron remnants exclusively.

Table 5-2 Model rate constants and FCR at baseline and with lipid-lowering therapy

	Baseline	Cerivastatin	Fenofibrate	Combination
k(2,1), pools/h	0.0246 ± 0.027	0.0164 ± 0.008	0.0398 ± 0.0176	0.0307 ± 0.0183
k(3,1), pools/h	0.0169 ± 0.0187	0.0456 ± 0.0277	0.0721 ± 0.0404	0.0391 ± 0.0212
k(2,3), pools/h	5.492 ± 2.830	7.841 ± 4.591	6.471 ± 4.563	3.215 ± 0.464
k(0,2), pools/h	1.211 ± 0.6147	1.619 ± 0.4554	1.590 ± 0.6060	1.245 ± 0.5455
FCR, pools/h	0.0416 ± 0.0381	0.0621 ±0.0332	0.1119 ± 0.0533	0.06989 ± 0.0171

Table 5-3 Model rate constants and FCR: ANOVA

	Tukey's multiple comparison test						ANOVA
	Baseline P vs.			Cerivastatin P vs.		Fenofibrate P vs.	
	Cer [1]	Feno [2]	Com [3]	Feno	Com	Com	
k(2,1), pools/h	NS [4]	NS	NS	NS	NS	NS	0.29
k(3,1), pools/h	NS	<0.05	NS	NS	NS	NS	0.02
k(2,3), pools/h	NS	NS	NS	NS	NS	NS	0.31
k(0,2), pools/h	NS	NS	NS	NS	NS	NS	0.50
FCR, pools/h	NS	<0.05	NS	NS	NS	NS	0.04

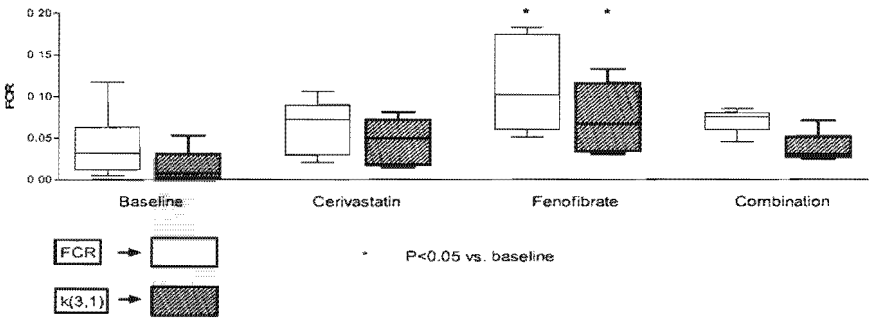
[1] Cer=Cerivastatin

[2] Feno= Fenofibrate

[3] Com= Combination therapy

[4] NS= Not significant (P>0.05)

Figure 5-29 Changes in FCR and k(3,1)



5.3 Discussion

5.3.1 Kinetic studies in dysbetalipoproteinaemia

Only a limited number of kinetic studies have been performed in dysbetalipoproteinaemic subjects. Many of these studies used experimental designs, sampling schedules and mathematical models that are currently regarded as suboptimal (383). Table 5.4 lists a selection of kinetic studies in dysbetalipoproteinaemia, emphasizing more recent studies.

Table 5-4 Kinetic studies in dysbetalipoproteinaemia

Author	Year	N [1]	Labelling [2]	Administration [3]	Tracer	Tracee	Sampling [4]	Drug [5]	Model [6]	Comment	Ref
Turner	1984	11	Exogenous	Bolus	^{131}I- VLDL+ IDL and ^{125}I-LDL	apoB in lipoprotein fractions	90h	Y	Exponential	1	(384)
Packard	1986	6	Exogenous	Bolus	^{131}I-VLDL2+ ^{125}I-VLDL1	apoB in lipoprotein fractions	336h	Y	Multicompartmental	2	(307)
Stalenhoef	1986	3	Exogenous	Bolus	^{125}I-TGRL	apoB in lipoprotein fractions	24h	N	No modelling	3	(385)

Author	Year	N [1]	Labelling [2]	Administration [3]	Tracer	Tracee	Sampling [4]	Drug [5]	Model [6]	Comment	Ref
Cortner	1987	5	Endogenous	Bolus	Vit A	Retinyl-esters in lipoproteins	12h	N	Multicompartmental	4	(386)
Vega	1988	3	Exogenous	Bolus	^{131}I- LDL+ ^{125}I-VLDL	apoB in lipoprotein fractions	336h	Y	Multicompartmental	5	(313;387)
Haffner	1989	7	Exogenous	Bolus	^{125}I - chylomicrons	apoB in lipoprotein fractions	72h	N	Residence time only	6	(387)
Gylling	1995	5	Endogenous	Bolus	Vit A	Retinyl-esters in lipoproteins	24h	Y	No modelling	7	(317)

Author	Year	N [1]	Labelling [2]	Administration [3]	Tracer	Tracee	Sampling [4]	Drug [5]	Model [6]	Comment	Ref
Mann	1995	1	Exogenous	Bolus	^{131}I- apoE3+ ^{125}I-apoE (Lys146→Glu)	Plasma activity	148h	N	Area under curve	8	(200)
Tremblay	2006	2	Endogenous	PCI [7]	D3-leucine	apolipoproteins	12h	Y	Multicompartmental	9	(388)

Legend

1 Refers to the number of subjects with dysbetalipoproteinaemia included in the study

2 Describes whether labelling was endogenous or exogenous

3 Describes whether label was administered as a bolus or as an infusion

4 Duration of sampling

5 Refers to whether patients were studied on and off lipid-lowering therapy(Y=lipid-lowering therapy, N= no lipid-lowering therapy) see comments for details

6 Briefly describes the type of data analysis

7 Primed constant infusion

Comments:

1 A large and detailed study of lipoprotein kinetics in dysbetalipoproteinaemia. Two patients were studied on and off therapy with gemfibrozil.

2 A very detailed study of apoB metabolism incorporating a complex multicompartmental model. Patients were studied prior to and after treatment with bezafibrate.

3 Two of the patients studied were homozygous for the apoE (R145C) mutation.

4 This was a study of chylomicron remnant metabolism with retinyl palmitate labelling.

5 In this small study patients were studied prior to and after therapy with lovastatin.

6 This was a study of chylomicron metabolism using iodinated autologous chylomicrons.

7 This study examined vitamin A and squalene clearance and cholesterol synthesis in response to lovastatin.

8 This study examined the plasma kinetics of apoE (Lys146Glu) in a single patient.

9 The two dysbetalipoproteinaemic patients in this study were also heterozygous for LDL-receptor mutations. They were studied before and after fenofibrate therapy.

5.3.2 Lipoprotein kinetics in dysbetalipoproteinaemia

5.3.2.1 Chylomicron metabolism

Despite varying methodologies all studies of chylomicron metabolism in dysbetalipoproteinaemia report delayed catabolism of chylomicrons or their remnants. Iodinated postprandial chylomicrons had a circulatory residence time of 17.4 ± 3.5 h in dysbetalipoproteinaemic subjects compared to 4.8 ± 2.1 h in patients with Type IV hyperlipidaemia and 5.9 ± 1.6 h in normolipidaemic subjects (387). The delayed clearance of chylomicrons in this study was not attributable to the expanded TG pool. In a study of chylomicron remnant metabolism following Vitamin A labelling the half-time of retinyl palmitate clearance from the chylomicron remnant fraction (which also contained hepatic VLDL and small nascent chylomicrons) was 611.9 ± 419.9 minutes in dysbetalipoproteinaemic subjects compared to 14.1 ± 9.7 minutes in normolipidaemic controls and 50.7 ± 20.8 minutes in Type IV hyperlipidaemia (386). Another study confirmed delayed clearance of vitamin A from plasma following an oral load in dysbetalipoproteinaemic subjects. Lovastatin did not increase postprandial vitamin A clearance significantly (317). Stalenhoef used apoB48 as a chylomicron marker and the half-life of injected labelled apoB48 was prolonged by a factor of 23-138 (385) in dysbetalipoproteinaemic patients, including a patient with "normolipidaemic dysbetalipoproteinaemia". As discussed previously none of these studies can accurately distinguish between the effects of defective lipolysis and impaired hepatic binding. However, the study by Redgrave (365) utilizing an artificial chylomicron remnant-like emulsion that does not require lipolytic conversion, does suggest that defective hepatic remnant binding accounts largely for delayed chylomicron remnant catabolism.

5.3.2.2 VLDL, IDL and LDL metabolism

Dysbetalipoproteinaemic patients have an approximately threefold larger VLDL pool size (PS), with lower production rates (PR) and markedly lower FCR (approximately 30% of control) than normolipidaemic controls (384). In another study of FH heterozygotes (FH HTZ), FH HTZ with apoE2/E2 (FHdysß) and normolipidaemic controls VLDL PS was highest in the FHdysß group and this finding was mainly attributable to a marked reduction (-76 % compared to FH HTZ) in FCR (388).

IDL PS is increased in dysbetalipoproteinaemia (± 4.8 fold) and FCR is reduced (41% of control). PR was not significantly different between dysbetalipoproteinaemia and controls (384). FHdysß have larger IDL PS than both FH HTZ and controls. The large PS is due to reduced FCR and increased PR (388). In dysbetalipoproteinaemia the mean conversion time of IDL to LDL was 18.7 h compared to 3.8 h in controls and the mean conversion rate (%/h) was also significantly lower (384).

LDL PS and PR were significantly lower (57% and 66% of control) in dysbetalipoproteinaemia. Although mean LDL FCR was higher in dysbetalipoproteinaemia the difference from controls was not statistically significant (384). In FHdysß subjects the LDL PS was smaller (56%) than in FH HTZ and this was mainly due to a greater LDL FCR (225%) (388).

5.3.2.3 Changes in lipoprotein kinetics with lipid-lowering therapy

5.3.2.3.1 Fibrate therapy

Packard and colleagues treated six dysbetalipoproteinaemic patients with bezafibrate. VLDLC was reduced and plasma HDLC increased, while LDLC was unchanged. The reduction in plasma VLDL was mainly ascribed to inhibition of synthesis of both large and small subfractions of VLDL. Catabolism of large VLDL also increased. Although plasma LDLC levels were unchanged the LDLC FCR fell by about 50% but this was compensated for by decreased synthesis of LDL from small VLDL and IDL (307). Turner reports changes in kinetic parameters in two patients treated with gemfibrozil. In both patients the PR of VLDL and IDL decreased with no changes in FCR (384). The kinetic changes observed by Tremblay in response to fenofibrate in the FHdysß subjects are somewhat different. Chylomicron (apoB48), VLDL and IDL PS decreased significantly from baseline with treatment and this decrease was largely due to increases in FCR with only modest reductions in PR (388). In contrast to the study by Packard LDL PR increased significantly, while LDL FCR increased in one patient and decreased in another patient. The authors speculate that LDL FCR may have decreased in the one patient due to a larger increase in LDL PS and subsequent saturation of LDL-receptor pathways. Unfortunately the study by Tremblay did not include any subjects with dysbetalipoproteinaemia only and it is therefore not possible to compare changes in kinetic parameters directly with other studies.

5.3.2.3.2 Statin therapy

The only report on changes in lipoprotein kinetics in dysbetalipoproteinaemic subjects treated with statins refers to three patients treated with lovastatin (313). VLDLC and LDLC levels were significantly reduced, but the modelling techniques employed did

not allow the authors to differentiate whether the changes were due to inhibition of synthesis of apoB-containing lipoproteins or increased activity of LDL-receptors and rapid removal of newly synthesized lipoproteins. The latter mechanism would decrease the input rates into later compartments and the observed decrease in LDL FCR could be explained by saturation of LDL-receptors by the more rapidly removed lipoproteins. Conceivably both mechanisms may also operate simultaneously (313).

5.3.2.3.3 Comparison of breath test results with previously published literature

There are no published reports of the effects of lipid-lowering treatment on breath test results in dysbetalipoproteinaemia. In FH patients 40 mg of simvastatin did not alter the FCR of the breath test (372), similarly there was no change in FCR when postmenopausal women with Type II DM were treated with 40 mg of pravastatin (368). In viscerally obese men atorvastatin 40 mg improved the FCR of the chylomicron remnant-like emulsion, after a previous study had documented impaired chylomicron remnant catabolism in viscerally obese men compared to non-obese men (374). The improved FCR was ascribed to decreased cholesterol synthesis and subsequent upregulation of LDL-receptors and decreased secretion of hepatic apoB lipoproteins. Increased LDL-receptor availability and decreased competition of chylomicron remnants with apoB100 lipoproteins results in greater catabolism of the former (373).

Chylomicron catabolism is markedly delayed in dysbetalipoproteinaemia for many reasons. High levels of apoE inhibit LPL activity, defective apoE binds poorly to hepatic lipoprotein receptors and high concentrations of apoB100-containing

remnants compete for hepatic lipoprotein receptors. Defective apoE is of course not altered by lipid-lowering therapy and an alternative explanation is therefore necessary for improved chylomicron remnant catabolism. As the breath test is not reliant on LPL activity (365) upregulation of the LPL gene by fibrates does not provide a direct answer. However, upregulation of lipolysis by fibrates reduces circulating remnant lipoprotein and therefore apoE concentrations. As high apoE levels increase VLDL production (73;178), reductions in circulating apoE further reduce VLDL secretion. Decreased competition for hepatic lipoprotein receptors, such as LRP and HSPG to which apoE2 can still bind may result in improved chylomicron remnant clearance.

Lovastatin did not improve postprandial vitamin A clearance in dysbetalipoproteinaemia (317), but in another study atorvastatin did reduce plasma levels of apoB48 (315). Upregulation of LDL-receptor activity can not explain improved chylomicron remnant clearance directly as apoB48/apoE2 lipoparticles do not bind well to hepatic LDL-receptors. In dysbetalipoproteinaemia the effect of statins on chylomicron remnant clearance must therefore again be indirect and due to decreased competition at other hepatic lipoprotein receptors. However, improvements in chylomicron remnant clearance, as assessed by the breath test, have only been demonstrated when relatively high dose (atorvastatin 40 mg/day) statins have been used (373). More potent statins upregulate LDL-receptor activity and decrease lipoprotein production more profoundly than less potent statins. An alternative explanation for the reduction seen in circulating apoB48 in dysbetalipoproteinaemic subjects treated with atorvastatin is that apoB48 production may be reduced by statins. In cultured transformed enterocytes atorvastatin accelerates the degradation of apoB48 (389) due to limited intracellular cholesterol availability. Even if statins do

not affect chylomicron production, reduced VLDL production has been attributed to statin therapy and this would reduce competition at lipoprotein receptors.

In the present study an increased chylomicron remnant FCR was seen with fenofibrate but not with cerivastatin. The statin dose (cerivastatin 0.4 mg) had less potency than atorvastatin 40 mg/day, which is the only statin dose at which an increase in breath test FCR has been observed thus far. It must be acknowledged that the number of subjects studied was smaller than planned and that none of the patients completed all three treatment arms as planned. In some patients it was not possible to obtain an ideal fit of the model to the observed data, due to outlying data points. As discussed above inadequate sample collection, exercise, consumption of carbonated beverages or failure to close the sampling tube tightly can influence tracer concentration significantly.

Clearly, the currently available information regarding changes in lipoprotein flux brought about by statins and fibrates in dysbetalipoproteinaemic subjects is discrepant in parts. There are significant differences in the potency of the studied drugs as well as the kinetic study protocols. A lipoprotein turnover study in dysbetalipoproteinaemic patients treated with various lipid-lowering drugs, with endogenous labelling of lipoproteins utilizing stable isotopes and state of the art computer based modelling techniques, would help to resolve many of these issues.

6 Chapter Six: Diagnostic issues in dysbetalipoproteinaemia

6.1 VLDL-compositional analysis

As described in section 1.6.2 there is no universally accepted diagnostic test for dysbetalipoproteinaemia. Analysis of VLDL-composition has, however, replaced detection of ß-VLDL as the phenotypic test of choice in almost all studies published on dysbetalipoproteinaemia in the last twenty years.

The GSH lipid clinic experience of partial VLDL-compositional analysis was evaluated. The laboratory has performed a large number of VLDL-compositional analyses over the years. These have all been performed by a single technician according to the same protocol and with the same equipment (see methods section for details).

6.1.1 VLDL-compositional analysis cohort

Data were available on 1109 VLDL compositional analyses in 779 individuals.

Table 6-1 Number of VLDL compositional analyses per individual patient

Number of VLDL compositional analyses	Number of individuals
1	566
2	152
3	43
4	14
5	7
6	2
7	1

VLDL-compositional analysis was performed on specimens with a very wide range of lipid concentrations. At the time of VLDL-compositional analysis only TC and TG was determined routinely.

Table 6-2 Lipid concentrations at time of VLDL-compositional analysis

	Mean	Median	SD	Range
TG (mmol/L)	4.19	3.30	3.78	0.10-62.00
TC (mmol/L)	6.79	6.30	2.75	1.90-40.60

6.1.1.1 ApoE genotyping

For the 779 individuals in whom VLDL-compositional analysis had been performed apoE genotyping data were available in 771. Many patients were genotyped

retrospectively (as part of this thesis), as the standard practice in the early years of the clinic had been to only genotype patients with cholesterol-enriched VLDL. In some cases VLDL-compositional analysis and genotyping had been performed on request of clinicians at other hospitals. These patients were included in the analysis of the diagnostic utility of VLDL-compositional analysis, but could obviously not be included in the clinical database. In a few instances no DNA was available for testing.

Table 6-3 ApoE isoforms in patients who underwent VLDL-compositional analysis

ApoE Genotype	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4
n	71	102	30	348	190	20
%	9	13	4	46	25	3

As there is a high local prevalence of the apoE (R145C) mutation all patients that were not apoE2 homozygotes were screened for this mutation where possible.

Table 6-4 ApoE (R145C) mutational screening

Patients "at risk"	690
Patients tested	684
Mutation detected	41
Mutation not detected	643

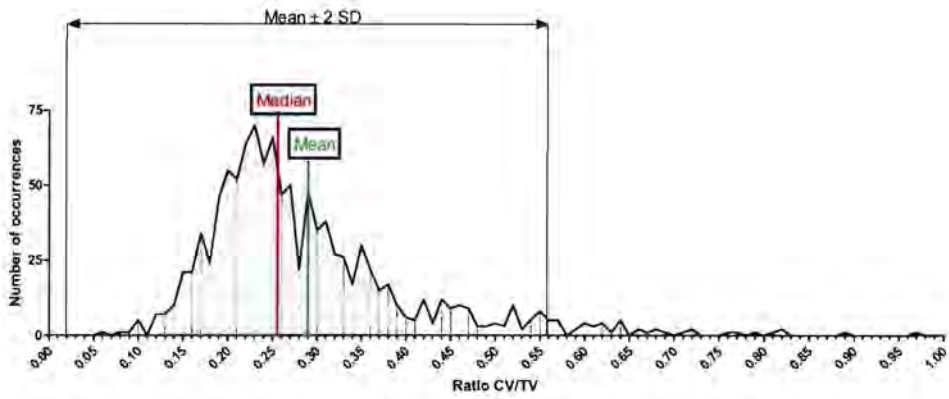
Of the 779 patients that had undergone VLDL-compositional analysis **117** (71 apoE2 homozygotes+ 41 apoE (R145C) mutation carriers + 5 apoE (K146Q) mutation carriers) were found to have a genotype associated with dysbetalipoproteinaemia. Of the remaining patients, **646** did not have an identified apoE mutation linked to the development of dysbetalipoproteinaemia. For the purposes of analyzing the diagnostic efficiency of VLDL-compositional analysis these patients were classified as "genonegative" in distinction to the group of genopositive patients. This approach obviously has its limitations, as there may be other undetected mutations in apoE in the genonegative group. As there is no absolute diagnostic gold standard for dysbetalipoproteinaemia it is not unreasonable to compare the diagnostic performance of a phenotypic test against that of even a limited genotypic test. The Hixson (237) fragment, which includes the receptor binding domain of apoE where most described mutations are localized, was also sequenced in all patients with cholesterol-enriched VLDL and no known apoE mutation. Sequencing did not identify any additional apoE mutations.

6.1.2 VLDL-cholesterol enrichment ratios

6.1.2.1 Ratio CV/TV

The ratio of VLDLC to VLDL-TG (CV/TV) ranged from 0.06-1.76 with a mean ratio of 0.29 and a median of 0.26. Results were not distributed symmetrically, as can be seen in figure 6.1.

Figure 6-1 Frequency distribution histogram of ratio CV/TV



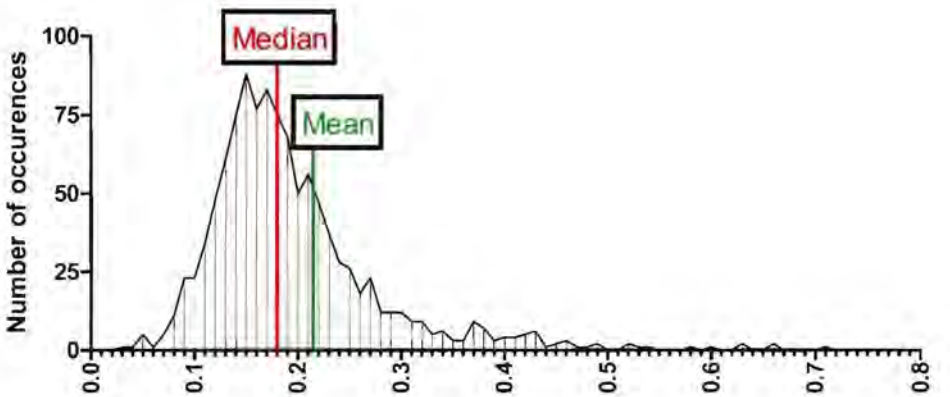
Ratios of CV/TV>1.0 are not shown on this graph

The ratio did not correlate significantly with either TG, as reported by Albers (229), or TC (data not shown).

6.1.2.2 Ratio CV/TP

The ratio of VLDLC to TG (CV/TP) ranged from 0.03 to 9.4. The mean was 0.22 with a median of 0.18. Only 7 ratios were higher than 0.7 and in 3 cases the ratio CV/TV was very much lower than would be expected considering the CV/TP ratio. In many cases the plasma TG levels were also very low and it is likely that the ratios were artefactually high. Figure 6.2 shows the frequency distribution of the ratio CV/TP.

Figure 6-2 Frequency distribution histogram of ratio CV/TP



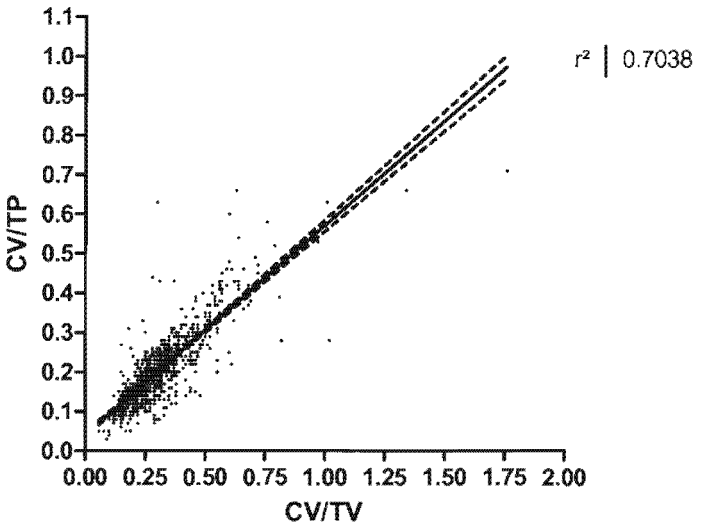
Ratios >0.8 are not shown on this graph

The ratio did not correlate significantly with either TG or TC (data not shown).

6.1.2.3 Relationship between ratios of VLDL cholesterol-enrichment

Both the ratios discussed above aim to describe the degree to which VLDL is cholesterol-enriched. VLDLC is common to both ratios and it is therefore not surprising that both ratios correlate well (r^2 =0.70 by linear regression) with each other. The ratio CV/TP is almost invariably lower than the ratio CV/TV.

Figure 6-3 Correlation of ratio CV/TV with ratio CV/TP



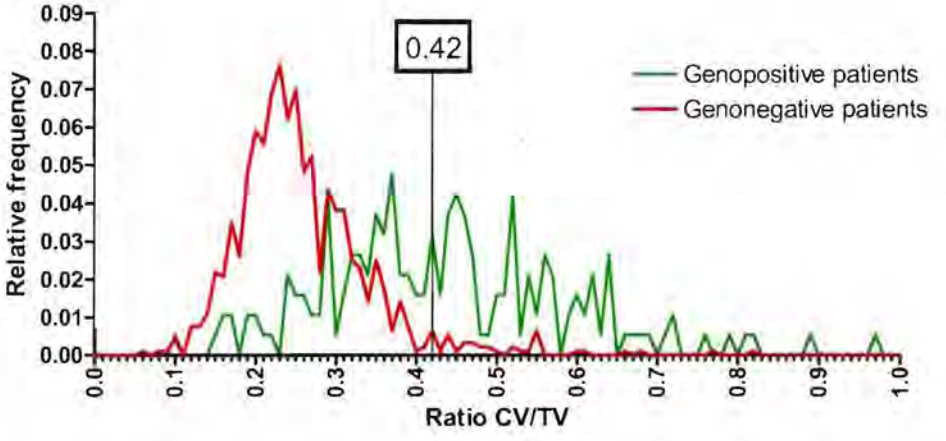
Ratios of CV/TP >0.75 were excluded from this analysis

6.1.3 Diagnostic performance of VLDL-compositional analysis

6.1.3.1 Ratio CV/TV

The ratio CV/TV was clearly higher in genopositive patients (0.45 ± 0.19 vs. 0.26 ± 0.09 for genopositive vs. genonegative, P<0.0001 by Mann Whitney test), but there was significant overlap between the two groups

Figure 6-4 Relative frequency distribution of ratio CV/TV in genopositive and genonegative patients



The graph does not show ratios > 1.0. One of the proposed diagnostic thresholds of 0.42 is also indicated on the graph.

To eliminate the possibility that the degree of hyperlipidaemia may have influenced the observed ratio, the ratios obtained in specimens matched for lipid values were compared. There were 160 specimens from genopositive patients that could be tightly matched with specimens from genonegative patients. The ratio remained significantly higher in the genopositive patients (0.46 ± 0.18 vs. 0.25 ± 0.07 for genopositive vs. genonegative, P<0.0001 by Mann Whitney test).

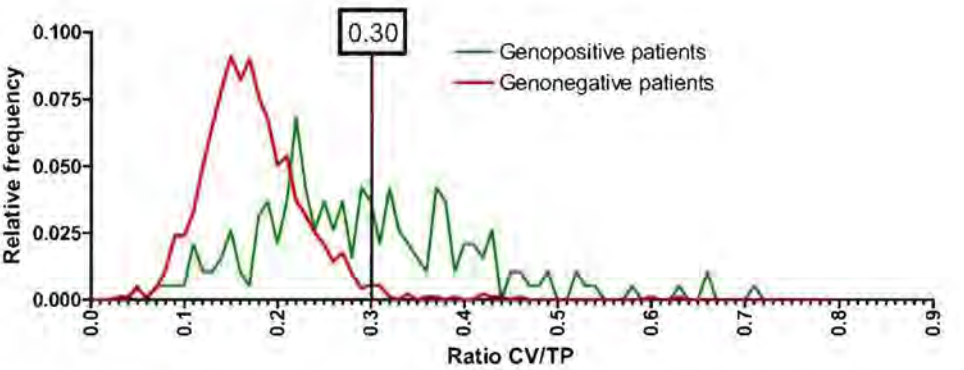
The ratios found in apoE2 homozygotes and apoE (R145C) carriers also overlapped broadly, but on average apoE2 homozygotes had higher ratios (0.47 ± 0.18 vs. 0.42 ± 0.21 for apoE2 vs. apoE (R145C), P=0.01 by Mann Whitney test) when all tests were considered. When only VLDL-compositional analyses with matching lipid values

were compared, there was no longer a statistically significant difference (0.42 ± 0.12 vs. 0.41 ± 0.11 for apoE2 vs. apoE (R145C), P=0.98 by Mann Whitney test)

6.1.3.2 Ratio CV/TP

The ratio CV/TP was higher in genopositive than genonegative patients (0.32 ± 0.26 vs. 0.19 ± 0.36 for genopositive vs. genonegative, P<0.0001 by Mann Whitney test). Once again there was significant overlap between the groups.

Figure 6-5 Relative frequency distribution of ratio CV/TP in genopositive and genonegative patients



The graph does not show ratios > 0.75. One of the proposed diagnostic thresholds of 0.30 is also indicated on the graph.

Comparing only ratios obtained for specimens matched for plasma lipids the difference between genopositive and genonegative patients remained highly statistically significant (0.33 ± 0.27 vs. 0.17 ± 0.05 for genopositive vs. genonegative, P<0.0001 by Mann Whitney test).

When all analyses were considered the CV/TP ratio was higher in apoE2 homozygotes than apoE (R145C) carriers (0.34 ± 0.30 vs. 0.27 ± 0.12 for apoE2 vs. apoE (R145C), P=0.003 by Mann Whitney test), but when analyses were matched for lipids the difference was no longer obvious (0.27 ± 0.10 vs. 0.27 ± 0.09 for apoE2 vs. apoE (R145C), P=0.96 by Mann Whitney test).

6.1.3.3 Concordance of ratios

As the two ratios discussed above strongly correlate with each other, it is important to know in what proportion of cases VLDL-compositional analysis would be classified as positive or negative by both ratios. This question was examined using "high" (CV/TV>0.42 and CV/TP>0.30) and "low" (CV/TV >0.35 and CV/TP> 0.25) diagnostic thresholds.

Figure 6-6 Concordance of ratio CV/TV and CV/TP when using "high" threshold

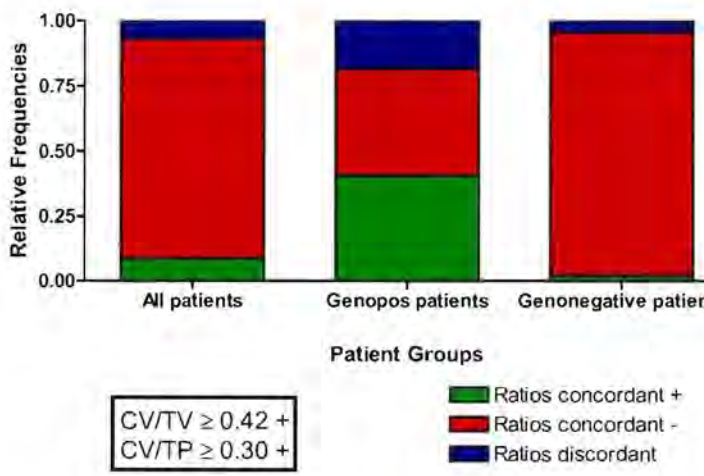
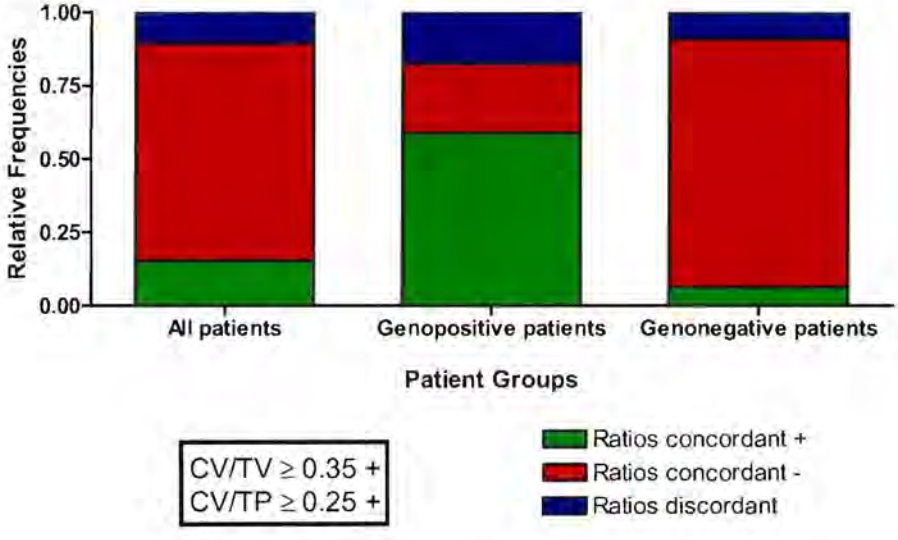


Figure 6-7 Concordance of ratio CV/TV and CV/TP when using "low" threshold



Using the "high" diagnostic threshold fewer spins are discordant, considering all patients, than when "low" criteria are used. Genopositive patients have a higher incidence of discordant spins than genonegative patients, as they have higher ratios and therefore a higher likelihood of ratios that lie close to the diagnostic limits. The higher concordance of "high" diagnostic thresholds in the total patient group has to be traded off against reduced diagnostic sensitivity and increased rates of discordance in the genopositive cohort.

6.1.3.4 Concordance of VLDL-compositional analysis for individual patients

Many patients had VLDL-compositional analysis performed on more than one occasion as diagnostic suspicion often remained high despite an initial negative test. In other patients VLDL-compositional analysis was repeated to investigate the effect of lipid-lowering medication or other interventions.

Patients that had at least two VLDL-compositional analyses were included in this analysis. The clinical practice at the lipid laboratory is to classify VLDL-compositional analysis as positive if either the ratio CV/TV or the ratio CV/TP meets the "high" diagnostic criteria. This criterion was applied to all analyses and patients in who all spins were either classified as positive or negative were labelled concordant.

Table 6-5 Concordance of VLDL-compositional analysis in individual patients

Group	Spins concordant	Spins discordant
All patients	172	47
Genopositive patients	26	21
Genonegative patients	146	26

Genopositive patients were more likely to have discordant spins than genonegative patients ($P<0.0001$ by Fisher's exact test). In most instances VLDL-cholesterol enrichment decreased below the diagnostic level with the institution of lipid-lowering medication. In genonegative patients the commonest scenario was for negative VLDL-compositional analysis to remain negative with repeated testing.

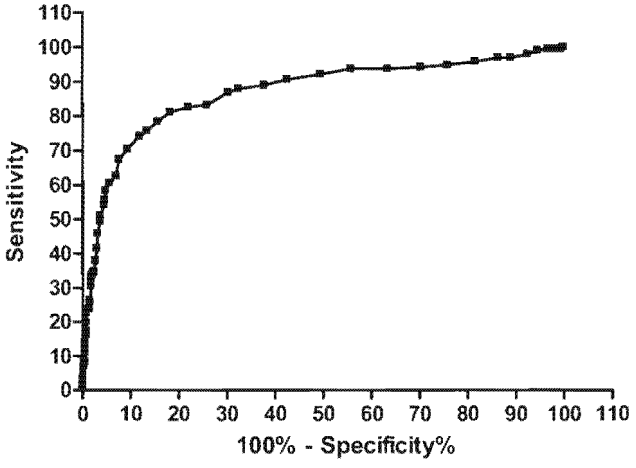
6.1.3.5 Receiver-operator characteristic curve (ROC) analysis

ROC analysis is useful in examining the tradeoff between sensitivity and specificity when setting cutoff values for diagnostic tests. The area under the ROC curve quantifies the ability of the test to truly distinguish between affected and non-affected individuals. If the area is 0.5 (the minimum value) the test has no diagnostic value at all, while an area of 1.0 indicates a perfect test. An area of 0.8 for instance indicates that a randomly selected patient, with the disease being tested for, will have a higher test value than 80% of randomly selected controls. ROC analysis relies on the true diagnosis having been established by a gold-standard diagnostic test. As previously discussed there is no single such test for dysbetalipoproteinaemia, but in this analysis genotyping is used as the gold-standard comparator. The ROC analysis therefore describes the diagnostic utility of VLDL-compositional analysis in identifying apoE2 homozygotes or apoE (R145C) mutation carriers.

6.1.3.5.1 Ratio CV/TV

The AUC for the ratio CV/TV was 0.87 (95% CI 0.84-0.90) if all analyses performed were included in the data set. Figure 6.8 shows the ROC curve and sensitivity and specificity at selected cutoff values.

Figure 6-8 ROC curve for ratio CV/TV (all plasma lipid values)



Area	0.8681
Std. Error	0.01678
95% confidence interval	0.8352 to 0.9010

Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.3150	81.05	74.75% to 86.36%	81.83	79.18% to 84.27%	4.46
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.3550	70.53	63.49% to 76.91%	90.64	88.57% to 92.45%	7.54
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.3850	60.53	53.19% to 67.53%	94.45	92.77% to 95.84%	10.91
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.4150	54.21	46.85% to 61.44%	95.54	94.00% to 96.78%	12.15
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.4250	51.05	43.71% to 58.36%	96.19	94.74% to 97.33%	13.40

As is to be expected, sensitivity decreases and specificity increases as the diagnostic threshold is raised. At a threshold of 0.355 sensitivity is 71% while specificity is 91%. Raising the diagnostic threshold to 0.415 increases specificity to 96%, but this occurs at the cost of marked reduction in sensitivity to 54%.

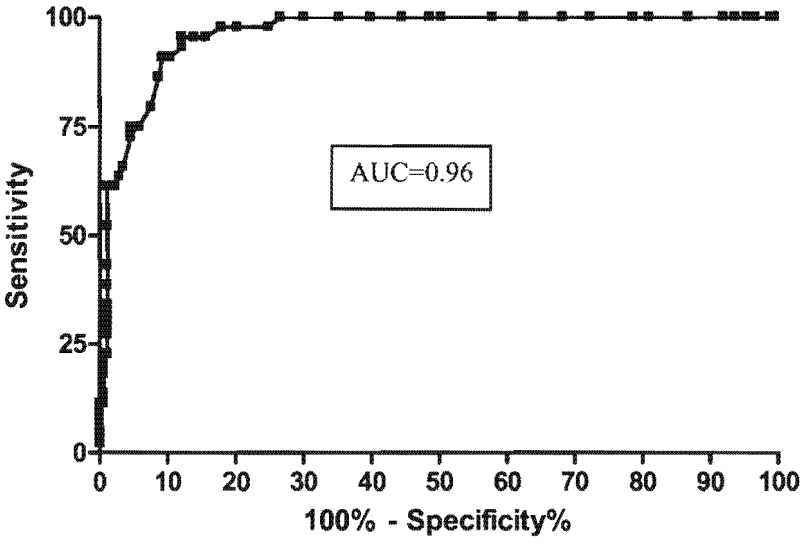
The effects on sensitivity and specificity of restricting the analysis to samples with plasma lipids within certain limits were explored.

Table 6-6 ROC analysis of ratio CV/TV with plasma lipid criteria

Lipid criteria (mmol/L)	Diagnostic threshold										AUC
	0.315		0.355		0.385		0.415		0.425		
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	
TG>1.5	83	82	74	91	64	94	56	96	53	96	0.88
TG>2.0	85	82	75	91	68	93	58	96	54	96	0.89
TG>2.5	84	81	74	91	65	94	59	95	55	96	0.88
TG>5.0	80	85	69	94	64	95	56	96	51	97	0.86
TG>7.5	61	90	50	94	44	95	33	95	28	96	0.73
TC>5.2	87	80	80	89	74	93	68	95	65	95	0.89
TC>7.5	88	69	83	82	80	88	77	90	75	91	0.87
TC>10.0	80	53	71	71	67	80	63	80	63	82	0.74
TG>2.5 + TC>5.2	87	80	79	90	73	93	66	95	62	96	0.89
TG>2.5 + TC>7.5	94	66	88	80	86	86	84	88	82	90	0.96
TG = 2.5-10 + TC>7,5	100	64	98	80	95	86	93	88	90	90	0.96

Figure 6.9 illustrates the ROC curve obtained when only specimens with a TC>7.5 mmol/L and a TG in the range of 2.5-10 mmol/L are analyzed.

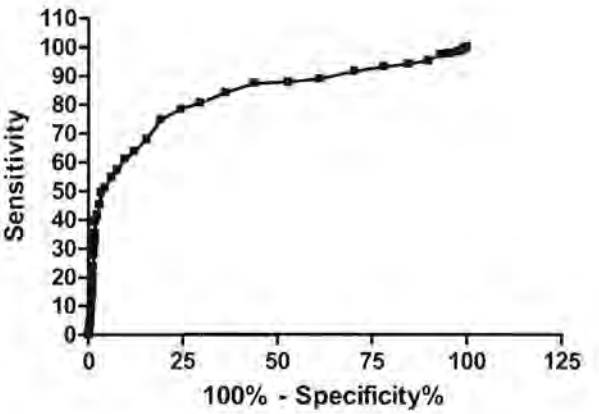
Figure 6-9 ROC curve for ratio CV/TV (TC >7.5 and TG range 2.5-10.0)



6.1.3.5.2 Ratio CV/TP

The AUC for the ratio CV/TP was 0.83 (95% CI 0.79-0.86) when all analyses were included in the calculation.

Figure 6-10 ROC curve for ratio CV/TP (all lipid values)



Area under the ROC curve	
Area	0.8260
Std. Error	0.01963
95% confidence interval	0.7875 to 0.8645
P value	< 0.0001

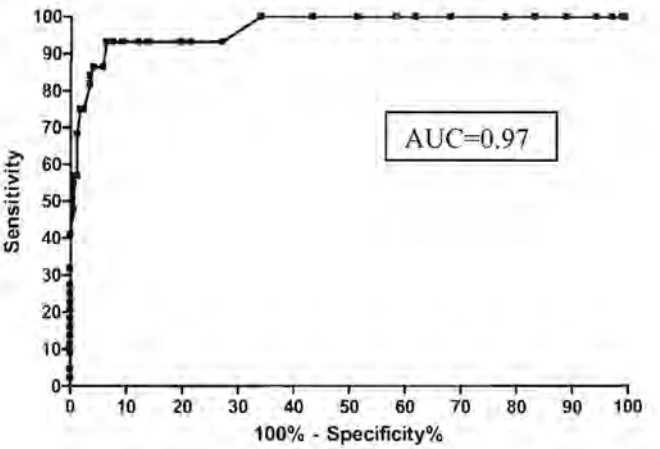
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.2450	61.05	53.73% to 68.03%	90.34	88.23% to 92.18%	6.32
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.2550	57.37	50.00% to 64.50%	92.43	90.51% to 94.06%	7.57
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.2850	49.47	42.16% to 56.81%	96.60	95.20% to 97.68%	14.54
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.3050	41.58	34.49% to 48.94%	97.59	96.37% to 98.48%	17.22
Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 0.3250	35.26	28.49% to 42.51%	98.24	97.16% to 98.99%	20.08

The effects on sensitivity and specificity of restricting the analysis to samples with plasma lipids within certain criteria were also explored for the ratio CV/TP.

Table 6-7 ROC analysis of ratio CV/TP with plasma lipid criteria

Lipid criteria	Diagnostic threshold										AUC
	0.245		0.255		0.285		0.305		0.325		
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	
TG>1.5	67	90	63	93	54	97	45	98	38	99	0.87
TG>2.0	69	90	66	92	56	97	48	98	40	99	0.88
TG>2.5	70	89	70	91	60	97	50	98	41	98	0.88
TG>5.0	64	90	64	92	60	94	44	97	40	98	0.85
TG>7.5	50	90	50	92	44	95	44	96	28	96	0.76
TC>5.2	73	89	71	91	67	96	56	98	48	98	0.88
TC>7.5	80	83	80	87	78	93	70	94	68	96	0.90
TC>10.0	71	73	71	80	67	87	58	89	58	91	0.81
TG>2.5 + TC>5.2	74	88	72	91	67	96	56	98	47	98	0.88
TG>2.5 + TC>7.5	86	81	86	87	84	93	78	94	76	97	0.93
TG = 2.5-10 + TC>7.5	93	80	93	86	93	92	86	94	84	97	0.97

Figure 6-11 ROC curve for ratio CV/TP (TC >7.5 and TG range 2.5-10.0)



6.1.3.5.3 Defining a diagnostic threshold

Choosing a diagnostic cutoff level requires establishing a balance between sensitivity and specificity. High sensitivity, achieved by choosing a low numerical cutoff, must be traded for a reduction in specificity. The magnitude of this tradeoff varies according to the baseline plasma lipid levels at the time of VLDL-compositional analysis.

The sensitivity of the CV/TV ratio decreases by about 20% in specimens with TG values >7.5 mmol/L, irrespective of whether a low (0.355) or high (0.415) threshold is set. Specificity remains relatively unchanged at all TG values. For the CV/TP ratio the sensitivity decreases most with increasing TG values at a low threshold (0.255), while the decrease is smaller at the higher threshold of 0.305. Specificity is again relatively unaffected by increasing TG values.

Both ratios are the most sensitive, using either high or low cutoffs, when the TC is >7.5 mmol/L. If only specimens with TC >10 mmol/L are considered there is a reduction in both sensitivity and specificity.

The ratio CV/TV performs best overall if only specimens with a raised TC (>7.5 mmol/L) and moderately raised TG (2.5-10 mmol/L) are considered. In specimens meeting these lipid criteria a ratio of 0.355 is highly sensitive (98%) with a reasonable specificity of 80%. At a ratio of 0.415 93% of cases will still be detected and the specificity is high at 88%.

The CV/TP ratio also has the best overall performance in specimens with a TC>7.5 mmol/L and TG range from 2.5-10.0 mmol/L. The cutoff of 0.255 is associated with a sensitivity of 93% and a specificity of 86%. Increasing the threshold to 0.305 reduces sensitivity to 86% while specificity rises to 94%.

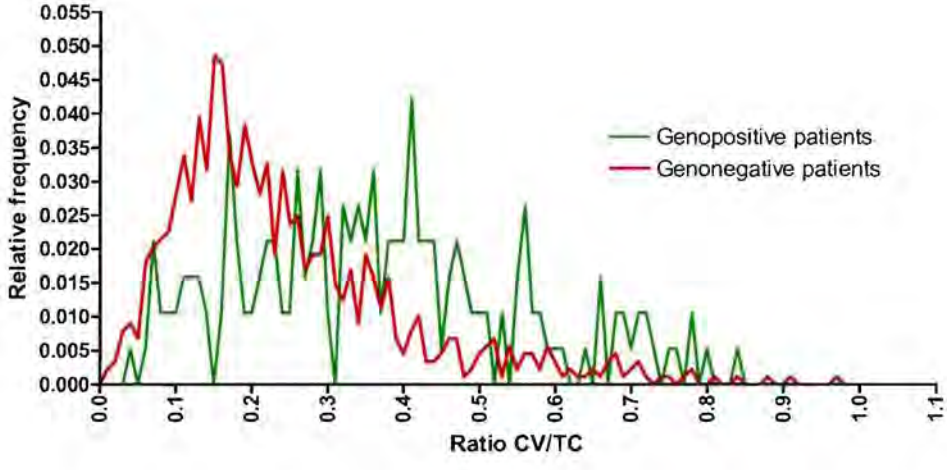
In summary increasing hypertriglyceridaemia reduces the diagnostic sensitivity of VLDL-compositional analysis, but if a positive result is obtained the chances of a false positive are very low. The test is most likely to be diagnostically useful in specimens with moderate hypertriglyceridaemia (2.5-10.0 mmol/L –data for the range 2.5-7.5 mmol/L is very similar and is not shown) and a TC > 7.5 mmol/L. Patients that have lipids within this range and an apoE mutation (apoE2 homozygosity or apoE (R145C) for the purposes of this analysis) will almost certainly be detected using the low diagnostic thresholds. If the high diagnostic thresholds are met the probabilities of detecting an apoE mutation are 90% or more.

The results of VLDL-compositional analysis should always be interpreted in the context of the patient's lipid levels. Dysbetalipoproteinaemia may be difficult to prove by ultracentrifugation when the dyslipidaemia is not (yet) severe.

6.1.3.6 Other ratios

Other ratios that could be potentially diagnostically useful were examined. The ratio of VLDLC to TC (CV/TC) was clearly higher in genopositive patients than genonegative patients (0.37 ± 0.21 vs. 0.26 ± 0.32 for genopositive vs. genonegative patients, P<0.0001 by Mann-Whitney test). The overlap between the two groups was, however, so large that it was not possible to suggest a cutpoint that would separate the two groups.

Figure 6-12 Relative frequency distribution of ratio CV/TC in genopositive and genonegative patients



The ratio of VLDL-TG to plasma TG was slightly, but statistically significantly, higher in genopositive than genonegative subjects (0.78 ± 0.84 vs. 0.76 ± 0.73 for genopositive vs. genonegative, P=0.03 by Mann Whitney test). The overlap between both ratios was, however, almost complete and this ratio therefore is of no diagnostic use (data not shown).

6.1.3.7 VLDL-compositional analysis in the context of previously published experience

Almost all literature on VLDL-compositional analysis was published in the period from 1970-1976 (207;229-232;390). Tables 6.8 and 6.9 list pertinent details regarding these studies.

Table 6-8 Proposed diagnostic cutoffs for VLDL-compositional analysis

Author	CV/TV	CV/TP	CV/TC	Comments	Reference
Wybenga	NA	NA	0.25-0.50	1	(390)
Hazzard	0.42	NA			(230)
Fredrickson		0.25 (s) [1] 0.30 (d) [2]			(207)
Mishkel	0.35	0.25		2	(231)
Vessby	NA	NA		3	(232)
Albers	0.38 [3]	0.24		4	(229)

[1] Suggestive of dysbetalipoproteinaemia

[2] Considered diagnostic

[3] TG dependant cutoff (> 0.38 x 0.00885 [TG in mmol/L])

Comments

1. Suggested diagnostic range
2. Ratios apply if TG<5.11 mmol/L and TC>5.2 mmol/l
3. The author proposes a composite index of VLDL and LDL composition, but the text provides no definite diagnostic cutoffs
4. TG dependant cutoff introduced.

Table 6-9 Details of previous studies on VLDL-compositional analysis

Author	Year	Subjects	Controls	Analyses		Diagnosis	Ratio CV/TV		Ratio CV/TP		Comment	Reference
				Subjects	Controls		Subject	Control	Subject	Control		
Wybenga	1972	NS	NS	55	152	ß-VLDL	NA	NA	NA	NA	1	(390)
Hazzard	1972	9	34	9	34	ß-VLDL	0.60 ± 0.11	0.31 ±0.08	NA	NA	2	(230)
Fredrickson	1975	59	123	3164 in total		ß-VLDL	NA	NA	Not given		3	(207)
Mishkel	1975	19	119	191	119	ß-VLDL	0.50 ± 0.13	0.29 ± 0.09	0.35 ± 0.10	0.20 ± 0.05	4	(231)
Vessby	1975	12	198			ß-VLDL	Not given		Not given		5	(232)
Albers	1976	43	191	198	191	ß-VLDL	Not given		Not given		6	(229)

Comments:

1. The author proposes that a ratio of VLDLC to TC be calculated. The ratio for dysbetalipoproteinaemia is said to range from 0.25-0.50 and 0.52-0.80 in Type IV hyperlipidaemia.
2. Controls included normolipidaemic individuals, patients with FH, Type IV and Type V hyperlipidaemia

3. The authors compare a large number of VLDL- compositional analyses in a relatively small group of patients. Some patients had >100 analyses. They calculate a mean CV/TP ratio and plot the distribution of this ratio.
4. The study included a large proportion of patients with mixed hyperlipidaemia.
5. The author proposes that a "III index" be calculated as follows: $\frac{\text{VLDLC/VLDL-TG}}{\text{LDLC/LDL-TG}}$ X10
6. The authors define a diagnostic ratio by creating a linear regression based cutline below which lie 95% of all ß-VLDL negative specimens. The false positive rate is therefore set at 5% by definition.

All previous studies defined dysbetalipoproteinaemia by the presence of ß-VLDL. As the UCT lipid laboratory did not electrophorese the ultracentrifugal supernatant routinely, no data on the sensitivity and specificity of ß-VLDL in relationship to dysbetalipoproteinaemia identified by genetic testing are available for this study. Fredrickson found discordant analyses for ß-VLDL in 12% of all subjects (207). In the Bayer study agarose electrophoresis of VLDL1 revealed a beta-migratory component in all genopositive patients at baseline. This was no longer the case when lipid-lowering therapy was given. ß-VLDL is therefore also not an ideal diagnostic standard for dysbetalipoproteinaemia as discordant results may be found and lipid-lowering therapy may result in the disappearance of ß-VLDL. Despite the limitations of this and previous analyses the suggested diagnostic cutoffs are remarkably similar.

6.2 ApoB as a diagnostic test

ApoB is readily and reliably quantified by automated methods and the test is widely available (391). Clinicians are measuring apoB levels more frequently and there are suggestions that apolipoprotein measurements should replace lipid measurements when assessing cardiovascular risk and the response to therapy (70;392;393). ApoB measurements have been incorporated in a few national lipid guideline statements (69).

Each lipoprotein contains one apoB molecule and plasma apoB levels reflect the total number of circulating atherogenic lipoproteins. Larger lipoproteins contain proportionally less apoB and more lipid than smaller lipoproteins. Remnant lipoproteins are larger than LDL. It can therefore be hypothesized that

dysbetalipoproteinaemic patients should have lower apoB levels relative to plasma cholesterol than patients in whom the predominant abnormality is increased circulating LDL. Additionally one may speculate that apoB levels should be even higher in patients with mixed hyperlipidaemia due to the presence of numerous small dense LDL particles.

ApoB levels and ratios derived from apoB and plasma lipids between patients with dysbetalipoproteinaemia and other forms of mixed hyperlipidaemia were thus compared.

6.2.1 Definition of patient groups studied

For this analysis the following definitions were used:

Dysbetalipoproteinaemia:

- apoE2 homozygosity
- CV/TV >0.42 and/or CV/TP>0.30

Mixed hyperlipidaemia

- apoE genotype: neither apoE2/E2 nor apoE (R145C)
- CV/TV<0.35 **and** CV/TP<0.25

Indeterminate VLDL-composition

- apoE genotype: neither apoE2/E2 nor apoE (R145C)

- CV/TV in range 0.35-0.42 **or** CV/TP in range 0.25-0.30

"Low" cutoffs were chosen to definitely exclude dysbetalipoproteinaemia to minimize the chance of falsely including dysbetalipoproteinaemic subjects in the mixed hyperlipidaemia cohort. Patients with indeterminate VLDL-composition were studied separately. Only apoE2 homozygotes were considered in this analysis, as this is the commonest genetic cause of dysbetalipoproteinaemia worldwide. This approach ensures maximum applicability at other centres.

6.2.2 Results

6.2.2.1 Lipids

There were 254 patients with mixed hyperlipidaemia, 57 with dysbetalipoproteinaemia and 22 with indeterminate VLDL composition. Dysbetalipoproteinaemic patients had higher levels of TC and TG than patients with mixed hyperlipidaemia or indeterminate VLDL composition. Patients with indeterminate VLDL-compositional analysis only differed significantly in their VLDL cholesterol enrichment from the mixed hyperlipidaemia group. Despite the higher levels of TC found in dysbetalipoproteinaemic subjects apoB levels were lowest in this group.

Table 6-10 Clinical characteristics and lipid values in patients with dysbetalipoproteinaemia and mixed hyperlipidaemia

	Dysßlipoproteinaemia	Mixed hyperlipidaemia	Indeterminate VLDL composition	P
Number of patients	57	254	22	
ApoE criterion	ε2 homozygosity	ε3 or ε4 allele present, not E (R145C)	Not ε2/ ε2 or E (R145C)	
VLDL composition criteria	CV/TV >0.42 or CV/TP >0.30	CV/TV < 0.35 and CV/TP <0.25	CV/TV 0.35-0.41 or CV/TP 0.25-0.29	
Age at presentation, years	50.1 ± 9.3	52.9 ± 11.8	54.43 ± 16.32	NS [1]
Male/Female,	29/28	101/153	7/15	NS [1]
Diabetes, %	17.5%	38.8%	36.4%	0.009
Total cholesterol, mmol/L	11.75 ± 4.4 [2,3]	7.94 ± 1.7	8.61 ± 1.7	<0.0001
Triglycerides, mmol/L	5.86 (4.89-7.00) [2,4]	4.32 (4.11-4.45) [4]	4.19 (3.59-4.88) [4]	<0.0001
HDLC, mmol/L	1.20 ± 0.4	1.14 ± 0.3	1.05 ± 0.4	NS [1]
ApolipoproteinB100, g/L	1.15 ± 0.48 [2,3]	1.61 ± 0.37	1.68 ± 0.52	<0.0001
Ratio TC/TG	2.1 ± 0.9	1.9 ± 0.6	2.2 ± 0.9	NS [1]
VLDL-C/VLDL-TG	0.50 ± 0.14 [2,3]	0.23 ± 0.05 [5]	0.38 ± 0.02	<0.0001
VLDL-C/Plasma-TG	0.35 ± 0.09 [2,3]	0.16 ± 0.04 [5]	0.24± 0.01	<0.0001
Ratio apoB/TC, g/mmol	0.10 ± 0.04 [2,3]	0.20 ± 0.03	0.19 ± 0.04	<0.0001

[1] NS, not significant

[2] P<0.05 vs. patients in whom dysbetalipoproteinaemia has been excluded

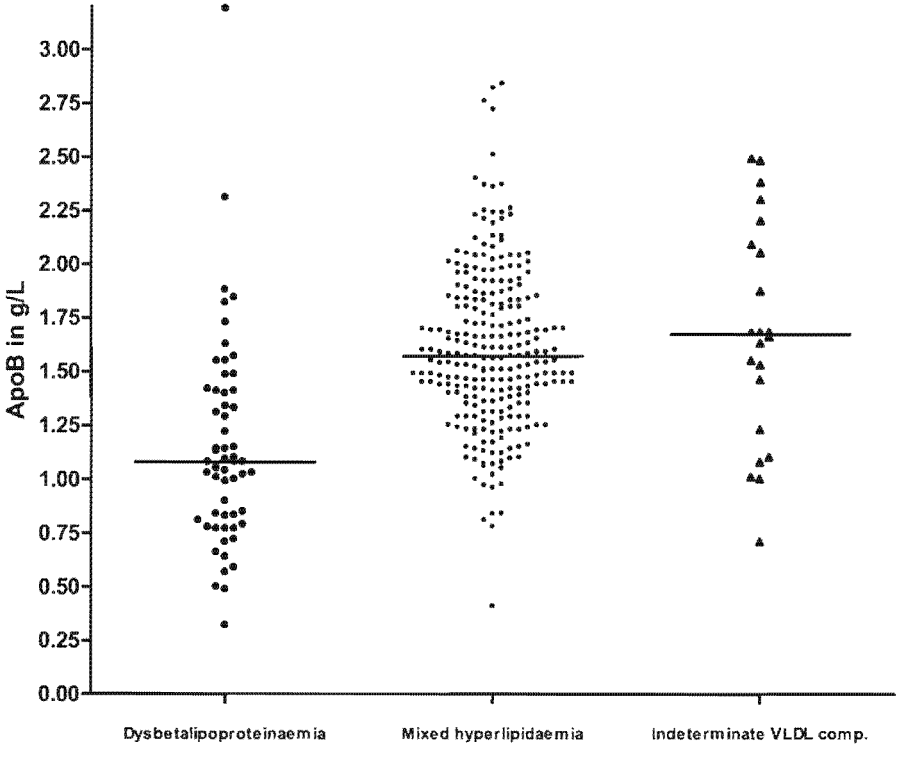
[3] P<0.05 vs. patients with indeterminate VLDL composition

[4] Geometric mean ± 95% confidence intervals

[5] P<0.05 vs. patients with indeterminate VLDL composition

Figure 6.13 illustrates that apoB levels are lower in dysbetalipoproteinaemic subjects, but that there is a wide overlap with the other groups.

Figure 6-13 ApoB levels

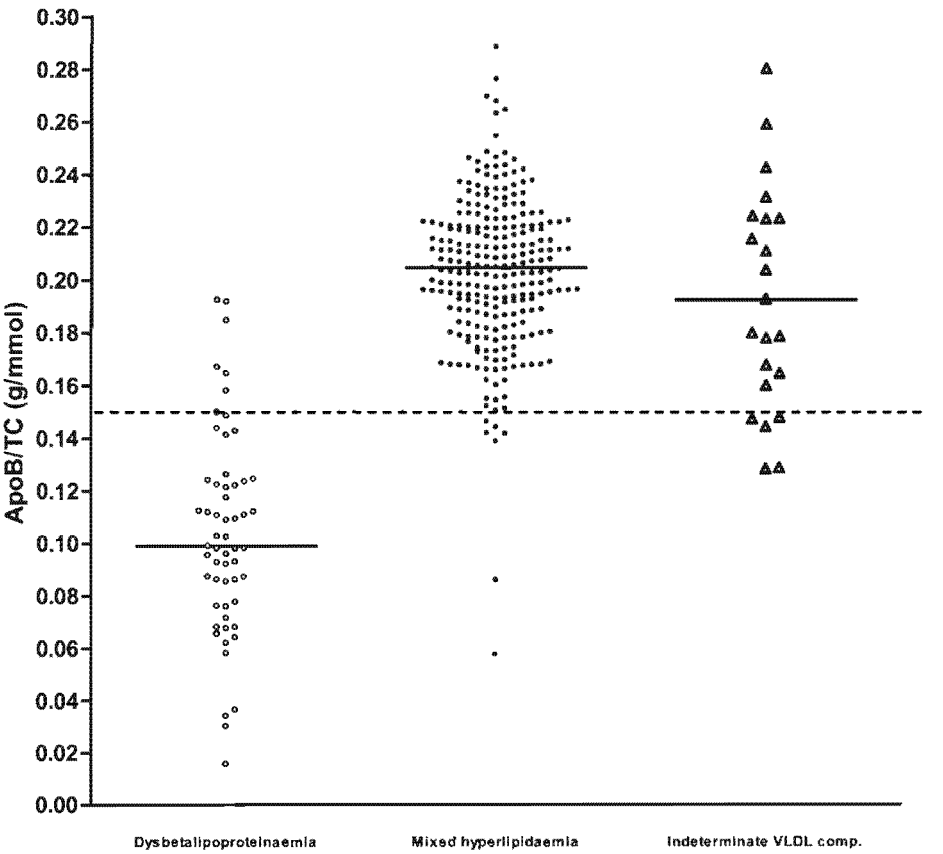


Legend: The solid lines indicate median values.

6.2.2.2 Ratio apoB/TC

Figure 6.14 illustrates the large differences in the apoB/TC ratio between the three groups.

Figure 6-14 ApoB/TC ratio

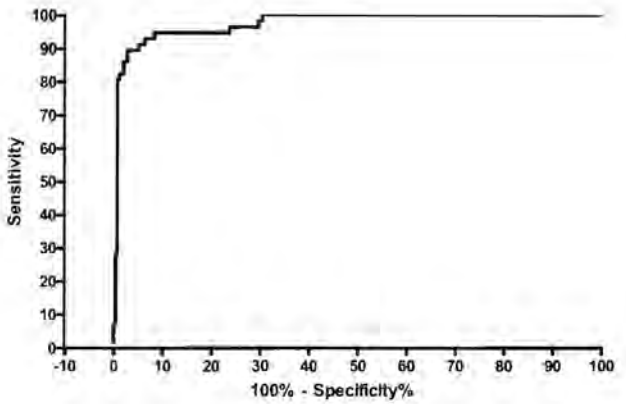


Legend: The solid lines indicate median values; the broken line indicates the proposed diagnostic threshold of 0.15

Comparing patients with dysbetalipoproteinaemia to those in whom the diagnosis has been definitely excluded (mixed hyperlipidaemia cohort) an apoB/TC cutoff value of 0.15 g/mmol identifies dysbetalipoproteinaemia with a sensitivity of 89% (CI 78-96) and a specificity of 97% (CI 94-99). The positive predictive value is 88% (CI 77-95) and the negative predictive value 98% (CI 95-99). No dysbetalipoproteinaemic patients with a ratio of >0.20 g/mmol were found. Including patients with indeterminate VLDL-composition in the mixed hyperlipidaemia cohort leaves the sensitivity unchanged, but the specificity decreases to 96% (CI 93-98) with a positive predictive value of 81% (CI 69-90) and a negative predictive value of 98% (CI 93-99). If patients with indeterminate VLDL-composition are considered to have dysbetalipoproteinaemia with unrecognized apoE mutations, the sensitivity of the test decreases to 71% (CI 60-81) with a specificity of 97% (CI 94-99). In this case the positive predictive value is 89% (CI 78-95) with a negative predictive value of 91% (CI 87-95).

The apoB/TC ratio performs well as a diagnostic test when evaluated by ROC analysis. The ROC AUC is 0.98 (CI 0.96-0.99) when considering patients with mixed hyperlipidaemia and dysbetalipoproteinaemia.

Figure 6-15 ROC curve for apoB/TC ratio for dysbetalipoproteinaemia and mixed hyperlipidaemia

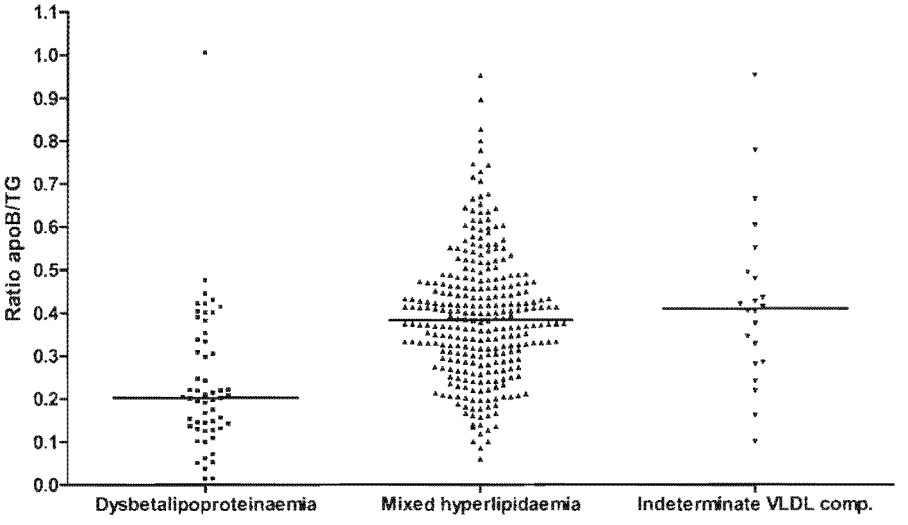


If patients with indeterminate VLDL-compositional analysis are included in the mixed hyperlipidaemia group the ROC AUC is 0.97 (CI 0.95-0.99). Classifying patients with indeterminate VLDL-composition as dysbetalipoproteinaemic reduces the ROC AUC to 0.87 (CI 0.81-0.93).

6.2.2.3 Ratio apoB/TG

The diagnostic utility of the apoB/TG ratio was also evaluated. It was significantly lower ($P<0.0001$ by ANOVA) in dysbetalipoproteinaemic patients (0.24 ± 0.16) than mixed hyperlipidaemia (0.39 ± 0.19) subjects and subjects with indeterminate VLDL composition (0.43 ± 0.20). The values, however, overlapped widely and this ratio was not diagnostically useful.

Figure 6-16 Ratio apoB/TG



The lines indicates median values

6.2.3 Discussion of utility of apoB as a diagnostic test

There are few screening tests for dysbetalipoproteinaemia suitable for general use. Non-denaturing polyacrylamide gradient gel electrophoresis of plasma prestained for lipoproteins is both sensitive and specific (227). Although the technique is inexpensive and not technically demanding, it is not available in routine clinical laboratories. The apoB/apoE ratio has been previously evaluated in 40 dysbetalipoproteinaemic patients and 48 controls matched for lipid values (235). Diagnostic sensitivity was 95% with a specificity of 88%. The apoB/apoE ratio is therefore somewhat more sensitive than the apoB/TC ratio in detecting

dysbetalipoproteinaemia, but apoE measurements are generally not available from routine chemical pathology laboratories in South Africa.

A precipitation-based screening method for dysbetalipoproteinaemia has also been described (236). Dextran sulfate/MgCl2 used in first generation direct LDL assays precipitates both LDL and remnants. The combination of high precipitate cholesterol and triglycerides identifies dysbetalipoproteinaemia with high sensitivity but has poor specificity (30%). Third generation direct LDLC assays have largely replaced first generation assays and may overestimate LDLC in patients with dysbetalipoproteinaemia (394;395). Some of the dysbetalipoproteinaemic subjects included in this series were initially evaluated in laboratories that perform direct LDLC measurements (various methods) and unpublished observations indicate that their measured LDLC is often much lower than the LDLC calculated by the Friedewald formula.

It has been suggested that measuring TC adds little to cardiovascular risk assessment when HDLC and LDLC are measured directly (396). This approach is valid for most patients but will underestimate atherosclerotic risk in conditions where abnormal non-measured lipoproteins such as remnants accumulate. ApoB levels are an important component of cardiovascular risk assessment (69;70). Elevated levels of apoB are associated with higher numbers of circulating atherogenic lipoproteins and higher cardiovascular risk. In dysbetalipoproteinaemia the situation is somewhat reversed and apoB levels lower than the 75th North American population percentile (397) are associated with very high cardiovascular risk. In dysbetalipoproteinaemia high TC

and TG indicate high risk, once again highlighting that apoB and lipid measurements are not identical but complementary parameters for risk assessment (393).

The ratio of apoB/TC can be calculated rapidly when faced with a patient with mixed hyperlipidaemia. It best identifies patients that are unlikely to be dysbetalipoproteinaemic. Using this approach the number of patients undergoing further testing for dysbetalipoproteinaemia can be reduced. The ratio should not be calculated or used diagnostically in the setting of other lipoprotein phenotypes. ApoB determination may in the future be routinely included in the assessment of dyslipidaemia and it is important to understand the additional diagnostic information provided by this measurement, but also the limitation of relying solely on apoB.

6.3 *Suggested diagnostic strategy for dysbetalipoproteinaemia*

Based on the data presented above diagnostic strategies for dysbetalipoproteinaemia should be adapted to the clinical setting.

If large numbers of samples need to be screened for the dysbetalipoproteinaemic phenotype, e.g. during an epidemiological study, PGGE is the method of choice. This method allows screening of large numbers of samples at a low cost. The required sample volume is small and samples may be frozen. Lipid measurement is required to ascertain whether the characteristic electrophoretic pattern seen is associated with hyperlipidaemia or not. This means that a substantial proportion of the individuals who have apoE mutations without overt hyperlipidaemia will also be detected.

Confirmation of the diagnosis in suspicious samples will then require apoE genotyping with or without VLDL-compositional analysis.

Dysbetalipoproteinaemia should be considered in all patients referred to the clinic with mixed hyperlipidaemia. A TC: TG ratio of 2:1 is suggestive, but the ratio is so variable that its absence should not be used to exclude the diagnosis. The presence of PVD should increase diagnostic suspicion further. Clinically palmar crease xanthomata are the most useful physical sign.

If an apoB measurement is available, the ratio apoB/TC should be calculated. A ratio of <0.15 g/mmol is highly suggestive of dysbetalipoproteinaemia and further investigations should be pursued. If the ratio is >0.2 dysbetalipoproteinaemia is very unlikely. PGGE may be used similarly in this setting.

All patients with mixed hyperlipidaemia that do not have clearly negative screening tests should be investigated further. The likelihood of detecting dysbetalipoproteinaemia is maximized if apoE genotype (common isoforms) and VLDL-compositional analysis are both performed. If other apoE mutations are known to be locally prevalent or the patient has suggestive ancestry, these mutations should be included in the work-up. Phenotypic testing helps identify patients with unusual mutations that may require further genetic evaluation.

7 Chapter Seven: Conclusions

7.1 Clinical aspects of dysbetalipoproteinaemia

The prevalence of dysbetalipoproteinaemia was 3.15% in patients attending a tertiary lipid service and therefore selected for severe hyperlipidaemia. The diagnosis was made in patients from all ethnic groups, but was particularly common in Africans, likely due to an increased likelihood of referral in the presence of cutaneous xanthomata. Homozygosity for apoE2 was the commonest molecular cause of dysbetalipoproteinaemia, but the apoE (R145C) mutation accounted for about one quarter of patients with known apoE mutations. In about 27% of the cohort no mutations were identified in apoE by limited sequencing (Hixson fragment sequenced).

The mean age at presentation in females was 6.25 years higher than in males; a finding that is consistent with the known protective effects of the premenopausal state. Occasionally patients present during childhood or early adolescence, usually in the face of severe precipitating metabolic stressors. Cutaneous xanthomata (eruptive or tuboeruptive) lead to earlier diagnosis and referral, while palmar crease and tendinous xanthomata usually go unrecognized.

Yellow palmar crease xanthomata are characteristic of dysbetalipoproteinaemia. LpX is the only other lipid disorder in which lipid infiltration of the palmar creases was found, but the colour of the infiltrate is white rather than yellow. Palmar crease

xanthomata are found in about 25% of patients. ApoE2 homozygosity, high levels of TC and highly cholesterol-enriched VLDL are risk factors for palmar crease xanthomata. Other cutaneous xanthomata (excluding xanthelasmata) are found in 23% of patients. Their presence is strongly associated with increasing severity of hyperlipidaemia, especially hypertriglyceridaemia. Tendinous xanthomata are uncommon and only occur in 17% of patients. Their presence is not predicted by lipid values. The prevalence of tendon xanthomata is much lower in dysbetalipoproteinaemia than in FH, where they are found in 80% of patients attending the lipid clinic at GSH (253). Xanthelasmata occur more commonly in females than in males, but bear no relationship to lipid values in dysbetalipoproteinaemia. The prevalence of arcus cornealis increases with age.

The physical signs of dyslipidaemia tend to cluster in patients and there were 11 patients in whom tendinous, palmar crease and tuboeruptive xanthomata occurred concurrently.

Cardiovascular complications are frequent in dysbetalipoproteinaemia. At least 44% of the cohort had manifest IHD at their last visit to the clinic. As follow-up was incomplete this figure likely underestimates the true prevalence of IHD. For unknown reasons remnant lipoprotein accumulation is strongly associated with the development of atherosclerosis in the peripheral vasculature. This is borne out by the striking difference in prevalence rates for PVD between subjects with FH (4%) and dysbetalipoproteinaemia (28%) attending the same clinic. This finding is more remarkable when taking into consideration that prevalence rates for IHD were nearly identical (43 % in FH, 44% in dysbetalipoproteinaemia). The higher prevalence of

alcohol abuse are risk factors for severe hypertriglyceridaemia. Hypercholesterolaemia tends to be severe with mean untreated values of more than 11.5 mmol/L. HDLC decrease with increasing hypertriglyceridaemia and is also lower in apoE (R145C) mutation carriers.

ApoB levels are raised in dysbetalipoproteinaemia, but not to the extent expected considering the markedly elevated levels of TC. Patients without identified apoE mutations have higher mean apoB levels and higher ratios of apoB/TC.

Cholesterol-enriched VLDL is characteristic of dysbetalipoproteinaemia and was one of the criteria used to select patients for inclusion in this review. Patients without an identified apoE mutation therefore had to have at least one ratio (CV/TV or CV/TP) exceeding previously published diagnostic thresholds. We found that the ratio CV/TV at a threshold of 0.42 was more frequently diagnostic of dysbetalipoproteinaemia in genonegative patients than the ratio CV/TP (threshold 0.30). In genopositive patients the situation was the reverse.

7.2.2 Electrophoresis in dysbetalipoproteinaemia

Dysbetalipoproteinaemia gets its alternative name of Type III hyperlipidaemia from the characteristic paper (and subsequently agarose) electrophoretograms seen in this condition. Agarose gel electrophoresis is, however, a poor diagnostic test for dysbetalipoproteinaemia. Characteristic electrophoretograms were only seen in 32% of all patients and 41% of patients with known apoE mutations. Broad-beta bands are

also occasionally seen in patients in whom the diagnosis of dysbetalipoproteinaemia can not be established either by genotyping or repeated VLDL compositional analysis.

In the Bayer study electrophoresis of ultracentrifugally derived lipoprotein fractions was performed on agarose gels. Although the method and density cutpoints used in the Bayer study differ from the original method described by Fredrickson (14), all patients with identified apoE mutations had a beta-migrating component in their VLDL1 fraction at baseline. Only 4 patients without identified apoE mutations were studied, but none of their VLDL1 had a beta-migrating component.

Agarose gel electrophoresis is therefore only diagnostically useful in dysbetalipoproteinaemia if combined with ultracentrifugation to isolate VLDL. This additional step adds extra work, cost and complexity. Frozen samples are also unsuitable for agarose gel electrophoresis, which is inconvenient for an infrequently requested investigation.

PGGE performs better than agarose gel electrophoresis in the diagnosis of dysbetalipoproteinaemia. We found electrophoretic patterns very suggestive of dysbetalipoproteinaemia in 70% of all patients and 80% of all patients with identified apoE mutations. Subjects with the apoE (R145C) mutation were more likely to have visible LDL-sized particles than apoE2 homozygotes, especially if they had TG values in the lower two quartiles.

PGGE is also not entirely specific for dysbetalipoproteinaemia and we were unable to prove the diagnosis in a small cohort of patients with a highly suggestive A pattern.

Some of these patients were extensively investigated with genotyping and repeated VLDL compositional analyses. In many patients with A patterns little clinical information was available, but the specimens were known to originate from clinical settings that may be associated with the phenotypic expression of dysbetalipoproteinaemia.

7.3 Treatment of dysbetalipoproteinaemia

The treatment of dysbetalipoproteinaemia was analyzed in two different settings. Treatment responses were studied in the "real-world situation" of a busy lipid clinic and during the controlled conditions of a clinical trial.

At the lipid clinic fibrates were the preferred agent for patients with dysbetalipoproteinaemia and 82% of patients received a fibrate. In about half of these patients a statin was given concurrently. Increasing length of follow-up at the clinic was associated with increasing rates of combination therapy. Many patients that were initially well-controlled on a single agent (usually a fibrate) later required an additional agent as they aged, gained more weight, became diabetic or lost their initial enthusiasm for dietary modification.

Most patients responded very well to lipid-lowering therapy, which was often prescribed in submaximal dosages due to fiscal constraints. Poor compliance was the commonest reason for the failure to achieve adequate control of lipid levels. The best median lipid values achieved were a TG of 2.0 mmol/L and a TC of 5.05 mmol/L. Patients with known apoE mutations achieved greater reductions in TC than those in

whom no mutations had been identified. Diabetes and abdominal obesity, as defined by the WHR, were associated with less favourable therapeutic responses.

The clinical trial to prospectively evaluate the efficacy of three treatment strategies in dysbetalipoproteinaemia could unfortunately not be completed as planned due to the worldwide withdrawal of cerivastatin from the market. Nevertheless, some insights were gained.

Statins and fibrates are useful drugs classes in dysbetalipoproteinaemia. Cerivastatin and fenofibrate were equally effective in lowering the TC, but fenofibrate was much better at lowering TG than cerivastatin. The combination of both drugs achieved the lowest lipid levels, but in most instances the difference between the combination and fenofibrate alone was not statistically significant. Lipid-lowering therapy did not change HDLC significantly in this study.

Based on clinical experience and the results of the Bayer study fibrates are preferred as the initial drug class in dysbetalipoproteinaemia as they achieve better TG reductions than statins and are therefore more likely to be successful as monotherapy. Fibrates also act primarily on the less dense lipoprotein fractions in which remnant lipoproteins are found. Fenofibrate did not reduce LDLC significantly in our study. In fact, LDLC increased in some patients, but in dysbetalipoproteinaemia LDLC values are low and remnant lipoproteins are the main concern. Statins can in most instances be safely added to fibrates if required.

7.4 Diagnosis of dysbetalipoproteinaemia

Awareness and knowledge of dysbetalipoproteinaemia are essential prerequisites for its diagnosis. Because dysbetalipoproteinaemia is an unusual disorder of lipoprotein metabolism few physicians are aware of it. The diagnosis is therefore usually not suspected and further investigations are not undertaken. The lipid clinic at GSH has been able to collect a large cohort of dysbetalipoproteinaemic patients due to the high awareness of the disorder in its staff and a low threshold for performing specialized investigations. A drug policy that required all lipid-lowering medications dispensed in the public sector to be initiated at a lipid clinic meant that most patients were initially evaluated in the untreated state.

From a clinical point of view, palmar crease xanthomata are useful diagnostic pointers as a mutation in apoE will be found in almost all patients with this physical sign. This physical sign should thus be included in medical curricula. The absence of palmar crease xanthomata can, however, not be used to exclude the diagnosis. Further investigations are therefore necessary whenever dysbetalipoproteinaemia is a diagnostic possibility.

The apoB/TC ratio and PGGE are useful initial screening tests. Both tests are inexpensive and can be used to rule out dysbetalipoproteinaemia. The demonstration of a mutation known to be predictive of dysbetalipoproteinaemia in a patient with mixed hyperlipidaemia can be considered diagnostic. Definite exclusion of apoE mutations, however, requires sequencing of the entire apoE gene. VLDL compositional analysis is useful to characterize the lipid phenotype and determine the need for further DNA analysis. The sensitivity and specificity of VLDL compositional

analysis varies according to the degree of hyperlipidaemia present at the time of analysis. Patients on lipid-lowering therapy have less cholesterol-enriched VLDL.

Assays of apoE could not be evaluated in this study. While high levels of apoE are characteristic of dysbetalipoproteinaemia severe hypertriglyceridaemia of other causes may also be associated with high concentrations of apoE.

7.5 Changes in lipoprotein fractions associated with lipid-lowering medication

Lipid-lowering therapy reduces plasma lipids significantly. The bulk of the changes seen in plasma lipids are accounted for by reduced lipid masses within the VLDL1 and VLDL2 fractions. Lipid-lowering therapy does reduce lipid concentrations within other lipoprotein fractions as well, but these fractions contribute relatively little to the total lipoprotein mass in dysbetalipoproteinaemia.

VLDL1 TG reduction accounts for between 61-71% of plasma TG reduction, depending on the lipid lowering agent chosen. VLDL2 accounts for a further 18-20% of TG reduction. For TC the figures are 44-60% in VLDL1 and 22-31 % in VLDL2.

Lipid-lowering therapy is associated with significant compositional changes within VLDL1. The cholesterol content of VLDL1 is reduced with particular reduction of CE. Relatively more TG and less CE is found in the lipoprotein core. The ratio of FC to PL in the lipoprotein shell also decreases, but the changes were not statistically significant. The composition of the other lipoprotein fractions remains unchanged except for a reduction in LDL TG content.

7.6 *Patients without identified apoE mutations*

For the purposes of this study both phenotypic and genotypic diagnostic criteria were used. Dysbetalipoproteinaemia was diagnosed if either of the criteria was fulfilled. We chose to include "genonegative" patients in this series because the standard genotyping performed at the laboratory only screens for the common apoE isoforms and two autosomal dominant mutations.

Thirty-seven patients with no known apoE mutation were included in this series. Sequencing the binding region of the apoE gene failed to reveal a mutation in any of these patients. The genonegative patients share many similarities with the genonegative patients but there are also important differences.

7.6.1 Similarities between genonegative and genopositive patients

Genopositive and genonegative patients presented at equivalent ages with a severe mixed hyperlipidaemia of similar magnitude. Overweight and obesity were very common, irrespective of whether an apoE mutation had been identified or not. Genopositive and genonegative patients had similar rates of atherosclerotic complications, including similar rates of PVD.

VLDL cholesterol-enrichment was comparable between genopositive and genonegative patients as assessed by the ratio CV/TV but the ratio CV/TP was significantly lower in genonegative patients.

7.6.2 Differences between genonegative and genopositive patients

Genonegative patients had higher levels of apoB, higher ratios of apoB/TC and less cholesterol-enriched VLDL when assessed by the ratio CV/TP. Broad-beta bands on agarose gel electrophoresis were unusual in genonegative patients and PGGE patterns also differed significantly from those seen in patients with identified apoE mutations. LDL-sized particles were seen much more frequently in genonegative patients.

Palmar crease xanthomata, cutaneous xanthomata and tendinous xanthomata were found infrequently (or very infrequently for palmar crease xanthomata) in genonegative patients. About half (46%) of genonegative patients were diabetic, a much higher rate than found amongst the genopositive patients. Genonegative patients were observed to respond less favourably to therapy with smaller deceases in TC than those observed in patients with identified mutations in apoE.

7.6.3 Conclusions

Some of the main clinical differences between genopositive and genonegative patients are the high prevalence of diabetes and low prevalence of physical signs such as cutaneous and tendinous xanthomata in genonegative patients. The lipid phenotype of genonegative patients is characterized by higher levels of apoB and the frequent presence of small LDL-sized particles on PGGE. These patients therefore have small dense LDL in the presence of excess circulating TGRL, while dysbetalipoproteinaemia is characterized by low levels of LDL due to decreased LDL formation and accumulation of remnant lipoproteins (50).

However, the group of genonegative patients is heterogeneous. On review of all the medical and laboratory records of the genonegative patients it is possible to separate and group patients by several criteria.

The presence of a tendinous xanthoma is generally taken to be indicative of a genetic disorder of lipoprotein or sterol metabolism. Four genonegative patients had tendinous xanthoma. For two of these patients little follow-up data are available and VLDL-compositional analysis was not repeated. One patient has had repeated VLDL compositional analyses with one positive and three "borderline" results. (*Note added in proof: A mutation in the LDL-receptor (Q357X) has subsequently been found in this patient*). The other patient with tendinous xanthomata underwent two VLDL compositional analyses with a positive and negative result.

One genonegative patient was found to have palmar crease xanthomata. This patient has undergone 4 VLDL compositional analyses with consistently positive results. The response to fibrate monotherapy has been excellent, with recurrent severe mixed hyperlipidaemia during periods of non-compliance. This patient clearly requires further investigation, including an analysis of apoE in lipoproteins and sequencing of the entire apoE gene.

There are further patients in whom no palmar crease xanthomata were found, but VLDL compositional analysis has been positive on multiple occasions. In these patients the clinical presentation and response to therapy has resembled dysbetalipoproteinaemia strongly.

There were four genonegative patients with renal disease characterized by heavy proteinuria. Positive VLDL compositional analysis in this setting may be a "false positive" or occur if overproduced lipoproteins are not cleared rapidly enough.

There is another large group of patients characterized by diabetes and mixed hyperlipidaemia. In many of these patients VLDL compositional analysis was only performed on a single occasion and the result may well be "false positive". Where repeat VLDL compositional analysis was available or could be requested it tended to be negative, though not universally so. It is likely that many of these patients have a lipoprotein disorder such as FCH with a prominent increase in TGRL secondary to poorly controlled diabetes.

The group of genonegative patients therefore likely includes subjects with true dysbetalipoproteinaemia but as yet undiscovered mutations in apoE, patients with FH and moderately severe hypertriglyceridaemia, patients with FCH and hypertriglyceridaemia as well as secondary hyperlipidaemia with remnant accumulation. Some of the genonegative subjects may have had HL deficiency, but no specific investigations were done to confirm or refute this diagnosis. In particular HDL size was not assessed routinely to screen for large HDL particles that are often seen in HL deficiency.

7.7 Limitations of this study

7.7.1 Dysbetalipoproteinaemia cohort

The GSH lipid clinic sees patients referred with severe hyperlipidaemia or physical stigmata of dyslipidaemia. There is therefore a referral bias towards more severe clinical problems. This may have biased the cohort studied to include the most extreme phenotypic manifestations of dysbetalipoproteinaemia. Establishing the true phenotypic spectrum of dysbetalipoproteinaemia would require a population-based study.

The analysis of the GSH lipid clinic experience is based on data collected retrospectively. The analysis is therefore limited by the problems known to be associated with retrospective data collection:

- Data is not complete in all subjects
- Diagnoses were not made according to predefined criteria
- Length of follow-up varies widely and some patients have no follow-up information available
- The treating clinicians were free in their choice of initial treatment and subsequent further modifications of therapy.

The impact of many of these potential problems was limited by the fact that almost all patients were evaluated using a standardized clerking sheet and patients were seen by a small number of highly experienced clinicians with little staff turnover.

Dysbetalipoproteinaemic patients have markedly elevated levels of apoE (5). None of the patients reported here underwent determination of apoE levels. Therefore no data on apoE levels are available. For this reason it was impossible to establish whether

there is any correlation between apoE levels, the degree of hyperlipidaemia, treatment response or apoE mutation. Analysis of apoE levels in genonegative patients may also have been instructive. Low apoE levels in the setting of cholesterol-enriched VLDL may also have indicated the possibility of apoE deficiency.

The analysis also included patients with no identified apoE mutations. As outlined above these patients differ in several important aspects from patients with known apoE mutations. Inclusion of these patients may therefore have skewed some results. This issue has been addressed by presenting results for the whole cohort and by mutational status.

7.7.2 Clinical trial

The Bayer study was a well designed clinical trial to evaluate three treatment strategies in dysbetalipoproteinaemia. The major limitation of the trial was its premature termination. As a result of this fewer patients than planned were enrolled and none of the patients completed the entire trial. This obviously limits the statistical power of the trial and the planned analysis using ANOVA with repeated measures could not be performed. All ancillary investigations including analysis of changes in lipoprotein composition, chylomicron remnant metabolism (breath test) and electrophoresis were affected likewise.

The Bayer study protocol also included measurement of apolipoproteins (apoB, apoE, apoCIII) and lipoparticles as secondary outcome variables. These analyses were to be performed on batched samples following completion of the study. Unfortunately the study sponsor did not give consent for these parameters to be measured, even in the

subset of patients that had at least completed two treatment arms. The apolipoprotein and lipoparticle data could have yielded useful insights into remnant metabolism and the distribution of apoE amongst various lipoprotein classes.

Patients could be included in the Bayer study based on a single positive VLDL compositional analysis. As a result of this 4 patients, of the patients with sufficient data for analysis, had no identified apoE mutations. Some of these genonegative patients may have had false positive results on VLDL compositional analysis and therefore may have diluted the treatment effect seen in "true" dysbetalipoproteinaemia.

7.8 The role of a lipid clinic in the diagnosis and management of dysbetalipoproteinaemia

Lipid clinics are tertiary referral centers for patients with severe or unusual dyslipidaemia. They offer specialized diagnostic services and management advice to patients. Lipid clinics also engage in basic, clinical and therapeutic research. Another function is the training of medical professionals, both at an undergraduate and postgraduate level.

There is little knowledge about dysbetalipoproteinaemia amongst physicians, including internists and many endocrinologists. The disorder is therefore generally not recognized and investigated appropriately. Referral of patients with severe mixed hyperlipidaemia to a lipid clinic may therefore benefit the patient in multiple ways. Identifying the lipid phenotype is helpful in choosing lipid-modifying therapy. Additionally patients with dysbetalipoproteinaemia need to be screened carefully for

possible precipitating disorders. Once the diagnosis of dysbetalipoproteinaemia has been confirmed and the apoE mutation identified it is also possible to provide genetic advice regarding the risk of siblings and children developing dysbetalipoproteinaemia. Genetic advice can then be complemented by screening at risk individuals for apoE mutations. Collection and collation of information relevant to the local context is also much easier if patients are seen at a central referral facility at least once.

Dysbetalipoproteinaemia generally responds well to therapy and patients stabilized on treatment can therefore be followed-up by their general practitioner or primary health care clinic as long as a reliable supply of lipid-lowering medication and intermittent monitoring of treatment response can be assured. Unfortunately both the public and the private health care system in South Africa fail to provide these basic essentials unacceptably often.

7.9 Future work on dysbetalipoproteinaemia in Cape Town

The atherogenic nature of dysbetalipoproteinaemia can be studied by carotid IMT using a standardized ultrasound protocol recently introduced in Cape Town. Currently all traceable dysbetalipoproteinaemic patients are undergoing ultrasound evaluation. Ultrasound screening in families known to carry the apoE (R145C) mutation is of particular interest to assess whether there are early changes in IMT in adolescence before overt hyperlipidaemia sets in.

Diagnostic criteria for VLDL composition need to be established for the local population. There are plans to recruit a cohort of normolipidaemic patients to undergo

both VLDL-compositional analysis and IMT screening. The former will provide a better range of expected results, while the latter will assist in building experience of IMT and comparing age-matched values with previously published studies. This benchmarking exercise is important if IMT measurements are to be included in future risk assessments of patients with intermediate risk.

The effect of ART on South African individuals genetically predisposed towards developing dysbetalipoproteinaemia is of interest. A collaboration with researchers from the Division of Endocrinology at UCT, who are conducting research on the metabolic effects associated with ART, is underway. A large cross-sectional survey of patients on ART has been initiated to study the metabolic effects of ART. The lipid laboratory will be evaluating lipid data, performing GGE and apoE genotyping. This study will therefore provide further data on the population prevalence of the apoE (R145C) mutation, the risk of hyperlipidaemia developing when exposed to metabolic stressors and the role of common apoE isoforms in ART induced dyslipidaemia.

8 Reference List

(1) Utermann G. Genetic polymorhisms in apolipoprotein E. Impact on plasma lipoprotein metabolism. In: Crepaldi G, Tiengo G, Baggio G, editors. Diabetes, Obesity and Hyperlipidaemias-III. Amsterdam: Elsevier, 1985: 1.

(2) Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. Nature 1977; 269(5629):604-607.

(3) Connelly PW, Maguire GF, Lee M, Little JA. Plasma lipoproteins in familial hepatic lipase deficiency. Arteriosclerosis 1990; 10(1):40-48.

(4) Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS et al. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. Arterioscler Thromb 1993; 13(5):720-728.

(5) Mahley RW, Rall S. Type III Hyperlipoproteinemia (Dysbetalipoproteinaemia) :The Role of Apolipoprotein E in Normal and Abnormal Lipoprotein Metabolism. In: Scriver A, Beadet W, Sly S, Valle D, editors. The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw- Hill, 1995: 1953-1980.

(6) Borrie P. Type 3 hyperlipoproteinaemia. Br Med J 1969; 2(658):665-667.

(7) Morganroth J, Levy RI, Fredrickson DS. The biochemical, clinical, and genetic features of type III hyperlipoproteinemia. Ann Intern Med 1975; 82(2):158-174.

(8) Blom DJ, Byrnes P, Jones S, Marais AD. Dysbetalipoproteinaemia--clinical and pathophysiological features. S Afr Med J 2002; 92(11):892-897.

(9) Stuyt PM, Van 't LA. Clinical features of type III hyperlipoproteinaemia. Neth J Med 1983; 26(4):104-111.

(10) Feussner G, Wagner A, Kohl B, Ziegler R. Clinical features of type III hyperlipoproteinemia: analysis of 64 patients. Clin Investig 1993; 71(5):362-366.

(11) McGinley J, JONES H, Gofman J. Lipoproteins and xanthomatous diseases. J Invest Dermatol 1952; 19(1):71-82.

(12) Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins--an integrated approach to mechanisms and disorders. N Engl J Med 1967; 276(3):148-156.

(13) Fredrickson DS, Levy R, Lees R. Fat Transport in Lipoproteins-An Integrated Approach to Mechanisms and Disorders. N Eng J Med 1967; 276(4):215-224.

(14) Fredrickson DS, Levy RI, Lindgren FT. A comparison of heritable abnormal lipoprotein patterns as defined by two different techniques. J Clin Invest 1969; 47(11):2446-2457.

(15) Masket BH, Levy RI, Fredrickson DS. The use of polyacrylamide gel electrophoresis in differentiating type 3 hyperlipoproteinemia. J Lab Clin Med 1973; 81(5):794-802.

(16) Havel RJ, Chao Y, Windler EE, Kotite L, Guo LS. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. Proc Natl Acad Sci U S A 1980; 77(7):4349-4353.

(17) Tan MH, Havel RJ, Gerich JE, Soeldner JS, Kane JP. Pancreatic alpha and beta cell function in familial dysbetalipoproteinemia. Horm Metab Res 1980; 12(9):421-425.

(18) Schneider WJ, Kovanen PT, Brown MS, Goldstein JL, Utermann G, Weber W et al. Familial dysbetalipoproteinemia. Abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits, and cows. J Clin Invest 1981; 68(4):1075-1085.

(19) Chait A, Brunzell JD, Albers JJ, Hazzard WR. Type-III Hyperlipoproteinaemia ("remnant removal disease"). Insight into the pathogenetic mechanism. Lancet 1977; 1(8023):1176-1178.

(20) Sandison AT. Degenerative vascular diseases. Diseases in Antiquity: A Survey of the Diseases, Injuries and Surgery of Early Populations. Sprinfield: Charles C Thomas, 1967: 474-488.

(21) Ignatowski A. Über die Wirkung des tierischen Eiweißes auf die Aorta und die parenchymatösen Organe des Kaninchen. Virchows Arch Pathol Anat 1909; 198:248-270.

(22) Windaus A. Über den Gehalt normaler und atheromatöser Aorten an Cholesterin und Cholesterinestern. Hoppe Seylers Z Physiol Chem 1910; 67:174-176.

(23) Anitschkow NN. Ueber die Veränderungen der Kaninchenaorta bei experimenteller Cholesterinsteatose. Beitr Pathol Anat 1913; 56:379-404.

(24) Anitschkow NN, Chalatow S. Ueber experimentelle Cholesterinsteatose und ihre Bedeutung für die Entstehung einiger pathologischer Prozesse. Zentralbl Allg Pathol 1913; 24:1-9.

(25) Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part I. J Lipid Res 2004; 45(9):1583-1593.

(26) Oliver MF. Serum cholesterol--the knave of hearts and the joker. Lancet 1981; 2(8255):1090-1095.

(27) McMichael J. Fats and atheroma: an inquest. Br Med J 1979; 1(6157):173-175.

(28) The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA 1984; 251(3):351-364.

(29) The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984; 251(3):365-374.

(30) Oliver MF. Consensus or nonsensus conferences on coronary heart disease. Lancet 1985; 1(8437):1087-1089.

(31) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994; 344(8934):1383-1389.

(32) Oliver MF. Statins prevent coronary heart disease. Lancet 1995; 346(8987):1378-1379.

(33) Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part II: the early evidence linking hypercholesterolemia to coronary disease in humans. J Lipid Res 2005; 46(2):179-190.

(34) Steinberg D. Thematic review series: the pathogenesis of atherosclerosis: an interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia. J Lipid Res 2005; 46(10):2037-2051.

(35) Steinberg D. Thematic review series: The Pathogenesis of Atherosclerosis. An interpretive history of the cholesterol controversy, part IV: The 1984 Coronary Primary Prevention Trial ends it--almost. J Lipid Res 2006; 47(1):1-14.

(36) Erkelens DW, Sherwood LM. Familial hypercholesterolemia, tendinous xanthomas, and Frans Hals. JAMA 1989; 262(15):2092.

(37) Gofman J, Lindgren FT, Elliot H. Ultracentrifugal studies of lipoproteins of human serum. J Biol Chem 1949; 179:973-979.

(38) Hazzard WR, Porte D, Jr., Bierman EL. Abnormal lipid composition of chylomicrons in broad-beta disease (Type III hyperlipoproteinemia). J Clin Invest 1970; 49(10):1853-1858.

(39) Sata T, Havel RJ, Jones AL. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. J Lipid Res 1972; 13(6):757-768.

(40) Havel RJ, Kane JP. Primary dysbetalipoproteinemia: predominance of a specific apoprotein species in triglyceride-rich lipoproteins. Proc Natl Acad Sci U S A 1973; 70(7):2015-2019.

(41) Utermann G, Jaeschke M, Menzel J. Familial hyperlipoproteinemia type III: deficiency of a specific apolipoprotein (apo E-III) in the very-low-density lipoproteins. FEBS Lett 1975; 56(2):352-355.

(42) Utermann G, Canzler H, Hees M, Jaeschke M, Muhlfellner G, Schoenborn W et al. Studies on the metabolic defect in Broad-beta disease (hyperlipoproteinaemia type III). Clin Genet 1977; 12(3):139-154.

(43) Utermann G, Vogelberg KH, Steinmetz A, Schoenborn W, Pruin N, Jaeschke M et al. Polymorphism of apolipoprotein E. II. Genetics of hyperlipoproteinemia type III. Clin Genet 1979; 15(1):37-62.

(44) Utermann G, Steinmetz A, Weber W. Genetic Control of Human Apolipoprotein E Polymorphism:Comparison of One- and Two-Dimensional Techniques of Isoprotein Analysis. Human Genet 1982; 60:344-351.

(45) Zannis VI, Breslow JL. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. Biochemistry 1981; 20(4):1033-1041.

(46) Zannis VI, Breslow JL, Utermann G, Mahley RW, Weisgraber KH, Havel RJ et al. Proposed nomenclature of apoE isoproteins,apoE genotypes,and phenotypes. J Lipid Res 1982; 23:911-914.

(47) Rall SC, Jr., Weisgraber KH, Innerarity TL, Mahley RW. Structural basis for receptor binding heterogeneity of apolipoprotein E from type III hyperlipoproteinemic subjects. Proc Natl Acad Sci U S A 1982; 79(15):4696-4700.

(48) Rall SC, Jr., Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. J Biol Chem 1982; 257(8):4171-4178.

(49) Weisgraber KH, Rall SC, Jr., Mahley RW. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 1981; 256(17):9077-9083.

(50) Mahley RW, Huang Y, Rall SC, Jr. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. J Lipid Res 1999; 40(11):1933-1949.

(51) Tabas I. Cholesterol in health and disease. J Clin Invest 2002; 110(5):583-590.

(52) Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109(9):1125-1131.

(53) Porter FD. Malformation syndromes due to inborn errors of cholesterol synthesis. J Clin Invest 2002; 110(6):715-724.

(54) Unger RH. Lipotoxic diseases. Annu Rev Med 2002; 53:319-336.

(55) Garg A. Acquired and inherited lipodystrophies. N Engl J Med 2004; 350(12):1220-1234.

(56) Berliner JA, Watson AD. A role for oxidized phospholipids in atherosclerosis. N Engl J Med 2005; 353(1):9-11.

(57) Wenger DA, Coppola S, Liu SL. Insights into the diagnosis and treatment of lysosomal storage diseases. Arch Neurol 2003; 60(3):322-328.

(58) Macheboeuf M. Recherches sur les phosphoaminolipides et les sterides du serum et plasma sanguins. Bull Soc Chim Biol 1929; 11:268-293.

(59) Cohn EJ, Strong EL, Hughes WL, Mulford DJ, Ashworth JN, Melin M et al. Preparation and properties of serum and plasma lipoproteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc 1946; 68:459-475.

(60) Havel RJ, Kane J. Introduction: Structure and metabolism of plasma lipoproteins. In: Scriver A, Beadet W, Sly S, Valle D, editors. The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw-Hill, 1995: 1841-1852.

(61) Rassart E, Bedirian A, Do CS, Guinard O, Sirois J, Terrisse L et al. Apolipoprotein D. Biochim Biophys Acta 2000; 1482(1-2):185-198.

(62) Thomas EA, Copolov DL, Sutcliffe JG. From pharmacotherapy to pathophysiology: emerging mechanisms of apolipoprotein D in psychiatric disorders. Curr Mol Med 2003; 3(5):408-418.

(63) Wolfrum C, Poy MN, Stoffel M. Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. Nat Med 2005; 11(4):418-422.

(64) Xu N, Dahlback B. A novel human apolipoprotein (apoM). J Biol Chem 1999; 274(44):31286-31290.

(65) O'Brien PJ, Alborn WE, Sloan JH, Ulmer M, Boodhoo A, Knierman MD et al. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low concentrations compared with other apolipoproteins. Clin Chem 2005; 51(2):351-359.

(66) Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC et al. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 2001; 294(5540):169-173.

(67) Merkel M, Heeren J. Give me A5 for lipoprotein hydrolysis! J Clin Invest 2005; 115(10):2694-2696.

(68) Chen SH, Habib G, Yang CY, Gu ZW, Lee BR, Weng SA et al. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science 1987; 238(4825):363-366.

(69) Genest J, Frohlich J, Fodor G, McPherson R. Recommendations for the management of dyslipidemia and the prevention of cardiovascular disease: summary of the 2003 update. CMAJ 2003; 169(9):921-924.

(70) Sniderman AD. Applying apoB to the diagnosis and therapy of the atherogenic dyslipoproteinemias: a clinical diagnostic algorithm. Curr Opin Lipidol 2004; 15(4):433-438.

(71) Whitfield AJ, Barrett PH, van Bockxmeer FM, Burnett JR. Lipid disorders and mutations in the APOB gene. Clin Chem 2004; 50(10):1725-1732.

(72) Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res 1999; 40(1):1-16.

(73) Huang Y, Liu XQ, Rall SC, Jr., Taylor JM, von Eckardstein A, Assmann G et al. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. J Biol Chem 1998; 273(41):26388-26393.

(74) Shore VG, Shore B. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. Biochemistry 1973; 12(3):502-507.

(75) Han X. The role of apolipoprotein E in lipid metabolism in the central nervous system. Cell Mol Life Sci 2004; 61(15):1896-1906.

(76) Horsburgh K, McCarron MO, White F, Nicoll JA. The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. Neurobiol Aging 2000; 21(2):245-255.

(77) Mahley RW, Huang Y. Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. Curr Opin Lipidol 1999; 10(3):207-217.

(78) Mahley RW, Rall SC, Jr. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000; 1:507-537.

(79) Zannis VI, McPherson J, Goldberger G, Karathanasis SK, Breslow JL. Synthesis, intracellular processing, and signal peptide of human apolipoprotein E. J Biol Chem 1984; 259(9):5495-5499.

(80) Wernette-Hammond ME, Lauer SJ, Corsini A, Walker D, Taylor JM, Rall SC, Jr. Glycosylation of human apolipoprotein E. The carbohydrate attachment site is threonine 194. J Biol Chem 1989; 264(15):9094-9101.

(81) Mazzone T, Papagiannes E, Magner J. Early kinetics of human macrophage apolipoprotein E synthesis and incorporation of carbohydrate precursors. Biochim Biophys Acta 1986; 875(2):393-396.

(82) Mazzone T, Pustelnikas L, Reardon CA. Post-translational regulation of macrophage apoprotein E production. J Biol Chem 1992; 267(2):1081-1087.

(83) Duan H, Lin CY, Mazzone T. Degradation of macrophage ApoE in a nonlysosomal compartment. Regulation by sterols. J Biol Chem 1997; 272(49):31156-31162.

(84) Lucas M, Mazzone T. Cell surface proteoglycans modulate net synthesis and secretion of macrophage apolipoprotein E. J Biol Chem 1996; 271(23):13454-13460.

(85) Heeren J, Beisiegel U. Intracellular metabolism of triglyceride-rich lipoproteins. Curr Opin Lipidol 2001; 12(3):255-260.

(86) Heeren J, Grewal T, Jackle S, Beisiegel U. Recycling of apolipoprotein E and lipoprotein lipase through endosomal compartments in vivo. J Biol Chem 2001; 276(45):42333-42338.

(87) Heeren J, Weber W, Beisiegel U. Intracellular processing of endocytosed triglyceride-rich lipoproteins comprises both recycling and degradation. J Cell Sci 1999; 112 (Pt 3):349-359.

(88) Elshourbagy NA, Liao WS, Mahley RW, Taylor JM. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. Proc Natl Acad Sci U S A 1985; 82(1):203-207.

(89) Boyles JK, Pitas RE, Wilson E, Mahley RW, Taylor JM. Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. J Clin Invest 1985; 76(4):1501-1513.

(90) Newman TC, Dawson PA, Rudel LL, Williams DL. Quantitation of apolipoprotein E mRNA in the liver and peripheral tissues of nonhuman primates. J Biol Chem 1985; 260(4):2452-2457.

(91) Wilson C, Wardell MR, Weisgraber KH, Mahley RW, Agard DA. Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. Science 1991; 252(5014):1817-1822.

(92) Weisgraber KH, Rall SC, Jr., Mahley RW, Milne RW, Marcel YL, Sparrow JT. Human apolipoprotein E. Determination of the heparin binding sites of apolipoprotein E3. J Biol Chem 1986; 261(5):2068-2076.

(93) Weisgraber KH. Apolipoprotein E: structure-function relationships. Adv Protein Chem 1994; 45:249-302.

(94) Lalazar A, Mahley RW. Human apolipoprotein E. Receptor binding activity of truncated variants with carboxyl-terminal deletions. J Biol Chem 1989; 264(15):8447-8450.

(95) Raussens V, Slupsky CM, Sykes BD, Ryan RO. Lipid-bound structure of an apolipoprotein E-derived peptide. J Biol Chem 2003; 278(28):25998-26006.

(96) Lund-Katz S, Zaiou M, Wehrli S, Dhanasekaran P, Baldwin F, Weisgraber KH et al. Effects of lipid interaction on the lysine microenvironments in apolipoprotein E. J Biol Chem 2000; 275(44):34459-34464.

(97) Li X, Kan HY, Lavrentiadou S, Krieger M, Zannis V. Reconstituted discoidal ApoE-phospholipid particles are ligands for the scavenger receptor BI. The amino-terminal 1-165 domain of ApoE suffices for receptor binding. J Biol Chem 2002; 277(24):21149-21157.

(98) Choy N, Raussens V, Narayanaswami V. Inter-molecular coiled-coil formation in human apolipoprotein E C-terminal domain. J Mol Biol 2003; 334(3):527-539.

(99) Aggerbeck LP, Wetterau JR, Weisgraber KH, Wu CS, Lindgren FT. Human apolipoprotein E3 in aqueous solution. II. Properties of the amino- and carboxyl-terminal domains. J Biol Chem 1988; 263(13):6249-6258.

(100) Westerlund JA, Weisgraber KH. Discrete carboxyl-terminal segments of apolipoprotein E mediate lipoprotein association and protein oligomerization. J Biol Chem 1993; 268(21):15745-15750.

(101) Perugini MA, Schuck P, Howlett GJ. Self-association of human apolipoprotein E3 and E4 in the presence and absence of phospholipid. J Biol Chem 2000; 275(47):36758-36765.

(102) Dong LM, Wilson C, Wardell MR, Simmons T, Mahley RW, Weisgraber KH et al. Human apolipoprotein E. Role of arginine 61 in mediating the lipoprotein preferences of the E3 and E4 isoforms. J Biol Chem 1994; 269(35):22358-22365.

(103) Hixson JE, Cox LA, Borenstein S. The baboon apolipoprotein E gene: structure, expression, and linkage with the gene for apolipoprotein C-1. Genomics 1988; 2(4):315-323.

(104) Wozniak MA, Itzhaki RF, Faragher EB, James MW, Ryder SD, Irving WL. Apolipoprotein E-epsilon 4 protects against severe liver disease caused by hepatitis C virus. Hepatology 2002; 36(2):456-463.

(105) Lin WR, Wozniak MA, Esiri MM, Klenerman P, Itzhaki RF. Herpes simplex encephalitis: involvement of apolipoprotein E genotype. J Neurol Neurosurg Psychiatry 2001; 70(1):117-119.

(106) Wozniak MA, Faragher EB, Todd JA, Koram KA, Riley EM, Itzhaki RF. Does apolipoprotein E polymorphism influence susceptibility to malaria? J Med Genet 2003; 40(5):348-351.

(107) Wozniak MA, Riley EM, Itzhaki RF. Apolipoprotein E polymorphisms and risk of malaria. J Med Genet 2004; 41(3):145-146.

(108) Aucan C, Walley AJ, Hill AV. Common apolipoprotein E polymorphisms and risk of clinical malaria in the Gambia. J Med Genet 2004; 41(1):21-24.

(109) Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002; 155(6):487-495.

(110) Kamboh MI, Sepehrnia B, Ferrell RE. Genetic studies of human apolipoproteins. VI. Common polymorphism of apolipoprotein E in blacks. Dis Markers 1989; 7(1):49-55.

(111) Howard BV, Gidding SS, Liu K. Association of apolipoprotein E phenotype with plasma lipoproteins in African-American and white young adults. The CARDIA Study. Coronary Artery Risk Development in Young Adults. Am J Epidemiol 1998; 148(9):859-868.

(112) Kataoka S, Robbins DC, Cowan LD, Go O, Yeh JL, Devereux RB et al. Apolipoprotein E polymorphism in American Indians and its relation to plasma lipoproteins and diabetes. The Strong Heart Study. Arterioscler Thromb Vasc Biol 1996; 16(8):918-925.

(113) Schaefer EJ, Lamon-Fava S, Johnson S, Ordovas JM, Schaefer MM, Castelli WP et al. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. Arterioscler Thromb 1994; 14(7):1105-1113.

(114) Assmann G, Schmitz G, Menzel HJ, Schulte H. Apolipoprotein E polymorphism and hyperlipidemia. Clin Chem 1984; 30(5):641-643.

(115) Lehtimaki T, Moilanen T, Viikari J, Akerblom HK, Ehnholm C, Ronnemaa T et al. Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study. J Lipid Res 1990; 31(3):487-495.

(116) Luc G, Bard JM, Arveiler D, Evans A, Cambou JP, Bingham A et al. Impact of apolipoprotein E polymorphism on lipoproteins and risk of myocardial infarction. The ECTIM Study. Arterioscler Thromb 1994; 14(9):1412-1419.

(117) Cattin L, Fisicaro M, Tonizzo M, Valenti M, Danek GM, Fonda M et al. Polymorphism of the apolipoprotein E gene and early carotid atherosclerosis defined by ultrasonography in asymptomatic adults. Arterioscler Thromb Vasc Biol 1997; 17(1):91-94.

(118) Evans AE, Zhang W, Moreel JF, Bard JM, Ricard S, Poirier O et al. Polymorphisms of the apolipoprotein B and E genes and their relationship to plasma lipid variables in healthy Chinese men. Hum Genet 1993; 92(2):191-197.

(119) Eto M, Watanabe K, Makino I. Increased frequencies of apolipoprotein epsilon 2 and epsilon 4 alleles in patients with ischemic heart disease. Clin Genet 1989; 36(3):183-188.

(120) Hanis CL, Hewett-Emmett D, Douglas TC, Bertin TK, Schull WJ. Effects of the apolipoprotein E polymorphism on levels of lipids, lipoproteins, and apolipoproteins among Mexican-Americans in Starr County, Texas. Arterioscler Thromb 1991; 11(2):362-370.

(121) Tanyanyiwa DT. ApolipoproteinE variants, plasma lipids, lipoproteins and dysbetalipoproteinaemia during pregnancy in Zimbabwean women. University of Cape Town, 2005.

(122) Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 1991; 49(2):338-349.

(123) Srinivasan SR, Ehnholm C, Wattigney W, Berenson GS. Apolipoprotein E polymorphism and its association with serum lipoprotein concentrations in black versus white children: the Bogalusa Heart Study. Metabolism 1993; 42(3):381-386.

(124) Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 1985; 37(2):268-285.

(125) Hixson JE. Apolipoprotein E polymorphisms affect atherosclerosis in young males. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb 1991; 11(5):1237-1244.

(126) Ilveskoski E, Perola M, Lehtimaki T, Laippala P, Savolainen V, Pajarinen J et al. Age-dependent association of apolipoprotein E genotype with coronary and aortic atherosclerosis in middle-aged men: an autopsy study. Circulation 1999; 100(6):608-613.

(127) Gerdes LU, Gerdes C, Kervinen K, Savolainen M, Klausen IC, Hansen PS et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction : a substudy of the Scandinavian simvastatin survival study. Circulation 2000; 101(12):1366-1371.

(128) Menzel HJ, Kladetzky RG, Assmann G. Apolipoprotein E polymorphism and coronary artery disease. Arteriosclerosis 1983; 3(4):310-315.

(129) Eichner JE, Kuller LH, Orchard TJ, Grandits GA, McCallum LM, Ferrell RE et al. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol 1993; 71(2):160-165.

(130) Lenzen HJ, Assmann G, Buchwalsky R, Schulte H. Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. Clin Chem 1986; 32(5):778-781.

(131) Hobbs HH, White AL. Lipoprotein(a): intrigues and insights. Curr Opin Lipidol 1999; 10(3):225-236.

(132) Tan CE, Foster L, Caslake MJ, Bedford D, Watson TD, McConnell M et al. Relations between plasma lipids and postheparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women. Arterioscler Thromb Vasc Biol 1995; 15(11):1839-1848.

(133) Gibbons GF, Wiggins D, Brown AM, Hebbachi AM. Synthesis and function of hepatic very-low-density lipoprotein. Biochem Soc Trans 2004; 32(Pt 1):59-64.

(134) Adiels M, Boren J, Caslake MJ, Stewart P, Soro A, Westerbacka J et al. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. Arterioscler Thromb Vasc Biol 2005; 25(8):1697-1703.

(135) Fraser R, Dobbs BR, Rogers GW. Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. Hepatology 1995; 21(3):863-874.

(136) Le Couteur DG, Fraser R, Cogger VC, McLean AJ. Hepatic pseudocapillarisation and atherosclerosis in ageing. Lancet 2002; 359(9317):1612-1615.

(137) Stow JL, Kjellen L, Unger E, Hook M, Farquhar MG. Heparan sulfate proteoglycans are concentrated on the sinusoidal plasmalemmal domain and in intracellular organelles of hepatocytes. J Cell Biol 1985; 100(3):975-980.

(138) Hamilton RL, Wong JS, Guo LS, Krisans S, Havel RJ. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. J Lipid Res 1990; 31(9):1589-1603.

(139) Sanan DA, Fan J, Bensadoun A, Taylor JM. Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. J Lipid Res 1997; 38(5):1002-1013.

(140) Mahley RW, Ji ZS, Brecht WJ, Miranda RD, He D. Role of heparan sulfate proteoglycans and the LDL receptor-related protein in remnant lipoprotein metabolism. Ann N Y Acad Sci 1994; 737:39-52.

(141) Ji ZS, Brecht WJ, Miranda RD, Hussain MM, Innerarity TL, Mahley RW. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. J Biol Chem 1993; 268(14):10160-10167.

(142) Shimano H, Namba Y, Ohsuga J, Kawamura M, Yamamoto K, Shimada M et al. Secretion-recapture process of apolipoprotein E in hepatic uptake of chylomicron remnants in transgenic mice. J Clin Invest 1994; 93(5):2215-2223.

(143) Kounnas MZ, Chappell DA, Wong H, Argraves WS, Strickland DK. The cellular internalization and degradation of hepatic lipase is mediated by low density lipoprotein receptor-related protein and requires cell surface proteoglycans. J Biol Chem 1995; 270(16):9307-9312.

(144) Ji ZS, Lauer SJ, Fazio S, Bensadoun A, Taylor JM, Mahley RW. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. J Biol Chem 1994; 269(18):13429-13436.

(145) Hegele RA, Little JA, Connelly PW. Compound heterozygosity for mutant hepatic lipase in familial hepatic lipase deficiency. Biochem Biophys Res Commun 1991; 179(1):78-84.

(146) Hegele RA, Vezina C, Moorjani S, Lupien PJ, Gagne C, Brun LD et al. A hepatic lipase gene mutation associated with heritable lipolytic deficiency. J Clin Endocrinol Metab 1991; 72(3):730-732.

(147) Connelly PW. The role of hepatic lipase in lipoprotein metabolism. Clin Chim Acta 1999; 286(1-2):243-255.

(148) Zambon A, Deeb SS, Bensadoun A, Foster KE, Brunzell JD. In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity. J Lipid Res 2000; 41(12):2094-2099.

(149) Shafi S, Brady SE, Bensadoun A, Havel RJ. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. J Lipid Res 1994; 35(4):709-720.

(150) Mulder M, Lombardi P, Jansen H, Van Berkel TJ, Frants RR, Havekes LM. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. J Biol Chem 1993; 268(13):9369-9375.

(151) Beisiegel U, Weber W, Bengtsson-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. Proc Natl Acad Sci U S A 1991; 88(19):8342-8346.

(152) Mortimer BC, Beveridge DJ, Martins IJ, Redgrave TG. Intracellular localization and metabolism of chylomicron remnants in the livers of low density lipoprotein receptor-deficient mice and apoE-deficient mice. Evidence for slow metabolism via an alternative apoE-dependent pathway. J Biol Chem 1995; 270(48):28767-28776.

(153) Willnow TE, Sheng Z, Ishibashi S, Herz J. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. Science 1994; 264(5164):1471-1474.

(154) Bu G, Geuze HJ, Strous GJ, Schwartz AL. 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. EMBO J 1995; 14(10):2269-2280.

(155) Herz J, Goldstein JL, Strickland DK, Ho YK, Brown MS. 39-kDa protein modulates binding of ligands to low density lipoprotein receptor-related

protein/alpha 2-macroglobulin receptor. J Biol Chem 1991; 266(31):21232-21238.

(156) Ji ZS, Dichek HL, Miranda RD, Mahley RW. Heparan sulfate proteoglycans participate in hepatic lipaseand apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. J Biol Chem 1997; 272(50):31285-31292.

(157) Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. J Clin Invest 1998; 101(3):689-695.

(158) Huang Y, Schwendner SW, Rall SC, Jr., Mahley RW. Hypolipidemic and hyperlipidemic phenotypes in transgenic mice expressing human apolipoprotein E2. J Biol Chem 1996; 271(46):29146-29151.

(159) Hazzard WR, Warnick GR, Utermann G, Albers JJ. Genetic transmission of isoapolipoprotein E phenotypes in a large kindred: relationship to dysbetalipoproteinemia and hyperlipidemia. Metabolism 1981; 30(1):79-88.

(160) Carmena R, Roy M, Roederer G, Minnich A, Davignon J. Coexisting dysbetalipoproteinemia and familial hypercholesterolemia. Clinical and laboratory observations. Atherosclerosis 2000; 148(1):113-124.

(161) Hopkins PN, Wu LL, Schumacher MC, Emi M, Hegele RM, Hunt SC et al. Type III dyslipoproteinemia in patients heterozygous for familial hypercholesterolemia and apolipoprotein E2. Evidence for a gene-gene interaction. Arterioscler Thromb 1991; 11(5):1137-1146.

(162) Huang Y, Schwendner SW, Rall SC, Jr., Sanan DA, Mahley RW. Apolipoprotein E2 transgenic rabbits. Modulation of the type III hyperlipoproteinemic phenotype by estrogen and occurrence of spontaneous atherosclerosis. J Biol Chem 1997; 272(36):22685-22694.

(163) Kushwaha RS, Hazzard WR, Gagne C, Chait A, Albers JJ. Type III hyperlipoproteinemia: paradoxical hypolipidemic response to estrogen. Ann Intern Med 1977; 87(5):517-525.

(164) Falko JM, Schonfeld G, Witztum JL, Kolar J, Weidman SW. Effects of estrogen therapy on apolipoprotein E in type IIi hyperlipoproteinemia. Metabolism 1979; 28(11):1171-1177.

(165) Toto RD, Vega GL, Grundy SM. Mechanisms and treatment of dyslipidemia of renal diseases. Curr Opin Nephrol Hypertens 1993; 2(5):784-790.

(166) Lenhard JM, Croom DK, Weiel JE, Winegar DA. HIV protease inhibitors stimulate hepatic triglyceride synthesis. Arterioscler Thromb Vasc Biol 2000; 20(12):2625-2629.

(167) Davies PJ, Berry SA, Shipley GL, Eckel RH, Hennuyer N, Crombie DL et al. Metabolic effects of rexinoids: tissue-specific regulation of lipoprotein lipase activity. Mol Pharmacol 2001; 59(2):170-176.

(168) Wilson C, Mau T, Weisgraber KH, Wardell MR, Mahley RW, Agard DA. Salt bridge relay triggers defective LDL receptor binding by a mutant apolipoprotein. Structure 1994; 2(8):713-718.

(169) Ji ZS, Fazio S, Mahley RW. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. J Biol Chem 1994; 269(18):13421-13428.

(170) Huang Y, Liu XQ, Rall SC, Jr., Mahley RW. Apolipoprotein E2 reduces the low density lipoprotein level in transgenic mice by impairing lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins. J Biol Chem 1998; 273(28):17483-17490.

(171) Thuren T, Sisson P, Waite M. Activation of hepatic lipase catalyzed phosphatidylcholine hydrolysis by apolipoprotein E. Biochim Biophys Acta 1991; 1083(2):217-220.

(172) Thuren T, Wilcox RW, Sisson P, Waite M. Hepatic lipase hydrolysis of lipid monolayers. Regulation by apolipoproteins. J Biol Chem 1991; 266(8):4853-4861.

(173) Weisgraber KH. Apolipoprotein E distribution among human plasma lipoproteins: role of the cysteine-arginine interchange at residue 112. J Lipid Res 1990; 31(8):1503-1511.

(174) Steinmetz A, Jakobs C, Motzny S, Kaffarnik H. Differential distribution of apolipoprotein E isoforms in human plasma lipoproteins. Arteriosclerosis 1989; 9(3):405-411.

(175) de Knijff P, Van den Maagdenberg AM, Stalenhoef AF, Leuven JA, Demacker PN, Kuyt LP et al. Familial dysbetalipoproteinemia associated with apolipoprotein E3-Leiden in an extended multigeneration pedigree. J Clin Invest 1991; 88(2):643-655.

(176) Horie Y, Fazio S, Westerlund JR, Weisgraber KH, Rall SC, Jr. The functional characteristics of a human apolipoprotein E variant (cysteine at residue 142) may explain its association with dominant expression of type III hyperlipoproteinemia. J Biol Chem 1992; 267(3):1962-1968.

(177) Kuipers F, Jong MC, Lin Y, Eck M, Havinga R, Bloks V et al. Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E-deficient mouse hepatocytes. J Clin Invest 1997; 100(11):2915-2922.

(178) Huang Y, Ji ZS, Brecht WJ, Rall SC, Jr., Taylor JM, Mahley RW. Overexpression of apolipoprotein E3 in transgenic rabbits causes combined hyperlipidemia by stimulating hepatic VLDL production and impairing VLDL lipolysis. Arterioscler Thromb Vasc Biol 1999; 19(12):2952-2959.

(179) Kypreos KE, Li X, van Dijk KW, Havekes LM, Zannis VI. Molecular mechanisms of type III hyperlipoproteinemia: The contribution of the carboxy-terminal domain of ApoE can account for the dyslipidemia that is associated with the E2/E2 phenotype. Biochemistry 2003; 42(33):9841-9853.

(180) Ghiselli G, Schaefer EJ, Gascon P, Breser HB, Jr. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. Science 1981; 214(4526):1239-1241.

(181) Kurosaka D, Teramoto T, Matsushima T, Yokoyama T, Yamada A, Aikawa T et al. Apolipoprotein E deficiency with a depressed mRNA of normal size. Atherosclerosis 1991; 88(1):15-20.

(182) Lohse P, Brewer HB, III, Meng MS, Skarlatos SI, LaRosa JC, Brewer HB, Jr. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to

a premature stop codon in the apolipoprotein E gene. J Lipid Res 1992; 33(11):1583-1590.

(183) Schaefer EJ, Gregg RE, Ghiselli G, Forte TM, Ordovas JM, Zech LA et al. Familial apolipoprotein E deficiency. J Clin Invest 1986; 78(5):1206-1219.

(184) de Villiers WJ, van der Westhuyzen DR, Coetzee GA, Henderson HE, Marais AD. The apolipoprotein E2 (Arg145Cys) mutation causes autosomal dominant type III hyperlipoproteinemia with incomplete penetrance. Arterioscler Thromb Vasc Biol 1997; 17(5):865-872.

(185) Lohse P, Mann WA, Stein EA, Brewer HB, Jr. Apolipoprotein E-4Philadelphia (Glu13----Lys,Arg145----Cys). Homozygosity for two rare point mutations in the apolipoprotein E gene combined with severe type III hyperlipoproteinemia. J Biol Chem 1991; 266(16):10479-10484.

(186) Lohse P, Rader DJ, Brewer HB, Jr. Heterozygosity for apolipoprotein E-4Philadelphia(Glu13----Lys, Arg145----Cys) is associated with incomplete dominance of type III hyperlipoproteinemia. J Biol Chem 1992; 267(19):13642-13646.

(187) Suehiro T, Yoshida K, Yamano T, Ohno F. Identification and characterization of a new variant of apolipoprotein E (apo E-Kochi). Jpn J Med 1990; 29(6):587-594.

(188) Pocovi M, Cenarro A, Civeira F, Myers RH, Casao E, Esteban M et al. Incomplete dominance of type III hyperlipoproteinemia is associated with the rare apolipoprotein E2 (Arg136-->Ser) variant in multigenerational pedigree studies. Atherosclerosis 1996; 122(1):33-46.

(189) Wardell MR, Brennan SO, Janus ED, Fraser R, Carrell RW. Apolipoprotein E2-Christchurch (136 Arg----Ser). New variant of human apolipoprotein E in a patient with type III hyperlipoproteinemia. J Clin Invest 1987; 80(2):483-490.

(190) Feussner G, Albanese M, Mann WA, Valencia A, Schuster H. Apolipoprotein E2 (Arg-136-->Cys), a variant of apolipoprotein E associated with late-onset dominance of type III hyperlipoproteinaemia. Eur J Clin Invest 1996; 26(1):13-23.

(191) Rall SC, Jr., Newhouse YM, Clarke HR, Weisgraber KH, McCarthy BJ, Mahley RW et al. Type III hyperlipoproteinemia associated with apolipoprotein E phenotype E3/3. Structure and genetics of an apolipoprotein E3 variant. J Clin Invest 1989; 83(4):1095-1101.

(192) Havel RJ, Kotite L, Kane JP, Tun P, Bersot T. Atypical familial dysbetalipoproteinemia associated with apolipoprotein phenotype E3/3. J Clin Invest 1983; 72(1):379-387.

(193) Richard P, de Zulueta MP, Beucler I, De Gennes JL, Cassaigne A, Iron A. Identification of a new apolipoprotein E variant (E2 Arg142-->Leu) in type III hyperlipidemia. Atherosclerosis 1995; 112(1):19-28.

(194) Smit M, de Knijff P, Kooij-Meijs E, Groenendijk C, Van den Maagdenberg AM, Gevers Leuven JA et al. Genetic heterogeneity in familial dysbetalipoproteinemia. The E2(lys146----gln) variant results in a dominant mode of inheritance. J Lipid Res 1990; 31(1):45-53.

(195) Rall SC, Jr., Weisgraber KH, Innerarity TL, Bersot TP, Mahley RW, Blum CB. Identification of a new structural variant of human apolipoprotein E,

E2(Lys146 leads to Gln), in a type III hyperlipoproteinemic subject with the E3/2 phenotype. J Clin Invest 1983; 72(4):1288-1297.

(196) Mulder M, van der BH, de Knijff P, Braam C, van den MA, Leuven JA et al. Triglyceride-rich lipoproteins of subjects heterozygous for apolipoprotein E2(Lys146-->Gln) are inefficiently converted to cholesterol-rich lipoproteins. Atherosclerosis 1994; 108(2):183-192.

(197) de Knijff P, Van den Maagdenberg AM, Boomsma DI, Stalenhoef AF, Smelt AH, Kastelein JJ et al. Variable expression of familial dysbetalipoproteinemia in apolipoprotein E*2 (Lys146-->Gln) Allele carriers. J Clin Invest 1994; 94(3):1252-1262.

(198) Moriyama K, Sasaki J, Matsunaga A, Arakawa F, Takada Y, Araki K et al. Apolipoprotein E1 Lys-146----Glu with type III hyperlipoproteinemia. Biochim Biophys Acta 1992; 1128(1):58-64.

(199) Mann WA, Gregg RE, Sprecher DL, Brewer HB, Jr. Apolipoprotein E-1Harrisburg: a new variant of apolipoprotein E dominantly associated with type III hyperlipoproteinemia. Biochim Biophys Acta 1989; 1005(3):239-244.

(200) Mann WA, Lohse P, Gregg RE, Ronan R, Hoeg JM, Zech LA et al. Dominant expression of type III hyperlipoproteinemia. Pathophysiological insights derived from the structural and kinetic characteristics of ApoE-1 (Lys146-->Glu). J Clin Invest 1995; 96(2):1100-1107.

(201) Hoffer MJ, Niththyananthan S, Naoumova RP, Kibirige MS, Frants RR, Havekes LM et al. Apolipoprotein E1-Hammersmith (Lys146-->Asn;Arg147-->Trp), due to a dinucleotide substitution, is associated with early manifestation

of dominant type III hyperlipoproteinaemia. Atherosclerosis 1996; 124(2):183-189.

(202) Wardell MR, Weisgraber KH, Havekes LM, Rall SC, Jr. Apolipoprotein E3-Leiden contains a seven-amino acid insertion that is a tandem repeat of residues 121-127. J Biol Chem 1989; 264(35):21205-21210.

(203) Yanagi K, Yamashita S, Hiraoka H, Ishigami M, Kihara S, Hirano K et al. Increased serum remnant lipoproteins in patients with apolipoprotein E7 (apo E Suita). Atherosclerosis 1997; 131(1):49-58.

(204) Greenow K, Pearce NJ, Ramji DP. The key role of apolipoprotein E in atherosclerosis. J Mol Med 2005; 83(5):329-342.

(205) Saito T, Matsunaga A, Oikawa S. Impact of lipoprotein glomerulopathy on the relationship between lipids and renal diseases. Am J Kidney Dis 2006; 47(2):199-211.

(206) Jimi S, Uesugi N, Saku K, Itabe H, Zhang B, Arakawa K et al. Possible induction of renal dysfunction in patients with lecithin:cholesterol acyltransferase deficiency by oxidized phosphatidylcholine in glomeruli. Arterioscler Thromb Vasc Biol 1999; 19(3):794-801.

(207) Fredrickson DS, Morganroth J, Levy RI. Type III hyperlipoproteinemia: an analysis of two contemporary definitions. Ann Intern Med 1975; 82(2):150-157.

(208) Oikawa S, Suzuki N, Sakuma E, Saito T, Namai K, Kotake H et al. Abnormal lipoprotein and apolipoprotein pattern in lipoprotein glomerulopathy. Am J Kidney Dis 1991; 18(5):553-558.

(209) Oikawa S, Matsunaga A, Saito T, Sato H, Seki T, Hoshi K et al. Apolipoprotein E Sendai (arginine 145-->proline): a new variant associated with lipoprotein glomerulopathy. J Am Soc Nephrol 1997; 8(5):820-823.

(210) Matsunaga A, Sasaki J, Komatsu T, Kanatsu K, Tsuji E, Moriyama K et al. A novel apolipoprotein E mutation, E2 (Arg25Cys), in lipoprotein glomerulopathy. Kidney Int 1999; 56(2):421-427.

(211) Konishi K, Saruta T, Kuramochi S, Oikawa S, Saito T, Han H et al. Association of a novel 3-amino acid deletion mutation of apolipoprotein E (Apo E Tokyo) with lipoprotein glomerulopathy. Nephron 1999; 83(3):214-218.

(212) Ando M, Sasaki J, Hua H, Matsunaga A, Uchida K, Jou K et al. A novel 18-amino acid deletion in apolipoprotein E associated with lipoprotein glomerulopathy. Kidney Int 1999; 56(4):1317-1323.

(213) Ishigaki Y, Oikawa S, Suzuki T, Usui S, Magoori K, Kim DH et al. Virus-mediated transduction of apolipoprotein E (ApoE)-sendai develops lipoprotein glomerulopathy in ApoE-deficient mice. J Biol Chem 2000; 275(40):31269-31273.

(214) Saito T, Oikawa S, Sato H, Sasaki J. Lipoprotein glomerulopathy: renal lipidosis induced by novel apolipoprotein E variants. Nephron 1999; 83(3):193-201.

(215) Saito T, Ishigaki Y, Oikawa S, Yamamoto TT. Etiological significance of apolipoprotein E mutations in lipoprotein glomerulopathy. Trends Cardiovasc Med 2002; 12(2):67-70.

(216) Arai T, Yamashita S, Yamane M, Manabe N, Matsuzaki T, Kiriyama K et al. Disappearance of intraglomerular lipoprotein thrombi and marked improvement of nephrotic syndrome by bezafibrate treatment in a patient with lipoprotein glomerulopathy. Atherosclerosis 2003; 169(2):293-299.

(217) Hoffmann M, Scharnagl H, Panagiotou E, Banghard W, Wieland H, Marz W. Diminished LDL receptor and high heparin binding of apolipoprotein E2 Sendai associated with lipoprotein glomerulopathy. J Am Soc Nephrol 2001; 12(3):524-530.

(218) Sakatsume M, Kadomura M, Sakata I, Imai N, Kondo D, Osawa Y et al. Novel glomerular lipoprotein deposits associated with apolipoprotein E2 homozygosity. Kidney Int 2001; 59(5):1911-1918.

(219) Saito T. Abnormal lipid metabolism and renal disorders. Tohoku J Exp Med 1997; 181(3):321-337.

(220) Brewer HB, Jr., Zech LA, Gregg RE, Schwartz D, Schaefer EJ. NIH conference. Type III hyperlipoproteinemia: diagnosis, molecular defects, pathology, and treatment. Ann Intern Med 1983; 98(5 Pt 1):623-640.

(221) Seidel D, Greten H. [Hyperlipoproteinemia Type 3. The possibility of an immunological diagnosis]. Clin Chim Acta 1970; 30(1):31-36.

(222) Weiland H, Seidel D. Improved techniques for assessment of serum lipoprotein patterns. II. Rapid method for diagnosis of type 3 hyperlipoproteinemia without ultracentrifugation. Clin Chem 1973; 19(10):1139-1141.

(223) Wikinski RL, Schreier LE, Rosental SB. New method for isolating and quantifying intermediate and beta-very-low-density lipoprotein cholesterol. Clin Chem 1991; 37(11):1913-1916.

(224) Godolphin WJ, Stinson RA. Isoelectrofocusing of human plasma lipoproteins in polyacrylamide gels: diagnosis of type III hyperlipoproteinemia ("broad beta" disease). Clin Chim Acta 1974; 56(1):97-103.

(225) Papadopoulos NK, Herbert PN. The beta-lipoprotein doublet in type 3 hyperlipoproteinemia. Clin Chem 1977; 23(6):978-981.

(226) Chew MY, Gutman JM, Green J. A case of type III hyperlipoproteinaemia studied by acrylamide gel gradient electrophoresis. J Clin Pathol 1978; 31(6):599-601.

(227) Blom DJ, Byrnes P, Jones S, Marais AD. Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia. J Lipid Res 2003; 44(1):212-217.

(228) Shepherd J, Packard CJ, Dryburgh FJ, Third JL. Diagnosis of type III hyperlipoproteinemia by chromatography of plasma lipoproteins on columns containing agarose. Clin Chem 1975; 21(13):1887-1889.

(229) Albers JJ, Warnick GR, Hazzard WR. Type III hyperlipoproteinemia: a comparative study of current diagnostic techniques. Clin Chim Acta 1977; 75(2):193-204.

(230) Hazzard WR, Porte D, Jr., Bierman EL. Abnormal lipid composition of very low density lipoproteins in diagnosis of broad-beta disease (type 3 hyperlipoproteinemia). Metabolism 1972; 21(11):1009-1019.

(231) Mishkel MA, Nazir DJ, Crowther S. A longitudinal assessment of lipid ratios in the diagnosis of type III hyperlipoproteinaemia. Clin Chim Acta 1975; 58(2):121-136.

(232) Vessby B. Studies on the serum lipoprotein composition in 50-year-old men. A suggestion of chemical criteria for diagnosis of hyperlipoproteinaemia type III (Broad-beta disease). Clin Chim Acta 1976; 69(1):29-42.

(233) Wang T, Nakajima K, Leary ET, Warnick GR, Cohn JS, Hopkins PN et al. Ratio of remnant-like particle-cholesterol to serum total triglycerides is an effective alternative to ultracentrifugal and electrophoretic methods in the diagnosis of familial type III hyperlipoproteinemia. Clin Chem 1999; 45(11):1981-1987.

(234) Blom DJ, O'Neill FH, Marais AD. Screening for dysbetalipoproteinemia by plasma cholesterol and apolipoprotein B concentrations. Clin Chem 2005; 51(5):904-907.

(235) Marz W, Feussner G, Siekmeier R, Donnerhak B, Schaaf L, Ruzicka V et al. Apolipoprotein E to B ratio: a marker for type III hyperlipoproteinaemia. Eur J Clin Chem Clin Biochem 1993; 31(11):743-747.

(236) Nauck M, Glatt L, Marz W, Feussner G, Wieland H. Simple precipitation-based method for the screening of type III hyperlipoproteinemia. Clin Chem 1999; 45(6 Pt 1):909-911.

(237) Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990; 31(3):545-548.

(238) Civeira F, Cenarro A, Ferrando J, Puzo J, Garcia-Otin AL, Mozas P et al. Comparison of the hypolipidemic effect of gemfibrozil versus simvastatin in patients with type III hyperlipoproteinemia. Am Heart J 1999; 138(1 Pt 1):156-162.

(239) Guyton JR. Treatment of type III hyperlipoproteinemia. Am Heart J 1999; 138(1 Pt 1):17-18.

(240) van Dam M, Zwart M, de Beer F, Smelt AH, Prins MH, Trip MD et al. Long term efficacy and safety of atorvastatin in the treatment of severe type III and combined dyslipidaemia. Heart 2002; 88(3):234-238.

(241) Shepherd J. Mechanism of action of bile acid sequestrants and other lipid-lowering drugs. Cardiology 1989; 76 Suppl 1:65-71.

(242) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18(6):499-502.

(243) Beaumont JL, Carlson LA, Cooper GR, Fejfar Z, Fredrickson DS, Strasser T. Classification of Hyperlipidaemias and Hyperlipoproteinaemias. Bull World Health Organ 1970; 43:891-908.

(244) Gambert P, Bouzerand-Gambert C, Athias A, Farnier M, Lallemant C. Human low density lipoprotein subfractions separated by gradient gel electrophoresis : composition,distribution,and alterations induced by cholesteryl ester transfer protein. J Lipid Res 1990; 31:1199-1210.

(245) Lindgren FT, Jensen LC, Hatch FT. The isolation and quantative analysis of serum lipoproteins. In: Nelson GJ, editor. Blood lipids and lipoproteins :quantitation,composition and metabolism. New York: John Wiley and Sons Inc., 1972: 181-207.

(246) Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. Biochem J 1991; 273(Pt 1):229-231.

(247) Emi M, Wu LL, Robertson MA, Myers RL, Hegele RA, Williams RR et al. Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics 1988; 3(4):373-379.

(248) Hsia SH, Connelly PW, Hegele RA. Restriction isotyping of apolipoprotein E R145C in type III hyperlipoproteinemia. J Investig Med 1995; 43(2):187-194.

(249) Marais AD, Burton R, Anthony J, Jones S, Henderson HE. High prevalence of the R145C variant of apolipoproteinE (apoE) in blacks. 8th LASSA Conference . 2004. Durban.

Ref Type: Generic

(250) Abera A. Microsatelite markers and haplotye analysis of the R145C mutation of the apoE gene in dysbetalipoproteinaemia. University of Cape Town, 2004.

(251) Ollila J, Lappalainen I, Vihinen M. Sequence specificity in CpG mutation hotspots. FEBS Lett 1996; 396(2-3):119-122.

(252) Kaysen GA, de Sain-van der Velden MG. New insights into lipid metabolism in the nephrotic syndrome. Kidney Int Suppl 1999; 71:S18-S21.

(253) Firth JC. Familial hypercholesterolaemia - the Cape Town experience. University of Cape Town, 2005.

(254) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285(19):2486-2497.

(255) Plasma lipid distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. The Lipid Research Clinics Program Epidemiology Committee. Circulation 1979; 60(2):427-439.

(256) Vermeer BJ, van Gent CM, Goslings B, Polano MK. Xanthomatosis and other clinical findings in patients with elevated levels of very low density lipoproteins. Br J Dermatol 1979; 100(6):657-666.

(257) Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem 2001; 47(9):1579-1596.

(258) Blom DJ. Polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinaemia (Type III hyperlipidaemia). University of Cape Town, 2003.

(259) Steyn K, Levitt NS, Hoffman M, Marais AD, Fourie JM, Lambert EV et al. The global cardiovascular diseases risk pattern in a peri-urban working-class community in South Africa. The Mamre study. Ethn Dis 2004; 14(2):233-242.

(260) Lister RK, Youle M, Nair DR, Winder AF, Rustin MH. Latent dysbetalipoproteinaemia precipitated by HIV-protease inhibitors. Lancet 1999; 353(9165):1678.

(261) Shahmanesh M, Jaleel H, DeSilva Y, Ross JD, Caslake M, Cramb R. Protease inhibitor related type III hyperlipoproteinaemia is common and not associated with apolipoprotein-E E2/E2 phenotype. Sex Transm Infect 2001; 77(4):283-286.

(262) Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. Trends Mol Med 2006; 12(7):324-332.

(263) Alfire ME, Treem WR. Nonalcoholic fatty liver disease. Pediatr Ann 2006; 35(4):290-299.

(264) Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982; 23(1):97-104.

(265) McNamara JR, Jenner JL, Li Z, Wilson PW, Schaefer EJ. Change in LDL particle size is associated with change in plasma triglyceride concentration. Arterioscler Thromb 1992; 12(11):1284-1290.

(266) Wakatsuki A, Ikenoue N, Okatani Y, Fukaya T. Estrogen-induced small low density lipoprotein particles may be atherogenic in postmenopausal women. J Am Coll Cardiol 2001; 37(2):425-430.

(267) Festa A, D'Agostino R, Jr., Mykkanen L, Tracy R, Howard BV, Haffner SM. Low-density lipoprotein particle size is inversely related to plasminogen activator inhibitor-1 levels. The Insulin Resistance Atherosclerosis Study. Arterioscler Thromb Vasc Biol 1999; 19(3):605-610.

(268) Austin MA, Krauss RM. Genetic control of low-density-lipoprotein subclasses. Lancet 1986; 2(8507):592-595.

(269) Bosse Y, Feitosa MF, Despres JP, Lamarche B, Rice T, Rao DC et al. Detection of a major gene effect for LDL peak particle diameter and association with apolipoprotein H gene haplotype. Atherosclerosis 2005; 182(2):231-239.

(270) Cromwell WC, Otvos JD. Low-density lipoprotein particle number and risk for cardiovascular disease. Curr Atheroscler Rep 2004; 6(5):381-387.

(271) Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ et al. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. Atherosclerosis 1994; 106(2):241-253.

(272) Watanabe A, Yoshimura A, Wakasugi T, Tatami R, Ueda K, Ueda R et al. Serum lipids, lipoprotein lipids and coronary heart disease in patients with xanthelasma palpebrarum. Atherosclerosis 1981; 38(3-4):283-290.

(273) Winder AF. Relationship between corneal arcus and hyperlipidaemia is clarified by studies in familial hypercholesterolaemia. Br J Ophthalmol 1983; 67(12):789-794.

(274) Yadav D, Pitchumoni CS. Issues in hyperlipidemic pancreatitis. J Clin Gastroenterol 2003; 36(1):54-62.

(275) Toskes PP. Hyperlipidemic pancreatitis. Gastroenterol Clin North Am 1990; 19(4):783-791.

(276) Standards of medical care in diabetes--2006. Diabetes Care 2006; 29 Suppl 1:S4-42.

(277) Effect of dietary fat and ethanol on lipemia. Nutr Rev 1970; 28(6):162-163.

(278) Pathogenesis of alcohol-induced hypertriglyceridemia. Nutr Rev 1987; 45(7):215-216.

(279) Feingold KR, Krauss RM, Pang M, Doerrler W, Jensen P, Grunfeld C. The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low density lipoprotein subclass pattern B. J Clin Endocrinol Metab 1993; 76(6):1423-1427.

(280) Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. Am J Med 1989; 86(1):27-31.

(281) Grunfeld C, Kotler DP, Shigenaga JK, Doerrler W, Tierney A, Wang J et al. Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. Am J Med 1991; 90(2):154-162.

(282) Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. Body shape changes in HIV-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 19(3):307-308.

(283) Carr A, Miller J, Law M, Cooper DA. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. AIDS 2000; 14(3):F25-F32.

(284) Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998; 12(7):F51-F58.

(285) Purnell JO, Zambon A, Knopp RH, Pizzuti DJ, Achari R, Leonard JM et al. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. AIDS 2000; 14(1):51-57.

(286) Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N Engl J Med 2005; 352(1):48-62.

(287) Riddle TM, Kuhel DG, Woollett LA, Fichtenbaum CJ, Hui DY. HIV protease inhibitor induces fatty acid and sterol biosynthesis in liver and adipose tissues due to the accumulation of activated sterol regulatory element-binding proteins in the nucleus. J Biol Chem 2001; 276(40):37514-37519.

(288) Miserez AR, Muller PY, Spaniol V. Indinavir inhibits sterol-regulatory element-binding protein-1c- dependent lipoprotein lipase and fatty acid synthase gene activations. AIDS 2002; 16(12):1587-1594.

(289) Caron M, Auclair M, Vigouroux C, Glorian M, Forest C, Capeau J. The HIV protease inhibitor indinavir impairs sterol regulatory element- binding protein-1 intranuclear localization, inhibits preadipocyte differentiation, and induces insulin resistance. Diabetes 2001; 50(6):1378-1388.

(290) Liang JS, Distler O, Cooper DA, Jamil H, Deckelbaum RJ, Ginsberg HN et al. HIV protease inhibitors protect apolipoprotein B from degradation by the proteasome: a potential mechanism for protease inhibitor-induced hyperlipidemia. Nat Med 2001; 7(12):1327-1331.

(291) Christeff N, Nunez EA, Gougeon ML. Changes in cortisol/DHEA ratio in HIV-infected men are related to immunological and metabolic perturbations leading to malnutrition and lipodystrophy. Ann N Y Acad Sci 2000; 917:962-970.

(292) Christeff N, Melchior JC, de Truchis P, Perronne C, Nunez EA, Gougeon ML. Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: correlation between dyslipidaemia and steroid hormone alterations. AIDS 1999; 13(16):2251-2260.

(293) Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse- transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. Lancet 1999; 354(9184):1112-1115.

(294) Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio MA et al. Cardiovascular disease risk factors in HIV patients--association with

antiretroviral therapy. Results from the DAD study. AIDS 2003; 17(8):1179-1193.

(295) Behrens G, Schmidt HH, Stoll M, Schmidt RE. ApoE genotype and protease-inhibitor-associated hyperlipidaemia. Lancet 1999; 354(9172):76.

(296) Furberg CD, Pitt B. Withdrawal of cerivastatin from the world market. Curr Control Trials Cardiovasc Med 2001; 2(5):205-207.

(297) Williams D, Feely J. Pharmacokinetic-pharmacodynamic drug interactions with HMG-CoA reductase inhibitors. Clin Pharmacokinet 2002; 41(5):343-370.

(298) Edwards JE, Moore RA. Statins in hypercholesterolaemia: a dose-specific meta-analysis of lipid changes in randomised, double blind trials. BMC Fam Pract 2003; 4:18.

(299) Ose L, Luurila O, Eriksson J, Olsson A, Lithell H, Widgren B. Efficacy and safety of cerivastatin, 0.2 mg and 0.4 mg, in patients with primary hypercholesterolaemia: a multinational, randomised, double-blind study. Cerivastatin Study Group. Curr Med Res Opin 1999; 15(3):228-240.

(300) Stuyt PM, Demacker PN, van't Laar A. A study of the hypolipidemic effect of estrogen in type III hyperlipoproteinemia. Horm Metab Res 1986; 18(9):607-610.

(301) de Beer F, Smelt AH, van Vark LC, Hoogerbrugge N, Havekes LM, Gevers Leuven JA. The effect of tibolone on the lipoprotein profile of postmenopausal

women with type III hyperlipoproteinemia. J Intern Med 2002; 251(2):148-155.

(302) Hoogwerf BJ, Bantle JP, Kuba K, Frantz ID, Jr., Hunninghake DB. Treatment of type III hyperlipoproteinemia with four different treatment regimens. Atherosclerosis 1984; 51(2-3):251-259.

(303) Stuyt PM, Stalenhoef AF, Demacker PN, Van 't LA. A comparative study of the effects of acipimox and clofibrate in type III and type IV hyperlipoproteinemia. Atherosclerosis 1985; 55(1):51-62.

(304) East CA, Grundy SM, Bilheimer DW. Preliminary report: treatment of type 3 hyperlipoproteinemia with mevinolin. Metabolism 1986; 35(2):97-98.

(305) Hoogwerf BJ, Peters JR, Frantz ID, Jr., Hunninghake DB. Effect of clofibrate and colestipol singly and in combination on plasma lipids and lipoproteins in type III hyperlipoproteinemia. Metabolism 1985; 34(10):978-981.

(306) Zhao SP, Smelt AH, Leuven JA, Vroom TF, Van der LA, 't Hooft FM. Changes of lipoprotein profile in familial dysbetalipoproteinemia with gemfibrozil. Am J Med 1994; 96(1):49-56.

(307) Packard CJ, Clegg RJ, Dominiczak MH, Lorimer AR, Shepherd J. Effects of bezafibrate on apolipoprotein B metabolism in type III hyperlipoproteinemic subjects. J Lipid Res 1986; 27(9):930-938.

(308) Lussier-Cacan S, Bard JM, Boulet L, Nestruck AC, Grothe AM, Fruchart JC et al. Lipoprotein composition changes induced by fenofibrate in dysbetalipoproteinemia type III. Atherosclerosis 1989; 78(2-3):167-182.

(309) Kuo PT, Wilson AC, Kostis JB, Moreyra AB, Dodge HT. Treatment of type III hyperlipoproteinemia with gemfibrozil to retard progression of coronary artery disease. Am Heart J 1988; 116(1 Pt 1):85-90.

(310) Houlston R, Quiney J, Watts GF, Lewis B. Gemfibrozil in the treatment of resistant familial hypercholesterolaemia and type III hyperlipoproteinaemia. J R Soc Med 1988; 81(5):274-276.

(311) Fruchart JC, Davignon J, Bard JM, Grothe AM, Richard A, Fievet C. Effect of fenofibrate treatment on type III hyperlipoproteinemia. Am J Med 1987; 83(5B):71-74.

(312) Feussner G, Kurth B, Lohrmann J. Comparative effects of bezafibrate and micronised fenofibrate in patients with type III hyperlipoproteinemia. Eur J Med Res 1997; 2(4):165-168.

(313) Vega GL, East C, Grundy SM. Lovastatin therapy in familial dysbetalipoproteinemia: effects on kinetics of apolipoprotein B. Atherosclerosis 1988; 70(1-2):131-143.

(314) Stuyt PM, Mol MJ, Stalenhoef AF, Demacker PN, Van 't LA. Simvastatin in the effective reduction of plasma lipoprotein levels in familial dysbetalipoproteinemia (type III hyperlipoproteinemia). Am J Med 1990; 88(1N):42N-45N.

(315) Ishigami M, Yamashita S, Sakai N, Hirano K, Hiraoka H, Nakamura T et al. Atorvastatin markedly improves type III hyperlipoproteinemia in association with reduction of both exogenous and endogenous apolipoprotein B-containing lipoproteins. Atherosclerosis 2003; 168(2):359-366.

(316) Illingworth DR, O'Malley JP. The hypolipidemic Effects of Lovastatin and Clofibrate Alone and in Combination in Patients with Type III Hyperlipoproteinemia. Metabolism 1990; 39(4):403-409.

(317) Gylling H, Relas H, Miettinen TA. Postprandial vitamin A and squalene clearances and cholesterol synthesis off and on lovastatin treatment in type III hyperlipoproteinemia. Atherosclerosis 1995; 115(1):17-26.

(318) Feussner G, Eichinger M, Ziegler R. The influence of simvastatin alone or in combination with gemfibrozil on plasma lipids and lipoproteins in patients with type III hyperlipoproteinemia. Clin Investig 1992; 70(11):1027-1035.

(319) Stuyt PM, Demacker PN, Van 't LA. Effect of infusion of normal plasma on lipoproteins in type III hyperlipoproteinemia. Horm Metab Res 1981; 13(12):715-716.

(320) Fruchart JC, Davignon J, Bard JM, Grothe AM, Richard A, Fievet C. Effect of fenofibrate treatment on type III hyperlipoproteinemia. Am J Med 1987; 83(5B):71-74.

(321) Larsen ML, Illingworth DR, O'Malley JP. Comparative effects of gemfibrozil and clofibrate in type III hyperlipoproteinemia. Atherosclerosis 1994; 106(2):235-240.

(322) Civeira F, Pocovi M, Cenarro A, Casao E, Vilella E, Joven J et al. Apo E variants in patients with type III hyperlipoproteinemia. Atherosclerosis 1996; 127(2):273-282.

(323) Zhao SP, Smelt AH, Van den Maagdenberg AM, Van Tol A, Vroom TF, Gevers Leuven JA et al. Plasma lipoproteins in familial dysbetalipoproteinemia associated with apolipoproteins E2(Arg158-->Cys), E3-Leiden, and E2(Lys146-->Gln), and effects of treatment with simvastatin. Arterioscler Thromb 1994; 14(11):1705-1716.

(324) Gylling H, Miettinen TA. Postabsorptive metabolism of dietary squalene. Atherosclerosis 1994; 106(2):169-178.

(325) Knopp RH, Illingworth DR, Stein EA, Ginsberg HN, Broyles FE, Behounek BD. Effect of Pravastatin in the Treatment of Patients with Type III Hyperlipoproteinemia. Am J Ther 1996; 3(11):755-762.

(326) Dallongeville J, Boulet L, Davignon J, Lussier-Cacan S. Fish oil supplementation reduces beta-very low density lipoprotein in type III dysbetalipoproteinemia. Arterioscler Thromb 1991; 11(4):864-871.

(327) Holmquist L. Surface modification of Beckman Ultra-Clear centrifuge tubes for density gradient centrifugation of lipoproteins. J Lipid Res 1982; 23(8):1249-1250.

(328) Markwell MA, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978; 87(1):206-210.

(329) Lipid Research Clinics Program. Lipid and Lipoprotein Analysis. Manual of Laboratory Operations. Washington DC: U.S. Government Printing Office, 1979.

(330) Warnick GR, Wood PD. National Cholesterol Education Program recommendations for measurement of high-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. Clin Chem 1995; 41(10):1427-1433.

(331) Nauck M, Neumann I, Marz W, Wieland H. A new liquid homogeneous assay for the determination of HDL-cholesterol. A comparison to precipitation with phosphotungstic acid/MgCl2 and a lyophilized homogeneous assay. Clin Chem Lab Med 1999; 37(5):537-543.

(332) Roberts WL, Leary ET, Lambert TL, Moulton L, Goestch JL. Falsely low direct HDL-cholesterol results in a patient with dysbetalipoproteinemia. Clin Chem 2000; 46(4):560-562.

(333) Lackner KJ, Schmitz G. beta-VLDL of patients with type III hyperlipoproteinemia interferes with homogeneous determination of HDL-cholesterol based on polyethylene glycol-modified enzymes. Clin Chem 1998; 44(12):2546-2548.

(334) Heeschen C, Hamm CW, Laufs U, Snapinn S, Bohm M, White HD. Withdrawal of statins increases event rates in patients with acute coronary syndromes. Circulation 2002; 105(12):1446-1452.

(335) Heeschen C, Hamm CW, Laufs U, Bohm M, Snapinn S, White HD. Withdrawal of statins in patients with acute coronary syndromes. Circulation 2003; 107(3):e27.

(336) McGowan MP. There is no evidence for an increase in acute coronary syndromes after short-term abrupt discontinuation of statins in stable cardiac patients. Circulation 2004; 110(16):2333-2335.

(337) Kesaniemi YA, Grundy SM. Influence of gemfibrozil and clofibrate on metabolism of cholesterol and plasma triglycerides in man. JAMA 1984; 251(17):2241-2246.

(338) Caroli-Bosc FX, Le Gall P, Pugliese P, Delabre B, Caroli-Bosc C, Demarquay JF et al. Role of fibrates and HMG-CoA reductase inhibitors in gallstone formation: epidemiological study in an unselected population. Dig Dis Sci 2001; 46(3):540-544.

(339) Stahlberg D, Reihner E, Rudling M, Berglund L, Einarsson K, Angelin B. Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients: reduced activity of cholesterol 7 alpha-hydroxylase. Hepatology 1995; 21(4):1025-1030.

(340) Roglans N, Vazquez-Carrera M, Alegret M, Novell F, Zambon D, Ros E et al. Fibrates modify the expression of key factors involved in bile-acid synthesis and biliary-lipid secretion in gallstone patients. Eur J Clin Pharmacol 2004; 59(12):855-861.

(341) Mukhtar RY, Reckless JP. Statin-induced myositis: a commonly encountered or rare side effect? Curr Opin Lipidol 2005; 16(6):640-647.

(342) Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol 2006; 97(8A):52C-60C.

(343) Tolman KG. The liver and lovastatin. Am J Cardiol 2002; 89(12):1374-1380.

(344) Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet 2005; 366(9500):1849-1861.

(345) Steinmetz J, Morin C, Panek E, Siest G, Drouin P. Biological variations in hyperlipidemic children and adolescents treated with fenofibrate. Clin Chim Acta 1981; 112(1):43.

(346) Day AP, Feher MD, Chopra R, Mayne PD. The effect of bezafibrate treatment on serum alkaline phosphatase isoenzyme activities. Metabolism 1993; 42(7):839-842.

(347) Ganotakis E, Tsimihodimos V, Bairaktari E, Rizos E, Athyros V, Seferiades C et al. Effects of various fibrates on serum alkaline phosphatase activity. Atherosclerosis 2002; 165(1):187-188.

(348) Papadakis JA, Ganotakis ES, Jagroop IA, Winder AF, Mikhailidis DP. Statin + fibrate combination therapy fluvastatin with bezafibrate or ciprofibrate in high risk patients with vascular disease. Int J Cardiol 1999; 69(3):237-244.

(349) Broeders N, Knoop C, Antoine M, Tielemans C, Abramowicz D. Fibrate-induced increase in blood urea and creatinine: is gemfibrozil the only innocuous agent? Nephrol Dial Transplant 2000; 15(12):1993-1999.

(350) Hottelart C, El Esper N, Achard JM, Pruna A, Fournier A. [Fenofibrate increases blood creatinine, but does not change the glomerular filtration rate in patients with mild renal insufficiency]. Nephrologie 1999; 20(1):41-44.

(351) Hottelart C, El Esper N, Rose F, Achard JM, Fournier A. Fenofibrate increases creatininemia by increasing metabolic production of creatinine. Nephron 2002; 92(3):536-541.

(352) Ansquer JC, Foucher C, Rattier S, Taskinen MR, Steiner G. Fenofibrate reduces progression to microalbuminuria over 3 years in a placebo-controlled study in type 2 diabetes: results from the Diabetes Atherosclerosis Intervention Study (DAIS). Am J Kidney Dis 2005; 45(3):485-493.

(353) Desager JP, Hulhoven R, Harvengt C. Uricosuric effect of fenofibrate in healthy volunteers. J Clin Pharmacol 1980; 20(10):560-564.

(354) Harvengt C, Heller F, Desager JP. Hypolipidemic and hypouricemic action of fenofibrate in various types of hyperlipoproteinemias. Artery 1980; 7(1):73-82.

(355) Feher MD, Hepburn AL, Hogarth MB, Ball SG, Kaye SA. Fenofibrate enhances urate reduction in men treated with allopurinol for hyperuricaemia and gout. Rheumatology (Oxford) 2003; 42(2):321-325.

(356) Zhang W, Doherty M, Bardin T, Pascual E, Barskova V, Conaghan P et al. EULAR Evidence based recommendations for gout - part ii management: report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 2006.

(357) Daskalopoulou SS, Tzovaras V, Mikhailidis DP, Elisaf M. Effect on serum uric acid levels of drugs prescribed for indications other than treating hyperuricaemia. Curr Pharm Des 2005; 11(32):4161-4175.

(358) Grodzicki T, Palmer A, Bulpitt CJ. Incidence of diabetes and gout in hypertensive patients during 8 years of follow-up. The General Practice Hypertension Study Group. J Hum Hypertens 1997; 11(9):583-585.

(359) Rapado A. Relationship between gout and arterial hypertension. Adv Exp Med Biol 1974; 41:451-459.

(360) Frank O. Observations concerning the incidence of disturbance of lipid and carbohydrate metabolism in gout. Adv Exp Med Biol 1974; 41:495-498.

(361) Diagnosis and classification of diabetes mellitus. Diabetes Care 2006; 29 Suppl 1:S43-S48.

(362) Redgrave TG, Mortimer BC, Martins IJ. Receptor-ligand interactions in the hepatic uptake of chylomicron remnants studied with genetically manipulated mice. Z Gastroenterol 1996; 34 Suppl 3:105-107.

(363) Redgrave TG, Martins IJ, Mortimer B-C. Measurement of expired carbon dioxide to assess the metabolism of remnant lipoproteins. J Lipid Res 1995; 36(12):2670-2675.

(364) Martins IJ, Redgrave TG. A 13CO2 breath test to assess the metabolism of triglyceride-rich lipoprotein remnants in mice. J Lipid Res 1998; 39(3):691-698.

(365) Redgrave TG, Watts GF, Martins IJ, Barrett PH, Mamo JC, Dimmitt SB et al. Chylomicron remnant metabolism in familial dyslipidemias studied with a remnant-like emulsion breath test. J Lipid Res 2001; 42(5):710-715.

(366) Watts GF, Barrett PH, Marais AD, Dane-Stewart CA, Martins IJ, Dimmitt SB et al. Chylomicron remnant metabolism in familial hypercholesterolaemia studied with a stable isotope breath test. Atherosclerosis 2001; 157(2):519-523.

(367) Phan CT, Mortimer BC, Martins IJ, Redgrave TG. Plasma clearance of chylomicrons from butterfat is not dependent on saturation: studies with butterfat fractions and other fats containing triacylglycerols with low or high melting points. Am J Clin Nutr 1999; 69(6):1151-1161.

(368) Ng TW, Watts GF, Stuckey BG, Ching HL, Chan DC, Uchida Y et al. Does pravastatin increase chylomicron remnant catabolism in postmenopausal women with type 2 diabetes mellitus? Clin Endocrinol (Oxf) 2005; 63(6):650-656.

(369) Martins IJ, Redgrave TG. Obesity and post-prandial lipid metabolism. Feast or famine? J Nutr Biochem 2004; 15(3):130-141.

(370) Martins IJ, Tran JM, Redgrave TG. Food restriction normalizes chylomicron remnant metabolism in murine models of obesity as assessed by a novel stable isotope breath test. J Nutr 2002; 132(2):176-181.

(371) Dane-Stewart CA, Watts GF, Barrett PH, Stuckey BG, Mamo JC, Martins IJ et al. Chylomicron remnant metabolism studied with a new breath test in

postmenopausal women with and without type 2 diabetes mellitus. Clin Endocrinol (Oxf) 2003; 58(4):415-420.

(372) Dane-Stewart CA, Watts GF, Mamo JC, Barrett PH, Martins IJ, Dimmitt SB et al. Effect of Simvastatin on markers of triglyceride-rich lipoproteins in familial hypercholesterolaemia. Eur J Clin Invest 2002; 32(7):493-499.

(373) Chan DC, Watts GF, Barrett PH, Martins IJ, James AP, Mamo JC et al. Effect of atorvastatin on chylomicron remnant metabolism in visceral obesity: a study employing a new stable isotope breath test. J Lipid Res 2002; 43(5):706-712.

(374) Watts GF, Chan DC, Barrett PH, Martins IJ, Redgrave TG. Preliminary experience with a new stable isotope breath test for chylomicron remnant metabolism: a study in central obesity. Clin Sci (Lond) 2001; 101(6):683-690.

(375) Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis 1994; 106(1):83-97.

(376) Hugh P, Barrett R. Kinetics of triglyceride rich lipoproteins: chylomicrons and very low density lipoproteins. Atherosclerosis 1998; 141 Suppl 1:S35-S40.

(377) Smith D, Watts GF, Dane-Stewart C, Mamo JC. Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest 1999; 29(3):204-209.

(378) Stalenhoef AF. Chylomicron apolipoprotein B catabolism. Diabetologia 1984; 27(6):609-610.

(379) Haffner SM, Foster DM, Kushwaha RS, Hazzard WR. Retarded chylomicron apolipoprotein-B catabolism in type 2 (non-insulin-dependent) diabetic subjects with lipaemia. Diabetologia 1984; 26(5):349-354.

(380) Ehnholm C, Mahley RW, Chappell DA, Weisgraber KH, Ludwig E, Witztum JL. Role of apolipoprotein E in the lipolytic conversion of beta-very low density lipoproteins to low density lipoproteins in type III hyperlipoproteinemia. Proc Natl Acad Sci U S A 1984; 81(17):5566-5570.

(381) Zilversmit DB, Morton RE, Hughes LB, Thompson KH. Exchange of retinyl and cholesteryl esters between lipoproteins of rabbit plasma. Biochim Biophys Acta 1982; 712(1):88-93.

(382) Schoeller DA, Klein PD, Watkins JB, Heim T, MacLean WC, Jr. 13C abundances of nutrients and the effect of variations in 13C isotopic abundances of test meals formulated for 13CO2 breath tests. Am J Clin Nutr 1980; 33(11):2375-2385.

(383) Barrett PH, Chan DC, Watts GF. Thematic review series: patient-oriented research. Design and analysis of lipoprotein tracer kinetics studies in humans. J Lipid Res 2006; 47(8):1607-1619.

(384) Turner PR, Cortese C, Wootton R, Marenah C, Miller NE, Lewis B. Plasma apolipoprotein B metabolism in familial type III dysbetalipoproteinaemia. Eur J Clin Invest 1985; 15(2):100-112.

(385) Stalenhoef AF, Malloy MJ, Kane JP, Havel RJ. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in patients with familial dysbetalipoproteinemia. J Clin Invest 1986; 78(3):722-728.

(386) Cortner JA, Coates PM, Le NA, Cryer DR, Ragni MC, Faulkner A et al. Kinetics of chylomicron remnant clearance in normal and in hyperlipoproteinemic subjects. J Lipid Res 1987; 28(2):195-206.

(387) Haffner SM, Kushwaha RS, Hazzard WR. Metabolism of chylomicrons in subjects with dysbetalipoproteinaemia (type III hyperlipoproteinaemia). Eur J Clin Invest 1989; 19(5):486-490.

(388) Tremblay AJ, Lamarche B, Ruel IL, Hogue JC, Deshaies Y, Gagne C et al. Effects of fenofibrate on apolipoprotein kinetics in patients with coexisting dysbetalipoproteinemia and heterozygous familial hypercholesterolemia. Atherosclerosis 2006; 188(1):203-212.

(389) Pal S, Allister E, Thomson A, Mamo JC. Cholesterol esters regulate apoB48 secretion in CaCo2 cells. Atherosclerosis 2002; 161(1):55-63.

(390) Wybenga DR, Ibbott FA, Winkelman JW. The indirect confirmation of hyperlipoproteinemia phenotypes II, 3 and IV. Clin Chim Acta 1972; 40(1):121-127.

(391) Marcovina SM, Albers JJ, Kennedy H, Mei JV, Henderson LO, Hannon WH. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International Reference Material. Clin Chem 1994; 40(4):586-592.

(392) Barter PJ, Ballantyne CM, Carmena R, Castro CM, Chapman MJ, Couture P et al. Apo B versus cholesterol in estimating cardiovascular risk and in guiding

therapy: report of the thirty-person/ten-country panel. J Intern Med 2006; 259(3):247-258.

(393) Sniderman AD, St Pierre AC, Cantin B, Dagenais GR, Despres JP, Lamarche B. Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk. Am J Cardiol 2003; 91(10):1173-1177.

(394) Esteban-Salan M, Guimon-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Rio E. Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients. Clin Chem 2000; 46(8 Pt 1):1121-1131.

(395) Miller WG, Waymack PP, Anderson FP, Ethridge SF, Jayne EC. Performance of four homogeneous direct methods for LDL-cholesterol. Clin Chem 2002; 48(3):489-498.

(396) Okada M, Ishida R. Direct measurement of low-density-lipoprotein cholesterol is more effective than total cholesterol for the purpose of lipoprotein screening. Prev Med 2001; 32(3):224-229.

(397) Bachorik PS, Lovejoy KL, Carroll MD, Johnson CL. Apolipoprotein B and AI distributions in the United States, 1988-1991: results of the National Health and Nutrition Examination Survey III (NHANES III). Clin Chem 1997; 43(12):2364-2378.

(398) Polk SL. Definitions and demographics of obesity: diagnosis and risk factors. Anesthesiol Clin North America 2005; 23(3):397-403, v.

(399) Behn A, Ur E. The obesity epidemic and its cardiovascular consequences. Curr Opin Cardiol 2006; 21(4):353-360.