EFFECTS OF APOLIPOPROTEIN 
AND LOW DENSITY 
LIPOPROTEIN RECEPTOR GENE 
POLYMORPHISMS ON LIPID 
METABOLISM, AND THE LIPID 
RISK FACTORS OF CORONARY 
ARTERY DISEASE

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LIPID RISK FACTORS OF CORONARY
ARTERY DISEASE

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Abstract

To facilitate the diagnosis of hypercholesterolemia, we wanted to create a simple and rapid method for diagnosing familial hypercholesterolemia in a homogenous population. The PCR method for the FH-Helsinki mutation detected 25 FH-Helsinki positive patients, two of whom had no clinical signs of FH, but had a positive family history for the disease. The method is exceptionally useful in Northern Finland, where 62% of the FH patients carry the FH-Helsinki mutation.

The role of polymorphisms and mutations of the apo B particle as etiologic factors of hypercholesterolemia was studied in a population of moderately hypercholesterolemic individuals. The catabolism of the patients’ own LDL was compared to that of a healthy and normocholesterolemic donor, and no major differences were observed. However, the presence of the XbaI cutting site was associated with elevated cholesterol values and a slightly lowered LDL catabolic rate. Patients homozygous for the EcoRI cutting site also had a slow LDL catabolic rate and slightly elevated cholesterol values. The MspI and Ins/del polymorphisms of the apo B particle were not associated with variations in LDL catabolism.

The e 4 allele of apolipoprotein E was slightly more frequent in our hypercholesterolemic population than in the average population. The lipid values did not differ significantly between the apo E phenotypes in moderately hypercholesterolemic individuals, nor could we detect any differences in the catabolic rates of their LDL according to the apo E phenotype. apo E 2/2 were excluded from the study. In our population of CAD patients, the frequency of the e 4 allele was lower than in CAD populations from Southern Finland (0.23 vs. 0.32), suggesting that apo E 4 is not so strongly associated with coronary disease in Northern Finland as in other populations. The E 4 phenotype was associated with slightly smaller LDL cholesterol reductions by colestipol and lovastatin treatment compared with patients with the phenotype 2/3.

The lipid risk factors of male and female CAD patients were studied in a group of patients admitted to one ward of the Oulu University Hospital. We found the males to have the typical high LDL cholesterol and low HDL cholesterol lipid pattern, but women with two- or three-vessel CAD had high LDL and low HDL cholesterol associated with high VLDL lipids, and hypertension, diabetes or smoking.

Pharmacological treatment of hypercholesterolemia was studied by comparing lovastatin to colestipol, and in a separate study where a new drug, enprostil was used. Enprostil, whose main effect is on the gastrointestinal tract, would be a useful alternative for long-term treatment of hypercholesterolemia. Unfortunately, however, gastrointestinal side-effects limit its long-term use. Colestipol reduced plasma LDL cholesterol and elevated plasma HDL cholesterol and triglycerides, but it, too, caused gastrointestinal side-effects. Lovastatin proved to be the most effective cholesterol-lowering drug with the least side-effects, and statins have now been established as the most widely used hypocholesterolemic drugs.

Keywords: hypercholesterolemia, male and female, treatment.
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### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AMI</td>
<td>acute myocardial infarction</td>
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<tr>
<td>apo(a)</td>
<td>apolipoprotein(a)</td>
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<tr>
<td>apo A</td>
<td>apolipoprotein A</td>
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<tr>
<td>apo B</td>
<td>apolipoprotein B</td>
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<tr>
<td>apo E</td>
<td>apolipoprotein E</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass grafting</td>
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<td>CAD</td>
<td>coronary artery disease</td>
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<tr>
<td>CETP</td>
<td>cholesteryl ester transfer protein</td>
</tr>
<tr>
<td>CoA</td>
<td>coenzyme A</td>
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<tr>
<td>FCR</td>
<td>fractional catabolic rate</td>
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<tr>
<td>FDB</td>
<td>familial defective hypercholesterolemia</td>
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<td>FH</td>
<td>familial hypercholesterolemia</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HMG</td>
<td>hydroxymethylglutaryl</td>
</tr>
<tr>
<td>IDL</td>
<td>intermediate density lipoprotein</td>
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<tr>
<td>KI</td>
<td>potassium iodide</td>
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<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
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<tr>
<td>Lp(a)</td>
<td>lipoprotein(a)</td>
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<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
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<tr>
<td>VLDL</td>
<td>very low density lipoprotein</td>
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1. Introduction

Coronary artery disease (CAD) is still the main cause of death among men aged over 45 years and women aged over 65 in Europe. The risk factors for CAD are elevated blood cholesterol, male sex, family history of CAD, cigarette smoking, hypertension, diabetes mellitus and severe obesity. It has been postulated that all the other risk factors would be cholesterol-dependent (Roberts 1992), as atherosclerotic events are rare in populations with an average serum total cholesterol level below 3.9 mmol/l. Decreases in the risk factors, such as lowering of blood cholesterol, have been shown to associate with a decrease in mortality from ischaemic heart disease in men and women (Scandinavian Simvastatin Study Group 1994, Shepherd et al. 1995).

Lipoproteins are the particles responsible for lipid transport in the blood. Apolipoprotein B (apo B) is the main protein constituent of LDL and also the main ligand for the LDL receptor in the liver. Apo B is highly polymorphic, and several mutations and polymorphisms of the apo B gene have been described (Humphries et al. 1995), some of which are associated with variations in blood lipid levels. Another particle associated with atherosclerosis (Dahlén et al. 1986, Armstrong 1986, Frick et al. 1987), myocardial infarction (Murai et al. 1986, Rhoads et al. 1986, Dahlén et al. 1975) and stroke (Murai et al. 1986, Woo et al. 1991) is lipoprotein(a) (Lp(a)), which is formed when an LDL particle is attached to an apolipoprotein(a) (Lipid Research Clinics Program 1984a).

Apolipoprotein E (apo E) is a lipoprotein that interferes with the lipid metabolism by affecting the intestinal absorption of cholesterol and by modifying the catabolism of LDL and its precursors, and it can also modify the reverse cholesterol transport (Kinoshita et al. 1993, Martin et al. 1993, Huang et al. 1995). Apo E is a polymorphic lipoprotein with three common alleles: E2, E3 and E4.

Cholesterol is transported from the tissues to the liver by high density lipoprotein (HDL) to be excreted from the body. Apolipoprotein A1 (apo A1), the main protein constituent of HDL, is the primary acceptor for cholesterol in HDL. The cholesteryl ester transfer protein (CETP) is bound to the apo A1-HDL particles and mediates the exchange of HDL cholesteryl esters with the triglycerides of apo B containing lipoproteins (Tall 1995).
Familial hypercholesterolemia (FH) is a disease characterised by a lifelong elevation of the blood levels of low density lipoprotein (LDL) cholesterol due to defective functioning of the LDL receptors (Brown & Goldstein 1986). These patients develop coronary disease 10-20 years earlier than the rest of the population (Thompson 1996).

The clinical diagnosis of FH can be difficult in patients with moderately elevated cholesterol values, and tests to detect the genetic errors of FH are therefore urgently needed. The inherited differences other than FH in plasma lipids and lipid-transporting proteins, and their influence on individual plasma lipid levels and eventually on the hypocholesterolemic efficacy of drugs could facilitate the choice of an ideal diet-drug combination for each hypercholesterolemic individual.
2. Review of the literature

2.1. Familial hypercholesterolemia

Familial hypercholesterolemia is one of the most common monogenic inherited diseases, the frequency of the heterozygous form being about 1:500 in most populations (Goldstein & Brown 1983). FH is caused by a diminished number of functional LDL receptors on the cell membranes (Brown & Goldstein 1986). Five different classes of mutations have been described, each of which includes several different gene defects. Class 1 mutations disrupt the synthesis of LDL receptors, resulting in a complete absence of detectable receptors, in class 2 mutations the transport of the receptor to the cell surface is blocked or obstructed, class 3 receptors bind LDL defectively, class 4 receptors are unable to cluster in the coated pits, and class 5 mutated receptors fail to recycle to the cell surface or deliver LDL to the endosomes (Hobbs et al. 1990). More than 150 different mutations causing FH have been described (Hobbs et al. 1992). The FH-Helsinki mutation belongs to class 4, and it is caused by a large 9500-bp deletion extending from intron 15 to exon 18 (Aalto-Setälä et al. 1989a). The FH North Karelia mutation results in a receptor-negative or binding-defective LDL receptor due to a 7 nucleotide deletion in exon 6 (Koivisto et al. 1992). Approximately one-third of the FH patients in Finland have the FH-Helsinki (Aalto-Setälä et al. 1988a), and one-third the FH-North Karelia (Koivisto et al. 1992) mutation. The other FH-mutations described in Finland include the FH Espoo (Koivisto et al. 1993), FH Jalasjärvi (Koivisto & Kontula 1996), FH Turku (Koivisto et al. 1995) and FH Pori (Koivisto et al. 1995).

The clinical diagnostic criteria for FH are a serum cholesterol concentration above 9 mmol/l after adequate dietary intervention, when secondary hypercholesterolemia has been excluded, and FH or early onset coronary artery disease in at least one first-degree relative (males < 45 years, females < 55 years) or tendon xanthomata. Tendon xanthomata is an age-related phenomenon, with only 7% of FH heterozygotes below the age of 19 years exhibiting xanthomata compared to >80% of patients older than 30 years (Thompson et al. 1989).
2.2. Apolipoprotein B

Apo B exists in human plasma as two isoforms, apo B-48 and apo B-100. Apo B-100 is the major physiological ligand for the LDL receptor. It is the largest monomeric protein sequenced so far, containing 4536 amino acid residues (Chen et al. 1986, Law et al. 1986). Its gene has been mapped on the short arm of chromosome 2, with an approximate length of 43 kilobases and 29 exons (Ludwig et al. 1987). The LDL-binding domain of the molecule is proposed to be located between the residues 3129 and 3532 (Knott et al. 1986). Apo B-100 is synthesised in the liver and is required for the assembly of very low density lipoproteins (VLDL). It does not interchange between lipoprotein particles, as do the other lipoproteins, and it is found in IDL and LDL particles after the removal of the apolipoproteins A, E and C (Young 1990).

Apo B-48 is present in chylomicrons and chylomicron remnants and plays an essential role in the intestinal absorption of dietary fats (Kane 1983). Apo B-48 is synthesised in the small intestine. It comprises the N-terminal 48% of apo B-100 and is produced due to postranscriptional apo B-100 mRNA editing at codon 2153, which creates a stop codon in the intestine instead of a glutamine in the liver (Chen et al. 1987).

Mutations occurring in the apo B gene can alter blood cholesterol levels. Most of the mutations lower blood cholesterol levels due to the production of truncated apo B. The mechanisms by which blood cholesterol is lowered are not yet fully understood. Two mutations in the apo B gene have been associated with elevated blood cholesterol. The apo B-3500 Arg→Gln substitution causes familial defective hypercholesterolemia (FDB) due to defective binding of LDL to its receptor (Vega & Grundy 1986, Soria et al. 1989).

The prevalence of the mutation in the general population in Central Europe is 1/204-1/700 (Innerarity et al. 1990, Tybjaerg-Hansen et al. 1990, Schuster et al. 1990). The highest prevalence has been reported from Switzerland (Miserez et al. 1994), and so far apo B-3500 has not been found in Finland (Hämäläinen et al. 1990). Another mutation in the LDL receptor binding area causing apo B-3531 Arg→Cys has been described to cause moderate hypercholesterolemia due to defective binding of LDL to its receptor (Pullinger et al. 1995).

Several restriction fragment length polymorphisms (RFLP) in the Apo B gene have been defined (Humphries & Talmud 1995). The most widely studied of these is the XbaI polymorphism in exon 26, which does not result in an amino acid substitution. In some populations the presence of the XbaI cutting site is associated with hypercholesterolemia in both normolipemic (Berg 1986, Talmud et al. 1987, Aalto-Setälä et al. 1988) and hypercholesterolemic (Leren et al. 1988, Aalto-Setälä et al. 1989) individuals. The absence of the XbaI cutting site was associated with higher triglyceride levels in one study (Deeb et al. 1986). Several studies have failed to reveal any association between the XbaI polymorphism and lipid values (Hegele et al. 1986, Aburatani et al. 1988, Rajput-Williams et al. 1988, Darnfors et al. 1989, Gajra et al. 1994,) and in one study the association of the presence of the XbaI cutting site with elevated cholesterol and triglyceride levels was only observed in patients with peripheral artery disease (Monsalve et al. 1988).

The EcoRI restriction fragment length polymorphism in exon 29 is associated with an amino acid change Gln → Lys84154. Most studies have revealed no association between the EcoRI polymorphism and cholesterol or triglyceride levels (Ma et al. 1987, Dunning et al. 1988, Jenner et al. 1988, Aburatani et al. 1988, Peacock et al. 1992). An associa-
tion between elevated triglycerides and the absence of the EcoRI cutting site has been reported in coronary heart disease patients (Paulweber et al. 1990, Tybjaerg-Hansen et al. 1991) and in healthy males (Paulweber et al. 1990).


The apo B signal peptide contains a leucine-alanine-leucine insertion/deletion polymorphism affecting the amino acids 14-16 producing signal peptides with 24 or 27 amino acids (Boerwinkle & Chan 1989). The ins allele has been associated with elevated serum triglycerides (Tikkanen & Heliö 1992), low serum cholesterol and apo B (Hansen et al. 1993), and coronary artery disease (Peacock et al. 1992) in some populations, whereas in others the del allele has been connected with elevated total and LDL cholesterol but not with myocardial infarction (Bohn et al. 1994). No association between the polymorphism and lipids was detected in Asian patients, but the del allele was associated with coronary artery disease (Wu et al. 1994). A strong linkage disequilibrium between the XbaI and ins/del polymorphisms has been reported (Hansen et al. 1993).

2.3. Apolipoprotein E

Apolipoprotein (apo) E is a 34-kDa protein consisting of 299 amino acids. It is a protein constituent of chylomicrons, very low density lipoproteins and HDL and VLDL remnants (Mahley 1988). On these particles, apo E serves as a ligand for uptake by lipoprotein receptors (Davignon et al. 1988, Mahley 1988,Mahley et al. 1990). Apo E is polymorphic with three common alleles: E2, E3 and E4 (Zannis et al. 1982), which are associated with variations in the blood lipid concentrations. The phenotype E2/2 is associated with type III hyperlipopemia, and E4 is associated with elevated serum total and LDL cholesterol concentrations compared to E2 and E3 (Ehnholm et al. 1986, Utermann 1987, Davignon et al. 1988). The serum concentrations of apo E are higher in individuals with E 3/3 than in individuals with E4, and highest in individuals with E2 (Berglund et al. 1993, Luc et al. 1994).

Apo E polymorphism modifies plasma lipids, at least in Caucasians, partly by affecting the efficiency of cholesterol absorption, so that individuals with E2 absorb less cholesterol than individuals with E4 (Kesäniemi et al. 1987), and individuals with the E4 allele respond better to changes in dietary cholesterol and saturated fatty acids than those without the E4 allele (Lehtimäki et al. 1995). The apo E polymorphism also modifies the metabolism of LDL. Individuals with the apo E phenotype 2/2 catabolise LDL faster than others, and normolipemic apo E 4 homozygotes catabolise LDL at a slower rate than apo E 3 homozygotes (Demant et al. 1991).

The E4 phenotype has been associated with an increased risk of CAD either directly (Kuusi et al. 1989) or via elevated atherogenic lipoprotein levels (Stuyt et al. 1991). The apo E phenotype distribution among CAD patients and myocardial infarction survivors is controversial. Some studies propose a higher frequency of apo E 4 in CAD patients (Nieminen et al. 1992, Wang et al. 1995), or myocardial infarction (AMI) survivors (Cumming and Robertson 1984), whereas others fail to detect any difference (Stuyt et al. 1991, Utermann et al. 1984).
2.4. Reverse cholesterol transport

2.4.1. HDL

HDL is responsible for most of the reverse cholesterol transport in man, as it is the only particle capable of receiving cholesterol from the peripheral cells (Funke 1997).

2.4.2. Apolipoprotein A1

Apolipoprotein A1 is the major lipoprotein in HDL and the primary acceptor for unesterified cholesterol from the peripheral tissues (von Eckardstein et al. 1993, Forte et al. 1993). The plasma apo A1 concentration is lower in smokers than in non-smokers (Dullaart et al. 1994), and exercise training increases the plasma level of apo A1 (Foger et al. 1994). Simvastatin has been reported to increase the plasma apo A1 concentration, especially in patients with nonfamilial hyperlipoproteinemia type II a (Homma et al. 1995). Several epidemiological studies have revealed an inverse relationship between coronary artery disease and the plasma apo A1 concentration (Stampfer et al. 1991, Buring et al. 1992, Amouyel et al. 1993).

2.4.3. Cholesteryl ester transfer protein

The cholesteryl ester transfer protein (CETP) is a glycoprotein synthesised mainly in the liver, but also in the intestine, spleen and adrenal glands. In plasma, CETP is predominantly bound to HDL containing apo-A1-only particles (Bruce et al. 1995). CETP mediates the exchange of HDL cholesteryl esters with triglycerides of apo B containing lipoproteins, and it plays a central role in the reverse cholesterol transport from the peripheral tissues to the liver (Bruce & Tall 1995). CETP gene expression is upregulated in response to increased dietary cholesterol or endogenous hypercholesterolemia (Tall 1995), and its concentration is elevated in hyperlipidemia (Lagrost et al. 1995). CETP plasma levels correlate with total cholesterol, VLDL cholesterol and apo E concentrations (McPherson et al. 1991), and its activity correlates with elevated LDL cholesterol and reduced HDL cholesterol in type IIa and IIb hypercholesterolemia (Tato et al. 1995a). Dietary correction of the lipoprotein changes results in a reduction of the plasma CETP concentration and an increase in HDL cholesterol in chylomicronemia and dysbetalipoproteinemia (McPherson et al. 1991). Alcohol consumption reduces the plasma CETP activity and concentration (Hannuksela et al. 1992). Genetic deficiency of CETP is associated with elevated HDL cholesterol and apolipoprotein (apo) A1 and decreased LDL cholesterol and apo B (Sakai et al. 1995).

It has been suggested that the increase in CETP associated with hypercholesterolemia is secondary to the elevation of serum cholesterol rather than its cause (Bruce & Tall 1995). CETP activity is higher in smokers than in non-smokers (Dullaart et al. 1991, Freeman et al. 1993), and short-term exercise training reduces CETP mass and activity.
(Seip et al. 1993, Foger et al. 1994). However, endurance athletes have higher CETP activity than controls (Gupta et al. 1993), and females have higher CETP levels than males (Marcel et al. 1990), and the prevalence of coronary heart disease is lower in males with low HDL and high CETP activity compared to men with low HDL and normal CETP activity (Tato et al. 1995b), suggesting that although CETP reduces the HDL levels, it seems to have a dominantly anti-atherogenic action \textit{in vivo} (Tall 1995).

### 2.4.4. Apolipoprotein E

The apo E phenotype can modify the reverse cholesterol transport in several mechanisms. Plasma of individuals with apo E 3 is capable of receiving more cholesterol from fibroblasts than plasma of individuals homozygous for E2 or E4 (Huang et al. 1995). Apo E enhances the cholesteryl ester and triglyceride exchange between VLDL and HDL, possibly by enhancing the affinity of CETP for VLDL (Kinoshita et al. 1993). The apo E genotype influences the plasma CETP and HDL cholesterol responses to dietary cholesterol. Normocholesterolemic males with the genotype E 2/3 showed no change in HDL cholesterol, but had an increase of 37% in the CETP response after cholesterol feeding, the respective changes for apo E 3/3 being +4% and +18% and for apo E 4/3 +12% and +9% (Martin et al. 1993).

### 2.5. Lipoprotein(a)

Lp(a) was first discovered in human plasma by Berg (Berg & Mohr 1963). Lipoprotein(a) is synthesised by the liver (Edelstein et al. 1994), it consists of an LDL particle covalently attached to apolipoprotein(a) (apo(a)), and it can also contain apo AI and apo E (Blanco-Vaca et al. 1994). Apo (a) is highly homologous to plasminogen, and the size variation of apo (a) is due to the number of kringle 4 type 2 copies present in the molecule (Gaw & Hobbs 1994). The plasma concentrations of Lp(a) are about 90% genetically determined (Utermann et al. 1987, Sandholzer et al. 1991, Austin et al. 1992, Snyder et al. 1994, Keesler et al. 1994, Rainwater 1995), being inversely related to the number (12-51) of kringle 4 type 2 repeats (Lackner et al. 1991) and the size of the apo (a) protein (Utermann et al. 1987). The catabolism of Lp(a) is less well known. Lp(a) can be degraded by the LDL receptor, but the affinity of Lp(a) for the receptor is low (Snyder et al. 1994). A receptor for Lp(a) has been characterised on human macrophages (Keesler et al. 1994).

High plasma levels of lipoprotein(a) have been positively associated with atherosclerosis (Frick et al. 1978, Dahlén et al. 1986, Armstrong 1986), myocardial infarction (Dahlén et al. 1975, Rhoads et al. 1986, Murai et al. 1986) and stroke (Murai et al. 1986, Woo et al. 1991) in numerous cross-sectional studies. The atherogenity of Lp(a) is at least partly associated with an elevated concentration of LDL cholesterol (Maher & Brown 1995), and a reduction of LDL cholesterol without a change in the Lp(a) concentration associates with slowing down of CAD progression (Thompson et al. 1995). The plasma Lp(a) concentration is higher in individuals with than those without the apo E 4 allele (Ukkola et al. 1993a, de Andrade et al. 1995). Diet has no effect on the serum concentrations of Lp(a), and so far,
neomycin and nicotinic acid are the only hypocholesterolemic drugs with an Lp(a) lowering effect (Gurakar et al. 1985, Carlson et al. 1989, Angelin 1997).

2.6. Association of plasma lipids and lipoproteins with coronary artery disease

Elevated LDL cholesterol and apo B concentrations are associated with an increased risk of coronary artery disease (Lipid Research Clinics Program 1984a, Multiple Risk Factor Intervention Trial Research Group 1986, Canner et al. 1986, Frick et al. 1987, Maher et al. 1997). Conversely, HDL is considered to be antiatherogenic, and elevated serum HDL cholesterol levels are associated with a reduced coronary risk (Gordon et al. 1989). A high total cholesterol-HDL cholesterol ratio is postulated to be a good marker of the other risk-factors for CAD: hypertension, diabetes, smoking and AMI, suggesting that the decrease in HDL cholesterol might be a secondary phenomenon (de Backer et al. 1998, Luria et al. 1991). The role of elevated plasma triglycerides as a risk factor for CAD is controversial, because, although significant in univariate analyses, the triglycerides are usually eliminated by HDL in multivariate statistical tests (Lechleitner et al. 1990, Austin 1991).

Coronary artery disease manifests about ten years later in life in women than in men. One of the preventive mechanisms has been postulated to be the lower triglyceride and higher HDL cholesterol levels in women than in men, which difference becomes less marked after menopause, when the LDL cholesterol levels of women rise (Thompson 1996). Family histories of coronary artery disease and hypertension are more prevalent in women than in men with angiographically verified coronary artery disease, and diabetes is more prevalent in women with CAD than in women with normal coronary arteries (Sullivan et al. 1994). In women, triglycerides have been reported to be an independent CAD risk factor (Nieminen et al. 1992). Premature onset of CAD in men is postulated to be associated with a genetically determined trait characterised by abnormalities in the metabolism of triglyceride-rich lipoproteins and HDL (Richards et al. 1989, Criqui et al. 1993), the trait being more apparent in female than in male relatives of patients with coronary artery disease.

2.7. Pharmacological treatment of hypercholesterolemia

The increased risk of CAD associated with an elevated serum cholesterol level (Multiple Risk Factor Intervention Trial Research Group 1986, Martin et al. 1986) can be reduced by lowering the blood cholesterol concentration (Lipid Research Clinics Program 1984b, Frick et al. 1987, Brown et al. 1990). In diet-resistant hypercholesterolemia, pharmacological treatment should be started at an early age in order to prevent premature coronary artery disease. However, the treatment of hypercholesterolemia is occasionally difficult, as moderate hypercholesterolemia gives no symptoms, and the patients often experience side-effects of cholesterol-lowering drugs (Illingworth 1988, Blane 1989, Simons 1993).
The ideal drug would be efficacious in lowering plasma lipid levels with no subjective side-effects, would have a documented effect on the clinical endpoint, and would be safe on a long-term basis (Olsson & Mölgaard 1988).

2.7.1. Enprostil

Enprostil is a synthetic prostaglandin E2 analogue developed for the treatment of peptic ulcer (Schwartz & Saito 1989). Analyses of data from studies on patients receiving enprostil have demonstrated a significant lowering of serum cholesterol, LDL cholesterol and apo B, while the ratio of HDL cholesterol to total cholesterol has increased or remained unchanged (Schwartz et al. 1988). Additional studies have demonstrated a suppression of alimentary lipemia by enprostil in diabetics (Reaven et al. 1988) and healthy volunteers (Schwartz & Saito 1989).

2.7.2. RS-86505-007

RS-86505-007 is an optic isomer of enprostil. In vitro animal studies suggest that RS-86505-007 may interfere with the active and passive transport of compounds in the small intestine mediated by specific receptor interaction (Grass et al. 1990), suggesting that the site of lipid-lowering action by the drug might be the gastrointestinal tract.

2.7.3. Colestipol

Colestipol prevents the passive and active reabsorption of bile acids, leading to hepatic depletion of bile acids. As a consequence, the hydroxymethylglutaryl (HMG) coenzyme A (CoA) reductase is activated and the LDL receptors are up-regulated, resulting in enhanced catabolism of LDL precursors and a lowering of the LDL cholesterol level. Triglyceride production is increased due to the release of feedback inhibition by bile acids on phosphatidic acid phosphatase (Gaw et al. 1996).

2.7.4. Statins

Statins inhibit the HMG CoA reductase, thus reducing the intrahepatic pool of free cholesterol and reciprocally up-regulating the LDL receptors (Dujovne 1997). They have also been reported to reduce the VLDL apo B production (Watts et al. 1995) and the esterification rate of cholesterol (Homma et al. 1995), and to enhance the catabolism of VLDL prior to its conversion to LDL (Sehayek et al. 1994). Outcome studies with statins (Scandinavian Simvastatin Survival Group 1994, Shepherd et al. 1995, Sacks et al. 1996) have shown reductions in cardiovascular morbidity and all-cause mortality in hypercholesterolemic people.
3. Purpose of the present research

The purpose of the research was to simplify the diagnosis of familial hypercholesterolemia, to study the role of apolipoprotein B and E polymorphisms in hypercholesterolemia and its treatment, and to compare the lipid risk factors of CAD in men and women. More specifically, the aims were to

1. develop a PCR method for the diagnosis of the FH-Helsinki mutation
2. study the metabolism of apolipoprotein B in moderate hypercholesterolemia and the association of its polymorphisms with the plasma lipid and lipoprotein levels
3. compare the lipid risk factors of angiographically verified CAD in men and women
4. compare the efficacy of hypocholesterolemic drugs in patients with different apo B and E polymorphisms and the effect of statin and bile-binding resin treatment on CETP activity
4. Methods

4.1. Patients

The studies were approved by the Ethical Committee of the University of Oulu. All the patients gave their informed consent to the study.

4.1.1. Study I

For study I, a total of 149 consecutive patients with serum cholesterol concentrations above 8 mmol/l referred to the Lipid Clinic of the Oulu University Central Hospital were examined. The presence of tendon xanthomata, a family history of high cholesterol levels, and heart disease or sudden deaths in the first-degree relatives were recorded. The clinical work was completed before the result of the DNA analysis was obtained. Thirty-nine patients with type IV or V hyperlipidemia (fasting plasma triglycerides >4.0 mmol/l) or secondary hyperlipidemia were excluded. The diagnosis of FH was based on the following criteria: serum total cholesterol above 8 mmol/l, LDL cholesterol above the 90th percentile, and tendon xanthomata in the patient or one first-degree relative. The plasma lipids of the study subjects are shown in Table 1.

Table 1. Demographic data and lipid concentrations in patients with primary hypercholesterolemia. Study I. The results are expressed as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>FH patients (N=37)</th>
<th>Non-FH patients (N=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>44±13</td>
<td>54±9</td>
</tr>
<tr>
<td>Sex(male/female)</td>
<td>15/22</td>
<td>22/51</td>
</tr>
<tr>
<td>Tot. Cholesterol mmol/L</td>
<td>9.85±1.37**</td>
<td>8.8±0.62</td>
</tr>
<tr>
<td>LDL cholesterol mmol/l</td>
<td>7.95±1.33**</td>
<td>6.4±0.73</td>
</tr>
<tr>
<td>HDL cholesterol mmol/l</td>
<td>1.28±0.27**</td>
<td>1.64±0.46</td>
</tr>
<tr>
<td>Triglycerides mmol/l</td>
<td>1.38±0.57*</td>
<td>1.69±0.76</td>
</tr>
</tbody>
</table>

FH = familial hypercholesterolemia * = P<0.05 ** = P<0.001
4.1.2. Study II

Forty-nine non-FH patients, 20 women and 29 men, were recruited from the Oulu University Lipid Clinic for study II. All patients were ambulatory throughout the study and they received information on a low-fat, low-cholesterol diet before the study. The patients discontinued their hypocholesterolemic drugs at least two weeks before the study. The patients received 1.0 g of KI daily by mouth in divided doses for 3 days before and throughout the study to inhibit the uptake of radio iodine by the thyroid.

The homologous LDL was obtained from two healthy male volunteers, in whom diseases transmitted through blood transfusion had been excluded. The total cholesterol of donor 1 was 4.62 mmol/l, triglycerides 0.75 mmol/l, and fractional catabolic rate (FCR) for autologous LDL 0.32 pools/day while the values of donor 2 were 4.51 mmol/l, 0.94 mmol/l and 0.37 pools/day, respectively. The FCR of donor 1’s LDL in donor 2 was 0.33 pools/day.

4.1.3. Study III

The patients included in study III had been consecutively referred to one ward of the Oulu University Hospital Department of Medicine for selective coronary angiography because of chest pain or suspected coronary artery disease. The plasma lipid profile and apolipoprotein E phenotype were not determined in 28 patients. In addition, four patients on lipid-lowering therapy were excluded from the study. 122 patients, 95 males and 27 females, with all the data was available were included in the study.

The control group comprised clients of the State Occupational Health Service Station in Oulu, who had volunteered for a survey of lipid values and maximal exercise electrocardiography. Five of the controls were taking beta-blocking agents for hypertension, and this treatment was stopped two days before the examination. The exercise electrocardiography revealed three controls with ST-depressions of more than 1 mm that were planar or down-sloping and persisted for 0.08 seconds after the J point. Their coronary angiographies were normal, and they were included in the control group. The demographic characteristics and medication schedules of the male and female controls and patients with different extensions of coronary artery disease are shown in the Tables 2 and 3.

Table 2. Demographic characteristics and medication of male controls and patients with different extensions of coronary artery disease. Study III.

<table>
<thead>
<tr>
<th></th>
<th>Controls N=33</th>
<th>&lt;50% stenosis N=10</th>
<th>1-vessel stenosis N=19</th>
<th>2-vessel stenosis N=18</th>
<th>3-vessel stenosis N=51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>46 (34-63)</td>
<td>46 (29-58)</td>
<td>51 (37-66)</td>
<td>51 (35-60)</td>
<td>53 (38-64)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9%</td>
<td>11%</td>
<td>21%</td>
<td>35%</td>
<td>25%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0%</td>
<td>11%</td>
<td>10%</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Smokers</td>
<td>36%</td>
<td>56%</td>
<td>78%</td>
<td>88%</td>
<td>84%</td>
</tr>
<tr>
<td>β-blocking agents</td>
<td>6%</td>
<td>38%</td>
<td>94%</td>
<td>92%</td>
<td>78%</td>
</tr>
<tr>
<td>Diuretics</td>
<td>0%</td>
<td>13%</td>
<td>18%</td>
<td>14%</td>
<td>22%</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>0%</td>
<td>63%</td>
<td>72%</td>
<td>64%</td>
<td>73%</td>
</tr>
</tbody>
</table>
27

Table 3. Demographic characteristics and medication of female controls and patients with different extensions of coronary artery disease. Study III.

<table>
<thead>
<tr>
<th></th>
<th>Controls N=27</th>
<th>&lt;50% stenosis N=8</th>
<th>1-vessel stenosis N=6</th>
<th>2-vessel stenosis N=7</th>
<th>3-vessel stenosis N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>45 (36-59)</td>
<td>47 (37-60)</td>
<td>54 (44-61)</td>
<td>55 (47-65)</td>
<td>59 (55-65)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15%</td>
<td>25%</td>
<td>33%</td>
<td>57%</td>
<td>43%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>29%</td>
<td>29%</td>
</tr>
<tr>
<td>Smokers</td>
<td>20%</td>
<td>25%</td>
<td>0%</td>
<td>14%</td>
<td>57%</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blocking agents</td>
<td>11%</td>
<td>63%</td>
<td>83%</td>
<td>83%</td>
<td>100%</td>
</tr>
<tr>
<td>Diuretics</td>
<td>0%</td>
<td>25%</td>
<td>17%</td>
<td>67%</td>
<td>85%</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>0%</td>
<td>50%</td>
<td>83%</td>
<td>50%</td>
<td>86%</td>
</tr>
</tbody>
</table>

4.1.4. Study IV

Patients of either sex between the ages of 18 and 63 were screened for study IV in four locations in Northern Finland. The inclusion criterion for lovastatin treatment was a total plasma cholesterol >5 mmol/l during an adequate diet and colestipol treatment. 203 patients were screened for the study and 150 patients started the lovastatin treatment. Ten patients were taking colestipol less than 20 g/day due to obstipation or abdominal discomfort, one patient had a cholesterol value lower than 5 mmol/l with lovastatin 20 mg daily, one patient had headache with lovastatin 20 mg/day, one patient had slightly elevated creatine kinase with lovastatin 20 mg/day, and one patient discontinued the study during the lovastatin treatment. The flowchart of study IV is shown in Fig. 1, and the demographic characteristics of the patients are given in Table 4. Familial hypercholesterolemia was defined as plasma cholesterol > 8 mmol/l with tendon xanthomata or a family history of severe hypercholesterolemia and/or coronary artery disease at an early age. Thirty-six patients had the CETP activity measured, half of them were males and half females, 26 had polygenic hypercholesterolemia and ten had FH. Ten patients had undergone coronary artery bypass grafting (CABG) or AMI, seven had the clinical diagnosis of angina pectoris, and 19 had no clinical sign of coronary artery disease.
Altogether 209 patients were screened for study V by local occupational health services. Twenty-five patients were taking concomitant medications during the active treatment phase: ten β-blocking agents, nine diuretics, seven calcium antagonists, four aspirin, nine nitrates and three angiotensin convertase inhibitors in different combinations with a stable regimen during the whole study. Demographic data on the patients and controls participating in the active treatment phase are shown in Table 5 and the flowchart in Fig. 2. Only patients with adequate compliance were included in the efficacy analyses. When assessing the influence of the apo B and apo E polymorphisms on drug efficacy and the effect of RS-86505-007 on Lp(a) concentrations, all patients receiving the active drug 6 µg tid for whom DNA was available were included.
Table 5. Demographic and clinical characteristics of patients and controls. Study V.

<table>
<thead>
<tr>
<th></th>
<th>RS-86505-007 3 μg tid</th>
<th>RS-86505-007 6 μg tid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>active drug N=24</td>
<td>active drug N=26</td>
</tr>
<tr>
<td></td>
<td>placebo N=12</td>
<td>placebo N=13</td>
</tr>
<tr>
<td>Male/female</td>
<td>(10/14)</td>
<td>(10/16)</td>
</tr>
<tr>
<td>(range)</td>
<td>(33-68)</td>
<td>(41-72)</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>53</td>
<td>58</td>
</tr>
<tr>
<td>(range)</td>
<td>(33-68)</td>
<td>(35-61)</td>
</tr>
<tr>
<td>BMI, kg/m² (SD)</td>
<td>25 (2)</td>
<td>24 (4)</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>21 (n)</td>
<td>24 (n)</td>
</tr>
<tr>
<td>type II a (n)</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>type II b (n)</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

4.2. Laboratory analyses

4.2.1. Lipid and protein analyses

The lipid and lipoprotein samples were drawn after a 12-hour fast. The values were determined from EDTA blood, from which plasma and cells were separated by routine centrifugation at 2000 rpm for ten minutes. The plasma lipoprotein fractions were isolated by ultracentrifugation in a Beckman 60 Ti rotor (Beckman Instruments, Palo Alto, CA) as described by Havel et al. (Havel et al. 11955). VLDL was isolated at a density of 1.006 g/ml. The cholesterol value was determined enzymatically by using the Boehringer Man-
nheim CHOD-PAP method and the triglycerides by using the Boehringer Mannheim
GPO-PAP method and a Gilford analyser (Gilford Instruments Laboratories Inc., Ober-
lin, Ohio), and HDL cholesterol was determined from the VLDL-free fraction after pre-
cipitating IDL and LDL with heparin-manganese. The protein concentrations of the frac-
tions were measured by the method of Lowry et al. (Lowry et al. 1951). The amount of
apolipoprotein B was determined after isopropanol precipitation (Holmquist & Carlson
1977). In the RS-86505-007 study, the LDL cholesterol was determined from the for-

tula of Friedewald et al. (Friedewald 1972).

4.2.2. Lipoprotein fractionation

The plasma lipid and lipoprotein analyses were carried out on blood samples collected into
EDTA tubes after a 12-hour fast. The plasma lipoprotein fractions were isolated by ultra-
centrifugation in a Beckman 60 Ti rotor (Beckman Instruments, Palo Alto, CA) as described
by Havel et al. (Havel et al. 1955), VLDL at a density of 1.006 g/ml, IDL at 1.006-1.019 g/
ml and LDL at 1.019-1.063 g/ml, using a fixed angle TFT rotor at 108000 x g for 18 hours
at +15°C, and HDL at a density of 1.210 for 48 hours. A separate plasma sample was drawn
simultaneously, from which VLDL was isolated as described above and HDL cholesterol
was determined after precipitation of apolipoprotein B containing lipoproteins with heparin-
manganese. LDL cholesterol (including the IDL fraction) was calculated by subtracting the
HDL cholesterol value from the VLDL infranatant cholesterol value. These lipid values
were used in the lipid comparisons, and also to compensate for the losses of material in
serial ultracentrifugations while calculating the apolipoprotein B concentration for LDL
production. The lipoprotein particle compositions were calculated using the lipid and pro-
tein concentrations obtained from the serial ultracentrifugations.

4.2.3. LDL isolation

LDL for the turnover studies was isolated from 80 ml of plasma with sequential ultracen-
trifugations. First, the plasma was adjusted to a density of 1.019 g/ml and centrifuged at
59000 x g and +15°C for 18 h. The infranatant was then adjusted to a density of 1.063 and
centrifuged as above. The supernatant was recentrifuged after adjustment with NaCl-
NaBr solution (1:1) to a density of 1.070 at 35000 x g and +15°C for 18h. The protein
concentration was determined from 100 µl of LDL, and the rest was dialysed against
0.9% saline, pH 7.4, for 1.5 hours with three changes of dialysate.

4.2.4. Radioiodination

Radiolabeling of LDL was carried out using a modification (Bilheimer et al. 1972) of the
iodine monochloride method of McFarlane (McFarlane 1958). The excess iodine was
removed by column chromatography (PD-10 Sephadex G-25M, Pharmacia, Uppsala,
Sweden) and EDTA saline dialysis. Precipitation with 10% trichloroacetic acid showed that 94.0 (2.2) % (mean and SD) of the $^{125}$I counts and 96.2 (2.4)% of the $^{131}$I counts were bound to protein. The lipid labelling was 10.3 (3.7)% for $^{125}$I and 12.2 (5.4)% for $^{131}$I, as determined with a chloroform-methanol solvent (2:1) by using the method of Folch et al. (Folch et al. 1957).

After labelling, LDL was sterilised by filtration through a 0.45 µm filter. In half of the turnover studies, autologous LDL was labelled with $^{125}$I and homologous LDL with $^{131}$I, and vice versa.

### 4.2.5. Kinetic analysis

The fractional catabolic rates, determined as the intravascular pool of LDL catabolised per day, for autologous and homologous LDL were calculated from the plasma decay curves using the Matthews method (Matthews 1957) adapted for LDL turnover studies (Langer et al. 1972). In the model, two exponential equations are fitted to each plasma decay curve using an interactive curve-peeling program (W.F. Beltz & T.E. Carew, unpublished method) on a VAX-VMS Computer. The LDL apo B production rates were calculated from the autologous FCR, pool volume and LDL apo B concentration and expressed as milligrams per kilogram of body weight per day.

### 4.2.6. Lipoprotein(a) measurement

The lipoprotein(a) concentration was measured by using the Pharmacia Apolipoprotein(a) RIA 100 assay system. The assay is a solid-phase, two-site immunoradiometric assay that uses two monoclonal antibodies directed towards different epitopes of apolipoprotein(a). The standard curve range was 16.8–840 U apolipoprotein(a)/l. The apo(a) values were multiplied by 0.7 and divided by ten to get the Lp(a) concentrations in mg/dl.

### 4.2.7. Apolipoprotein E phenotyping

The apo E phenotypes were determined from plasma after delipidation using isoelectric focusing and immunoblotting techniques (Menzel & Uterman 1986).

### 4.2.8. Apolipoprotein A1 measurement

The apolipoprotein A1 concentration was determined nephelometrically by measuring the turbidity of the apo A1 antigen-antibody complex with the Turbox method (Orion Diagnostica).
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4.2.9. Cholesteryl ester transfer protein activity

CETP activity was determined as described by Savolainen *et al.* (Savolainen *et al.* 1990), using the method of Groener (Groener *et al.* 1986), where VLDL+LDL-free plasma is incubated with radioactively labelled LDL and unlabelled HDL. CETP activity is expressed as nmol of cholesteryl esters transferred from LDL to HDL per hour per ml of plasma.

4.2.10. Apolipoprotein B polymorphisms

The apo B polymorphisms were detected by using the polymerase chain reaction (PCR). Blood samples for the apo B polymorphisms were collected into EDTA tubes and stored at -20°C until analysed. DNA was prepared by the salting-out method described by Miller *et al.* (Miller *et al.* 1988). A primer pair (5’ primer, nucleotides 7336→7359, 5’ACCAAGGCCACAGTGCTGAT3’; 3’ primer, nucleotides 7665→7642, 5’CTCTACCAATGCTTTCTACAGTTTAG3’) was used to amplify a 330 base-pair product containing the XbaI polymorphism site (Thr2488) of the apo B gene (Priestley *et al.* 1985, Blackhart *et al.* 1986), and another pair of primers (5’ primer, nucleotides 12295→12318, 5’ATCGACGTAGTTCCAGAAAGCA3’; 3’ primer, nucleotides 12684→12661, 5’GAAAGGAATGTTAACACTAGGCTTT3’) was used to amplify a 390 base-pair product containing the EcoRI polymorphism site (Glu4154→Lys) at the apo B gene as described by Ukkola *et al.* (Ukkola *et al.* 1993b). This method with a 5’ primer, nucleotides 10587→10602 5’ACCTGTACCTTCTCATGATCTC3’; and a 3’ primer, nucleotides 11178→11154 5’ATCCTCTACACCTTTGCTTAGACT3’) was also used to amplify a 592 base-pair product containing the MspI polymorphism site Arg3611→Gln in the apo B gene. The ins/del polymorphism was detected as described by Boerwinkle (Boerwinkle *et al.* 1991b), except for the gel electrophoresis, which was done on 2% Agarose+6% NuSieve gel.

The apo B-3531 Arg→Cys mutation was detected using the NsiI restriction site polymorphism described by Pullinger *et al.* (Pullinger *et al.* 1995) and the MspI oligonucleotides described above. The apo B 3500 G to A mutation was detected using the method described by Tybjaerg-Hansen *et al.* (Tybjaerg-Hansen *et al.* 1990), with the exception of the allele-specific oligonucleotides used to detect the presence of the G to A mutation at position 10699, which were two basepairs longer than those used by Tybjaerg-Hansen (5’AGCACACGGTCTTCA3’ for the normal allele and 5’ AGCACACAGTCTTCA3’ for the mutant allele). The filters were exposed to x-ray films (Kodak X-Omat AR) for 24 hours at -70°C.

DNA amplification was carried out in a final volume of 50 µl using 0.25 µg of genomic DNA and 20 nmol of each of the primers. The 4 deoxynucleotides (dATP, dCTP, dGTP, dTTP) were present at a final concentration of 200 µmol/l. The reaction buffer was that recommended by the manufacturer. The amplification reaction was started by the addition of 0.5 units of Taq polymerase (Dynazyme, Finnzymes OY, Espoo, Finland). Annealing, extension and denaturing were carried out at 3 temperatures using an automatic thermal cycler (Perkin-Elmer Cetus, CT, USA). After the first cycle of 5 min at
95°C, 1 min at 60°C and 2 min at 72°C, thirty-nine cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C were carried out for each reaction. The PCR products were digested at 37°C for 1 h using 25 µl of each PCR amplified product with 10 units of the restriction enzyme (New England Biolabs, Inc., Beverly, MA, USA). The digestion products were electrophoreses at 75 V for 1.5 h through a 1.5 percent agarose gel stained with ethidium bromide and visualised by ultraviolet light.

### 4.2.11. FH-Helsinki and North Karelia

Oligonucleotides were synthesised with the methoxyphosphoamidite method on an Applied Biosystems 380A DNA synthesiser. The oligonucleotides were deprotected by treatment in 25% (w/v) ammonia, recovered by precipitation with ethanol, and finally dissolved in 10 mM TRIS-HCl, 1mM EDTA, pH 7.5, at a concentration of 20 µmol/µl and stored at -20°C until used. The first two oligonucleotide primers, LR05 5’d(CAGCTCACAGCGGTAAGGACACAGC) and LR06 5’d(ACTCTGAACTGAGAAAGTGCAAGGAG), span the positions 2193 to 2218 within exon 15 of the coding strand of the LDL receptor gene and the positions 3391 to 3416 within exon 18 of the non-coding strand, respectively. In the presence of the FH-Helsinki mutation in the LDL receptor gene, the PCR technique yields a product 391 base pairs in length, whereas in the absence of the mutation, the product would be over 9000 bp long and thus beyond the scope of the amplification reaction. The other two primers, AE03 5’d(AGACGCGGGCGACACGGCTGTTCAAGGA) and AE04 5’d(CCTCGCGGGCCCCGGCCTGGTACACT), correspond to sequences of the apolipoprotein E gene and serve to control the adequacy of the PCR amplification. The PCR product of the apo E gene is 244 bp in length. Another pair of oligonucleotides was designed to detect the FH-North Karelia mutation. The 5’ primer was a sequence of intron 5 and exon 6 (5’CTCTGGCTCTCACAGTGACACTCT3’). The 3’ primer (5’ATTCTGACTCACCACACTCTTTTCA3’) was designed to match the mutated gene, so that the last three (or four) nucleotides of the 3’ end of the primer were located immediately after the deletion site.

### 4.2.12. Polymerase chain reaction

Target sequences were amplified in 100µl of reaction mixture containing 0.5-1.0µg genomic DNA, 20 nmol of each deoxyribonucleotide, 10 mmol/l TRIS-HCl, pH 8.3, 0.01% (w/v) gelatine, 50 mmol/l KCl, 1.5 mmol/l MgCl2, 100 pmol of each amplification primer, and 2.5 units of Taq polymerase (Ampli-Taq, Perkin Elmer cetus, Norwalk, CT, USA). The first cycle comprised a denaturing step at 94°C for 5 min, annealing at 60°C for 5 min and primer extension at 70°C for 2 min in a programmable thermal cycler (Perkin Elmer Cetus). The subsequent 39 cycles consisted of 1 min denaturation at 94°C, 30 s annealing at 60°C, and 30 s elongation at 70°C. The primer extension of the 40th cycle was extended to 10 min.
4.2.13. Gel electrophoresis

The PCR product (18 µl) was fractionated by gel electrophoresis in 1.5%(w/v) agarose gel in 40 mM TRIS-acetate, pH 7.8, 1 mM EDTA, until the bromophenol blue marker dye reached the 5 cm level. A 123 bp DNA ladder (GIBCO/BRL, Grand Island, NY) was used as a standard to provide estimates of molecular size. The DNA-ethidium-bromide complex was visualised by ultraviolet fluorescence and photographed to give a permanent record.

4.2.14. Selective coronary angiography

Coronary angiography was performed using the Judkins method and analysed according to the clinical routine of the hospital. A luminal narrowing of 50% or more was defined as a significant lesion, and the patients have been referred to as the severe CAD group. Patients with lesions with less than 50% luminal narrowing were considered not to have significant coronary artery disease and they have been referred to as the moderate CAD group. Three-vessel disease involved significant lesions in all the three vessels; the left main or the left anterior descending artery with its diagonal branches, the left circumflex artery with its marginal branches, and the right coronary artery. If two of the major branches or the left main coronary artery were involved, the patient was defined as having two-vessel disease, and if only one of the branches was involved, the patient was defined as having one-vessel disease.

4.2.15. Additional laboratory analyses

The haematologic tests were done in the local hospital laboratories. Urine was checked semi-quantitatively for sugar and protein. The values for liver function (SGOT, SGPT, GGTP and alkaline phosphatase), thyroid function (total T4, free thyroxin, thyroid stimulating hormone), plasma total proteins, serum glucose, fructosamine, uric acid, urea, creatinine, albumin, haptoglobin, iron, total iron binding capacity, transferrin, lactate dehydrogenase, phosphorous, calcium, potassium and bilirubin were determined at the Calab laboratories in Stockholm, Sweden. Blood pressure was measured manually by one of the two nurses after at least 15 minutes’ rest in a sitting position.

4.3. Statistical methods

All statistical analyses were performed using the SAS program (SAS Institute Inc., Cary, NC, USA).

In study I, all values were expressed as mean ± standard deviation (SD). The statistical significance of the results was calculated using Student's t-test.
When comparing the effects of the polymorphisms in study II, the values were expressed as mean ± standard deviation, except for Lp(a), for which the median and range were given. The quantitative variables were compared using the t-test or the analysis of variance with Tukey’s Studentized Range test, while Lp(a) and triglycerides were analysed with the nonparametric tests of Wilcoxon or Kruskal-Wallis. Proportional data were compared using the χ² test.

In study III, the values for total, LDL and HDL cholesterol were expressed as mean ± standard deviation and those for Lp(a), total triglycerides, VLDL cholesterol and VLDL triglycerides as median and range. The quantitative variables were compared using the t-test. For multiple pairwise comparisons, analysis of variance was used, followed by pairwise comparisons with Tukey’s Studentized Range test. Proportions were compared using the χ²-test or the Cochran-Mantel-Haenszel test. Logarithmic transformation was applied to plasma total triglycerides, VLDL triglycerides and VLDL cholesterol because of their skewed distributions. Due to the different age distributions of the patient groups, the values of total cholesterol, LDL cholesterol, HDL cholesterol and plasma total triglycerides were age-adjusted for the statistical analyses by linear regression. Stepwise logistic procedure and ranked stepwise logistic procedure were used to compare the lipid risk profiles of the male and female controls and the patients with CAD and different extensions of CAD, respectively. Age, smoking status, hypertension and apolipoprotein E phenotype were forced as parameters into each model, and the lipids included were lipoprotein(a), HDL cholesterol, LDL cholesterol, VLDL cholesterol and VLDL triglycerides.

In study IV, the lipid values were expressed as mean (SD). The quantitative variables were compared using the t-test or the analysis of variance with Tukey’s Studentized Range test, and the analysis of covariance was performed using the general linear models procedure. Logarithmic transformation was applied to the triglycerides due to their skewed distributions. The relationships between variables were measured by the Pearson correlation. Due to the small number of patients, the apo E 2/3 and 3/3 phenotypes were grouped as one group and 3/4 and 4/4 as one group for the statistical comparisons involving CETP.

In study V, the sample size for evaluating the cholesterol-lowering effect of RS-86505-007, 24 for each group, was based on the following power calculations: a 11 % detectable difference between the groups in mean total cholesterol and a 12 % difference in the LDL cholesterol level, and 4 % within groups for total cholesterol and 5 % for LDL cholesterol. The means of the three measurements of total cholesterol, HDL cholesterol and triglycerides made during the diet period were used as the baseline values in the analysis. Each of the sections of the trial (3 µg and 6 µg dose groups) was analysed separately, the main analysis being the comparison of RS-86505-007 and placebo with respect to the change from the baseline to the end of treatment. Patients with triglyceride values higher than 3.95 mmol/l were excluded from the analyses of HDL and LDL cholesterol. The percentage changes were tested using a two-way analysis of variance with a model including the centre, the treatment and their interactions. All statistical tests were two-tailed and considered to be significant with a 95 % confidence interval.

Paired t-test and the analysis of variance were used in the comparisons of lipid changes between the polymorphism groups. The Lp(a) concentrations were compared with the t-test.
5. Results

5.1. Detection of FH Helsinki by polymerase chain reaction

Plasma lipids and the presence of tendon xanthomata in patients with or without the FH-Helsinki mutation detected by the PCR method are shown in Table 6. Out of the 37 patients in the FH group, 30 had tendon xanthomata. The FH-Helsinki mutation was present in 25 (23%) of the 110 patients, 23 of whom had clinically confirmed FH.

Table 6. Plasma lipids and presence of tendon xanthomata in patients with or without the FH-Helsinki mutation.

<table>
<thead>
<tr>
<th></th>
<th>FH patients</th>
<th>Non-FH patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FH-Helsinki+</td>
<td>FH-Helsinki-</td>
</tr>
<tr>
<td>No.of patients</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>Xanthomata</td>
<td>83%(19)</td>
<td>79%(11)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10.10±1.59</td>
<td>9.44±0.80</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>8.24±1.45</td>
<td>7.47±0.95</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.26±0.30</td>
<td>1.33±0.23</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.35±0.62</td>
<td>1.44±0.49</td>
</tr>
</tbody>
</table>

5.2. Catabolism of LDL

The mean(SD) FCR for autologous LDL was 0.29(0.06) and that for homologous LDL 0.27 (0.05) pools/day. Twelve patients cleared homologous LDL 0.8 to 19.3 % faster than their own, while all the others cleared their own LDL 1.0-21.6% faster than homologous LDL.

The proportion of individuals with the different apo B and apo E genotypes catabolizing homologous LDL faster than their own is shown in Fig. 3, and the LDL decay curves of the patients with the greatest differences between autologous and homologous LDL are shown in Fig. 4.
Fig. 3. Numbers of patients catabolising autologous LDL faster than homologous LDL according to the XbaI, EcoRI, Apo E, Ins/del and MspI genotypes.

Fig. 4. Difference between the catabolic rates of autologous and homologous LDL.
5.3. Association of the apo B structure with plasma cholesterol metabolism

5.3.1. Mutations

Neither the apo B-3500 mutation nor the apo B-3531 mutation were detected in this population.

5.3.2. Polymorphisms

5.3.2.1. XbaI

The effects of the XbaI polymorphism of apolipoprotein B on the lipid values, lipoprotein composition, LDL apo B contents and production, and autologous and homologous FCR are shown in Table 7. The XbaI genotype -/- was associated with lower plasma levels of total, IDL and LDL cholesterol compared to the other genotypes, the differences being statistically significant between the homozygous XbaI -/- and the heterozygous XbaI +/- genotypes. Also, the chemical composition of the apo B containing lipoprotein particles was associated with XbaI RFLP. The protein-cholesterol ratio was high and the cholesterol-triglyceride ratio low in XbaI -/- homozygotes, and their apo B concentration was also low. There was a tendency towards higher autologous LDL FCR in the patients with XbaI -/- compared with the other genotypes.
Table 7. Lipid values, lipoprotein composition, and LDL apoB metabolism by XbaI genotype.

<table>
<thead>
<tr>
<th></th>
<th>XbaI -/-</th>
<th>XbaI +/-</th>
<th>XbaI +/+</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>10/4</td>
<td>17/12</td>
<td>2/4</td>
<td>0.51</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 (3.0)</td>
<td>27.5 (3.5)</td>
<td>26.7 (3.6)</td>
<td>0.65</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tot</td>
<td>7.03* (0.82)</td>
<td>8.11* (1.09)</td>
<td>7.96 (0.85)</td>
<td>0.006</td>
</tr>
<tr>
<td>VLDL</td>
<td>1.08 (0.75)</td>
<td>1.37 (0.84)</td>
<td>0.84 (0.33)</td>
<td>0.23</td>
</tr>
<tr>
<td>IDL</td>
<td>0.37 (0.18)</td>
<td>0.65* (0.4635)</td>
<td>0.39 (0.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL</td>
<td>4.35* (0.68)</td>
<td>5.05* (0.92)</td>
<td>5.29 (0.74)</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL</td>
<td>1.37 (0.40)</td>
<td>1.16 (0.30)</td>
<td>1.43 (0.32)</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tot</td>
<td>1.98 (1.06)</td>
<td>2.25 (1.17)</td>
<td>1.62 (0.50)</td>
<td>0.36</td>
</tr>
<tr>
<td>VLDL</td>
<td>1.08 (0.73)</td>
<td>1.20 (0.82)</td>
<td>0.75 (0.40)</td>
<td>0.42</td>
</tr>
<tr>
<td>IDL</td>
<td>0.12 (0.04)</td>
<td>0.15 (0.08)</td>
<td>0.11 (0.03)</td>
<td>0.21</td>
</tr>
<tr>
<td>LDL</td>
<td>0.37 (0.09)</td>
<td>0.49 (0.19)</td>
<td>0.36 (0.07)</td>
<td>0.04</td>
</tr>
<tr>
<td>Prot/chol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.81 (0.21)</td>
<td>0.68 (0.16)</td>
<td>0.70 (0.10)</td>
<td>0.08</td>
</tr>
<tr>
<td>IDL</td>
<td>0.66 (0.15)*</td>
<td>0.53 (0.16)*</td>
<td>0.56 (0.14)</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL</td>
<td>0.65 (0.11)</td>
<td>0.70 (0.12)</td>
<td>0.60 (0.06)</td>
<td>0.10</td>
</tr>
<tr>
<td>HDL</td>
<td>3.39 (0.73)</td>
<td>3.79 (0.79)</td>
<td>3.23 (0.40)</td>
<td>0.14</td>
</tr>
<tr>
<td>Chol/trigly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.30 (0.05)</td>
<td>0.36 (0.10)</td>
<td>0.37 (0.08)</td>
<td>0.06</td>
</tr>
<tr>
<td>IDL</td>
<td>1.38 (0.43)*</td>
<td>1.93 (0.73)*</td>
<td>1.51 (0.34)</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL</td>
<td>5.44 (1.74)</td>
<td>5.04 (1.91)</td>
<td>6.49 (0.98)</td>
<td>0.20</td>
</tr>
<tr>
<td>Lipoprotein (a)ξ (mg/dl)</td>
<td>24 (2.85)</td>
<td>14 (0.5-143)</td>
<td>28 (2-87)</td>
<td>0.54</td>
</tr>
<tr>
<td>LDL apoB (mg/dl)</td>
<td>111* (19)</td>
<td>146* (28)</td>
<td>121 (12)</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL apo B production (mg/kg/day)</td>
<td>15.1 (4.5)</td>
<td>16.5 (3.9)</td>
<td>13.9 (2.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>LDL apoB FCR (pool/day)</td>
<td>0.30 (0.05)</td>
<td>0.28 (0.06)</td>
<td>0.28 (0.02)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

ξ median and range. Triglyceride and Lp(a) comparisons are non-parametric (Kruskal-Wallis). * difference between the groups is statistically significant. FCR=fractional catabolic rate.

5.3.2.2. EcoRI

The EcoRI genotype +/+ was associated with higher total, VLDL and LDL cholesterol than the genotypes +/- and -/-, but the differences were not statistically significant. The chemical composition of the lipoprotein particles was quite similar in the genotype groups, the only difference being the higher cholesterol-triglyceride ratio in the subjects with the EcoRI +/+ genotype. The autologous FCR of LDL apo B was significantly lower in the subjects with the EcoRI +/+ genotype compared to EcoRI +/-, 0.27 (0.05) and 0.31 (0.06) pools/day, respectively.
5.3.2.3. MspI and ins/del

The MspI and ins/del polymorphisms were not associated with variations in the lipid or apo B concentrations, lipoprotein particle composition or catabolism of LDL apo B.

5.3.2.4. Influence of XbaI and EcoRI on the hypolipidemic capacity of RS-86505-007

The influence of the apolipoprotein B gene XbaI and EcoRI polymorphisms on the lipid-lowering capacity of RS-86505-007 was studied in patients receiving the drug 6 µg i.d.

Patients homozygous for the XbaI restriction site tended to have smaller reductions in total and LDL cholesterol than those who had only one or no allele with the cutting site, although the differences were not statistically significant. No differences were detected in the lipid changes of patients with or without the EcoRI restriction site.

5.4. Apolipoprotein E

5.4.1. Allele distribution

The apolipoprotein E allele distribution of the individuals in study IV is given in Table 4, and the allele frequencies of the male and the female CAD patients and controls are given in Tables 8 and 9 respectively. This series included no patients with the apo E phenotype 2/2, and all the CAD patients with an ε 2 allele were smokers.

Table 8. Lipid values and apolipoprotein E gene frequencies of male controls and patients with different extensions of coronary artery disease.

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=33)</th>
<th>&lt;50% stenosis (N=10)</th>
<th>1-vessel stenosis (N=19)</th>
<th>2-vessel stenosis (N=18)</th>
<th>3-vessel stenosis (N=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>5.6 (0.9)</td>
<td>5.8 (1.2)</td>
<td>6.6 (1.1)</td>
<td>6.1 (1.1)</td>
<td>6.4 (1.3)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.7 (0.9)</td>
<td>3.7 (1.2)</td>
<td>4.5 (0.9)*</td>
<td>4.8 (0.7)*</td>
<td>4.5 (1.1)*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.2 (0.2)</td>
<td>1.1 (0.3)</td>
<td>1.1 (0.2)*</td>
<td>1.0 (0.2)*</td>
<td>1.0 (0.3)*</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5*</td>
<td>0.5</td>
<td>0.6*</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>1.4</td>
<td>1.8*</td>
<td>1.7*</td>
<td>1.8*</td>
<td>2.80 (0.029)</td>
</tr>
<tr>
<td>LDL triglycerides</td>
<td>0.9</td>
<td>1.2*</td>
<td>1.0*</td>
<td>1.1*</td>
<td>4.48 (0.002)</td>
</tr>
<tr>
<td>Lp(a)&gt;35mg/dl (%)</td>
<td>15</td>
<td>20</td>
<td>28</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

The values for total, LDL and HDL-cholesterol are expressed as mmol/l, mean and (SD), and those for total triglycerides, VLDL cholesterol and VLDL triglycerides as mmol/l, median and range. For the statistical analyses, total cholesterol, LDL cholesterol, HDL cholesterol and total triglycerides were age-adjusted. Logarithmic transformation was done for the analyses of total triglycerides, VLDL cholesterol and VLDL triglycerides. * p<0.05 compared to controls.
Table 9. Lipid values and apolipoprotein E gene frequencies of female controls and patients with different extensions of coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>Controls N=27</th>
<th>&lt;50% stenosis N=8</th>
<th>1-vessel stenosis N=6</th>
<th>2-vessel stenosis N=7</th>
<th>3-vessel stenosis N=7</th>
<th>F-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>5.6 (1.1)</td>
<td>6.3 (2.2)</td>
<td>6.2 (1.2)</td>
<td>7.4 (1.4)</td>
<td>6.8 (1.2)</td>
<td>1.85</td>
<td>0.135</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.6 (1.0)</td>
<td>4.1 (1.1)</td>
<td>4.4 (1.1)</td>
<td>5.0 (1.0)</td>
<td>4.4 (1.3)</td>
<td>1.89</td>
<td>0.127</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.6 (0.4)</td>
<td>1.3 (0.4)</td>
<td>1.1 (0.2)*</td>
<td>1.3 (0.7)</td>
<td>1.1 (0.3)*</td>
<td>4.47</td>
<td>0.003</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.2 (0.1-0.5)</td>
<td>0.3 (0.1-2.2)</td>
<td>0.3 (0.2-1.1)</td>
<td>0.7* (0.2-1.2)</td>
<td>1.0* (0.5-2.3)</td>
<td>8.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>0.9 (0.4-1.9)</td>
<td>1.5 (0.5-5.2)</td>
<td>1.4 (1.0-2.9)</td>
<td>2.0* (0.9-3.1)</td>
<td>2.6* (1.5-5.6)</td>
<td>8.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>0.4 (0.1-1.1)</td>
<td>0.7 (0.2-2.3)</td>
<td>0.7* (0.5-1.8)</td>
<td>1.6* (0.3-2.1)</td>
<td>1.7* (1.2-3.7)</td>
<td>11.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lp(a) &gt;35mg/dl (%)</td>
<td>7</td>
<td>12</td>
<td>13</td>
<td>33</td>
<td>0</td>
<td>3.67</td>
<td>0.45</td>
</tr>
<tr>
<td>ε4</td>
<td>0.19</td>
<td>0.13</td>
<td>0</td>
<td>0.29</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε3</td>
<td>0.80</td>
<td>0.88</td>
<td>1.0</td>
<td>0.71</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values for total, LDL, and HDL cholesterol are expressed as mmol/l, mean and (SD), and those for total triglycerides, VLDL cholesterol and VLDL triglycerides as mmol/l, median and range. For the statistical analyses, total cholesterol, LDL cholesterol and total triglycerides were age-adjusted. Logarithmic transformation was done for the analyses of total triglycerides, VLDL cholesterol and VLDL triglycerides. δ: p<0.05 compared to 1vessel stenosis, ξ p<0.05 compared to moderate disease, * p<0.05 compared to controls.

5.4.2. Association of apo E phenotypes with lipid and lipoprotein concentrations

No statistically significant differences in the plasma total cholesterol, HDL cholesterol or triglyceride values of the CAD patients or the controls were observed in the different apo E groups (phenotypes 2/3 and 2/4, 3/3, and 4/3 and 4/4).

The apo E allele 4 was associated with higher cholesterol concentrations in VLDL and IDL, higher total plasma triglycerides and higher triglyceride concentration in VLDL, IDL and LDL. The protein-cholesterol ratio of LDL was higher and the cholesterol-triglyceride ratio lower in the individuals with an apo E 4 allele. Also, the apo B concentrations of the subjects with at least one apo E 4 allele were higher than in those in the subjects with the apo E 3/3 phenotype.
5.4.3. Fractional catabolic rate of LDL in individuals with different apo E phenotypes

We were unable to detect a difference in the FCR of LDL between patients having the apo E 4 allele and those homozygous for apo E 3. However, in three of the four patients with apo E 4/4, the autologous particle was cleared at a slower rate than the homologous apo E 3/3.

5.4.4. Influence of apo E polymorphism on hypolipidemic drug response

The influence of the apo E polymorphism on the lipid-lowering capacity of RS-86505-007 was studied in patients receiving the drug 6 µg tid. The patients with the apo E phenotype 3/3 had larger reductions in total and LDL cholesterol compared to those with at least one ε 4 allele, but these differences were not statistically significant (Table 10).

Table 10. Plasma total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol concentrations before treatment and their mean reductions during treatment in patients receiving RS-86505-007 6 µg tid according to apolipoprotein E polymorphisms.

<table>
<thead>
<tr>
<th>Apo E</th>
<th>E 3/3 (N=14)</th>
<th>E 4/*1 (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tot.chol.</td>
<td>8.09(0.61)</td>
<td>7.78(1.08)</td>
</tr>
<tr>
<td>Δcholesterol</td>
<td>1.49(0.95)</td>
<td>0.61(1.50)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.44(0.70)</td>
<td>1.37(0.46)</td>
</tr>
<tr>
<td>Δtriglyceride</td>
<td>0.26(0.46)</td>
<td>0.13(0.31)</td>
</tr>
<tr>
<td>HDL chol.</td>
<td>1.59(0.44)</td>
<td>1.63(0.21)</td>
</tr>
<tr>
<td>ΔHDL</td>
<td>0.02(0.22)</td>
<td>0.14(0.29)</td>
</tr>
<tr>
<td>LDL chol.</td>
<td>5.85(0.63)</td>
<td>5.53(1.07)</td>
</tr>
<tr>
<td>ΔLDL</td>
<td>1.35(1.00)</td>
<td>0.42(1.32)</td>
</tr>
</tbody>
</table>

The values are expressed as mmol/l (SD). The differences are not statistically significant.
1 E 4/* = subjects with E4/3 or E 4/4, 2Δ= change due to treatment.

The lipid concentrations of the patients did not differ statistically significantly in the apo E groups before or after colestipol and lovastatin treatment. Hypertriglycerideremia was only associated with a reduced HDL cholesterol level in the patients lacking the apo E 4.
5.5. Cholesteryl ester transfer protein

5.5.1. Activity in different sexes and different types of hypercholesterolemia

CETP activity was higher in females (201 (53) nmol/h/l) than in males (162 (45) nmol/h/l) with p=0.03, but no differences were detected between the sexes during colestipol or lovastatin treatment. The CETP activities or changes during the drug treatment were similar in non-FH and FH patients and in patients with type II a and II b hypercholesterolemia.

5.5.2. Effect of apo E phenotypes on cholesteryl ester transfer protein activity and drug response

The CETP activity was 183 (59) nmol/h/ml in the apo E 2/3 and 3/3 patients and 179 (43) nmol/h/ml in the patients with the E4 allele. Colestipol reduced plasma CETP activity by 19.5% in the patients with apo E 2/3 or 3/3, and by 13.3% (p=0.41) in the patients with apo E 3/4 or 4/4, the corresponding reductions for lovastatin being 19.4% and 20.0% (p=0.99). The best correlations between the CETP basal activity and the lipids were observed with LDL and total cholesterol after lovastatin treatment in the patients with apo E 2/3 or 3/3 (Table 11). The CETP change correlated well with the HDL cholesterol change during colestipol, but not lovastatin treatment in both the patients with the apo E 4 allele and those without it (Fig. 5).

Table 11. Pearson correlation of lipids and their changes with the cholesteryl ester transfer protein basal activity according to apo E phenotype

<table>
<thead>
<tr>
<th></th>
<th>Apo E 2/3 and 3/3</th>
<th>Apo E 4/3 and 4/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.52 p=0.01</td>
<td>0.13 p=0.65</td>
</tr>
<tr>
<td>Colestipol</td>
<td>0.51 p=0.02</td>
<td>0.04 p=0.90</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.65 p=0.001</td>
<td>0.57 p=0.03</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.49 p=0.02</td>
<td>0.07 p=0.81</td>
</tr>
<tr>
<td>Colestipol</td>
<td>0.42 p=0.06</td>
<td>-0.09 p=0.77</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.65 p=0.0009</td>
<td>0.23 p=0.42</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.26 p=0.24</td>
<td>0.17 p=0.56</td>
</tr>
<tr>
<td>Colestipol</td>
<td>0.16 p=0.48</td>
<td>0.45 p=0.11</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.36 p=0.10</td>
<td>0.32 p=0.27</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.27 p=0.22</td>
<td>0.13 p=0.65</td>
</tr>
<tr>
<td>Colestipol</td>
<td>0.21 p=0.36</td>
<td>0.16 p=0.59</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>-0.001 p=0.99</td>
<td>0.44 p=0.11</td>
</tr>
</tbody>
</table>
5.6. Lipoprotein(a)

5.6.1. Effect of Lp(a) on the severity of CAD

To evaluate the effect of Lp(a) on the severity of CAD, the population was divided into two groups according to the Lp(a) value (Lp(a) ≥ 35 mg/dl and Lp(a) < 35 mg/dl). Twelve percent of the controls, 17% of the patients with < 50% stenosis and 29% of the patients with > 50% stenosis had high (≥ 35 mg/dl) Lp(a) ($\chi^2 = 5.482$ and $p = 0.019$).
5.6.2. Effect of apo E phenotype on Lp(a) concentration

High Lp(a) concentrations were most frequent among the patients with the apo E 3/4 or 4/4 phenotype, whilst none of the controls with the apo E 2/3 or 2/4 phenotype had an Lp(a) concentration higher than 35 mg/dl (Table 12).

Table 12. Apolipoprotein E phenotypes in controls and patients with high lipoprotein(a)

<table>
<thead>
<tr>
<th></th>
<th>Controls Lp(a)≥35mg/dl (%)</th>
<th>Patients Lp(a)≥35mg/dl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E 3/4 or 4/4</td>
<td>13</td>
<td>38</td>
</tr>
<tr>
<td>E 3/3</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>E 2/3 or 2/4</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E 3/4 or 4/4</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>E 3/3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>E 2/3 or 2/4</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

* Cochran-Mantel-Haenszel statistics value=11.6 p=0.001, ** Cochran-Mantel-Haenszel statistics value=2.4 p=0.11.

5.6.3. Effect of RS-86505-007 on Lp(a) concentration

The effect of RS-86505-007 on the Lp(a) concentration was studied in patients receiving the higher 6 µg dose. The median Lp(a) concentration was 11.9 mg/dl (range 1.4-44) after the diet phase and 13.1 mg/dl (range 1.4-49) after drug treatment, but the difference was not statistically significant.

5.7. Coronary artery disease

5.7.1. Plasma lipids of male CAD patients

The men with CAD had a more atherogenic plasma lipid profile than the controls. The patients had higher plasma total cholesterol (6.3 vs. 5.6 mmol/l, p=0.03), lower HDL cholesterol (1.0 vs. 1.2 mmol/l, p<0.001) and higher LDL cholesterol (4.5 vs. 3.7 mmol/l, p=0.003), but no difference could be detected between the Lp(a) concentrations (11.7 vs. 6.6 mg/dl). The patients also had higher plasma total triglyceride (1.7 vs. 1.3 mmol/l), VLDL cholesterol (0.5 vs. 0.3 mmol/l) and VLDL triglyceride (1.1 vs. 0.7 mmol/l) concentrations than their controls (p < 0.001 for all the differences). The lipid concentrations of the male patients with different extensions of CAD and the controls are given in Table 8. The LDL-to-HDL cholesterol ratio was higher in all the patient groups than in the controls (Fig. 6).
Fig. 6. The LDL-to-HDL cholesterol ratio (± SD) of the controls and the male and female patients with different extensions of coronary artery disease. C=controls, <50%=<50% luminal stenosis, 1V=one-vessel disease, 2V=two-vessel disease 3V=three-vessel disease. The women with one-, two- or three-vessel disease differ from the controls at p<0.05. The men with two- and three-vessel disease differ from the men with <50% stenosis at p<0.05.

5.7.2. Plasma lipids of female CAD patients

The women with CAD also had a more atherogenic plasma lipid profile than the controls. The patients had higher plasma total cholesterol (6.7 vs. 5.6 mmol/l, p=0.04), lower HDL cholesterol (1.2 vs. 1.6 mmol/l, p<0.001) and higher LDL cholesterol (4.5 vs. 3.6 mmol/l, p=0.02), but no difference could be detected between the Lp(a) concentrations (7.6 vs. 9.7 mg/dl). The patients had significantly higher plasma total triglyceride (1.8 vs. 0.9 mmol/l), VLDL cholesterol (0.5 vs. 0.2 mmol/l) and VLDL triglyceride (1.1 vs. 0.4 mmol/l) concentrations than their controls, with p < 0.001 for all the differences. The lipid concentrations of the female patients with different extensions of CAD and the controls are given in Table 9. The female patients with severe CAD also had a higher LDL-to-HDL cholesterol ratio than the controls (Fig. 6).

5.7.3. Differences between male and female CAD patients

HDL cholesterol was lower in all the male patient groups compared to the corresponding female groups. The female patients with severe CAD had higher plasma total triglyceride, VLDL triglyceride and VLDL cholesterol values than the males, and the female patients with three-vessel disease had the highest VLDL cholesterol and VLDL triglyceride concentrations. There was no clustering of diabetes, hypertension or smoking in the male
two- and three-vessel disease patients, whereas three of the women with two- or three-vessel disease had hypertension and four had diabetes with hypertension, five were ex-smokers and only three patients had no other known CAD risk factors.

The stepwise logistic procedure suggests that HDL and LDL cholesterol discriminate both male and female controls from patients with different extensions of CAD. The effect of VLDL cholesterol is abolished in the presence of smoking and hypertension, but VLDL triglycerides remain significant in females after adjustment for age, smoking, hypertension and apolipoprotein E phenotype (Table 13).

Table 13. Ranked stepwise logistic procedure on the lipids of male and female controls and patients as explanatory factors for the extension of CAD

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.03</td>
<td>0.007-0.13</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.7</td>
<td>1.17-2.86</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>1.01</td>
<td>1.0-1.02</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.12</td>
<td>0.02-0.75</td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>45.5</td>
<td>2.14-964</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.6</td>
<td>0.84-3.04</td>
</tr>
</tbody>
</table>

Age, smoking, hypertension and apolipoprotein E phenotype were forced into the model. LDL cholesterol, HDL cholesterol, lipoprotein(a), VLDL cholesterol and VLDL triglycerides have been included in the stepwise procedure.

5.8. Treatment of hypercholesterolemia

5.8.1. RS-86505-007

The lower dose of RS-86505-007, 3 µg three times daily, showed a trend towards lower total and LDL cholesterol. RS-86505-007 at a dose of 6 µg three times daily produced a highly significant decreases in total and LDL cholesterol in patients with good compliance at all time points, resulting in mean reductions of 14.6 % (5% confidence interval 1.4-23.8, p=0.03) for total cholesterol and 18.5 % (95% confidence interval 2.1-28.8, p=0.03) for LDL cholesterol at six weeks. There were no consistent trends for HDL cholesterol or triglycerides.

Compliance with and side-effects of RS-86505-007 are shown in Table 14. At both dose levels, around two thirds of the side-effects were related to the gastrointestinal system. The higher dose of the drug compared to the lower dose doubled the percentage of patients reporting side-effects (81% in the drug-treated vs. 46% in the placebo group). In addition, the frequency of side-effects per patient was higher in the drug-treated group (53 side-effects in 21 patients) than in the placebo group (9 side-effects in 6 patients). Four patients
dropped out prematurely due to side-effects, one in the 3 µg and three in the 6 µg group due to gastrointestinal disturbances, and one patient also had moderate menorrhagia.

Table 14. Compliance and adverse events.

<table>
<thead>
<tr>
<th></th>
<th>RS-86505-007 3 µg tid</th>
<th>RS-86505-007 6 µg tid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor compliance*</td>
<td>25 %</td>
<td>33 %</td>
</tr>
<tr>
<td></td>
<td>31 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Events¹</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Mild</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Dropouts due to side-effects</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Consumption of less than 78% of capsules delivered
¹ The severity of adverse events was not recorded in 3 patients in the 3 µg group and 4 patients in the 6 µg group

5.8.2. Lovastatin and colestipol

Lovastatin lowered both total and LDL cholesterol more effectively than colestipol, but the drugs were equally effective in elevating HDL cholesterol, whereas triglycerides were slightly elevated during the colestipol treatment and lowered during the lovastatin treatment (Table 15). The patients also lost some weight during the colestipol treatment, but started gain.

Table 15. Lipid values of patients at baseline and after colestipol and lovastatin treatment.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Colestipol</th>
<th>Lovastatin</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>8.89(1.62)</td>
<td>6.54(1.36)</td>
<td>5.87(1.11)</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>6.78(1.73)</td>
<td>4.32(1.46)</td>
<td>3.81(1.18)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.39(0.37)</td>
<td>1.46(0.45)</td>
<td>1.48(0.37)</td>
<td>0.44</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.59(0.58)</td>
<td>1.78(0.80)</td>
<td>1.31(0.58)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The values are expressed as mean (SD). * significance for the difference between colestipol and lovastatin treatment.
6. Discussion

6.1. Detection of FH-Helsinki by polymerase chain reaction

The clinical diagnosis of FH is based on an elevated LDL cholesterol level and tendon xanthomata and supported by the existence of CAD in a first-degree relative. In accordance with the previously published data (Thompson et al. 1989), 83% of our FH patients had tendon xanthomata. However, the diagnosis of FH can be difficult, especially in adolescents who have not yet developed tendon xanthomata. Since the subjects would benefit from therapeutic interventions, a simple and rapid diagnostic test is needed.

The PCR method is most applicable in homogenous populations with a founder gene mutation. The FH-Helsinki mutation is a founder mutation in Finland and responsible for almost half of the FH cases in Finland (Aalto-Setälä et al. 1988a, Aalto-Setälä et al. 1989a). Another PCR method with two pairs of oligonucleotides has been applied to the diagnosis of FH-Helsinki, whereas the method described here involves only one pair of oligonucleotides. The results show that the prevalence of the FH-Helsinki mutation is even higher in Northern than in Southern Finland, 62% and 33%, respectively, supporting the use of the test in this area.

6.2. Catabolism of LDL

The various apo B polymorphisms and apo E phenotypes were not related to the slow clearance of autologous LDL, and, overall, no major differences between autologous and homologous LDL were observed among the study patients.

6.2.1. Apo B mutations

Three patients cleared homologous LDL markedly (9.0-19.3 %) faster than their own LDL, but this difference was considerably smaller than in the patients with FDB, who clear autologous LDL at a 48% slower rate than homologous LDL (Vega & Grundy 1986,
Neither the apo B-3500 nor the apo-B-3531 mutation was detected in our patients, and this study confirms the earlier findings that FDB is not a common cause of hypercholesterolemia in Finland (Hämäläinen et al. 1990).

6.2.2. Apo B polymorphisms

6.2.2.1. XbaI

Our study confirms the earlier associations between the presence of the XbaI cutting site and elevated cholesterol values, and part of the effect might be due to the slightly lower LDL catabolic rate in the individuals with the XbaI cutting site. It has been demonstrated earlier that the XbaI polymorphism modifies dietary fat and cholesterol responses in such a way that individuals having the XbaI cutting site are more responsive to a low-fat, low-cholesterol diet than those lacking the cutting site (Tikkanen et al. 1992). This could, in part, explain the differences in the lipoprotein composition associated with the XbaI polymorphism. Patients treated with the prostaglandin E analogue RS-86505-007 who were homozygous for the presence of the XbaI restriction site of the apolipoprotein B gene tended to have smaller reductions in total and LDL cholesterol, although the concentrations before the drug treatment were similar. However, the response to the drug is different from the dietary effect, hypercholesterolemic patients homozygous for the restriction site being more resistant to the drug. The difference in response could be due to different sites of action. Medication interfering with fat and cholesterol absorption may alter the formation of lipoprotein particles in the intestine, whereas the interaction between the apo B polymorphism and the diet most probably occurs at the level of the removal of LDL particles from the circulation.

6.2.2.2. EcoRI

An association between elevated triglycerides and the absence of the EcoRI cutting site has been reported in coronary heart disease patients (Paulweber et al. 1990, Tybjaerg-Hansen et al. 1991) and in healthy males (Paulweber et al. 1990). In our population, individuals homozygous for the EcoRI restriction site had slow LDL catabolic rates associated with high LDL and total cholesterol values. We suggest that the polymorphism could have an effect on cholesterol metabolism, at least in hypercholesterolemic individuals.

6.2.2.3. MspI

The MspI polymorphism has not been associated with variations in serum lipid concentrations (Hegele et al. 1986, Xu et al. 1989, Genest et al. 1990). Our results confirm these findings.
6.2.2.4. Ins/del

We were unable to confirm any of the lipid associations reported earlier and found no differences in the concentration or production of apo B, which suggests that the polymorphism is not associated with changes in the processing or post-translational modification of apo B that would affect the catabolism of LDL.

6.3. Apolipoprotein E

6.3.1. Allele frequency

The frequency of the ε 4 allele was slightly higher in our hypercholesterolemic group (0.26) than in the average population (Ehnholm et al. 1986). The baseline plasma lipid concentrations did not, however, differ significantly between the apo E phenotypes, a finding that has been reported earlier in a large sample of dyslipidemic men (Mänttäri et al. 1991). The frequency of the ε 2 allele of apo E, which is 0.03 in patients with > 50% stenosis, is lower than the ε 2 frequencies reported in other coronary artery disease series (Nieminen et al. 1991), and also lower than reported in any population studies (Hallman et al. 1991). The ε 3 frequency, 0.75, is in accordance with other Northern European populations, but lower than in most voluntary populations (Tiret et al. 1994). The present ε 4 frequency exceeds that of the other populations (Davignon et al. 1988, Hallman et al. 1991). Compared with CAD patients in southern Finland, the ε 3 frequency is higher (0.75 vs. 0.63) and the ε 4 frequency lower (0.23 vs. 0.32)(Kuusi et al. 1989). There were no statistically significant differences in the lipid concentrations between the apo E phenotype groups in patients with > 50% and < 50% stenosis, suggesting that the elevated plasma lipids of coronary patients in this series were not mediated by the apo E phenotypes. Accordingly, our results suggest that the apo E 4 phenotype is not so strongly associated with CAD in Northern Finland as in other populations.

6.3.2. Influence of apo E polymorphism on plasma lipids and lipoproteins

Individuals with apo E 4 absorb cholesterol most efficiently, and their FCR is low (Miettinen et al. 1992). Our results on the effect of apo E 4 on VLDL and IDL lipids and the apo B concentration are in accordance with the earlier studies. However, we did not detect significant differences in the plasma total cholesterol concentrations or the LDL fractional catabolic rates between our patients with apo E 4 and apo E 3/3. This could be due to some yet unknown genetic or environmental effect, since similar results on normolipemic individuals have been reported (Savolainen et al. 1991). The patients were also on a low-fat, low-cholesterol diet, which may have masked the differences between the apo E 3 and apo E 4 individuals.
6.3.3. Influence of apo E polymorphism on the efficacy of hypolipidemic drugs

We found a tendency towards smaller LDL cholesterol reductions by colestipol and lovastatin in the patients with the apo E 4 allele than in the patients with the apo E phenotype 2/3. The finding is in accordance with the earlier reports, where the apo E 4 allele has been associated with smaller reductions in total and LDL cholesterol during lovastatin treatment in hypercholesterolemia (O’Malley & Illingworth 1990) in non FH (Ojala et al. 1991) and FH patients (Carmena et al. 1993) compared to E 2 and E 3.

During treatment with the prostaglandin E analogue RS-86505-007, the reductions in total and LDL cholesterol and triglyceride concentrations tended to be smaller in the patients with apo E 4 than in those with apo E 3/3. This finding suggests that the absorption of cholesterol is not so easily affected by such medical interventions in patients with apo E 4 than in patients with apo E 3.

The plasma HDL cholesterol concentration decreased during colestipol and lovastatin treatment in the individuals with the apo E 4/4 phenotype in contrast to an elevation in the individuals with the other phenotypes. The colestipol-induced change in HDL cholesterol correlated well with the change in CETP activity regardless of the apo E phenotype, whereas no correlation could be detected during lovastatin treatment. Thus, the variation in HDL cholesterol in patients with different apo E phenotypes cannot be explained by apo E-induced variation in CETP activity.

The apo E phenotype response to a pharmaceutical lowering of cholesterol seems to differ from the diet-induced change, which has been equal (Boerwinkle et al. 1991a, Martin et al. 1993) in many and more profound in some (Mänttäri et al. 1991, Lehtimäki et al. 1995) studies on patients with the E 4 allele. A low-fat, low-cholesterol diet also induces larger (Mänttäri et al. 1991, Savolainen et al. 1991) reductions in total and LDL cholesterol in patients with the E 4 allele than in those without, the HDL responses being similar in the phenotype groups (Mänttäri et al. 1991).

6.4. Cholesteryl ester transfer protein activity

6.4.1. Difference between the sexes

Females have been reported to have both higher (Marcel et al. 1990) or similar (Savolainen et al. 1990) CETP levels compared with males, and we found the basal CETP activity to be high in females.

6.4.2. Effect of apo E polymorphism

CETP activity correlated well with the pre-treatment total and LDL cholesterol concentrations and with the lovastatin-induced decrease in LDL cholesterol, but only in the patients without the apo E 4 allele. The effect of apo E 4 on lipoprotein metabolism seems to mask
the correlation between CETP activity and LDL cholesterol concentration as well as the drug-induced lowering of LDL cholesterol. One mechanism by which apo E might increase the CETP-mediated cholesteryl ester and triglyceride transfer between VLDL and HDL can be the enhanced affinity of CETP for VLDL (Kinoshita et al. 1993).

6.5. Lipoprotein(a)

6.5.1. Lp(a) and CAD

The association between coronary artery disease, high Lp(a) values (Frick et al. 1978, Dahlén et al. 1986) and the ε 4 allele (Davignon et al. 1988) has been well established. The previously well-known (Brown et al. 1990, Ukkola et al. 1993a) high prevalence of ε 4 in the patients with high Lp(a) concentrations in this study suggests that the simultaneous presence of these risk factors confers susceptibility to CAD. The present results also suggest that the combination of a low Lp(a) value and the ε 2 allele, both of which are considered antiatherogenic (Nieminen et al. 1992, Sandholzer et al. 1992, Schaefer et al. 1994) does not protect individuals from CAD in the presence of smoking, although the number of patients with the ε 2 allele was too small to warrant definite conclusions.

6.5.2. Effect of drug treatment on Lp(a) concentration

RS-86505-007 had no effect on the Lp(a) concentration, a result that could be expected, since the serum Lp(a) concentration is largely genetically determined by a single gene attributable to differing numbers of kringle 4 repeats (Boerwinkle 1992).

6.6. Difference between male and female coronary artery disease

6.6.1. Lipids

The plasma total cholesterol, LDL and HDL cholesterol values and the plasma triglyceride values of patients with coronary artery disease were similar to those of other CAD populations (Kauppinen-Mäkelin & Nikkilä 1988, Nieminen et al. 1992). The lipid profile of the coronary patients was more atherogenic than that of the controls, and the patients with > 50% stenosis had an even more atherogenic lipid profile than those with < 50% stenosis. The decline in HDL cholesterol with the severity of CAD was typical of men and the increase in plasma triglycerides of women. It has been postulated that a high plasma triglyceride concentration together with small dense LDL particles is associated with premature CAD (Coresh et al. 1993, Ballantyne 1998), the atherogenic LDL particles originating from VLDL (Teng et al. 1986). An increase in total triglycerides, a decrease in HDL cholesterol (Höstmark et al. 1990) and an association of elevated total
and LDL cholesterol and total triglycerides and low HDL cholesterol (Bolibar et al. 1995) with the increasing extension of CAD have been reported, and a lowering of VLDL cholesterol and triglycerides has been reported to associate with a slowing down of the progression of coronary arteriosclerosis in young AMI survivors (Ericsson et al. 1996).

6.6.2. Risk factors of males

The male patients with two- and three-vessel stenosis also had higher VLDL cholesterol and VLDL triglycerides than the controls, but the differences were not so clear as in females, and the correlation with the increasing severity of CAD was weaker than in women. The differences in the HDL cholesterol values between the male patients with one-, two- or three-vessel disease were not statistically significant, although there was a trend towards lower HDL cholesterol concentrations in the patients with three-vessel disease compared with one- or two-vessel disease. VLDL cholesterol and triglyceride concentrations have been reported to discriminate male AMI survivors from controls, and LDL cholesterol has been claimed to be the best discriminator of AMI patients with different extensions of coronary atheromatosis, but not of coronary stenosis (Hamsten et al. 1986).

6.6.3. Risk factors of females

VLDL triglycerides and cholesterol were the only lipids discriminating the female controls from the patients with three-vessel stenosis without any overlap. All the diabetic female patients had two- or three-vessel disease, as did seven of the nine hypertensive women. It seems that women with two- or three-vessel stenosis often have multiple risk factors combined to a special lipid profile with high VLDL lipids and low HDL cholesterol, whereas their LDL and total cholesterol values are comparable to those of other CAD patients. This finding is in accordance with the results reported by Johansson and co-workers on female AMI survivors (Johansson et al. 1988). The association of VLDL lipids with the severity of CAD in women appears strong, but as this is a cross-sectional study, no causal relationship can be established on the basis of the present results.

6.7. Treatment of hypercholesterolemia

6.7.1. RS 86505-007

The effect of RS-86505-007 on plasma lipids is favourable: it lowers LDL cholesterol without any effect on HDL cholesterol, and thus improves the LDL-to-HDL ratio. No firm conclusions on the effect of RS-86505-007 on elevated plasma triglycerides can be
drawn from our results, since the patients had normal or only slightly elevated plasma triglyceride concentrations. Gastrointestinal side-effects, however, are frequent and would limit the use of the drug in long-term treatment of hypercholesterolemia.

6.7.2. Colestipol and lovastatin

The changes in plasma lipids were more favourable after lovastatin treatment than after colestipol treatment. Lovastatin lowered LDL cholesterol more effectively than colestipol, and it also lowered the triglycerides, which were elevated after colestipol treatment. The variation in the responses to colestipol and lovastatin treatments was different in the patients with different apo E phenotypes. This could be due to the different mechanisms of action of the drugs, lovastatin being able to modify the structure of the lipoprotein particles (Homma et al. 1995) and colestipol acting mainly by enhancing the loss of cholesterol in bile (Gaw et al. 1996). Statins reduce the CETP-mediated transfer of cholesterol esters from HDL to VLDL and LDL by lowering the plasma CETP activity (Kleinveld et al. 1993) without modifying the plasma CETP mass, suggesting that the reduction in cholesterol transfer results from a reduction in the LDL acceptor particles (Guerin et al. 1995). The apo E phenotype seems to modify the interaction between LDL cholesterol and CETP activity, but does not affect the LDL-lowering efficacy of the drugs.
7. Conclusions

The diagnosis of familial hypercholesterolemia can be achieved by the PCR method alone for the majority of FH patients in a homogenous population, such as that of Northern Finland.

Deficient binding of the LDL particle to the apo B-E receptor is not a major cause of hypercholesterolemia in Northern Finland. The slow catabolism of LDL in hypercholesterolemia does not seem to be primarily due to modulations in the LDL particle, since the homologous LDL particles from normocholesterolemic individuals were also catabolised slowly. However, some of the apo B and the apo E polymorphisms are associated with variations in the composition of lipoprotein particles, the catabolic rate of apo B, and the plasma lipid concentrations. The apo E 4 allele seems to associate with smaller changes in plasma lipids during hypocholesterolemic drug treatment.

Male and female CAD patients seem to have different risk profiles. High LDL cholesterol and low HDL cholesterol are the most important risk factors in men. Women with two- or three-vessel disease also have low HDL cholesterol and high LDL cholesterol, but associated with high VLDL lipids and other risk factors, such as hypertension, diabetes or smoking.

The capacity of RS-86505-007 to lower LDL cholesterol is moderate. As its potential site of action is the gastrointestinal tract, it would be a good long-term treatment alternative for other hypolipidemic drugs, and also a useful drug to combine with drugs acting in the liver. Unfortunately, side-effects seem to limit its clinical use. Lovastatin is more potent than colestipol in lowering plasma total and LDL cholesterol. Colestipol treatment results in a slight elevation of plasma triglycerides, compared to a decrease during lovastatin treatment, and the drugs have similar effects on CETP and apo A I. Side-effects, on the other hand, are more frequent during colestipol than lovastatin treatment. Of the drugs used in these studies, lovastatin seems to have the best hypocholesterolemic effect and to be the most palatable. As statins have also been shown to prevent cardiovascular events, they are now the most widely used hypocholesterolemic drugs.
8. References


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