Regression of Atherosclerosis:

The Clinical and Metabolic Response to Cholesterol-Lowering

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Abstract

A large number of studies have established that raised cholesterol levels increase the probability of the development of atherosclerotic vascular disease, and that reducing serum cholesterol will result in fewer cardiac events in the treated population, both in those with and without evidence of pre-existing coronary disease. More direct evidence that this is due to alteration of the progression of the atheromatous plaques has resulted from angiographic studies demonstrating the halting of progression or even regression of the stenotic lesions. Some workers have found a relationship between the extent of lowering of the serum lipoproteins and the likelihood of regression, although it has not been clear whether this continues to hold true at the lower extremes, nor whether there may be a threshold level which requires to be achieved before regression may take place.

The principal purpose of these studies was to investigate the effects of applying very intensive lipid-lowering therapy, including LDL-apheresis, in a group of patients with coronary artery disease and moderately severe hypercholesterolaemia to achieve sub-normal lipoprotein levels, and comparing the effects of such treatment with those achieved in another group of subjects treated with drug therapy to the currently recommended therapeutic targets for such patients. The studies involved the measurement of lipids and lipoproteins before and after apheresis and at regular intervals throughout the two-year study period. ApoB metabolism was assessed at baseline and following completion of the treatment period, and the data analysed using a multicompartmental mathematical model. The patients were assessed non-invasively by exercise electrocardiography at regular intervals, and by thallium scintigraphy at baseline and at annual intervals. The principal end-point was the proportion of arterial segments undergoing regression or progression in each group assessed by computer-assisted analysis of coronary angiograms performed at baseline and on completion of the intervention.

The results from these studies demonstrated radical differences in lipoprotein concentration and composition during treatment. There was increased catabolism of LDL precursors with diminished flux of apoB which may reflect up-regulation of the LDL-receptor, but a rapid return to pre-treatment lipid levels indicated the effects on lipoprotein metabolism were transient. There was a reduction in the progression of coronary disease in the majority of lesions, with a small number in each group undergoing definite regression. There were significant differences in the changes in exercise tolerance with treatment, and the likely mechanisms for this are discussed. The thallium scans demonstrated no difference between the groups in the number of segments with improved perfusion, but were shown to have some value in the non-invasive assessment of predicting angiographic changes in the proximal segments, particularly in the right coronary artery.

The findings are put into the context of the recent publications on cholesterol reduction in coronary disease; implications for clinical management are drawn, and areas of potential future research are highlighted.

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Author's Declaration

The work presented in this thesis was performed solely by me, except where the assistance of others is acknowledged.

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Graeme Tait

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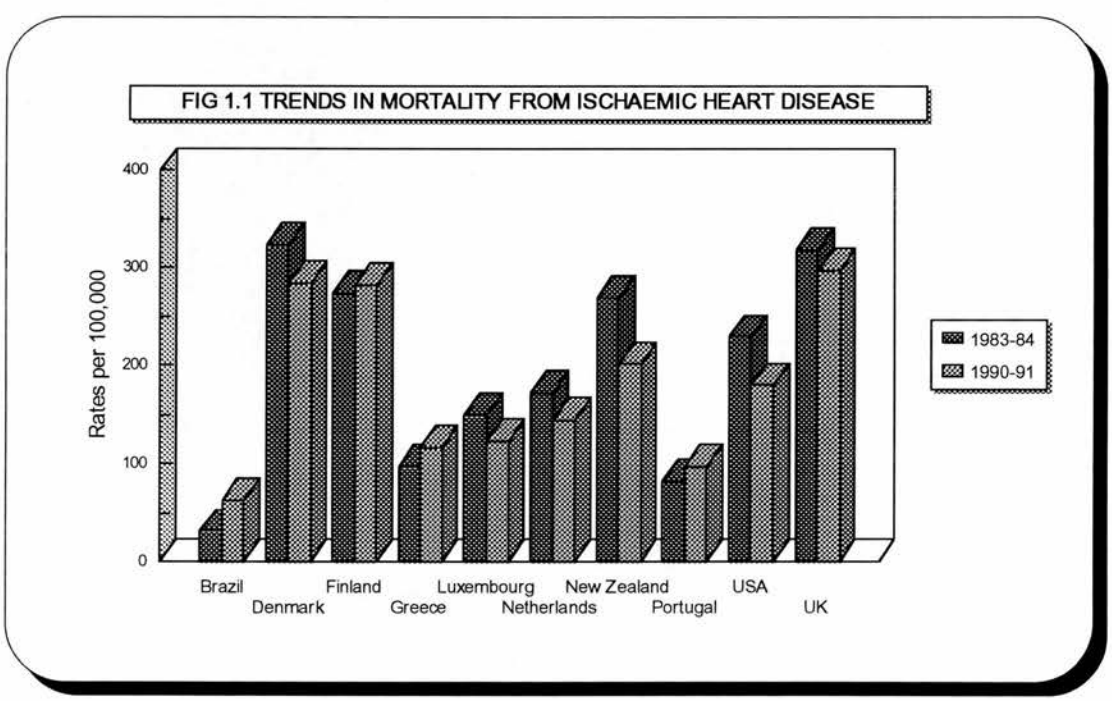
1.1 CORONARY ARTERY DISEASE

1.1.1 Incidence

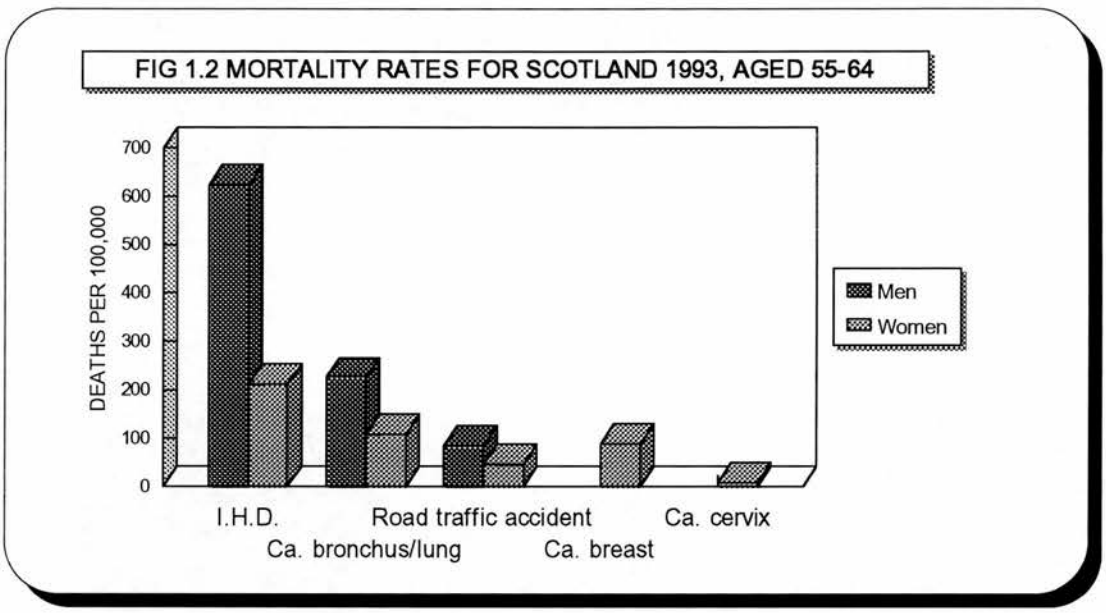
Coronary artery disease in industrialized societies is the principal cause of premature death, and imposes an enormous burden both on health service resources and on national economic loss [National Forum for Coronary Heart Disease Prevention, 1988].

The description of its commonest presentation - angina pectoris - was first recorded two hundred years ago by William Heberden [Heberden 1772], and its mechanism of imbalance between myocardial oxygen supply and demand was recognised soon after [Parry 1799]. Despite its recognition, it appears (judging by the paucity of publications) to have been an uncommon entity until the present century. From the 1920's there has been a dramatic increase in the incidence of coronary disease in most Western communities which has been described as an epidemic. In the United Kingdom the incidence appears to have peaked around 1980 and may now be exhibiting a slow downward trend, as has been observed in other countries over the past two decades (Fig 1.1).

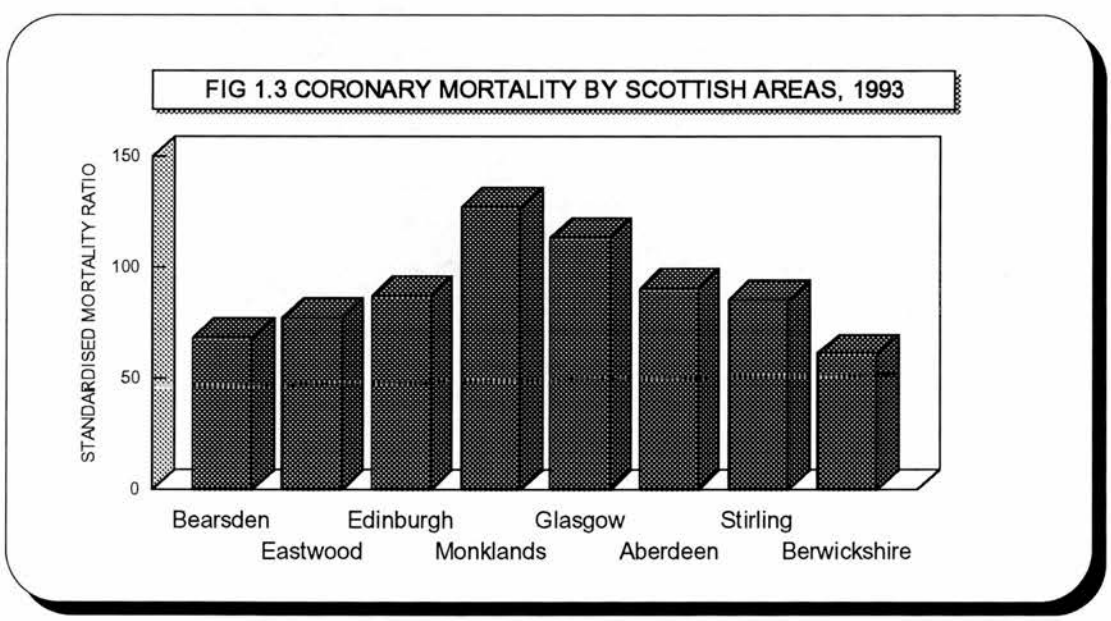


[Figures from Britannica World Data 1986 & 1994]

Despite these changes in incidence, mortality rates from coronary heart disease in Britain remain among the highest in the world. The impact of this condition may be illustrated by a comparison with mortality rates from other common causes of death including those which have received particular attention from screening programmes (Fig1.2, [Government Statistical Service 1994]).



Within the United Kingdom there are well-recognised areas which have an incidence of coronary disease consistently above the national average, and the West of Scotland in particular is an example (see Figure 1.3 [Government Statistical Service 1994]).



1.1.2 Pathology and pathogenesis

Ischaemic heart disease is usually due to obstruction of the coronary arteries, most commonly by atherosclerotic plaque. This process appears to have its origins in childhood, when "fatty streaks" may be observed even before ten years of age [Stary 1983]. These consist mainly of lipid-filled macrophages and smooth muscle cells, and appear as an area of yellowish discolouration of the arterial intima. These early lesions have a predilection to occur at the sites at which the more advanced lesion, the fibrous plaque, also appear, and are believed to be the precursors of the latter lesions [McGill 1984]. Fibrous plaques are white, and - unlike the fatty streak - usually elevated, often protruding into the lumen of the artery where they may compromise blood flow. Microscopically they consist of large numbers of intimal smooth muscle cells and macrophages containing a variable amount of lipid, the origin of the cells confirmed by monoclonal antibody studies [Tsukada *et al* 1986].

The cellular element is surrounded by proteoglycan and collagen and elastin fibres, and are usually covered by a fibrous cap. The relative contribution to the plaque by fibrous tissue and lipid-rich material varies between lesions even in the one individual, but there is a relationship between the constitution of the plaques and serum lipid levels, hypercholesterolaemic subjects tending to have greater amounts of lipid within the lesions [Ross *et al* 1984].

The distribution of atherosclerosis in humans follows a general pattern: the abdominal aorta is usually more extensively involved than the thoracic aorta, particularly around the ostia of its branches. The coronary arteries tend to be among the worst affected, usually in their more proximal part. It is thought that the characteristics of the blood flow [Caro *et al* 1969, Caro *et al* 1971, Chien 1976] may be of primary importance in determining the site, extent, and severity of the lesions.

The pathogenesis of atherosclerosis is not completely understood, but the "respose-to-injury" hypothesis was widely accepted for several years [Ross & Glomset 1976]. This view states that localised endothelial damage is the initiating event, and was suggested by the experimental observations that deliberate

denudation of the endothelium results in the aggregation of platelets which release a growth factor (platelet-derived growth factor, PDGF) which induces the proliferation of smooth muscle cells characteristic of the early fibrous plaque [Sheppard & French 1971, Ross *et al* 1974]. However, subsequent studies showed that the precursor fatty streak develops under an intact endothelium [Davies *et al* 1976], suggesting an alternative mechanism for the initiation of most lesions.

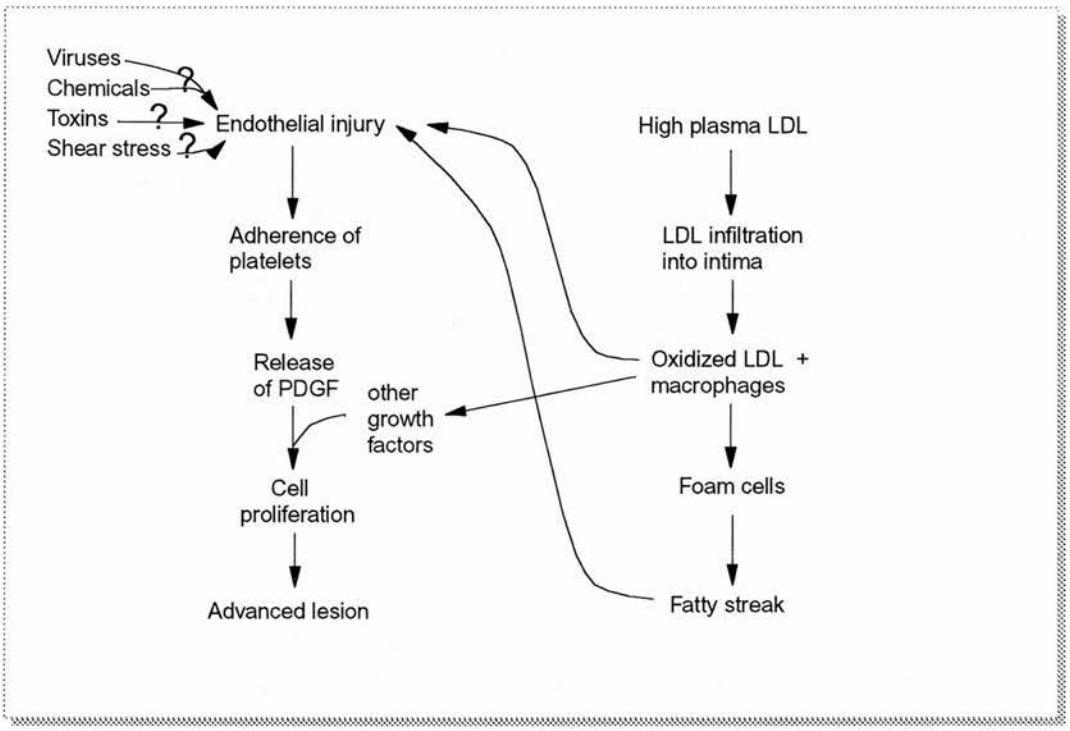
The majority of lipid-laden foam cells in the early human fatty streak have been shown to be derived from monocytes [Aqel *et al* 1984]. In experimental studies in non-human primates the adhesion of circulating monocytes to the arterial endothelium may be observed within days of commencing an atherogenic diet [Faggiotto *et al* 1984]. Following adhesion these monocytes may be seen to migrate into the subendothelial space where they may undergo transformation into macrophages, secreting further chemoattractants, recruiting more platelets and monocytes, and producing free radicals with consequent local intimal damage. Additionally, both the activated macrophage and the endothelial cell may release growth factors which stimulate the migration and proliferation of fibroblasts and smooth muscle cells leading to the development of a proliferative intimal lesion. Proliferating smooth muscle cells themselves have been shown to produce growth factors in some species, suggesting that the atherosclerotic process may then proceed even if the initial stimulus to its formation is no longer present [Hansson *et al* 1989]. Platelets may also contribute to the endothelial-cellular interaction by adhesion to exposed foam cells or connective tissue [Faggiotto *et al* 1984], with subsequent aggregation, formation of mural thrombi, and release of platelet-derived growth factor and other mitogens.

Alternative theories regarding the pathogenesis of atherosclerosis have been proposed. These include a monoclonal origin of cells in an atherosclerotic lesion, with transformation of a single cell by mutagens such as viruses or chemicals. A number of viruses have been shown to be associated with atherosclerotic-like lesions in experimental animals, and evidence of virus antigens have been demonstrated by electron microscopy and immunofluorescence studies in humans with established atherosclerotic disease [Cunningham & Pasternak 1988, Melnick *et al* 1983, Melnick *et al* 1990]. The apparent epidemic nature of coronary disease might, according to this hypothesis, be explained on the basis of food-chain

transmission, and the recent decrease in coronary mortality ascribed to improved sanitation and food hygiene [Mozar *et al* 1990]. In addition to their mere presence in arterial smooth muscle cells in individuals with atherosclerosis, which clearly need not be causative (particularly since such antigens have been identified in only a minority of coronary patients [Melnick *et al* 1983]), there is also evidence of alteration in cellular lipid metabolism and lipid composition and content in human arterial smooth muscle cells [Hajjar & Grant 1986]. Chemicals such as homocysteine may also be associated with increased vascular risk [Mudd & Levy 1983]. While this is of importance in small numbers of patients with inborn errors of methionine metabolism [Harker *et al* 1976, Wilcken & Dudman 1989] and has been reported to be elevated in patients suffering an acute myocardial infarction [Olszewski & Szostak 1988], it is unlikely to be an aetiological factor for most subjects with atherosclerosis [Wilcken *et al* 1983, Dudman *et al* 1990]. The finding of complement antigens in atherosclerotic lesions suggest the possibility of a role for other infectious agents or immunological stimuli [Hollander *et al* 1979]. The observation however that fatty streaks contain few antigens of complement components compared to fibrous plaques [Hansson *et al* 1979, Vlaicu *et al* 1985] suggests that these are more involved in the growth and propagation of the plaque rather than its initiation [Seifert & Kazatchkine 1988]. Neuropsychological factors may also be important, and result in direct pathological changes in the arterial wall, rather than only an indirect effect mediated by behavioural changes [Gutstein 1990].

It is likely that there is more than one initiating event and that the atherosclerotic process may result from a number of types of endothelial injury, including release of cytotoxic factors from a subendothelial collection of modified macrophages, as supported by the observation of cellular retraction over lipid-laden foam cells in animal models [Gerrity 1981, Faggiotto & Ross 1984]. The progression to the more advanced lesion might then proceed mostly due to cellular proliferation and release of growth factors irrespective of the nature of the original injury, although the process is likely to be accelerated in the presence of factors which cause continuing endothelial damage. A 'unifying hypothesis' is presented schematically in fig 1.4 (adapted from Steinberg 1988).

Figure 1.4 Hypothesis of initiation of atherosclerosis



1.1.3 Clinical Manifestations of Ischaemic Heart Disease

Despite the continuing debate about the aetiology and pathogenesis of the atherosclerotic process, the end result is reduction of the arterial lumen by plaques which reduce the myocardial blood supply and are liable to superimposed thrombus formation. The major epicardial coronary arteries, in which atherosclerosis is most often seen to occur, normally offer little resistance to blood flow. Regulation of blood flow is determined in health by vasomotor tone, which may be influenced by neural control, circulating catecholamines, pharmacological, myogenic, and endothelial factors. The normal coronary flow reserve in health means that a reduction of as much as 80% in luminal diameter of a proximal artery may not induce myocardial ischaemia at rest due to dilatation of the resistance vessels distal to the obstruction. However any increase in oxygen demand thereafter will inevitably result in myocardial hypoxia. If the obstruction is less severe there remains some capacity for further dilatation, and the occurrence of ischaemia depends on the extent to which demand is increased. A reduction of

less than 40% of the luminal diameter is usually not associated with reduction in blood flow even at maximal exercise. Thus although blood flow is little affected with mild degrees of stenosis, since flow is inversely proportional to the fourth power of the radius, it decreases precipitously with more severe degrees of narrowing.

In addition to the ischaemia which results when oxygen demand exceeds the ability of the coronary vessels to maintain adequate flow because of fixed arterial obstruction, it may also arise as a consequence of transient reduction of blood flow caused by platelet aggregates or coronary vasospasm. In this way symptoms of myocardial ischaemia may be present at more modest workloads than can be achieved without any symptoms at other times due to these alterations in coronary flow reserve [Maseri *et al* 1985].

One dramatic - and sometimes unheralded - presentation of coronary artery disease is myocardial infarction. The relationship between atherosclerotic narrowing and myocardial necrosis was not fully understood from earlier post-mortem studies (which suffered from an obvious selection bias) [Roberts & Jones 1980, Silver *et al* 1980], or from studies employing coronary angiography in those who had survived one month [Betriu *et al* 1982]. Studies with angiography being performed acutely have demonstrated total occlusion in the artery subtending transmural infarcts in about 90% of cases [DeWood *et al* 1980]. Plaques associated with total occlusion are seen to be more complex and irregular at autopsy than those in arteries not associated with myocardial infarction [Levin & Fallon 1982], and often show evidence of plaque fissuring or rupture [Falk 1983, Davies & Thomas 1985, Onodera *et al* 1989]. Angiographic appearances suggestive of plaque rupture have also been identified in most lesions associated with unstable angina or myocardial infarction, and this finding is uncommon in vessels of patients with chronic stable angina and in noninfarct-related arteries in MI patients [Wilson 1986]. This has been graphically confirmed by angioscopy which has allowed direct inspection of the fissuring plaque [Mizuno *et al* 1991]: occlusive thrombus was observed in all 14 patients with acute myocardial infarction, while the thrombus in unstable angina was more likely to be mural. Xanthomatous plaques were seen in 50% of those with acute coronary syndromes, compared with only 8% of those with previous myocardial infarction

and 15% with chronic stable angina, suggesting that lipid-rich plaques may be more liable to undergo fissure or rupture.

Despite these advances in our understanding, the exact sequence leading from coronary atherosclerosis to myocardial infarction is not clear, but appears to involve a dynamic interaction between obstructing lesion, vasospasm, platelet activation and thrombotic mechanisms [Willerson *et al* 1984, Epstein & Palmeri 1984].

Much attention has been focused on the dramatic presentation of acute coronary insufficiency syndromes, including sudden cardiac death and acute myocardial infarction. However most of the morbidity associated with coronary disease is due to chronic ischaemic heart disease. In addition to the common clinical syndrome of chronic stable angina, the prevalence of significant coronary artery disease in asymptomatic males aged 50 - 70 years with a normal exercise test has been estimated in one population to be 2 - 3% [Diamond & Forrester 1979]. The lack of symptoms does not correlate well with a lesser severity of disease as "silent ischaemia" may be associated with advanced obstructive lesions [Singh 1986].

1.1.4 Natural History

Long-term follow-up studies have shown the average mortality in chronic stable angina to be approximately 4 per cent per annum [Kannel 1973]. The likelihood of fatal events for any individual with symptomatic coronary disease however is quite variable, and depends on left ventricular function and the severity, location and extent of coronary disease; for example, single vessel involvement with normal ventricular function has an annual mortality of around 2%, compared to 7% for single vessel disease with severely impaired LV function, 5% p.a. for three-vessel disease with a normal ventricle or 12% p.a. for those with triple-vessel disease and a left ventricular ejection fraction below 35% [Mock 1982].

Although surgical intervention is superior to medical management in the alleviation of symptoms, and confers an initial improvement in survival in selected groups, i.e.,

those with 3-vessel disease or left main coronary artery stenosis [Chaitman *et al* 1981, Kaiser *et al* 1985], the long-term survival for most patients is not significantly improved [Veterans Affairs Study 1992, Alderman *et al* 1990] (although it is important to recognise the improvements in event-free survival in surgically-managed patients during the period spanned by these studies [Muhlbaier *et al* 1992]). One reason for this is the progression of the atherosclerotic process in the bypass grafts, which may be related to risk factors, such as serum cholesterol levels, identified at the time of surgery [Palac *et al* 1982].

There is a lack of predictability of the progression of any individual lesion: it is recognised that coronary artery disease tends to be progressive, and that severe lesions are more likely to progress than mild ones [Bruschke *et al* 1981]. Although chronic occlusion occurs more commonly with severe stenoses, 87% of severe lesions in a 3-year follow-up study did not occlude [Bissett *et al* 1990]; of those patients who did develop chronic occlusion only 25% had clinical evidence of myocardial infarction. In another study in which serial angiography was performed on three occasions only 19% of patients demonstrated progression of disease during both intervals, and in only 28% of those was the progression in the same lesion [Bruschke *et al* 1989].

Predicting which lesion may cause infarction is no less difficult. It is commonly assumed that the "culprit lesion" will be the one identified at angiography as the most severe, and intervention is frequently undertaken on the basis of this assumption. However recent evidence suggests that this is not the case: a group of patients who had undergone angiography shortly before a myocardial infarct had this repeated at the time of the event, and in only 34% was the artery with the most severe lesion at the time of the initial angiogram the infarct-related vessel and the degree of stenosis in the culprit lesion was less than 70% in no less than 97% of patients [Little 1988]. It is therefore likely to be the morphology of the plaque and its propensity to fissure or rupture which is of greater importance in determining its natural history rather than simply its severity [Davies & Thomas 1985, Lendon *et al* 1991].

1.2 THE ASSOCIATION BETWEEN CHOLESTEROL AND CORONARY DISEASE

1.2.1 Total and Low Density Lipoprotein Cholesterol

1.2.1.1 Epidemiology

Since Anitschow described cholesterol as being a prerequisite for the development of atherosclerosis [Anitschow 1913] the relationship between hypercholesterolaemia and coronary disease has been one of the most intensively studied. The evidence from a large number of cross-sectional and longitudinal studies is entirely consistent, and indicate a direct association between cholesterol levels and incidence of coronary disease. One of the early studies to show this relationship between populations was the Seven Countries Study [Taylor *et al* 1970], which related the incidence of coronary disease to the population mean total cholesterol. Severity of atherosclerosis was related to the level of serum cholesterol in the International Atherosclerosis Project [Holman *et al* 1958]. International cardiovascular mortality is also correlated with mean population serum cholesterol, 45% of the interpopulation variation due to differences in cholesterol levels (and 55% of the variation attributable to variation in the total cholesterol/HDL cholesterol ratio) [Simons 1986].

Studies within populations followed longitudinally have also consistently found the same correlation between serum cholesterol and the likelihood of developing coronary disease [Keys *et al* 1972, Heller *et al* 1984, Shaper *et al* 1985, Gouldbourt *et al* 1985, Kannel *et al* 1986, Rose & Shipley 1986, Livshits *et al* 1989, Pekkanen *et al* 1990, Gouldbourt & Yaari 1990, Wilson *et al* 1991, Shipley *et al* 1991, Assmann & Schulte 1992, Isles *et al* 1992], and indicate a curvilinear association with a significant rise in coronary events being associated with cholesterol levels above 5.2 mmol/l. Some smaller studies have failed to confirm the association of total cholesterol with coronary events, but document the increased risk associated with raised low density lipoprotein cholesterol levels [Hargreaves *et al* 1991]. It has recently been proposed that the strength of the association has been underestimated by failing to take account of regression dilution bias and the 'surrogate dilution effect' [Law *et al* 1994].

Most of this data relates the risk of a first cardiac event to the serum cholesterol level, and it had previously been debated whether the lipids were of prognostic significance in survivors of myocardial infarction. It is established that one of the main determinants of their survival is left ventricular function (see Section **1.1.4**), but there is also observational data demonstrating a powerful predictive effect of total cholesterol and LDL cholesterol levels on cardiac mortality. In a cohort of middle-aged men followed up from the Lipid Research Clinics Prevalence Study the ten-year risk of death from cardiovascular disease for those with pre-existing cardiovascular disease was 3.8% in those with a total cholesterol of < 5.2 mmol/l and 19.6% if total cholesterol > 6.2 mmol/l. This compares with rates of 1.7% and 4.9% respectively for those without pre-existing cardiovascular disease [Pekkanen *et al* 1990]. This is consistent with the strength of this relationship after the first cardiac event seen in other studies [Shaper *et al* 1985, Frost *et al* 1987, Phillips *et al* 1988], and demonstrates an even greater potential for benefit from intervening in this group of individuals.

One of the criticisms of the epidemiological data is the lack of information on women, and that it may be a weaker risk factor for coronary disease in women [Khaw 1993, Crouse 1989]. Some studies have shown that total cholesterol is not significantly related to total or cardiac mortality in women, while serum triglycerides is predictive [Bengtsson *et al* 1993]. This study however looked only at mortality rather than other cardiac events, and followed only 1462 subjects. Other studies using much larger populations with longer duration of follow-up however yield data entirely consistent with that available for men. 45 000 Swedish women were followed up for 18 - 20 years, and showed a highly significant positive relationship between coronary heart disease mortality and baseline blood cholesterol levels [Törnberg 1989]. Others confirm that the relationship between total cholesterol concentration and coronary deaths is at least as strong for women as men, and the relative risks may be slightly greater than those for men even if the absolute and attributable risks may be lower [Stensvold *et al* 1993, Isles *et al* 1992].

1.2.1.2 Familial Hypercholesterolaemia

The importance of elevated cholesterol as a risk factor for the development of coronary disease is nowhere better illustrated than in familial hypercholesterolaemia. This is inherited as an autosomal dominant disorder, with a gene frequency in most populations of 1 in 500 [Brown & Goldstein 1976]. In the classical condition there is a deficiency in the number of cell-surface receptors for LDL. Thus cholesterol concentrations in heterozygotes range from 8 to 12 mmol/l, while the levels in homozygotes are usually above 14 and may be as high as 30 mmol/l [Thompson *et al* 1989, Allen *et al* 1980].

Homozygotes may present with premature atherosclerosis in childhood or adolescence, and untreated the life expectancy is only 17.7 years [Thompson *et al* 1989], with no apparent protective effect from gender [Seftel *et al* 1980]. Some series report a less ominous prognosis, with 50% survival at age 50 - perhaps a consequence of a predominant 'receptor-defective' status in this particular population [Haitas *et al* 1990]. The receptor status is clearly of importance in determining survival, since 26% of 'receptor-negative' subjects die from coronary disease before the age of 25 years, compared to just 4% of 'receptor-defective' individuals [Thompson 1990]. Recent advances in molecular genetics have demonstrated a variety of defects affecting the LDL-receptor gene [Hobbs *et al* 1992]. These defects give rise to differences in plasma cholesterol concentrations resulting in variability of severity of coronary heart disease expression and life expectancy [Moorjani *et al* 1993], and account for the spectrum of survival reported in earlier observational studies.

The incidence of coronary disease is increased more than ten-fold in heterozygotes, so that at least 50% of affected men have died or had a myocardial infarct before the age of 60 [Stone *et al* 1974], and in untreated familial hypercholesterolaemia it was the cause of death in about 70% of subjects [Mabuchi *et al* 1986]. The risk for women is also greatly increased: first manifestations of coronary disease were seen in 5.4% of men by the age of 30, 51% by 50 and 85% by 60 years, compared to 0%, 12% and 58% respectively in women [Slack 1969], the mean age of onset being delayed by about 9 years compared to male heterozygotes [Heiberg 1975, Mabuchi *et al* 1989]. The rates of

coronary heart disease were approximately 20 years earlier than those experienced in the same kindreds with normal cholesterol values [Stone *et al* 1974].

1.2.1.3 Cholesterol-Lowering Intervention Trials

The epidemiological data provide evidence of an association between cholesterol levels and its long-term consequences. It requires a trial of intervention however before any causal relationship may be implied, and much time, effort and money has been expended in attempts to influence coronary disease by lowering serum cholesterol levels. These have been performed almost exclusively in middle-aged men, both in groups free of clinical manifestations of vascular disease (primary prevention) or following myocardial infarction, utilised either dietary modification or pharmacotherapy or a combination, varied with regard to length of follow-up, and employed differing end-points (clinical events, mortality, and angiographic). There are many difficulties inherent in such studies, including the relatively brief period of intervention compared to the decades over which the disease process has evolved, the presence of confounding variables, the relatively modest reductions in cholesterol levels achievable with available therapies, the numbers required to demonstrate the effects of the intervention - particularly on mortality, and the adequacy of surrogate endpoints.

Trials of dietary therapy (Tables Ii and Iii)

There is only one randomised trial of diet in the primary prevention of coronary disease as a unifactorial intervention which has reported CHD incidence as an endpoint [Frantz *et al* 1989]. Although there was a reduction in cholesterol in the experimental group of 15% and a polyunsaturated/saturated fat intake of 1.6 (compared to 0.3 in controls) in a study of 9057 institutionalized men and women conducted over 4.5 years, there was no significant reduction in cardiovascular events or total mortality. However the mean duration of time on the diets was only 384 days, with only 17% of the subjects consuming the diets for two years.

The Lifestyle Heart Trial [Ornish *et al* 1990] incorporated exercise and stress management techniques as well as dietary measures into their intervention group,

TABLE 1i. RANDOMISED TRIALS OF DIETARY THERAPY

Trial	Primary/ secondary	M/F	Age range (years)	Mean age	Follow-up (years)	Mean TC at baseline (mmol/l)	Treatment group n	Control group n
Frantz 1975	Primary	M + F	<30 - >70	---	5	5.40	4922	4853
Dayton 1969	Secondary	M	> 55	66	< 8	6.06	424	422
Leren 1966	Secondary	M	30 - 64	56	5	7.67	206	206
MRC 1968	Secondary	M	< 60	---	2 - 7	7.05	199	194
MRC 1965	Secondary	M	< 65	---	6	6.81	123	129
Rose 1965	Secondary	M + F	< 70	55	2	6.74	54	26
Woodhill 1978	Secondary	M	30 - 59	49	2 - 7	7.31	231	237

Adapted from Holme 1990

TABLE 1ii. OUTCOME OF DIETARY TRIALS

Trial	TC difference (%)	No. deaths treatment n	No. deaths control n	Odds ratio (95% CL)	No. CHD events treatment n	No. CHD events control n	Odds ratio (95% CL)
Frantz 1975	13	268	256	1 (0.867-1.233)	134	129	1 (0.804-1.304)
Dayton 1969	13	174	177	0.978 (0.733-1.267)	60	88	0.682 * (0.494-0.941)
Leren 1966	14	44	51	0.764 (0.498-1.237)	61	81	0.755 (0.544-1.049)
MRC 1968	16	28	32	0.853 (0.479-1.437)	40	39	1 (0.644-1.553)
MRC 1965	6	20	24	0.874 (0.444-1.627)	43	44	1.038 (0.674-1.560)
Rose 1965	4	6	1	2.890 (0.472-12.67)	13	4	1.744 (0.546-4.097)
Woodhill 1978	5	39	28	1.429 (0.901-2.533)	0	0	---

* $p < 0.05$

Adapted from Holme 1990

but did not include pharmacological reduction of cholesterol levels. Smoking intervention was also planned, though only one of the 28 subjects in the treatment group smoked on admission to the study, and she stopped at baseline. The endpoint in this study was however angiographic changes rather than CHD events and will be discussed further below (Sect **1.5.2**).

The Oslo diet-heart study also eschewed lipid-lowering drug therapy, but combined rigorous dietary changes with smoking intervention [Hjermann *et al* 1981]. In this study 1232 severely hypercholesterolaemic (but otherwise healthy) middle-aged men were followed for 7.5 years. Cholesterol was lowered by 13% in the intervention group, and 25% in this group reported that they had stopped smoking compared to 17% in the control group. A significant reduction in nonfatal and fatal myocardial infarction of 42% was observed, with a decrease in cardiac deaths of 56%. Follow- up at 15 years reveals this ultimately results in a significant decrease in total mortality.

Both the MRFIT [Multiple Risk Factor Intervention Trial Research Group 1982] and the WHO multifactorial study [WHO Collaborative Group 1983] included dietary lipid reduction in the intervention groups, while using drugs to lower blood pressure when indicated. The cholesterol reductions achieved in these very large studies were only 2% and 1% respectively. MRFIT showed a non-significant decrease in cardiac events with a non-significant excess of total mortality, while the WHO trial - involving 49,784 men followed for 5 - 6 years - revealed a statistically significant increase in overall mortality. A follow-up study in MRFIT after 10.5 years did result in a significant reduction in all-cause mortality, but no difference in cardiac death rate [Multiple Risk Factor Intervention Trial Research Group 1990]. Another large randomised study of over 10,000 men subjected to reductions in cholesterol, weight, and blood pressure over ten years also failed to demonstrate any significant change in coronary mortality [Wilhelmsen *et al* 1986]. Although reductions in cholesterol, blood pressure and smoking habit were achieved in the intervention group there was also a reduction in risk factors in the control group, and within the intervention group the successes were diluted by an excess mortality amongst non-participants.

Since the cardiac event rate in hypercholesterolaemic subjects is greater after the first cardiovascular event (Sect **1.2.1.1**), it might be anticipated that the effects of lipid intervention might be more readily seen in this group. A number of trials of dietary treatment in such patients have been carried out. These have involved smaller numbers of subjects, but some have reduced mean cholesterol levels by approximately the same extent as the drug trials. One of these, the Los Angeles Veterans Administration Study, randomised 846 men with a mean age of 65 to receive a different proportion of their fat intake as polyunsaturates [Dayton *et al* 1969]. The event rate in this study was particularly high - 41% mortality in each group over the eight year follow-up period. Although this was not significantly different between the groups, there was a significant reduction in combined endpoints of nonfatal and fatal atherosclerotic events from 20.9% to 14.2%, a reduction of 32%. In another study in Finland which achieved a 14% reduction in cholesterol by diet, coronary events were 53% lower in men and 34% in women, although neither the cardiac events nor total mortality overall was significantly different [Turpeinen 1979]. The baseline characteristics of the participants and the results of these and smaller dietary studies are presented in Tables 1i - ii.

Trials of Drug and Surgical Therapy (Tables 1iii and 1iv).

Several trials of primary prevention using drug therapy are now completed and others are in progress. The first was a WHO trial started in 1965, in which middle-aged men with cholesterol levels in the upper tertile were randomised to receive clofibrate or placebo, and compared to a group of equal size with serum cholesterol levels in the lowest tertile who were also given placebo [Committee of Principal Investigators 1978]. Cholesterol was lowered by just 9%; nonfatal and fatal myocardial infarctions combined were reduced by 20% (from 3.93% to 3.13%), due almost entirely to the reduction in nonfatal infarcts. However there was an excess of noncardiac deaths in this study due to cancer and deaths from violence and total mortality was significantly increased, although there was no difference in age-standardized mortality in these categories between the intervention group and the second control group. The adverse mortality outcome continued long after discontinuation of the drug intervention [Committee of Principal Investigators 1980].

TABLE 1iii. RANDOMISED CLINICAL TRIALS OF NON-DIETARY THERAPY

Trial	Primary/ secondary	M/F	Age range (years)	Mean age	Follow-up (years)	Mean TC at baseline (mmol/l)	Treatment group n	Control group n
WHO 1978	Primary	M	30 - 59	45	5.3	6.42	5331	5296
LRC-CPPT 1984	Primary	M	35 - 59	47	7 - 10	7.23	1906	1900
Frick 1987	Primary	M	30 - 59	47	5	7.49	2051	2030
Brown 1990	Mixed	M	< 62	47	2.5	6.95	74	46
SSP 1971	Secondary	M + F	40 - 69	52	6	6.89	264	273
CDP 1975	Secondary	M	30 - 64	54	4.5 - 8	6.45	2222	2789
Newcastle 1979	Secondary	M + F	< 65	52	5	6.45	244	253
Dorr 1978	Secondary	M + F	> 18	54	3	7.95	1149	1129
Carlson 1988	Secondary	M + F	< 70	59	5	6.40	279	276
Buchwald 1990	Secondary	M + F	30 - 64	51	7 - 10	6.49	421	417

Adapted from Holme 1990

TABLE 1iv. OUTCOME OF NON-DIETARY TRIALS

Trial	TC difference	No. deaths		Odds ratio	No. CHD events		Odds ratio
		treatment	control	(95% CL)	treatment	control	(95% CL)
	(%)	n	n		n	n	
WHO 1978	9	128	87	1.462 * (1.119-1.921)	167	208	0.791 * (0.640-0.977)
LRC-CPPT 1984	9	68	71	0.955 (0.679-1.337)	155	187	0.811 (0.669-1.022)
Frick 1987	10	45	42	1.060 (0.694-1.624)	56	83	0.658 * (0.481-0.936)
Brown 1990	39	1	0	---	5	11	0.73 * (0.23-0.90)
SSP 1971	14	34	38	0.934 (0.574-1.520)	59	76	0.778 (0.535-1.131)
CDP 1975	8	554	709	0.981 (0.857-1.108)	596	839	0.892 * (0.804-0.990)
Newcastle 1979	13	27	48	0.583 * (0.331-0.883)	55	89	0.646 * (0.467-0.897)
Dorr 1978	10	37	48	0.751 (0.487-1.158)	--	--	---
Carlson 1988	13	61	82	0.744 * (0.524-1.017)	83	127	0.647 * (0.472-0.909)
Buchwald 1990	23	49	62	0.783 (0.611-1.950)	82	125	0.650 * (0.453-0.979)

* $p < 0.05$ Adapted from Holme 1990

Between 1973 and 1976 the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) screened over 480,000 men from twelve lipid clinics across the U.S.A. to find 3806 middle-aged men with a plasma cholesterol above the 95th percentile, LDL cholesterol of at least 4.9 mmol/l, triglycerides below 3.5 mmol/l, and free from heart disease, hypertension and diabetes. They were randomized after a period of dieting to receive either cholestyramine 24g daily or placebo and followed for seven to ten years. Total cholesterol was reduced by 9% compared to the control group, and LDL cholesterol by 13%. The combined endpoint of coronary death or nonfatal myocardial infarction was reduced from 8.6% to 7%, a reduction of 19%, which was significant. Total mortality was reduced also by 7%, but this failed to achieve statistical significance [Lipid Research Clinics Program 1984a]. The power of the study was hampered by the small cholesterol-lowering effect due to a minority of the subjects being able to tolerate the full dose of the resin and fewer deaths than expected in the control group. An accompanying analysis [Lipid Research Clinics Program 1984b] showed a dose-response relationship between cholesterol reduction and coronary events: those individuals who were able to tolerate the prescribed dose of cholestyramine experienced a 19% reduction in cholesterol and a 39% reduction in coronary events. The study also documented significant reductions in secondary endpoints, such as development of angina, new positive exercise tests and number of subjects referred for coronary surgery.

The Helsinki Heart Study entry criteria were very similar to the LRC-CPPT, but specified the elevation in cholesterol at baseline as non-HDL cholesterol, and the trial attempted to test the efficacy of simultaneously raising HDL while lowering non-HDL cholesterol [Frick *et al* 1987]. 19,000 men were screened, and randomised to gemfibrozil 1.2g or placebo if the non-HDL was persistently above 5.2 mmol/l and there was no clinical evidence of heart disease. Men with hypertension and "mild" non-insulin-dependent diabetes were not excluded from this study. Total cholesterol in the study group was lowered by 10%, non-HDL cholesterol by 14%, and triglycerides by 43%; HDL-cholesterol increased over the study period by a mean of 10%. Despite a dropout rate of 30% in both groups over the five years of the trial cardiac end points were reduced by 34% from 4.14% to 2.73%, due to fewer nonfatal infarctions with no change in coronary mortality.

Although the Familial Atherosclerosis Treatment Study was designed as an angiographic study - and discussed further below (Sect **1.5.2**) - it employed a randomised design and described clinical endpoints in addition to the angiographic changes [Brown *et al* 1990]. The subjects were included irrespective of their personal history of vascular events, so included 45% with a history of previous myocardial infarction. The authors randomized 146 men with elevated apoprotein B levels, angiographic evidence of coronary disease, and a family history of vascular disease to one of three treatment groups: combined colestipol and niacin, combined lovastatin and colestipol, or placebo (or colestipol only if LDL cholesterol above 90th percentile). LDL cholesterol fell by 32% while HDL rose by 43% in the colestipol /niacin group, and changes for the lovastatin/resin group were 46% and 15% respectively. Treatment in one of the active arms reduced the incidence of clinical events (death, myocardial infarction, or new ischaemia requiring peripheral or coronary bypass or angioplasty) by 73% (p=0.01).

One multifactorial primary prevention study from Finland utilised drug therapy for hyperlipidaemia (clofibrate or probucol) and hypertension when indicated, in addition to advice about smoking, exercise, and diet. Despite a fall in predicted risk from risk factor scores, the intervention group suffered more myocardial infarctions and cardiac deaths [Miettinen *et al* 1985]. Follow up for ten years after the intervention period has revealed progressively increasing cardiac deaths and total mortality in the group allocated to special intervention compared with controls, and that this adverse result is statistically significant [Strandberg *et al* 1991].

Clofibrate has featured prominently also in secondary prevention trials. In the Stockholm Ischaemic Heart Disease Secondary Prevention Study consecutive survivors of myocardial infarction were randomised to receive open clofibrate and niacin or no lipid-lowering therapy [Carlson & Rosenhamer 1988]. Cholesterol was reduced by 13% and triglycerides by 19%; there were significant reductions in cardiac events, cardiac deaths and total mortality. A sizable subgroup of patients with reductions in triglycerides of at least 30% experienced a 60% reduction in CHD mortality. Interestingly in this study decrease in IHD deaths was directly related to the degree of triglyceride lowering, but was not related to the decrease in cholesterol levels.

Two other trials of clofibrate therapy were carried out in the UK in middle-aged men and women [Research Committee of SSP 1971, Group of Physicians of Newcastle 1971]. The former showed reduced mortality and combined events in those with angina and in those with recent MI with preceding angina; in the second of these studies there were significant reductions in cardiac events and also in total mortality. Although the degree of cholesterol-lowering in each study was similar, in neither study was this related to the reduction in events.

The Coronary Drug Project, carried out between 1966 and 1975, assessed the efficacy and safety of five different drug regimens in the secondary prevention of coronary disease in 8341 male survivors of a myocardial infarction in 53 centres in the US and Puerto Rico [CDP Research Group 1975]. The groups assigned to two doses of oestrogen and that to dextrothyroxine were discontinued prematurely because of adverse events, particularly adverse trends in total mortality. The groups taking clofibrate, niacin or placebo continued to the planned end of the study period, a mean follow-up of 6.2 years. There was no benefit in either active treatment group with regard to overall mortality on study completion compared to the placebo group, but patients in the niacin arm had a significantly lower incidence of nonfatal myocardial infarction. A follow-up study nine years after termination of the trial - initiated to detect any adverse trends prompted by excess cancer mortality in the low-dose oestrogen group, and also the adverse results on total mortality in the WHO clofibrate trial - revealed an 11% reduction in total mortality in the niacin group compared to placebo ($p = 0.0004$) [Canner *et al* 1986].

Although compliance may have been a limiting factor in achieving the reductions in cholesterol which had been anticipated in several of the trials, this was not a feature of the POSCH Study (Program on the Surgical Control of the Hyperlipidemias). They randomised 838 survivors of a first myocardial infarct to either diet alone, or dietary instruction plus partial ileal bypass surgery, to test whether the consequent cholesterol reduction would favourably alter coronary mortality. In addition to serial coronary angiography, they reported on mortality and cardiac events after a follow-up of 7 to 14.8 years. Despite reductions in total mortality of 21% and in cardiac mortality of 27%, these failed to reach statistical significance. The combined endpoint of coronary deaths and myocardial infarction however was reduced by 35%, a result which is highly significant, the more so

considering that by ten years 31.5% of the control group were taking lipid-lowering medication [Buchwald *et al* 1990].

Overview analyses of the randomised unconfounded trials involving cholesterol reduction as the sole intervention both in secondary and primary prevention studies show that a reduction in coronary heart disease of 21% has been achieved, irrespective of the method used to reduce the cholesterol [SMAC Report 1990, pp 29-31]. The intervention studies thus give results consistent with the epidemiological data relating cholesterol levels to coronary disease, and help provide evidence for a causal relationship.

1.2.1.4 Animal Studies

Atherosclerosis affects man to a much greater extent than other animals, at least in part due to the greater cholesterol levels encountered compared to other species. Several animal models have been studied in experimental atherosclerosis, but many of these are limited by anatomical and metabolic differences. Nonhuman primates appear to be the most suitable for study, and readily develop lesions that are similar in structure and distribution to human disease [Faggiotto & Ross 1984]. Arterial lesions which are induced in rhesus monkeys by feeding an atherogenic diet may be shown to regress when the diet is altered to maintain the cholesterol level at a lower value [Clarkson *et al* 1984]. Studies in genetically hyperlipidaemic rabbits - metabolically resembling familial combined hyperlipidaemia - which spontaneously develop atherosclerosis, have shown that normalising cholesterol levels by administration of an HMG CoA-reductase inhibitor almost completely prevents the development of disease [La Ville *et al* 1989]. The extent of arterial disease in this study was shown to be directly related to the concentration of LDL cholesterol.

1.2.2 The Association with High Density Lipoprotein

Several epidemiological studies have reported an inverse relationship between serum HDL-cholesterol and cardiovascular events [Livshits *et al* 1989, Gouldbourt

et al 1985, Castelli *et al* 1986, Heiss *et a*l 1980, Simons 1986, Tyroler *et al* 1988, MRFIT 1982, Hargreaves *et al* 1991, Assmann & Schulte 1992]. This relationship has been found to hold for men both with and without evidence of cardiac disease at the time of recruitment [Pekkanen *et al* 1990, Phillips *et al* 1988]. In some populations the predictive value of HDL was higher than that for total cholesterol [Livshits *et al* 1989, Nikkilä *et al* 1990, Hargreaves *et al* 1991], and the HDL/TC ratio has been shown to be of value in others [Gouldbourt *et al* 1985, Simons 1986, Nikkilä *et al* 1990]. Within populations at high risk of coronary events the lipoprotein fraction with the greatest predictive value is HDL [Manninen et al 1990]. Some studies reported a lack of a relationship [Keys *et al* 1984, Levy & Klimov 1987, Pocock *et al* 1986, Shaper *et al* 1985] between HDL and coronary events, although in some cases this may be due to analytical differences [Gordon *et al* 1989], and the conclusions from one study were subsequently revised [Pocock *et al* 1989]. There are also reports which demonstrate no clear relationship between HDL level and risk of coronary disease when comparing high- and low-risk populations [Kesteloot *et al* 1985, Lewis *et al* 1978], and also a lack of risk associated with reduced HDL levels in populations with low serum LDL [Robinson & Williams 1979, Sacks *et al* 1975]. Amongst survivors of myocardial infarction, one of the commonest dyslipidaemias is isolated low HDL, and this has been found also in patients with desirable levels of total cholesterol undergoing coronary angiography [Miller *et al* 1990].

There is a dichotomy in the effects of low HDL levels resulting from genetic causes since Tangier disease is not associated with increased coronary risk [Schaefer *et al* 1980], but both familial apolipoprotein A-I deficiency and apoC-III deficiency result in severe premature atherosclerosis [Norum *et al* 1982, Schaefer *et al* 1982, Matsunaga *et al* 1991]. Some mutations in apoA-I are associated with low HDL but not with increased atherosclerosis [Funke *et al* 1991], and other investigators report very low HDL levels (< 0.38 mmol/l) without known genetic abnormality or evidence of premature atherosclerosis [Rader *et al* 1993].

Comparisons with other species indicate that many animals that are resistant to the development of atherosclerosis have HDL as the predominant lipoprotein, and certain strains which are susceptible to diet-induced atheroma have lower levels than those which are resistant [Chapman 1986, Lusis *et al* 1983].

The effects of intervention with diet or drugs suggest that raising serum HDL may be beneficial [Blankenhorn *et al* 1990, Manninen *et al* 1988, Levy *et al* 1984], perhaps particularly for those with low HDL levels at baseline [Manninen *et al* 1990], although some agents which consistently lower HDL levels may have antiatherogenic effects [Yamamoto *et al* 1986]. The infusion of homologous HDL into cholesterol-fed rabbits however has been shown to reduce established atherosclerotic lesions [Badimon *et al* 1990].

There are therefore several strands of evidence to suggest that high HDL levels are protective, and have anti-atherogenic activity. However it is not a good predictor of risk between populations, there is not a clear biochemical mechanism, and genetic disorders resulting in low HDL levels are not universally associated with increased risk of atherosclerosis.

1.2.3 The Association with Lipoprotein(a)

Lp(a) has been described as an independent risk factor for coronary artery disease [Rhoads *et al* 1986, Rosengren *et al* 1990), although the associated risk may be dependent on the co-existence of elevated LDL cholesterol [Armstrong *et al* 1986]. It has been shown to be less discriminating than apoB or apoA-I, but does explain much of the association between parental history of ischaemic heart disease and coronary risk [Durrington *et al* 1988]. There is evidence of a relationship between the serum Lp(a) level and the extent of angiographically-defined coronary artery disease [Armstrong *et al* 1986, Frick *et al* 1978, Dahlen *et a*l 1986, Hearn 1990] - including the development of disease in aortocoronary saphenous vein grafts [Hoff *et al* 1988], and much to support an association with myocardial infarction [Dahlen *et al* 1975, Rhoads *et al* 1986, Durrington *et al* 1988, Berg *et al* 1977, Kostner *et al* 1981]. In patients with heterozygous familial hypercholesterolaemia the median level of Lp(a) was two to three times greater in those with symptomatic coronary heart disease compared to patients without evidence of disease and the apo(a) level was the most significant variable distinguishing between the groups [Seed *et al* 1990, Wiklund *et al* 1990].

1.3 LIPOPROTEIN METABOLISM

1.3.1 Absorption and Synthesis of Cholesterol

Cholesterol is a solid alcohol, and is the main sterol present in man. It is ubiquitous in the tissues, being an integral part of cell membranes. It also serves as precursor for steroid hormones and for bile salts, required for the absorption of fats and fat-soluble vitamins. Thus every cell requires constant access to a pool of cholesterol. This pool may be expanded from two sources: either preformed sterol is absorbed from dietary sources, or the cholesterol is synthesized *de novo* from acetyl-CoA in a variety of tissues, principally the liver (although almost all cells retain the capacity for cholesterol synthesis).

The typical Western diet contains 0.2 - 1.0 grams of cholesterol per day, and 70 - 160 grams of fat which contains a high proportion of saturated animal fat, the mean polyunsaturated:saturated fat ratio being 0.24 [Miettinen & Kesäniemi 1989]. In order to be absorbed cholesterol is solubilised by formation of mixed micelles, which contain unesterified cholesterol, fatty acids, monoglycerides, phospholipids and conjugated bile salts. 25 - 75% of the ingested cholesterol is absorbed [Miettinen & Kesäniemi 1989], maximum absorption occurring in the middle and terminal ileum [Stein 1987]. After its absorption into the enterocyte, the cholesterol is re-esterified within this cell and re-assembled into large chylomicron particles, which enter the venous circulation via the lymphatic system [Dietschy & Wilson 1970].

Cholesterol absorption increases linearly with the dietary cholesterol ($r=0.836$, $p < 0.001$), but is negatively correlated with body mass index. There is also a strong positive association with total fat intake, but no relationship between cholesterol absorption and the P:S ratio [Miettinen & Kesäniemi 1989]. Fractional cholesterol absorption was correlated with serum total cholesterol, LDL and HDL, although only HDL_2 cholesterol was significantly associated with absolute dietary cholesterol absorption.

The requirements for cholesterol synthesis are very variable, and depend on the intracellular cholesterol pool. When this is high, the synthetic enzymes are

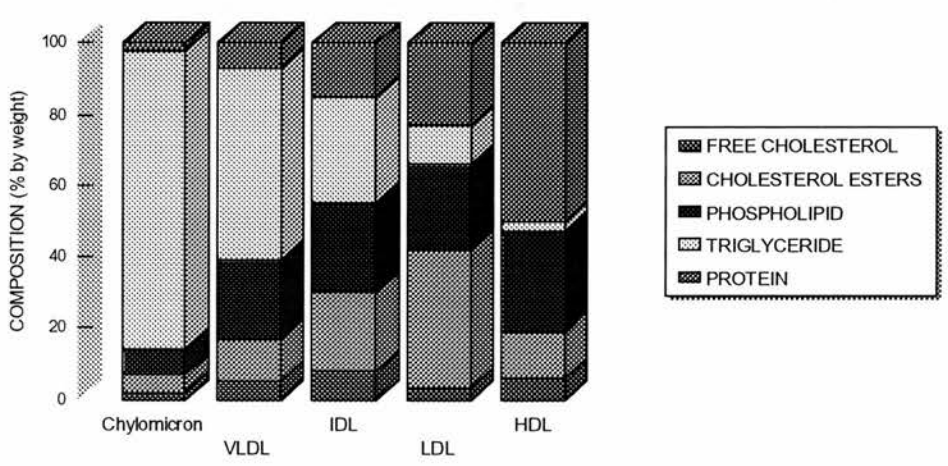
suppressed, particularly hydroxymethyl-glutaryl Coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis. The synthetic rate is highly dependent on the absorption of dietary cholesterol - a highly significant negative correlation is observed ($r = -0.643$, $p < 0.001$).

Calculation of the regression equations show that for every increase of 1 mg/kg/day in absorption of dietary cholesterol, synthesis is reduced by about 2.2 mg/kg/day [Miettinen & Kesäniemi 1989], although the response is non-linear.

1.3.2 Structure of Lipoproteins

Since lipids are insoluble in plasma, their transport to the tissues where they are required demands some hydrophilic adaptation. They are thus incorporated into complex micellar structures consisting of an outer layer of water-soluble elements - protein, phospholipid and free cholesterol - surrounding an inner hydrophobic core of cholesterol esters and triglycerides. The lipoproteins consist of a series of these structures, which continuously undergo hydrolysis and exchange constituents both from the core and from the particle coat. The lipoproteins have a distinctive composition, both in terms of their lipid content and the associated apoproteins (See Fig1.5 - adapted from Stein 1987).

FIG 1.5 LIPOPROTEIN COMPOSITIONS



Chylomicrons are large triglyeride-packed particles with small amounts of cholesterol, phospholipid and protein. The proteins in the nascent particle, apo AI and apo B, both differ from the mature forms found in the circulation. The apo AI is secreted as a proprotein which has an attached oligopeptide which is cleaved in the circulation [Bojanovski *et al* 1985]. The apo B differs from that of hepatic origin in being only 48% of the molecular weight of the latter species (hence 'B_{48}'). Immunological study shows that apo B_{48} represents the N-terminal half of apo B_{100}, so that the receptor-binding domain is absent [Hospattanker *et al* 1986]. On entering the circulation the chylomicron rapidly acquires apoproteins C and E, largely by transfer from HDL, which are vital for its metabolism [Havel *et al* 1973].

The liver secretes lipoproteins chiefly in the very low density range, the so-called 'VLDL' being the principal transporters of endogenously derived triglyeride. Although these particles exist as a continuum, they may be separated into major classes on the basis of their physical and chemical properties by their size, electrophoretic mobility, or flotation density in a centrifugal field (Table 1v).

Large VLDL (or 'VLDL 1') contains relatively more triglyceride than the smaller species, and may undergo hydrolysis in the circulation to small VLDL ('VLDL 2'). Each has one apo B_{100} moiety associated with it, and also a significant amount of apo C and apo E. The function of the C apoproteins appears to be as a cofactor for lipoprotein lipase, and inhibiting the binding of VLDL to hepatic receptors. Apo E exists as three major isoforms (E_2, E_3, and E_4) and is essentially a ligand for the B/E receptor.

Intermediate density lipoprotein ('IDL') are smaller particles than VLDL, contain a higher proportion of apoprotein - more of which is apoB - and smaller amounts of triglyeride. The density spectrum of 'low density lipoprotein' may be arbitrarily subdivided into at least four subspecies of slightly varying composition and metabolic behaviour. The associated apoprotein is virtually exclusively apoB, and almost half of the composition of the particle by weight is esterified cholesterol.

While the formation and fate of LDL is reasonably well understood, less is known about high density lipoprotein (HDL). It consists of small heterogeneous particles which are rich in apoprotein AI and AII, with small amounts of apo C and apo E.

TABLE 1v. PROPERTIES OF SERUM LIPOPROTEINS

Measurement	Chylomicron	VLDL	IDL	LDL	HDL
Hydrated density (g/ml)	0.93	0.97	1.003	1.034	1.121
Solvent density for isolation (g/ml)	< 1.006	< 1.006	1.006 - 1.019	1.019 - 1.063	1.063 - 1.21
Molecular weight	(0.4-30) x E+9	(5-10) x E+6	(3.9 - 4.8) x E+6	2.75 x E+6	(1.75 - 3.6) x E+
Diameter (nm)	> 70	25 - 70	22 - 24	19.6 - 22.7	4 - 10
Electrophoretic mobility	Origin	Pre-beta	broad-beta	beta	alpha

It was found that the LDL of some individuals contained an antigen - Lp(a) - not found in others [Berg 1963]. Further support that this was a distinct lipoprotein species came with the description of an electrophoretic band moving between the beta and prebeta lipoproteins [Dahlen *et al* 1975]. This lipoprotein has since been widely investigated, and has been shown to consist of an LDL-like particle with an associated apoprotein, apo(a) [Gaubatz *et al* 1983], thought to be linked to the apoB-100 by a disulphide bond. Apo(a) exhibits structural homology with plasminogen, consisting largely of repeating sequences of one of the plasminogen kringles [Eaton *et al* 1987]. Its average molecular mass varies widely between individuals due to a series of genetic isoforms, and the expression of these isoforms, at least in part, influence the serum concentration of Lp(a) [Utermann *et al* 1987].

1.3.3 Metabolic Pathways of Lipoproteins

The 'remnant' of the chylomicron particle, having undergone hydrolysis by the action of hepatic lipase and endothelium-bound lipoprotein lipase and exchange of components with the circulating lipoproteins, is taken up by the liver by a receptor protein, LDL receptor-related protein (LRP). This is thought to be specific for the remnant particle, but is not yet fully established [Brown *et al* 1991]. This recognises and binds the apoE (transferred to the particle with apoC from HDL) constituent of the remnant particle, which is degraded within the hepatocyte lysosomes.

Cholesterol derived either from exogenous or endogenous pathways may be excreted in bile, stored within the cell, or secreted with triglycerides in VLDL. The control of the secretion process is poorly understood, although there is some evidence to show a degree of diurnal variation [Parker *et al* 1982] as well as hormonal influences [Taskinen *et al* 1990]. Increased availability of fatty acids (either dietary or newly-released from adipose tissue) determines the rate of synthesis of triglycerides. VLDL is secreted as heterogeneous particles of varying size and composition [Hamilton 1983], containing apoB-100 and also apoproteins C and E which are augmented by transfer from HDL. Increased triglyceride production results in the production of larger VLDL particles with an increased triglyceride/protein ratio [Melish *et al* 1980].

VLDL catabolism is similar to that of chylomicrons in that they undergo hydrolysis in the circulation by lipoprotein lipase using the same cofactor, apoC-II. Studies of radiolabelled apoB in VLDL indicate that the apoprotein moiety is associated with a spectrum of particles, with the apoB being transferred progressively from $VLDL_1$, (Sf 60-400) through '$VLDL_2$' (Sf 20-60) and IDL (Sf 12-60) to LDL [Berman *et al* 1978]. During this process triglyceride is released to the peripheral tissues by lipolysis [Nilsson-Ehle *et al* 1980], but also exchanged for cholesterol ester from HDL by the action of cholesterol ester transfer protein [Eisenberg 1985]. ApoC and phospholipid from the surface of the VLDL also exchange with apoE from HDL [Berman *et al* 1978]. The formation of IDL from VLDL is analogous to the metabolism of chylomicrons, and IDL particles are commonly referred to also as 'remnants'. A schema for lipoprotein metabolism is illustrated in Fig 1.6.

Both lipoprotein lipase and hepatic triglyceride lipase play a role in VLDL metabolism. Studies in individuals with enzyme deficiencies show that the former has a greater affinity for large, triglyceride-rich particles while the hepatic lipase favours smaller VLDL. Thus patients with reduced lipoprotein lipase activity accumulate large particles rich in triglycerides and smaller lipoproteins of greater density disappear rapidly from plasma, while those with hepatic lipase deficiency exhibit reduced clearance of small VLDL and IDL, with low LDL levels [Carlson *et al* 1986, Demant *et al* 1988, Demant *et al* 1991].

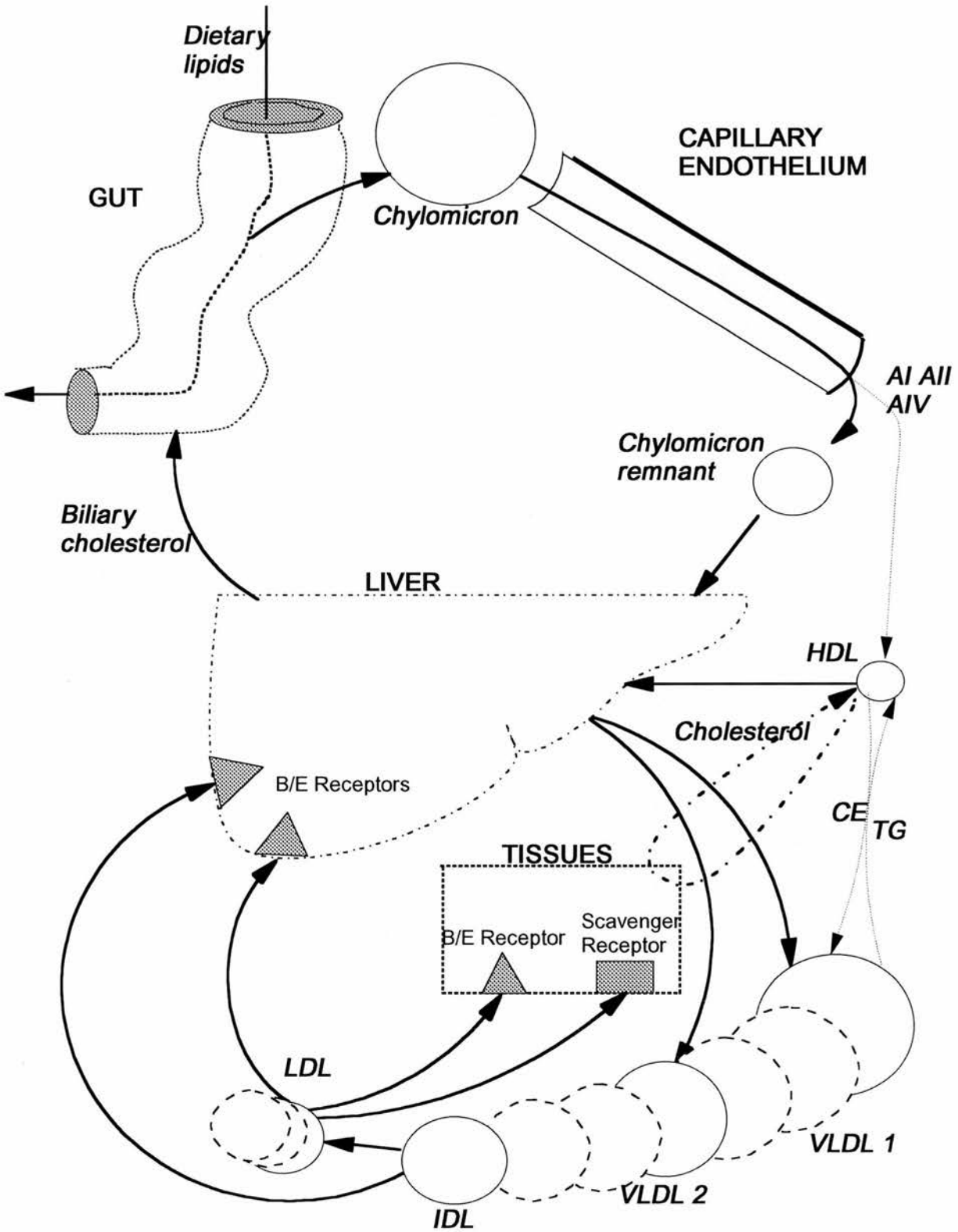


FIG 1.6 LIPOPROTEIN METABOLISM

VLDL metabolism is also affected by the apoE component, which exists in three distinct polymorphisms. These have differing affinities for the apo B/E receptor sites, and this significantly alters the clearance rates of the lipoproteins [Demant *et al* 1991]. This is best exemplified by type III hyperlipidaemia ('remnant hyperlipidaemia'), in which individuals with E2/E2 genotype develop accumulation of VLDL with a high cholesterol/triglyceride ratio.

Studies in most individuals show that a considerable proportion of the apoB in VLDL does not complete the transformation into LDL [Packard *et al* 1984], due to significant removal of VLDL remnants from the circulation. There appears to be heterogeneity in the metabolism of the VLDL, with a greater part of the LDL being derived from relatively rapid transformation of smaller VLDL [Demant *et al* 1988].

The weight of evidence suggests that LDL is formed mostly by the metabolism of VLDL; it has been postulated that there may be direct hepatic synthesis of LDL, and that this may be of importance in some hypercholesterolaemic states [Janus *et al* 1980, James *et al* 1989]. As noted above, the density range of LDL encompasses a number of subfractions separable by density gradient ultracentrifugation and gradient gel electrophoresis [Krauss & Burke 1982, Griffin *et al* 1990]. Elevated triglyceride levels and a predominance of $VLDL_1$ appears to give rise to an increased proportion of smaller, more dense LDL, while the more bouyant LDL subfractions may arise from the metabolism of small VLDL.

The metabolic fate of LDL has been extensively investigated. Tracer kinetic studies [Shepherd *et al* 1979, Kesaniemi *et al* 1983, Slater *et al* 1984] have shown that most of the LDL is removed from the circulation by the apoB/E receptor [Goldstein & Brown 1977], and that this is deficient in heterozygous familial hypercholesterolaemia and absent in the classical homozygous state. In normals the activity of this receptor is closely regulated by the intracellular pool of sterols. Receptor production and expression is increased when the requirement for cholesterol rises, and is down-regulated (with HMG CoA reductase activity) when sterol pools are replete [Clarke *et al* 1987]. Since its discovery, a large number of different mutations of the apoB/E receptor have been described which give rise to familial hypercholesterolaemia [Hobbs *et al* 1992]. Binding to the receptor may also be impaired due to defects in the apoproteins [Innerarity *et al* 1990], with consequent

reduction in receptor-mediated clearance. It is likely that minor alterations in structure of either the receptor or its ligand account for some of the variation in lipoprotein levels in moderate 'non-familial' hypercholesterolaemia.

LDL not removed by these specific receptors is catabolised by 'scavenger' pathways by macrophages; it is thought these preferentially take up modified LDL, since these cells have little affinity for native LDL [Goldstein & Brown 1977]. An increase in the proportion of LDL removed by this pathway has considerable potential for atherogenicity [Steinberg *et al* 1989].

Lp(a) appears to be synthesised in and secreted from the liver. Little is known of the influences affecting its synthetic rate, and the metabolic fate of apo(a) is not known. The Lp(a) level in plasma is inversely correlated with the size of the apo(a) protein, which is determined by the apo(a) genotype.

Nascent HDL particles are produced by the liver. The factors controlling this process and the production of its apoproteins have not been elucidated. The role of HDL in the transport of cholesterol and its interactions with the apoB-containing lipoproteins is reviewed in **1.4.3**.

1.3.4 Kinetic Studies of Lipoprotein Metabolism

Early attempts to study lipid metabolism employed labelled precursors and gave information only about whole body turnover rates. More recently investigators have employed exogenous labelling of apoproteins with regular sampling of blood following re-injection to calculate the rate at which the isotope disappears from plasma, the fractional catabolic rate (FCR), expressed as pools per day. Although having been applied previously to lipoproteins, its potential was only appreciated after the description of the synthetic and catabolic rates of LDL apoB in 1972 [Langer *et al* 1972] and the technique has since been used to investigate lipoprotein kinetics in a number of different conditions.

The validity of the method has been rigorously examined, and assumes that the labelling process does not *per se* alter the metabolism of the tracee, that the site of catabolism is in rapid eqilibrium with the plasma compartment, and that the pool of

the lipoprotein under study is in steady state. To date, it is generally accepted that iodination of the protein moiety of lipoproteins does not influence the immunological, electrophoretic or flotation characteristics, and does not affect the properties on chromatography [Langer *et al* 1972, Eisenberg *et a*l 1973, Kissebah *et al* 1982, Nestel *et al* 1983].

Labelling of LDL apoB with radioiodine by the iodine mono-chloride method [Mcfarlane 1958] has shown that virtually all the radioactivity is associated with the apoB. The radioactivity remains associated with the LDL fraction following re-injection, and the plasma decay curve gives an estimate of LDL FCR. Since VLDL contains significant quantities of other apoproteins (particularly C and E) these also acquire some of the label. Following re-injection, the apoB is transferred to other lipoprotein fractions while the non-apoB components are transferred mostly to HDL. The specific activity of apoB however is calculated in each lipoprotein fraction after subfractionation by density-gradient ultracentrifugation and chemical separation of the apoprotein (see **3.1.1**), so that all the radioactivity detected is associated with apoB. In view of the heterogeneity of behaviour observed within VLDL, $VLDL_1$ and $VLDL_2$ are labelled separately.

Most of the lipoprotein fractions display bi-exponential kinetics when plotted on semi-logarithmic paper, and simple regression may be used to determine the lines of best fit to the exponentials. The FCR can then be calculated by the method of Matthews [Matthews 1957]. This is however not adequate to analyse all the relationships between fractions in VLDL metabolism, and the specific radioactivity curves obtained may best be analysed by multicompartmental modelling which generates a number of kinetic parameters to describe quantitatively the metabolism of each of the fractions. The model was first proposed in 1975 [Phair *et al*], and since then has increased in complexity as it has been adapted to incorporate new experimental observations. The model used for the analysis in the present studies is shown in fig. 3.15 (p 138).

In the modelling process each fraction is considered to consist of one or more discrete metabolic compartments whose size is constant due to equilibration of transfer rates into and out of the compartment. Transfer between compartments is assumed to follow first-order kinetics, and can be described by a linear function:

$$\mathbf{R(b,a) = L(b,a) \times M(a)},$$

where **R(b,a)** is the flux from compartment **a** to **b** in mg/day, **L(b,a)** is the rate constant for this flux in pools/day, and **M(a)** is the mass of apoB in **a** (mg).

The shape of the appearance and decay curves determines the number of exponentials required for a reasonable fit of the data. The total flux out of a compartment is equal to the product of the mass and the sum of the individual rate constants:

$$\mathbf{R(x,a) = [L(b,a) + L(c,a) + L(d,a)] \times M(a)}$$

The series of differential equations generated from the observed data can be solved simultaneously using the SAAM (Simulation, Analysis And Modelling) computer programme [Berman & Weiss 1978, Berman *et al* 1983]. This plots calculated radioactivity curves by a 'least-squares' fit function; the individual rate constants may then be adjusted manually to improve the approximation of the observed and calculated data. When the sum of squares for the residual differences between the data sets is achieved in this fashion, the programme will further improve the fit in an iterative manner until the overall sum of squares reaches a minimum value within the constraints imposed.

1.3.5 Effects of Intervention on Lipoprotein Metabolism

1.3.5.1 Effects of bile-acid sequestrant resins

Enhanced receptor-mediated clearance of LDL (increased LDL FCR) has been described in heterozygous FH [Shepherd *et al* 1980] and in rabbits [Slater *et al* 1980] as a result of resin therapy. Other investigations in normolipaemic animals [Witztum *et al* 1985, Huff *et al* 1985] and in non-FH individuals [Gaw 1992] have failed to show increases in the catabolic rate. In the latter study, there was a reduction in direct $VLDL_1$ catabolism, increased synthesis and pool size of $VLDL_2$, decreased IDL apoB pool size, and a reduction in LDL pool size (due to reduction in precursors and in total LDL synthesis, without significant change in LDL catabolic rate).

It had been thought that the upregulation of the LDL receptor resulting from depletion of the intracellular sterol pool would be manifest in an increased FCR for LDL. The LDL precursors however may also interact with the apoB/E receptor, and the reduction in the IDL pool leading to decreased flux into LDL may explain the apparent paradox.

1.3.5.2 Effects of HMG Co-A reductase inhibitors

Using the same methods in a similar group of patients with moderate hypercholesterolaemia, it was shown that the reduced LDL pool size was accounted for by an increase in direct catabolism of VLDL and IDL with reduced LDL synthesis from its precursors as well as enhanced LDL FCR [Gaw *et al* 1993]. Earlier studies in FH also described the dual action of suppression of synthesis and increased receptor uptake of LDL [Bilheimer *et al* 1983], and this has also been seen in patients with non-familial (or 'polygenic') hypercholesterolaemia [Grundy & Vega 1985]. Other reports however [Vega *et al* 1990] show no effect on catabolism, but reduced VLDL apoB synthesis only.

1.3.5.3 Effects of a resin-statin combination

This powerful combination has been shown to normalise the cholesterol levels in many patients with FH [Weisweiler 1988, Leren *et al* 1988, Illingworth 1984] and result in regression of coronary disease [Brown *et al* 1990]. Kinetic tracer studies in FH has shown the reductions in LDL to be attributed to increased LDL clearance [Bilheimer *et al* 1983, Vega *et al* 1989], while the results in others show both increased clearance and reduced synthesis [Grundy *et al* 1985]. Studies in non-FH reveal the same heterogenous response as with the drugs used singly, but the marked reduction in the LDL pool size was accounted for by a combination of decreased production (particularly direct synthesis) and increased clearance [Vega & Grundy 1987, Gaw 1992]. There were also significant increases in HDL levels but, as reported elsewhere, no alteration in Lp(a).

1.3.5.4 Effects of a nicotinic acid derivative

Seven patients with non-familial hypercholesterolaemia have been studied during treatment with Acipimox [Gaw 1992]. There was a reduction in pool sizes of $VLDL_1$, $VLDL_2$ and LDL; direct $VLDL_2$ synthesis was increased, and there was increased fractional transfer from this compartment to IDL and LDL. Direct catabolism of IDL was increased, while direct LDL synthesis fell. The main changes could be accounted for by reduced synthesis rather than alterations in catabolism, and the reduction in transfer from IDL to LDL is consistent with the known suppression of hepatic lipase by these agents.

1.3.5.5 Effects of LDL-apheresis

While the above studies can be performed while the subject is taking drug therapy, the same techniques for studying metabolism are not usually applied during apheresis treatment since the patient is not in a steady state. It has been shown however that the marked abrupt change in plasma pool sizes did not alter the clearance of LDL either in a normal volunteer or in an FH homozygote [Thompson *et al* 1981].

1.4 PATHOPHYSIOLOGY OF ATHEROSCLEROSIS

As described above (**1.1.2**), atherosclerosis is a chronic process involving changes in all layers of the arterial wall and much of the natural history of this process is subclinical, manifest only in the final stages. Its relationship with several risk factors is incontrovertible, although the pathogenesis remains incompletely understood. It is clear from the epidemiological and clinical evidence that markedly raised levels of low density lipoprotein cholesterol are neither necessary nor sufficient to result in atherosclerotic disease. The putative initiating factors have been reviewed in Sect **1.1.2**, and the role of lipid factors in the progression of the disease process will now be examined.

1.4.1 The Role of Low Density Lipoprotein

Although atherosclerosis consists of cellular and connective tissue elements as well as a lipid component, and that in some lesions the first two may predominate, the effects of lipid on the endothelium, monocytes, thrombocytes and smooth muscle cells are of central importance to the process. It has been observed that hypercholesterolaemia in animal models fed a high-fat diet leads within a few days to attachment of large numbers of monocytes to the surface of the arterial endothelium [Faggiotto *et al* 1984]. These cells migrate to cellular junctions and enter the subendothelial space, where they are converted into scavenger cells. The lipid may enter the subendothelium in large quantities in the presence of elevated plasma levels resulting within weeks in the establishment of fatty streaks. The same process is observed in animals with more moderate lipid levels, albeit at a slower rate [Masuda & Ross 1990a].

Some investigators have proposed another means by which elevated LDL levels may predispose to endothelial dysfunction [Jackson & Gotto 1976]. They have described an alteration of the viscosity of the endothelial cell membrane by changes in the cholesterol/phospholipid ratio, resulting in the endothelium being less malleable and more susceptible to cell retraction particularly at sites of altered shear stress such as arterial bifurcations.

Hypercholesterolaemia itself leads to alterations in normal endothelial function, including impairment of production of endothelium-derived nitric oxide, even before the formation of atherosclerotic lesions [Osborne, Siegman *et al* 1989, Zeiher *et al* 1991, Chowienczyk *et al* 1992]. It may also stimulate the endothelial cells to produce increased amounts of growth factor, particularly 'platelet-derived growth factor' (PDGF), which binds avidly to cells derived from connective tissue, is a highly potent mitogen and vasoconstrictor, and increases binding of LDL to cells by increasing the number of LDL receptors [Heldin *et al* 1981, Grotendorst *et al* 1982, Ross *et al* 1986, Witte & Cornicelli 1980].

The fatty streaks continue to expand by the same processes of monocyte adherence, subendothelial migration and lipid accumulation. As they expand, the surface becomes more irregular while the intima is increasingly infiltrated with smooth muscle cells which assume the characteristics of foam cells. After several months of hypercholesterolaemia, retraction of the endothelium occurs at localised areas overlying the fatty streaks, with a predilection for branch points and bifurcations. The exposure of the underlying macrophages results in the adherence of platelets and the formation of microthrombi, and ultimately the fibrous cap [Faggiotto & Ross 1984, Masuda & Ross 1990b]. These plaques in the animal model are indistinguishable from those found in human arteries. The degree of change observed at various anatomical sites have been shown to correlate with the level of plasma cholesterol and the duration of hyperlipidaemia [Masuda & Ross 1990b].

It may be noted that the platelets themselves are altered in hypercholesterolaemia: they are smaller, have an increased cholesterol/phospholipid ratio [Shattill *et al* 1977], exhibit increased TXA_2 production, and are hyperaggregable with thrombin and TXA_2-mimetics [Gross *et al* 1991, DiMinno *et al* 1986].

While elevated serum levels of LDL are associated with accelerated atherosclerosis, there exist several subclasses of LDL which can be differentiated on grounds of size by gradient gel electrophoresis, or of density by density gradient ultracentrifugation [Griffin *et al* 1990, Austin, Brunzell *et al* 1990, Austin, King *et al* 1990]. These vary in composition, the small dense particles being particularly enriched with cholesteryl ester. There is evidence that a

preponderance of such particles increases the risk of coronary disease. Such a subclass distribution is associated with raised serum triglycerides and apolipoprotein B, raised VLDL and IDL mass, and reduced HDL-cholesterol, HDL2 mass, and plasma apolipoprotein A-I. This has been termed the 'atherogenic lipoprotein phenotype' [Austin, King *et al* 1990] and may be inherited as a single-gene trait.

1.4.2 The Role of Lipoprotein(a)

The physiological role of Lp(a) remains undetermined. In spite of the sequence homology with plasminogen, evidence is lacking for a role in coagulation or fibrinolytic pathways. Due to amino acid substitution the usual cleavage site of plasminogen by streptokinase is unaffected [Eaton *et al* 1987]. It does however inhibit the binding of plasminogen to immobilised fibrin and fibrinogen, and impairs the generation of plasmin by tissue plasminogen activator in the presence of fibrin [Hajjar *et al* 1989, Harpel *et a*l 1989, Rouy *et a*l 1991, Loscalzo *et al* 1990]. It also impedes access of plasminogen to the endothelial cell surface receptors [Hajjar *et al* 1989], and it has been suggested that it may induce plasminogen activator inhibitor expression at this site [Etingin *et al* 1991].

It is clearly possible that Lp(a) may promote thrombosis and impair fibrinolysis at atheromatous plaques, and thus be a risk factor for myocardial infarction. It may also participate in atherogenesis by its binding to the fibrinogen of the evolving plaque or to fibronectin, which is also present in early lesions [Mbewu & Durrington 1990]. Lp(a) has been demonstrated in biopsies from the aortic wall of patients undergoing coronary bypass surgery; the quantity of apo(a) in the biopsies is strongly correlated with the serum Lp(a) level, and elevated Lp(a) levels also are associated with increased aortic deposition of apoB, most of which is co-localised extracellularly with apo(a) [Rath *et al* 1989]. Similar pathological findings have been described in resected aorto-coronary bypass grafts, with co-localisation of apo(a) and apoB in areas of atherosclerosis [Cushing *et al* 1989]. It has also been shown that in this group the tissue apo(a)/apoB ratio is higher than the corresponding plasma ratio, suggesting that Lp(a) has a propensity for deposition in the vascular wall. In addition to binding to fibrinogen, it has recently been demonstrated that Lp(a) may bind by a receptor to monocyte-derived

macrophages [Zioncheck *et al* 1991], which is distinct from the apoB/E receptor and probably also from the scavenger receptor, and this may represent a significant route of entry into the vessel media. This is supported by evidence that Lp(a) accumulates in plaques more readily than apoB not associated with apo(a) [Smith & Cochran 1990].

In summary, Lp(a) is bound to fibrin at sites of vascular injury. It appears to be able to traverse the endothelium, and may bind either to fibrin, the glycosaminoglycan matrix [Bihari-Varga *et al* 1988] or to specific receptors on the macrophages. Because of its binding in the subendothelial space, its residence time is prolonged compared to LDL; this may enhance the likelihood of oxidative modification, with the recruitment of other cells involved in the atherosclerotic process.

1.4.3 The Role of HDL

The widely held view of the function of the HDL fraction is the transport of cholesterol from the periphery, including the arterial wall, to the liver via lipid exchange with VLDL. This 'reverse cholesterol transport system' was first suggested by Glomset and subsequently demonstrated in vitro [Glomset 1968, Fielding *et al* 1983]. Cholesterol is taken up by HDL or apoA-I from macrophages and other cells within the arterial wall and esterified by the action of lecithin cholesterylacyl transferase, for which A-I is a cofactor. The apo-E-enriched HDL particle may then deliver the cholesterol ester directly to the liver via the apoE receptor, or may exchange the ester for triglyceride from VLDL by the action of cholesteryl ester transfer protein [Tall 1986], the triglyceride then being removed by lipases, allowing the HDL to take up further cholesterol in a complex series of metabolic rearrangements [Franceschini *et al* 1991, Reichl & Miller 1989] (see Fig 1.6). This indirect pathway of transferring free cholesterol to the liver via lower density lipoproteins appears to play a major role in humans, who have a high concentration of LDL. However other pathways that operate in other species may also do so in man, including individuals with familial CETP deficiency; such individuals accumulate in plasma large, apoE-rich particles with high affinity for the apoB/E receptor which can directly deliver cholesterol to hepatocytes.

It is readily appreciated that a summary measure of the activity of this process is elusive. Although it has been shown that most HDL particles probably efflux from the arterial wall through the vasa vasorum and postulated that the atherosclerosis-protective effect of high HDL levels is a function of the increased flux of the HDL through the arterial wall [Nordestgaard *et al* 1990], this takes no account of variation in function of the acceptor particles. For instance, the peripheral mobilisation of cholesterol from the tendon xanthomata of patients with familial hypercholesterolaemia treated with probucol [Yamamoto *et al* 1986] takes place in the face of reduced HDL cholesterol, and is associated with an increase in plasma CETP [Sirtori *et al* 1988]. In most circumstances however it does appear that the inverse relation between HDL levels and the propensity to develop atherosclerosis may be causal. This is further supported by animal experiments demonstrating not only the inhibition of development of fatty streaks [Badimon *et al* 1989], but also the induction of regression in cholesterol-fed rabbits by the infusion of homologous HDL [Badimon *et al* 1990].

Other possible anti-atherogenic roles have been proposed for HDL. Both HDL and apoA-I inhibit the formation of aggregates of LDL - normally avidly phagocytosed by macrophages - in response to vortexing or incubation with phospholipase C [Khoo *et al* 1990]; it has been proposed that the high concentrations of LDL present in the arterial intima, particularly at the site of lesions [Smith & Ashall 1983], might lead to LDL aggregates and that HDL may have a role at this site to prevent their uptake by macrophages.

Certain classes of HDL may also directly inhibit uptake of LDL [Carew *et al* 1976]. Other workers have shown that HDL can prevent the oxidative modification of LDL [Parthasarathy *et al* 1987, Klimov *et al* 1989], and ameliorate the cytotoxic effect of oxidised LDL on vascular smooth muscle and endothelial cells [Hessler *et al* 1979]. The transmigration of monocytes induced by oxidative modification of LDL may be abolished by HDL [Navab 1992]. There is also considerable evidence for the role of triglyceride-rich lipoproteins in the development of atherosclerosis (see **1.4.4**), and so it may be of importance that HDL inhibits the cytotoxicity of lipolytic surface remnants to macrophages [Chung *et al* 1989].

1.4.4 Role of Triglyceride-rich Lipoproteins

Most epidemiological studies have demonstrated a significant correlation between serum triglyceride levels and incidence of coronary disease, but this usually becomes non-significant on multivariate analysis after correcting for HDL levels [NIH Consensus Development Panel 1993]. Recent studies have shown triglycerides may be an independent predictor of coronary and total mortality for women, and may be a weak risk factor for coronary heart disease also in men in some populations [Carlson & Böttiger 1985, Lapidus *et al* 1985, Tverdal *et al* 1989, Stensvold *et al* 1993]. Although triglycerides ceased to be an independent risk factor in the PROCAM study [Assmann & Schulte 1992] after correction for HDL, hypertriglyceridaemia indicated a substantial additional risk for those subgroups with LDL-cholesterol > 4.9 mmol/l and LDL/HDL ratio > 5, the relative risk of a triglyceride level above 3 mmol/l in the latter subgroup being 2.5. Similar findings were documented in the Helsinki Heart Study, and treatment reduced CHD events by more than 70% in this subgroup [Huttunen *et al* 1991]. Other groups have however found an association between triglycerides and coronary disease only in subjects with low cholesterol levels [Criqui *et al* 1993], and the place of serum triglycerides in screening and in management of dyslipidaemias remains unresolved [Garber & Avins 1994].

The severity of coronary disease assessed angiographically has been found to be greatest in those patients with elevated triglycerides, low HDL-cholesterol levels (particularly low HDL_2 fraction) and low lipoprotein lipase activity [Breier *et al* 1989]. Another study showed a significant correlation between LDL-triglycerides and angiographic severity of coronary disease in a group of MI survivors, and that the latter was also related to the degree of susceptibility to LDL oxidation [Regnström *et al* 1992]. Elevated triglycerides have been shown to be associated with the formation of smaller, more dense LDL subfractions - which have a lower affinity for the LDL receptor and which are more susceptible to oxidative modification - as well as increased mass of very low density lipoproteins and decreased HDL-cholesterol, HDL_2 , and plasma apoA-I [Austin, King *et al* 1990, Griffin *et al* 1994].

It is possible that the variability of the triglyeride levels and the heterogeneity of the triglyceride-rich particles have combined to mask the association between triglyceride levels and coronary heart disease. Certainly the strong inverse correlation with HDL-cholesterol has consistently reduced the importance of the triglycerides after statistical correction for HDL. Such statistical analyses have their critics [Abbott & Carroll 1984]; the metabolism of the triglyceride-rich particles is closely related to that of both LDL and HDL (see fig 1.6), and "to dismiss an association between coronary heart disease and one class of lipoprotein on statistical grounds ignores the fact that this may not be the way the arteries see it" [Nestel 1990].

1.4.5 Role of Oxidative Modification

Atherosclerosis occurs in familial hypercholesterolaemia (FH) homozygotes at an early age even in the absence of other risk factors because of the several-fold increase in cholesterol levels, as it does in certain animal models [Goldstein *et al* 1983]. The lipid accumulating in the fatty streaks which initiate the formation of the plaque does so within foam cells derived from transformed circulating monocytes or from medial smooth muscle cells [Faggiotto *et al* 1984]. It was recognised [Steinberg *et al* 1989] that mechanisms other than the LDL-receptor were likely to be involved, since receptor-negative homozygote FH patients and Watanabe heritable hyperlipidaemic rabbits do develop foam cells; further, normal monocytes and monocyte-derived macrophages in culture cannot be converted into foam cells even with very high concentrations of native LDL [Brown & Goldstein 1983].

This alternative uptake of LDL was first described by Brown and Goldstein [Goldstein *et al* 1979], demonstrating that chemically modified LDL could be avidly taken up by a specific saturable receptor which did not recognise native LDL. Subsequent work has established that such modification may be mediated by endothelial cells, smooth muscle cells and macrophages themselves [Henriksen *et al* 1981 & 1983, Morel *et al* 1984] and that cell-mediated oxidation results in modified LDL which can be taken up by a high-affinity receptor distinct from the acetyl-LDL receptor [Sparrow *et al* 1989]. Such modified-LDL have been shown to

have reduced affinity for the LDL receptor, demonstrate chemotactic activity for circulating monocytes, and are cytotoxic. A schema for the role of oxidised LDL in the formation of foam cells has been proposed (Fig 1.7).

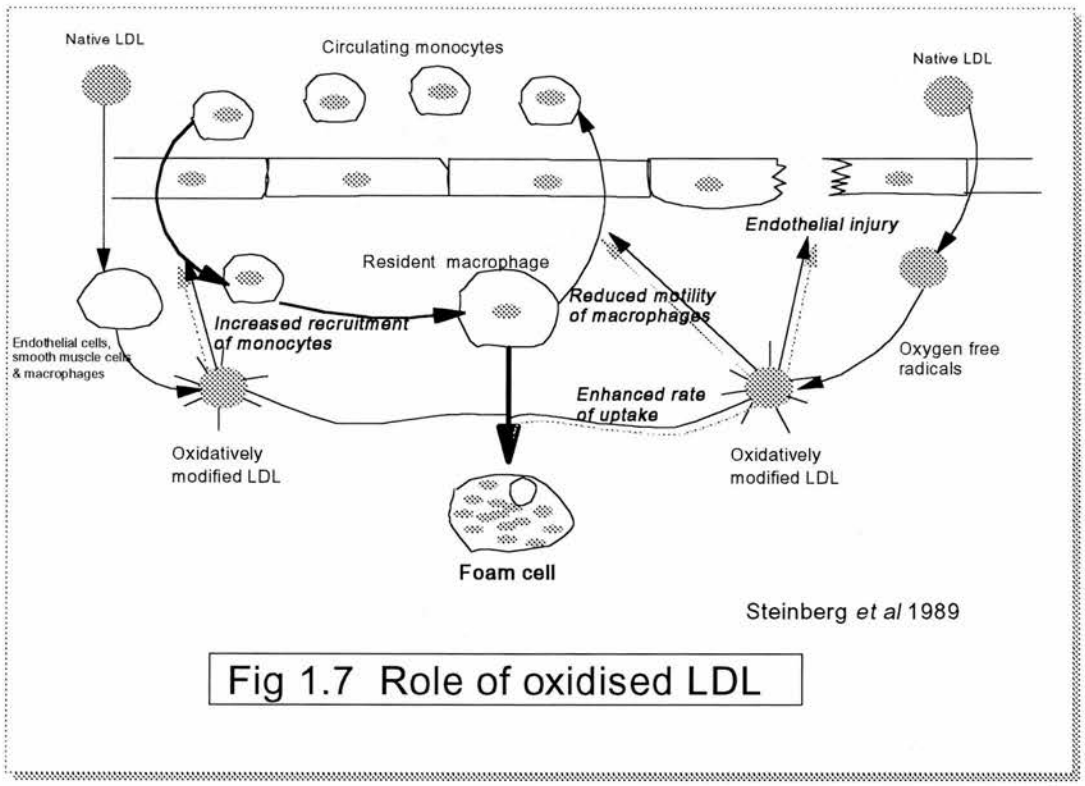


Fig 1.7 Role of oxidised LDL

Under normal circumstances oxygen metabolism generates only small amounts of free radicals, and these are rapidly removed by serum and tissue antioxidants. However the presence of hypoxia can accelerate oxyradical formation, overcome local defence mechanisms and cause tissue damage [Crawford & Blankenhorn 1991]. Abnormalities of oxygen distribution may result from increased wall thickness, abnormal wall composition, or increased metabolism, and experimental evidence suggests that within the media or thickened intima there is a zone where oxyradical production is favoured.

There is now increasing evidence that oxidation of LDL occurs in vivo and that the resulting modified lipoproteins are involved in atherogenesis [Witztum & Steinberg 1991, Steinberg *et al* 1989]: antibodies against oxidised LDL show immunostaining in atherosclerotic areas of rabbit aorta, but not in normal areas [Palinski *et al* 1989]; LDL extracted from human atherosclerotic lesions demonstrate the

physical, chemical and biological properties of LDL oxidatively modified in vivo [Ylä-Herttuala *et al* 1989]; LDL from aortic lesions reacts with antibodies to modified LDL [Palinski *et al* 1989, Ylä-Herttuala *et al* 1989]; plasma from both WHHL rabbits and humans contain antibodies to various forms of oxidised LDL [Palinski *et al* 1989, Rosenfeld *et al* 1990]; anti-oxidants reduce the progression of atherosclerosis in WHHL rabbits due to its inhibition of oxidation [Kita *et al* 1987, Nagano *et al* 1989, Nagano *et al* 1992]; lipid peroxides in plasma are increased in subjects with significant atherosclerotic disease [Stringer *et al* 1989]; the antibody titres to epitopes of oxidised LDL may be an independent predictor of the progression of atherosclerosis [Salonen *et al* 1992]; the susceptibility to oxidation of low density lipoprotein is associated with severity of atherosclerosis in young survivors of myocardial infarction [Regnstrom *et al* 1992]; and, antioxidants reduce plasma lipid peroxides in hypercholesterolaemic patients [Paterson *et al* 1992].

The described cytotoxicity of the oxidised lipoproteins [Cathcart *et al* 1985] offers one potential way in which the formation of subendothelial foam cells may lead to the development of more complex lesions, since the oxidation products may cause loss of the overlying endothelial cells. This would lead to the adherence and aggregation of platelets, the generation of growth factors, leading to further attraction of monocytes and recruitment and retention of other cells involved in the growth of the plaque. Other post-secretory modifications may be as important as oxidation and contribute to foam cell formation; for example, glycation in diabetes.

A schematic representation of some of the main factors believed to be involved in the formation of atherosclerosis is shown in figure 1.8.

1.4.6 Interaction of Lipids with Other Factors

There are many individuals at risk of developing atherosclerosis without significant abnormality of their serum lipid values. However if the final common pathway in the initiation of the lesion is foam cell formation, factors other than hyperlipidaemia may operate in various ways to promote lipid uptake into the arterial wall.

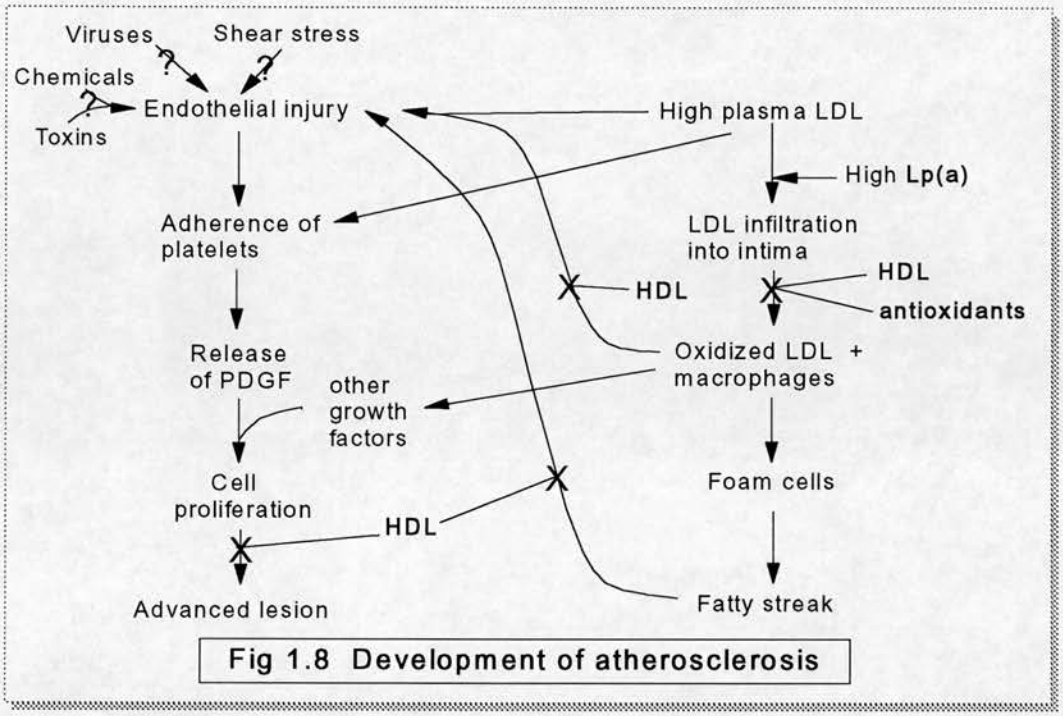


Fig 1.8 Development of atherosclerosis

Smoking is a powerful risk factor for cardiovascular disease [Kannel *et al* 1986, Shaper *et al* 1985, Reid *et al* 1976], and its effects may not be limited to those who choose to smoke [Hole *et al* 1989, He *et al* 1994]. The adverse effects may be seen in the earliest stages of the development of atherosclerosis [PDAY Research Group 1990], and the increased risk of cardiac events persists for some years after cessation [Robinson *et al* 1989, Rosenberg *et al* 1990]. Some studies however show little increased risk in smokers, particularly in women [Skrabanek 1992].

Rats exposed to smoke inhalation have a reduced HDL level and increased VLDL-cholesterol, similar to that seen in human smokers [Maida & Howlett 1990], in whom these effects are reversed by cessation [Freeman *et al* 1994]. In addition LDL from smokers is more susceptible to oxidative modification, and contains less antioxidant [Harats *et al* 1989, Scheffler *et al* 1990, Salonen *et a*l 1991], although thiobarbituric acid reactive substances (TBARS) are not different in plasma or LDL in smokers compared to non-smokers. Peritoneal macrophages metabolise labelled LDL conditioned by smooth muscle cells, this process occurring at twice the rate when LDL is obtained from smokers [Harats *et al* 1989]. The reduction in antioxidants may be, at least in part, attributed to differences in dietary intake [Margetts & Jackson 1993].

In addition to its effects on lipids, smoking may influence the atherogenic process through influences on other factors. These include effects on clotting factors, platelet function and plasma fibrinogen: smokers have increased platelet aggregation and prolonged bleeding time [Meade *et al* 1987]; fibrinogen levels are significantly higher than in non-smokers, and the levels may remain elevated for up to five years after giving up [Kannel *et al* 1987]. Fibrinogen has itself been demonstrated to independently predict risk of cardiovascular events [Ernst 1991]. Smoking also affects the response to antihypertensive therapy, since treated smokers have twice the mortality of non-smokers [Langford *et al* 1986], and directly affects endothelial cell function as evidenced by the impaired production or release of prostacyclin [Nowak *et al* 1987].

Hypertension is another major modifiable factor which is independently related to the risk of cardiovascular disease [Kannel *et al* 1986, Shaper *et al* 1985, Reid *et al* 1976, Tverdal 1987]. The lack of significant reduction in the incidence of myocardial infarction or coronary mortality in the antihypertensive drug trials has been both surprising and disappointing, and these trials and their implications for management are summarised by McInnes [McInnes 1991]. It has been suggested that the drug treatment of hypertension may actually contribute to atherogenesis, and thereby explain the lack of reduction in coronary events in virtually all the blood pressure-lowering trials; many of the agents used have a deleterious effect on lipoprotein metabolism and it may be this rather than the hypertension per se which is responsible for the lack of benefit in the treatment groups [Lardinois & Neuman 1988, Freis 1989, Kaplan 1989].

Direct mechanisms by which hypertension contributes to the development of atherosclerosis is not established. Increasing the transmural pressure in animal aortas with intact endothelium leads to significantly greater deposition of labelled LDL and decreases efflux from the wall [Curmi *et al* 1990] and this may be due to increased endothelial permeability, or pressure-driven convection which is supported by the uniform distribution of labelled albumin across the media at higher pressures compared to the greater concentration of the larger LDL on the luminal side of the media.

More important than the pressure effects or the adverse effects of therapy may be the metabolic abnormalities increasingly recognised to be associated with hypertension. There is an increased incidence of hypertension in subjects with hyperlipidaemia, and it has been shown [Williams *et al* 1988, Hunt *et al* 1988] that familial hypertension may also be associated with either the fully expressed or a partially expressed 'atherogenic lipoprotein phenotype' [Austin, King et al 1990], discussed above in **1.4.1**. This has been termed 'familial dyslipidemic hypertension'.

It has been demonstrated that insulin resistance is a common finding in essential hypertension, and that the degree of reduction in insulin-mediated glucose uptake is proportional to the severity of the hypertension [Ferrannini *et al* 1987]. Interestingly therapy with thiazide diuretics or beta-adrenergic blocking agents exaggerated these metabolic defects compared to untreated patients [Swislocki *et al* 1989]. Insulin levels are not associated with blood pressure independent of body mass index (BMI) or plasma glucose, but the simultaneous elevation of BMI, glucose and insulin is strongly associated with blood pressure [Cambien *et al* 1987]. These authors also showed a positive correlation between plasma insulin and serum triglycerides and a negative association with HDL cholesterol which are independent of plasma glucose and BMI, findings confirmed also in an elderly population [Ostlund *et al* 1990]. In a recent study of European University students, plasma insulin level was one of the factors which was found to be significantly increased in offspring of men with premature myocardial infarction compared to age- and sex-matched controls. An imbalance in the action of insulin and the counter-regulatory hormones (as in the insulin-resistant state) have been postulated to explain the effects of such diverse factors as diabetes, obesity, hypertension, stress and altered lipoprotein metabolism on atherosclerosis and their interaction [Brindley & Rolland 1989]. Interestingly, increased lipoprotein (a) concentrations have been found in patients with impaired glucose tolerance, with a five-fold increase in the proportion with levels > 30 mg/dl [Davies *et al* 1992]. Although a causal link is not established, the association may contribute to the mechanisms whereby glucose intolerance may accelerate coronary disease.

There are a number of other 'minor' risk factors for atherosclerosis whose role is incompletely understood. Some of these however do interact with lipids and a

possible pathogenic role may be suggested. For example, the progression of carotid atherosclerosis in men with elevated LDL levels may be accelerated in the presence of high serum copper levels (which acts as a pro-oxidant) and reduced serum selenium concentrations (which is a cofactor for glutathione peroxidase, an enzyme which scavenges free radicals) [Salonen *et al* 1991].

1.5 REVERSIBILITY OF ATHEROSCLEROSIS

1.5.1 Experimental studies

The early recognition that lipid is one of the major components of atherosclerotic lesions led to the search for suitable animal models for the disease. Anitschow [Anitschow 1933] first demonstrated in rabbits that it was possible to regress diet-induced atherosclerosis on withdrawal of the cholesterol-rich diet. Subsequently many studies have confirmed these results in other vertebrate models, including fowl [Horlick & Katz 1949], dog [DePalma *et al* 1977], swine [Fritz *et al* 1976], and non-human primates [Clarkson *et al* 1984, Eggen *et al* 1987, Wissler & Vesselinovitch 1990]. The disease which develops in these models may vary in several respects from that in humans; such differences include the propensity to develop spontaneous atherosclerosis, the sensitivity to the induction of disease by diet, the distribution of disease in the aorta and involvement of different calibre of arteries, and the histological appearances [Wissler & Vesselinovitch 1989]. The non-human primate models develop lesions that closely mimic the progressive human atherosclerotic disease, although the rabbit strains which develop disease spontaneously are a useful model for human familial hypercholesterolaemia.

Several studies using rhesus monkeys indicated that there is depletion of foam cells and disappearance of extracellular lipid in animals fed an atherogenic diet over a short period when cholesterol is then removed from the diet and plasma cholesterol concentrations return to normal [Eggen *et al* 1974, Stary 1978]. Further work demonstrated regression of established disease induced by fat-feeding over a more prolonged period in both cynomolgus and rhesus monkeys, with reduction in plaque size in both aorta and coronary arteries (Armstrong *et al* 1970, Armstrong & Megan 1972, Armstrong & Megan 1975, Eggen *et al* 1987], although some workers have found significant differences in resistance to regression between the macaques [Wissler & Vesselinovitch 1990]. Disease which had been established in rhesus monkeys even longer has been demonstrated to be partly reversible: Clarkson and colleagues followed a 38-month period of atherosclerosis induction with a low cholesterol diet for 24 - 48 months. They found that the incidence and extent of regression was determined by the cholesterol level during

the regression phase and also the length of this period [Clarkson *et al* 1984]. Intensive lipid-lowering therapy (using a combination of an HMGCoA reductase inhibitor and a bile acid sequestrant resin) given over a shorter period of time was found to induce regression of disease in Watanabe Heritable Hyperlipidemic (WHHL) rabbits, an animal model deficient in LDL receptors and thus resembling familial hypercholesterolaemia [Shiomi *et al* 1990].

In addition to the accumulation of lipid deposits, the atherosclerotic process involves a loss of elasticity of the involved vessels [Blankenhorn & Kramsch 1989]. This is more difficult to measure, but is associated with increased collagen and elastin [Greenwald & Berry 1978]; reversal of fibrous plaques in animal models is associated with reduction of calcium, collagen and elastin [Kramsch 1985]. The elasticity of the aorta - reflected by the aortic pressure pulse wave velocity - is reduced in animals fed an atherogenic diet for a prolonged period, and this is restored after cessation of cholesterol-feeding [Farrar *et al* 1980]. Similar experiments indicate that the reversal of the sclerosis may be mediated by EDRF: the intimal lesion formation reduces production or delivery of EDRF; an increase in EDRF production by reduction of cholesterol may account for reduction in sclerosis without the vessel architecture returning completely to normal [Harrison *et al* 1987, Heistad *et al* 1987).

1.5.2 Evidence for Regression of Atherosclerosis in Humans

Observational studies of human autopsies suggested that subjects who had been deprived nutritionally showed much less evidence of atherosclerosis than those who had been well nourished [Vartainen & Kanerva 1947, Wilens 1947]. There was however no reliable means of assessing the extent of atherosclerosis in living subjects until the development of angiography. Early attempts to demonstrate the efficacy of cholesterol-lowering in man to induce regression of atheroma using repeated angiography were disappointing. Favourable trends were seen in non-randomised trials: nine of twenty-eight patients treated with hypolipidaemic agents over a period of seven years showed no evidence of progression of disease, while only one of thirteen untreated patients with coronary disease with

similar risk factor profiles at baseline failed to progress over a five year follow-up [Nikkila *et al* 1984]. There was no evidence of regression in either group. In thirty-nine patients treated by intensive dietary therapy in the Leiden Intervention Trial, there was progression of lesions in twenty-one patients and stabilisation in the remainder, the extent of progression being correlated with the mean lipid levels during the period of intervention [Arntzenius *et al* 1985].

A randomised (but unblinded) study of femoral atherosclerosis in twenty-four patients with claudication was described, with 10 patients treated with diet, niacin, cholestyramine or clofibrate for an average of 19 months [Duffield *et al* 1983]. Treatment reduced LDL-cholesterol by 28%, triglycerides by 45% and resulted in a rise of 41% in HDL. There was a significant reduction in the number of femoral segments exhibiting disease progression by nonquantitative analysis but no regression was seen, and the number of subjects in the trial was too small for analysis by treatment group .

The first placebo-controlled double-blind angiographic study of lipid-lowering therapy in coronary artery disease was performed in just 40 patients with established disease [Cohn *et al* 1975]. 16 of these were treated for one year, with reductions of only 6% in total cholesterol and 16% in triglycerides. There was no difference between the groups in the incidence of progression.

Early trials with larger numbers of subjects which were randomised, controlled and double blind failed to show a significant treatment effect. The National Heart, Lung and Blood Institute (NHLBI) Type II Study tested the effects of lipid-lowering intervention with diet and a fixed dose of a resin against the effects of diet alone [Brensike *et al* 1984]. This showed a non-significant reduction in the number of subjects with progression of disease and no difference in regression. This study however was designed to detect differences in a study population of 250 subjects. Only 143 were recruited, and of these only 116 underwent repeat angiography through death, drop-out or refusal to complete the trial. In addition the angiograms were analysed by a panel-reading method, which is less sensitive and less reliable at detecting and quantifying changes in lesions than computerised methods [Blankenhorn *et al* 1992, Reiber *et al* 1984). On the other hand the initial design was for repeat angiography at 24 months, but this was changed to five years after

the initial two-year angiograms failed to show any significant change. However post-trial analysis showed a significant inverse relationship between HDL/LDL and angiographic change when the subjects were analysed independent of treatment group [Levy *et al* 1984].

Following these inconclusive results have been other studies which together demonstrate convincingly that alteration of the serum lipids may result in favourable changes in the angiographic appearances (Tables 1vi and 1vii).

The first of these was the Cholesterol-Lowering Atherosclerosis Study (CLAS) which recruited 188 nonsmoking men between 40 and 59 years of age, who had undergone coronary bypass surgery on average four years previously [Blankenhorn *et al* 1987]. Following a pre-randomisation assessment of their tolerance to the study medication, they underwent coronary, femoral and carotid angiography before being assigned to the treatment group (taking colestipol and niacin) or placebo. The total cholesterol level before the trial ranged from 4.8 to 9.1 mmol/l, mean LDL-cholesterol was 4.4 mmol/l, and HDL 1.1 mmol/l. In the treatment group there was an average reduction of 26% in total cholesterol and 43% in LDL, while HDL rose by an average of 37%. 162 subjects completed the study and had repeat angiography after two years; these were assessed by panel-reading. The treatment group showed significant reduction in progression of disease in the native coronary arteries and in the percentage of patients with new lesions in both the native arteries and the bypass grafts, and the coronary 'global change score' showed regression in 16.2% of subjects in the treated group compared to 2.4% in the controls and progression in 39% compared to 61%. The benefits were observed for both those above and below a baseline total cholesterol of 6.2 mmol/l. Subsequent analysis of a randomly selected number of film-pairs by computerised quantitative methods confirmed the angiographic benefits of treatment and showed a highly significant correlation between the change in percent diameter stenosis by this method and the change in global score obtained by the initial panel-reading [Blankenhorn *et al* 1992].

61% of the CLAS placebo group exhibited progression over two years compared with a rate of progression in the placebo group of the NHLBI type II study of 49% over five years. Thus it may appear that the differences between the CLAS groups

were largely because of acceleration of disease in the placebo group rather than a beneficial effect of treatment. However the patients in CLAS were nearly ten years older, had more severe disease at entry, and did not appear to have mixed regression and progression of lesions within individuals which complicated interpretation of the NHLBI study. Follow-up for a further two years in a subgroup of the original cohort showed a slight increase in the likelihood of regression in the treatment group, and significantly more subjects exhibiting non-progression in the treatment group compared to controls, with far fewer new lesions [Cashin-Hemphill *et al* 1990].

Another angiographic study examined the effects of combination drug therapy in patients with familial hypercholesterolaemia, mostly using a combination of niacin and colestipol as in the CLAS study [Kane *et al* 1990]. Differences from CLAS include a lower average age (41.7 vs 54.2 years), higher lipid levels (9.65, 7.2, 1.3 and 1.38 mmol/l in Kane for TC, LDL, HDL and triglycerides respectively, vs 6.3, 4.4, 1.14 and 1.72 mmol/l in CLAS), inclusion of women and smokers, exclusion of patients with previous revascularisation, and use of quantitative coronary angiography as opposed to a 'global change score' by a panel of viewers. Of note also is that only 3 of the 97 patients entering the study had symptomatic coronary disease; there were however an average of 6.1 lesions per patient of greater than 10% area stenosis on visual inspection.

LDL fell by 38% in the treatment group, and HDL rose 28%, both effects being smaller than those achieved in CLAS despite almost half of the treatment group having lovastatin added to their drug regimen when this became available during the course of the study. There was an overall reduction in the mean (per patient) percent area stenosis in the treatment group in contrast to an increase in the controls, the difference being statistically significant, albeit of small magnitude. The changes in the lesions in the women were no different from that observed in the male subjects. The change in the mean percent area stenosis was correlated with on-trial LDL levels ($r = 0.282$, $P = 0.018$).

The St Thomas' Atherosclerosis Regression Study (STARS) was similar to the NHLBI study in comparing the effects of diet plus fixed dose of cholestyramine against diet alone [Watts *et al* 1992], and recruited 90 men with coronary heart

disease and total cholesterol levels above 7.2 mmol/l. Although the diet was more rigorous in STARS, the dose of resin used was 16gm compared to 24gm and the period of intervention was three years rather than five. On the other hand, STARS used a computerised method of evaluating the angiograms, and also included a 'usual care' group for comparison. The group treated by diet alone experienced a fall of 14% in total cholesterol, 16% in LDL, and 16% in TC/HDL. The other intervention group achieved respective reductions of 25%, 36% and 24%, while there was no significant change in any of the serum lipids in the usual care group. The mean width of the arterial segments decreased by 0.13 mm in the usual care group, and was slightly increased in the other groups - by 0.01mm in the dietary group and 0.08mm in the drug-treated group. Progression or regression was defined by an overall measure of the change in mean arterial width of the segment (MAWS) per patient of 2 SD, 46% of the control group being classified as showing progression. There was a significant reduction in disease progression and more patients exhibited regression in the treatment groups compared to the controls, although there was no significant increase in regression when analysed by lesion. Although some of the differences may be ascribed to the 'usual care' group being older by 5 years on average, and that the mean diameter stenosis was lower in this group compared to the intervention groups (while the "most striking changes in diameter stenosis were seen in the worst affected segments"), there were highly significant negative associations between the change in MAWS and the average on-trial LDL and LDL/HDL ratio. In addition, despite the relatively small numbers in the groups and the short intervention period there was a significant reduction in total cardiac events in the intervention groups compared to the controls.

Two other recent studies investigating regression of coronary disease angiographically have also shown a significant reduction in clinical events. The first randomised 146 men at high risk for cardiovascular events because of family history and elevated serum apolipoprotein-B levels into three treatment groups, comparing a 'usual-care' group on diet +/- colestipol with two treatment groups receiving either lovastatin and colestipol or lovastatin and niacin [Brown *et al* 1990]. Treatment was given over two and a half years, and resulted in reductions of 46% and 32% in LDL and rises in HDL of 15% and 43% in the two active intervention groups respectively. Progression of disease (taken as a 10-point

difference in percent diameter stenosis) without evidence of regression was seen in 46% of the conventionally treated group and in 21% and 25% of the drug-treated groups; 11% of the usual care subjects showed regression compared to 32% and 39% in the intensively treated groups (p for trend < 0.005). Regression of lesions was independently correlated with a reduction in LDL cholesterol, a rise in HDL and a fall in systolic blood pressure. Adverse clinical events (myocardial infarction, revascularization or death) was reduced from 19.2% to 6.5% and 4.2%.

The second studied 838 survivors of myocardial infarction over 9.7 years, the subjects being randomised to undergo partial ileal bypass surgery or to a control group on dietary therapy only [Buchwald *et al* 1990]. Total cholesterol was 19% lower in the intervention group, LDL 34% lower and HDL 6% higher at ten years compared to the controls. Angiograms were analysed by a panel-reading method, using the global score as used in the CLAS trial [Blankenhorn *et al* 1987]. Definite disease progression was seen in 85% of controls at ten years and in 55% in the surgery group while regression occurred in 3.8% and 6.4% respectively (P for trend 0.0002). There were non-significant reductions in total and cardiac mortality (the primary endpoints for the trial), while a significant reduction in total mortality of 36% was observed in a retrospective subgroup analysis of subjects with an ejection fraction above 50% at baseline. There was a reduction of 35% when the two endpoints of coronary deaths and nonfatal myocardial infarctions were combined ($p < 0.001$), and also significant reductions in the surgical group in subsequent coronary artery bypass grafting and coronary angioplasty.

The latter study also illustrates that the natural history of the atherosclerotic process may be modified by the alteration of the lipid milieu irrespective of the means used to accomplish this change. One group of investigators have studied the short term effects of more comprehensive lifestyle changes, but without the use of cholesterol-lowering drugs or surgery [Ornish *et al* 1990]. The programme included a low-fat diet, aerobic exercise, relaxation techniques, and group therapy, and the effects were analysed by quantitative angiography after one year. Total cholesterol was reduced by 24% in the treatment group and 5% in controls ($p < 0.02$), LDL fell by 37% and 6% respectively ($P < 0.01$), while HDL fell by 3% in each group (N.S.). Fat and cholesterol intake was drastically reduced in the intervention group and was associated with a mean fall in body weight of 11.1kg

(12.2%) in contrast to a rise of 1.4kg in controls. The analysis of the angiograms per lesion indicates a reduction in the mean percent diameter stenosis by 2.2 percentage points in the treatment group and an increase of 4.4 points in controls, this difference within one year of intervention in a small study population being highly significant (P = 0.001). The changes in the angiographic appearances were directly correlated with the "overall adherence score".

A further study employing only diet and exercise in men with coronary disease divided the 113 subjects into a 'control' group, to receive the AHA-I diet and exercise advice, and an 'intervention' group which followed the AHA-III diet and were given a group and home exercise programme. The differences in lipids between the groups were modest, with a difference of just 12% in LDL, but quantitative coronary angiography at one year showed a significant decrease in progression in the more intensively treated group, with regression in 15% [Schuler *et al* 1992].

The inclusion of diet and exercise was added to lipid-lowering therapy in the 'special intervention group' to achieve an LDL level of less than 2.85mmol/l in the Stanford Coronary Risk Intervention Project (SCRIP), while the control group received 'usual care' by their own physicians [Alderman *et al* 1991]. During the four years of the study, LDL was lowered by 28% from baseline in the treatment group compared to 9% in the controls; HDL rose by 14% compared to 7%, while the respective reductions in triglycerides were 18% and 4%. The results were analysed in the treatment group according to the LDL subfraction pattern (types 'A' and 'B'): there was no difference in the treatment group between type A and type B individuals in terms of the changes in LDL or HDL. However there was a 70% reduction in serum triglycerides in the type B sub-group compared to just 10% in the type A subjects. The 'usual care' group sustained a mean decrease of approximately 0.035mm in mean arterial diameter of the diseased segments irrespective of their LDL phenotype. There was no difference between the controls and the degree of progression seen in the treated group when the latter were type A, but the response in the treated type B subjects was significantly different both from the controls and from the treated type A subgroup.

The most recent angiographic studies to be reported have all used fixed dose schedules of HMGCoA reductase inhibitors with control groups taking placebo and diet. The Monitored Atherosclerosis Regression Study (MARS) treated 270 subjects (men and women) with at least one coronary lesion greater than 50% diameter stenosis [Blankenhorn *et al* 1993]. Lovastatin in a dose of 40mg twice daily for two years was compared with placebo. There was a reduction in LDL of 38%, triglycerides were reduced by 18%, and HDL increased by 7%. There was a non-significant difference in minimum lumen diameter between the two groups. 'Regression ' was seen in 23% in the treatment group compared to 11% of controls ($p = 0.05$), while progression occurred in 47% and 65% respectively ($p = 0.007$). A significant difference in minimum lumen diameters was seen between the groups for those lesions > 50% at baseline.

The Canadian Coronary Atherosclerosis Intervention Trial (CCAIT) also compared lovastatin and placebo over a two-year period [Waters *et al* 1994]. Although the reduction in LDL was 29%, there was a significant improvement of 0.04mm in minimum lumen diameter, with less progression and fewer new lesions. Neither CCAIT nor MARS showed any significant reductions in clinical events.

The Multicentre Anti-Atheroma Study has recently reported the results of four years of intervention with simvastatin 20mg or placebo in 381 men and women with at least two-vessel coronary disease (and at least 5 coronary segments suitable for analysis in two projections), and total cholesterol levels of 5.5 - 8.0 mmol/l. The study did not exclude smokers, but patients who had previously undergone bypass surgery were not entered. Compared with placebo, the treatment group had a mean reduction in total cholesterol of 23%, LDL 34% and triglycerides 18%; HDL was increased by 9%. The mean lumen diameter per patient decreased by an average of 0.02mm in the treatment group and 0.08 mm in the controls ($p < 0.05$); there were also significant benefits of therapy on minimum lumen diameter and percent diameter stenosis. There was no correlation between the degree of change in the serum lipids and change in lumen diameter. 23% of the simvastatin-treated patients showed angiographic progression, compared to 32% in the placebo group, while the proportion undergoing regression was 18.6% and 12% respectively (combined $p = 0.02$). Only 1.8% of lesions from both groups underwent > 15% decrease in diameter stenosis. There

were no differences in clinical outcomes between the groups, although when definite angiographic progression is combined with cardiac events and interventions, there is a risk reduction with treatment of 29% (95% C.I. 5 - 47).

All these studies show a reduction in the progression of disease during long-term treatment with the statins. Like MARS, STARS, and FATS, MAAS demonstrated the greatest effects on those lesions with an initial diameter stenosis of greater than 50%, although in the CCAIT the greatest benefit was in the less severe lesions. Despite the larger numbers in the statin trials, the reduction in clinical events seen in FATS was not replicated - although they were not designed to have the power to show this.

The angiographic studies extend our understanding of the benefits of lipid reduction documented in the primary and secondary prevention trials. They have been regarded as a surrogate endpoint for clinical events by some, although in the light of the statin trials, it is not certain whether clinical benefit will ultimately be obtained in those who show the greatest angiographic change. They have however shown that atherosclerosis may be stabilised in the majority of lesions in individuals with moderate hypercholesterolaemia and that regression can occur in a small number.

TABLE 1vi. RANDOMISED LIPID-LOWERING CORONARY ANGIOGRAPHIC TRIALS

Trial	Intervention	M/F	Age (range)	n Treat/Con	Interval (years)	Lipid results at baseline TC	TG (mmol/l)	LDL	HDL
Cohn 1975	Clofibrate vs placebo	-	av 49	16/24	1	6.59	2.16	-	-
Brensike 1984 (NHLBI Type II)	Diet+resin vs diet+placebo	94/22	21 - 55	59/57	5	8.35	1.86	6.50	1.01
Blankenhorn 1987 (CLAS I)	Resin,niacin+diet vs diet	162/0	40 - 59	80/82	2	6.32	1.73	4.39	1.14
Buchwald 1990 (POSCH)	PIB surgery vs 'usual'	763/75	30 - 64	421/417	av 9.7	6.49	2.29	4.62	1.04
Ornish 1990	Diet + lifestyle	36/5	35 - 75	22/19	1	6.09	2.41	4.11	1.16
Brown 1990 (FATS)	Diet+/-resin vs resin+niacin or (2) resin+statin	146/0	< 62	46/36,38	2.5	6.99	2.37	4.9	0.98
Kane 1990 (SCOR)	Diet + resin vs resin+/-niacin+/-statin	31/41	19 - 72	40/32	2.2	9.66	1.38	7.23	1.26
Watts 1992 (STARS)	"Usual care" vs diet or (2) diet + resin	90/0	< 66	24/26,24	3	7.23	2.28	4.85	1.2
Schuler 1992	AHA-I diet vs AHA-III diet + exercise program	113/0	35 - 68	56/57	1	6.07	2.1	4.25	0.92
Blankenhorn 1993 (MARS)	Statin vs placebo	247/23	< 70	123/124	2	5.99	1.8	4.06	1.1
Haskell 1994 (SCRIP)	"Usual care" vs exercise, diet, weight loss +/- drugs (LDL-C goal < 2.8 mmol/l)	259/41	< 75	119/145	4	5.95	1.81	4.1	1.09
Waters 1994 (CCAIT)	Statin vs placebo	269/62	27 - 70	165/166	2	6.46	2.2	4.44	1.07
MAAS 1994	Statin vs placebo	336/45	30 - 67	193/188	4	6.39	1.88	4.42	1.1
Sacks 1994 (HARP)	Placebo vs statin+/-niacin+/-resin+/-fibrate (TC/HDL goal < 2.0 and TC < 4.1 mmol/l)	70/9	30 - 75	40/39	2.5	5.48	1.89	3.56	1.08

TABLE 1vii. OUTCOME OF CORONARY ANGIOGRAPHIC TRIALS

(*= p < 0.05, **= p < 0.01, ***= p < 0.001)

Trial	Group	% change				QCA	Change in				
		TC	TG	LDL	HDL		% D. S.	MinAWS (mm)	MAWS	Progression (% patients/% lesions)	Regression (% patients/% lesions)
Cohn 1975	Rx	-3	-13.7	-	-	NO	-	-	-	69 / -	0
	Con	+3	+2.3	-	-		-	-	-	63 / -	0
Brensike 1984	Rx	-17	+28	-26	+8	NO	-	-	-	32 / 11	6.8 / 3.1
	Con	-1	+26	-5	+2		-	-	-	49 / 12	7.1 / 1.4
Blankenhorn 1987	Rx	-26	-22	-43	+37	NO	-	-	-	38.8 / -	16.2 ** / -
	Con	-4	-5	-5	+2		-	-	-	61 / -	2.4 / -
Buchwald 1990	Rx	-28	+4.9	-41	+4.8	NO	-	-	-	48 ***/ -	14.4 / -
	Con	-9	-13	-11	-1.0		-	-	-	77 / -	6.3 / -
Ornish 1990	Rx	-24	+22	-37	-3	YES	-2.2 ***	-	-	18 / -	82 / -
	Con	-5	-9	-6	-3		+3.4	-	-	53 / -	42 / -
Brown 1990	Rx 1	-23	-29	-32	+41	YES	-1.1	+0.04	-	25 / -	39 / -
	Rx 2	-34	-9	-45	+16		-0.3	-0.002	-	21 / -	32 / -
	Con	-4	+15	-7	+3		+2.0	-0.05	-	46 / -	11 / -
Kane 1990	Rx	-31	-22	-39	+25.5	YES	-1.4	-	-	20 / -	32.5 / -
	Con	-9	4.5	-12	-0.6		+1.8	-	-	41 / -	12.5 / -
Watts 1992	Rx 1	-14	-20	-16	0	YES	-1.1	+0.03 *	+0.003	15 / -	38 / -
	Rx 2	-25	+0.5	-36	-4		-1.9 **	+0.117 ***	+0.103 ***	12 / -	33 / -
	Con	-2	+1.3	-3	-0.8		+5.8	-0.232	-0.201	46 / -	4 / -
Schuler 1992	Rx	-10	-24	-8	+3	YES	-1 **	-0.01 *	-	20 / -	30 / -
	Con	0	-17	+2	0		+3	-0.13	-	42 / -	4 / -
Blankenhorn 1993	Rx	-32	-22	-45	+8.5	YES	+1.6	-	-	47 / -	23 / -
	Con	-2	+3.5	-3	+2		+2.2	-	-	65 / - **	11 / - *
Haskell 1994	Rx	-16	-19	-23	+12	YES	+1.2 *	-0.08 ***	-0.09 *	50 / 29 **	20 / 14 **
	Con	-1.5	-0.6	-5	+5		+3.6	-0.2	-0.17	50 / 41	10 / 12
Waters 1994	Rx	-21	-8	-29	+7	YES	+1.7 *	-0.05 **	-	42 / 6.8 *	19 / -
	Con	-1	-4	-2	+3		+2.9	-0.09	-	56 / 9.4	13 / -
MAAS 1994	Rx	-22	-12.5	-31	+7	YES	+1.0 **	-0.04 **	-0.02 *	23 / 3.1	18.6 / 1.8
	Con	+0.3	+4.3	+0.7	-2.7		+3.6	-0.13	-0.08	32.3 / 4.0	12.0 / 1.8
Sacks 1994	Rx	-26	-20	-38	+13	YES	+2.1	-0.12	-	33 / 23	13 / 13
	Con	+2	+1	+3	0		+2.4	-0.17	-	38 / 28	15 / 13

1.6 PLASMA EXCHANGE THERAPY AND LDL-APHERESIS

1.6.1 History and Development

Physical removal of cholesterol as a treatment for hyperlipidaemia was first reported in 1967 [De Gennes *et al* 1967]. In a patient with homozygous familial hypercholesterolaemia a 40% reduction in plasma total cholesterol was achieved by the removal of 400 mls of plasma every 2-3 days over some months. The advent of continuous-flow cell separators made this a more practicable procedure, and its use was demonstrated by Turnberg *et al.* (1972) for the treatment of the hypercholesterolaemia of primary biliary cirrhosis. The principle was then applied to the treatment of homozygous familial hypercholesterolaemia by Thompson *et al* (1975).

The extracorporeal circuit for continuous-flow plasma exchange is illustrated diagrammatically in Figure 1.9. Venous blood is separated by centrifugation or a hollow-fibre membrane separator; plasma is collected and discarded, to be replaced by a suitable plasma substitute. This is most frequently 5% human albumin solution, which has superseded the use of fresh frozen plasma and plasma protein fraction because of the theoretical possibility of anaphylaxis or the transmission of viral agents.

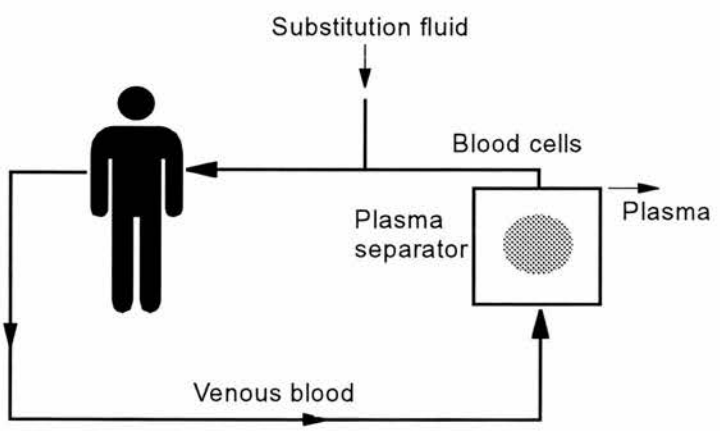
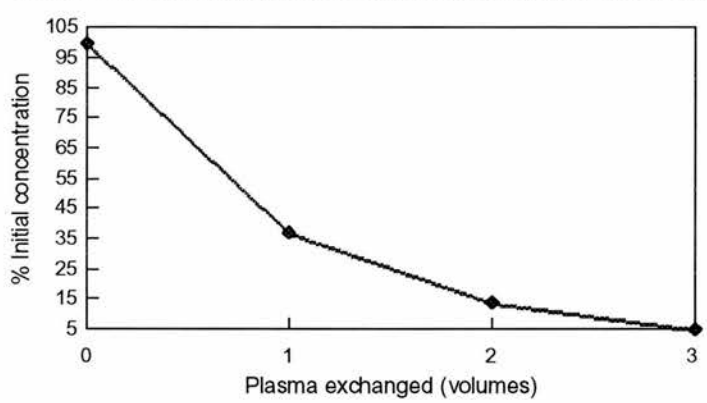


Fig 1.9 Continuous-flow plasma exchange

In view of the above drawbacks, the cost and limited availability of the replacement solutions, and the non-specific loss of other plasma proteins (including HDL), more selective techniques of LDL removal would seem preferable. Plasma lipoproteins had been shown to adhere to glass powder [Carlson 1960], and Lupien *et al* (1976) demonstrated the affinity of the apoprotein B-containing lipoproteins for heparin-agarose. The principles of on-line blood separation were combined with those of batch affinity chromatography to produce selective continuous removal of LDL [Burgstaler *et al* 1980]. Subsequent additions to the circuit have included regenerable affinity columns in series, with automated devices to divert the plasma through each of the two columns in rotation [Mabuchi *et al* 1987]. In this way the volume of plasma treated may be almost infinite, although the rate of reduction in plasma constituents will gradually diminish with increasing plasma volumes treated (see Figure 1.10). One such system is illustrated in Figure 1.11 (p 79) [Kaneka MA-01 circuit]: blood is obtained from venepuncture with a wide-bore cannula, and separated by a membrane separator (or centrifugal cell separator). The plasma is then pumped through the affinity columns, and the LDL-depleted plasma returned with the cells to be re-infused.

FIG 1.10 PLASMA COMPONENTS DURING PLASMA EXCHANGE



1.6.2 Methods of LDL-apheresis

Immunoadsorption

Stoffel and Demant first described an in-vivo technique specific for apoprotein B-containing lipoproteins in a swine model, preparing from the serum of sheep

immunised against pig LDL anti- LDL antibodies which were bound to sepharose [Stoffel & Demant 1981]. The method was adapted for clinical application using monospecific anti-human LDL antibodies from sheep immune serum [Stoffel *et al* 1981]. The columns may be used in pairs, and desorbed off-line. Following treatment, the columns may be stored under sterile conditions and re-used many times without loss of adsorptive capacity.

Double Membrane Filtration

This technique was first described in 1980, but did not find clinical application for six years [Mabuchi *et al* 1986]. After separation of the blood by the primary filter which has an average pore diameter of 0.2 microns, the secondary filter with a pore diameter of 0.03 - 0.06 microns fractionates plasma proteins according to their size. A slight modification to the technique involves the use of a cell separator instead of the primary filter.

Heparin Extracorporeal LDL Precipitation ('HELP')

Plasma obtained by filtration in this system is mixed continuously with heparin which forms a precipitate with apoprotein B at low pH. The precipitate is filtered out, and the filtrate passed through an adsorber to remove excess heparin before being dialsyed to restore physiological pH and electrolyte balance [Eisenhauer *et al* 1987]. In addition to removing LDL, HELP also eliminates fibrinogen and C4, but has no significant effect on HDL.

Precipitation with Dextran Sulphate

Dextran sulphate has been shown also to precipitate apoprotein B containing lipoproteins; the dextran sulphate may be covalently linked to an inert matrix, such as porous cellulose, and packed into columns through which plasma is pumped [Yokoyama *et al* 1985]. As in the system illustrated in Figure 1.11, two columns may be employed alternately in an automated device with off-circuit regeneration by hypertonic saline.

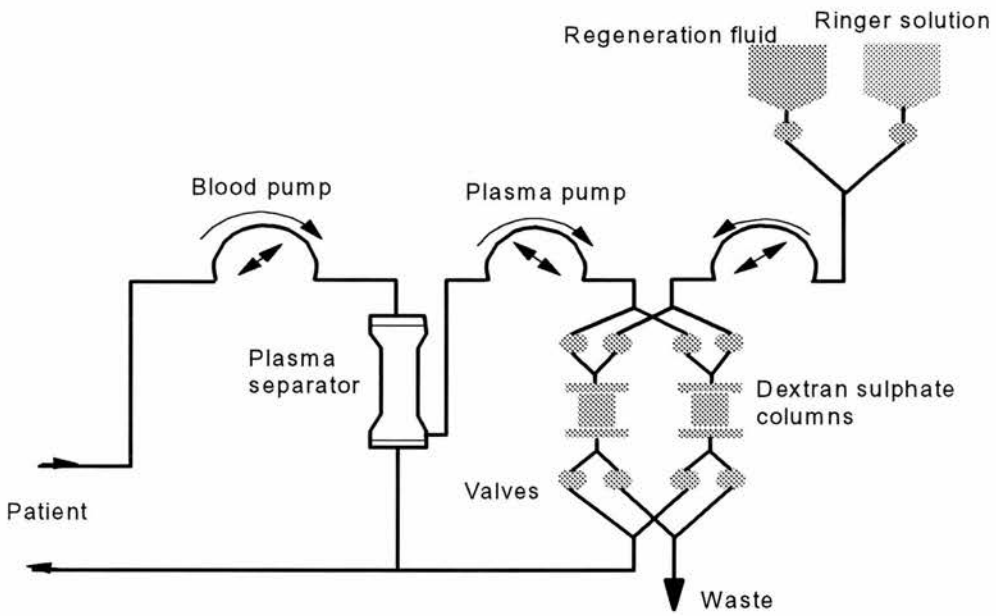


Fig 1.11 LA-15 LDL-apheresis system

Of the alternative methods available, the cheapest and most simple is the double membrane (or, 'cascade') filtration. It is however the least selective, and has the disadvantage of requiring protein replacement if performed at intervals of less than two weeks. HELP is highly effective, and the removal of fibrinogen in addition may well be advantageous. The system though is more complex, and the time required to set up is greater. Immunoadsorption and dextran sulphate are also both highly selective and appear equally effective. Immunoadsorption columns require more time to prepare, and since they require to be re-used need more thought regarding proper storage and sterilisation between treatments than the alternatives. The dextran sulphate columns are presently available only as single-use devices; while this ensures their sterility and reduces preparation time, it also makes them expensive to use. Whichever method of removal is adopted, it is the degree to which cholesterol is lowered and the interval between treatments which determine the effects.

1.6.3 Effects of Apheresis

The rapid decrease in cholesterol levels produced by plasma exchange or LDL-apheresis is followed by a comparatively slow rebound phase. The slope of

the rebound curve of the plasma cholesterol is determined by its fractional catabolic rate [Apstein *et al* 1978], which is uninfluenced by the rapid decrease in LDL pool size [Thompson *et al* 1981]. The mean cholesterol concentration during repeated apheresis sessions may be calculated by integrating the area under the rebound curve joining each set of post-treatment and pre-treatment values. However this necessitates frequent blood sampling, and if the interval between treatments is less than the time taken for the pretreatment value to be reached, the arithmetic mean may be used as an approximation for the calculated average. Very low time-averaged levels may be maintained by shortening the time interval between treatments, although protein losses would limit this approach with the less specific techniques such as plasma exchange or cascade filtration.

Treatment with double membrane filtration reduces total and LDL cholesterol by approximately 55%. Complement components, fibrinogen, and immunoglobulins are all significantly reduced. Similar reductions in total cholesterol are observed during HELP treatment, but albumin and immunoglobulins are not reduced to the same degree. Dextran sulphate and immunoadsorption have been reported to reduce LDL cholesterol acutely by 54 - 80%, and time-averaged LDL by 40 - 70%. HDL may be decreased by these methods by 10 - 20%, perhaps partly a dilutional effect, although a time-averaged twofold rise in HDL has been documented [Parker *et al* 1986]. This contrasts with the long-term effects of double membrane filtration on HDL levels, which are reduced by about 15%.

No major side effects have been reported with any of the techniques described. All have been associated with occasional chills, shivering and episodes of hypotension in a small minority of treatments, but on the whole therapy has been well-tolerated.

1.6.4 Clinical Benefits of Apheresis

Longterm effects of regular plasma exchange have been most striking in homozygous familial hypercholesterolaemia, in which the progression of atherosclerosis is usually inexorable. Exchange of 2 - 4 litres at 2-weekly intervals over more than two years in two homozygotes resulted in a reduction of 45% in mean total cholesterol, associated with reduction in tendon xanthomata and

stabilisation of coronary lesions. Over the same period two other homozygotes were treated monthly; they experienced a reduction in cholesterol levels of 33%, but had an increase in pressure gradient across the aortic valve and no xanthoma regression [Thompson *et al* 1980]. Thompson has shown an improvement in life expectancy in homozygotes undergoing longterm plasma exchange compared to untreated siblings: age at death of the untreated subjects was 17.7 year (SD 3.8), compared to current age or age at death in the treated group of 25.7 (SD 3.7), $p < 0.001$ [Thompson *et al* 1989].

The manifestations of atherosclerosis have also been shown to improve by long term weekly treatment with LDL-apheresis. In eleven subjects with familial hypercholesterolaemia, xanthomata of skin and tendons resolved or were significantly reduced [Borberg *et al* 1988]. After a mean of 35 months severity of angina was significantly improved, associated with a mean increase in exercise capacity of 48%. At angiography, of 23 stenoses identified in this group of patients, 10 showed evidence of regression while only one appeared to have progressed.

A larger series of patients with familial hypercholesterolaemia treated regularly by LDL-apheresis and evaluated by interval angiography has recently been described [Tatami *et al* 1992]. These include 7 homozygotes, who had been treated for several years and had undergone 2 coronary angiograms on average four years apart. Although there are a number of methodological problems, the authors document regression in a significant proportion of the lesions analysed, including several in homozygous patients in whom the pre-apheresis LDL cholesterol averaged 10 mmol/l.

Experience with plasma exchange therapy and LDL-apheresis has shown that it may safely be carried out over prolonged periods. The acute perturbations in the plasma lipids are profound, and there is a highly significant reduction from baseline when this is applied regularly. It enables the maintenance of exceptionally low plasma lipid levels, and its effects may be further augmented by the addition of lipid-lowering drugs. It is well-established in the management of patients with homozygous familial hypercholesterolaemia, and has proved of value also in heterozygotes. Its application in non-familial hypercholesterolaemia has not been examined previously.

2.1 RATIONALE

Evidence for a possible threshold effect

2.1.1 Epidemiological evidence

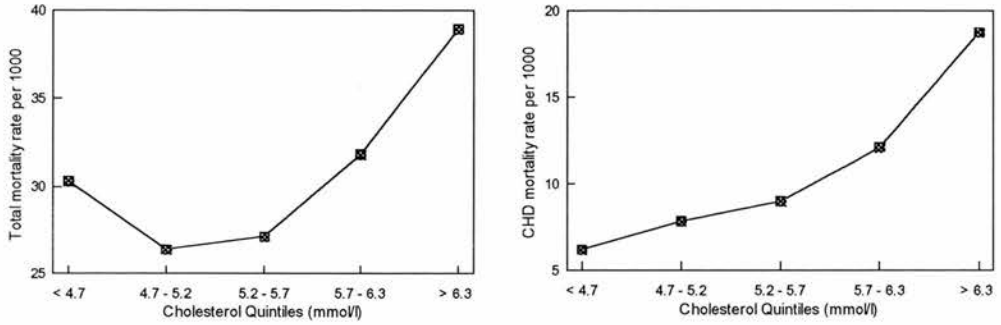
The evidence linking the incidence of coronary disease, and particularly coronary mortality, to serum total or low-density lipoprotein cholesterol is convincing and consistent. Coronary heart disease and cholesterol are strongly correlated between populations [Keys *et al* 1984] and within populations (see Sect. **1.2.1.1**).

The table showing the 6-year age-specific coronary mortality from follow-up of subjects screened for the Multiple Risk Factor Intervention Trial illustrates that the relative risk for subjects in the top quintile are high compared to those with the lowest serum cholesterol [Kannel *et al* 1986]. The curves however are clearly non-linear (Fig 2.1), and the attributable risk between the first and the second quintiles even among the men aged 55 to 57 years is only 1.7 deaths/1000. At these levels of cholesterol there is virtually no increase in relative risk, including among those with the highest absolute risk of cardiac death (ie. cigarette smokers in the older age groups) unless significant hypertension is also present (see Table 2ii).

TABLE 2i. 6-yr CARDIAC MORTALITY BY AGE AND SERUM CHOLESTEROL

AGE	No.	Mean TC *(mmol/l)*	6-yr CHD mortality (per 1000)			
			TC < 4.7	TC 4.7 - 5.2	Q2/Q1	Q5/Q1
35 - 39	65,664	5.34	0.6	0.8	1.3	8.2
40 - 44	70,328	5.53	1.4	2.6	1.9	6.1
45 - 49	77,068	5.64	3.1	4.5	1.5	4.1
50 - 54	77,290	5.68	6.2	7.9	1.3	3.0
55-57	35,034	5.68	10.2	11.9	1.2	2.4
TOTAL	325,384	5.57	3.7	4.9	1.4	5.2

FIG 2.1 6-YR TOTAL AND CARDIAC MORTALITY IN MRFIT FOR MEN AGED 50 - 54 YRS



Kannel et al 1986

TABLE 2ii.

RATIO OF 6-yr CARDIAC MORTALITY IN 1st AND 2nd CHOLESTEROL QUINTILES IN MEN AGED 46-57 YEARS BY SMOKING AND Bp QUINTILE

	DIASTOLIC Bp				
	< 76	76 - 80	81- 85	86 - 91	> 91
NON-SMOKERS	1.1	1.1	1.9	1.2	1.1
SMOKERS	1.1	1.0	1.0	0.8	2.3

Similar results are seen in the other large prospective epidemiologic studies including the Framingham study [Kannel *et al* 1971], the British Regional Heart Study [Shaper *et al* 1985], the Whitehall study [Rose & Shipley 1986], and a study of Israeli civil servants [Gouldbourt *et al* 1985]. The latter two studies used death from coronary heart disease as an endpoint, rather than incidence of coronary heart disease. In the Whitehall study the relative risk of ten-year coronary mortality was 1.9 in the top quintile compared to the lowest quintile, but only 1.2 for the second quintile to the lowest. Fifteen-year follow-up of ten thousand men in Israel (in whom the distribution of serum cholesterol levels was virtually identical to the MRFIT screenees) showed that the relative risk of coronary death in this population was not increased in either the second or third quintiles for total cholesterol, although the follow-up period was considerably longer than in MRFIT and the mean age at entry 50.6 years compared to 46.1 years. A later follow-up of

this group at twenty-three years also confirmed this trend of slowly rising risk for coronary mortality at low cholesterol levels and rapidly escalating risk with higher levels, the curve rising only beyond the third decile (total cholesterol > 4.8 mmol/l) [Goldbourt & Yaari 1990].

Although the relative risks between the study populations were very similar, the absolute incidence of coronary mortality was greater in the U.S. than in Israel. Other comparisons between populations have reported similar findings: predictions of incidence of coronary disease in American men derived from regression coefficients in Europeans (and vice versa) were highly correlated with observed incidence, although there was a significant difference in absolute risk [Keys *et al* 1972].

Most of the epidemiological data accrue from observation only in men. Although the absolute and attributable risks of serum cholesterol for coronary death is much less in middle-aged women than in men, a similar curvilinear distribution is seen in women with a relative risk of 1.2 for those in the second quintile and 1.8 for the fifth (compared to 1.1 and 1.7 for men in this study) [Isles *et al* 1992, DJ Hole - personal communication].

TABLE 2iii. AGE-ADJUSTED 12-yr INCIDENCE OF M.I. (RATE/1000)

	HDL Quartile	TOTAL CHOLESTEROL QUARTILE			
		1st	2nd	3rd	4th
Men	1st	171	170	129	143
	4th	22	131	87	109
Women	1st	94	76	126	131
	4th	13	0	23	20

(Adapted from Wilson 1990)

HDL-cholesterol is also of considerable importance as assessed by epidemiological data. Twelve year follow-up of the Framingham data show that for women there was a statistically significant effect of HDL-cholesterol levels within each quartile of total cholesterol [Wilson 1990] (Table 2iii). This inverse relationship was strongest for those in the top quartile (total cholesterol 6.9-10.7

mmol/l), with a relative risk of 6.6 for those in the lowest quartile of HDL compared to those in the fourth. This trend was significant in men only for those in the lowest quartile for total cholesterol [Castelli *et al* 1986, Abbott *et al* 1988].

Other studies - the Lipid Research Clinics (LRC) Prevalence Mortality Follow-up Study, the LRC Coronary Primary Prevention Study, and MRFIT - which examined separately the effects of HDL levels also demonstrate that crude coronary event rates were generally highest in those with HDL-cholesterol below 1.04 mmol/l and lowest in those with levels above 1.3 mmol/l for both men and women [Gordon *et al* 1989].

The Israeli civil servants study [Goldbourt *et al* 1985, Livshits *et al* 1989] found HDL to be a better predictor of cardiac mortality than total cholesterol, and that describing the HDL as a proportion of the total cholesterol is the best of all the parameters (see Table 2iv).

TABLE 2iv.
15-yr CHD RATE BY QUINTILES OF HDL and TC/HDL RATIO

QUINTILE	HDL *(mmol/l)*	Cardiac mortality *(per 1000)*	Relative risk
I	< 0.75	88	2.3
II	0.75 - 0.85	77	2.0
III	0.86 - 0.96	66	1.7
IV	0.97 - 1.11	46	1.2
V	> 1.11	38	1
	TC/HDL *ratio*	**Cardiac mortality** *(per 1000)*	**Relative risk**
I	> 7.1	99	3.7
II	7.1 - 6.7	70	2.6
III	5.4 - 6.6	62	2.3
IV	4.3 - 5.3	45	1.7
V	< 4.3	27	1

Adapted from Gouldbourt 1985

In keeping with the other studies above, the TC/HDL ratio in the Israeli study was of particular value in accurately predicting coronary events in the group with a "normal" total cholesterol.

The PROspective CArdiovascular Munster (PROCAM) Study screened 19 698 subjects for the prevalence of cardiovascular risk factors, and have followed longitudinally 7220 men between 40 and 65 years who had no history of previous myocardial infarction or stroke. 73 of the 2815 subjects who had completed four years of follow-up to 1989 had sustained a non-fatal MI or coronary death. The single most predictive parameter was HDL cholesterol - more than 64% of those who had a cardiac event had HDL below 0.9 mmol/l compared to just 18% of those without cardiac disease. The relative risk was further enhanced by the substitution of HDL with TC/HDL [Assmann & Schulte 1992].

2.1.2 Phylogenetic

With the exception of a few species with strains which may exhibit metabolic disorders, man is virtually alone in developing atherosclerosis spontaneously. The incidence of atherosclerotic disease is roughly inversely proportional to the average serum cholesterol. Below a level of approximately 3.4 mmol/l, and LDL below 1 mmol/l, the development of atherosclerosis is very uncommon.

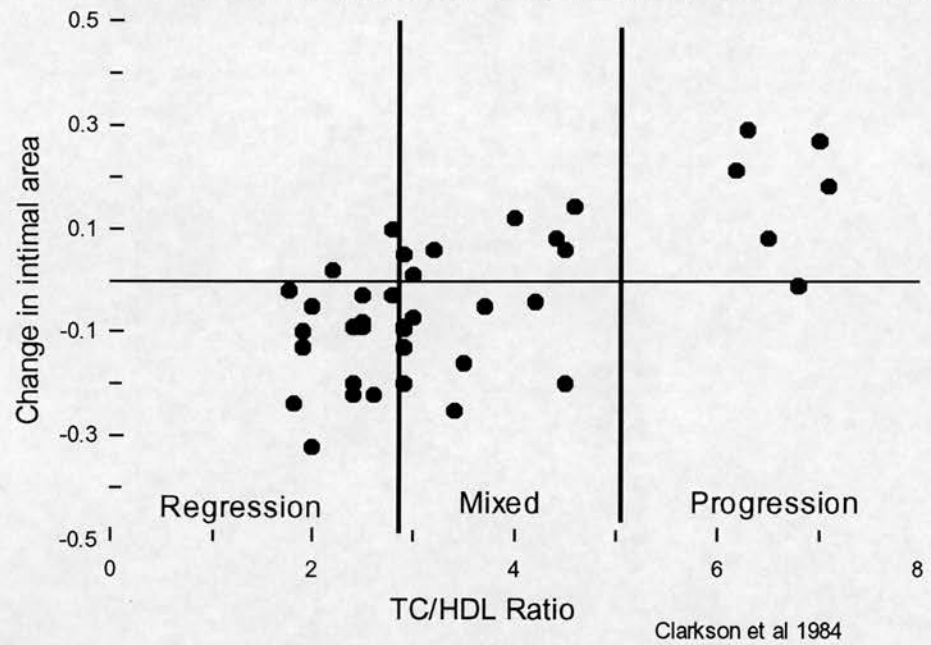
2.1.3 Animal Studies

Many species can be induced to develop atherosclerosis in response to fat feeding, although it is readily apparent that their lipoprotein metabolism and the susceptibility to atherosclerosis differs significantly from man. Non-human primates readily develop atherosclerosis on high-fat diets. The restoration of the normal diet after the disease has developed affords us the opportunity to test the 'threshold hypothesis', ie. that a particular level of cholesterol is required to consistently achieve regression of established disease. One elegant study of regression in diet-induced atherosclerosis was carried out by Clarkson and colleagues in rhesus monkeys [Clarkson *et al* 1984].

Those animals in which the TC/HDL ratio remained above 5 exhibited almost universal progression of disease, whereas regression was the rule when TC/HDL was lowered to 2.8 or less (Fig 2.2). Other studies in non-human primates have

shown that regression will occur when the plasma cholesterol level is lowered below 150 mg/dl (3.9 mmol/l) [Eggen *et al* 1987], although with significant variability between animals when the atheromatous lesions are produced by maintenance of moderately elevated lipid levels for prolonged periods. It is also of interest that while the component of the lesion showing the greatest change is the cholesteryl esters, both collagen and elastin in the plaques are also reduced [Wissler & Vesselinovitch 1989, 1990].

FIG 2.2 TC/HDL RATIO AND REGRESSION OF ATHEROSCLEROSIS



Clarkson et al 1984

2.1.4 Intervention Studies

The plasma lipoprotein concentrations required to induce egress of cholesterol from plaques in man is not known. A few studies have angiographically documented reduced progression of disease in most lesions with a minority of lesions undergoing regression, but none of the intervention studies previously published have achieved the goals outlined in the above animal studies.

Nikkilä demonstrated significant differences in the occurrence of progression between subjects above and below the median cholesterol value (6.30 mmol/l), and between those with HDL levels above and below the median (1.10 mmol/l)

[Nikkilä *et al* 1984]. There were clear trends in lipid levels between subjects when grouped for the degree of progression, (including LDL/HDL ratio) but the correlation between proportional increase in luminal narrowing and lipid variables was significant only for HDL-C ($r = -0.36$, $p < 0.05$). No regression was observed in the treatment group in this uncontrolled study.

In the uncontrolled Leiden Intervention Trial there was a mean progression of coronary lesions of 4.5 percentage points in diameter stenosis over two years ($p < 0.001$) despite a reduction in total cholesterol and TC/HDL ratio of 10% which was achieved by diet alone [Arntzenius *et al* 1985]. There was however a significant association between disease progression and the on-trial and baseline TC/HDL ratio. Subjects with a baseline TC/HDL ratio below the median of 6.9 who reduced this by at least 2% during the study had no evidence of disease progression. In subjects with baseline TC/HDL above 6.9, those in whom TC/HDL was lowered by 13 percent to below this value had no progression whereas for those in whom this parameter remained above 6.9 (even if it had been reduced by 8%) exhibited decrease in mean luminal diameter.

Following adjustment for baseline inequalities between randomised groups in the NHLBI Type II Coronary Intervention Study, there was a significant reduction in progression of lesions greater than 50% stenosed at baseline in the intervention group. Analyses which included all lesions showed trends in favour of the treatment group, but overall these were non-significant [Brensike *et al* 1984]. Analysis of the relationship between changes in lipid values and likelihood of progression however, demonstrate that the subjects with the greatest decrease in TC/HDL ratio had progression rates of less than 50% of those with the least decrease. The mean decrease in the TC/HDL ratio in the treatment group was 23% (from a mean ratio of 7.7 to 5.9).

In the other randomised placebo-controlled angiographic trial available before commencement of this study [Blankenhorn *et al* 1987], the reduction in the serum lipids were much greater than in previous studies and the on-trial lipids approached the levels at which regression might be expected (table 2v).

TABLE 2v. LIPID LEVELS DURING ANGIOGRAPHIC TRIALS

STUDY	GROUP	BASELINE LEVELS				ON-TRIAL LEVELS				% CHANGE			
		TC	LDL	HDL	TC/HDL	TC	LDL	HDL	TC/HDL	TC	LDL	HDL	TC/HDL
NHLBI	C	7.6	5.9	1.0	7.6	7.5	5.7	1.0	7.5	-1	-5	+2	-1
	I	8.0	6.3	1.0	7.7	6.6	4.6	1.1	5.9	-17	-26	+8	-18
CLAS	C	6.3	4.4	1.1	5.6	6.0	4.1	1.2	5.2	-4	-5	+2	-6
	I	6.4	4.4	1.2	5.5	4.6	2.5	1.6	3.0	-26	-43	+37	-47

C = Controls, I = Intervention group

From Brensike 1984 & Blankenhorn 1987

The angiographic results were not presented as correlations with changes in serum lipids on an individual basis, but demonstrate a significant reduction in coronary global score in the treatment group for both those above and below a baseline total cholesterol level of 6.25 mmol/l. Despite the impressive reductions in lipids maintained for two years, progression continued in 39% of the treatment group (Table 2vi).

TABLE 2vi. OUTCOME OF ANGIOGRAPHIC TRIALS

STUDY	GROUP	No.	No. (%) of SUBJECTS WITH :		
			REGRESSION	UNCHANGED	PROGRESSION
NHLBI	C	57	4 (7%)	24 (37%)	29 (51%)
	I	59	4 (7%)	31 (53%)	24 (41%)
CLAS	C	80	2 (2%)	29 (36%)	49 (61%)
	I	82	13 (16%)	37 (45%)	32 (39%)

C = Controls, I = Intervention group

It cannot be concluded from the above that atherosclerosis in man will necessarily respond to lipid lowering at the same levels as in the experimental models. Apart from possible species differences, human disease is often far advanced before resulting in symptoms and the lipid component may be less accessible due to greater degrees of fibrosis and calcification. It remains unclear whether there is a threshold for serum cholesterol (or TC/HDL) which requires to be achieved or whether the extent of reduction is the more important - the NHLBI study did

suggest a threshold for non-progression even when the TC/HDL ratio was reduced to a greater extent in some individuals above that level than in others below it; on the other hand the CLAS results demonstrate as much benefit for those at the lower end of the cholesterol distribution as those with higher levels, and suggest that the proportional reduction in lipids is of greater importance for the induction of regression.

The central thesis of these studies is that the achievement and maintenance of extremely low cholesterol levels (< 3.4 mmol/l) and TC/HDL ratio (< 2.8) will increase the frequency of regression and stabilisation of human atherosclerotic plaques than currently recommended targets for therapy (total cholesterol < 5.2 mmol/l) in this group. The importance of this goal is clear from the observation that "patients whose lesions do not show progression have improved chance of survival and lower rates of myocardial infarction" [Moise *et al* 1985].

These goals were applied in a group of patients with coronary disease and severe hypercholesterolaemia in a pilot study in New York [Saal *et al* 1989]. The proposed lipid levels were achieved in the majority of their subjects using combination drug therapy and regular LDL-pheresis. The subjects reported an improvement in their angina with universal improvement in objective exercise testing after treatment for > 1.5 years, and without any serious adverse event in up to 4 years of therapy.

2.2 DESIGN

2.2.1 Patient recruitment, selection and exclusion criteria

Participants in these studies were recruited from various sources within Glasgow Royal Infirmary. The principal source was the pool of patients undergoing diagnostic coronary angiography for investigation of angina. All of the patients in our institution undergoing angiography had blood drawn for fasting lipoprotein profile, and the angiograms of those with hypercholesterolaemia were reviewed. Those with at least two significant stenoses in the major epicardial arteries and who were deemed unsuitable for re-vascularisation procedures were considered for the study, and this identified 15 of the 22 participants. I also identified individuals attending the lipoprotein clinic with possible angina or with a bad risk factor profile; if these patients had evidence of significant reversible ischaemia on an exercise treadmill test, they were further investigated by coronary angiography using the study protocol. In addition all patients attending the 'Chest Pain Clinic' had fasting lipid levels checked, and those who proceeded to angiography following a positive exercise test were investigated according to the study protocol. Patients who had undergone coronary artery bypass surgery or angioplasty more than 12 months previously were considered suitable.

The lipid levels were checked after a minimum of three months on a low-cholesterol diet and on no lipid-lowering drugs. To be suitable the total cholesterol had to be less than 10.5 mmol/l and above 7 mmol/l with a TC/HDL ratio of at least 4.5, or above 6.5 mmol/l with a TC/HDL ratio exceeding 6. Fasting triglycerides were required to be below 2.8 mmol/l. The patients - who had to be below the age of 60 at the time of the baseline angiogram - were required to be non-smokers or to have given up the habit at least six months before study entry. The fasting blood sugar had to be less than 6.5 mmol/l, and there was to be no evidence of renal or hepatic dysfunction. Other exclusion criteria included uncontrolled hypertension, dysrhythmias, intractable cardiac failure, coagulation disorders and any other condition which might limit life expectancy. An ejection fraction less than 30% on ventriculography was considered unsuitable.

2.2.2 Patients - Baseline Characteristics

Using the above criteria I identified 22 patients who were suitable, whose general practitioner and cardiologist both agreed to their participation, and who gave informed consent. They were aged between 37 and 60 years, and 20 of them were men.

TABLE 2vii. PARTICIPANTS' THERAPY AND PAST HISTORY

	GROUP 1	GROUP 2
AGE (mean (SEM))	50.6 (2.3)	52.9 (2.3)
HISTORY OF MI	4	4
HISTORY OF CABG	3	2
PREVIOUS PTCA	0	0
EX-SMOKER	8	7
HYPERTENSION	0	2
HYPOLIPIDAEMIC THERAPY	1	4
PROBABLE FAM. HYPERCHOL.	3	2
ON ASPIRIN	7	8
BETA-BLOCKER	6	8
CALCIUM ANTAGONIST	10	6
NITRATES	8	1
No of ANTI-ANGINAL DRUGS = 1	1	3
No of ANTI-ANGINAL DRUGS = 2	4	6
No of ANTI-ANGINAL DRUGS = 3	5	0

TABLE 2viii. BASELINE LIPID RESULTS

	GROUP 1 *MEAN (SEM)*	GROUP 2 *MEAN (SEM)*
TOTAL CHOLESTEROL	8.01 (0.45)	8.23 (0.45)
TRIGLYCERIDES	2.13 (0.15)	1.89 (0.18)
VLDL-C	1.01 (0.10)	0.90 (0.13)
LDL-C	5.84 (0.42)	6.27 (0.41)
HDL-C	1.16 (0.08)	1.09 (0.04)
TC/HDL	7.32 (0.77)	7.72 (0.59)
LDL/HDL	5.38 (0.77)	5.84 (0.55)

The first twenty patients were allocated at random to one of the two treatment groups. It had initially been intended to have a third group of patients to be

assigned to 'usual care', but the recruitment rate was much slower than planned and time constraints prohibited extension of the recruitment phase.

2.2.3 Study protocol

The selection of the patients for the studies was performed if, following three months on a cholesterol-lowering diet, the mean of at least two samples for lipoprotein analysis fulfilled the criteria outlined above, the other entry criteria were met and there were no grounds for exclusion. The proposed subjects were required to consent to allocation to any of the three planned treatment groups, and those who agreed to participate conditional on their allocation to any particular group were excluded. Since those assigned to regular apheresis would require regular venous cannulation, the ease of venous access was also considered at this time before their consent to the study was sought. The patients then underwent tracer studies of lipoprotein metabolism. During this time they were required to complete 7-day weighed dietary records and undergo treadmill exercise testing, bicycle ergometry and coronary angiography (if not performed according to the study protocol within the previous three months).

Following assessment of the dietary records all the patients were instructed by a qualified dietitian on a suitable diet to achieve their ideal body weight, restrict daily intake of cholesterol to a maximum of 200 mg/day, the fat intake to no more than 25% of total calories, and maintain a polyunsaturated:saturated fat ratio greater than 1.

The patients were then allocated to one or other of the treatment groups: one group ('Group 1') was to maintain a time-averaged total cholesterol of 3.4 mmol/l or below and a TC/HDL ratio of 2.8 (with a secondary goal of a time-averaged LDL level of 1.5 mmol/l); another active intervention group ('Group 2') was to maintain an average total cholesterol of 5.2 mmol/l (with a secondary goal of TC/HDL of 3.5 - 4.0), and a third to be assigned to 'usual care' without a defined lipid goal, the treatment being prescribed by the patients' own doctor or cardiologist. This process was random, in that the patients were not selected by age, sex, angiographic results or lipoprotein levels. The first six patients to complete the baseline tests were allocated to the lower treatment goal, since this was the

maximum number of patients which could be readily accommodated weekly on a single apheresis machine. The next six were allocated to the more conservative goal, and it had initially been planned to have the third six allocated to 'usual care'. This was abandoned when the recruitment rate failed to meet original expectations, and the treatment allocation altered to have the remaining eight subjects assigned alternately in pairs. According to this amended randomisation schedule, patient #14 was to be treated by LDL-apheresis. Although ease of venous access had been considered satisfactory at the time of study entry, regular sampling during the tracer studies proved difficult. We attempted apheresis on a single occasion in this individual, but failed to obtain satisfactory blood flow rates despite repeated repositioning of the venous cannulae. Having undergone extensive baseline investigation and prepared for regular intensive therapy, rather than exclude him from the study he was assigned to the other active treatment group. Patient #15 took his place in the apheresis schedule, #16 allocated as planned to group 2, and the remaining pairs allocated alternately to each group as planned.

The subjects in group 1 were commenced on weekly LDL-apheresis without concomitant lipid-lowering drugs for four weeks to assess the effects of apheresis alone in patients with non-familial hypercholesterolaemia. At the fourth treatment they were commenced on either cholestyramine, or pravastatin, depending partly on previous tolerance of the resin but also in order to compare their effects when added to regular apheresis. For both treatment groups cholestyramine was commenced at one sachet daily for the first week and increased by increments of one sachet daily at intervals of one week, to an initial maximum of four sachets. The pravastatin was initiated at 20 mg daily. After four weeks if the primary lipid targets were not being maintained the dosages were increased (cholestyramine gradually to eight sachets daily, and pravastatin to 40mg) and unaltered for a further four weeks.

The other agent was later added to the first if one drug alone was insufficient, the resin being added in increments of two sachets daily and the HMG CoA reductase inhibitor at doses of 20, 40 and 80 mg. When 80mg of pravastatin was reached together with the maximum tolerated dose of the resin, Acipimox (a nicotinic acid derivative) was added incrementally until the serum goals were achieved.

For the patients in group 1, LDL-apheresis was performed weekly using the Kaneka 'MA-01' system (Kaneka Chemical Industries, Osaka, Japan) [Mabuchi *et al* 1987]. Blood is obtained by peristaltic pump following forearm venepuncture with a wide-bore fistula needle, and the plasma separated by a polysulfone membrane separator (effective pore size 200 microns). The plasma is then pumped through one of two columns containing dextran sulphate covalently bound to an inert cellulose matrix, the columns used in rotation and regenerated off-line by rinsing with hypertonic saline. The lipoprotein-depleted plasma is then returned to the patient with the blood cells (Fig 1.11, p 79). We attempted to treat approximately 5000 mls plasma on each occasion.

Blood was taken before and after each apheresis session in the patients in group 1, although alterations in therapy were made at intervals of not less than one month according to the recent trends. Lipoproteins were measured monthly in group 2. For both groups therapy was adjusted throughout the study to maintain the primary goals only as much as possible; further alterations were not made in group 2 if the total cholesterol was 5.2 mmol/l or less even if the TC/HDL ratio was higher than desired. Similarly in group 1, I aimed to achieve the time-averaged LDL goal as much as possible, but adjusted therapy only on the basis of the total cholesterol and TC/HDL levels. If these were exceeded after one month, the patient was counselled appropriately by the dietitian or myself, and drug therapy adjusted only if results after a further month remained above target levels.

The lipid goals were maintained for a period of two years. During this time the response to the treatment was assessed subjectively by the completion of questionnaires at intervals (the Nottigham Health Profile), and objectively by exercise tests on the treadmill at 6-monthly intervals and by thallium scanning once yearly. At the completion of the two-year period all the subjects underwent repeat coronary angiography according to the study protocol, and after stopping apheresis and drug therapy for one month had further tracer studies of lipoprotein metabolism.

2.2.4 Statistical considerations

The primary endpoint of greatest importance was the demonstration of a significant difference in the proportion of patients in groups treated to different lipid goals showing angiographic evidence of regression. The standard for comparison was chosen to be the lipid goals widely recommended for the management of patients with established coronary disease, and the expected proportion of patients treated to this goal was estimated from CLAS data to be approximately 10%. The sample size required to show a 20% difference between the two treatment groups in the proportion of patients demonstrating a reduction in the global coronary score with 80% power on a one-sided test at the 95% level was calculated to be 100. A 30% regression rate was felt to be the minimum worthwhile to justify the more aggressive regime with extracorporeal therapy.

To achieve this recruitment a multicentre study was planned with colleagues in four centres in the U.S., and the USSR Cardiological Research Centre in Moscow. Regrettably the joint study did not proceed due to funding difficulties, and the twenty Glasgow patients alone were studied. This number is clearly too small to detect a significant difference between two active treatment schedules. It may however be sufficient to establish the feasibility of such alternative therapy and to demonstrate its safety in the treatment of individuals with advanced coronary disease. It will also extend the lower limits of previously achieved lipid levels and enable us to examine whether the relationship between the extent to which cholesterol is lowered and the rate of disease progression continues to hold at these extremes.

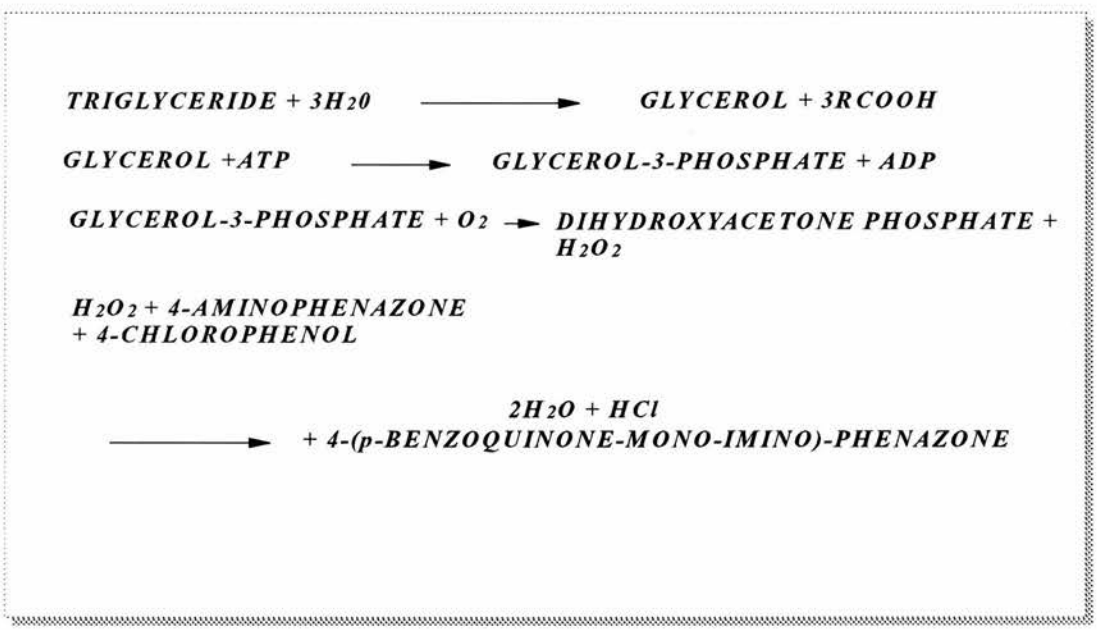
3.1 EFFECTS ON LIPIDS AND LIPOPROTEINS

3.1.1 Methods of measurement

3.1.1.1 Lipoproteins

Plasma was collected from each subject (after a 14-hour overnight fast) from a large antecubital vein with minimal venous occlusion, into disodium-EDTA containers. This was spun immediately, and the plasma separated by pipette leaving approximately 0.5 mls above the buffy coat. The plasma was stored at +4°C and analysed usually within 24 hours, and always within 72 hours, of venepuncture.

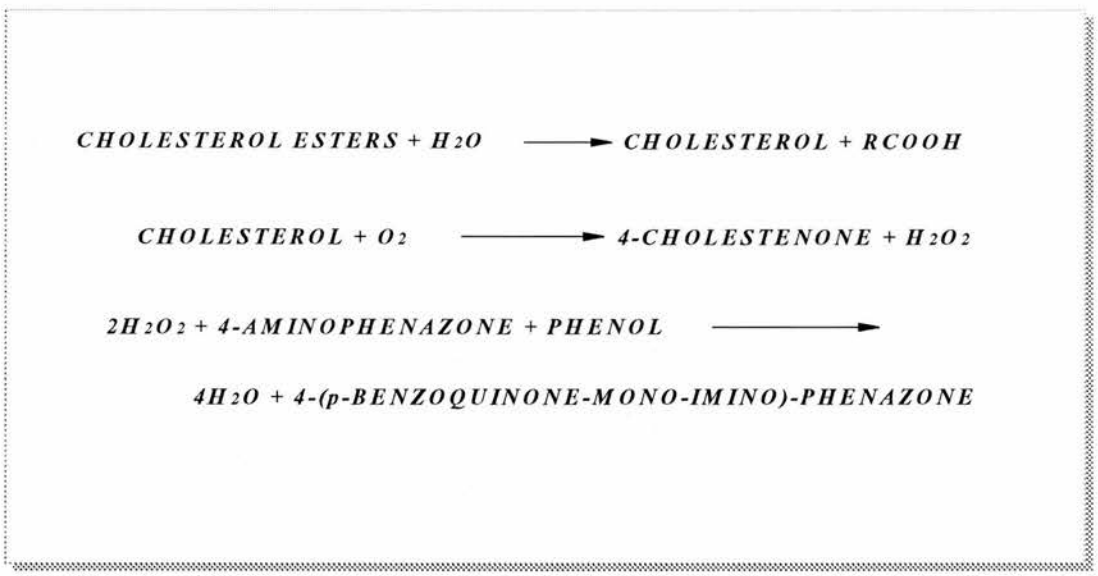
Triglycerides were measured in whole plasma using a colorimetric method (Boehringer Kit No 704113), which utilises the following principle:



The resulting colour changes were then measured on a Hitachi 704 auto-analyser at 505nm.

Lipoprotein classes were prepared by ultracentrifugation and selective precipitation by standard methodology [Lipid Research Clinics Program Manual of Laboratory

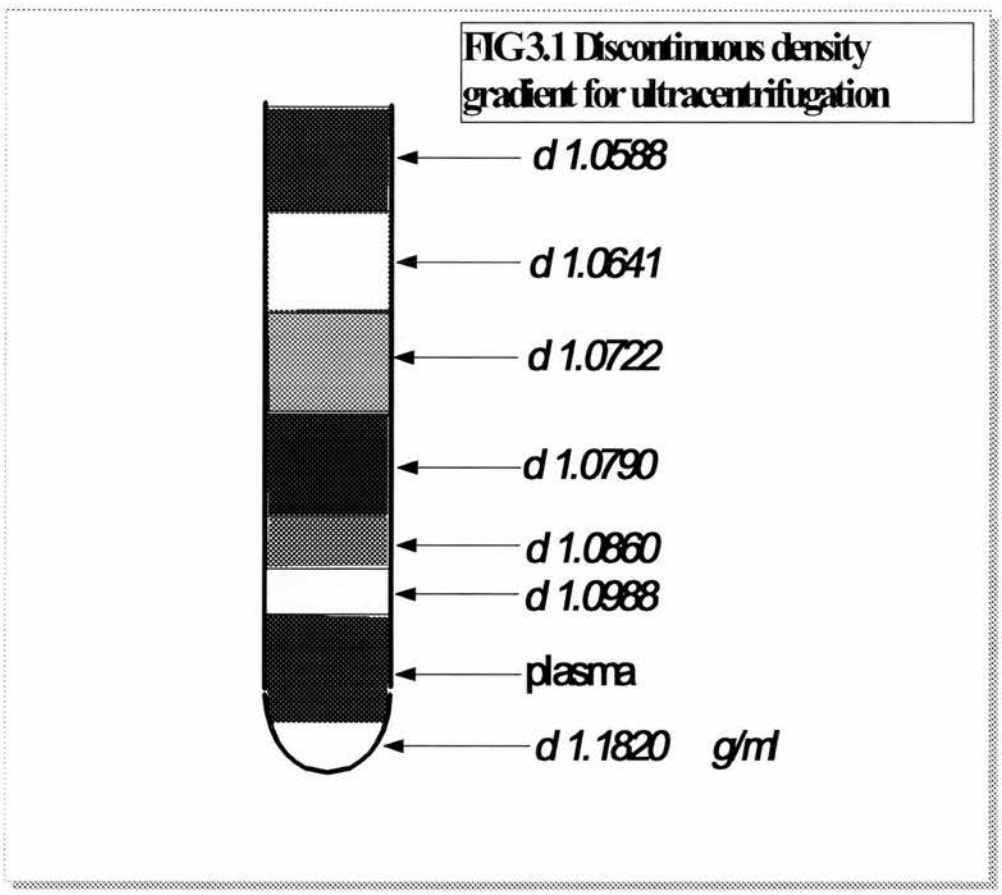
Operations, 1984]. A 5-ml aliquot of plasma was overlaid with 2 mls of d 1.006 g/dl density solution in a Beckman Ultra-clear tube which was capped and centrifuged overnight at 4°C in a Beckman 50.4 rotor at 35000 rpm. VLDL were measured in the supernatant in a standard volume, and the bottom fraction was transferred to a volumetric flask and made up to 5 mls. The IDL and LDL in an aliquot of the bottom fraction was then precipitated by a heparin/Mn^{2+} reagent and separated by centrifugation in a Beckman centrifuge at 10000rpm for 30 minutes. HDL were measured in the supernatant, and the cholesterol in the IDL-LDL fractions (density range 1.006 - 1.063) calculated by subtraction of the VLDL- and HDL- from the total cholesterol. Cholesterol was determined in each fraction by an enzymatic colorimetric assay (Boehringer kit No 704121) according to the following principle:



To calculate the 'time-averaged' lipoprotein levels during the period of apheresis I have used the arithmetic mean of the post-treatment value from one week and the pre-apheresis value of the following week. This may under-estimate the value obtained by integrating the area under the individual rebound curve by about 10% [Apstein *et al* 1972], but is a reasonable estimate and more readily calculated.

3.1.1.2 Lipoprotein composition

The apoprotein B-containing lipoproteins were obtained by sequential ultra centrifugation of plasma. 2 ml aliquots were adjusted to d 1.182g/ml by the addition of 0.341g NaCl and placed on 0.5 mls d 1.182 g/ml solution in six Beckman Ultra-Clear™ tubes before being overlaid with a discontinuous density gradient using a Technicon Autoanalyzer II gradient-mixer (Technicon (Ireland) Ltd, Swords, Co. Dublin) to produce a density range 1.0988 - 1.0588g/ml (Figure 3.1).



The tubes were placed in the buckets of a Beckman swing-out SW40 rotor, and centrifuged at 23°C in a Beckman L8-60 ultracentrifuge under conditions previously described [Lindgren *et al* 1972]. After removal of the top 1 ml containing the $VLDL_1$ (Sf 60-400), the gradient was relayered with d 1.058g/ml solution and recentrifuged. Further centrifugation steps yielded $VLDL_2$ (Sf 20-60), IDL (Sf 12-20) and finally LDL (Table 3i).

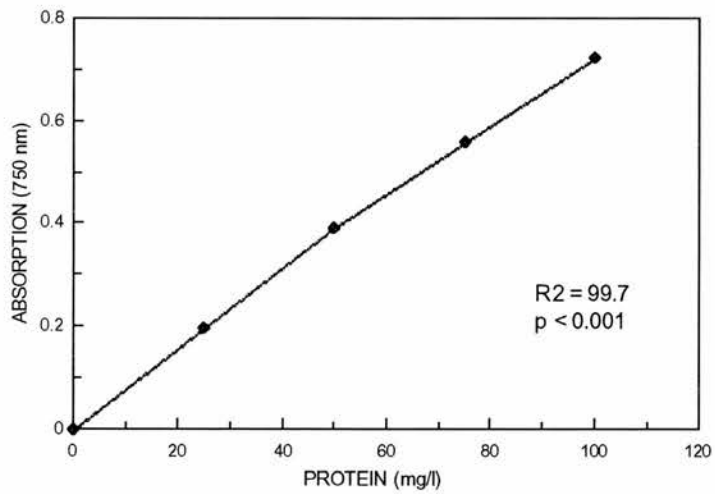
TABLE 3i SEPARATION OF THE SERUM LIPOPROTEINS BY CUMULATIVE FLOTATION ULTRACENTRIFUGATION

FRACTION sf	**SPEED** rpm*1000	**TIME** hrs:mins	**^W2t**	**TOTAL W2t**	**SAMPLE** mls
VLDL 1 (60-400)	39	01:38	1.03 x 1000	1.03 x 1000	1.0
VLDL 2 (20-60)	18.5	15:41	2.12 x 1000	3.15 x 1000	0.5
IDL (12-20)	39	02:35	1.63 x 1000	4.78 x 1000	0.5
LDL (0-12)	30	21:10	7.52 x 1000	12.3 X 1000	1.0

For each lipoprotein, the cholesterol content (esterified and free), triglycerides, phospholipid and protein concentration was measured. Free cholesterol was determined on a Centrichem Encore centrifugal analyser (Baker Instruments) by an enzymatic colorimetric assay (Boehringer Kit No 310328) in which the first step in the reactions outlined above resulting from the action of cholesterol esterase is omitted. Esterified cholesterol was calculated by the difference of total less free cholesterol. Phospholipid was determined by an enzymatic assay (Boehringer Kit No 691844) on the Encore analyser which measures the liberation of choline by phospholipase D. Total protein was measured in each fraction by the method of Lowry [Lowry *et al* 1951]: suitable aliquots - adjusted for each fraction according to the protein content to ensure the result lay in the middle of the standard curve: 400 µl for VLDL1, 300 µl VLDL2 , 50 µl IDL and 25 µl for LDL - adjusted to 0.4mls with deionised water were mixed with 2mls of freshly-constituted working Biuret reagent (100mls 2% Na2CO3 in 0.1M NaOH, 1ml 2% Na K tartrate in H_2O and 1ml 1% CuSO4 in H_2O) to which had been added 1 µg/ml of sodium dodecyl sulphate (Eastman Fine Chemicals) [Curry *et al* 1978]. To these samples were added 200 µl Folin-Ciocalteu reagent (BDH) diluted 1:1 with deionised water, and immediately vortexed. The standard curve in the range 0-100 µg/ml was prepared simultaneously by taking duplicate aliquots of 0, 25, 50, 75 and 100 µl of a stock human serum albumin standard (1mg/ml) adjusted to a final volume of 400 µl. Quality control of the standards was assured by the routine inclusion of control samples of human serum albumin, using 100 µl aliquots of 150 and 300 mg/l

adjusted to a final volume of 400 µl. Absorbances were measured after 30 minutes at 750nm on a Beckman DU-70 spectrophotometer; the regression line was obtained after entering the data from the standard curve into a statistics software package, 'Minitab' (Minitab Inc. 1989), and the protein concentration calculated. An example of a curve is shown in figure 3.2; the coefficient of variation of protein assay by this method was 7.1%, while the mean square of the correlation coefficient for 62 curves was 0.9904 (S.D. 0.006).

FIG 3.2 EXAMPLE OF LOWRY STANDARD CURVE



The apo-B content of each of the fractions was derived from the difference between the total protein content (method described above) and the TMU-soluble protein [Kane *et al* 1975]. 250 µl aliquots of each fraction were incubated with equal amounts of warmed tetramethylurea (Sigma Chemical Co. Ltd, Poole, Dorset). The precipitated apoproteins were discarded while 80 µl of the supernatant obtained following centrifugation at 3000 rpm for 30 minutes were subjected to a modified Lowry assay, in which the standards were made up to a final volume of 400 µl with deionised water and 40 µl of TMU. The protein content was then assayed by the method outlined above.

The mass of each of the components of the lipoprotein was calculated per 100mg, and the proportion of each expressed as a percentage.

The statistical analysis of compositional data poses particular difficulties. Standard tests are not valid for their analysis since the proportions of the components are subject to a unit-sum constraint, and different statistical techniques have been developed to analyse such data. These have been utilised and validated in a number of settings (such as geology and medical diagnosis) where statistical models have been sought, and the techniques are expounded in a monograph on statistics and applied probability [Aitchison 1986].

The methods employed reject the inadequacies of crude variances and covariances, and formulates compositions as a covariance structure of logarithms of ratios of components. The resulting 'compositional variation array' provides a useful descriptive summary of the pattern of variability of the composition. Since the apoprotein B is a subcomponent of the protein content, I have expressed the compositions as consisting of five parts: free cholesterol (FC), cholesterol ester (CE), triglycerides (TG), phospholipids (PL), and protein (P). The compositional variation array then consists of a symmetrical 5 x 5 table, with the logratio means displayed on the lower triangle and the logratio variances set out in the upper triangular array:

FIG 3.3 THE COMPOSITIONAL VARIATION ARRAY

	FC	CE	TG	PL	P
FC	*	var{log(FC/CE)}	var{log(FC/TG)}	var{log(FC/PL)}	var{log(FC/P)}
CE	m{log(CE/FC)}	*	var{log(CE/TG)}	var{log(CE/PL)}	var{log(CE/P)}
TG	m{log(TG/FC)}	m{log(TG/CE)}	*	var{log(TG/PL)}	var{log(TG/P)}
PL	m{log(PL/FC)}	m{log(PL/CE)}	m{log(PL/TG)}	*	var{log(PL/P)}
P	m{log(P/FC)}	m{log(P/CE)}	m{log(P/TG)}	m{log(P/PL)}	*

where m{log(**x**/**y**)} and var{log(**x**/**y**)} are, respectively, the mean and the variance of logarithm of ratio of **x**:**y**.

In addition to summarising a number of related compositions (eg., pre-treatment and post-treatment) into a meaningful form, the format above is used to specify matrices for subsequent analysis of the covariance structure. For ease of visualisation, and comparison with previously published data, the crude means of the components are also given, although not used for analytical purposes.

The compositions are converted from the format above into additive logratio compositions and then analysed by multivariate statistical procedures, after validation of the distributional assumption of logistic normality. The initial hypothesis tested is that both the means and the covariances for the two composition sets being compared are equal; if this is rejected at the 0.05 level, the model (fig 3.4) calculates the maximum likelihood ratios for rejecting one or both of the alternative hypotheses that either the means or the covariances are equal; the null hypothesis - that the compositions are not different - is rejected only if this complete 'lattice of hypotheses' rejects all of the individual steps.

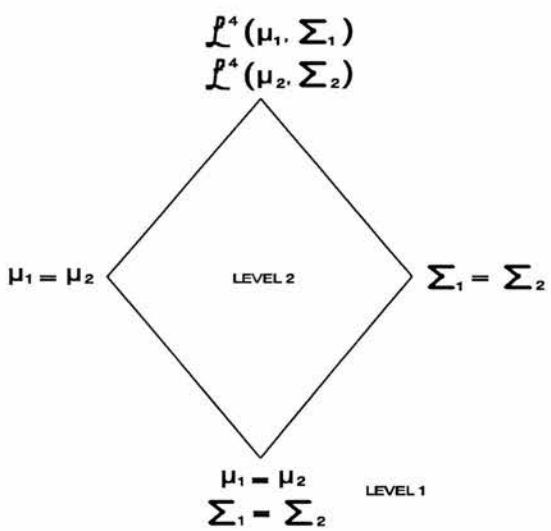


FIG 3.4. LATTICE OF HYPOTHESES TO TEST DIFFERENCES BETWEEN COMPOSITIONAL DATA

Since the logratio cannot be calculated if one of the components is zero, some device to replace these values is required. The zero values were substituted with a value representing a fifth of the maximum rounding-off error for the measurement of that parameter (see discussion, 3.1.3).

3.1.1.3 HDL Subfractions

Plasma concentrations of HDL_2 and HDL_3 were estimated by analytical ultracentrifugation using a Beckman L8-70 centrifuge incorporating an ultraviolet scanner, and an AnF rotor with double sector centrepiece [Shepherd *et al* 1984].

3.1.1.4 Lipoprotein(a)

Plasma Lp(a) was measured using 'Innotest Lp(a)', a commercially available kit (Innogenetics SA, Belgium) [Dagen *et al* 1991]. This utilises a mouse monoclonal anti-Lp(a) antibody, and a second (labelled with a peroxidase) sheep anti-apoB polyclonal antibody which binds to the apo B moiety of the antibody/Lp(a) complex. Inter- and intra-assay variances were 2.3% and 4.5% respectively.

3.1.2 Results

3.1.2.1 Serum Lipids

The baseline serum lipids for each individual were derived from a mean of at least eight samples taken after an overnight fourteen-hour fast during the initial apoprotein B turnover studies. Patients initially fulfilling the entry criteria (Sect **2.2**) on the basis of a mean of at least two lipoprotein profiles before commencing the trial were included even if the results given in the table deviated slightly below these criteria. There were no significant differences between the groups for any of the parameters (Table 3ii).

The main determinant of the degree of reduction achieved acutely with apheresis was the volume of plasma treated. The percentage reduction for successive treatments was similar within each individual subject, but after the first week the

TABLE 3ii BASELINE LIPIDS BY SUBJECT AND TREATMENT GROUP

SUBJECT	TC	TG	VLDL	LDL	HDL	TC/HDL	LDL/HDL
GROUP 1							
1	7.42	2.20	1.00	4.72	1.70	4.38	2.78
2	7.65	2.36	1.04	5.34	1.27	6.05	4.23
3	9.49	2.46	1.33	7.10	1.06	9.01	6.74
4	6.91	1.93	0.70	4.88	1.28	5.45	3.86
5	10.66	2.39	1.14	8.66	0.87	12.36	10.03
6	9.15	3.00	1.70	6.61	0.84	10.89	7.85
7	6.81	1.69	0.76	4.93	1.13	6.07	4.39
8	6.73	1.76	0.96	4.60	1.17	5.78	3.95
9	8.30	2.31	0.99	6.08	1.23	6.85	5.02
10	6.72	1.20	0.49	5.18	1.05	6.41	4.95
MEAN	**7.98**	**2.13**	**1.01**	**5.81**	**1.16**	**7.32**	**5.38**
SD	**1.30**	**0.48**	**0.32**	**1.24**	**0.23**	**2.44**	**2.08**
GROUP 2							
11	9.83	1.61	0.65	8.14	0.99	9.92	8.22
12	8.73	2.57	1.73	6.13	0.88	9.95	6.98
13	7.16	1.27	0.58	5.48	1.10	6.52	4.99
14	9.35	2.82	1.07	6.85	1.42	6.61	4.83
15	6.88	1.60	0.69	5.00	1.17	5.91	4.27
16	6.77	1.48	0.78	4.78	1.21	5.62	3.96
17	7.82	2.10	0.75	5.90	1.16	6.75	5.10
18	9.25	1.33	0.68	7.56	1.01	9.26	7.56
19	10.04	2.75	1.56	7.48	1.00	10.04	7.45
20	6.56	1.36	0.53	5.04	1.00	6.57	5.05
MEAN	**8.24**	**1.89**	**0.90**	**6.24**	**1.09**	**7.72**	**5.84**
SD	**1.28**	**0.58**	**0.40**	**1.14**	**0.15**	**1.74**	**1.46**

VLDL,LDL and HDL are expressed as cholesterol concentration in mmol/l; TC and TG are also in mmol/l, while TC/HDL and LDL/HDL are numeric ratios.

inter-subject variation was also small. Pre- and post-apheresis lipid profiles were obtained on a regular basis for the first weeks of treatment in six individuals (#05-10). These show that the pre-apheresis total cholesterol (TC) and LDL-cholesterol (LDL) were significantly reduced from baseline after just one week, and that after week 3 there were no further significant changes in these variables when apheresis was continued as sole therapy (Fig 3.5). The time-averaged levels of TC and LDL after the first treatment fell by 41.7% and 51% to 4.6 and 2.85 mmol/l respectively ($P < 0.001$), with further significant falls after the second treatment before reaching a new steady-state. There were no significant changes in pre-treatment or time-averaged HDL levels with apheresis alone, but acute reductions of up to 29% were observed.

Rebound curves were plotted from a number of treatment sessions in eight of the patients undergoing apheresis. These show that the initial recovery in LDL levels was rapid after the first 12 hours following apheresis, reaching 63% of the pre-apheresis value by the third day, before the rate of rebound slowed. HDL levels were little changed at the end of the procedure, but fell in the ensuing hours before recovering rapidly (Fig 3.6).

Since none of the subjects achieved the primary lipid targets on apheresis alone, drug therapy was added. Six patients were commenced initially on a resin - either cholestyramine ('Questran' and 'Questran A', Bristol Myers-Squibb, Staines, Middlesex, UK) or colestipol ('Colestid', Upjohn Ltd, Crawley, Sussex) - at increasing doses. Compared to the results on apheresis alone, there was a steady decrease in the pre-apheresis total and LDL-cholesterol which reached statistical significance after four weeks, and resulted in a further reduction in time-averaged LDL (Fig 3.7) as well as marked falls in TC/HDL and LDL/HDL. The remaining four apheresis patients - together with two patients who had initially been commenced on a resin which had been discontinued because of side-effects - were commenced on pravastatin (Bristol Myers-Squibb, Princeton, New Jersey, USA). This was associated with significant reductions in both pre- and post-apheresis total cholesterol and LDL levels compared to results in steady-state on apheresis alone, the additional reductions in pre-treatment TC and LDL being 20% and 25% respectively and 29% for post-apheresis LDL (Fig 3.8).

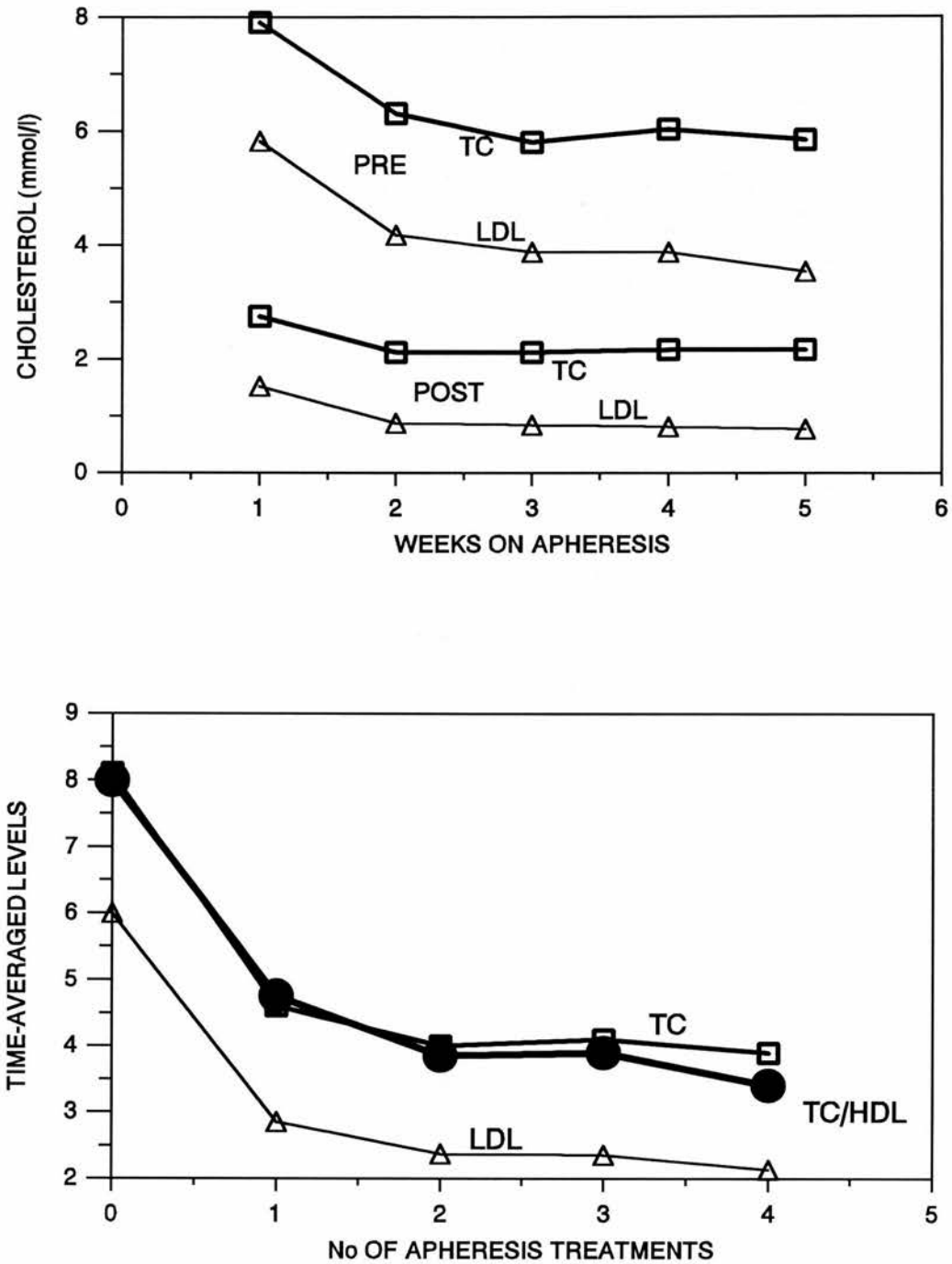


FIG 3.5. CHOLESTEROL LEVELS ON APHERESIS ONLY

Data represent means, with T=0 the pre-treatment values (n=6).

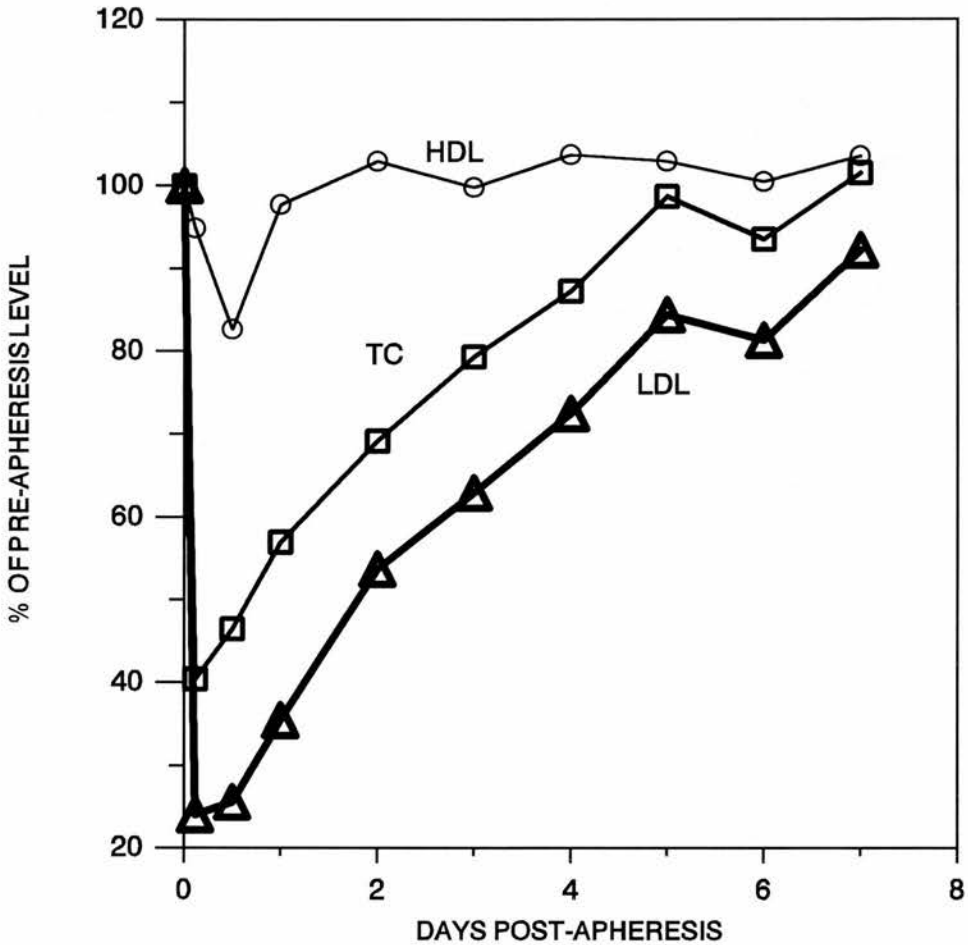


FIG 3.6. REBOUND OF LIPOPROTEINS FOLLOWING APHERESIS THERAPY.

Data represent means of several apheresis treatments from 8 subjects before introduction of lipid-lowering drug therapy.

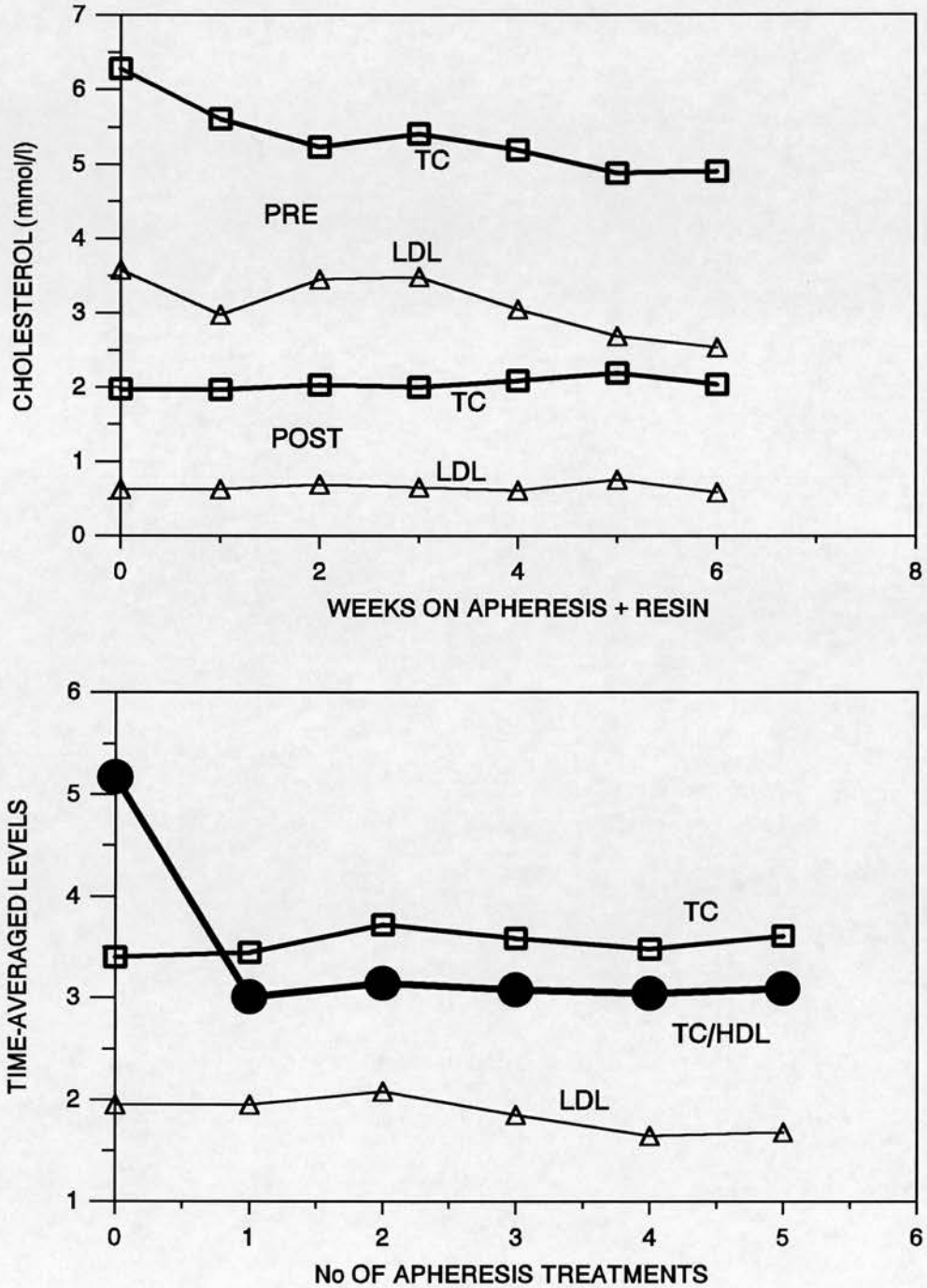


FIG 3.7. CHOLESTEROL LEVELS ON APHERESIS AND BILE-ACID SEQUESTRANT RESIN

Data represent means, with T=0 the apheresis treatment immediately prior to introduction of resin (n=4).

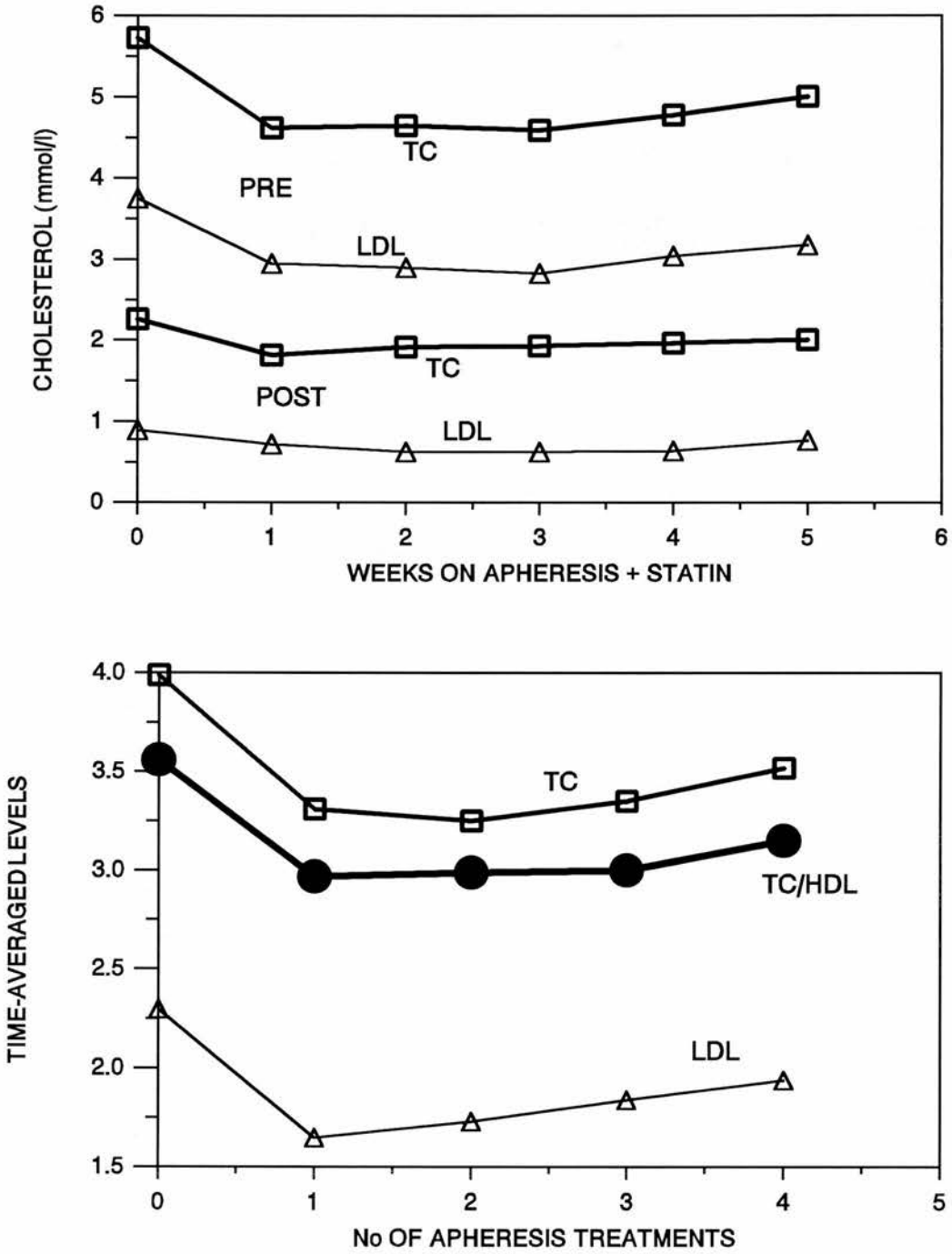


FIG 3.8. CHOLESTEROL LEVELS ON APHERESIS AND HMGCoA REDUCTASE INHIBITOR

Data represent means, with T=0 the apheresis treatment immediately prior to introduction of pravastatin (n=6).

None of the subjects achieved both primary lipid goals on apheresis alone. Of those in whom a resin was used as sole adjunctive therapy, only one reached these targets (patient #07), but he was unable to tolerate any of the resin preparations due to gastric upset. Three subjects (#07, 08 & 09) consistently achieved the primary goals on the addition of pravastatin to apheresis, the first two of these also reaching the secondary objective of a time-averaged LDL level below 1.5 mmol/l. The remainder were commenced on combination therapy: the introduction of a second drug reduced pre-apheresis total cholesterol from 5.51 mmol/l (SD 0.51), achieved with apheresis and a single agent, to 4.76 mmol/l ($P < 0.001$). This resulted in a reduction of the time-averaged TC to 3.4 mmol/l (Fig 3.9). Two subjects with familial hypercholesterolaemia required the addition of a third agent, acipimox, a nicotinic acid derivative ('Olbetam', Farmitalia Carlo Erba, St. Albans, Herts, UK). Although the percentage additional reductions achieved were not of large magnitude, the triple drug combination permitted the achievement of the TC target level in one subject (#05), and enabled the other,(#06), to reach both primary goals and the secondary LDL goal rather than only the TC level as achieved with the two-drug combination (fig 3.10).

A resin alone was sufficient to maintain the more conservative TC goal of group 2 in only one of those subjects; another one was maintained satisfactorily on statin as sole therapy, while five required the combination of the two agents. The remainder were not able to maintain the target cholesterol value despite treatment with the maximum tolerated doses of these drugs, and to this combination acipimox was added.

Seven of the nine patients to complete the study in group 1 maintained the total cholesterol goal and eight the time-averaged TC/HDL goal for at least 50% of the period of intervention. Eight maintained at least one of the primary lipid targets on 70% of occasions or more, including the first fourteen weeks (the mean time to achieve the treatment goals). Six of these nine subjects averaged an LDL level of 1.5 mmol/l or less while on their optimal drug combination. Six of the patients in group 2 maintained a total cholesterol of 5.2 mmol/l or less 50% of the time, and this was achieved on at least 70% of occasions in five. The mean levels at baseline and during intervention are illustrated in table 3iii and fig 3.11.

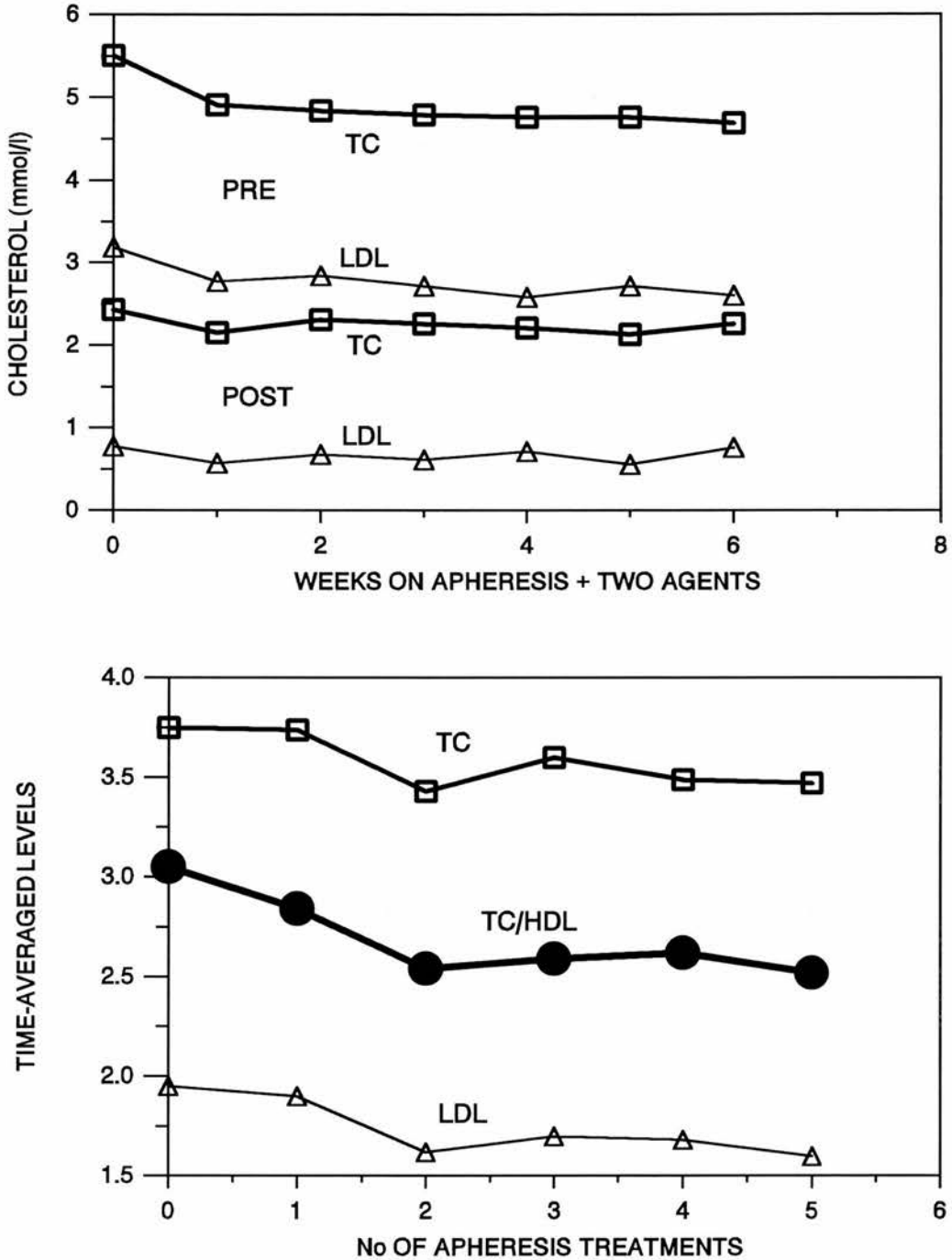


FIG 3.9. CHOLESTEROL LEVELS ON APHERESIS AND RESIN/STATIN COMBINATION

Data represent means, with T=0 the apheresis treatment immediately prior to the addition of a second agent (n=8).

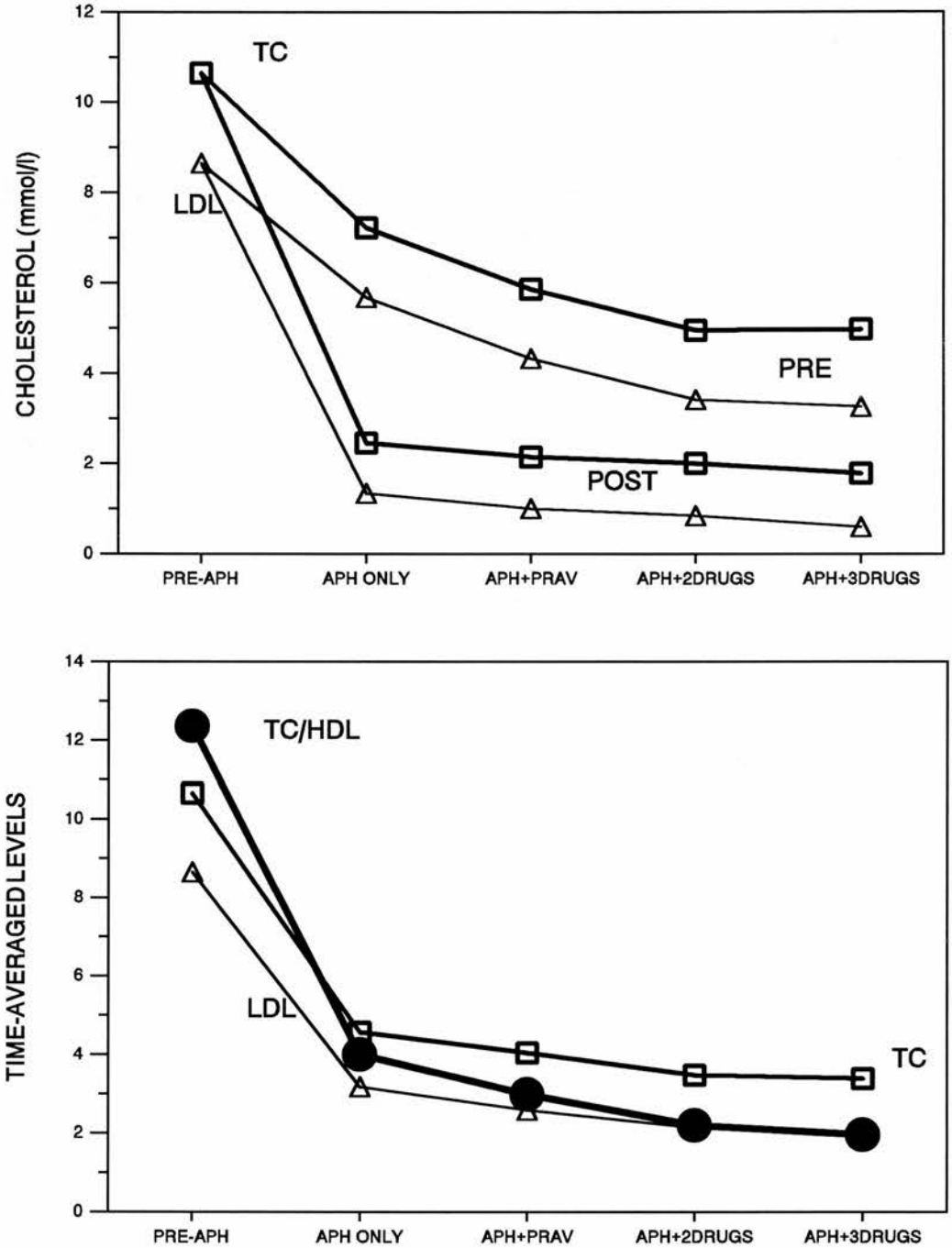


FIG 3.10a. EFFECTS OF DIFFERENT TREATMENT REGIMENS ON CHOLESTEROL LEVELS IN SUBJECT #05 WITH FAMILIAL HYPERCHOLESTEROLAEMIA

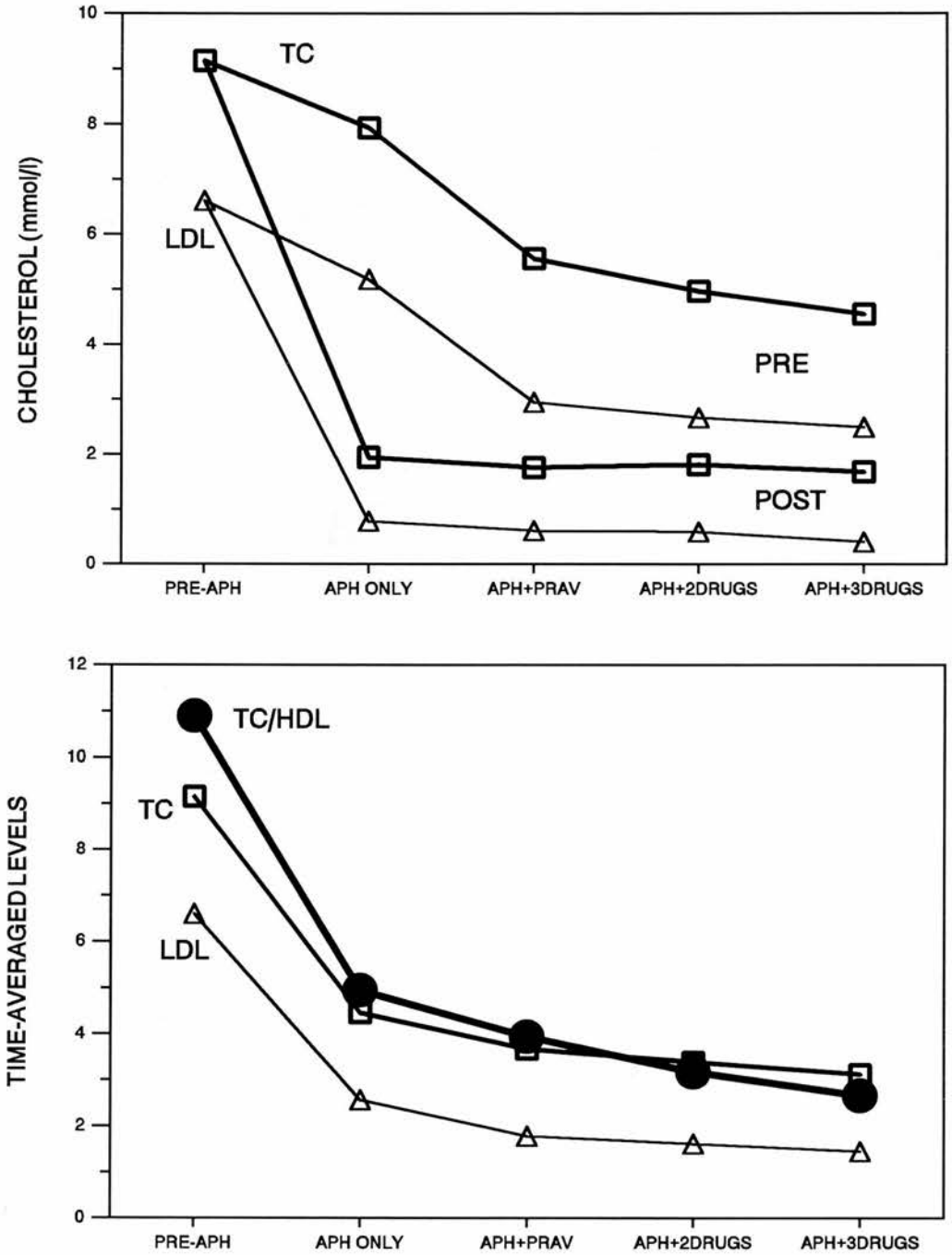
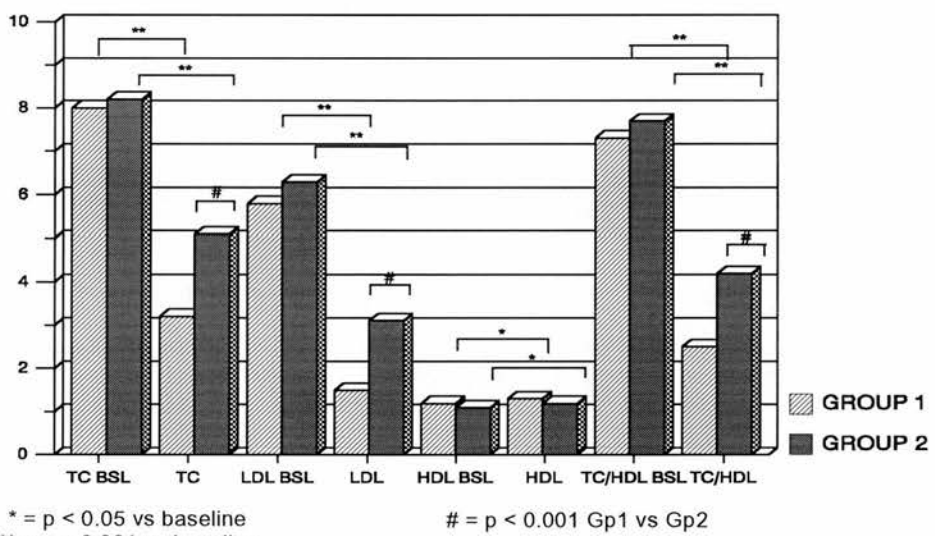


FIG 3.10b. EFFECTS OF DIFFERENT TREATMENT REGIMENS ON CHOLESTEROL LEVELS IN SUBJECT #06 WITH FAMILIAL HYPERCHOLESTEROLAEMIA

TABLE 3iii. LIPID RESULTS DURING INTERVENTION

	GROUP 1			GROUP 2		
	BASELINE	ON THERAPY	CHANGE	BASELINE	ON THERAPY	CHANGE
	mean (SD)	*mean (SD)*	*mean*	*mean (SD)*	*mean (SD)*	*mean*
TC (mmol/l)	8.0 (1.3)	3.2 (0.3)	-59%	8.2 (1.3)	5.38 (0.43)	-34%
LDL (mmol/l)	5.8 (1.2)	1.6 (0.2)	-74%	6.2 (1.1)	3.43 (0.45)	-44%
HDL (mmol/l)	1.16 (0.23)	1.3 (0.26)	+12%	1.1 (0.15)	1.21 (0.2)	+11%
TC/HDL	7.3 (2.4)	2.5 (0.3)	-60%	7.7 (1.7)	4.59 (0.81)	-40%

FIG 3.11 EFFECT OF INTERVENTION ON LIPID LEVELS



* = p < 0.05 vs baseline
** = p < 0.001 vs baseline
\# = p < 0.001 Gp1 vs Gp2

3.1.2.2 Compositions of apoprotein B-containing lipoproteins

The crude averaged lipoprotein compositions are tabulated in table 3iv, and the treatment groups at baseline compared in table 3v. The latter suggests that the groups are well-matched at baseline, although the variability of the subjects' compositions cannot be appreciated from the data in this format.

TABLE 3iv. BASELINE LIPOPROTEIN COMPOSITION

	MEAN % COMPOSITION					
	FC	CE	TG	PL	TOT PROT	APO B
VLDL1	2.54	14.40	57.83	14.86	10.37	6.23
VLDL2	5.84	25.66	33.21	19.40	15.89	12.69
IDL	8.49	37.10	11.19	22.20	21.03	17.60
LDL	10.04	37.75	4.67	20.19	27.35	23.85

TABLE 3v. BASELINE COMPOSITION BY TREATMENT GROUP

		MEAN % COMPOSITION					
		FC	CE	TG	PL	TOT PROT	APO B
VLDL1	GROUP 1	3.08	12.38	59.66	14.88	9.99	5.26
	GROUP 2	2.05	16.22	56.18	14.83	10.71	6.91
VLDL2	GROUP 1	6.25	24.48	33.93	19.54	15.80	11.37
	GROUP 2	5.47	26.72	32.56	19.28	15.97	13.61
IDL	GROUP 1	9.28	35.24	10.61	23.14	21.73	15.29
	GROUP 2	7.78	38.77	11.71	21.36	20.39	19.22
LDL	GROUP 1	9.28	38.29	4.50	19.49	28.45	23.62
	GROUP 2	10.73	37.27	4.82	20.83	26.35	24.04

The variation array of each lipoprotein from the two groups are contrasted in Table 3vi. These show that there are no marked differences between the groups either in the logratio means or in the variances. Statistical analysis using the Behrens Fisher iterative method [Aitchison 1986] confirmed there were no significant differences.

TABLE 3vi. VARIATION ARRAYS FOR BASELINE COMPOSITIONS

	GROUP 1					GROUP 2				
VLDL1	FC	CE	TG	PL	P	FC	CE	TG	PL	P
FC	*	7.52	5.03	4.29	5.99	*	6.06	4.50	4.60	5.23
CE	-2.33	*	0.72	0.99	0.50	-3.20	*	0.19	0.16	0.15
TG	-4.08	-1.75	*	0.10	0.07	-4.50	-1.30	*	0.01	0.11
PL	-2.65	-0.33	1.43	*	0.28	-3.17	0.04	1.33	*	0.09
P	-2.27	0.06	1.81	0.38	*	-2.82	0.39	1.69	0.35	*
VLDL 2										
FC	*	0.11	0.10	0.08	0.14	*	2.59	2.40	2.27	2.45
CE	-1.39	*	0.05	0.04	0.07	-1.98	*	0.11	0.05	0.07
TG	-1.72	-0.33	*	0.02	0.03	-2.18	-0.20	*	0.02	0.04
PL	-1.17	0.22	0.55	*	0.07	-1.67	0.31	0.52	*	0.04
P	-0.95	0.44	0.78	0.22	*	-1.47	0.51	0.71	0.20	*
IDL										
FC	*	0.53	0.36	0.10	0.20	*	0.08	0.20	0.05	0.05
CE	-1.39	*	0.05	0.28	0.12	-1.63	*	0.08	0.01	0.03
TG	-0.21	1.18	*	0.14	0.06	-0.41	1.22	*	0.08	0.07
PL	-0.98	0.40	-0.78	*	0.09	-1.04	0.60	-0.63	*	0.02
P	-0.93	0.45	-0.73	0.05	*	-0.99	0.64	-0.58	0.05	*
LDL										
FC	*	0.18	0.14	0.04	0.08	*	0.05	0.16	0.02	0.13
CE	-1.44	*	0.04	0.09	0.06	-1.26	*	0.16	0.01	0.10
TG	0.70	2.14	*	0.07	0.06	0.85	2.11	*	0.15	0.28
PL	-0.76	0.68	-1.46	*	0.08	-0.68	0.58	-1.53	*	0.09
P	-1.15	0.30	-1.85	-0.38	*	-0.89	0.36	-1.74	-0.22	*

Compositional analysis of the lipoproteins was performed on serum taken immediately before and after apheresis on 59 occasions from patients #1-6. The crude means and variation arrays are presented in Tables 3vii and 3viii respectively. The changes in composition with apheresis are highly significant for all the lipoproteins ($P < 0.00001$). The biggest change was in the proportion of free cholesterol, which was greatest in the $VLDL_1$ fraction.

Although the lipoprotein species appear distinct, and the compositions between the classes at baseline were highly significantly different, the variation arrays for VLDL1 and VLDL2 post-apheresis appear very similar. The lack of difference was confirmed on analysis only between these two lipoproteins, and may be attributable to the variances of the components in the post-apheresis VLDL2 being rather higher than expected.

TABLE 3vii. LIPOPROTEIN COMPOSITIONS PRE- AND POST-APHERESIS

PRE-APH	FC	CE	TG	PL	PROT	APO B
V1	2.97	10.79	59.81	14.95	11.48	3.69
V2	5.09	21.74	39.19	18.71	15.27	9.80
IDL	6.11	35.66	15.00	20.22	23.01	16.54
LDL	8.27	39.34	4.91	20.47	27.01	18.36

POST-APH	FC	CE	TG	PL	PROT	APO B
V1	0.62	15.46	57.59	12.21	14.11	3.49
V2	2.77	23.09	38.64	17.82	17.67	8.56
IDL	2.63	37.40	17.32	18.19	24.47	13.19
LDL	4.06	42.40	6.96	18.09	28.48	17.67

TABLE 3viii. VARIATION ARRAYS PRE- AND POST-APHERESIS

	PRE-APHERESIS					POST-APHERESIS				
VLDL 1	FC	CE	TG	PL	P	FC	CE	TG	PL	P
FC	*	9.70	6.75	5.64	6.53	*	1.04	1.54	0.95	0.83
CE	-1.79	*	3.94	4.49	3.08	-0.47	*	0.86	0.51	0.37
TG	-3.93	-2.14	*	1.86	1.13	-0.55	-0.08	*	0.59	0.37
PL	-2.52	-0.72	1.42	*	1.40	-0.46	0.02	0.09	*	0.3
P	-2.25	-0.46	1.68	0.26	*	-0.49	-0.02	0.06	-0.04	*
VLDL 2										
FC	*	2.7	2.84	2.54	4.02	*	1.31	1.03	0.98	1.01
CE	-1.86	*	0.16	0.09	1.12	-0.46	*	0.13	0.14	0.14
TG	-2.48	-0.62	*	0.05	0.99	-0.52	-0.07	*	0.02	0.01
PL	-1.74	0.12	0.74	*	1.07	-0.44	0.01	0.08	*	0.03
P	-1.42	0.45	1.06	0.32	*	-0.44	0.02	0.08	0.00	*
IDL										
FC	*	2.78	2.06	1.39	1.60	*	1.05	1.09	0.99	1.06
CE	-1.92	*	1.70	1.25	1.25	-0.54	*	0.06	0.04	0.07
TG	-1.10	0.81	*	0.22	0.23	-0.47	0.08	*	0.02	0.04
PL	-1.47	0.44	-0.37	*	0.05	-0.48	0.07	-0.01	*	0.04
P	-1.59	0.32	-0.49	-0.12	*	-0.50	0.04	-0.03	-0.02	*
LDL										
FC	*	0.33	1.22	0.12	0.28	*	0.87	1.05	0.78	0.86
CE	-1.64	*	0.45	0.10	0.21	-0.39	*	0.17	0.01	0.01
TG	0.56	2.20	*	0.89	0.99	-0.18	0.21	*	0.17	0.18
PL	-1.00	0.64	-1.55	*	0.12	-0.30	0.09	-0.12	*	0.01
P	-1.26	0.38	-1.82	-0.27	*	-0.35	0.04	-0.17	-0.05	*

Compositional analysis was performed also on specimens taken at 12 months and 24 months in both groups, and these results are tabulated in Tables 3ix-xii. In both treatment groups the two-year compositions of VLDL and IDL reveal a relative reduction in esterified cholesterol, with an increase in the proportion of triglycerides. The free cholesterol component is more affected at one year, but appears to increase after further therapy. Compositions analysed on average two months after withdrawal of therapy demonstrate no significant alteration from pretreatment results, except for LDL (when treatment groups are combined), although there is no significant change in the LDL composition at two years compared to baseline. As expected there were differences in composition after therapy was discontinued compared to the two-year specimens, except in $VLDL_1$. In LDL, the contribution of total cholesterol was virtually identical to that on treatment and beforehand, but a greater proportion was present in the non-esterified form.

This impression from the data is confirmed by analysis of the amalgamation matrices of LDL when free and esterified cholesterol are combined (Table 3xiv). By contrast, the compositions of VLDL2 and IDL at two years remain significantly different from baseline even when the cholesterol components are amalgamated.

TABLE 3ix. LONG-TERM COMPOSITION CHANGES - GROUP 1

	% COMPOSITION						
	FC	CE	TG	PL	PROT	APO B	p * vs baseline
BASELINE							
VLDL 1	3.08	12.38	59.66	14.88	9.99	5.26	
VLDL 2	6.25	24.48	33.93	19.54	15.80	11.37	
IDL	9.28	35.24	10.61	23.14	21.73	15.29	
LDL	9.28	38.29	4.50	19.49	28.45	23.62	
I YEAR							
VLDL 1	2.39	7.98	59.95	17.62	12.05	8.39	N.S.
VLDL 2	4.58	18.40	40.77	18.43	17.83	14.52	< 0.05
IDL	4.90	30.50	19.72	19.34	25.54	22.36	<0.0001
LDL	4.84	38.85	6.17	19.70	30.44	28.16	N.S.
2 YEAR							
VLDL 1	4.31	7.09	63.34	16.11	9.15	4.87	N.S.
VLDL 2	6.88	16.14	43.07	19.05	14.86	11.41	< 0.01
IDL	7.90	33.61	18.07	19.60	20.82	18.64	< 0.01
LDL	8.64	37.56	6.28	20.46	27.07	22.79	N.S.
POST-Rx							
VLDL 1	4.61	10.00	58.85	17.21	9.32	5.68	N.S.
VLDL 2	7.94	20.97	35.45	21.18	14.46	11.85	N.S.
IDL	8.43	37.93	11.19	22.33	20.11	19.16	N.S.
LDL	12.40	34.80	4.75	21.13	26.92	23.42	N.S.

TABLE 3x. LONG-TERM COMPOSITION CHANGES - GROUP 2

	% COMPOSITION						
	FC	CE	TG	PL	PROT	APO B	p * vs baseline
BASELINE							
VLDL 1	2.05	16.22	56.18	14.83	10.71	6.91	
VLDL 2	5.47	26.72	32.56	19.28	15.97	13.61	
IDL	7.78	38.77	11.71	21.36	20.39	19.22	
LDL	10.73	37.27	4.82	20.83	26.35	24.04	
1 YEAR							
VLDL 1	1.36	14.35	60.32	14.11	9.86	6.64	< 0.05
VLDL 2	4.81	21.76	38.24	18.98	16.21	14.08	N.S.
IDL	6.05	32.34	18.37	20.64	22.60	20.34	< 0.01
LDL	5.68	37.77	6.89	18.99	30.67	29.06	< 0.01
2 YEAR							
VLDL 1	4.05	9.15	60.43	16.99	9.37	5.35	< 0.05
VLDL 2	5.30	20.83	38.02	20.90	14.95	11.36	0.05
IDL	11.36	36.01	15.87	22.13	19.59	17.26	< 0.05
LDL	7.58	39.30	5.22	20.49	27.41	26.38	N.S.
OFF-Rx							
VLDL 1	4.15	9.65	59.95	17.58	8.66	5.00	N.S.
VLDL 2	6.41	25.64	34.33	19.26	14.36	11.34	N.S.
IDL	9.02	37.60	11.74	22.98	18.65	17.44	N.S.
LDL	13.07	35.19	4.55	21.81	25.39	22.04	N.S.

TABLE 3xi. VARIATION ARRAYS DURING TREATMENT

GROUP 1

	12 MONTHS					24 MONTHS				
VLDL 1										
	FC	**CE**	**TG**	**PL**	**P**	**FC**	**CE**	**TG**	**PL**	**P**
FC	*	9.04	8.66	8.16	9.64	*	0.72	0.44	1.47	0.44
CE	-2.1	*	5.64	4.83	5.82	-0.1	*	0.09	0.99	0.09
TG	-4.8	-2.7	*	0.32	0.19	-0.3	-0.3	*	0.74	0.00
PL	-3.5	-1.4	1.28	*	0.41	-0.1	-0.0	0.22	*	0.69
P	-3.1	-1.1	1.65	0.37	*	-0.1	-0.1	0.19	-0.0	*
VLDL 2										
FC	*	0.19	0.10	0.06	0.05	*	0.47	0.11	7.10	0.08
CE	-1.4	*	0.16	0.06	0.14	-0.8	*	0.23	8.75	0.23
TG	-2.2	-0.8	*	0.04	0.04	-1.9	-1.1	*	7.93	0.02
PL	-1.4	-0.0	0.79	*	0.03	-0.2	0.60	1.64	*	7.31
P	-1.4	0.01	0.83	0.04	*	-0.8	0.01	1.06	-0.6	*
IDL										
FC	*	5.13	4.02	4.49	4.26	*	0.41	0.26	7.46	0.18
CE	-2.4	*	0.15	0.05	0.20	-1.5	*	0.25	8.80	0.13
TG	-2	0.42	*	0.05	0.07	-0.9	0.62	*	7.70	0.18
PL	-2	0.43	0.01	*	0.07	-0.2	1.33	0.72	*	7.10
P	-2.2	0.17	-0.3	-0.3	*	-1	0.45	-0.2	-0.9	*
LDL										
FC	*	8.42	9.36	8.6	7.41	*	0.26	0.77	0.28	0.21
CE	-3.3	*	0.05	0.10	0.09	-1.6	*	0.20	0.07	0.02
TG	-1.4	1.86	*	0.04	0.15	0.32	1.88	*	0.18	0.28
PL	-2.6	0.68	-1.2	*	0.13	-0.9	0.62	-1.3	*	0.07
P	-3	0.26	-1.6	-0.4	*	-1.2	0.33	-1.6	-0.3	*

TABLE 3xii. VARIATION ARRAYS DURING TREATMENT

GROUP 2

	12 MONTHS					24 MONTHS				
VLDL 1										
	FC	**CE**	**TG**	**PL**	**P**	**FC**	**CE**	**TG**	**PL**	**P**
FC	*	1.23	0.90	0.91	0.98	*	0.72	0.25	0.22	0.17
CE	-0.5	*	0.03	0.03	0.02	-0.8	*	0.24	0.23	0.43
TG	-0.6	-0.2	*	0.00	0.01	-2.8	-2	*	0.05	0.09
PL	-0.5	-0.0	0.15	*	0.01	-1.5	-0.7	1.28	*	0.08
P	-0.4	0.03	0.18	0.04	*	-0.90	-0.1	1.89	0.61	*
VLDL 2										
FC	*	0.15	0.16	0.05	0.02	*	0.42	0.27	0.24	0.16
CE	-1.5	*	0.34	0.10	0.07	-1.5	*	0.07	0.05	0.13
TG	-2.1	-0.6	*	0.08	0.18	-2.1	-0.6	*	0.05	0.04
PL	-1.4	-0.1	0.67	*	0.04	-1.5	-0.0	0.60	*	0.09
P	-1.2	0.26	0.85	0.17	*	-1.1	0.33	0.94	0.34	*
IDL										
FC	*	0.13	0.14	0.08	0.23	*	0.37	0.37	0.24	0.18
CE	-1.7	*	0.01	0.02	0.04	-1.8	*	0.11	0.03	0.12
TG	-1.2	0.57	*	0.01	0.04	-1	0.84	*	0.12	0.15
PL	-1.3	0.45	-0.1	*	0.07	-1.3	0.49	-0.4	*	0.14
P	-1.4	0.37	-0.20	-0.1	*	-1.20	0.62	-0.2	0.14	*
LDL										
FC	*	0.13	0.35	0.09	0.18	*	0.19	0.24	0.12	0.10
CE	-1.9	*	0.16	0.01	0.05	-1.7	*	0.09	0.02	0.02
TG	-0.2	1.74	*	0.13	0.09	0.35	2.04	*	0.08	0.07
PL	-1.3	0.69	-1.1	*	0.05	-1	0.65	-1.40	*	0.02
P	-1.7	0.21	-1.5	-0.5	*	-1.3	0.36	-1.7	-0.3	*

TABLE 3xiii. COMPOSITIONAL CHANGES (GROUPS COMBINED)

	FC	CE	TG	PL	PROT	APO B	P vs 2-yr	P vs pre-Rx
BASELINE								
V1	2.54	14.40	57.83	14.86	10.37	6.23		
V2	5.84	25.66	33.21	19.40	15.89	12.69		
IDL	8.49	37.10	11.19	22.20	21.03	17.60		
LDL	10.04	37.75	4.67	20.19	27.35	23.85		
1 YR								
V1	1.94	10.77	60.12	16.08	11.09	7.63		
V2	4.68	19.87	39.66	18.67	17.12	14.33		
IDL	5.40	31.30	19.13	19.91	24.26	21.48		
LDL	5.21	38.38	6.48	19.39	30.54	28.56		
2 YR								
V1	4.18	8.12	61.88	16.55	9.26	5.11		N.S.
V2	6.09	18.49	40.55	19.98	14.90	11.38		< 0.001
IDL	7.15	34.81	16.97	20.86	20.20	17.95		< 0.001
LDL	8.11	38.43	5.75	20.47	27.24	24.58		N.S.
POST-Rx								
V1	4.40	9.84	59.36	17.39	9.02	5.36	N.S.	N.S.
V2	7.22	23.15	34.93	20.28	14.41	11.61	< 0.05	N.S.
IDL	8.71	37.78	11.45	22.64	19.43	18.36	< 0.001	N.S.
LDL	12.71	34.98	4.66	21.45	26.20	22.78	0.001	< 0.05

TABLE 3xiv. VARIATION ARRAYS FOR AMALGAMATED COMPOSITIONS

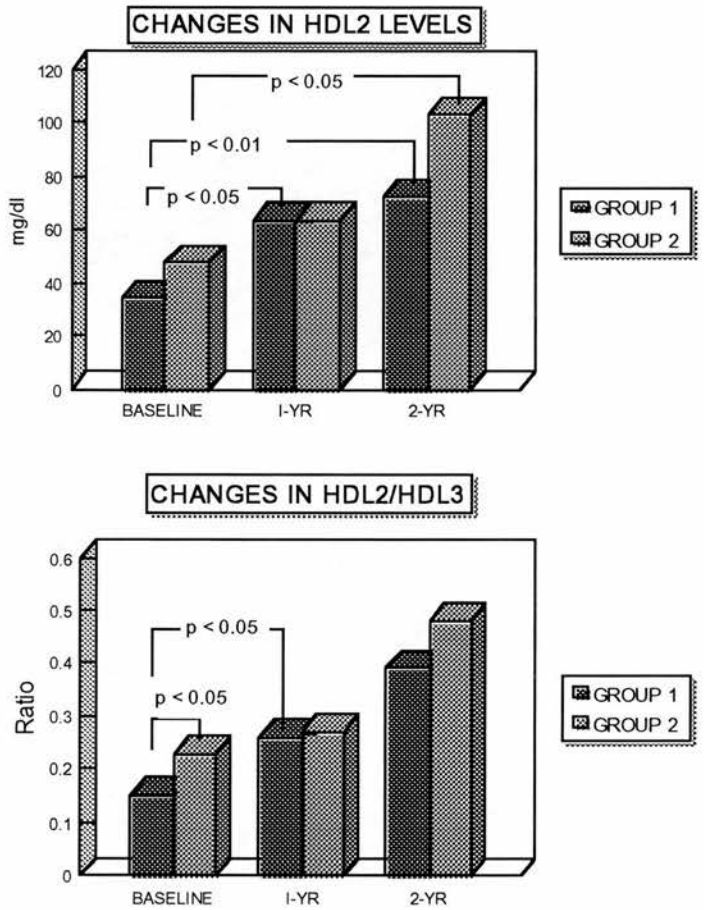
	BASELINE				2 YR				POST-Rx			
VLDL 2	TC	TG	PL	PROT	TC	TG	PL	PROT				
TC	*	0.07	0.03	0.06	*	0.07	3.97	0.06				
TG	-0.06	*	0.02	0.03	-0.51	*	4.01	0.03				
PL	0.48	0.53	*	0.05	0.62	1.12	*	3.68				
PROT	0.69	0.74	0.21	*	0.50	1.00	-0.12	*				
					p < 0.0001 vs baseline							
IDL	TC	TG	PL	PROT	TC	TG	PL	PROT				
TC	*	0.06	0.04	0.03	*	0.14	4.19	0.07				
TG	1.43	*	0.11	0.07	0.93	*	3.95	0.15				
PL	0.73	-0.70	*	0.05	1.11	0.18	*	3.66				
PROT	0.78	-0.65	0.05	*	0.74	-0.19	-0.19	*				
					p < 0.001 vs baseline							
LDL	TC	TG	PL	PROT	TC	TG	PL	PROT	TC	TG	PL	PROT
TC	*	0.09	0.03	0.07	*	0.16	0.03	0.01	*	0.10	0.01	0.03
TG	2.36	*	0.10	0.17	2.16	*	0.13	0.17	2.37	*	0.10	0.11
PL	0.87	-1.50	*	0.09	0.83	-1.33	*	0.04	0.80	-1.57	*	0.02
PROT	0.57	-1.8	-0.30	*	0.54	-1.62	-0.29	*	0.60	-1.76	-0.20	*
					p = N.S. vs baseline				p = N.S. vs baseline p = N.S. vs 2 yrs			

3.1.2.3 HDL subfractions

HDL_2 was lower in Group 1 at baseline, 34.3 mg/100ml (SD 14.26) vs 47.7 mg/100ml (SD 16.36), although this difference was not statistically significant ($p = 0.081$); HDL_3 was similar in each group. The HDL_2/HDL_3 ratio was significantly lower in the apheresis group (see Fig 3.12) before the intervention period.

The subfractions were measured in fasting plasma from subjects in both treatment groups after one year and after two years of therapy. There was an increase of more than 100% in HDL_2 over the two years from 41.0 (SD 17.2) to 88.8 mg/dl (SD 52.3), ($P < 0.001$). The increase was greater in the apheresis subjects, so that the initial non-significant difference between the groups at baseline was abolished. HDL_3 did not alter in either group, while the baseline difference in HDL_2/HDL_3 between the groups was no longer evident on repeat sampling at one year or beyond.

FIG 3.12 HDL SUBFRACTION CHANGES DURING TREATMENT



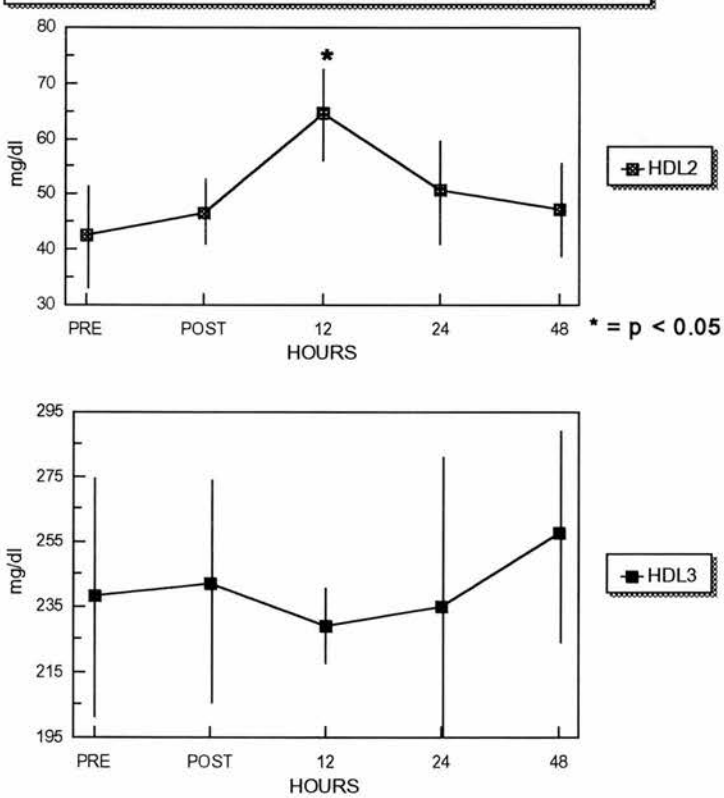
HDL subfractions were measured before and after approximately 50 apheresis treatments, and results are available for 47 (Table 3xv). The mean increase in HDL_2 level (treatments averaged for each individual since these are not independent) was 4.1 mg/dl (SEM 2.4), which was not statistically significant. Changes in HDL_3 were even more variable, although there was a trend towards a decrease following apheresis. HDL_2/HDL_3 increased from a mean of 0.215 (SEM 0.021) to 0.245 (SEM 0.029), which was significant ($p < 0.05$).

TABLE 3xv. HDL SUBFRACTION CHANGES WITH APHERESIS

	PRE-APH	POST-APH	MEAN DIFF	P
	MEAN (S.E.M.)			
HDL2	53.7 (6.2)	57.8 (8.1)	4.1 (2.42)	0.12
HDL3	248.1 (8.0)	235.5 (11.2)	-12.5 (8.7)	0.18
HDL2/HDL3	0.22 (0.02)	O.24 (0.03)	0.03 (0.01)	0.04

Following eight apheresis procedures the subfractions were measured to determine the rate of recovery. This showed that at 12 hours (at the nadir of the total HDL-cholesterol), HDL_2 had increased significantly above the pre-treatment level (see fig 3-13).

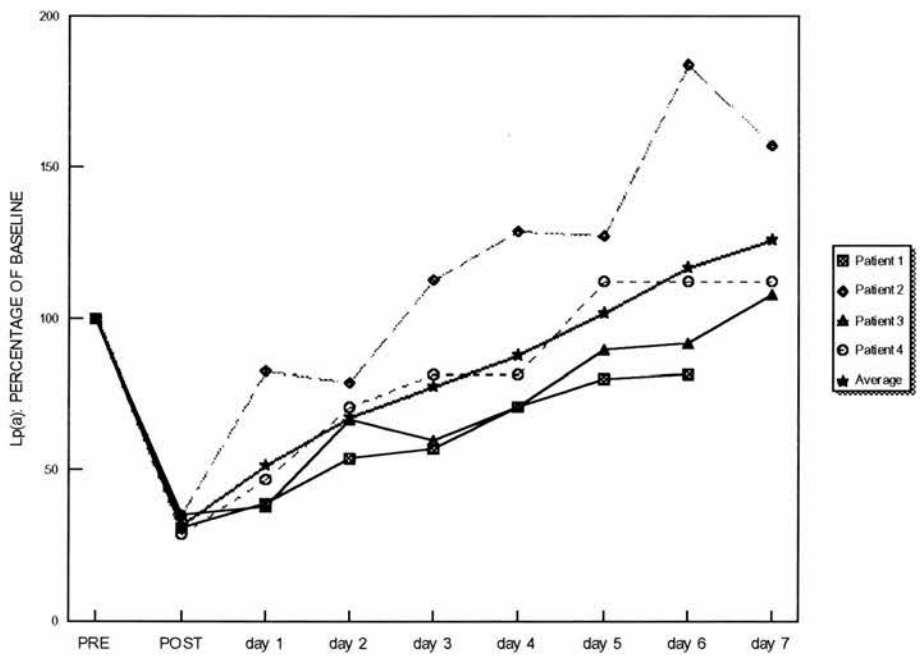
FIG 3-13. HDL SUBFRACTION REBOUNDS POST-APHERESIS



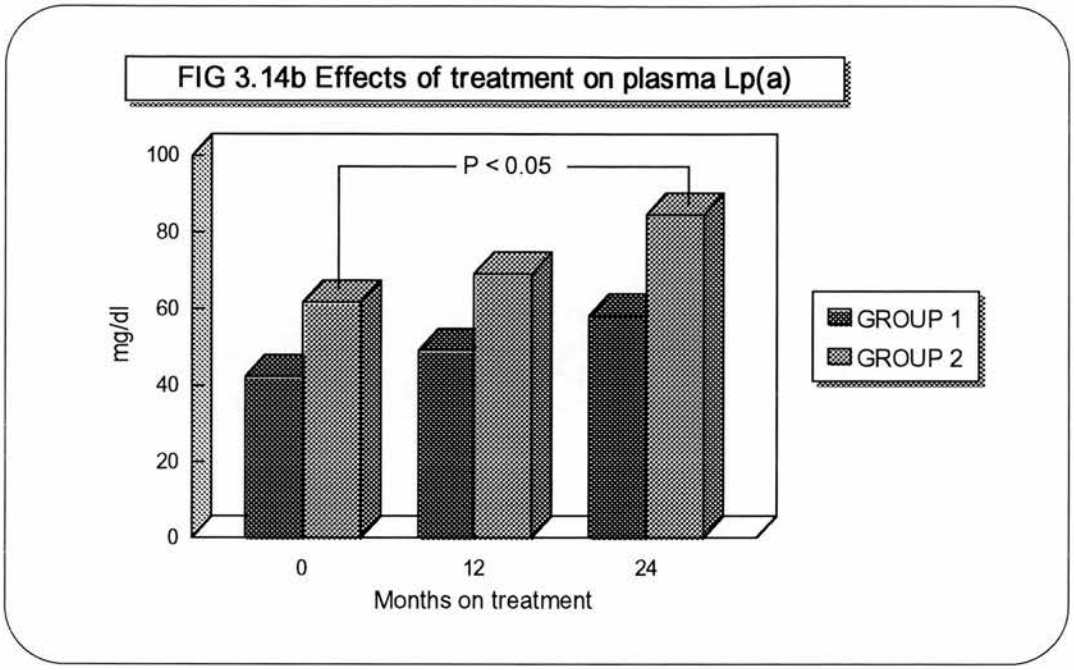
3.1.2.4 Lp(a)

Lp(a) was measured in all the subjects on several occasions before and after apheresis sessions. The mean acute reduction during apheresis was 70%, and this was correlated with the reductions in LDL ($r = 0.59$, $P < 0.02$). The rate of rebound of the lipoprotein following apheresis was studied in four subjects, all of whom were taking lipid-lowering drugs at the time. The pre-apheresis levels were reached by day 3 in one subject, day 5 in another, and by day 7 in all cases (Fig14a).

FIG 3.14a. REBOUND OF Lp(a) FOLLOWING LDL-APHERESIS



Lp(a) levels were measured at baseline, and repeated at one year and two years. The range in the cohort was 5 - 167 mg/dl, and the median levels were very similar in the two groups (Fig 14b). There was no significant reduction in the levels over time in the apheresis group. The group treated with drugs alone however showed a rise in Lp(a), and this change was significant after two years of therapy.



3.1.3 Discussion

The reduction in lipids achieved in the drug-treated group (Table 3iii) were comparable with that seen in the active-treatment groups in all the angiographic regression studies (Table 1vii), the mean reduction in TC and LDL being 34% and 44% respectively. The additional effects of LDL-apheresis resulted in even greater reductions from baseline, with highly significant differences between the groups during the period of intervention. No study reported to date has maintained serum lipids at these levels, which were achieved consistently in the whole group, and permitted the attainment of the specified primary lipid goals.

Simvastatin has been reported to slow the rate of rebound of lipoproteins and to enhance the acute reduction with plasmapheresis in patients with familial hypercholesterolaemia [Thiery *et al* 1988], but in other FH patients no additional effects of resins was seen during regular plasma exchange [Keller *et al* 1985]. The present studies demonstrated that both agents will significantly reduce serum total cholesterol and LDL during repeated LDL-apheresis in non-familial hypercholesterolaemia and that these effects are additive.

The acute reductions in lipids (up to 93% reduction from pre-apheresis levels in LDL-cholesterol) resulted in alteration in composition of the lipoproteins; the overall effect was a reduction in free cholesterol, with a relative increase in the proportions of cholesterol ester and protein. It is not clear whether this may in itself be beneficial, but may imply that less cholesterol per lipoprotein particle is available for deposition in the tissues.

The analysis of the compositions immediately following apheresis was complicated by the frequent finding of undetectable quantities of free cholesterol, particularly in the VLDL fractions. Alternatives for dealing with the problem of zero values include amalgamating the zero with another component, replacing the zero with a trace value, or excluding the composition as a probable outlier. With few exceptions the zero values recorded occurred in the free cholesterol component, and most of these following apheresis. Amalgamation (most reasonably with esterified cholesterol, as in Table 3xiv) would result in a loss of sensitivity, while the notion of outliers may be dismissed in view of the frequency of this finding post-apheresis. The most plausible explanation is a quantitative one, rather than qualitative, ie. it is likely that the zero denotes not that the part is completely absent, but that the accuracy of the measurement process is unable to quantify the proportion present. It has been shown that replacement of the zero with a trace value related to the accuracy of the measurement and/or rounding process is reasonable, providing that the replacement value lies in the range of 0.2 - 2 X the maximum rounding-off error. These minimum and maximum trace values were substituted in turn into the compositions taken pre- and post-apheresis, and the variation of the zero replacement procedure was shown to have no effect on the significance probability. It is therefore justified to use the minimum replacement value for these datasets.

Free cholesterol was reduced in the LDL fraction in both treatment groups at 1 and 2 years of treatment (Tables 3ix - 3xii), while the largest change in VLDL and IDL was the reduced proportion of cholesterol ester. The lipoproteins were relatively enriched with triglycerides, an effect which may increase the atherogenicity of the particles, leading to more dense LDL which binds less avidly to the apoB/E receptor.

The increase in HDL_2 and HDL_2/HDL_3 ratio both acutely following apheresis and in both groups in the longer term may represent an increase in uptake of free cholesterol. LCAT activity has been shown to increase by 40% after LDL-apheresis after an initial reduction, and it has been suggested that these compositional changes may induce mobilization of tissue cholesterol [Franseschini *et al* 1988].

Lp(a) accumulates in the arterial wall and is thought likely to contribute to the development of atherosclerosis [Rath *et al* 1989]. It is associated with an increased risk of coronary artery disease [Armstrong *et al* 1986, Seed *et al* 1990, Wiklund *et al* 1990, Genest *et al* 1991], increases the risk of vein graft stenosis after bypass surgery [Hoff *et al* 1988, Cushing *et al* 1989], and is associated with accelerated coronary artery disease in transplant recipients [Barbir *et al* 1992]. Although it is known that none of the drugs employed cause any reduction in Lp(a) levels [Vessby *et al* 1982, Thiery *et al* 1988, Leren *et a*l 1988, Wiklund *et al* 1990], the rise in group 2 was unexpected. This may be a spurious finding rather than a true 'treatment effect', but a significant increase in Lp(a) levels with both lovastatin [Jürgens *et al* 1989] and simvastatin [Kostner *et al* 1989] has been reported, which may be due to genetic differences in the response to this class of drugs [Berg & Leren 1989]; other studies have not replicated this finding with lovastatin and pravastatin [Jacob *et al* 1990] or with simvastatin [Crook *et al* 1992], and in Kostner's paper the increase reported was primarily due to a marked increase in Lp(a) levels in one subject. Significant reductions in this lipoprotein are seen in hyperlipidaemic patients treated with nicotinic acid [Carlsson *et al* 1989], although this is less pronounced with the nicotinic acid derivative, acipimox, used in a minority of patients in this study, and the response of Lp(a) in the subjects receiving this did not differ from the remainder. Sustained-release bezafibrate has been demonstrated in one study to lower Lp(a) levels to a significant degree, particularly in those with higher baseline values [Bimmermann *et al* 1991].

There has been an observed acute reduction in Lp(a) with LDL-apheresis [Eisenhauer *et al* 1987, Armstrong *et al* 1989, Ritter *et al* 1990], as would be expected due to the binding and elimination of the apoB moiety. Acute reductions in Lp(a) have been reported with both HELP and immunoabsorption, with a highly significant correlation with the fall in LDL and apoB levels [Ritter *et al* 1990]. The ensuing rebound post-apheresis appears to follow that of LDL-cholesterol,

although the degree of recovery may be greater following immunoabsorption compared to HELP [Ritter *et al* 1990, Schenck *et al* 1988]. Over a period of two years Ritter *et al* reported a significant reduction from baseline in a small number of patients treated with either HELP or immunoabsorption: this appears to be true for two individuals with very high pre-treatment values of 880 and 760 U/l, but is probably not significant for the remaining six subjects who had a mean baseline level of 191 U/l compared to a two-year average for the eight patients of 219 U/l.

Since the weight of evidence points conclusively to Lp(a) being an independent risk factor for atherosclerosis, and the available therapies for cholesterol-lowering are largely ineffectual in reducing its serum concentrations, the reports that regular apheresis therapy may maintain significantly lower levels indicates a possible role for such treatment in high-risk individuals with high Lp(a) levels. The evidence for such an effect however is weak, and none of the subjects in the present investigation experienced reduction in Lp(a) over two years. The rebound curves for Lp(a) (Fig 3.14a) increase more quickly than those of LDL before the introduction of drug therapy (Fig 3.6), and suggests different control mechanisms for the synthesis and/or release of these lipoproteins.

The finding of an increase in the subjects treated by drugs alone is in keeping with other published reports, although these are not consistent. Most of these studies are in small groups of patients, and generally over a short time period. The Multicentre Anti-Atheroma Study [MAAS 1994] recently reported the effects of 20mg simvastatin over four years in a placebo-controlled study in 178 patients with coronary disease: no significant difference of treatment was observed. This would suggest that the findings in the present study and also in the previous reports are likely to arise only by chance, although a variation in response in Lp(a) to the different HMG CoA reductase inhibitors cannot be excluded on the current evidence.

3.2 LIPOPROTEIN METABOLISM

The metabolism of apolipoprotein B in VLDL and LDL was studied by established tracer techniques at baseline and on completion of the period of intervention. The results were analysed using multi-compartmental mathematical modelling.

3.2.1 Methods

(a) Preparation of VLDL:

Following an overnight fast, 200 - 250 mls citrated plasma was obtained from the patients by plasmapheresis. VLDL was obtained from this by ultracentrifugation in a Beckman Ti60 rotor for 24 hours at 39,000 rpm at 10°C. The density of 13 mls of the supernatant fraction was adjusted to 1.118 g/ml by the addition of 2.2165 mg of NaCl, and layered in six 2 ml aliquots over 0.5mls of d 1.182 g/ml solution of NaBr in Beckman SW40 rotor tubes which had been pre-coated with polyvinylalcohol. Above each aliquot was constructed a discontinuous density-gradient, from 1.099 to 1.058 g/ml, as illustrated in figure 3.1.

The gradient was centrifuged in a Beckman SW40 rotor for 1 hr 38 mins at 39,000 rpm and 23°C. The top 1 ml from each of the six tubes was removed by pipette, yielding the Sf 60-400 fraction ($VLDL_1$). This was replaced with 1 ml of d 1.059 g/ml solution, and the remaining samples centrifuged in the same rotor for a further 15 hrs 41 mins at 18,500 rpm and 23°C. $VLDL_2$ was subsequently obtained by removal of the top 0.5 ml of solution from each of the tubes.

(b) Radio-iodination of the VLDL subfractions:

Labelling of the VLDL species with radio-iodine was performed using the iodine monochloride method [MacFarlane *et al* 1958] as modified by Bilheimer [Bilheimer *et al* 1972]. To each of 2 ml aliquots of $VLDL_1$ and $VLDL_2$ were added 0.5 ml 1.0 M glycine (pH 10) and 1.0 - 2.0 mCi of I-131 (to the former) or I-125 (to $VLDL_2$). 6 µl of ICl (25mM in M NaCl) was then added and mixed gently. Both radio-iodinated

preparations were then dialysed at 4°C overnight to remove free iodide, using 3 x 2 litres of 0.15 M NaCl.

(c) Sterilisation of prepared material:

Following dialysis, the labelled lipoproteins were retrieved into sterile containers. Membrane filters with a pore size of 0.45 µm (Acrodiscs, Gelman Sciences) were primed with the patient's plasma, before the VLDL preparations were passed through them into sterile containers. These were then drawn up under aseptic conditions into sterile disposable graduated syringes.

(d) Patient preparation:

The patients were commenced on oral potassium iodate for three days before injection, and this was continued for 28 days to block thyroid uptake of radio-iodide. During the turnover study the patients maintained their usual diet and activities to maintain steady state conditions. A lipoprotein profile was obtained every 3 - 4 days during the turnover study to ensure this consistency.

(e) Patient injection and blood sampling:

The concentration of radioactivity for each isotope was calculated from counts from 10 µl aliquots of sterilised VLDL when compared against standards of I-131 and I-125. A suitable quantity was injected after an overnight fast via a large antecubital vein to provide 100-150 µCi of $VLDL_1$/I-131 and 50-100 µCi of $VLDL_2$/I-125. From the opposite arm regular 10ml venous blood samples were taken via a previously-sited indwelling cannula, which was flushed with physiological saline between samples. These were taken at 10 minutes, 30 minutes, 1, 1.5, 2, 3, 4, 6, 8, 10 and 14 hours after injection of the tracers, and then daily fasting specimens for twelve successive days. The samples were collected into K_2EDTA at a final concentration of 1mg/ml, the plasma separated by centrifugation at 2000rpm for 10 minutes and stored at 4°C.

(f) Re-isolation of tracer:

2ml aliquots of plasma obtained at each of the time-points above were adjusted to d 1.118 g/ml by the addition of 0.341 mg of NaCl, and layered over 0.5mls of d 1.182 g/ml solution of NaBr in pre-coated Beckman SW40 rotor tubes. Above each aliquot was constructed a discontinuous density-gradient, as in (a), above (figure 3.1).

The gradient was centrifuged in a Beckman SW40 rotor to isolate sequentially $VLDL_1$ and $VLDL_2$ as in (a) above. Following removal of the VLDL fractions, the IDL and LDL species were obtained by two further ultracentrifugation steps: IDL was removed in the top 0.5 mls after centrifuging for 2.58 hours at 39,000 rpm, and LDL in the top 1 ml after a further 21.17 hours at 30,000 rpm and 23°C (see Table 3i).

(g) Measurement of specific activity:

The apoB for each specimen (ie 24 X $VLDL_1$, $VLDL_2$, IDL and LDL) was precipitated by the addition of an equal volume (1ml to $VLDL_1$ and LDL, 0.5ml to $VLDL_2$ and IDL) of 1,1,3,3-tetramethylurea (TMU) at 37°C [Kane *et al* 1975]. The mixture was immediately vortexed and incubated at 37°C for one hour. The resulting precipitate was separated by low-speed centrifugation (2000 rpm for 30 minutes) from the TMU-soluble phase, and the latter removed by suction and discarded. The lipids were extracted from the apoprotein by the addition of organic solvents (ethanol:diethyl ether 3:1 v/v) which was kept overnight at -20°C, following which the pellet was re-formed by further centrifugation (2000 rpm for 20 minutes at 4°C) and the solvent removed and discarded. The residual apoprotein pellet was then washed with diethyl ether for two hours at -20°C; following removal of the ether by suction, the pellets were dried by incubation at 40°C.

The apoprotein pellets were solubilised in 1 ml of 0.5 M NaOH by overnight incubation at 37°C. The radioactivity present was measured over 5 minutes in a twin-channel automated gamma scintillation counter (Auto gamma 500C, Packard

Instrument Co, Pangbourne, Berks,UK), and the correction factor calculated for spillover of I-131 into the I-125channel (% spillover in I^{131} tracer = (cpm125 channel - background 125)/(cpm131 channel - background 131) x 100). Protein concentrations for each sample were determined by a modification of the method of Lowry [Lowry *et al* 1951] in which the NaOH is omitted from the Cu/alkali reagent.

(h) Calculation of pool sizes:

3 - 4 mls of plasma obtained from each of the days the lipoprotein profile was checked were pooled, and from this was prepared $VLDL_1$, $VLDL_2$, IDL and LDL by cumulative flotation ultracentrifugation, as described above. Apo-B concentration was measured by two methods: (i) the calculated difference between total protein and TMU-soluble protein measured by the methods of Lowry and Kane respectively (Lowry *et al* 1951, Kane *et al* 1975); (ii) the average concentration of apo B in each lipoprotein fraction during the period of study. The latter measurements were generally adopted in the calculations due to its greater reliability. Plasma volume was estimated as 4% of the body weight measured on the day of injection of the tracers, and the pool sizes for each lipoprotein fraction calculated as the product of the plasma concentration of apo B and the plasma volume. Correction for centrifugal losses was made by comparison of the recovered VLDL + IDL + LDL cholesterol to the "non-HDL cholesterol" measured in plasma.

(i) Data analysis:

The total radioactivity was calculated for each fraction as the specific activity multiplied by the average pool size, and expressed as a fraction of the total apo B radioactivity at 10 minutes after injection. This was plotted on a logarithmic scale as the ordinate axis in apo B decay curves. The decay curves and associated masses were used to model apo B metabolism, utilising a digital computer programme SAAM30 (Simulation, Analysis and Modeling) [Berman & Weiss 1978].

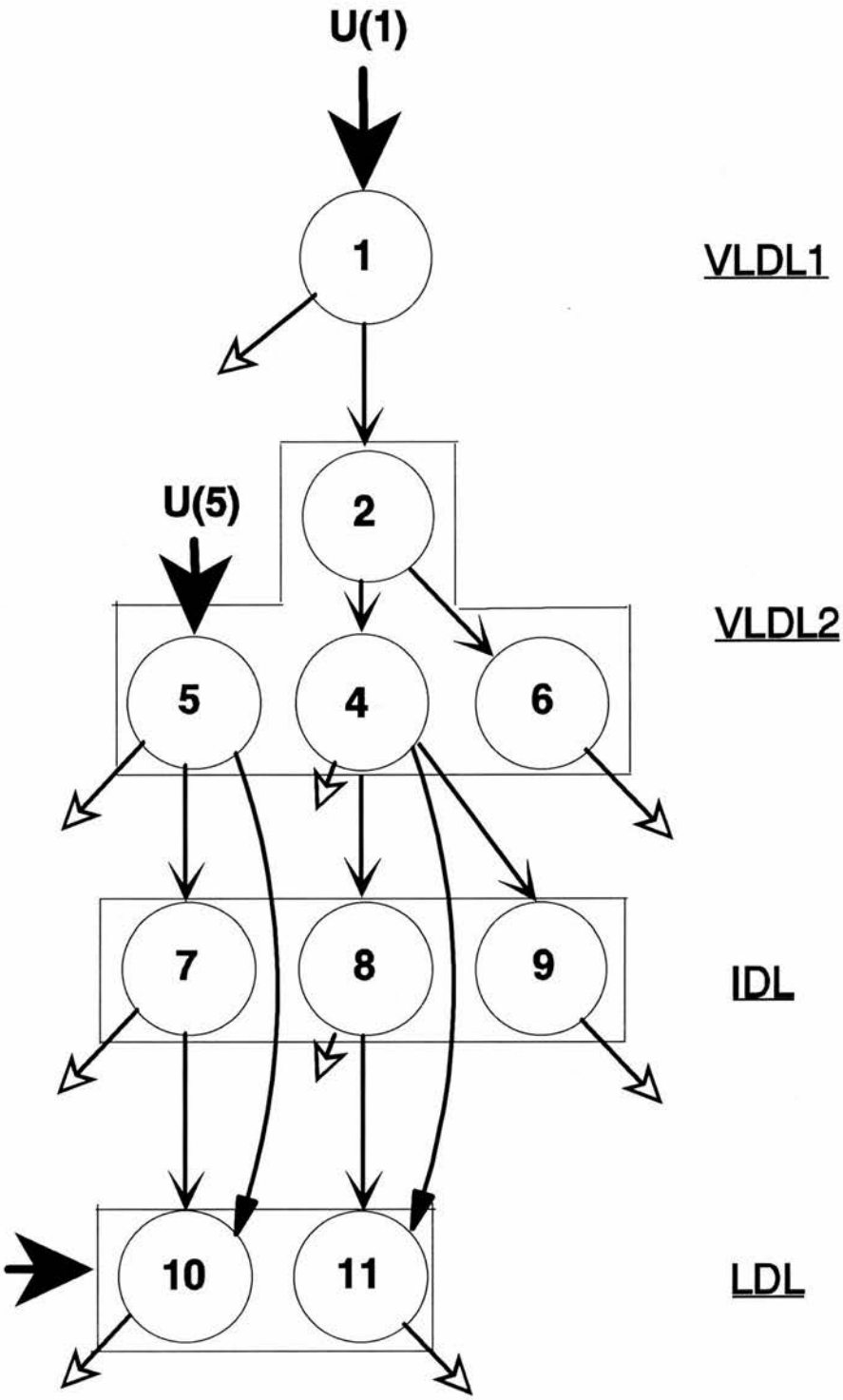


FIG 3.15. MODEL OF APO-B METABOLISM FROM VLDL

The model employed has been described previously to provide an acceptable fit for the observed data [Packard *et al* 1984, Demant *et al* 1991], and is illustrated in Fig 3.15.

In this model there is apo B synthesis into the $VLDL_1$ and $VLDL_2$ compartments, the latter required to account for the discrepancy between the amount of apo B observed in VLDL2 and the smaller mass known to be derived from large VLDL. In addition, directly labelled $VLDL_2$ appears to be metabolised at a different rate from that derived from labelled $VLDL_1$, and this pathway (-> 5 -> 7 -> 10) is distinct from the parallel pathway (2 -> 4 -> 8 -> 11). Pools of more slowly catabolized lipoproteins are proposed for $VLDL_2$ and IDL to account for the second exponential functions observed in the decay curves of these fractions.

Fractional catabolic rates (FCR) were calculated from the slope of the lines of the decay curves by a 'least squares' method. Absolute catabolic rates (ACR) were calculated from the plasma apo B concentration for the fraction, the plasma volume (PV)and the FCR:

$$\text{ACR } (mg/kg/day) = \frac{\text{FCR x PV } (l) \text{ x apoB } (mg/l)}{\text{body weight } (kg)}$$

3.2.2 Baseline results

The labelled apoB in $VLDL_1$ disappeared rapidly from this density range, and by 24 hours less than 1% of the injected radioactivity was present in this fraction (fig 3-16a). One-third of the apoB from $VLDL_1$ was transferred to $VLDL_2$; the peak of radio-activity of apoB derived from precursors was at 4 hours, and it took an average of 4 days to fall to 1%. The decay of directly labelled $VLDL_2$ was

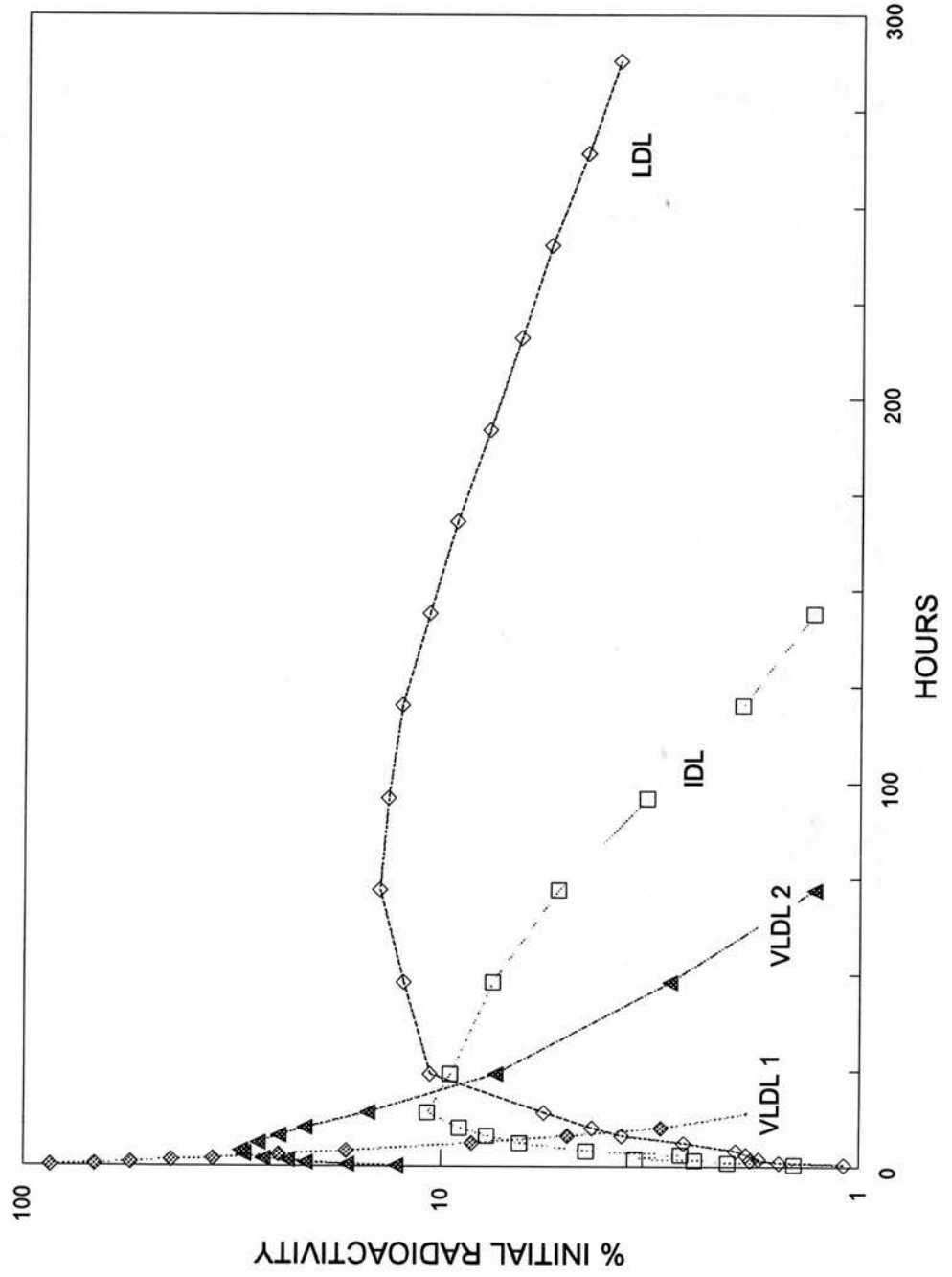


FIG 3.16a I-131 SPECIFIC ACTIVITY CURVES

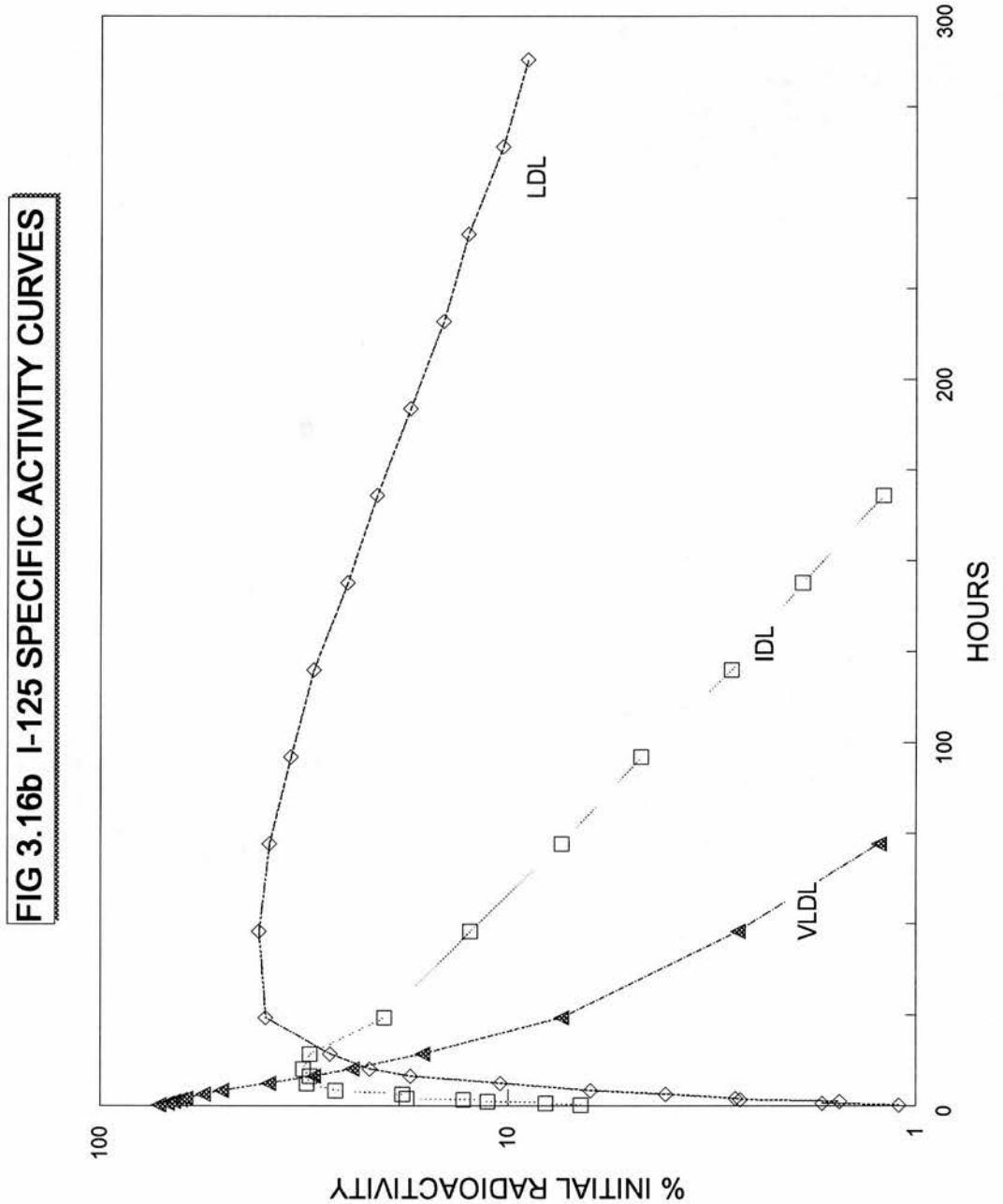


FIG 3.16b I-125 SPECIFIC ACTIVITY CURVES

considerably slower than the lighter VLDL species, and also took a mean of 96 hours to fall to less than 1% (see Fig 3.16b). A greater proportion of the apoB from $VLDL_2$ was transferred through IDL to LDL and the rate of transfer in this pathway was also quicker, although the proportion of apoB in $VLDL_2$ derived from $VLDL_1$ transferred to the higher density ranges was very similar to that which was directly labelled.

There was a wide variation between the patients in many of the kinetic parameters, and this was reflected in the range of values obtained for $VLDL_1$ synthesis, from 1.3 to 39.7 mg/kg/day; the mean value was 9.6 mg/kg/day (S.E.M. 2.09). The fractional transfer to $VLDL_2$ was less variable, with a mean value of 3.8 pools/day and S.E.M. of 0.43. The rate of direct $VLDL_1$ catabolism was on average twice the rate of transfer to $VLDL_2$ (see fig 3.17 and table 3xvi), and was positively correlated with the synthetic rate ($R = 0.76$, $P < 0.001$).

There was no correlation between the synthetic rate and the serum triglyceride level or the level of cholesterol in the VLDL fraction, and the rate of catabolism was independent of the plasma pool size. The latter was however significantly correlated with the synthetic rate ($R = 0.77$, $p < 0.001$).

Direct synthesis contributed two-thirds of the production of $VLDL_2$, and a considerably smaller proportion of this lipoprotein was catabolised directly compared to the $VLDL_2$ derived from $VLDL_1$. There was no correlation between the rates of direct synthesis of the two VLDL fractions.

The IDL pool was significantly correlated with the rate of transfer into this compartment ($R = 0.66$, $p < 0.001$), but not to the catabolic rate. The correlation between synthesis or transfer into LDL and the LDL pool was weak, neither was the latter significantly correlated with the FCR.

While the ratio of directly removed $VLDL_1$ to fractional transfer of $VLDL_1$ to $VLDL_2$ was 2:1, the ratio of directly catabolised $VLDL_2$ to transfer by delipidation was 1:3, and 1:9 for direct removal of IDL compared to conversion to LDL. There was a

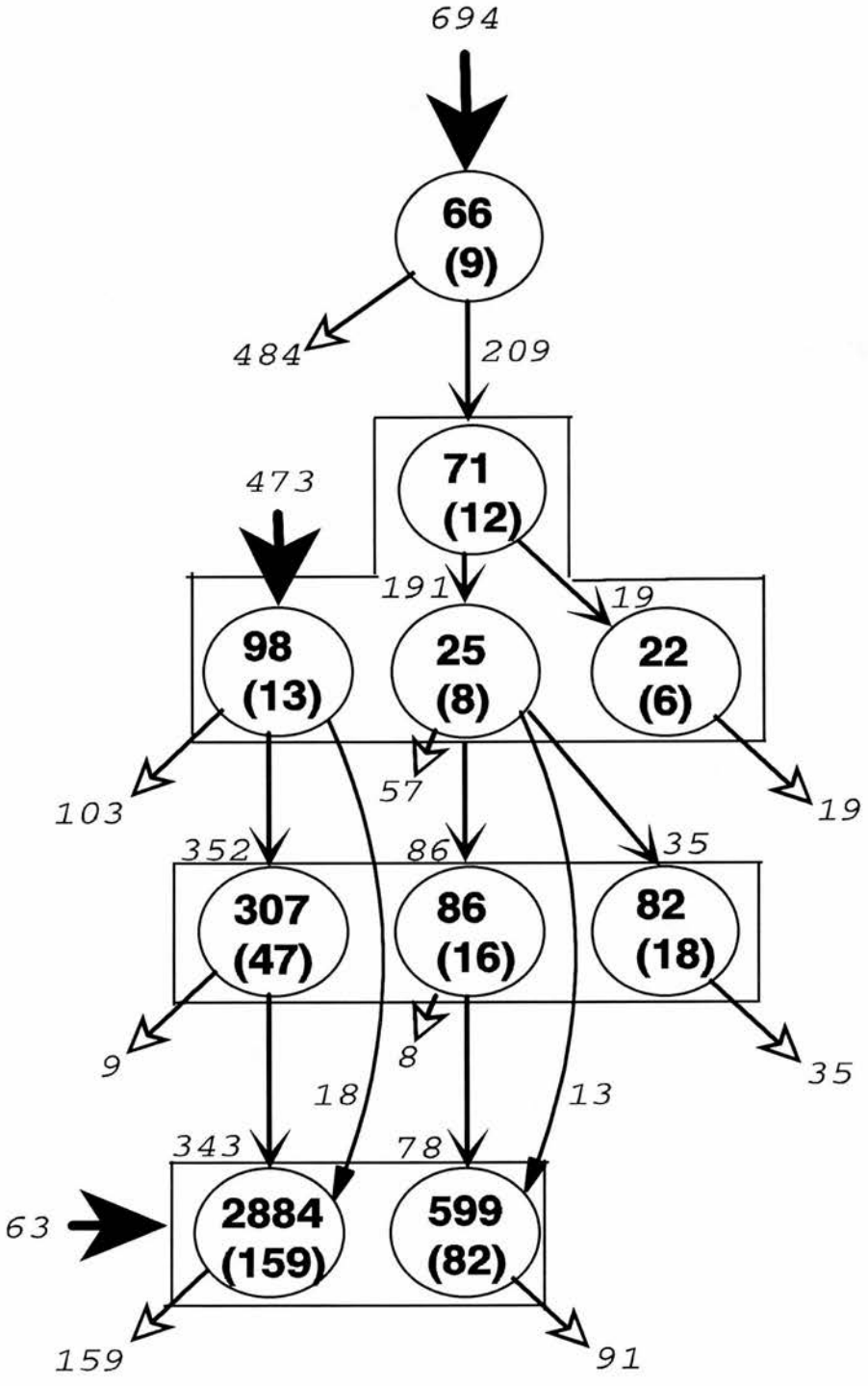


FIG 3.17. VLDL METABOLISM AT BASELINE

Nos. at arrows = transfer of apoB in mg/day; nos. in circles = mean pool size in mg (SEM); n = 17.

TABLE 3xvi(a) VLDL 1 APO-B METABOLISM AT BASELINE

PATIENT #	Syn Rate *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to VLDL 2 *(pools/day)*
01	1025	73	11.6	2.4
02	803	90	6.9	2.1
03	263	67	0.5	3.4
04	379	23	11.1	5.4
05	260	43	2.7	3.4
06	199	26	3.7	3.8
07	822	93	6.4	2.3
08	722	67	5.7	5.1
09	586	57	5.8	4.4
10	104	7	9.6	4.4
11	151	21	0.0	7.2
12	389	85	2.5	2.0
13	523	45	3.9	7.7
14	1037	82	8.1	4.6
16	1217	143	6.7	1.8
17	828	72	8.5	3.0
19	2484	123	18.3	1.9
MEDIAN	586	67	6.5	3.4
MEAN	694	66	6.6	3.8
SEM	139	9	1.1	0.4

TABLE 3xvi(b) VLDL 2 APO-B METABOLISM AT BASELINE

PATIENT #	Direct synthesis *(mg/day)*	Flux from VLDL1 *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to IDL & LDL *(pools/day)*
01	1019	175	170	4.5	2.4
02	912	186	227	1.3	3.5
03	531	227	349	0.2	1.8
04	1121	124	198	1.3	4.7
05	152	144	204	0.3	1.0
06	255	101	186	0.2	1.6
07	253	220	202	0.3	1.7
08	723	339	357	0.9	2.0
09	450	251	154	1.2	3.3
10	419	33	97	0.0	4.4
11	451	151	277	0.2	1.8
12	317	175	235	0.5	1.6
13	33	346	127	1.3	1.6
14	358	374	165	1.5	2.4
16	345	263	266	0.3	1.8
17	445	219	176	0.1	2.4
19	256	231	268	0.0	1.5
MEDIAN	420	219	202	0.3	1.8
MEAN	473	209	215	0.8	2.3
SEM	74	22	17	0.3	0.3

TABLE 3xvi(c) IDL APO-B METABOLISM AT BASELINE

PATIENT #	Flux from VLDL2 *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to LDL *(pools/day)*
01	407	617	0.1	0.5
02	792	452	0.1	1.6
03	644	720	0.0	0.9
04	930	995	0.0	0.9
05	196	435	0.0	0.4
06	303	300	0.0	1.0
07	343	436	0.0	0.7
08	703	541	0.2	1.1
09	505	270	0.5	1.4
10	427	253	0.1	1.6
11	503	359	0.0	1.4
12	378	409	0.0	0.9
13	209	337	0.2	0.4
14	402	314	0.1	1.1
16	486	551	0.3	0.5
17	418	325	0.1	1.2
19	395	754	0.1	0.4
MEDIAN	418	435	0.1	0.9
MEAN	473	475	0.1	1.0
SEM	48	49	0.0	0.1

TABLE 3xvi(d) LDL APO-B METABOLISM AT BASELINE

PATIENT #	Direct synthesis *(mg/day)*	Flux from IDL & VLDL2 *(mg/day)*	VLDL-derived plasma pool *(mg)*	Total apoB pool *(mg/day)*	FCR *(pools/day)*	Total apoB synthesis *(mg/day)*
01	111	355	2520	3307	0.1	2155.1
02	155	738	3856	4664	0.2	1869.6
03	49	660	3961	4253	0.2	842.9
04	0	914	3912	3713	0.2	1499.4
05	3	220	4874	4934	0.0	414.8
06	45	326	2901	3302	0.1	499.7
07	82	364	3191	3907	0.1	1156.4
08	116	627	4040	4788	0.2	1561.1
09	64	374	2474	2897	0.2	1099.5
10	140	433	2073	2741	0.2	662.8
11	24	510	3781	3961	0.1	626.6
12	21	370	3520	3718	0.1	726.3
13	4	138	2466	2528	0.1	559.2
14	13	441	4842	4984	0.1	1408.2
16	60	340	3383	3979	0.1	1621.5
17	12	559	3794	3875	0.1	1285.3
19	134	313	3625	5179	0.1	2873.8
MEDIAN	54	374	3625	3934	0.1	971.0
MEAN	63	452	3483	3855	0.1	1086.0
SEM	14	48	194	219	0.0	149.0

in the calculated mass of LDL apoB derived from VLDL compared to the measured masses, and it is necessary to postulate direct hepatic synthesis of about 10% to account for this. The contribution of Lp(a)-associated apoB was examined as a potential explanation for the difference between observed and calculated apoB mass in LDL: there was no correlation between plasma Lp(a) and the LDL pool, and no significant association with direct LDL synthesis. There was however a non-significant association between Lp(a) and total apoB production at baseline (R = -0.46, p = 0.07). The LDL apoB mass was related at baseline to a number of observed and calculated parameters, as tabulated in Tab 3xvii:

TABLE 3xvii CORRELATION OF LDL APO-B MASS WITH KINETIC PARAMETERS

	r	p
VLDL 1 SYNTHESIS	0.45	0.012
VLDL 2 SYNTHESIS	0.35	0.056
VLDL 1 FLUX TO VLDL 2	0.45	0.012
VLDL 2 POOL	0.57	0.001
VLDL 2 FLUX TO IDL	0.55	0.001
IDL POOL	0.48	0.006
TOTAL APO-B PRODUCTION	0.54	0.001

3.2.3 Effects of intervention

The repeat studies were performed four weeks after the last apheresis treatment, the achievement of a new steady-state confirmed by weekly lipoprotein profiles. The labelling of $VLDL_1$ in patient D was not successful, possibly due to the wrong pH, and there are therefore nine patients only from the apheresis group with complete datasets at baseline and post-intervention. Three of the drug-treated group did not undergo repeat metabolic studies due to time constraints, and the data on two others was incomplete. Comparisons of parameters at baseline and post-intervention are therefore derived from the fourteen patients with complete data at both time-points, and the statistical tests done on paired data from each individual (Table 3xviii).

TABLE 3xviii(a) VLDL1 METABOLISM

PATIENT #	Synthetic Rate *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to VLDL2 *(pools/day)*
		BASELINE		
01	1025	73	11.6	2.4
02	803	90	6.9	2.1
03	263	67	0.5	3.4
05	260	43	2.7	3.4
06	199	26	3.7	3.8
07	822	93	6.4	2.3
08	722	67	5.7	5.1
09	586	57	5.8	4.4
10	104	7	9.6	4.4
11	151	21	0.0	7.2
12	389	85	2.5	2.0
13	523	45	3.9	7.7
14	1037	82	8.1	4.6
16	1217	143	6.7	1.8
MEDIAN	**554**	**67**	**5.8**	**3.6**
MEAN	**579**	**65**	**5.3**	**3.9**
SEM	**98**	**9**	**0.9**	**0.5**
		REPEAT		
01	309	99	2.0	1.2
02	155	19	4.4	3.9
03	874	164	3.9	1.4
05	610	76	5.6	2.4
06	158	21	5.1	2.6
07	612	59	8.9	1.4
08	705	72	6.3	3.5
09	478	44	3.2	7.8
10	305	30	6.8	3.4
11	508	79	5.7	0.7
12	613	97	5.7	0.6
13	496	14	30.5	5.5
14	866	60	10.1	4.4
16	217	21	3.9	6.7
MEDIAN	**502**	**60**	**5.7**	**3.0**
MEAN	**493**	**61**	**7.3**	**3.3**
SEM	**64**	**11**	**1.9**	**0.6**
P	**N.S.**	**N.S.**	**N.S.**	**N.S.**

TABLE 3xviii(b) VLDL2 METABOLISM

PATIENT #	Direct synthesis *(mg/day)*	Flux from VLDL1 *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to IDL & LDL *(pools/day)*
		BASELINE			
01	1019	175	170	4.49	2.39
02	912	186	227	1.25	3.49
03	531	227	349	0.22	1.85
05	152	144	204	0.30	0.96
06	255	101	186	0.16	1.63
07	253	220	202	0.28	1.70
08	723	339	357	0.86	1.97
09	450	251	154	1.17	3.27
10	419	33	97	0.05	4.40
11	451	151	277	0.24	1.82
12	317	175	235	0.47	1.61
13	33	346	127	1.31	1.65
14	358	374	165	1.47	2.44
16	345	263	266	0.29	1.83
MEDIAN	**389**	**203**	**203**	**0.39**	**1.84**
MEAN	**444**	**213**	**215**	**0.90**	**2.20**
SEM	**74**	**26**	**20**	**0.31**	**0.25**
		REPEAT			
01	34	115	158	0.34	0.56
02	521	73	199	0.57	2.30
03	1317	233	241	4.05	2.22
05	399	180	222	0.26	2.26
06	39	53	114	0.26	0.42
07	440	84	88	1.48	3.67
08	305	254	190	0.98	1.82
09	175	339	123	2.09	1.91
10	1070	102	137	5.87	2.68
11	277	57	199	0.80	0.55
12	433	58	208	0.99	1.36
13	248	36	78	1.03	2.40
14	492	263	88	4.34	3.98
16	97	137	86	0.22	2.41
MEDIAN	**352**	**109**	**148**	**0.99**	**2.24**
MEAN	**418**	**142**	**153**	**1.66**	**2.04**
SEM	**99**	**26**	**15**	**0.48**	**0.29**
P	**N.S.**	**< 0.05**	**< 0.01**	**N.S.**	**N.S.**

TABLE 3xviii(c) IDL METABOLISM

PATIENT #	Flux from VLDL2 *(mg/day)*	Pool *(mg)*	Direct catabolism *(pools/day)*	Flux to LDL *(pools/day)*
		BASELINE		
01	407	617	0.12	0.54
02	792	452	0.12	1.63
03	644	720	0.00	0.90
05	196	435	0.01	0.44
06	303	300	0.00	1.01
07	343	436	0.04	0.75
08	703	541	0.18	1.12
09	505	270	0.49	1.39
10	427	253	0.06	1.63
11	503	359	0.00	1.40
12	378	409	0.02	0.91
13	209	337	0.21	0.41
14	402	314	0.15	1.13
16	486	551	0.34	0.54
MEDIAN	**417**	**422**	**0.09**	**0.96**
MEAN	**450**	**428**	**0.12**	**0.98**
SEM	**46**	**37**	**0.04**	**0.11**
		REPEAT		
01	89	224	0.00	0.40
02	460	291	0.03	1.54
03	537	310	0.00	1.73
05	501	378	0.00	1.33
06	48	199	0.03	0.22
07	323	198	0.02	1.62
08	347	318	0.00	1.09
09	236	254	0.25	0.68
10	366	260	0.74	0.67
11	109	312	0.01	0.34
12	283	272	0.01	1.03
13	189	159	0.11	1.07
14	352	183	0.55	1.36
16	207	190	0.50	0.59
MEDIAN	**303**	**257**	**0.02**	**1.05**
MEAN	**289**	**254**	**0.16**	**0.98**
SEM	**41**	**17**	**0.07**	**0.13**
P	**< 0.01**	**< 0.001**	**N.S.**	**N.S.**

TABLE 3xviii(d) LDL METABOLISM

PATIENT #	Direct synthesis *(mg/day)*	Flux from IDL & VLDL2 *(mg/day)*	VLDL-derived plasma pool *mg*	Total ApoB pool *mg*	FCR *(pools/day)*	Total apoB synthesis *(mg/day)*
			BASELINE			
01	111	355	2520	3307	0.14	2155
02	155	738	3856	4664	0.19	1870
03	49	660	3961	4253	0.17	843
05	3	220	4874	4934	0.05	415
06	45	326	2901	3302	0.11	500
07	82	364	3191	3907	0.11	1156
08	116	627	4040	4788	0.16	1561
09	64	374	2474	2897	0.15	1099
10	140	433	2073	2741	0.21	663
11	24	510	3781	3961	0.13	627
12	21	370	3520	3718	0.11	726
13	4	138	2466	2528	0.06	559
14	13	441	4842	4984	0.09	1408
16	60	340	3383	3979	0.10	1621
MEDIAN	**54**	**372**	**3451**	**3934**	**0.12**	**971**
MEAN	**63**	**421**	**3420**	**3855**	**0.13**	**1086**
SEM	**14**	**44**	**233**	**219**	**0.01**	**149**
			REPEAT			
01	1	93	1813	1839	0.05	344
02	0	473	4357	4286	0.11	676
03	83	546	3012	3471	0.18	2274
05	85	501	3349	3918	0.15	1094
06	3	57	1666	1768	0.03	200
07	62	388	2254	2616	0.17	1114
08	128	365	2382	3219	0.15	1139
09	53	172	2364	3092	0.07	706
10	0	179	4202	4118	0.04	1375
11	0	128	2873	2808	0.04	785
12	0	281	2931	2925	0.10	1046
13	264	183	883	2158	0.21	1008
14	461	269	1765	4787	0.15	1819
16	22	112	1848	2214	0.06	336
MEDIAN	**38**	**226**	**2373**	**3008**	**0.10**	**1027**
MEAN	**83**	**268**	**2550**	**3087**	**0.11**	**994**
SEM	**35**	**43**	**262**	**251**	**0.02**	**152**
P	**N.S.**	**< 0.01**	**< 0.05**	**< 0.01**	**N.S.**	**N.S.**

Analysis of the I-131 specific activity curves shows no differences in decay in the $VLDL_1$ fraction, activity falling below 1% of initial dose at 24 hours (Fig. 3.18). Peak activity in $VLDL_2$, IDL and LDL is lower in the repeat studies than at baseline in the drug-treated subjects, but in LDL only in the apheresis patients. A reduction in the amount of apoB entering the delipidation cascade is seen also in the I-125 curves (Figs. 3.19).

$VLDL_1$ apoB pool size, fractional transfer rate to $VLDL_2$, and direct fractional catabolic rate were unchanged by therapy. There was a mean reduction in synthesis rate of 15% which was not significant. The $VLDL_2$ pool was decreased from a mean of 215.4 mg to 152.5 mg ($p < 0.01$), due in part to a decrease of 34% in the amount of material derived from $VLDL_1$ ($p < 0.05$); the fractional catabolic rate increased from a mean of 0.9 to 1.7 pools/day, although this was not statistically significant due to the wide inter-individual variability. Direct synthesis of $VLDL_2$ was unaltered, as was the fractional transfer rate. The apoB pool size in IDL was also reduced by 41%, from 428.1 mg to 253.5 mg ($p < 0.001$). This was due to the reduced transfer of apoB from $VLDL_2$, as there was no significant change in the catabolism of this fraction. Similarly, there was no change in the LDL FCR, but a 20% reduction in the apoB pool ($p < 0.01$). There was no significant difference in direct LDL synthesis, but a 36% reduction in apoB derived from VLDL and IDL ($p < 0.01$).

The main differences in the post-intervention studies were the reductions in pool sizes in all the compartments beyond $VLDL_1$, an increase in direct catabolism of $VLDL_2$, and no change in total apoB synthesis. The metabolic changes are illustrated by group in Figs 3.20 - 3.23.

The reduction in $VLDL_2$ derived from $VLDL_1$ was greater in the drug-treated group than for the apheresis group (151.6 mg/day (SD 89) vs. 27.1 (SD 80.2), $p = 0.035$), but there was no other parameter in which the changes following intervention differed significantly between the two groups.

FIG 3.18 I-131 SPECIFIC ACTIVITY CURVES (GROUP 1)

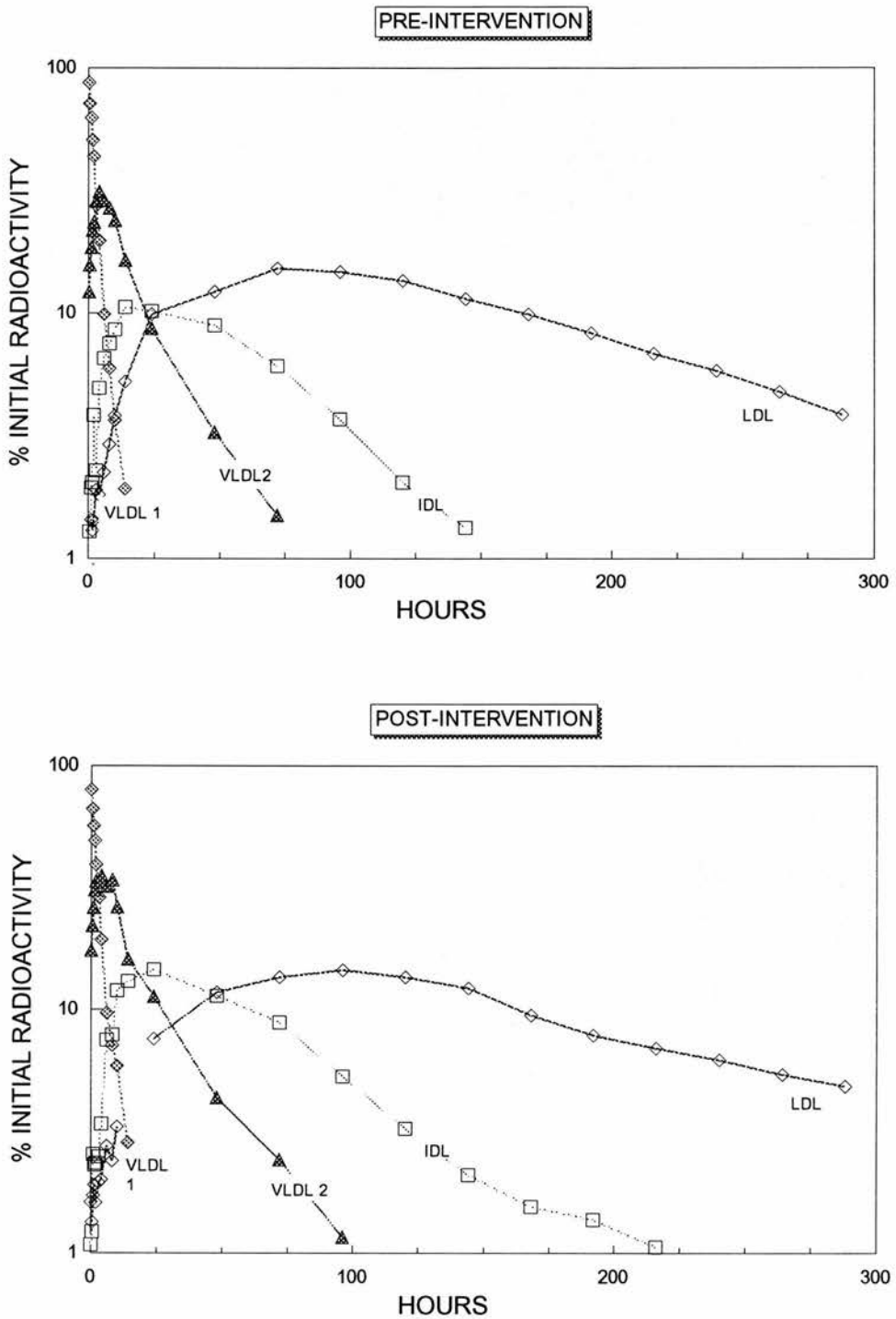
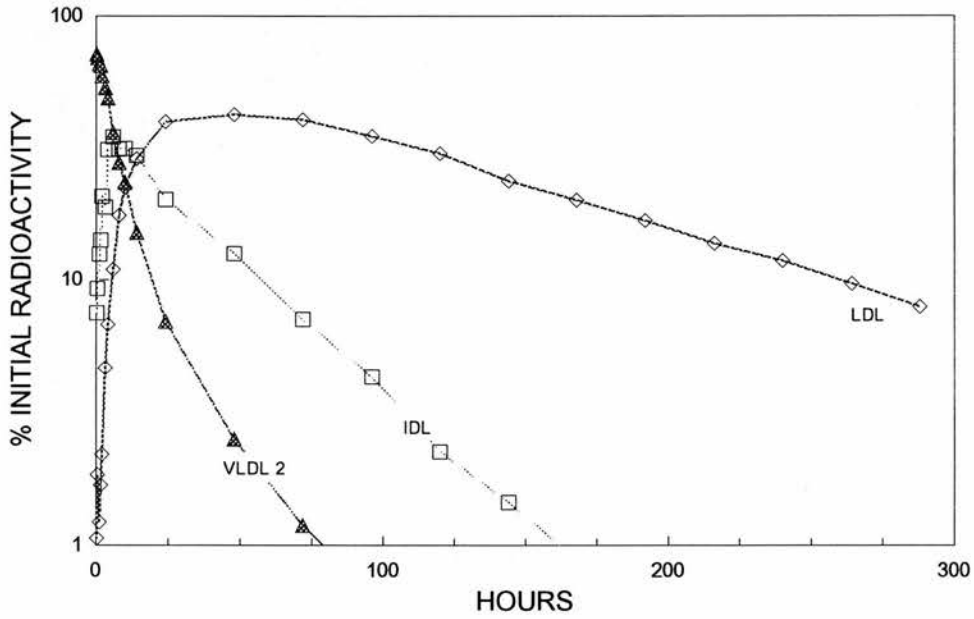
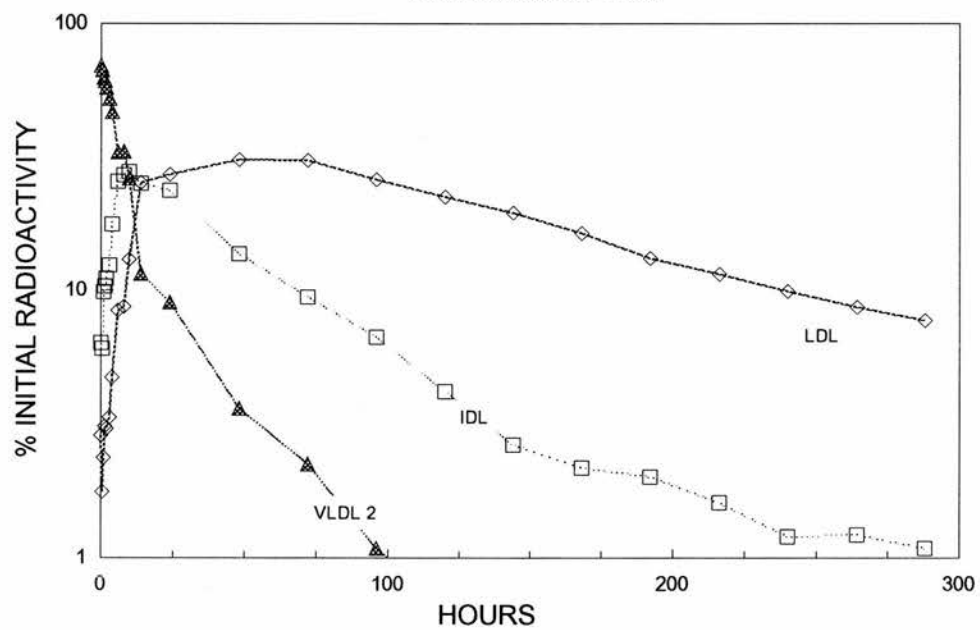


FIG 3.19 I-125 SPECIFIC ACTIVITY CURVES (GROUP 1)

PRE-INTERVENTION



POST-INTERVENTION



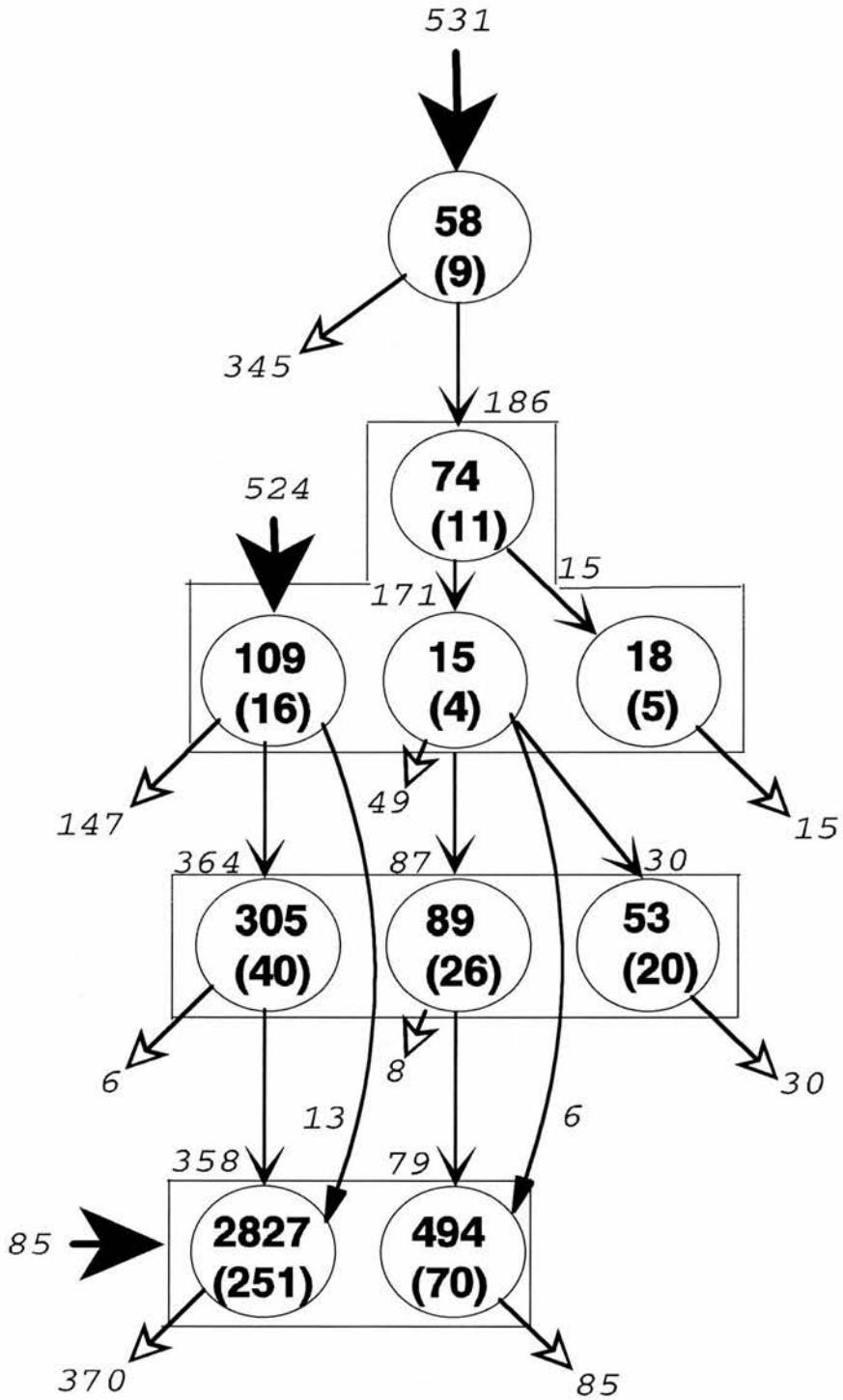


FIG 3.20 GROUP 1 BASELINE VLDL METABOLISM

Nos. at arrows = transfer of apoB in mg/day; nos. in circles = mean pool size in mg (SEM); n=9

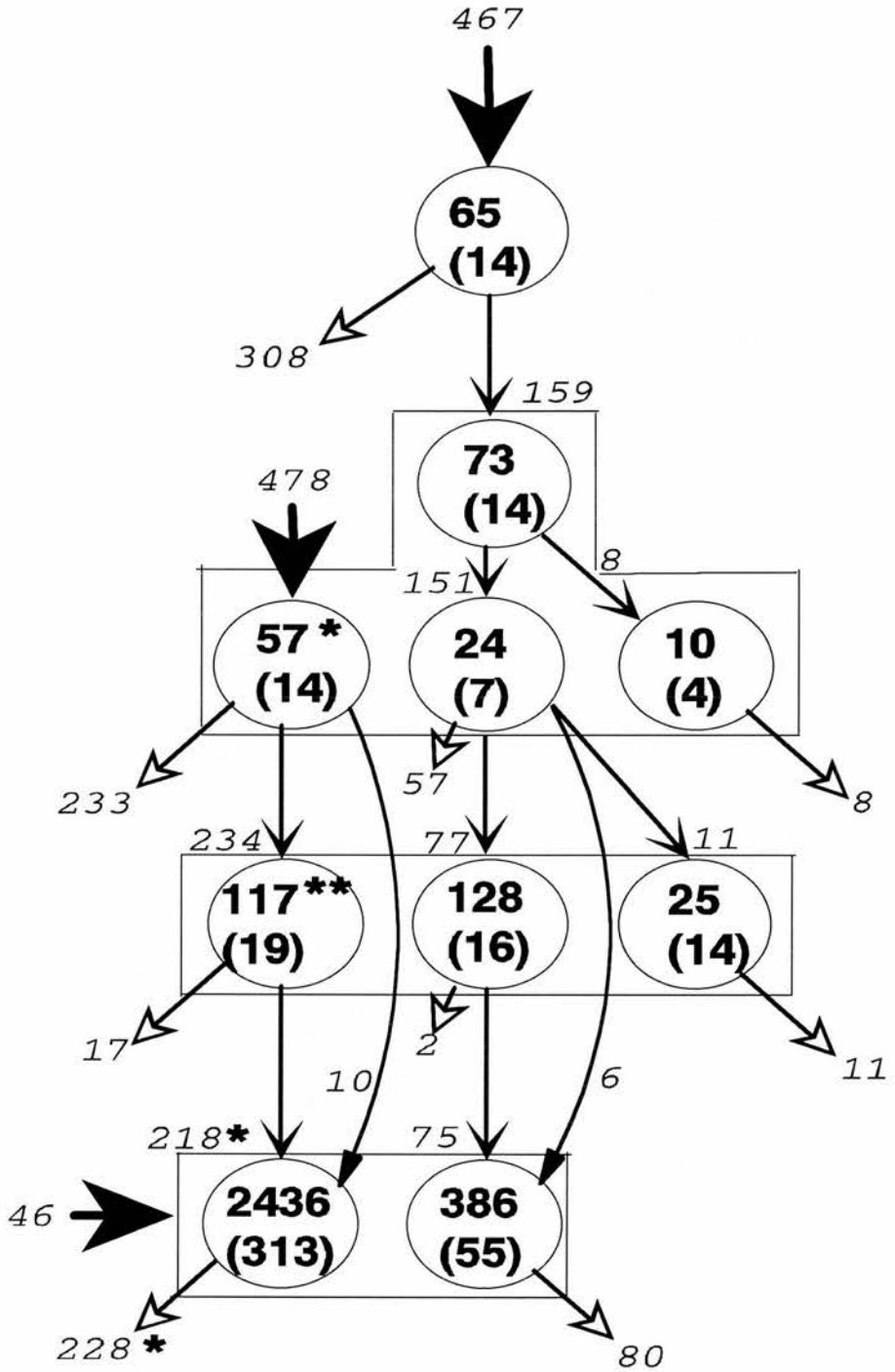


FIG 3.21. GROUP 1 VLDL METABOLISM POST-INTERVENTION

*Nos. at arrows = transfer of apoB in mg/day; nos. in circles = mean pool size in mg(SEM); n = 9. * p < 0.05, ** p < 0.01 vs pre-treatment.*

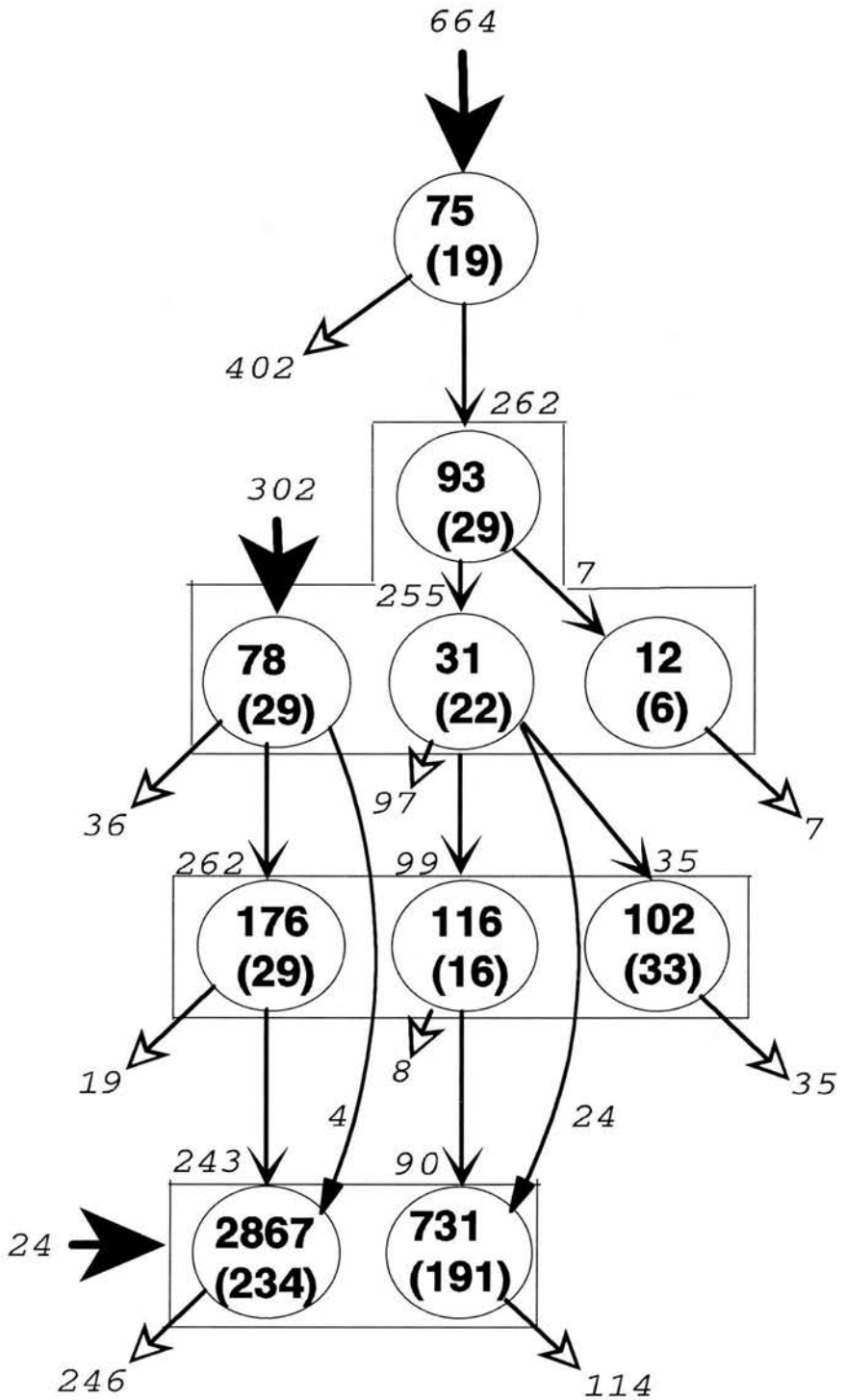


FIG 3.22. GROUP 2 BASELINE VLDL METABOLISM

Nos. at arrows = transfer of apoB in mg/day; nos. in circles = mean pool size in mg (SEM); n = 5.

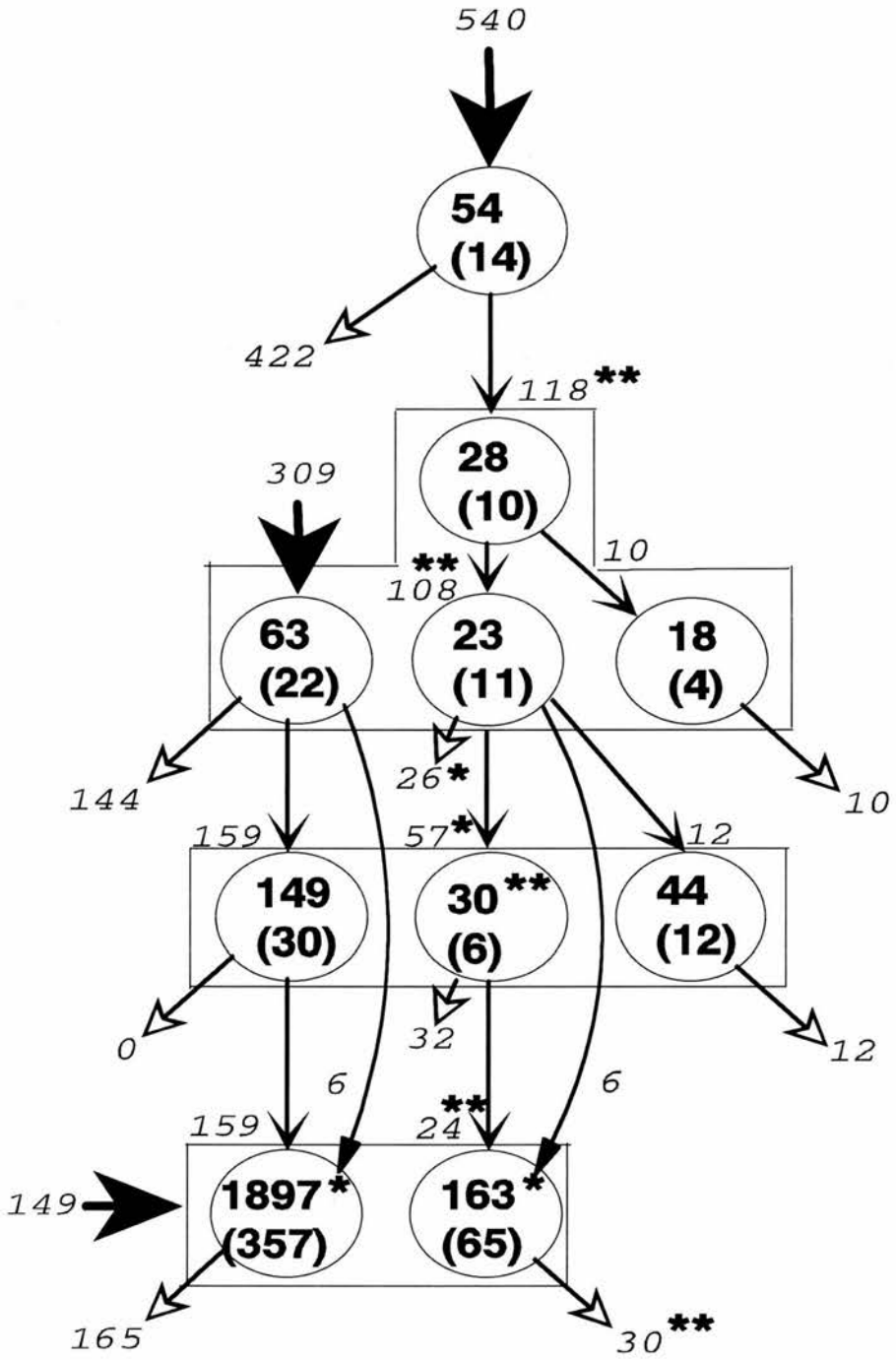


FIG 3.23. GROUP 2 VLDL METABOLISM POST-INTERVENTION

Nos. at arrows = transfer of apoB in mg/day; nos. in circles = mean pool size in mg (SEM); n = 5.
** $p < 0.05$, ** $p < 0.01$ vs pre-treatment.*

Changes in the parameters related to LDL apoB mass at baseline might be expected to contribute to an alteration of LDL levels. The differences in apoB pools and fluxes were therefore plotted against the observed alterations in LDL apoB mass. The correlations and 'p' values are tabulated in table 3xix:

TABLE 3xix. CORRELATION OF KINETIC PARAMETERS WITH LDL APO-B MASS

		r	p
Changes in:	VLDL 1 synthesis	0.28	N.S.
	VLDL 2 synthesis	0.49	0.075
	V1 -> V2 flux	0.34	N.S.
	VLDL 2 pool	0.63	0.015
	V2 -> IDL flux	0.25	N.S.
	IDL pool	0.57	0.035
	ApoB production	0.41	N.S.

Although there was a highly significant correlation between changes in $VLDL_1$ synthesis and direct $VLDL_2$ synthesis ($r = 0.68$, $p < 0.01$), the LDL apoB pool changes were not correlated with synthesis of the lighter VLDL species. The main determinants of the apoB pool following intervention were the masses of apoB in $VLDL_2$ and IDL, related to the synthesis of $VLDL_2$.

3.2.4 Discussion

The decay curves and calculated kinetic parameters obtained at baseline were similar to those previously noted in tracer studies of patients with similar lipoprotein levels, although some differences were observed. The masses of apoB in each of the $VLDL_1$, $VLDL_2$ and IDL pools were significantly smaller than in other hypercholesterolaemics, while the LDL apoB mass was at the upper end of observed values. All the pools other than $VLDL_1$ were however considerably increased compared to those obtained in studies of normocholesterolaemia [Gaw 1992]. Synthesis of $VLDL_1$ and transfer rates were not significantly different, but

the fractional catabolic rate was increased compared to other studies in hypercholesterolaemia. The other most striking differences in the present study were the markedly reduced FCRs of IDL and LDL (0.12 (SD 0.13) and 0.13 (0.05) respectively, compared to 0.40 (0.20) and 0.26 (0.06)), and increased fractional transfer of IDL to LDL. These unexpectedly low FCRs are not explained by an increased proportion of E4 homozygotes, and may reflect the inclusion of some familial hypercholesterolaemia heterozygotes (including some unrecognised) which other investigators have excluded from metabolic studies.

Some of the differences in other kinetic parameters may however be due to the apoE phenotypes [Demant *et al* 1991]; within this population, the E3/E3 phenotype was associated with significantly larger apoB pools in $VLDL_2$, IDL and LDL than other phenotypes (Table 3xx).

TABLE 3xx. BASELINE KINETIC PARAMETERS AND APO-E PHENOTYPES

	E3/E3 *(n=6)*	E4/E3 *(n=5)*	E4/E4 *(n=2)*	P
VLDL1 POOL	83.6 (33.5)	41.5 (34.2)	-	0.075
VLDL1 FTR	2.52 (0.68)	4.08 (1.11)	-	< 0.05
VLDL 2 from VLDL 1	195 (42.8)	-	360 (19.9)	< 0.01
	-	145.7 (89.2)	360 (19.9)	< 0.01
VLDL2 POOL	242 (61.3)	168 (43.6)	-	< 0.05
	242 (61.3)	-	146 (26.6)	< 0.05
IDL POOL	531 (121.5)	-	326 (15.7)	< 0.01
LDL POOL	4142 (603)	3312 (503)	-	< 0.05

There were few qualitative differences between the apoE phenotypes in the response to intervention. The changes in certain parameters however were more marked in those with particular phenotypes compared to others (Table 3xxi). The two E4/4 individuals were alone in exhibiting a significant increase in total apoB production, against the general trend of reduction in all the individuals with other phenotypes.

TABLE 3xxi. APO-E PHENOTYPES AND RESPONSE TO INTERVENTION

		E3/3 *(n=6)*	E4/3 *(n=4)*	E3/2 *(n=2)*	E4/E4 *(n=2)*
VLDL 2 from VLDL 1	Pre	195 (43)	146 (89)	240 (94)	360 (20)
	Post	132 (66) *	145 (131)	155 (140) **	150 (160)
IDL from VLDL 2	Pre	484 (210)	502 (252)	534 (156)	305 (136)
	Post	346 (180)	244 (141)	228 (168) **	270 (115)
IDL POOL	Pre	531 (122)	451 (313)	552 (198)	326 (16)
	Post	278 (66) ***	228 (34)	315 (4)	171 (17) *
LDL from VLDL/IDL	Pre	447 (204)	482 (244)	484 (159)	289 (215)
	Post	335 (201)	199 (138) *	246 (168)	226 (61)
LDL POOL	Pre	4142 (603)	3312 (503)	4643 (622)	3756 (1736)
	Post	3109 (961) ***	2898 (980)	3013 (290) *	3473 (1859)
Total ApoB Synthesis	Pre	1272 (704)	984 (402)	1687 (1129)	984 (600)
	Post	962 (721)	849 (513)	962 (250)	1413 (573) **

* $p < 0.1$ ** $p < 0.05$ *** $p < 0.01$

The effects of intervention were principally to increase the direct catabolism of VLDL2, and thereby to decrease apoB pools in IDL and LDL. Similar to the effects

seen during the course of treatment with simvastatin alone, there is little change in LDL FCR but increased LDL-receptor activity results in increased direct catabolism of precursors and a diminished influence of lipase-driven processes. At baseline 64% of apoB entering the delipidation cascade was recovered in LDL in the subjects treated by apheresis combined with drugs, compared to 48% following treatment. Since these measurements were made after re-attaining steady state off-treatment, the changes in receptor-mediated clearance during treatment are likely to have been even greater.

The observation that the most significant changes in kinetic parameters occurred in reduced flux rates between lipoproteins rather than in direct catabolism does however suggest some other mechanism in addition to LDL-receptor upregulation. Lipase activities were not measured, but the concomitant changes in lipoprotein compositions may suggest an alteration in enzyme activity in response to therapy. This is difficult to reconcile with the accepted mechanism of action of the drugs used in this study, which is to promote the removal of apoB/E-containing lipoproteins by direct catabolism. By combining LDL-apheresis (which should stimulate cholesterol synthesis in response to depletion of body cholesterol stores) with a cholesterol synthesis inhibitor the effects on upregulation of the receptors should be maximised, although extracorporeal therapy does not itself cause any alteration in LDL FCR [Thompson et al 1981].

3.3 EFFECTS ON OTHER LABORATORY PARAMETERS

3.3.1 Methods

3.3.1.1 Biochemical

A routine biochemical screen was performed at the same time as the lipoprotein analyses on a multi-channel SMAC autoanalyser (Technicon, Basingstoke, UK) to monitor the safety profile of the apheresis procedure and concomitant changes in non-lipid parameters during long-term combination lipid-lowering therapy. Blood was withdrawn after cannulation before apheresis treatment, and again immediately at the end of apheresis. This was placed in a plain glass container, and the serum separated after standing for ten minutes. The serum was stored at 4°C, and analysed within 24 hours. A routine screen was also performed at monthly intervals in the drug-treated group.

3.3.1.2 Haematological

A full blood count was obtained before and after each apheresis procedure in group 1, and at monthly intervals in the remainder. Blood was collected into EDTA bottles, and analysed in a routine fashion on a semi-automated Coulter counter (Coulter Electronics, Florida, USA) within 24 hours. The activated partial thromboplastin time was measured in each of the subjects undergoing apheresis before each of the first few procedures and again during the treatment to adjust the bolus and infusion rate of the administered heparin to maintain the APTT at approximately twice the control value. The prothrombin time was measured routinely in each of the subjects at baseline and at yearly intervals to test for effects on the coagulation system.

3.3.1.3 Haemorheological

Samples were taken at baseline and at annual intervals to assess effects of therapy on whole blood viscosity, plasma viscosity, fibrinogen and plasminogen levels. Blood was taken into EDTA bottles for viscosity measurements, and analysed immediately on a Coulter Harkness viscometer (Coulter Electronics,

Luton, UK) at 25°C. Blood for fibrinogen was collected with minimal venostasis into 1 ml 3.8% citrate solution, and immediately put on ice. This was then centrifuged at 4°C, separated and the plasma stored at -70°C for subsequent analysis. Samples were also taken pre- and post-apheresis in an identical fashion from all the group 1 subjects on a number of occasions to assess the acute effects of the procedure on these variables, and on a weekly basis following discontinuation of apheresis in a subset of the patients as described below.

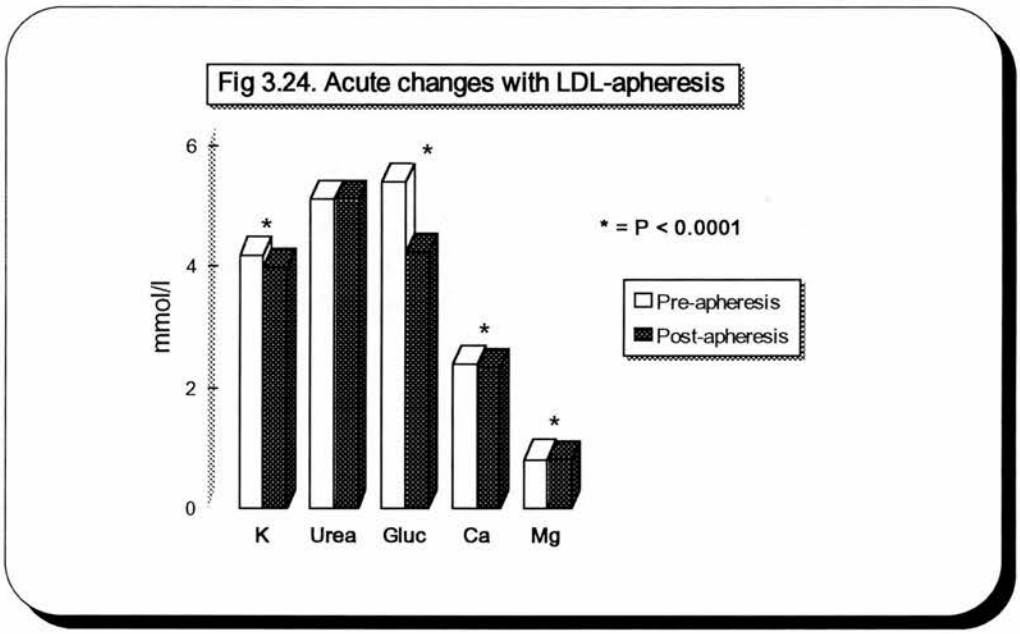
Following the premature death of one of the subjects (#06) within a few weeks of completion of apheresis, the effects of withdrawal from lipid-lowering treatment on platelet function was studied in those remaining on therapy to assess whether the platelets may become hyperaggregable when treatment is discontinued. Freshly obtained platelets were resuspended in normal serum and the maximum aggregation and the rate of aggregation in response to standard stimuli - arachidonate, ristocetin and collagen - was measured and compared to normal controls selected from young healthy members of hospital staff. These were performed immediately prior to the final apheresis procedure and again in the fasting state at weekly intervals for four weeks.

3.3.2 Effects of intervention

3.3.2.1 Biochemical parameters

A number of parameters were affected acutely by LDL-apheresis (Fig 3.24). There were no significant differences between pre- and post-apheresis serum creatinine, urea or sodium. Other cations were significantly reduced during the procedure, although there was no change over time in the pre-apheresis levels. Overall the reduction in Ca^{2+} was 2.37% (SD 2.72), which was consistent if only modest ($p < 0.0001$). The mean reduction in Mg^{2+} was 2.91% (SD 4.86, $p < 0.0001$), but did not vary significantly in three of the subjects. The mean intra-subject change in Mg^{2+} tended to be inversely related to the pre-apheresis levels. There was a trend in reduction of the pre-apheresis magnesium level, but this was of borderline significance statistically, the levels were not significantly different from baseline at 24 months, and the values for all the subjects remained within the reference limits. The mean reduction in K^{+} during the procedure was 3.81% (SD 9.26, $p < 0.0001$).

In nine of the subjects the average reduction was significant, varying from 1.9% to 9.9%; in the other patient there was a mean increase during apheresis from 4.15 mmol/l (SD 0.25) to 4.24 mmol/l (SD 0.27) ($p < 0.02$).

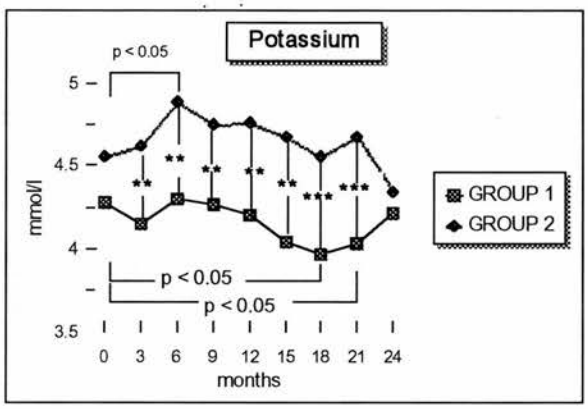


Plasma glucose fell during the procedure by an average of 19%, from 5.41 to 4.23 mmol/l ($p < 0.0001$). In some individuals the reduction in plasma glucose was consistently greater than for others, ranging from a mean fall of 5.9% to 32.6%. The mean percentage reduction was significantly related to the pre-apheresis glucose level ($r = 0.69$, $p < 0.05$).

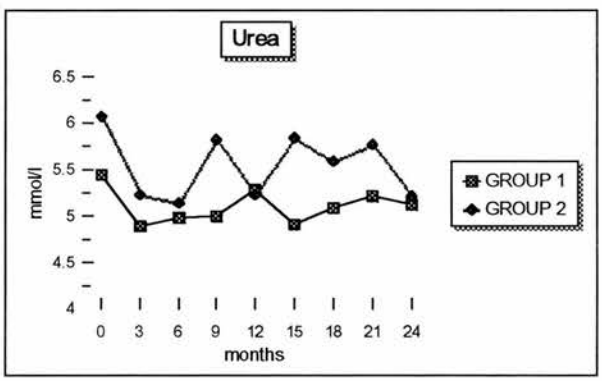
As has been described previously (Sect **1.6**) the procedure results in non-specific loss of plasma proteins. There was a reduction in total proteins from 66.0 to 57.8 g/l, a mean change of 12.3% ($p < 0.0001$). There was little inter-subject variability, and the percentage reduction was not related to either pre-apheresis levels or the number of procedures performed. The same pattern was observed for albumin and the globulin fractions, although the percentage decrease was slightly greater for the latter (mean reduction of 14.8%).

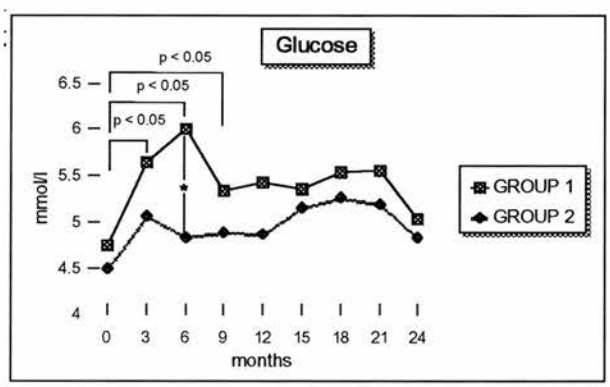
Long-term changes were assessed by comparing the measured parameters at three-monthly intervals in both treatment groups (Fig 3.25). Inter-group comparisons were made using unpaired t-tests, and paired t-tests were employed for changes within the groups.

FIG 3.25 LONG-TERM CHANGES IN BIOCHEMICAL PARAMETERS



* p < 0.05, ** p < 0.01,
*** p < 0.001 vs Group 2





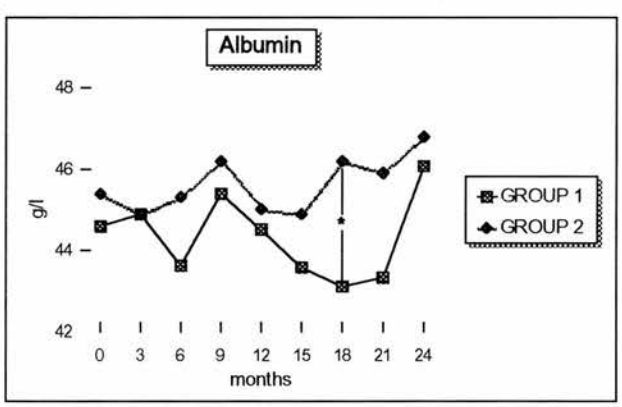
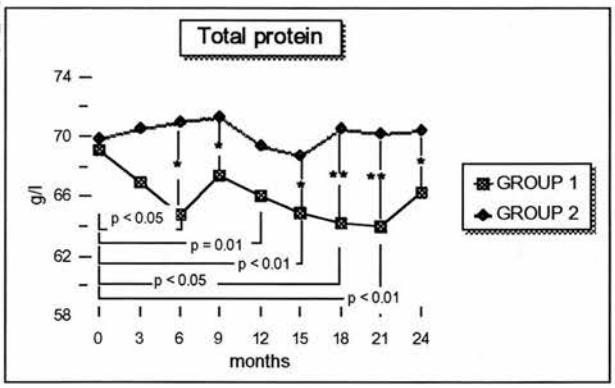
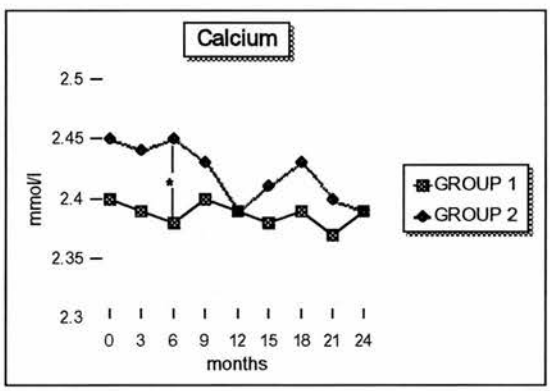
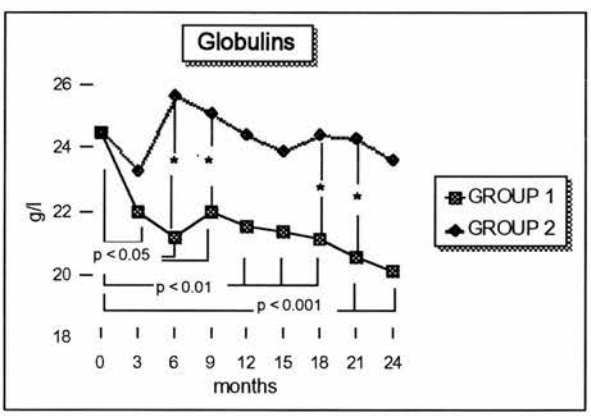


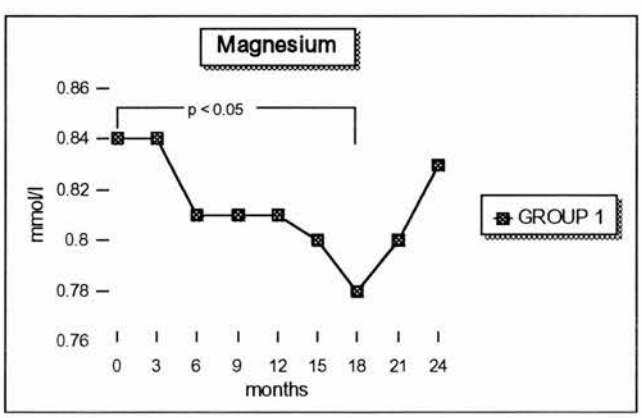
FIG 3.25 (Cont)



* p < 0.05, ** p < 0.01,
*** p < 0.001 vs Group 2







There was no change in the urea or creatinine levels in either group over time. Pre-apheresis plasma glucose levels were higher at each time-interval compared to baseline, and these differences were significant for the first year. There were however no statistically significant differences (after Bonferroni correction) between the groups.

The pre-apheresis serum potassium concentration was reduced compared to baseline (the differences being greater and statistically significant in the second year), while there were non-significant increases from baseline in group 2. The differences between the groups at each time-point other than at baseline and at 24 months averaged 0.51 mmol/l and were highly significant; the serum K^+ did not fall below the lower limit of normal in any of the subjects at any of the measurement times, however, and the differences between the groups was largely due to unexplained transient elevation in a number of the group 2 subjects. The pre-apheresis Ca^{2+} and Mg^{2+} levels tended to be slightly lower than at baseline, but the differences were not statistically significant either from baseline nor from the other treatment group.

No changes were seen in the albumin or globulin fractions or in the total serum proteins in group 2, and the pre-apheresis albumin did not alter significantly from baseline in the group 1 subjects. The globulin fraction however was progressively reduced compared to baseline in the apheresis group, the difference being highly significant, and resulted in a significant reduction also in total serum proteins in this group. The differences in globulin fraction and total proteins between the groups became significant after three months.

3.3.2.2 Haematological parameters

During each apheresis procedure, there were small changes in the haemoglobin concentration and packed cell volume. Although changes in the former were statistically significant overall, the mean increase was just 0.25% (Table 3xxii). For one individual (#03) the average rise was 0.5 g/dl (4%); for this subject and three others the individual changes in haemoglobin and haematocrit were statistically significant. Overall however the average increase in haematocrit was 0.72% (SD 4.72), which was not significant. Alterations in white cell count were variable,

ranging from a mean individual fall of 14.6% to a mean increase of 17.4%, and for all the procedures the mean fall was 2.3% (SD 18.5, N.S.). Platelet counts were more consistent: a highly significant reduction was observed in each of the individuals, the average change being 13.3% (S.D. 9.2, $p < 0.0001$).

TABLE 3xxii. ACUTE HAEMATOLOGY CHANGES DURING APHERESIS

	pre mean (SEM)	post mean (SEM)	p
Hb (g/dl)	13.45 (0.14)	13.47 (0.13)	< 0.05
Hct	0.392 (0.004)	0.395 (0.004)	N.S.
WCC (1000/ml)	6.31 (0.171)	6.18 (0.228)	N.S.
Plats (1000/ml)	311 (9.5)	269 (9.0)	< 0.001

Long-term changes in haematological parameters were assessed as described in **3.3.2.1**. Small reductions in pre-apheresis haemoglobin levels were noted in group 1 over the first few months, before increasing significantly above concentrations prior to commencing regular treatment (FIG 3.27). Analysis of our early experience with the first six patients showed more dramatic decreases - to the extent of requiring transfusion in two patients (#03 and #06) for worsening angina (FIG 3.26). All the subjects undergoing apheresis were maintained thereafter on oral iron supplements. In the drug-treated group there was a steady increase from baseline in haemoglobin concentration, even without enhanced iron intake. Long-term alterations in haematocrit mirrored closely those in haemoglobin levels, while pre-apheresis platelet levels remained unchanged despite the marked fluctuations seen during each procedure. There were no long-term changes in either group in coagulation.

FIG3.26 PRE-APHERESIS HAEMOGLOBIN

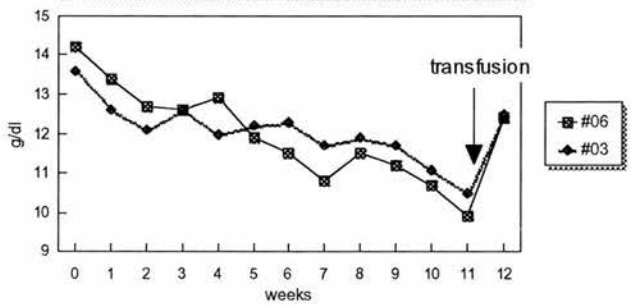
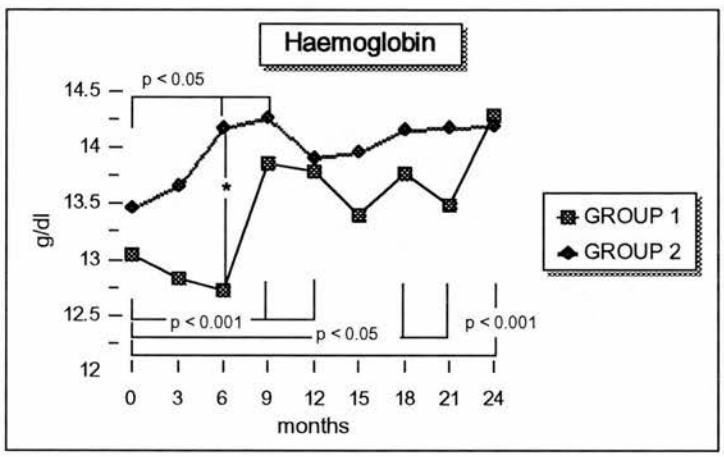
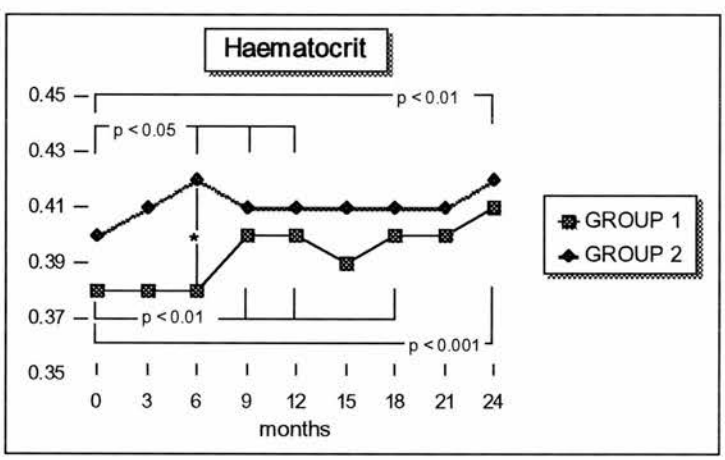
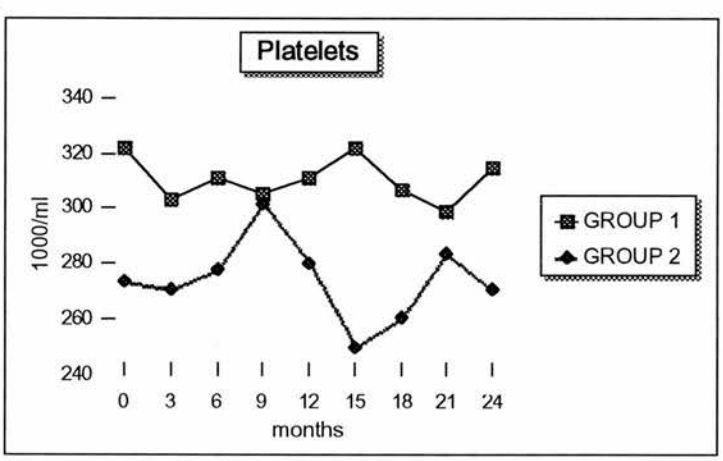


FIG 3.27 LONG-TERM CHANGES IN HAEMATOLOGICAL PARAMETERS







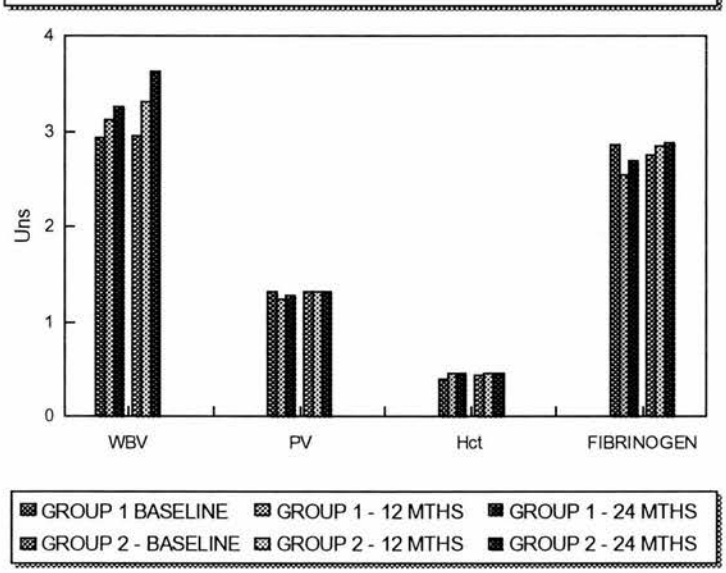
* p < 0.05, group 1 vs group 2

3.3.2.3 Haemorheological parameters

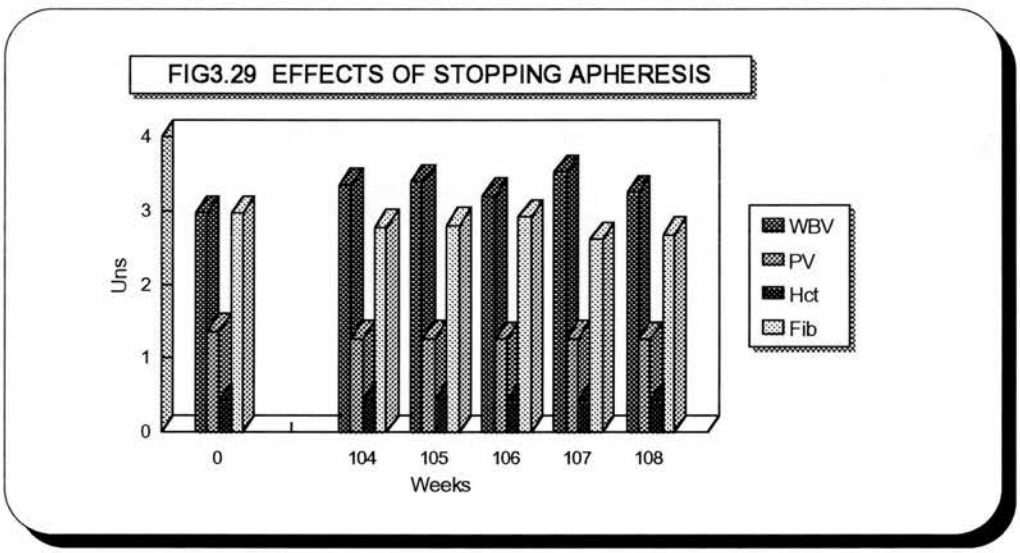
During apheresis sessions there was a reduction in fibrinogen of 39.9% (SEM 2.49), from an individual pre-apheresis average of 2.66 mg/dl (SEM 0.10) to 1.84 mg/dl (SEM 0.12) following the procedure. Whole blood viscosity and plasma viscosity were reduced on average by 10.8% (SEM 1.35) and 12.0% (SEM 1.23) respectively, and these changes were all highly significant ($p < 0.0001$). The reduction in plasma fibrinogen was significantly correlated with the volume of plasma processed ($R = 0.46$, $p < 0.02$); it was not, however, correlated with the fall in total or LDL-cholesterol, nor was there a significant association with the degree of reduction in plasma viscosity. Although whole blood and plasma viscosity fell by roughly the same extent, the changes in these parameters were not correlated. Although packed cell volume is the single greatest determinant of whole blood viscosity [Begg & Hearns 1966], there was no correlation between changes in the latter and the measured haematocrit.

In this study there were no significant alterations in pre-apheresis results at 12 or 24 months, neither were there any differences between the two treatment groups in any of the measured rheology parameters at any time-point (FIG 3.28).

FIG 3.28 LONG-TERM HAEMORHEOLOGICAL CHANGES



The change in haemorheology following withdrawal of apheresis treatment is shown in Fig 3.29. No consistent pattern emerged, and there was no statistically significant alteration in these parameters in this small group of subjects.



Compared to the controls, platelet aggregability during regular apheresis treatment was significantly diminished to all stimuli except at the highest concentrations. Over the ensuing weeks the response to collagen remained unchanged, with no increase in aggregability following withdrawal of lipid-lowering treatment. 3 of the 4 patients were however taking aspirin, and in the remaining patient - who avoided aspirin due to dyspepsia - aggregability exceeded that of the control group between 1-3 weeks after cessation of therapy.

3.3.3 Discussion

The acute effects of dextran sulphate adsorption on plasma proteins is well-documented [Schulzeck *et al* 1992, Nakajima *et al* 1988, Eriksson *et al* 1989] and compares favourably with other selective methods of LDL-removal [Olbricht 1993]. The results of the present study show that the long-term consequences of their slight reduction over time is not of clinical significance. It is also well-known that an acute reduction in serum cations is observed: these changes are of small magnitude, and unlikely to be of clinical consequence. The mild reduction in

magnesium levels after prolonged therapy was monitored closely in view of the underlying ischaemic heart disease in the subjects, but at no time did these fall outwith the reference range in any individual.

The acute reductions observed in plasma glucose are unlikely to be attributable to the apheresis procedure. Apart from fasting specimens at 12 and 24 months the apheresis patients were not routinely required to refrain from eating immediately prior to each treatment; the fall in plasma glucose is therefore likely to be due to physiological post-prandial reduction. The pre-treatment non-fasting state also accounts for the apparent decrease in glucose tolerance in this group over the study period. Analysis of only those specimens obtained in the fasting state show (Fig 3.25) a reduction from baseline at 12 months of 0.06 mmol/l (SD 0.8) , and an increase at 24 months of only 0.16 mmol/l (SD 0.7) (N.S.).

Minor fluctuations in haemoglobin during the apheresis procedure were accompanied by alterations of the same magnitude and direction as the haematocrit, and are attributed to dilution or (more commonly) concentration of plasma constituents due to net gain or loss of fluid. It is unclear why these changes were more marked or more consistent in some individuals than in others; those with the largest changes were not more prone to symptomatic hypotension, although the most profound episodes of hypotension were associated with significant haemoconcentration.

The fall in haemoglobin during the early weeks of apheresis is almost certainly due to frequent venous sampling: a fall in haemoglobin from screening values was seen in all subjects before commencement of apheresis or drug therapy due to frequent venesection during metabolic studies. This was associated with the development of a degree of iron deficiency in a number of patients, and was corrected by prescription of oral iron supplements. The occurrence of a hypochromic anaemia during repeated plasma exchange and LDL-apheresis has been reported, is accompanied by marked reductions in serum ferritin [Richter *et al* 1990, Lane *et al* 1993], and has been shown to respond to oral iron supplementation [Berger *et al* 1978, Saito *et al* 1988].

The reduction in platelets averaged 13.3% per procedure, but with reductions noted on occasion of up to 61% - despite a degree of haemoconcentration - and also increases of the same magnitude. This finding has not been a constant feature of LDL-apheresis in earlier reports, and most investigators have noted no significant alteration [Stefanutti *et al* 1988, Saito *et al* 1988]. There were however no significant changes over time in pre-apheresis values (Fig 3.27).

Acute reductions in fibrinogen levels have been documented with all types of LDL-apheresis, with the smallest changes being seen with the dextran sulfate method [Keller 1991], and the greatest with HELP. It has been previously shown that long-term treatment with LDL-apheresis does influence plasma fibrinogen levels [Schuff-Werner *et al* 1989, Seidel *et al* 1988], although this is more pronounced with the HELP system [Sühler *et al* 1990] and may not be significant with other methods [Richter *et al* 1990]. The absence of long-term effects on fibrinogen levels may be explained in part by the variability of fibrinogen levels: the plasma fibrinogen concentration was measured on two occasions in seven individuals during the pre-treatment phase. The means of the measurements of viscosity and fibrinogen were very constant, but the correlation between the individual results taken just 4-6 weeks apart was weak ($R = 0.55$), and non-significant.

Although haematocrit is a critical determinant of blood viscosity in larger vessels, *in vivo* viscosity in many parts of the circulation - particularly the microcirculation - is relatively independent of packed cell volume and more closely dependent on plasma viscosity and fibrinogen levels [Dormandy 1981]. Patients with hyperlipidaemia have higher plasma viscosities than controls, and viscosity has been shown to be significantly correlated with triglyceride and cholesterol levels [Leonhardt *et al* 1977, Seplowitz *et al* 1981]. Treatment with fenofibrate in patients with type II hyperlipidaemia was shown to significantly reduce whole blood viscosity and plasma viscosity, but had no effect on plasma fibrinogen levels; no correlation was seen between the fall in LDL or VLDL and change in viscosity measurements [Arntz *et al* 1990]. In another study treatment of a similar group of hyperlipidaemic patients with gemfibrozil resulted in significant increases in fibrinogen and plasma viscosity despite reductions of 12.5% and 28.4% in cholesterol and triglycerides respectively [Stringer *et al* 1990]. The 32% reduction

in cholesterol levels with lovastatin in a further study however was associated with lowering of plasma viscosity and red cell aggregation with no effects on fibrinogen levels [Koppensteiner *et al* 1990]. The marked reductions in lipid levels during apheresis using HELP, immunoadsorption or dextran sulfate columns has been shown to be associated with acute decreases in plasma viscosity and red cell aggregation rates [Schuff-Werner *et al* 1989, Sühler *et al* 1990, Agishi *et al* 1992]. Repeated apheresis with dextran sulfate in familial hypercholesterolaemia has been shown to result in decreased whole blood viscosity and enhanced peak peripheral blood flow [Rubba *et al* 1990], and this is thought to explain the rapid improvement in angina commonly experienced within weeks of commencing regular treatment [Keller 1991].

Platelet aggregability has been documented by some investigators to be increased in hyperlipidaemia [Carvalho *et al* 1974] , but this has not been found in other studies which also failed to show a change in aggregability during cholesterol-lowering therapy [Lowe *et al* 1982, Koppensteiner *et al* 1990]. The withdrawal of such therapy could potentially lead to an increased likelihood of thrombosis; the effects of aspirin however appear to be much greater in magnitude than any changes induced by alteration of the lipid milieu, and any effects on platelet adhesion of withdrawal of treatment or recurrence of elevated levels of lipids would appear to be transient.

During the apheresis procedures which were terminated prematurely because of profound hypotension, there were distinctive changes in both haematological and biochemical parameters. Each of these episodes occurred after the processing of 2000-3000 mls of plasma. They resulted in an impalpable brachial pulse with a systolic blood pressure of 70 mmHg or less, with or without loss of consciousness. On each occasion the subjects remained hypotensive for some hours after cessation of the procedure despite administration of fluids, unlike other treatments complicated by hypotension, which was transient and readily reversed. Changes in laboratory parameters are illustrated in FIG 3.30 : there were no significant alterations in serum potassium, urea or platelets compared to normal, while alterations in calcium and magnesium were generally only slightly more marked. The reductions in plasma proteins were more than two to three times greater than usual, with acute reductions of up to 40%. Reductions in total and LDL cholesterol

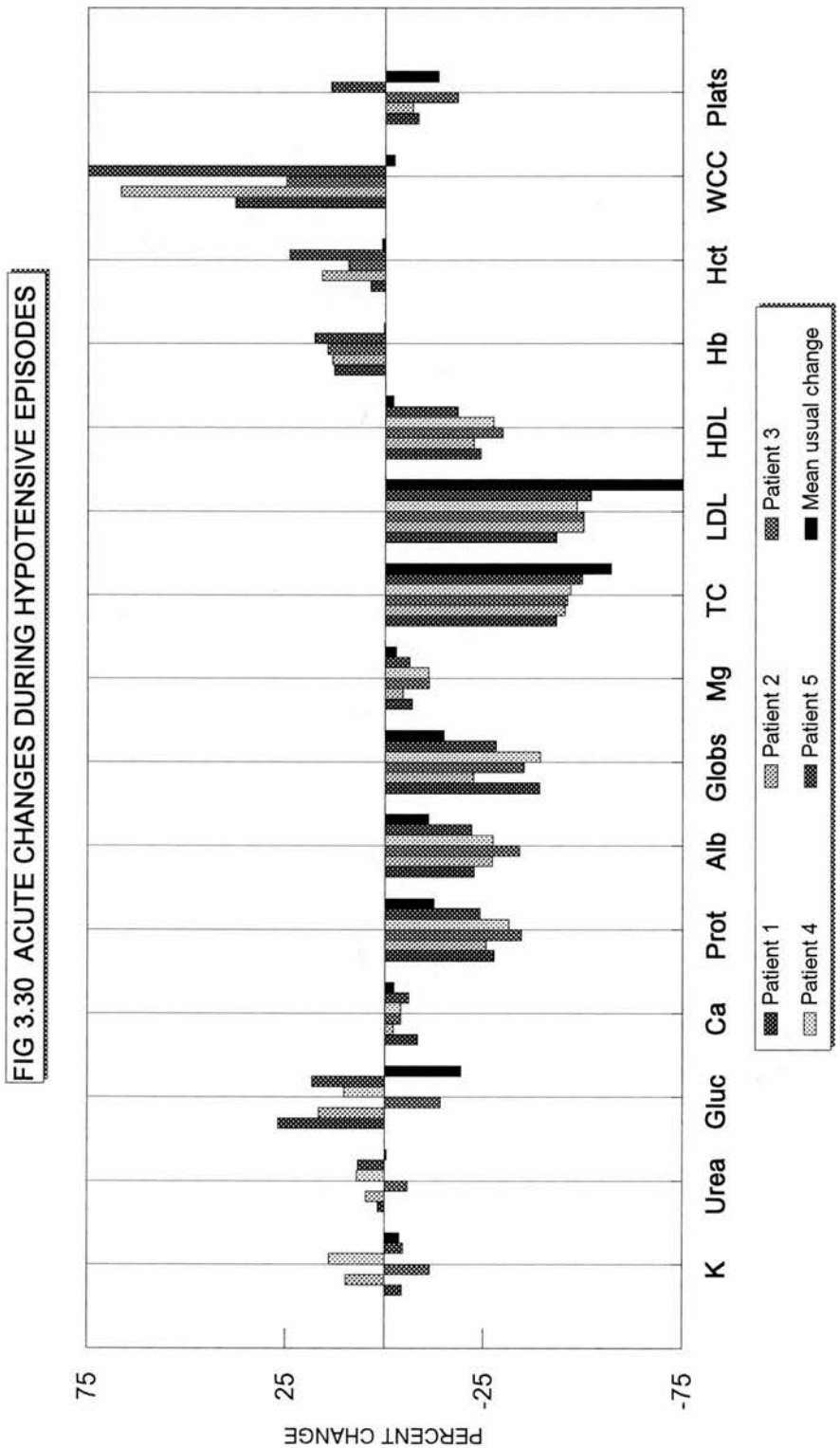
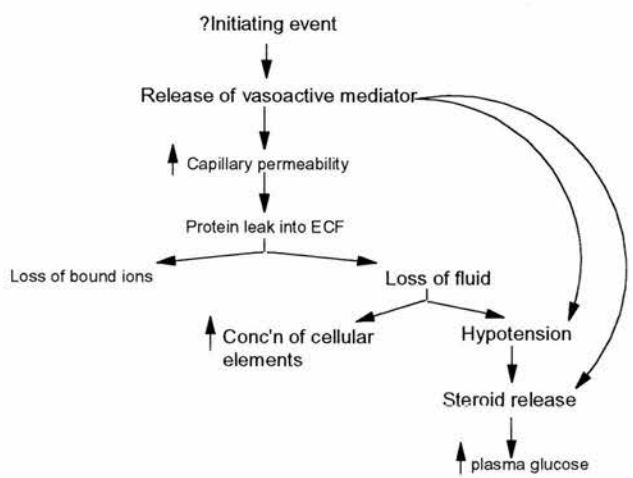


FIG 3.30 ACUTE CHANGES DURING HYPOTENSIVE EPISODES
PERCENT CHANGE
75
25
-25
-75
K
Urea
Gluc
Ca
Prot
Alb
Globs
Mg
TC
LDL
HDL
Hb
Hct
WCC
Plats
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Mean usual change

were less than average since the treatment was discontinued after processing only half the usual volume of plasma, but HDL fell by 18-30% compared to a mean of only 2% (SD 1) for all other procedures. Increases in haemoglobin and haematocrit were also strikingly different, with changes of up to 24% compared to procedure means of 0.25 and 0.72% respectively. The white cell count usually fell by an average of 2.3%, and although this parameter was normally widely variable (SD 18.5), in these instances the response appeared uniformly different to the norm.

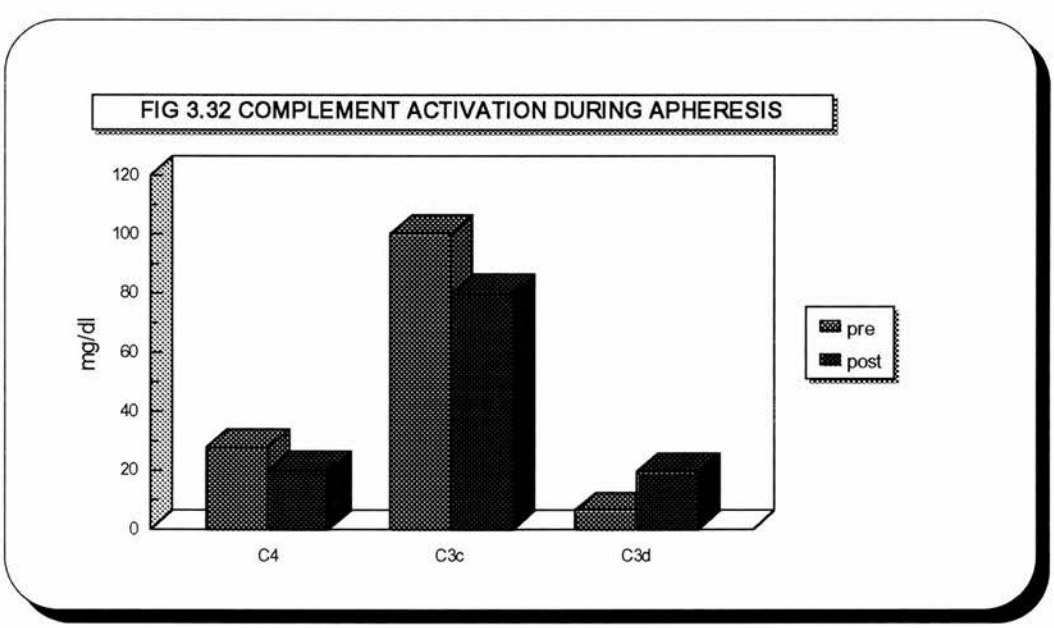
During these treatment sessions there was no evidence of malfunction of the apheresis equipment. There was no loss of plasma or blood, and it is clear that loss of either would not result in the changes observed. The clinical course and associated laboratory changes would be consistent with the extravasation of plasma proteins, including lipoproteins (or at least HDL); unbound ions would simply diffuse into the ECF with the fluid, while ions with protein-binding would suffer a greater drop in plasma concentration. Although the disproportionate increase in leucocytes is consistent with a response to an infective or immunological initiating event, the underlying cause remains unclear.

FIG 3.31 Hypothesis to explain hypotensive episodes



All of the episodes occurred within a two-month period, and the disposable components of the equipment originated from two identified lots from the supplier. These were tested bacteriologically for contamination, but cultures were sterile and pyrogen tests of both implicated disposables and unused samples from the same lots were negative. Further, disposables from these two lots were used without problem in fourteen other locations. Serum from each of the apheresis subjects were tested for antibodies to dextran sulphate (Dr. Otto Scheiner, University of Vienna, Austria), but these were not increased in those who had suffered such reactions .

Complement levels were measured during a number of apheresis procedures, including one of those complicated by hypotension. Initially these were measured from plasma which had been immediately separated and stored at +4°C until analysis, with a mean storage time of 15.7 days (SD 7) and a range of 6-30 days. It was however noted that the pre-apheresis samples showed evidence of activation, the mean C_{3d} level being twice the upper reference range, and later review of the data demonstrated correlation between the C_{3d} level and the time to analysis ($R = 0.75$, $P < 0.001$). Subsequent samples were thereafter immediately frozen and stored at -70°C. The pre-apheresis values for C_4, C_{3C} and C_{3d} were consistently within the reference ranges, and there was a clear statistically significant reduction in the first two components associated with an increase in C_{3d} during apheresis (FIG 3.32).



These results demonstrate that there is complement activation during routine procedures. Unfortunately the plasma taken at the time of one of the severe hypotensive episodes (which proved to be the last one) was not frozen immediately, and the results therefore are unreliable. It is not established whether the degree of complement activation is increased at the time of such episodes, nor whether this might play a role in its mechanism.

Bradykinin is a potent vasodilator and is generated by activation of the contact activation system [Parnes & Shapiro 1991], eg by dextran sulphate. It is usually rapidly inactivated by kininase II, which is identical to angiotensin-converting enzyme (ACE). Severe hypotensive reactions have been documented during apheresis with dextran sulphate columns in patients on ACE inhibitors, although none of the subjects in the present study were prescribed such agents [Olbricht *et al* 1992], and brochospasm was not a feature in any of the episodes of collapse.

3.4 EFFECTS ON SUBJECTIVE RESPONSE

3.4.1 Rationale

While the main outcomes of these studies have relatively 'hard' end-points which are readily assessed by measurements which can be defined in terms of distributions, means and standard deviations, the effects of the interventions experienced by the patients are more difficult to appreciate, far less quantify. The physician's perception of the patient's 'well-being' has been shown to be poorly correlated with subjective reporting [Slevin *et al* 1988].

Measurement of the 'quality of life' has become an important issue with regard to the management of chronic illness, and most studies of oncology patients incorporate some assessment of the effects of intervention on overall well-being rather than simply side-effects of therapy. Other areas which have been studied include arthritis, chronic respiratory disease and cardiac disease [Guyatt *et al* 1986]. There is increasing recognition of the importance of measuring outcome in terms other than simply of survival [Wiklund *et al* 1989, Rogers *et al* 1990].

The subjects in this study were not only limited to a variable degree by ischaemic symptoms, but also were required to attend more frequently than usual, to take more medication (sometimes on a four-times daily regimen) and 50% had to attend for a half-day weekly for LDL-apheresis. There was thus considerable interference with our patients' usual activities, and it was felt desirable to measure their perception of any changes in their general well-being resulting from their participation.

There may in addition be risks other than the physical restrictions imposed by participation in the study. Previous cholesterol-lowering studies have noted a slight excess of deaths due to suicide in the treatment groups [Muldoon *et al* 1990]. A number of hypotheses have been proposed to account for this [Engelberg 1992, Law *et al* 1994], but in view of the possible causal relationship between perturbations of cholesterol homeostasis and psychological disturbance it was considered prudent to monitor the impact of such profound cholesterol reduction.

3.4.2 Choice of measurement tool

A number of quality-of-life instruments have been developed for population screening for psychological disturbance (such as the General Health Questionnaire), but these are unlikely to be sensitive to small, but clinically important, changes. Other questionnaires have been developed and validated for specific clinical settings, some of which have a narrow range of applicability [Guyatt *et al* 1986]. The Nottingham Health Profile (NHP) was chosen on the basis of its simplicity and its known acceptability and reliability [Hunt *et al* 1980]. It has been previously validated in patients with cardiac disease, and is regarded as an appropriate tool to evaluate the effects of interventions in a pre/post test design and to monitor changes over time in the subjective health of patients with chronic illness [O'Brien *et al* 1987], and gives more weight than other measurement tools to the infuence of symptoms on lifestyle [Taylor 1987].

The NHP is self-administered and consists of two parts: the first is made up of 38 simple queries to which a yes/no response is required; areas covered by the statements are sleep, energy, emotions, pain, social isolation and physical mobility. The second part assesses a number of aspects of everyday life which are affected by health problems, incorporating employment, jobs around the home, personal relationships at home, sex life, hobbies, holidays, and social life. The queries from each section in Part I are in random order on the questionnaire, and responses are weighted according to severity (calculated from responses by 1200 people aged 18 to 74 about the relative seriousness of the statements employed - using Thurstone's Method of Paired Comparisons [McKenna *et al* 1981]).

3.4.3 Baseline results

Initial assessments were carried out soon after commencement of the study. Median scores were higher in every category in the group 1 subjects (Table 3xxiii), and their overall restriction of activies (reflected by the total weighted score) was significantly greater ($P < 0.05$, Wilcoxon rank sums). The only single category in which the difference in scores was significant was that of 'pain' ($P < 0.01$).

TABLE3xxiii. BASELINE QUESTIONNAIRE RESULTS

PART I

	GROUP	MEAN	S.D.	MEDIAN	Q. 3	P (Wilcoxon)
ENERGY	1	26.16	27.62	24.00	63.20	N.S.
	2	17.60	31.90	0.00	27.80	
EMOTIONAL	1	9.36	9.95	8.49	18.33	N.S.
	2	6.98	14.98	0.00	10.46	
SLEEP	1	22.72	30.80	12.57	45.11	N.S.
	2	14.06	25.04	0.00	20.64	
SOCIAL	1	4.27	13.49	0.00	0.00	N.S.
	2	0.00	0.00	0.00	0.00	
PHYSICAL	1	13.04	16.02	5.39	24.62	N.S.
	2	1.08	3.41	0.00	0.00	
PAIN	1	23.36	23.10	17.05	32.80	< 0.01
	2	1.58	3.48	0.00	1.46	
TOTAL	1	98.90	83.40	70.20	154.8	< 0.05
	2	41.30	69.10	18.30	45.30	

The weighted scores for the responses to enquiries regarding pain were strongly correlated to the total scores ($R = 0.71$, $P = 0.001$). With the exception of one individual in group 1 who responded in the affirmative to all statements inquiring about pain, including "I'm in constant pain" (although was able to exercise for 15 minutes on the treadmill protocol), the weighted scores for pain were also correlated with exercise duration on the treadmill at baseline ($R = 0.5$, $P < 0.05$).

The patients completed in addition an abbreviated version of the General Health Questionnaire, 'GHQ 28' [Goldberg & Hillier 1979]. The total scores from this were strongly correlated with the NHP scores ($R = 0.86$, $P < 0.0001$), although failed to discriminate physical limitation as well as the latter.

Six of the patients in group 1 felt that their condition interfered with their employment, and an equal number felt similarly restricted in terms of pursuit of hobbies. Fewer of the group 2 patients felt limited in these domains by their

symptoms, but none of the differences between the groups in this part of the test were significant (Table 3xxiv).

TABLE3xxiv. BASELINE QUESTIONNAIRE RESULTS - PART II

	No. AFFECTED		
	GROUP 1	GROUP 2	P (Fisher's)
EMPLOYMENT	6	3	N.S.
JOBS IN HOME	1	1	N.S.
SOCIAL LIFE	4	1	N.S.
FAMILY RELATIONSHIPS	1	1	N.S.
SEX LIFE	4	3	N.S.
HOBBIES	6	2	N.S.
HOLIDAYS	3	1	N.S.

3.4.4 Effects of intervention

The NHP was completed by all the patients again immediately prior to the end of the intervention period. Compared to the baseline responses, there were significant changes in reporting of problems. Group 1 had fewer restrictions due to pain and physical limitation than previously (Table 3xxv), although the changes in total scores just failed to achieve statistical significance. There was no evidence for any change in psychosocial disturbance.

The change in scores for pain were compared with percent change in exercise duration. The correlation co-efficient was 0.19 (N.S.), but the predictive value of a reduction in pain score being associated with an improvement in exercise tolerance was 87.5%, the relative risk being 4.0. Sensitivity and specificity were 87.5% and 100% respectively, although test accuracy was only 53%.

The patients in the group treated by apheresis tended to feel less restricted in most areas of their lives than they had previously and the others more so, although the

differences were not sufficiently striking to achieve levels of significance statistically.

TABLE 3xxv. CHANGES IN NOTTINGHAM HEALTH PROFILE SCORES

	GROUP	MEAN	S.D.	MEDIAN	Q. 3	P (Wilcoxon)
ENERGY	1	-18.40	21.99	-24.00	0.00	N.S.
	2	-5.20	38.90	0.00	0.00	
EMOTIONAL	1	3.53	23.93	0.00	3.54	N.S.
	2	-0.69	22.56	0.00	1.77	
SLEEP	1	-5.75	28.82	0.00	8.05	N.S.
	2	1.75	12.02	0.00	12.57	
SOCIAL	1	7.10	21.30	0.00	0.00	N.S.
	2	5.73	12.49	0.00	5.50	
PHYSICAL	1	-6.10	5.81	-10.57	0.00	< 0.05
	2	3.09	8.55	0.00	10.79	
PAIN	1	-14.26	17.82	-8.06	0.00	< 0.05
	2	4.77	13.94	0.00	7.18	
TOTAL	1	-33.90	52.40	-35.20	9.1	N.S.
	2	9.50	52.40	1.50	16.20	

3.4.5 Discussion

The assessment of perception of changes in symptoms and their severity is not frequently part of clinical practice. The self-completion at different times of a questionnaire requiring some consideration of exactly which activities may be associated with difficulty and whether they are coping with any emotional or psychological stress should be considerably more reliable than a straight enquiry about whether the patient is feeling "better" or "worse". The use of the weighted scores for the responses should also give a more representative picture of their limitations than simply a diary of their use of GTN or frequency of angina attacks.

The difference in reported limitation by pain between the groups at the time of the initial questionnaire was not anticipated but since the scores were correlated to exercise duration, this is likely to be a chance difference between the groups at baseline rather than a spurious finding attributable to an insensitive device.

The most important analysis however is the difference in the degree and direction of change. In this case the results are entirely consistent with the objective data: the patients subjected to the more rigorous intervention experienced a significant decrease in the restriction of their activities, resulting in fewer physical limitations and less pain during their normal activities than they had earlier reported. The poor correlation between the change in scores and the relative improvement in objective measures of exercise tolerance is readily understood. The items pertaining to pain inquire predominantly about fairly major limitation, and are unlikely to be sensitive to relatively small changes. This is suggested by 7 of the 19 patients reporting no difference in scores for pain, although three of these had increases of at least 50% in exercise duration. An additional series of questions specific to patients with chronic stable angina would undoubtedly improve the ability of the inquiry tool to detect changes of smaller magnitude, but would require rigorous testing of reliability and validity in suitable populations.

The findings of high specificity and sensitivity of the changes in weighted scores to pain to predict changes in exercise tolerance do not imply that questionnaires should be used as a surrogate for objective assessment. It does however demonstrate that the improvement in reported symptoms was due to demonstrable changes in the patients' performance (and conversely that the NHP was measuring what it purported to be).

The absence of evidence of any systematic change in emotional or psychological distress is of note. Although the number of subjects in the present study is very small, the changes in the serum lipids were profound and the period of follow-up of sufficient duration to give some small reassurance. It was observed that the responses were successful in identifying the individuals who had recently been bereaved or were experiencing significant marital disharmony. However it could be argued in the same way as for the pain scores that the NHP lacks sufficient discrimation to detect subtle psychological changes which may nonetheless be of clinical relevance.

The results support the contention that, despite major disruption of the patients' normal activities, there were no untoward effects on the quality of life. In particular there were no problems experienced in the incorporation of regular extracorporeal

therapy into the treatment regimen, and that marked alterations in serum lipids were not accompanied by significant psychological disturbance.

3.5 EXERCISE TESTING

3.5.1 Methods

The subjects were exercised on each occasion using a standard protocol, the Sheffield modification of the Bruce protocol, which entails increases of speed and/or gradient at intervals of three minutes. The procedures were performed to a symptom-limited maximum up to the end of stage 6 (13 METS*) of this protocol. The subjects were exercised in the morning either fasting or after a very light breakfast, without routine omission of their usual anti-anginal medication. The tests were performed on each occasion in the same room - and therefore with little change between tests in ambient temperature. Due to technical failure the treadmill employed for the tests was changed during the course of the study, but these were both maintained and calibrated on a regular basis by hospital physicists. The time to onset of symptoms was recorded, together with total exercise duration. Blood pressure was measured manually before exercise, towards the end of each three-minute stage, and at termination. A 12-lead ECG with the Mason-Likar modification was recorded standing before exercise, at the end of each stage, at maximal exercise, immediately on cessation of the test and at one-minute intervals thereafter until recovery of the exercise-induced changes.

The protocol was performed at baseline and repeated at 6-month intervals to completion of the study in an identical fashion, the technician and the attending doctor being blinded to the results of the previous tests.

The analysis was performed blinded to patient identity and sequence. The ST-segment was measured manually at 80 msec after the J-point. The 'maximum ST-depression' was calculated by aggregating the change from the resting recording in the displacement of the ST-segment from the isoelectric line in each of six leads: II, III, aVF, V4, V5 and V6. The ST depression at the 'maximal comparable workload' was calculated in an identical fashion, using the recordings at the lowest workload achieved at any of the time-points and comparing ECG changes obtained on each occasion at this level of exercise.

[* Note: 1 MET is equivalent to 3.5ml/min/kg body weight, the resting respiratory oxygen uptake for a 70kg man aged 40.]

Anti-anginal therapy was unchanged throughout the period of intervention, with three exceptions:

#05 Isosorbide mononitrate was increased from 20mg b.d. to 40 mg b.d. for a period of 5.5 months between the baseline test and the 6-month test. The dose at the time of each of the procedures, however, was constant.

#07 Atenolol 100 mg was added six weeks before the final exercise test. This was discontinued for three days before the test was performed.

#11 Adalat retard 20mg b.d. was being taken for each of the first three tests; this was discontinued (because of side-effect of headache) at the time of the 18-month examination, when he was receiving no anti-anginal therapy. Atenolol was prescribed (because of hypertension) following the fourth test, but was discontinued for 24 hours before the final exercise test. Rate-pressure product (see below) for this individual was only slightly altered, being 19.2 bpm.mmHg/10^3 and 18.0 at baseline and on study completion respectively.

3.5.2 Baseline Results

The exercise tests were performed after allocation to the treatment groups.The mean exercise time in group 1 was 10.3 minutes (SD 4.2), exercise being discontinued because of chest pain in 8, dyspnoea in 1, while the other patient managed to complete 18 minutes of the protocol and was stopped electively. Time to onset of symptoms was 6.5 minutes on average, and mean time to 1mm ST-depression (in any single lead) was 10.3 minutes. Maximum (aggregate) ST depression averaged 6.44mm (SD 3.6), including one subject with left bundle branch block.

There were no significant differences between the groups at baseline in any of the parameters measured above. Mean exercise duration in group 2 was 11.85 minutes (SD 4.2). Time to onset of symptoms and time to 1mm ST-depression were 7.3 minutes and 9.2 minutes respectively. ST segment changes were evaluated in all subjects, although 1 had left bundle branch block and another developed ST-elevation in response to exercise; the average maximum ST depression was 3.45mm (SD 3.5).

The principal reason for stopping the test was chest pain in only 3 subjects; 2 stopped with dyspnoea, 3 developed pain from arthritis or claudication before the onset of chest pain, 1 was stopped because of the development of severe ST depression, and the other reached the end of the prescribed sixth stage. Although three subjects in this group were limited more by locomotor or peripheral vascular causes, this did not adversely affect the mean exercise time of the group, since their exercise duration averaged 12.5 minutes; one of these subjects had bundle branch block, but the other two both showed evidence of ST-segment depression.

3.5.3 Effects of intervention

Patients treated by regular apheresis experienced an early benefit in exercise tolerance, and this was significantly different (using paired T-test) from baseline by six months (Fig 3-34). The improvement continued in this group up to 18 months, before levelling off. The average percentage increase in exercise duration in this group at completion of the study was 77% over baseline, a mean increase in exercise duration of 5.31 (SD 3.09) minutes ($P < 0.001$). There was no change in maximum ST-depression, and the rate-pressure product was also unchanged (Table 3xxvi). Time to 1mm ST-depression was increased, as was time to onset of symptoms. ST-depression at maximum comparable workload decreased from 5.5 (SD 4.1) mm to 2.1 (SD 3.4) mm ($p < 0.05$).

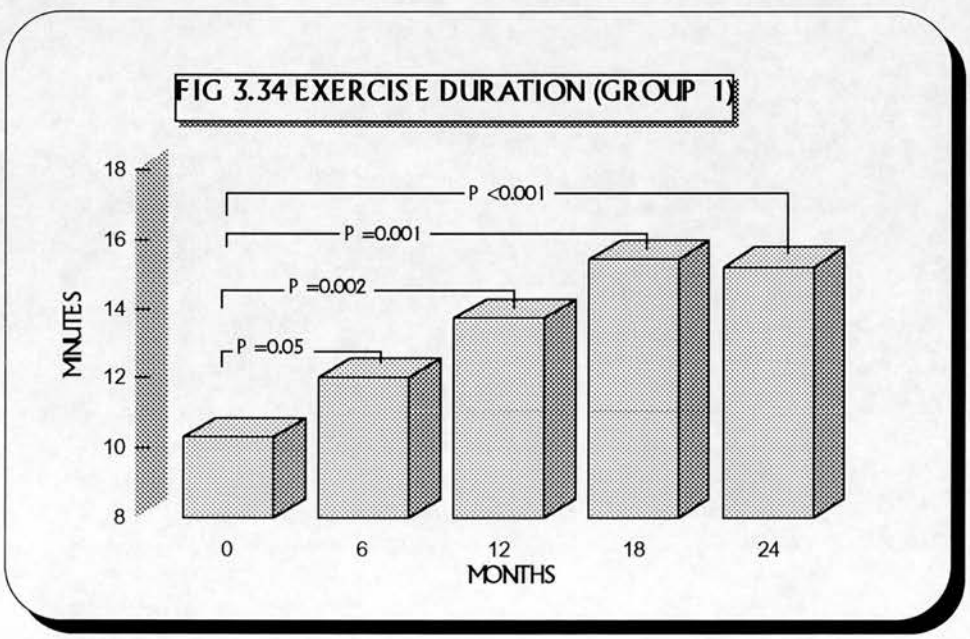


FIG 3.35 EXERCISE DURATION (GROUP 2)

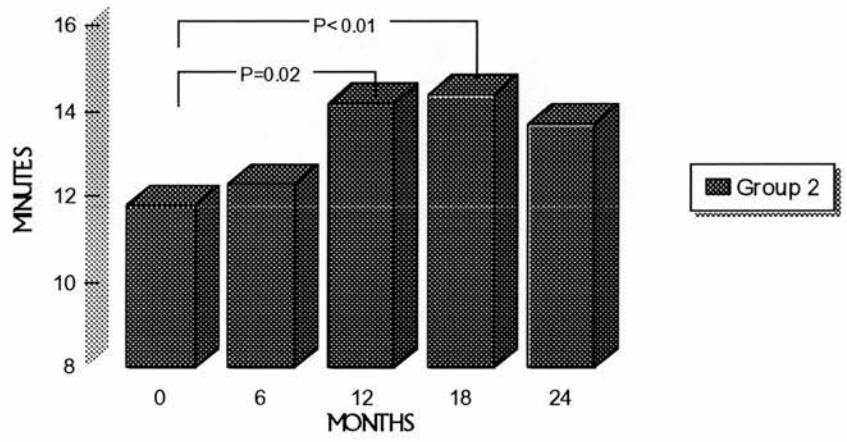
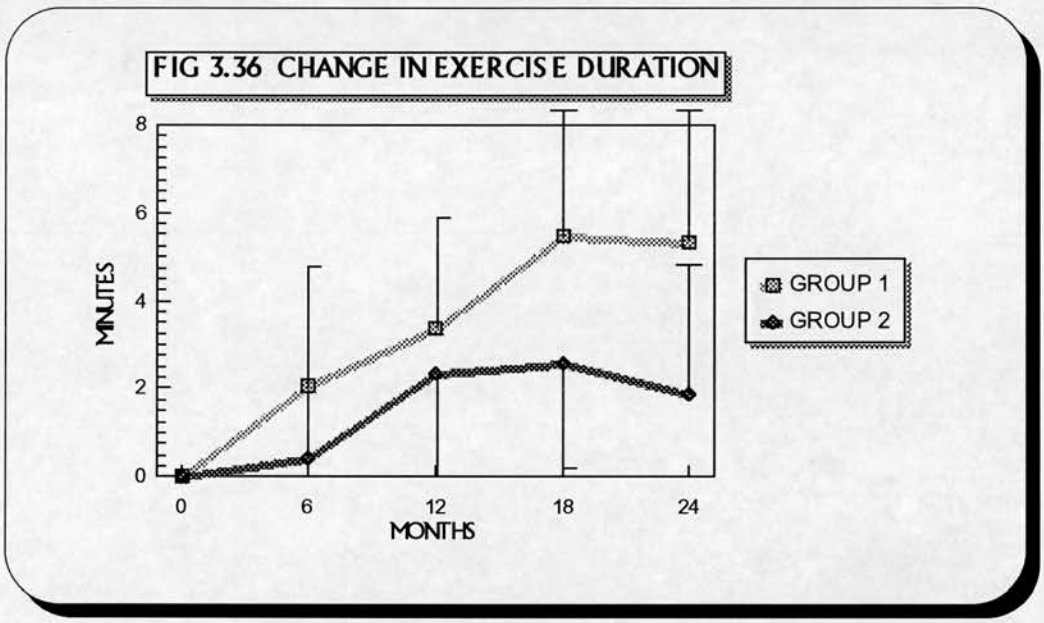


TABLE 3xxvi. CHANGES IN EXERCISE PARAMETERS

	Exercise duration	Onset of symptoms	Time to 1mm ST-dep	Max ST dep (1 lead)	Double product	Tot ST-dep @peak ex	ST-dep @max comp work
	(mins)	*(mins)*	*(mins)*	*(mm)*	*bpm.mmHg/E-3*	*(mm)*	*(mm)*
Group 1							
Baseline	10.3 (4.5)	6.5 (3.4)	9.8 (5.3)	1.78 (1.1)	19.7 (6.4)	6.4 (3.6)	5.5 (4.1)
6 mths	12.0 (4.0)	6.8 (1.8)	10.9 (5.2)	1.44 (1.3)	19.7 (4.8)	5.4 (4.1)	3.2 (4.7)
12 mths	13.7 (3.0)	11.1 (3.1	10.3 (3.6)	1.78 (1.1)	20.0 (6.0)	6.9 (4.4)	4.6 (4.0)
18 mths	15.4 (2.6)	12.5 (2.0	13.6 (3.9)	1.50 (0.9)	20.2 (6.4)	6.9 (5.0)	2.6 (3.6)
24 mths	15.2 (2.8)	12.0 (3.6	12.5 (4.4)	1.72 (0.8)	19.9 (5.6)	6.8 (4.0)	2.1 (3.4)
Group 2							
Baseline	11.8 (4.5)	-	9.2 (4.5)	1.20 (1.2)	14.8 (3.5)	3.4 (3.5)	3.0 (3.3)
6 mths	12.3 (3.6)	7.3 (1.7)	10.5 (3.3)	1.20 (1.3)	16.3 (3.9)	4.0 (4.0)	2.9 (3.8)
12 mths	14.2 (2.8)	10.5 (2.5	10.3 (4.5)	1.45 (1.5)	15.9 (4.4)	3.6 (4.2)	1.4 (2.6)
18 mths	14.4 (2.9)	9.3 (2.2)	11.2 (1.9)	1.70 (1.4)	15.6 (3.1)	4.8 (5.0)	1.7 (2.2)
24 mths	13.7 (2.8)	9.5 (2.3)	9.7 (3.1)	1.70 (1.8)	14.4 (2.6)	5.6 (7.2)	3.2 (4.8)

In Group 2 there were also improvements in exercise duration at each time-point compared to baseline, although these were more modest, and were significant only at 12 and 18 months (Fig 3.35). Mean percentage increase in exercise time at 24 months was 31%, the average increase 1.9 (SD 3.0) minutes (P = 0.078). There was a non-significant increase in maximum ST-depression from 3.45 (SD 3.5) mm to 5.6 (SD 7.2) mm, and no significant alteration of the double product (Table 3xxvi).

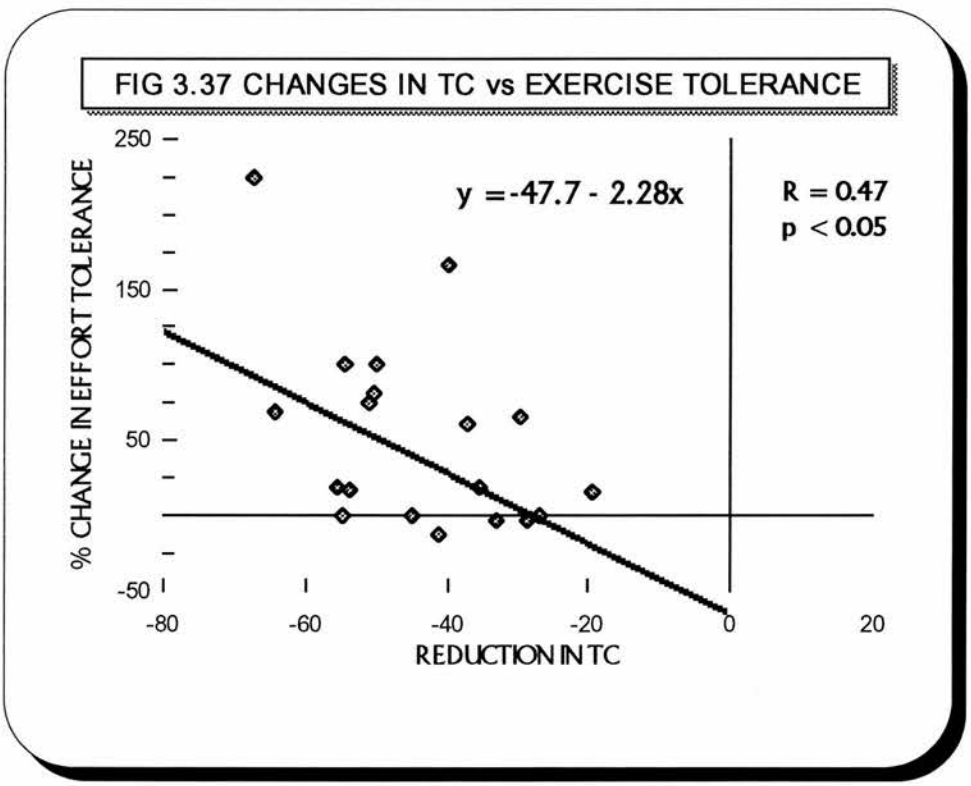


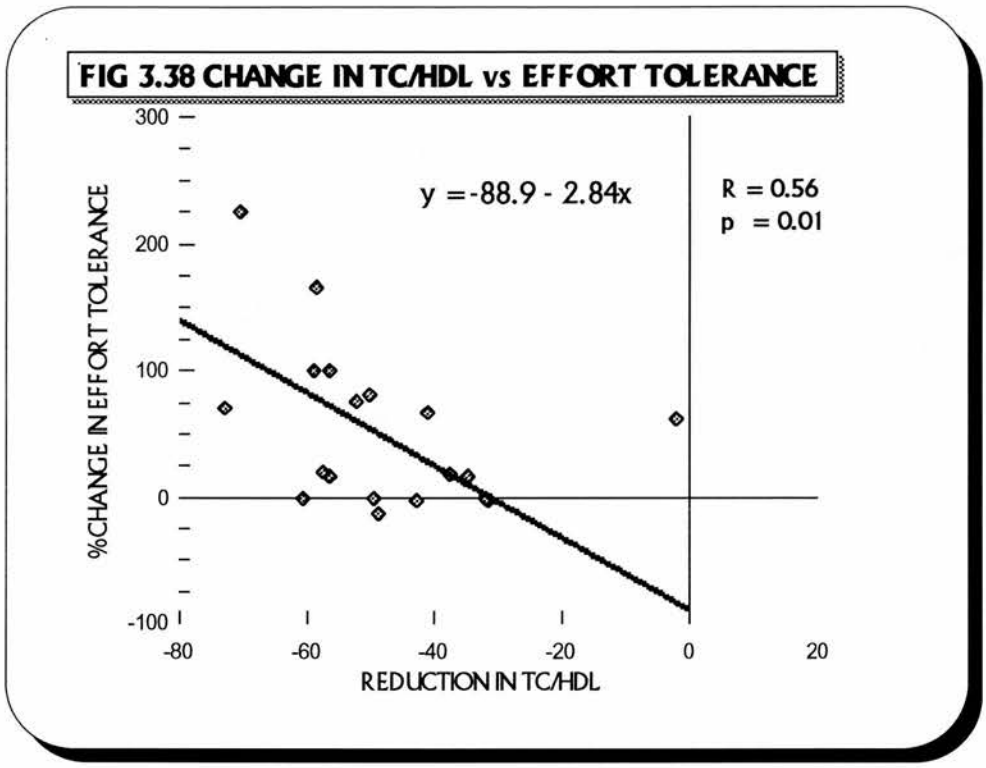
ST depression at maximum comparable workload was unchanged: 3.0 (SD 3.3) mm at baseline, and 3.2 (SD 4.8) mm at completion. The difference between the groups in changes in ST-depression at maximum comparable workloads was statistically significant (P = 0.05).

Eight of the nine patients in Group 1 to complete the study were limited by some manifestation of myocardial ischaemia (chest/arm pain, dyspnoea, fall in systolic blood pressure, or development of 5mm ST-depression) at the initial exercise test, while the other completed the full protocol with evidence of silent ischaemia. At the final test, only four continued to be limited by ischaemia; two others were able to complete the protocol, while the other two were limited by fatigue. In contrast, all five of the patients initially limited by ischaemia in group 2 continued to be so limited after two years, and two of the remaining five who had been stopped

previously by fatigue or other non-cardiac endpoint now experienced symptomatic ischaemia. Fig 3.36 clearly shows the differences in improvement in exercise tolerance between the treatment groups. This was significant at the 95% level at 24 months (Wilcoxon sum-ranks test).

When the two groups are pooled to examine the determinants of improvement in exercise tolerance there was a weak, but significant, correlation between the mean individual reduction in total cholesterol achieved and the percentage improvement in exercise duration (R = 0.47, P = 0.041) (Fig 3.37). This correlation was improved when the TC/HDL ratio was used rather than total cholesterol, the correlation coefficient in this case being 0.56 (P = 0.014) (Fig 3.38). The effects of blood viscosity changes were also examined: alterations of whole blood viscosity accounted for less than 20% of the variability in changes in exercise tolerance, and did not improve the fit of the regression model on multivariate analysis.





The best predictor of the change in exercise tolerance with treatment was the baseline exercise duration. There was a highly significant negative correlation between the duration of effort tolerance at baseline and percentage change during the study ($R = -0.84$, $P < 0.001$). The fit of the regression line was further improved by the addition of the TC/HDL ratio changes, the equation obtained,

% change in exercise duration =

74.6 - 10.5 (baseline ex duration) + 1.86 (% change TC/HDL)

accounting for 83% of the changes in exercise tolerance.

3.5.4 Discussion

The graded treadmill exercise test affords a means of testing objectively the effort tolerance in a physiological manner to which the subjects are accustomed. It is less likely to be limited by poor physical conditioning than is bicycle ergometry and the metabolic work required for any given workload is less dependent on body

weight, although the quality of the ECG recording may be impaired due to increased motion artefact. The results for an individual are quite reproducible over time, providing variables such as ambient room temperature, time of day, time interval from last meal, and medication and time interval from last administration are constant. Its use in evaluation of functional capacity in patients with coronary disease subjected to alteration in cardiovascular therapy is well-accepted [Schlant *et al* 1986].

Baseline results showed a small, non-significant difference in exercise tolerance between the groups. This is in accord with the greater limitation by pain that subjects in this group described (see Sect **3.4.3**), and also with the greater degree of ST depression at maximum workload. The double-product (DP) is an index of the myocardial perfusion requirement and characterizes cardiovascular performance. Thus the increase in exercise time without any change in DP or ST-depression at maximal exercise suggests that the coronary blood flow is increased (since oxygen extraction by the myocardium is already optimal); this is supported further by the reduction in ST-depression at maximum comparable workload. The subjects in Group 2 achieved their maximal exercise at a lower DP than those in Group 1, although the difference between the groups at baseline just failed to achieve significance ($P = 0.061$). The difference may be explained either in terms of greater severity of coronary disease in Group 2, or may be due to differences in drug therapy affecting response to exercise. It may be seen from Fig 2.1 that the use of anti-anginal drugs were dissimilar: only six of the apheresis patients were prescribed a beta-blocker at the outset compared to 8 subjects in group 2. The differences in use of calcium-antagonists and long-acting nitrates are even more striking, with a significantly greater number of subjects in group 1 taking each of these classes of agents (eg. only one subject in group 2 was prescribed an oral nitrate preparation compared to 8 in the apheresis group, $P = 0.006$ by Fisher's exact test). The differences in drug prescription were not attributable to ejection fraction, since there were no differences between the groups in this regard. Analysis of the initial coronary angiograms show there was no difference in the extent or severity of disease between the groups (see **3.7.2**).

Although the groups were not well-matched for concurrent drug therapy, the anti-anginal treatment was constant for all the subjects (with the exceptions

outlined in **3.5.1**) and comparison of the exercise tests appears to be valid with respect to the effects of the lipid-lowering intervention, since each individual acts as his own control.

The effects on exercise tolerance were dramatic: considering the two groups in combination, the average increase from baseline was 3.5 minutes (SD 3.4), $P < 0.001$ (paired t-test). This compares favourably with the effects of other interventions in coronary disease including coronary artery bypass surgery and coronary angioplasty, when an identical exercise protocol was repeated after the same time interval [RITA Trial 1993]. Large-scale primary and secondary prevention studies [Lipid Research Clinics Program 1984b, Buchwald *et al* 1990] have described a reduction in new positive exercise tests, and lipid-lowering therapies have been shown to improve haemorheology and blood flow in resistance vessels [Rubba *et al* 1990] by reducing the impaired endothelium-dependent vasodilation seen in hypercholesterolaemia [Leung *et al* 1993, Harrison *et al* 1987, Creager *et al* 1990, Chowienczyk *et al* 1992]. The documentation of such marked improvements in exercise tolerance by lowering cholesterol in subjects with coronary disease has not been described previously, although Brown and colleagues demonstrated a significant relationship between the degree of lipid reduction and the likelihood of disease regression [Brown *et al* 1990].

The degree of change in objective measurement of exercise capacity following prolonged treatment is striking in the apheresis group, and the differences from the baseline examination are highly significant. Although the 'p' value for the differences between the groups is considerably smaller (Fig 3-36), this is probably of greater consequence, as the alternative treatment strategy has been demonstrated to yield a significantly better outcome in this regard despite the small numbers and the unfavourable differences at baseline. The clinical importance of such variation in improvements is clearly demonstrated by the limiting symptoms on exercise, half of those in group 1 no longer having limiting symptomatic ischaemia while more subjects in group 2 were limited by angina on completion of the study than at baseline.

The strong negative correlation between baseline exercise tolerance and percentage change during treatment is unsurprising: individuals with 'normal'

exercise capacity can hardly improve on that, while the scope for improvement remains greatest for those who are most limited. Furthermore, the expression of any change as a percentage of the initial test duration will inevitably amplify the apparent benefit to those in this latter group. Notably however, the changes in lipid levels remained significantly correlated with improvements in exercise tolerance even after correction for baseline test duration.

The finding of a significant correlation between the degree of reduction in total cholesterol and improvement in exercise tolerance is an important one. There is now a large body of evidence that the reduction in 'coronary risk' is proportional to the extent of reduction in cholesterol levels, and the angiographic trials appears to support the suggestion that more profound alterations in lipid levels may be associated with a greater likelihood of regression. The relationship between cholesterol reduction and cardiac symptoms on an individual basis however has not been well-established.

The TC/HDL ratio has been demonstrated to be better correlated than total cholesterol with the occurrence of disease in epidemiological studies [Gouldbourt *et al* 1985, Assman & Schulte 1992] and a more specific marker than total cholesterol for progression/regression in intervention studies [Brensike *et al* 1984]. The finding in the present study that the changes in TC/HDL are also correlated with improvement in exercise tolerance is therefore consistent.

While the total exercise test duration is the most readily measured variable, the other parameters monitored (see Table 3xxvi) yield results which are wholly consistent. Time to onset of symptoms and to 1mm ST depression both increase to a greater extent in group 1. The rate-pressure product is fairly constant within each group, with consistently lower results for group 2, presumably reflecting the greater proportion of patients on ß-antagonists. The aggregate ST-depression at peak workload is unchanged in group 1 over the duration of the study, while the increase in values for this variable for the group 2 subjects suggest the increase in exercise time may be bought only at the expense of increased ischaemia and this would be consonant with the increase in maximum ST depression in any single lead in this group. Further support for a reduction in the degree of ischaemia is

obtained from the trends in the ST segment changes at maximum comparable workloads, which is significant for the apheresis-treated group only.

3.6 RADIONUCLEIDE IMAGING

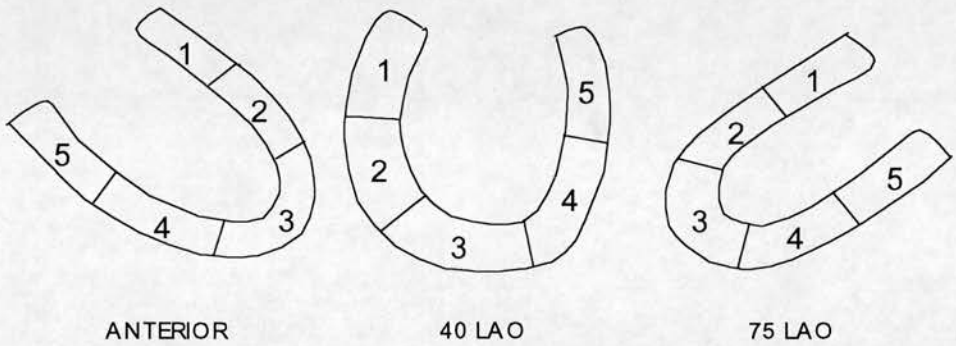
3.6.1 Methods

Thallium scintigraphy was performed after exercise on a bicycle ergometer at baseline, at one year, and on study completion. The procedure was performed in the morning on each occasion without witholding the subject's usual medication. The same equipment, including mechanically-braked bicycle which was calibrated at regular intervals, was employed for the duration of the study. A standard protocol was utilised starting at 50W with 50W increments at 3-minute intervals to a symptom-limited maximum [Martin *et al* 1987]. The patients were required to pedal at 50 rpm, and the test discontinued if they developed limiting pain or dyspnoea or if they were unable to maintain a cycling frequency above 40 rpm. A 12-lead electrocardiogram using the Mason-Likar modified limb-lead placement was obtained before exercise, at the end of each stage, at termination of the test and at one-minute intervals thereafter. A continuous ECG recording was obtained throughout exercise on a six-channel Siemens recorder. 60 MBq of thallium-201 was injected at peak exercise via a previously-sited intravenous cannula, and standard views obtained using a high sensitivity parallel collimator in the anterior, 40°- and 75°-left anterior oblique (LAO) projections using a mobile gamma camera (GE Portacamera IIC). A 25 keV photopeak centred on thallium's 80 keV X-ray emission peak was employed. Data gated to the electrocardiogram were acquired and stored on computer (LINK MAPS 2000). The patient was then rested for 3-4 hours and redistribution scans obtained in identical projections. The stored images were retrospectively reconstructed into representative cardiac cycles of eight frames in each projection, using an image size of 64 x 64, and subsequently analysed independently on study completion by two observers blinded to patient identity and scan sequence.

Each projection was divided into five equal segments (Fig 3.39) and, after normalisation of the linear-gray scale to the "hottest" pixel, the segments graded visually for perfusion: < 10%, 10-34%, 35-65%, 66-90% and > 90%. Segments were deemed to have a reversible perfusion defect if perfusion was impaired on the stress scan and normal during the re-distribution images, and a partially reversible perfusion defect if the segment was abnormal on exercise and improved

by at least one category on the reperfusion scan. Abnormal segments were classified as having 'fixed' defects if abnormal on exercise without changes at the redistribution scan, and 'reverse reperfusion' defects if the latter scan had diminished perfusion compared to the exercise image.

FIG 3.39 PROJECTIONS AND SEGMENTS FOR RADIONUCLEIDE SCANNING



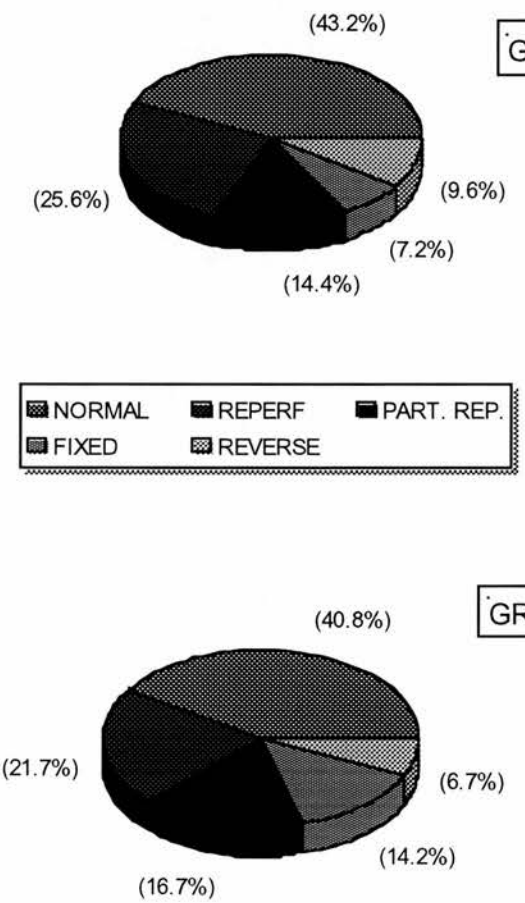
At the time of the redistribution thallium scan, patients were administered stannous pyrophosphate for in-vivo labelling with 600 MBq technetium 99-m pertechnetate which was injected 20 minutes later. A gated equilibrium blood pool ventriculogram was obtained using a single-plane collimator. Images were obtained in the 40° and 70° left anterior oblique views, and from these were derived ejection fractions for each ventricle, as well as total and segmental wall motion scores. Segments were scored 1-5, according to whether the motion was normal, slightly reduced, markedly reduced, immobile, or dyskinetic. Ejection fractions were calculated from a mean of four estimates measured in two planes.

The scores accorded by the individual observers (GWT/WM) for both perfusion and wall motion were well-correlated, with greater than 90% identical, and the remainder varying by no more than one group. Intra-observer variability of assessment of the area shaded on the perfusion scans was also examined: the identical score was assigned in 92% of segments, with less than 1% varying by more than one group.

3.6.2 Baseline Results

Of the nine patients to complete the study in group 1, the stress images obtained in the anterior and 70° LAO views for one subject (#04) were not traced in the computer storage system. There were five segments in each of three views for the remaining eight, and thus a total of 125 segments could be analysed. A redistribution scan was not performed on one (#14) of the group 2 patients, and in another (#19) no computer record of the redistribution scan was found. Records were complete for the remaining patients, giving 120 segments in total for this group.

FIG 3.40 BASELINE RESULTS FOR THALLIUM SCAN



There were 50 segments with reversible perfusion defects at baseline in group 1, 18 of which were partially reversible only; there were 9 fixed defects, 12 segments with reversed reperfusion, and 54 with normal perfusion on stress and at rest. The subjects in group 2 had 46 segments with reversible perfusion defects (20 only partially reversible); 17 abnormalities were fixed, eight reversed, and 49 segments were normal.

Left ventricular ejection fraction (LVEF) calculated from the blood pool studies showed a wide inter-subject variation with a range of 15 to 45%, and mean 29.7% (SEM 1.69). The groups were well-matched in this regard with no significant differences, neither were there any systematic differences between the groups in localised wall motion abnormalities. An overall wall motion score (WMS) was derived from the summation of the segmental scores assigned from the two LAO views, and this was highly significantly correlated with the LVEF ($R=0.83$, $P < 0.0001$).

Limited information was obtained from the exercise data, since only five of the patients were limited by symptoms of ischaemia and the remainder of the tests were discontinued due to fatigue. Total exercise duration on the protocol was recorded, together with time to 1mm ST-depression, maximum ST-depression, and rate-pressure product; there were no differences between the groups in any of the parameters recorded.

TAB 3xxvii EXERCISE PARAMETERS ON BICYCLE ERGOMETER

	EX TIME (mins)	TIME TO 1mm ST DEP (mins)	MAX ST DEP 1mm (mm)	DP bpm.mmHg /1000
GROUP 1				
BASELINE	6.0 (1.1)	4.7 (2.2)	1.1 (1.2)	18.3 (7.6)
12 MONTHS	5.6 (1.0)	4.1 (1.7) *	1.9 (1.6)	19.55 (6.2)
24 MONTHS	5.6 (0.7)	4.5 (0.8)	1.0 (0.9)	19.0 (7.3)
GROUP 2				
BASELINE	5.7 (1.1)	4.5 (1.3)	1.5 (1.3)	15.7 (4.0)
12 MONTHS	4.9 (1.1) *	4.2 (1.0)	1.6 (1.6)	15.3 (4.1)
24 MONTHS	5.2 (1.2)	3.6 (1.1)	1.4 (1.5)	14.9 (3.2)

* = $P < 0.05$, vs baseline

FIG 3.41 2-YR SCAN RESULTS BY BASELINE SCAN

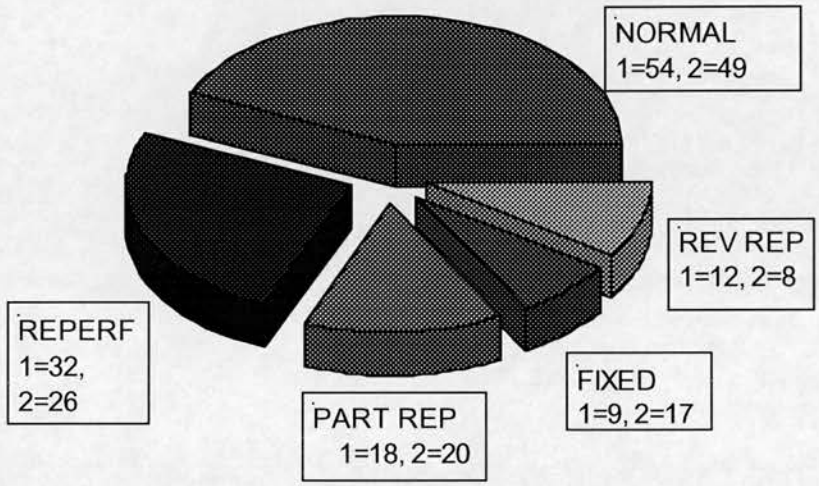


TABLE 3xxviii

		REPEAT SCAN					
		NORMAL	REPERF +/ -	PART. REP. +/ -	FIXED	REVERSE	TOTA
BASELINE SCAN							
NORMAL	GROUP 1	41	9	2	0	2	54
	GROUP 2	32	9	2	1	5	49
REPERF	GROUP 1	8	10 /6	4	1	3	32
	GROUP 2	10	9 /2	1	0	4	26
PART. REP.	GROUP 1	1	6	3 /1	3	4	18
	GROUP 2	4	3	3 /3	3	4	20
FIXED	GROUP 1	2	3	3	1	0	9
	GROUP 2	1	3	6	6	1	17
REVERSE	GROUP 1	3	4	1	4	0	12
	GROUP 2	3	1	0	1	3	8

3.6.3 Effects of intervention

Of the 32 segments with completely reversible perfusion defects at baseline in group 1, 8 were normal at the two-year scan, 1 had developed a fixed defect, 3 reverse reperfusion and 4 were now only partially reversible. 16 remained fully reversible: 10 had better perfusion on exercise at the repeat scan than at baseline,

while the others were worse or unchanged (see Table 3xxviii). 18 segments from patients in this group had had partially reversible perfusion defects at baseline; only 1 of these was normal at the final scan, and 6 had become completely reversible. 3 were either less ischaemic on exercise or were more reversible than previously, while one was worse. The remainder had developed either fixed or reversed reperfusion defects.

Similar results were seen in Group 2, with more than 50% of completely reversible lesions at baseline becoming normal or having improved perfusion on exercise, while the majority of partially reversible deficits remained incompletely reversible or developed fixed or reverse lesions (see Table). The proportion of segments showing improvement was not significantly different between the treatment groups. New reversible perfusion defects arose in 11 previously normal segments in each group.

A scoring system was devised to assess semi-quantitatively the changes in perfusion seen in individual segments from the baseline scan to that at completion, in an attempt to predict arteriographic changes in the coronary arteries supplying these territories. Each segment was assigned a value according to the degree of reduced perfusion at exercise and the extent of reversibility at both time points and the change assigned a value on a scale of -4 to +4; segments which were completely normal at baseline and developed a perfusion defect obtained a negative score, while those with reduced uptake initially and subsequently improved or normalised were accorded a positive score commensurate with the degree of improvement. The scoring system used for each permutation is documented in Appendix I. The scores were noted for each segment by group, and also aggregated for each individual:

TAB 3xxix THALLIUM SCORE - BY SEGMENT

	BETTER	WORSE	TOTAL
GROUP 1	44	29	+15
GROUP 2	48	25	+23

TAB 3xxx THALLIUM SCORE - BY SUBJECT

	BETTER	UNCHANGED	WORSE
GROUP 1	7	0	2
GROUP 2	5	1	2

LV ejection fraction did not change significantly in either group, although small increases were observed (30.3% (SEM 2.3) to 32.2% (3.7) in group 1, and 29.0% (3.0) to 30.4% (3.5) for group 2). Wall motion of individual segments did show minor changes but there were no systematic differences.

Exercise duration assessed by bicycle ergometer did not alter significantly in either group over the study, although there was a significant reduction in exercise time in group 1 at 12 months; this was not due to ischaemic symptoms, but there was a significant increase in maximum ST-depression noted at this point. At two years however, neither group showed any significant change in exercise duration, time to 1mm ST-depression, maximum ST-depression or rate-pressure product (Table 3xxvii).

3.6.4 Discussion

The purpose in obtaining this non-invasive imaging data was to evaluate the usefulness of perfusion scans in reliably assessing changes in blood flow consequent on regression of coronary artery lesions. It is recognised that thallium scintigraphy has greater sensitivity in the detection of coronary disease than electrocardiographic data alone obtained by exercise stress testing [Kotler & Diamond 1990], although the improvement in predictive accuracy compared to ECG-stress testing is small in patients not taking digoxin and with a normal resting ECG [Gibbons *et al* 1990]. It can be used to assess alterations in perfusion defects following interventions to achieve revascularisation (such as PTCA and

coronary bypass surgery) [Kiat *et al* 1988], but it was not known whether it might be sufficiently sensitive to detect changes in perfusion of a much smaller magnitude.

The data obtained confirms that for the duration of the study there was no new silent infarction in any of the participants. Both groups however had an approximately equal number of segments showing evidence of improved and diminished perfusion. Weighted (according to the extent of changes in perfusion) and unweighted scores showed no significant changes between the groups in either the proportion of subjects experiencing net benefit, or the number of segments per group demonstrating improvement (see Tables 3xxix and 3xxx).

Since there is a degree of subjectivity in the assessment of perfusion of each segment and some variation in adjustment to the visual display from the recorded data, quantifying the difference in reversibility between two pairs of scans will greatly amplify any systematic errors. Devising an arbitrary score to compare - what some see as - two other rather subjective assessments may be criticised as intrinsically flawed. However the intra-observer (GWT) and inter-observer variation (WM/GWT) in assessing perfusion was remarkably consistent and of sufficiently small magnitude to allow acceptably reproducible scoring. If the evaluation of the change in reversibility by the scoring system adopted is shown to be correlated either with angiographic change or some measure of clinical outcome, then it could be justifiably used as a useful surrogate for invasive assessment and I shall examine this following discussion of the angiographic results (Sect **3.7.4**, p 212).

Despite the lack of difference between the groups, it may be seen from the table that, of the 17 patients for whom the scans were analysed, 12 had an overall improvement in perfusion. Although it is possible that improved myocardial perfusion may have contributed to the benefits observed in exercise tolerance as exhibited in the serial treadmill tests, individual changes in thallium scores were not correlated with alterations in symptomatic status or exercise tolerance. This may be due to insufficient sensitivity of this approach to small changes in perfusion; however exercise time may remain unchanged even with marked improvements to all myocardial segments except one with the culprit lesion.

There were no significant correlations between the scores obtained and any of the lipid parameters, although a weak trend was evident when plotted against change in haematocrit ($R = 0.45$, $P = 0.084$).

3.7 CORONARY ANGIOGRAPHY

3.7.1 Methods

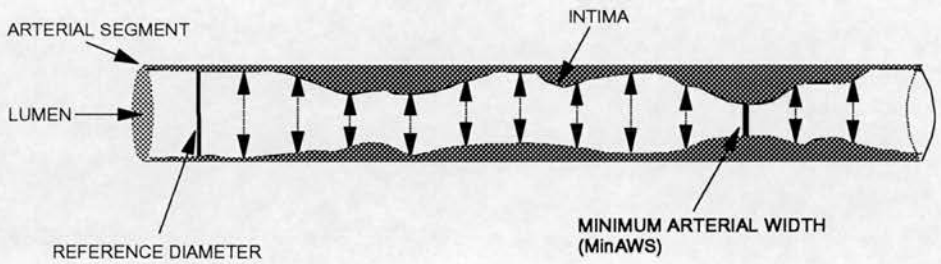
Coronary angiography was performed at the outset of the study and on completion in all the patients by a modified Judkin's technique under local anaesthesia. Patients were admitted on the evening prior to the procedure, or fasting on the morning of the angiogram. Patients were premedicated with 10mg oral diazepam before angiography, and received also their usual medication. Written informed consent was obtained before each investigation.

Biplane ventriculography was followed by selective coronary catheterisation using marked catheters (Cordis 7F, Miami, Florida, USA). Vasodilation was achieved by sublingual or intracoronary nitrates or intracoronary nifedipine according to the routine practice of the angiographer. If further doses were required during the procedure the dose and timing of administration was documented, and this was repeated at subsequent angiography at the same stage of the procedure. At least two orthogonal views were obtained of each coronary artery. The procedure was repeated after two years of intervention by the same angiographer using the same radiographic equipment. The views were replicated and performed in the same order as in the initial angiogram.

The films had all patient identifiers removed and replaced by a code number known only to non-study personnel. Film pairs were analysed blindly with a computerised method (CAAS system) by a trained technician [Reiber *et al* 1984]. The paired films were first viewed simultaneously by an experienced cardiologist who visually compared coronary segments to ensure adequate quality of angiography, matching of angulation, absence of foreshortening, and vessel overlap. Each segment [Austen *et al* 1975] suitable for analysis then had the edges detected by an automated method and the mean width of the segment overall measured from each film. The minimum arterial width and percent diameter stenosis was measured in both films for those segments in which there was a reduction of at least 20% of the reference diameter for part of its length in either film (Fig 3.42). The focal score and global score was calculated for each patient,

the former being the average of all minimum lumen diameters per patient, and the latter representing the average of all mean widths per patient.

FIG 3.42 ANGIOGRAPHIC MEASUREMENTS



MEAN ARTERIAL WIDTH OF SEGMENT (MAWS) = AVERAGE OF ALL DIAMETERS

% DIAMETER STENOSIS = MINIMUM ARTERIAL WIDTH / REFERENCE DIAMETER

FOCAL SCORE = AVERAGE OF ALL MinAWS PER PATIENT

GLOBAL SCORE = AVERAGE OF ALL MAWS PER PATIENT

3.7.2 Baseline results

133 segments in total were suitable for computer-assisted evaluation from the film pairs, 59 from group 1 with a mean segment width at baseline angiography of 2.47 mm (SD 0.71) and 74 from group 2, mean 2.49 mm (SD 0.82). Average minimal luminal diameter was 1.64 mm (SD 0.57) and 1.59 mm (SD 0.55) respectively, while percent diameter stenosis for the initial lesions ranged in group 1 from 20% to 64% with a mean of 34.4% (SD 11.1) and in group 2 from 12% to 67%, mean 35.8% (SD 13.7) (Table 3xxxi).

The focal score (the average for all minimum lumen diameters per patient) and global score (the average of all the mean arterial widths per patient) at baseline was strongly correlated with total cholesterol, LDL cholesterol, and TC/HDL ratio (Table 3xxxii). There was no significant correlation between the severity of angiographic disease and age in this selected population, nor between the extent of disease and anti-anginal therapy or haemorheological variables.

TABLE 3xxxi BASELINE ANGIOGRAPHIC RESULTS

#		Min AWS		%DIAMETER STENOSIS		M AWS		
GROUP 1	n	AV	SD	AV	SD	n	AV	SD
01	5	1.666	0.24	25.2	6.3	8	2.401	0.509
02	4	1.619	0.14	32.25	8.3	8	2.785	0.924
03	4	1.95	0.23	27.75	13.3	7	2.517	0.446
04	2	2.505	0.9	38.5	2.12	3	3.113	1.145
05	6	1.493	0.74	40.33	14	8	2.225	0.674
06	6	1.446	0.36	34.17	13.24	8	2.18	0.319
07	5	2.048	0.66	36	13.44	8	2.81	0.704
08	6	1.553	0.31	34	11.85	6	2.189	0.297
09	5	1.165	0.32	33.6	7.02	8	2.218	0.74
10	2	2.205	1.27	39	18.4	2	3.08	1.202
GROUP 2								
11	4	1.56	0.51	34	19.54	5	2.209	0.511
12	7	1.288	0.48	37.57	7.37	9	2.017	0.722
13	9	1.663	0.6	33.22	15.55	11	2.435	0.67
14	4	1.465	0.24	30.5	7.19	10	2.33	0.648
15	5	1.845	0.8	43.6	14.05	5	2.775	0.765
16	3	2.222	0.66	23.33	3.51	6	3.483	1.154
17	3	1.605	0.3	27.33	12.01	5	2.455	0.962
18	5	1.453	0.22	39.2	6.83	7	2.583	1.047
19	4	1.328	0.35	42	17.49	10	2.448	0.682
20	5	1.693	0.83	40	20.63	6	2.671	0.866

FIG3.43 FREQUENCY-DISTRIBUTION OF BASELINE LESIONS

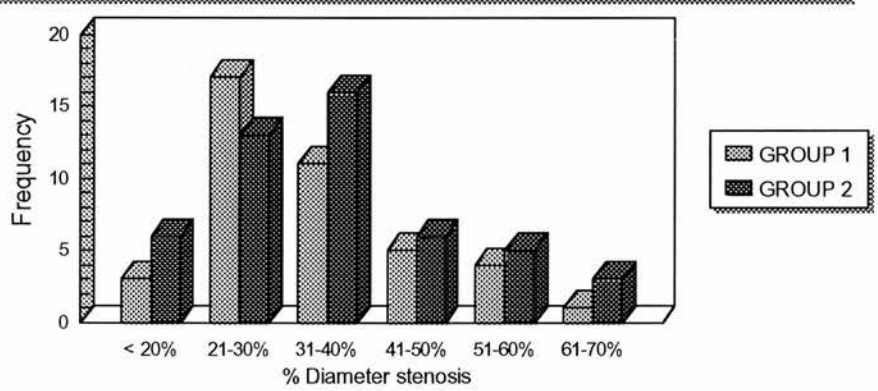


TABLE 3xxxii BASELINE ANGIOGRAPHY AND LIPID LEVELS

	FOCAL SCORE		GLOBAL SCORE	
	R	P	R	P
TC	-0.61	0.005	-0.61	0.006
LDL	-0.52	0.024	-0.52	0.025
HDL	0.27	N.S.	0.23	N.S.
TC/HDL	-0.50	0.03	-0.54	0.02

3.7.3 Effects of intervention

Follow-up angiography demonstrated a mean change in segment width of 0.015 mm (SD 0.39) in group 1 and -0.025 (SD 0.38) in group 2, a non-significant difference. Similar changes were observed for the minimal lesion diameter and percent diameter stenosis for the groups overall. Although there was no significant improvement or deterioration in the angiographic appearances on average, there were marked changes in the appearances of individual lesions. Certain individuals achieved statistically significant changes in their minimal luminal diameters or percent diameter stenosis, although within most subjects there was a mixed response to intervention (Tab 3xxxiii).The degree of 'progression' and 'regression' was similar for each group, a change of at least 8 percentage points in diameter stenosis being regarded as a definite change [Gibson *et al* 1992] (FIG3.44). Four of the nine apheresis patients had more lesions with an increase in mean arterial width of at least 0.17mm than a reduction by the same amount, four showed overall 'progression' by this criterion and one was unchanged. In the drug-treated group, six had more lesions altered in a favourable direction than had progressed, while only three had an excess of stenoses undergoing progressive change. Overall 40% of the lesions showed a significant alteration in the percent diameter stenosis, half of these in a favourable direction, and thus 80% of all stenoses were stabilised or made to regress over the two-year period.

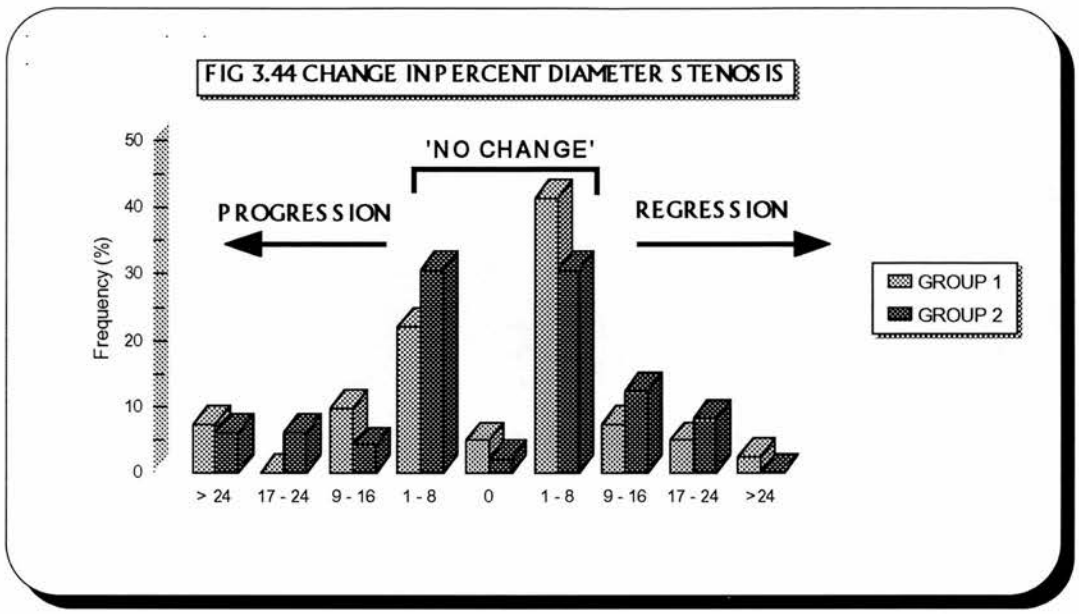
The severity of the stenosis at base-line did not affect the degree or direction of change in the lesions. A measure of roughness (or 'edge irregularity index') was calculated by a computer-derived algorithm. This showed no significant change in either group, and changes were not correlated with alterations in serum lipids. A weak positive correlation was noted between the roughness index and changes in mean segment width ($R = 0.24$, $P < 0.01$). The mean roughness change per patient, however was not related to the focal or global score measures.

TABLE 3xxxiii ANGIOGRAPHIC RESULTS POST-INTERVENTION

#		Min AWS		%DIAMETER STENOSIS			M AWS	
GROUP 1	n	AV	SD	AV	SD	n	AV	SD
01	5	1.744	0.22	26.4	9	8	2.372	0.29
02	4	1.622	0.13	28	5.9 *	8	2.879	1.02
04	2	2.345	0.9	35.5	0.7	3	2.978	1.06
05	6	1.629	0.72	37.2	10.6	8	2.208	0.7
06	6	1.302	0.22	37	9.8	8	1.971	0.23 ***
07	5	2.056	0.67	34.8	13.4	8	3.033	0.72 *
08	6	1.909	0.27 **	31.7	12.1	6	2.642	0.22 ***
09	5	1.239	0.39	39.8	15	8	2.232	0.72
10	2	1.67	2.37	59.5	57.3	2	2.06	2.91
GROUP 2								
11	4	1.987	0.91	26	22.1	5	2.415	0.75
12	7	1.318	0.35	34.7	10.9	9	2.109	0.78
13	9	1.49	0.49 *	37	12.6	11	2.327	0.64
14	4	1.57	0.38	26.2	7.6	10	2.314	0.64
15	5	2.001	1.44	45.2	35.6	5	2.692	1.65
16	3	2.408	0.72	27.7	10.7	6	3.421	1.08
17	3	1.36	1.46	50	43.7	5	2.06	1.4
18	5	1.436	0.21	42.8	3.6	7	2.451	0.96 *
19	4	1.345	0.43	38.3	22.5	10	2.519	0.83
20	5	1.593	0.42	45.8	11	6	2.724	0.83

* = P < 0.05, ** = P < 0.01, *** = P < 0.001 vs baseline (Table 3xxxi)

On combining the two study groups, no relationship was observed between the change in global score and improvement in exercise tolerance; neither was this related to patient age, extent of angiographic disease at baseline, or lipid levels at the outset of the trial or during treatment. There was a weak non-significant correlation ($R = 0.31$, $P < 0.2$) between the reduction in LDL/HDL ratio and change in global score. Within the group treated by apheresis there was a stronger correlation between the change in focal score and LDL/HDL ratio reduction (R =



0.60, P = 0.08); similarly, within group 2 there was a correlation between change in global score and TC/HDL ratio (R = 0.60) which just failed to achieve statistical significance (P = 0.067).

3.7.4 Correlation of scintigraphic and angiographic changes

3.7.4.1 Methods

As described in 3.6.1, the thallium images obtained at rest and redistribution were divided into five segments in each of three projections, and the perfusion assessed on each as normal (N, or greater than 95%), nearly normal (M, 65 - 95%) half normal (H, 35 - 65%), abnormal (B, 10 - 35%) or absent (A, or less than 10%). The rest and redistribution scans were then reviewed and graded for degree of reversibility of any perfusion deficits. These grades obtained for each segment from the baseline and the final scans were assigned a numerical value according to changes in the degree of reversibility (see Appendix). A summary measure of perfusion change for the area of distribution of each coronary artery was calculated by the summation of the values for the segments(S - see fig 3.39) subtended by the respective vessel:

Proximal RCA - Ant S4 + S5

Distal RCA - Ant S4 + S5, LAO-40 S3, + LAO-70 S3 + S4

Proximal LAD - Ant S1, LAO-40 S1, + LAO-70 S1 + S2

Distal LAD - Ant S2 + S3, LAO-40 S2, + LAO-70 S2

Prox LCx - LAO-40 S5 + LAO-70 S5

Distal LCx - LAO-40 S4 + LAO-70 S

The coronary arterial tree was divided into segments according to guidelines of the American Heart Association [Austen et al 1975]. The segments used for the proximal arteries were #1 for the right, #5 and #6 for the left anterior descending, and #11 for circumflex arteries. Changes in all three measures outlined above, namely minimum lumen diameter, mean absolute segment width and percent diameter stenosis were plotted against the scores of the changes in perfusion.

3.7.4.2 Results

TAB 3xxxiv CORRELATION OF THALLIUM SCORES WITH ANGIOGRAPHY

		CHANGE IN MAWS				
		ALL SEGS	**RCA**	**LAD**	**LCX**	**PROX SEGS**
CORRELATION	(R)	0.27 **	0.45 *	0.16	0.16	0.39 **
SENSITIVITY	(%)	56	17	74	45	69
SPECIFICITY	(%)	46	100	59	57	80
POS PRED VALUE	(%)	50	14	71	45	64
NEG PRED VALUE	(%)	61	50	62	40	75
TEST ACCURACY	(%)	42	35	57	26	49
RELATIVE RISK		1.15	2.0	1.88	0.76	2.57
		CHANGE IN MinAWS				
		ALL SEGS	**RCA**	**LAD**	**LCX**	**PROX SEGS**
CORRELATION	(R)	0.10	0.31	0	0.24	0.32
SENSITIVITY	(%)	52	33	64	44	50
SPECIFICITY	(%)	38	100	36	9	63
POS PRED VALUE	(%)	42	100	47	25	50
NEG PRED VALUE	(%)	36	45	36	17	50
TEST ACCURACY	(%)	29	33	35	18	29
RELATIVE RISK		0.83	1.83	1.04	0.30	1
		CHANGE IN %DS				
		ALL SEGS	**RCA**	**LAD**	**LCX**	**PROX SEGS**
CORRELATION	(R)	-0.12	-0.59 **	0.09	0.09	-0.39 *
SENSITIVITY	(%)	60	50	64	60	70
SPECIFICITY	(%)	48	100	33	18	70
POS PRED VALUE	(%)	47	100	47	38	70
NEG PRED VALUE	(%)	46	73	36	33	70
TEST ACCURACY	(%)	39	46	35	24	41
RELATIVE RISK		1.11	3.67	1.04	0.56	2.3

** =P <0.01 * =P <0.05

The sensitivity, specificity and predictive values for the perfusion change scores were determined for all the segments combined, for each artery separately and for only the proximal segments of all the arteries (Table 3xxxiv).

For all the angiographic measures, changes were most closely correlated with perfusion changes for the right coronary artery, and none were useful in assessment of changes in the circumflex artery. Changes in MLD were not reliably predicted by perfusion scores, and even for the proximal arterial segments relative risk was only 1.

The predictive value of perfusion changes was similar for percent diameter stenosis and mean segment width. The latter measure retained a significant correlation for all the segments and, although poorer for changes in RCA, was more powerful a predictor than changes in % diameter stenosis for lesions in the LAD distribution. The predictive accuracy of the thallium scores compared to the mean arterial width when applied to proximal segments (n=45) was 75% and 64% for progression and regression respectively, the score giving a relative risk of 2.57.

3.7.5 Discussion

Quantitative coronary angiography (QCA) directly assesses the effects of interventions, and at present is the most reliable means of studying progression and regression of disease despite the inherent difficulties due to both patient-related variables (such as vasomotor tone, presence of thrombus, vessel motion, and arterial geometry), and technical factors (such as comparability of angulation of the image intensifier, calibration and presence of pincushion distortion) [Gibson *et al* 1992, Selzer *et al* 1989, de Feyter *et al* 1991]. It has been suggested that the change seen at angiography is a useful surrogate endpoint for clinical events [Buchwald *et al* 1990]. Angiographic studies have clearly shown that the progression of the atherosclerotic process may be influenced by effective modification of serum lipids [Blankenhorn *et al* 1987, Brown *et al* 1990, Buchwald *et al* 1990, Kane *et al* 1990, Ornish *et al* 1990, Watts *et al* 1992, Blankenhorn *et al* 1993, Waters *et al* 1994, MAAS Investigators 1994], and that there does appear to be a relation between the extent to which cholesterol is lowered and the likelihood of plaque regression [Watts *et al* 1992, Brown *et al* 1990, Levy *et al* 1984].

The primary outcome measure in this study was the effects of lipid lowering on the angiographic appearances. Although there are in total 285 coronary arterial segments potentially analysable in this number of patients, the segments require to be completely comparable in each projection on two angiograms. The proportion of segments analysed in this study is similar to that obtained in others using QCA. These were selected entirely on technical grounds using film pairs by an observer blinded to the angiogram sequence, patient identity, and the nature of the intervention, and represent the fullest possible data available from the angiograms obtained. Whenever possible a mean of two or three projections were used for each segment, and multiple projections were used in 44.4% of the segments analysed.

There has been debate about which angiographic measures are the most relevant, and how best to present them. Prominent authorities highlight the relative merits of each of the measures, and recommend that percent diameter stenosis, minimum lumen diameter and mean segmental diameter should all be presented as both per-patient and per-lesion analysis [deFeyter *et al* 1991].

The significance of the correlations at baseline between the lipid levels and the angiographic scores were surprising, given that the patients were selected to have both severe hyperlipidaemia and advanced coronary disease. This would tend to support the strength of the link between cholesterol and heart disease, and increase the expectation that modification of the lipoproteins may result in alteration of the atheromatous plaques.

Analysis of the angiograms shows that only a minority of lesions may be induced to undergo regression. The extent to which this occurred did not differ between the groups, although within each there was a correlation between the degree of change in the TC/HDL ratio (the 'atherogenic index') and changes in the global score. The most striking finding was the marked reduction in progression in both groups, best appreciated by a comparison with results from control subjects in similar studies (Figure 3.45). Clearly the proportion of lesions exhibiting significant change is entirely dependent on the definition employed of 'regression' and 'progression'. The cut-off point requires to be related to the accuracy of the measurement, and its reliability in detecting small differences. Since blood flow is

FIG 3.45 RESULTS OF ANGIOGRAPHIC LIPID-LOWERING TRIALS

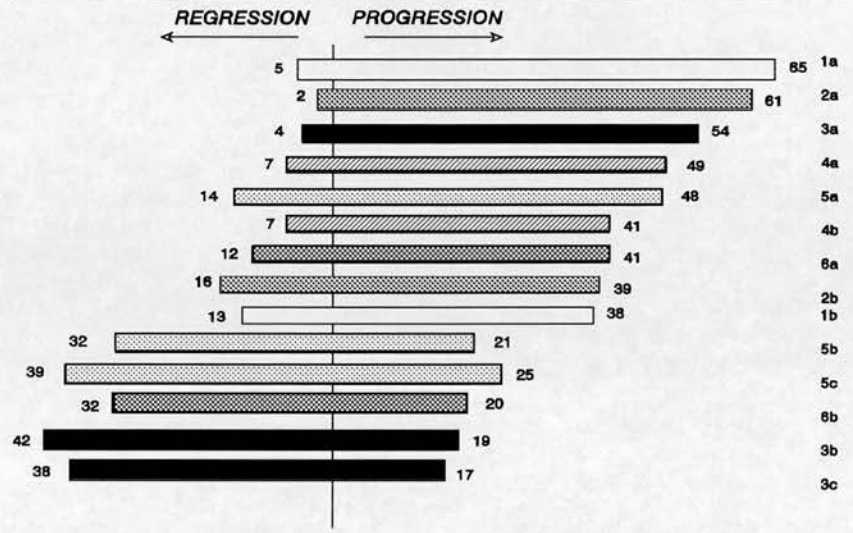


Fig 3.45 Trials used in the above figure are for percentage of patients exhibiting angiographic change. Groups marked 'a' are control groups, 'b' intervention groups, and 'c' where trials involved a second intervention group. 1 Buchwald et al 1990; 2 Blankenhorn et al 1987; 3 Watts et al 1992; 4 Levy et al 1984; 5 Brown et al 1990; 6 Kane et al 1990.

proportional to the fourth power of the radius of the vessel, small differences in the arterial lumen may well be of importance. In view of the number of other biological and technical variables present however, it is necessary to demand a difference in the measurements of at least 2 S.D. before accepting the variation to be definite.

One possible explanation of the lack of difference in response between the treatment groups is that there is in fact a threshold of cholesterol levels for inducing regression. This is quite possible since the relationship between the incidence of ischaemic heart disease and total cholesterol level is clearly curvilinear rather than linear, with a significant rise in the curve for most populations with cholesterol levels above 5.2 mmol/l (see Sect **2.1**). Previous intervention trials have documented a reduction in risk of 2.5% for each 1% reduction in cholesterol level [Holme 1990], but have not achieved large enough reductions to test whether this continues to hold true with more profound lowering. It could also be argued that the disease in our patients was too advanced to be readily reversed, and that more profound reductions may improve the probability of

regression in more lipid-rich plaques [Dollar *et al* 1991]. If this was the case however it might reasonably be expected that the younger patients in our study might have exhibited a greater tendency to regress, and this was not the case.

The failure of the more profound lipid lowering therapy to uniformly induce regression, despite achieving the TC/HDL ratio associated with this in animal models is probably related to the difference in composition of the plaques in those cholesterol-fed models and the advanced plaques in our subjects. Alternatively, we have perhaps missed a real difference in the effects of our lipid levels because of an inadequate number of subjects: the difference between the groups for change in focal score of 0.055mm in favour of group 1 is well within the 95% confidence intervals of a difference of 0.163mm in this direction, and a difference of 0.273mm in favour of group 2. The 95% confidence intervals for changes in the global score are equally wide (Table 3xxxv).

Since the correlation in changes in lesions within an individual however appears to be very weak, lesions may be regarded as behaving independently and reduce drastically the number of patients required to detect differences between treatment groups [Gibson *et al* 1992]. Assuming an average of 5 lesions per patient, and a standard deviation in measurement of mean segment width of 0.24mm [Watts *et al* 1992], there is 95% power at the 0.01 level to detect a difference of 0.2mm in the

TABLE 3xxxv. CHANGES IN ANGIOGRAPHIC PARAMETERS

		n	mean	median	S.D.	Q1	Q3	95% C.I.
Change in focal score	Group 1	9	-0.020	0.009	0.245	-0.152	0.101	} -0.273
	Group 2	10	+0.035	0.006	0.195	-0.118	0.164	} 0.163
Change in global score	Group 1	9	-0.115	-0.135	0.412	-0.250	0.159	} -0.39
	Group 2	10	-0.052	-0.072	0.168	-0.139	0.077	} 0.265
Change in MinAWS	Group 1	41	0.037	0.010	0.312	-0.100	0.227	} -0.137
	Group 2	49	0.018	-0.003	0.434	-0.175	0.238	} 0.176
Change in MAWS	Group 1	59	+0.015	0.050	0.389	-0.145	0.170	} -0.089
	Group 2	74	-0.029	0.005	0.379	-0.166	0.171	} 0.177
Change in % D.S.	Group 1	41	0.80	-1.0	13.94	-5.0	5.0	} -7.4
	Group 2	49	1.76	-1.0	16.9	-6.5	7.0	} 5.5

change in mean absolute width of the coronary segments - the difference described between the dietary intervention and 'usual care' groups in the STARS with reductions in total cholesterol of 2.0% and 14.2% respectively [Watts *et al* 1992] - with twenty patients. This would imply that there is sufficient power in the present data (Table 3xxxv) to conclude there is no difference in angiographic outcome between achieving a total cholesterol of 5.2 mmol/l using drug therapy and maintaining a lower level by the addition of regular LDL-apheresis in subjects with moderate hypercholesterolaemia and advanced coronary disease. This conclusion is supported by the lack of angiographic benefit seen in the intensively treated group of normocholesterolaemic patients in the Harvard Atherosclerosis Reversibility Project (HARP) [Sacks *et al* 1994]. Despite achieving similar percentage changes in serum lipids as in CLAS (although it may be significant that the rise in HDL was only 13% in HARP compared to 37%) [Blankenhorn et al 1987] to achieve the treatment goals of TC < 4.1 mmol/l and LDL/HDL < 2.0, there was no significant difference in angiographic outcome from the control subjects who maintained a mean total cholesterol level of 5.56 mmol/l for the 2.5 years of the study.

Further support for the "threshold hypothesis" comes from STARS - in which the degree of improvement with diet alone (and on-trial cholesterol of 6.17 mmol/l) was not significantly different from treatment with diet and cholestyramine (on-trial TC 5.56mmol/l) [Watts *et al* 1992] - and analysis of behaviour of lesions in subgroups in other studies: progression was reduced to a lesser extent in normocholesterolaemic subjects than in hypercholesterolaemic patients in CLAS ; in familial hypercholesterolaemic subjects reducing LDL from 5.2 to 2.6 mmol/l resulted in no greater improvement than in those in whom the LDL was lowered from 7.32 to 4.45 mmol/l [Kane *et al* 1990]; and, in CCAIT [Waters *et al* 1994] there was significant benefit for those with a baseline LDL above the median value of 4.5 mmol/l, but not for those below this level. In FATS there was angiographic improvement even in those with baseline levels below 4.1 mmol/l [Brown *et al* 1990], but the patients in this study were required to have elevated serum apoB at baseline which may affect the response to therapy.

A difference in progression or regression of lesions with varying severity of stenosis at baseline has been described [Stone *et al* 1993, Sacks *et al* 1994]. This may be partly due to "regression to the mean", but may also reflect genuine differences in the behaviour of these plaques. In the present analysis lesions initially greater than 40% diameter stenosis (n=34) had a mean decrease of 2 percentage points (SD 14.5), while 22 lesions with < 25% stenosis at baseline increased by a mean of 10% (SD 19.2) (P = 0.02). However the lesions with a baseline diameter stenosis less than 20% which did not progress above this level were not analysed in this way, and this is likely to have introduced some selection bias. The mean arterial width (MAWS) was examined for all segments which were suitable for analysis, and this demonstrates that there was no difference in the change in MAWS between segments with less than 20% diameter stenosis at baseline and those with an initial stenosis greater than 40%.

It is held by some observers that genuine 'regression' of an established lesion will be achieved only rarely due to the presence in the plaque of less reversible components. Stabilisation of the plaque may be a more readily attainable goal. The 'roughness index' may be a measure for this, since it incorporates some measure of the degree of irregularity of the lesion, including ulceration. Its usefulness however has not been established and results from this study do not suggest that even profound alterations in the cholesterol level have any effect. Indeed there was, surprisingly, a positive correlation between the degree of irregularity and increases in segment width.

The relationship between changes in the atherogenic index and the summary measures of lesion progression within each of the treatment groups, with a less clear trend for the groups combined, may indicate a difference in response to lipid modification dependent on the means used to achieve it. As previously discussed, there is a relationship between the degree of cholesterol reduction and both clinical events and extent of angiographic changes both within and between published studies. The extent of change per unit reduction in TC or TC/HDL does however differ, and changes achieved by dietary means appear to be greater than those achieved in studies in which pharmacotherapy was employed, which in turn are greater than those seen with physical interventions such as ileal bypass surgery.

Numerous studies have demonstrated the diagnostic value of thallium scintigraphy combined with either physical or pharmacological stress, and have documented the difficulties in relating the distribution of reversible perfusion defects to specific coronary lesions. The present study has not attempted to correlate the scintigraphic and angiographic findings at baseline, but sought to determine whether changes in lesions at angiography might be detectable as differences in perfusion, and whether it might be suitable as a non-invasive marker for disease progression or regression. Since the analysis is only semi-quantitative, it had been anticipated that it would be insufficiently sensitive to detect the relatively small changes expected.

The results however show not only a significant correlation between arteriographic changes and scores of changes in myocardial perfusion, but also indicate that the thallium scores may be of some value in predicting changes in individual lesions assessed angiographically. The overall accuracy of the scoring is reduced by a significant proportion of the thallium scores being zero. This might be improved by quantifying the regional myocardial uptake, so that absolute differences in perfusion might still be determined between scans rather than only differences relative to the hottest pixel. Another possibility which was examined was to adjust the zero scores to a positive or negative value depending on whether segmental wall motion had improved or deteriorated. Inserting 1 or -1 from regional wall motion scores (derived from either the moving reconstructed thallium image or from the blood pool scan) did increase the test accuracy, but at the expense of decreased sensitivity, specificity and predictive values.

The poorer predictive value of the scores for circumflex lesions might be anticipated, since the myocardial territory supplied is less well-visualised on the three projections of the thallium scan than are the other two arteries, and the low sensitivity of the technique in detecting circumflex artery lesions is well-recognised [Hargreaves & Muir 1992]. The degree of overlap of the territories of distribution of all three arteries due to right- or left-dominance and the extent of collateralisation is illustrated by the improvement in the predictive values for the scores when only the proximal arterial segments are considered.

The correlations found in this study would suggest that changes in perfusion and the degree of reversibility of perfusion defects may be of value in the non-invasive assessment of interventions which alter flow in the right coronary artery and possibly in the proximal segments of the left coronary artery.

4 GENERAL DISCUSSION

The studies cited in the section on the pathogenesis of atherosclerosis have focused for the purpose of the development of the thesis on the role of lipoproteins, while seeking to identify those other factors which act in concert with lipids and those aspects of the development of the disease process which may require the examination of alternative mechanisms. The nature of the plaque itself indicates that lipids are an essential component of the process, and the low incidence of disease in other animal species and of clinical endpoints in populations with high levels of other risk factors but lower median levels of cholesterol highlights the central role of lipoproteins. This recognises the existence of populations in which the prevalence of the precursor lesion, the 'fatty streak', is as high as in others but with a lower incidence of progression to plaque-formation, and does not deny the necessity of presence of other factors in addition to hypercholesterolaemia.

In attempting to find a unifying hypothesis for atherogenesis, it must be recognised that hypercholesterolaemia is not a prerequisite for the development of atherosclerosis, neither is the latter an inevitable consequence of the former. This is supported by the (uncommon) occurrence of the disease in subjects with none of the recognised risk factors. The variety of possible injurious agents which may initiate the process in different individuals however, are likely to have a final common pathway which is aggravated and accelerated by the presence of excess LDL which may undergo oxidative modification and accounts for the regression which may take place in lesions which occur in individuals with normal or even low levels of serum lipids.

Following the acceptance of the association between cholesterol levels and coronary disease both within and between populations, attempts to elucidate the causal nature of the relationship and the desire to reduce clinical events led to a considerable number of trials of intervention. As in other areas of research the early studies were flawed in design and failed to substantiate the hypothesis of a causal role for lipids. The dietary trials of unifactorial intervention (Tables 1i - 1ii) were largely ineffectual because of reductions in cholesterol levels being too small, too short a period of follow-up, too few participants, or having an event rate

which proved inadequately low. The first two of the three major primary prevention trials finally proved the 'cholesterol hypothesis' in significantly reducing the combined cardiac end-points (in the LRC-CPPT [Lipid Research Clinics Program 1984]) or coronary events (in the WHO trial [WHO Collaborative Group 1983]). The WHO clofibrate study however moderated the urge to adopt widespread use of clofibrate to prevent coronary disease by its unexpected and worrying finding of an increase in overall mortality in the treatment group. This has cast a long shadow over the treatment of raised lipid levels with drugs which has affected also the need for more intensive treatment of subjects with established disease, and this has only recently begun to be redressed.

The increase in non-cardiac deaths in the drug-intervention studies had no single statistically significant cause, lacked an association between the degree of cholesterol-lowering and the likelihood of an adverse event, tended to occur in non-compliers, and lacked a biologically plausible explanation. However the trends were consistent in all the studies, and consonant with the epidemiological findings of an increased overall mortality rate (including deaths from malignancy) at the lower end of the cholesterol distribution [Jacobs *et al* 1990, Neaton *et al* 1992]. Although it had been felt that the low cholesterol levels might result from some pathological process which was ultimately responsible for death, rather than the stimulus for the cause of death itself, some studies report the same relationship between total mortality and low cholesterol levels even after discounting the deaths occurring within the first few years of cholesterol measurement (eg Isles *et al* 1992). It is quite possible that the agents used in the intervention trials had untoward side-effects unrelated to their cholesterol-lowering activity in the same way as some of the benefits of treatment may be derived by other mechanisms [Research Committee of the Scottish Society of Physicians 1971, Group of Physicians of the Newcastle upon Tyne Region 1971], and it is noteworthy that the trials of dietary therapy were largely free of deleterious effects on mortality (Table 1ii), but this does not explain the epidemiological observations which should not be dismissed.

Although some individuals would appear to be resistant to the effects of prolonged hypercholesterolaemia, for those who develop the lesions of atherosclerosis it would seem that "whatever their level of cholesterol, it is too high *for them*". Since

the occurrence of a prior cardiac event is one of the strongest predictors of subsequent cardiovascular morbidity and mortality, the benefits of cholesterol-lowering in the secondary setting has always appeared to be more promising in yielding a favourable risk:benefit ratio. The drug treatment of moderate elevations of cholesterol in this population has consistently led to a reduction in cardiac events (Table 1iv); the prospect that this might lead ultimately to improved survival overall is supported by the long-term follow-up of the Coronary Drug Project [Canner *et al* 1988]. Although it has been argued that the lack of effect of treatment on total mortality of the other studies is unsurprising given that the studies were not designed to have the power to detect this, the results of the Program on the Surgical Control of Hyperlipidemias (POSCH, [Buchwald *et al* 1990]) set out with this objective in survivors of myocardial infarction. Although the reduction in total mortality failed to achieve statistical significance at ten years, the curves continue to diverge and further follow-up of this cohort of obligate adherents may yet yield this elusive goal (although the increasing use of effective therapy in the control group will reduce the apparent impact of this heroic intervention).

The recent publication of the Scandinavian Simvastatin Survival Study (4S) has altered irrevocably the approach to management of moderate hypercholesterol-aemia in patients with existing disease. The highly significant reduction in total mortality and all primary and secondary cardiac endpoints (in both men and women, and also in older age-groups) combined with the absence of increased non-cardiac deaths both justifies the enthusiasm for secondary prevention and reassures the sceptics that the benefits of cholesterol lowering (at least with some groups of agents) can be obtained without the spectre of simply changing the cause of death. Further studies using other HMG CoA reductase inhibitors in both primary and secondary settings should firmly substantiate the impressive findings of this trial.

Although a large-scale and prolonged clinical trial would be desirable to assess the effects of any agent used for primary or secondary prevention in order to evaluate the risk of unanticipated adverse events, the adoption of surrogate endpoints may be one method of predicting the effects of any therapy on atherosclerotic events and may be of value in selecting therapies for more rigorous clinical scrutiny. The

use of computerised methods for analysing coronary angiograms permits the study of a smaller number of patients over a shorter time period, using the changes in atherosclerotic plaques as the endpoint. Studies that have documented beneficial alterations in angiographic appearances and reduction in clinical events [Buchwald *et al* 1990, Brown *et al* 1990, Watts *et al* 1992] attest to the validity of this approach. The optimal means of presenting the results from such studies should include both per-patient analyses and per-lesion changes [deFeyter *et al* 1991], since the intraclass correlations have been shown to be low in all cases (0.05 - 0.2), indicating virtual independence of lesion changes from each other within individual patients [Sacks *et al* 1994, Watts *et al* 1992]. As discussed in Section **3.7.5** (pp 218 - 219), this would imply that the power of the present study was sufficient to detect a significant difference between the treatment groups and that it would be reasonable to conclude a lack of effect of the more intensive treatment schedule.

The biological significance of the angiographic findings analysed by computer-assisted edge-detection algorithms has been questioned. The absolute change in lesions was small: the mean change (S.D.) in minimum lesion diameter was -0.05mm (0.14) for the conventionally treated group in the Familial Atherosclerosis Treatment Study [Brown *et al* 1990] compared to -0.002mm (0.14) in the lovastatin-colestipol group and +0.04mm (0.12) for the statin-niacin group. The other studies demonstrating benefit have yielded changes of similar magnitude. The strength of the results however lie not so much in the magnitude of the response, as in the consistent relationship with the clinical outcome. It is likely that the alterations in the circulating lipids lead to a degree of resorption of the lipid component of the atherosclerotic plaque resulting in minimal change in the overall dimensions, but fundamentally altering its composition and reducing its propensity to rupture with the attendant complications.

Recent angiographic studies employing monotherapy with an HMG CoA reductase inhibitor [Blankenhorn *et al* 1993, Waters *et al* 1994, MAAS 1994] have shown significant reductions in serum lipids and angiographic evidence of enhanced regression and decreased progression (see Tables 1vi and 1vii, pp 74 - 75), but have failed to produce a significant reduction in clinical endpoints, despite intervention periods similar to that of FATS. The latter study also showed a

significant correlation between the changes in LDL and HDL with the alteration in arterial stenosis, and this was not a finding of the statin studies. It may be of note that the investigations yielding some of the best clinical results have employed niacin as part of the treatment regime. While this may be due in part to actions other than lipid-lowering (or HDL-raising), such as vasodilation, its effects on Lp(a) may also be of import. Niacin is the only cholesterol-lowering drug shown to have a beneficial effect on Lp(a) and while statins lower LDL, various reports indicate a rise in Lp(a). Although the benefits of reducing Lp(a) *per se* are as yet unproven, the evidence for its influence in promoting atherosclerosis is considerable. Its reduction is therefore likely to be beneficial: since LDL requires to undergo oxidative modification before uptake by the macrophage scavenger receptor, the presence of a bound modified particle in the subendothelial space may accelerate the oxidation of native LDL, and the presence of excess LDL would lead to more rapid formation of foam cells. In the absence of Lp(a) the residence time of LDL may not permit much modification of the particles in the absence of another oxidative source, even if the serum LDL concentration was high.

It is well-accepted that the architecture of the stenosis contributes only in part to the overall clinical importance of the lesion, and that endothelial dysfunction resulting in abnormal vasomotor tone will influence both the angiographic appearances and the vascular function. The impaired vasomotor tone seen in hypercholesterolaemia [Creager *et al* 1990] is reversed with effective lipid-lowering therapy in animal models as well as man [Osborne, Lento *et al* 1989]; the authors of the Lifestyle Heart Trial noted that *Improvements in functional status occurred after only one month*, which they attributed to alterations in *platelet-endothelial interactions, vasomotor tone, or other dynamic characteristics of stenoses* [Ornish *et al* 1990]. Such early symptomatic improvements in patients with coronary disease is an almost universal experience during regular therapy with LDL-apheresis [Keller 1991], including the present study. The benefits of rigorous reduction in lipids compared to more modest changes in serum cholesterol has not however been better-demonstrated than in the present investigations, and were greatest for those whose exercise capacity was most limited prior to treatment (see section **3.5**).

It had been thought that the early improvement in symptoms may be due to favourable alterations in haemorheology, as had been described elsewhere. As described in Sect 3.3.3 however, there was a small unexpected (but significant) increase in blood viscosity. The changes in blood viscosity were negatively correlated with percentage reduction in cholesterol, so that those with larger falls in TC had the smallest increases, or largest decreases, in whole blood viscosity. Despite more profound lipid reductions than previously documented maintained over a prolonged period (and despite acute changes in fibrinogen levels and viscosity during apheresis), there was not a significant reduction in the longterm in pre-apheresis haemorheological values; this is underlined by the absence of changes after withdrawal of therapy. It is likely therefore that the changes in endothelial function were responsible for much of the impressive benefit obtained during treatment.

The sustained symptomatic improvement following prolonged therapy gave grounds for optimism that regression would have occurred more frequently in the more intensively treated group. It is clear from the results (sect **3.7.3**) that however the terms are defined, the groups did not differ in the proportion of lesions in each in which 'progression' and 'regression' occurred. The results are remarkable however for the extent to which the lesions could be stabilised in both groups, despite the advanced nature of the disease before intervention and the severity of the hypercholesterolaemia. The results from other studies tend to suggest that this is a more readily attainable goal of therapy; even lesser reductions in the proportion of subjects exhibiting disease progression has been shown to be associated with reduced clinical endpoints [Buchwald *et al* 1990].

As previously stated, the multifactorial nature of atherosclerosis is well-recognised. However it is possible that for an individual only one factor is the 'determining factor', and correction of this dominant cause may be sufficient to arrest or reverse the process [Steinberg 1987]. For individuals with proven coronary disease and marked hypercholesterolaemia the management clearly should include effective lipid-lowering therapy.

Recent research has focused on the mechanisms involved in the uptake of lipid into the artery wall. It is now established that oxidation of LDL is central to this

process, and it is possible that the prevention of this step will prove more rewarding than cholesterol-reduction in the stabilisation of the atherosclerotic plaque. Nevertheless, as observed by Salonen and colleagues, *"..even if oxidation of LDL were the 'whole story'....it is obvious that if there were fewer LDL particles to be oxidised, there would be less atherosclerosis."* [Salonen *et al* 1992].

Interestingly the use of captopril following myocardial infarction is increasing following the publication of studies showing improved long-term mortality in this setting. While the reduction in mortality is related mainly to reduced progression to heart failure, a reduction in re-infarction has also been noted. The anti-oxidant activity of captopril (but not the non-sulfhydryl-containing angiotensin converting enzyme inhibitors) has been noted [Balla *et al* 1991], and may account for this hitherto unanticipated benefit.

LDL-apheresis has been shown to allow the attainment of any chosen lipid goal, and for those individuals unable to tolerate drug combinations or in whom the effects of such therapy is ineffective in resulting in the achievement of the desired lipoprotein levels (ie. total cholesterol < 5.2 mmol/l, LDL < 3.5 mmol/l) it may be a useful adjunct to conventional treatment. Its use in homozygous familial hypercholesterolaemia is well-established and uncontroversial. The widespread availability of effective drug therapy however limits its applicability to a small group of subjects. While most procedures were uncomplicated, the unpredictable nature of the small number of serious adverse reactions pose a hazard to those with impaired myocardial function and diminished coronary reserve.

While the documented benefits of effective cholesterol-lowering should not be denied to those who stand to gain most from its application, the majority of patients with familial hypercholesterolaemia will however be able to achieve normal or near-normal lipoprotein levels with effective combination drug therapy. Also, the advent of gene therapy in certain disorders is likely to be applied in FH patients within the next few years, and it is possible that extracorporeal removal of lipoproteins will be superseded by such definitive therapy.

In the meantime the evidence would suggest that even modest elevations of cholesterol in patients with proven coronary disease should be corrected by lipid-

lowering drugs and that it is safe to do so, but that extreme measures do not appear to yield significant additional benefit and that lowering lipid levels further in subjects with normal levels is not warranted. Within the latter group however there may be subgroups with an atherogenic lipoprotein phenotype who might benefit from lipid modification, and this requires to be studied further. Further work is also needed to ascertain whether the addition of anti-oxidant therapy confers any advantage, and whether the development of agents which have greater effect on Lp(a) (or haemostatic or thrombotic factors) might improve the clinical outcome for this high-risk group.

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APPENDIX

Determination of score for change in myocardial perfusion

1st scan		->	3rd scan		Score
Ex	***Redist***		***Ex***	***Redist***	
Normal			*Normal*		
N,	N		N,	N	0
Normal			*Reperfusion*		
N,	N		M,	N	-1
N,	N		H,	N	-2
N,	N		B,	N	-3
N,	N		A,	N	-4
Normal			*Partial reperfusion*		
N,	N		H,	M	-1
N,	N		B,	M	-1
N,	N		A,	M	-1
N,	N		B,	H	-2
N,	N		A,	H	-2

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
N,	N		A,	B	-3

Normal			Fixed			
N,	N		M,	M	}	
N,	N		H,	H	}	0
N,	N		B,	B	}	
N,	N		A,	A	}	

Normal			Reverse reperfusion		
N,	N		N,	M/H/B/A	0
N,	N		M/H/B	H/B/A	0

Reperfusion			Normal		
M,	N		N,	N	1
H,	N		N,	N	2
B,	N		N,	N	3
A,	N		N,	N	4

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Reperfusion			Reperfusion		
M,	N		M,	N	0
M,	N		H,	N	-1
M,	N		B,	N	-2
M,	N		A,	N	-3
H,	N		M,	N	1
H,	N		H,	N	0
H,	N		B,	N	-1
H,	N		A,	N	-2
B,	N		M,	N	2
B,	N		H,	N	1
B,	N		B,	N	0
B,	N		A,	N	-1
A,	N		M,	N	3
A,	N		H,	N	2
A,	N		B,	N	1
A,	N		A,	N	0

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Reperfusion			Partial reperfusion		
M,	N		H,	M	-1
M,	N		B,	M	-2
M,	N		A,	M	-3
M,	N		B,	H	-2
M,	N		A,	H	-3
M,	N		A,	B	-3
H,	N		H,	M	-1
H,	N		B,	M	-1
H,	N		A,	M	-2
H,	N		B,	H	-2
H,	N		A,	H	-2
H,	N		A,	B	-3
B,	N		H,	M	-1
B,	N		B,	M	-1
B,	N		A,	M	-1

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
B,	N		B,	H	-2
B,	N		A,	H	-2
B,	N		A,	B	-3
A,	N		H,	M	-1
A,	N		B,	M	-1
A,	N		A,	M	-1
A,	N		B,	H	-2
A,	N		A,	H	-2
A,	N		A,	B	-3

Reperfusion		Fixed			
M/H/B/A	N	M,	M	}	
M/H/B/A	N	H,	H	}	0
M/H/B/A	N	B,	B	}	
M/H/B/A	N	A,	A	}	

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Reperfusion			Reverse reperfusion		
M/H/B/A	N		N,	M/H/B/A	0
M/H/B/A	N		M/H/B	H/B/A	0
Partial reperf			Normal		
H,	M		N,	N	1
B,	M		N,	N	2
A,	M		N,	N	3
B	H		N,	N	3
A,	H		N,	N	4
A,	B		N,	N	4
Partial reperf			Reperfusion		
H,	M		M/H/B/A	N	1
B,	M		M,	N	2
B,	M		H/B/A	N	1

1st scan		->	3rd scan		Score
Ex	***Redist***		***Ex***	***Redist***	
A,	M		M,	N	3
A,	M		H,	N	2
A,	M		A/B	N	1
B,	H		M/H/B/A	N	2
A,	H		M,	N	3
A,	H		H/B/A	N	2
A,	B		M/H/B/A	N	3

Partial reperf		Partial reperfusion		
H,	M	H,	M	0
H,	M	B,	M/H	-1
H,	M	A,	M/H/B	-2
B,	M	H,	M	1
B,	M	B,	M	0
B,	M	A,	M	-1
A/B	M	A/B	H	-1
A/B	M	A,	B	-2

1st scan		->	3rd scan		Score
Ex	***Redist***		***Ex***	***Redist***	
A,	M/B/H		H,	M	2
A,	M		B,	M	1
A,	M		A,	M	0
B,	H		A/B/H	M	1
B,	H		B,	H	0
B,	H		A,	B/H	-1
A,	H		A/B	M	1
A,	B/H		B,	H	1
A,	H		A,	H	0
A,	H		A,	B	-1
A,	B		A/B	M	2
A,	B		A,	H	1
A,	B		A,	B	0

Partial reperf		Fixed			
A/B/H	B/H/M	H,	H	}	-1
A/B/H	B/H/M	A,	A,etc.	}	

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Partial reperf			Reverse reperfusion		
A/B/H	B/H/M		N,	M/H/B/A }	0
A/B/H	B/H/M		M/H/B	H/B/A }	
Fixed			Normal		
M,	M		N,	N }	2
H,	H,etc		N,	N }	
Fixed			Reperfusion		
A,	A }				
B,	B }		A/B/H/M	N	2
H,	H }				
M,	M }				
Fixed			Partial reperfusion		
A,	A }		A/B/H	B/H/M	1
B,	B, etc. }				

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Fixed			Fixed		
A,	A		B,	B	}
A,	A		H,	H	} 0
M,	M		A,	A, etc	}
Fixed			Reverse reperfusion		
A,	A		N,	M/H/B/A	} 0
B,	B,etc		M/H/B	H/B/A	}
Reverse reperf			Normal		
N,	M/H/B/A		N	N	0
Reverse reperf			Reperfusion		
N,	M/H/B/A		M/H/B/A	N	0
Reverse reperf			Partial reperfusion		
N,	M/H/B/A		H/B/A	M/H/B	0

1st scan		->	**3rd scan**		**Score**
Ex	***Redist***		***Ex***	***Redist***	
Reverse reperf			Fixed		
N,	M/H/B/A		M	M	} 0
M/H/B	H/B/A		H	H, etc.	}
Reverse reperf			Reverse reperfusion		
N,	M/H/B/A		N,	M/H/B/A	} 0
M/H/B	H/B/A		M/H/B	H/B/A	}