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PATHOPHYSIOLOGY OF ADIPOSE TISSUE METABOLISM AND ATHEROSCLEROSIS IN FAMILIAL COMBINED HYPERLIPIDEMIA

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ACADEMIC DISSERTATION

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List of original publications

The present thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Ylitalo K, Porkka KVK, Meri S, Nuotio I, Suurinkeroinen L, Vakkilainen J, Pajukanta P, Viikari JSA, Peltonen L, Ehnholm C, Taskinen M-R. Serum complement and familial combined hyperlipidemia. *Atherosclerosis* 1997;129:271-277.

II Ylitalo K, Pajukanta P, Meri S, Cantor RM, Mero-Matikainen N, Vakkilainen J, Nuotio I, Taskinen M-R. Serum C3 but not plasma acylation-stimulating protein is elevated in Finnish patients with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 2001;21:838-843.

III Ylitalo K, Large V, Pajukanta P, Reynisdottir S, Porkka KVK, Vakkilainen J, Nuotio I, Taskinen M-R, Arner P. Reduced hormone-sensitive lipase activity is not a major metabolic defect in Finnish FCHL families. *Atherosclerosis* 2000;153:373-381.

IV Ylitalo K, Nuotio I, Viikari J, Auwerx J, Vidal H, Taskinen M-R. C3, hormone-sensitive lipase and PPARγ mRNA expression in adipose tissue of FCHL patients. Submitted.

V Ylitalo K, Syvänne M, Salonen R, Nuotio I, Taskinen M-R, Salonen JT. Carotid artery intima-media thickness in Finnish families with familial combined hyperlipidemia. In press in *Atherosclerosis.*

In addition, some unpublished data are presented.

Abbreviations

aP2	adipocyte fatty acid binding protein gene
ACS	acyl-CoA synthetase
ANOVA	analysis of variance
Apo	apolipoprotein
ASP	acylation-stimulating protein
AUC	area under the curve
BMI	body mass index
CB	carotid bulb
CCA	common carotid artery
cDNA	complementary deoxyribonucleic acid
cAMP	cyclic adenosine monophosphate
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CV	coefficient of variation
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
EUFAM	European multicenter study on familial dyslipidaemias in patients with premature coronary heart disease
FABP-pm	plasma membrane fatty acid binding protein
FAT	fatty acid translocase
FATP	fatty acid transport protein
FFA	free fatty acid(s)
FW	far-wall
HDL	high density lipoprotein
HL	hepatic lipase
HSL	hormone-sensitive lipase
HyperapoB	hyperapobetalipoproteinemia
ICA	internal carotid artery
IDL	intermediate density lipoprotein
IMT	intima-media thickness
LDL	low density lipoprotein
lod	logarithm of odds
LPL	lipoprotein lipase
mRNA	messenger ribonucleic acid
MTP	microsomal transfer protein
NW	near-wall
OGTT	oral glucose tolerance test
PCR	polymerase chain reaction
PLTP	phospholipid transfer protein
PPAR	peroxisome proliferator-activated receptor
PPRE	peroxisome proliferator-activated receptor response element
RNA	ribonucleic acid
RT-cPCR	reverse transcription with competitive polymerase chain reaction
SBP	systolic blood pressure
SD	standard deviation
TC	total cholesterol
TG	triglycerides
TNF-α	tumour necrosis factor α
TZD	thiazolidinedione
VLDL	very low density lipoprotein

1 INTRODUCTION

Both observational and intervention studies have clearly established the important role of total and low density lipoprotein (LDL) cholesterol as risk factors for coronary heart disease (CHD) (Martin et al. 1986, The Scandinavian Simvastatin Survival Study Group 1994, Shepherd et al. 1995). A low level of serum high density lipoprotein (HDL) cholesterol is also associated with an increased risk of CHD (Gordon et al. 1989, Rubins et al. 1999). Growing evidence of the relationship between serum triglyceride (TG) concentration and CHD has accumulated in the 1990´s (Manninen et al. 1992, The BIP Study Group 2000).

Serum lipid and lipoprotein levels are determined by both genetic and environmental factors. Hereditary dyslipidemias may be due to single gene mutations as in familial hypercholesterolemia, or result from the combined effect of a few or several genes that may interact with each other and/or environmental factors. Familial combined hyperlipidemia (FCHL), the most common hereditary dyslipidemia, is an example of a complex lipid disorder that has a heterogeneous background. The current estimate of the prevalence of FCHL in Western populations varies between 0.3 and 2.0% (Goldstein et al. 1973, Grundy et al. 1987), which corresponds to 15 000-100 000 FCHL patients in Finland. FCHL is characterised by elevated serum levels of total cholesterol (TC) and/or triglycerides. Despite intensive research for almost three decades, the exact metabolic and genetic background of FCHL still remains to be solved.

Adipose tissue stores most of the body´s triglycerides and fatty acids, and FCHL is characterised by elevations of serum triglycerides and fatty acids. An elevated serum fatty acid level has several deleterious consequences: it reduces insulin sensitivity and increases glucose output from the liver. Increased fatty acid flux to the liver stimulates very low density lipoprotein (VLDL) apolipoprotein B (apoB) production resulting in a lipid profile typical of FCHL. In recent years adipose tissue has proved to be not only a passive energy store, but an active endocrine organ that synthesises and secretes several bioactive molecules (Spiegelman and Flier 1996). These observations have resulted in growing interest in abnormalities of adipose tissue metabolism as possible causes for FCHL.

The aim of this work was to examine whether selected key regulators of adipose tissue fatty acid metabolism play a central role in the pathogenesis of FCHL. Should abnormalities in regulators of adipose tissue fatty acid metabolism have a primary role in the pathogenesis in FCHL, these defects would also be expected to be reflected in the degree of atherosclerosis. To quantify early atherosclerosis, FCHL family members were subjected to carotid artery ultrasonography with intima-media thickness (IMT) measurements. Ultrasonographic examination was also carried out to assess the validity of the lipid criteria that are currently used for categorising the FCHL family members as affected or unaffected. Finnish FCHL families that were identified and carefully characterised as a part of the European multicenter study on familial dyslipidaemias in patients with premature coronary heart disease (EUFAM Study) provided unique material for this work.

2 REVIEW OF THE LITERATURE

2.1 Familial combined hyperlipidemia

Familial combined hyperlipidemia is the most common hereditary lipid disorder among patients with premature CHD. FCHL was first characterised in 1973 when Nikkilä and Aro (1973) described 101 patients who had survived myocardial infarction under the age of 50 years. They observed that in 24 (24%) of the 101 families, half of the family members were hyperlipidemic, and represented different lipid phenotypes, i.e. multiple type hyperlipidemia. Shortly after this report, Goldstein et al. (1973) reported a similar dyslipidemia they called familial combined hyperlipidemia, in 47 (27%) of 176 families of hyperlipidemic survivors of myocardial infarction under 60 years of age. The third group to characterise FCHL independently (in 1973) was Rose and co-workers (1973), who also considered the features associated with this lipid abnormality: obesity, impaired glucose tolerance and hyperuricemia. Genest et al. (1992) showed later that 14% of survivors of myocardial infarction below the age of 60 have FCHL. Estimates on the population prevalence of FCHL vary between 0.3 and 2.0% (Goldstein et al. 1973, Grundy et al. 1987), while 10-20% of patients with early-onset CHD may suffer from FCHL (Goldstein et al. 1973, Nikkilä and Aro 1973, Grundy et al. 1987, Genest et al. 1992).

2.1.1 Diagnostic criteria

FCHL diagnosis is still based on examination of whole families. FCHL diagnosis requires presence of different lipid phenotypes in affected first-degree relatives: elevated serum total cholesterol (Fredrickson´s lipid phenotype IIA), triglycerides (lipid phenotype IV), or both (combined lipid phenotype IIB) (Goldstein et al. 1973, Nikkilä and Aro 1973, Rose et al. 1973, Grundy et al. 1987). The lipid cut-off points used to define the phenotypes usually utilise age and gender-specific 90^{th} or 95^{th} population percentiles of TC and TG (Cullen et al. 1994, Bredie et al. 1996, Porkka et al. 1997). When the 90^{th} age and gender-specific percentiles for TC and TG were used in Finnish FCHL families, 45% of family members were categorised as affected. If the 95^{th} percentiles were utilised, only 20% of family members were affected (Porkka et al. 1997). In some studies lower percentiles have been used, or the definition has been based only, or in part, on reference values of TC and TG (Castro Cabezas et al. 1993b, Reymer et al. 1995, Karjalainen et al. 1998). The differences in diagnostic criteria are further accentuated by the use of serum apoB or LDL cholesterol as criteria (Castro Cabezas et al. 1993b, Ascaso et al. 1998). The existence of premature CHD in the family is also often required for FCHL diagnosis in addition to the lipid criteria (Castro Cabezas et al. 1993b, Reymer et al. 1995, Porkka et al. 1997).

2.1.2 Other characteristics

2.1.2.1 Elevation of apoB-containing particles

Elevations of serum TC and TG in FCHL are reflected in lipoproteins as an increased number of both VLDL and LDL particles. Since each of these particles contains one molecule of apoB, an increase in serum concentration of apoB is a

frequent finding in FCHL patients (Brunzell et al. 1983, Kwiterovich et al. 1987, Austin et al. 1990a, Genest et al. 1992). A high serum level of apoB is also a characteristic feature of hyperapobetalipoproteinemia (hyperapoB). The term hyperapoB was initially applied to conditions characterised by high levels of LDL apoB with normal levels of LDL cholesterol (Sniderman et al. 1980, Sniderman et al. 1982). As hyperapoB also predisposes patients to premature CHD (Sniderman et al. 1980, Sniderman et al. 1982, Kukita et al. 1985, Kwiterovich et al. 1993) and aggregates in families (Sniderman et al. 1985, Coresh et al. 1993), it is still not known whether these are two distinct entities or two overlapping syndromes (Grundy et al. 1987, Sniderman et al. 1992).

2.1.2.2 Small dense LDL

A tendency towards smaller and denser LDL particles in FCHL has been observed by several authors (Austin et al. 1990a, Hokanson et al. 1995, Bredie et al. 1996). Small, dense LDL particles are associated especially with high serum TG levels (McNamara et al. 1987, Austin et al. 1988, Austin et al. 1990b), and are thus particularly prevalent in subjects with lipid phenotypes IIB and IV. In the study by Austin et al. (1990a) FCHL family members with small and dense LDL particles also exhibited significantly lowered HDL cholesterol in addition to elevated plasma levels of apoB and TG.

The propensity towards smaller and denser LDL particles, which are prone to oxidation (Chait et al. 1993, Dejager et al. 1993, de Rijke et al. 1997), and show decreased binding affinity to LDL receptors (Galeano et al. 1994, Toyota et al. 1999) and increased binding to arterial wall proteoglycans (Anber et al. 1996), may in part explain the increased risk of CHD in FCHL (Austin et al. 1988, Lamarche et al. 1997).

2.1.2.3 Exaggerated postprandial hyperlipemia

Dietary fat is packed by intestinal enterocytes into chylomicrons. These large, TG-rich particles are secreted into the lymphatic system, from which they enter the venous circulation. The apoB-48 produced by enterocytes is the structural protein in chylomicrons. Other surface apoproteins of chylomicrons are A-I, A-IV, C-II, C-III and E (Gotto et al. 1986).

Several studies have shown that FCHL is associated with exaggerated postprandial lipemia. Castro Cabezas and co-workers (1993a, 1993c) have demonstrated delayed chylomicron and chylomicron remnant clearance in FCHL patients. When serum TG levels were lowered by drug treatment, chylomicron clearance improved, but did not return to the level of the controls (Castro Cabezas et al. 1993a). In a later study by the same authors the postprandial responses of apoB-100 and apoB-48 were measured separately to distinguish between lipoproteins of hepatic and intestinal origins (Castro Cabezas et al. 1994). ApoB-48 concentrations remained elevated compared with apoB-100 levels, suggesting that the exaggerated postprandial lipemia in FCHL is due to both overproduction of VLDL and defective clearance of TG-rich lipoproteins.

2.1.2.4 Insulin resistance and glucose intolerance

FCHL patients, especially if they are hypertriglyceridemic, often present with hyperinsulinemia, dependent on, or independent of, obesity (Hunt et al. 1989, Castro Cabezas et al. 1993b, Vakkilainen et al. 1998, Pihlajamäki et al. 2000a). Vakkilainen et al. (1998) showed that affected FCHL family members have impaired glucose tolerance compared with their unaffected relatives. This difference was also found in men, but not in women, with lipid phenotype IIA. A similar finding was reported by Ascaso et al. (1998) when results of the oral glucose tolerance test (OGTT) were compared between FCHL subjects and body mass index (BMI) matched normolipidemic control subjects.

Insulin resistance is associated with most of the typical features of FCHL: elevated serum TG, low HDL cholesterol and small, dense LDL particles (Reaven 1988, Byrne et al. 1994, Taskinen 1995). Insulin resistance in FCHL has been demonstrated in several studies using either the minimal model (Ascaso et al. 1998) or euglycemic clamp (Aitman et al. 1997, Bredie et al. 1997b, Karjalainen et al. 1998).

Impaired insulin action in FCHL has been shown to affect not only glucose uptake, but also the regulation of free fatty acid (FFA) metabolism (Aitman et al. 1997, Karjalainen et al. 1998). Karjalainen et al. (1998) performed euglycemic clamp on 58 FCHL family members and 72 control subjects. The suppression of FFA during hyperinsulinemia was impaired in all affected FCHL family members compared with controls. Importantly, a similar defect in FFA suppression was also seen in unaffected FCHL family members without hyperlipidemia. The result was later confirmed by the same group in 55 affected and 50 unaffected FCHL family members (Pihlajamäki et al. 2000a). Elevated fasting and postprandial FFA levels in FCHL have been previously reported by Castro Cabezas et al. (1993b).

2.1.3 Genetics of familial combined hyperlipidemia

When FCHL was first described in 1973 by three independent groups, Goldstein et al. (1973) suggested transmission of the FCHL trait by a single, autosomal dominant gene, whereas Nikkilä and Aro (1973) proposed a heterogeneous origin for this disorder. Further studies have suggested major genes acting on serum TG (Cullen et al. 1994), apoB (Jarvik et al. 1994, Bredie et al. 1997a), and LDL particle size (Bredie et al. 1996), or both apoB and LDL particle size (Juo et al. 1998).

Lipoprotein lipase (LPL) is one of the most extensively studied candidate genes in FCHL. Babirak et al. (1992) reported that up to a third of subjects with FCHL have reduced LPL activity associated with elevated serum TG and low HDL cholesterol. Several studies have established that LPL mutations do not represent a frequent cause for FCHL (Gagné et al. 1994, Nevin et al. 1994, Yang et al. 1995, Pajukanta et al. 1997), even if mutations in the LPL gene are able to modify plasma lipid levels in subjects with FCHL (Reymer et al. 1995, de Bruin et al. 1996, Hoffer et al. 1996, Hoffer et al. 1998).

As regards other lipolytic enzymes, hormone-sensitive lipase (HSL) and hepatic lipase (HL) have been ruled out as major FCHL loci in Finnish FCHL families

(Pajukanta et al. 1997). However, some reports support the role of HSL and HL as possible susceptibility or modifier genes for FCHL (Gehrisch et al. 1999, Allayee et al. 2000, Pihlajamäki et al. 2000b, Pihlajamäki et al. 2001).

The apoA-I/C-III/A-IV gene cluster on chromosome 11 is interesting with regard to FCHL because apo C-III inhibits the catabolism of TG-rich particles by LPL, and may thus influence serum TG levels (Ginsberg et al. 1986). An association between FCHL and polymorphisms in this cluster has been reported in several studies (Hayden et al. 1987, Wojciechowski et al. 1991, Tybjærg-Hansen et al. 1993, Xu et al. 1994, Dallinga-Thie et al. 1996, Dallinga-Thie et al. 1997, Ribalta et al. 1997, Groenendijk et al. 1999). However, no association between the gene cluster and FCHL was found in two studies, including the Finnish FCHL study (Marcil et al. 1996, Tahvanainen et al. 1998). Linkage studies on FCHL and the apoA-I/C-III/A-IV gene cluster have also produced conflicting results (Wojciechowski et al. 1991, Xu et al. 1994, Dallinga-Thie et al. 1997, Allayee et al. 1998, Wijsman et al. 1998). Recently, new genetic variants in apoA-I and apoC-III genes have been shown to be associated with plasma TG and apoC-III levels (Groenendijk et al. 2001). Moreover, specific haplotype combinations of these two variants were associated with elevated lipid levels in probands and spouses (Groenendijk et al. 2001).

Among other interesting loci identified recently are the lecithin:cholesterol acyltransferase and manganese superoxide dismutase (an enzyme affecting lipoprotein oxidation and possibly formation of small, dense LDL particles) loci (Aouizerat et al. 1999a). Polymorphisms in genes encoding intestinal fatty acid binding protein, β_3-adrenergic receptor and peroxisome proliferator-activated receptor (PPAR) -γ2 have been shown to modify lipid metabolism and insulin sensitivity in Finnish FCHL families, but none of these genes was likely to represent a major FCHL locus (Pihlajamäki et al. 1997, Pihlajamäki et al. 1998, Pihlajamäki et al. 2000c).

The first novel FCHL locus on chromosome 1q21-q23 was recently revealed by Pajukanta and co-workers in Finnish FCHL families (Pajukanta et al. 1998). This study emphasised the combined lipid phenotype as a diagnostic criterion. The linkage to chromosome 1 was later replicated in 12 Chinese and 24 German FCHL families (Pei et al. 2000), and 71 FCHL families of the Family Heart Study in the United States (Coon et al. 2000). The locus probably represents a novel, so far unknown gene. Aouizerat and co-workers (Aouizerat et al. 1999b) have reported a novel FCHL locus on chromosome 11 in Dutch FCHL families.

Four additional putative loci that influence serum TG (chromosomes 10 and 2), TC (chromosome 10), and apoB (chromosome 21) in FCHL have been suggested by Pajukanta et al. (Pajukanta et al. 1999).

Despite rapid technical advances in molecular genetics, dissecting the genetic basis of FCHL has proved extremely complicated. Nikkilä and Aro (1973) were remarkably farsighted in predicting, as long ago as 1973, that ”probably this disorder will appear to be heterogeneous”, and that ”it possibly represents a polymorphic expression of several genotypes”. Differences in diagnostic criteria (see chapter 2.1.1), uncertainty of the penetrance, and population differences have

in part prevented identification of the underlying genetic defects. Recognition of gene-environment and gene-gene interactions further complicates the elucidation of the genetic background of this complex disease.

2.2 Metabolic disturbances in familial combined hyperlipemia

Two main hypotheses that aim to explain the pathophysiology of FCHL are depicted in **Figure 1.** Below, numbers in parentheses refer to **Figure 1**. One concept considers the increased secretion of apoB-100-containing lipoproteins from the liver (1), with or without impaired clearance of triglycerides, the primary metabolic defect. The delayed clearance of TG-rich particles may be due to defective lipolysis of the TG-rich particles (2), and/or impaired uptake of remnant particles (3). The other hypothesis states that disturbances in adipose tissue metabolism - either reduced fatty acid uptake (4) or increased fatty acid mobilisation from adipose tissue (5) - are the initial metabolic perturbations that secondarily lead to overproduction of VLDL apoB.

2.2.1 Increased production of apoB-containing particles in the liver

Most kinetic studies have supported the concept that elevated serum apoB and TG levels in FCHL are due to abnormally high VLDL apoB production rate in the liver (Chait et al. 1980, Janus et al. 1980, Kissebah et al. 1981, Teng et al. 1986, Cortner et al. 1991, Venkatesan et al. 1993, Cuchel et al. 1997). In a study by Kissebah et al. (1984) LDL apoB synthesis remained elevated even after normalisation of plasma lipid levels by diet and fibrate treatment.

The hydrophobic lipid core of VLDL particles contains triglycerides and cholesteryl esters, surrounded by a hydrophilic surface layer of phospholipids, non-esterified cholesterol, and apolipoproteins C-I, C-II, C-III, E, and one molecule of apoB-100. ApoB-100 is essential for the assembly and secretion of VLDL. In normal subjects, more than 90% of apoB-100 is secreted in VLDL, whereas secretion to intermediate density lipoproteins (IDL) and LDL is increased in familial hypercholesterolemia (Fisher et al. 1991). ApoB gene expression does not vary widely even when secretion of apoB from liver cells *in vitro* changes markedly (Pullinger et al. 1989, Ginsberg 1995). The rate at which apoB-containing particles are secreted depends mostly on the proportion of apoB that is protected from post-translational degradation (Boren et al. 1993b, Wu et al. 1994). The ability of apoB to assemble a large enough lipid core determines whether it will be able to translocate and be secreted as a mature particle (Dixon et al. 1991, Boren et al. 1993a). Thus, protection against proteolysis is critically dependent on the availability of TG. There is evidence that other lipid components, cholesterol (Fungwe et al. 1992) and cholesteryl esters (Cianflone et al. 1990b, Cianflone et al. 1992), can also influence apoB and VLDL secretion, but the data are to some degree conflicting (Dashti 1992, Wu et al. 1994).

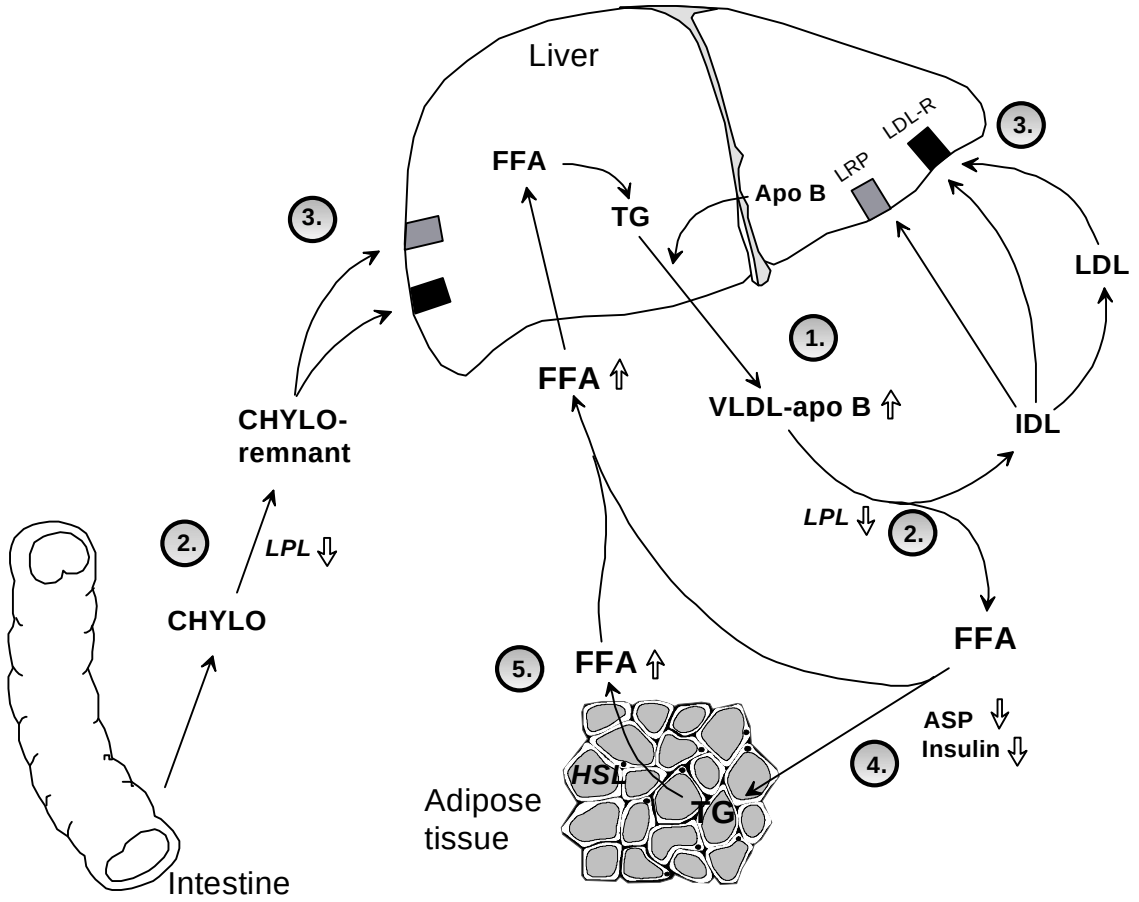

Figure 1. *Schematic illustration of the hypotheses aiming to explain the metabolic abnormalities in FCHL. See text (chapter 2.2) for explanation. CHYLO denotes chylomicrons; CHYLO-remnant, chylomicron remnants; LRP, LDL receptor-related protein and LDL-R, LDL receptor.*

Microsomal transfer protein (MTP) protects apoB from degradation by transferring core lipids, TG and cholesteryl esters to the translocating apoB peptide (Pan et al. 2000) (**Figure 2**). Cultured cells from subjects with abetalipoproteinemia lack MTP, and translocation of apoB is arrested (Du et al. 1996).

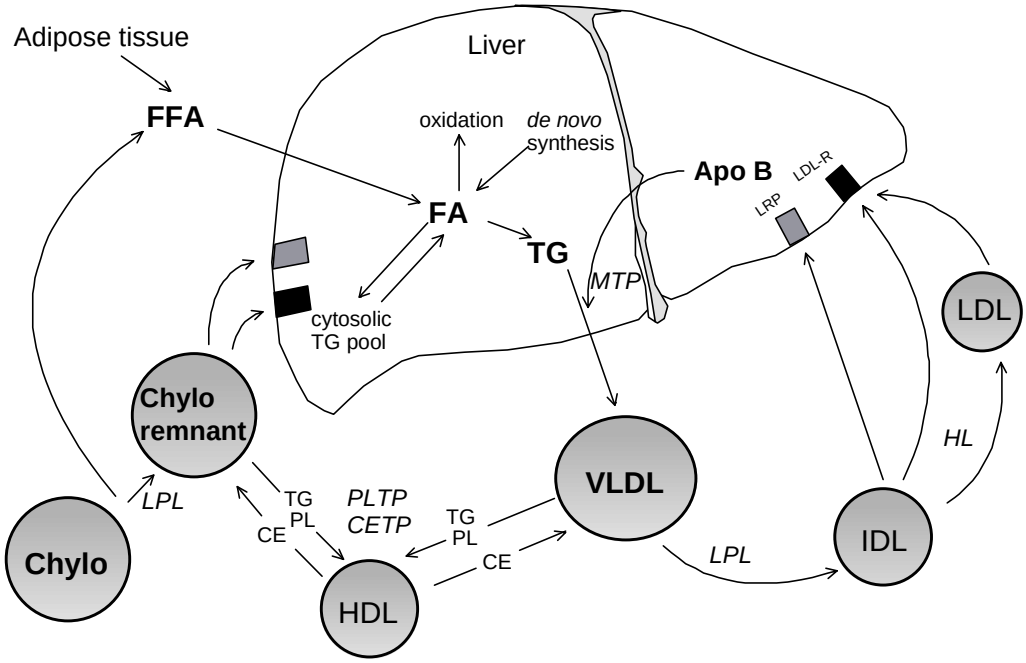

Figure 2. *Schematic illustration of VLDL production and intravascular metabolism of TG-rich particles. See text (chapter 2.2.1) for explanation. FA, fatty acid; CE, cholesteryl ester; PL, phospholipid; Chylo, chylomicron; LDL-R, LDL receptor and LRP, LDL receptor-related protein.*

2.2.1.1 Effect of fatty acids on VLDL apoB production

As discussed above, VLDL secretion is largely determined by the availability of its substrates, of which fatty acids appear to be the most important. Fatty acids for VLDL synthesis can originate from different sources: from lipoprotein remnants returned to the liver or from plasma non-esterified fatty acids, or they can be synthesised *de novo* (Gibbons and Wiggins 1995, Lewis 1997) (**Figure 2**). *De novo* synthesis of fatty acids appears to account for only a minor proportion of newly synthesised VLDL TG (Hellerstein et al. 1991, Aarsland et al. 1996). Non-esterified fatty acids delivered to the liver are not directly utilised for VLDL assembly, but also first enter the cytoplasmic TG pool. Most (up to 70%) VLDL TG origins from this cytoplasmic store, and undergoes lipolysis before re-esterification of fatty acids.

When Lewis and co-workers (1995) infused both heparin and Intralipid to induce an increase in plasma FFA in eight healthy males, they observed that elevation of plasma FFA *in vivo* acutely stimulates VLDL apoB production. Several authors have established that when oleic acid is added to HepG2 cell culture medium, both TG and apoB synthesis increase (Pullinger et al. 1989, Cianflone et al. 1990b, Dixon et al. 1991, Byrne et al. 1992, Wu et al. 1994). As the number of lipoprotein particles secreted from the liver is comparable to the amount of apoB that is secreted, VLDL production increases accordingly.

2.2.1.2 Effect of insulin on VLDL apoB production

The association between chronic hyperinsulinemia and increased VLDL production *in vivo* has been well described in both humans and animals (Reaven et al. 1981, Tobey et al. 1981, Steiner et al. 1984). Lewis (1997) stated in his review article that chronic insulin exposure may reduce fatty acid oxidation and stimulate TG esterification, and thus enhance *de novo* lipogenesis in spite of reduced FFA flux to the liver. On the other hand, acute hyperinsulinemia suppresses VLDL TG and VLDL apoB production. The evidence is derived from studies both *in vitro* (Durrington et al. 1982, Byrne et al. 1991) and *in vivo* in healthy subjects (Vogelberg et al. 1980, Lewis et al. 1994, Lewis et al. 1995, Malmström et al. 1997).

Insulin affects VLDL production primarily by limiting the availability of FFA. Insulin inhibits the action of HSL, which is the key lipolytic enzyme that mobilises fatty acids from adipose tissue. Recently, Malmström et al. (1998) studied whether insulin has a direct effect on VLDL production. VLDL apoB production was determined in healthy men during infusion of acipimox, an antilipolytic agent, or insulin. Only insulin suppressed the net production of VLDL apoB. Acipimox lowered the production of larger TG-rich VLDL1 particles, but at the same time the production of smaller VLDL2 particles was increased. The net VLDL production remained constant. They concluded that there is an insulin-dependent mechanism, other than FFA suppression, that affects VLDL production. Similar results have been reported by Lewis et al. (1995) in healthy male volunteers. Insulin-resistant individuals have been found to be resistant to the acute suppressive effect of insulin on VLDL production, despite a reduction in plasma FFA (Lewis et al. 1993, Malmström et al. 1997). However, the specific mechanisms by which insulin directly affects VLDL production are currently unknown.

2.2.2 Intravascular metabolism of triglyceride-rich lipoproteins

In the circulation, large VLDL1 particles are converted stepwise into smaller VLDL2, IDL and LDL particles. LPL, HL, and cholesterol ester transfer protein (CETP) have critical and complementary functions in the delipidation cascade (**Figure 2**).

LPL is the key enzyme in removing lipoprotein TG from circulation (Eckel 1989). LPL resides on the luminal side of endothelial cells, where it is bound to proteoglycans (Olivecrona and Bengtsson-Olivecrona 1990). LPL hydrolyses TG in chylomicrons and VLDL particles to monoglycerides and FFA, and thus converts VLDL into IDL. One third of FCHL patients may have reduced LPL activity (Babirak et al. 1992). Individuals heterozygous for LPL deficiency have reduced LPL activity and mass, and their lipid phenotype resembles that of FCHL (Babirak et al. 1989).

However, Olivecrona and Olivecrona (1995) concluded in their review article that most LPL heterozygotes have normal lipid levels, unless exposed to another predisposing condition such as diabetes.

HL is critical for conversion of IDL to LDL. In individuals with HL deficiency, VLDL is converted to IDL at a normal rate, but further conversion of IDL to LDL is significantly reduced (Demant et al. 1988). HL is also able to hydrolyse TG and phospholipids in HDL (Olivecrona and Bengtsson-Olivecrona 1990). Interestingly, HL seems to play a role in chylomicron and VLDL clearance by acting as a bridging protein to binding with LDL receptor-related protein and LDL receptor (Krapp et al. 1996, Medh et al. 1999). LPL also enhances the binding of apoE-containing lipoproteins to LDL receptor-related protein (Beisiegel et al. 1991).

Along the delipidation cascade, there is also exchange of lipid components between different lipoproteins. Plasma CETP facilitates the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins, whereas TG are transferred from TG-rich particles to LDL and HDL (Fielding et al. 1995). CETP mass concentration has been shown to be elevated in combined hyperlipidemia (McPherson et al. 1991, Tato et al. 1995). However, it seems that the accelerated net transfer of neutral lipids observed in combined hyperlipidemia results from an increase in VLDL concentration, whereas CETP mass is not a major determinant of CETP activity (Mann et al. 1991, Guérin et al. 1996). Increased CETP activity also enhances the exchange of HDL cholesteryl esters for VLDL triglycerides, which in turn generates TG-rich HDL, which is a good substrate for plasma lipases (Ginsberg 1996).

Phospholipid transfer protein (PLTP) plays a role in the transfer of phospholipids from TG-rich lipoproteins to HDL. PLTP may also participate in the reverse cholesterol transport (Lagrost et al. 1998). However, no strong evidence for a direct relationship between plasma PLTP and lipid levels has been offered, though in one study serum PLTP activity correlated with serum TG (Tahvanainen et al. 1999).

2.2.3 Clearance of triglyceride-rich particles from the circulation

VLDL production from the liver is increased in FCHL, as stated above. However, the number of apoB-containing particles in the circulation depends also on the rate of elimination. Aguilar-Salinas and co-workers (1997) observed decreased catabolic rates rather than increased production of VLDL and LDL apoB in FCHL kindred. Furthermore, the lipid lowering effect of pravastatin treatment in this study was due to an increase in the fractional catabolic rate of LDL apoB, whereas lovastatin therapy has been reported to reduce the production of apoB-containing lipoproteins (Arad et al. 1990, Cuchel et al. 1997).

VLDL and lipoprotein particles originating from VLDL can be directly removed from the circulation at any point of the lipolytic cascade. LDL receptor removes VLDL2, IDL, and LDL particles from plasma, but large VLDL1 must undergo conformational changes during lipolysis before apoB or apoE can bind to LDL receptors. Another catabolic route for large VLDL particles is *via* LDL receptor-related protein, which binds apoE (Beisiegel et al. 1989). VLDL receptor binds VLDL but not LDL (Takahashi et al. 1992). The natural sites of high VLDL receptor expression are the heart, skeletal muscle and adipose tissue, where fatty acids are actively

metabolised (Aalto-Setälä et al. 1998). Studies on knockout mice suggest that the role of VLDL receptor is not essential in TG clearance from plasma, since mice lacking the VLDL receptor had normal plasma lipid levels even when subjected to a high-fat diet (Frykman et al. 1995).

Intravascular lipolysis of chylomicrons results in formation of chylomicron remnants that reside in the same density range as VLDL remnants. In postprandial state, the increase in serum TG is attributed to both chylomicron and VLDL TG, the VLDL fraction representing about 80% of the increase in lipoprotein number (Genest et al. 1986, Karpe et al. 1993, Schneeman et al. 1993). VLDL and chylomicrons compete for LPL, and since chylomicrons have greater affinity for LPL than VLDL, large VLDL particles accumulate in plasma postprandially when the common removal pathway is saturated (Karpe et al. 1993, Schneeman et al. 1993). Chylomicron remnants are taken up by liver parenchyma cells through the LDL receptor and LDL receptor-related protein, which binds apoE, but not apoB-100 (Beisiegel et al. 1989).

The association between exaggerated postprandial lipemia and CHD has been observed by several authors, and has been reviewed by Karpe and Hamsten (1995). The latter proposed that it may not be chylomicrons as such that are atherogenic, but their metabolism may alter other lipoproteins, i.e. reduce HDL and skew the distribution of LDL towards smaller and denser species. Plasma CETP activity is enhanced when the level of TG-rich VLDL and chylomicron particles is increased (Mann et al. 1991, Guérin et al. 1996). The large, TG-enriched LDL particles are effectively hydrolysed by HL, thus generating smaller and denser LDL particles (Packard et al. 1997). A similar metabolic cascade results in conversion of TG-enriched HDL particles into denser species that are removed from the circulation more rapidly. A reduction in circulating HDL compromises reverse cholesterol transport that would give protection against atherosclerosis (Patsch 1998). The impaired clearance of TG-rich particles from the circulation results as such in a prolonged residence time of these particles in the circulation. Consequently, the uptake of these particles by macrophages is promoted, enhancing the formation of foam cells and atherosclerosis (Willnow 1997).

2.3 Adipose tissue fatty acid metabolism

Elevated serum FFA levels may result from reduced FFA uptake in peripheral tissues or from an increased rate of fatty acid release from adipose tissue.

2.3.1 Uptake of free fatty acids by adipose tissue

Most human cells are capable of taking up fatty acids, which are necessary for several cellular processes: as an energy source, as precursors for steroid hormones and intracellular messengers, and as components for biological membrane lipids. Significantly, FFA uptake comprises both the transportation of FFA into the cells and incorporation of FFA into TG. Several authors have suggested that defective FFA esterification into TG may be responsible for hypertriglyceridemia and hyperapoB (Carlson et al. 1976, Rubba 1978, Arner et al. 1982, Teng et al. 1988) . Furthermore, FFA uptake may affect the rate at which FFA are released from TG-rich particles in

the first place, since high local concentrations of fatty acids may detach LPL from endothelial cells (Saxena et al. 1989, Peterson et al. 1990).

2.3.1.1 Fatty acid transporters

It has now been shown that FFA uptake exhibits kinetic properties of both non-facilitated and protein-facilitated transport. Three proteins have been suggested to mediate FFA uptake into adipocytes: plasma membrane fatty acid binding protein (FABP-pm) (Stremmel et al. 1985, Isola et al. 1995), fatty acid translocase (FAT) (Abumrad et al. 1993) and fatty acid transport protein (FATP) (Schaffer et al. 1994).

Induction of FATP has been shown to increase FFA uptake in adipocyte-like 3T3-L1 cells (Martin et al. 1997). The messenger RNA (mRNA) expression of FATP and FABP-pm correlated strongly with FFA uptake in adipocytes from Zucker fatty rats (Berk et al. 1997). An increase in FAT and FABP-pm mRNA in adipose tissue of *ob*/*ob* mice as compared to control littermates has been reported (Memon et al. 1999). Acyl-CoA synthetase (ACS) catalyses activation of FFA into acyl-CoA derivatives, thus preventing the escape of fatty acids from the cell (Suzuki et al. 1990). ACS mRNA levels were found to increase in parallel with FAT and FABP-pm mRNA in *ob*/*ob* mice, further promoting the uptake of FFA (Memon et al. 1999). On the other hand, FAT and FABP-pm mRNA were up-regulated in the liver of *ob*/*ob* mice (Memon et al. 1999), which may provide more substrates for VLDL production. So far, no studies on fatty acid transporters or ACS and dyslipidemia have been published. Fatty acid transporters and ACS will be further discussed with PPARγ in chapter 2.3.3.1.

2.3.1.2 Effect of insulin on fatty acid uptake

It is known that insulin stimulates the net uptake of FFA into adipocytes. The mechanisms involve suppression of HSL and stimulation of LPL. FFA ”retention” is enhanced by direct stimulation of re-esterification (Campbell et al. 1992, Frayn et al. 1994). However, the effect of insulin may not be attributable to increased glucose uptake and glycerol-3-phosphate synthesis (Frayn et al. 1994).

2.3.1.3 Acylation-stimulating protein

In the late 1980s a novel regulator of FFA uptake, acylation-stimulating protein (ASP), was described by a group led by Sniderman and Cianflone (Cianflone et al. 1989b). ASP is a small, 76-amino acid basic plasma protein that stimulates TG synthesis *in vitro* in human adipocytes and skin fibroblasts (Cianflone et al. 1989b, Baldo et al. 1993). Further purification revealed that ASP is identical to C3a-desArg (Baldo et al. 1993), the inactive fragment of complement anaphylatoxin C3a (Hugli 1989). ASP is generated by interaction of three components of the alternative complement pathway: complement C3, factor B, and factor D (adipsin) (Baldo et al. 1993) (**Figure 3**). Each of these proteins can be produced by adipocytes (Choy et al. 1992, White et al. 1992). Expression of adipsin is significantly down-regulated in rodent obesity models (Flier et al. 1987). C3 will be further discussed in chapter 2.3.1.4.

ASP mediates its effect on TG synthesis (1) by increasing the activity of diacylglycerol acyltransferase, a key enzyme in TG synthesis (Yasruel et al. 1991), and (2) by stimulating the transport of glucose, a substrate for TG synthesis, by enhancing translocation of glucose transporters (GLUT 1, GLUT 3, GLUT4) to the cell membrane (Germinario et al. 1993, Maslowska et al. 1997b, Tao et al. 1997) (**Figure 3**). This effect is independent of, and additive to, that of insulin (Germinario et al. 1993). ASP has no direct effect on FFA transport across the plasma membrane, but stimulation of TG synthesis enhances the rate at which FFA enter adipocytes. In their recent report, van Harmelen and co-workers (1999) demonstrated that ASP may also increase the fractional re-esterification of FFA by stimulating phosphodiesterase 3, and to a lesser extent, phosphodiesterase 4.

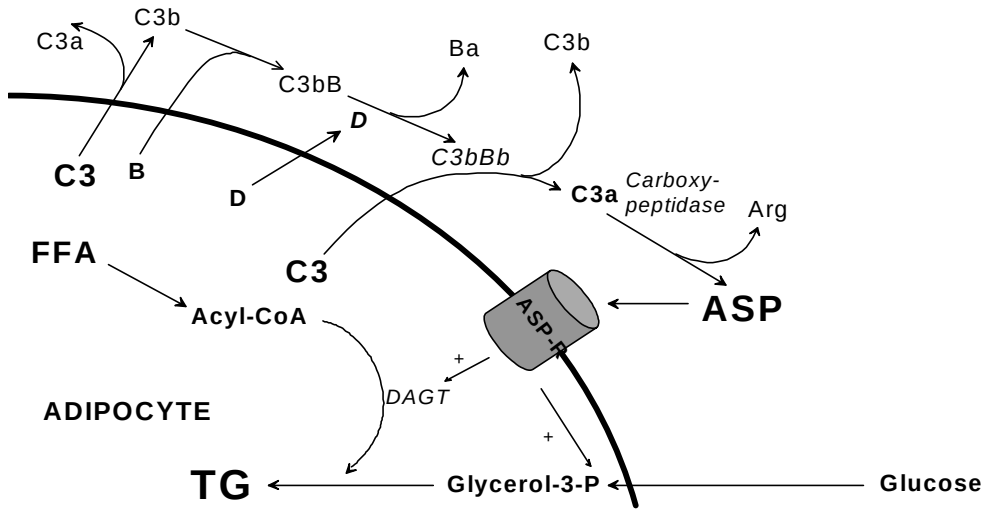

Figure 3. *Simplified illustration of ASP generation and activation of the alternative complement pathway. C3 in circulation undergoes continuous hydrolysis into a "C3b-like molecule", $C3_{(H2O)}$, which differs from C3b only by its inability to bind to surfaces. In the fluid phase, the C3b-like molecule binds factor B. Factor D cleaves factor B into Ba and Bb, thereby forming a priming C3-convertase $C3_{(H2O)}Bb$. $C3_{(H2O)}Bb$, and later, C3bBb, promote cleavage of C3 into C3a and C3b. C3b can bind to surfaces. Further fate of C3b is determined by the nature of the surface: it is either inactivated or forms another C3 convertase with factor B, thus initiating the amplification loop of the alternative pathway. ASP (C3a-desArg) is generated when plasma carboxypeptidases remove the C-terminal arginine from C3a. DAGT denotes diacylglycerol acyltransferase; glycerol-3-P, glycerol-3-phosphate, and Arg, arginine. + = stimulation.*

The signalling mechanisms by which ASP mediates its effects from the plasma membrane have not been elucidated. Baldo et al. (1995) suggested involvement of the protein kinase C pathway, but this was later contradicted by van Harmelen et al. (1999). So far there is only indirect evidence to support the existence of the putative ASP receptor. Radiolabelled ASP exhibited specific saturable binding to normal and

hyperapoB fibroblasts (Cianflone et al. 1990a). A decrease in the cell-surface concentration of ASP receptors has been reported to be responsible for lowered ASP stimulation of TG synthesis in fibroblasts from subjects with hyperapoB and high plasma ASP concentration (Zhang et al. 1998). On the other hand, according to Kildsgaard and co-workers (1999), it is not unlikely that a highly cationic peptide such as ASP would bind non-specifically, in a concentration-dependent manner, to anionic surface molecules on fibroblasts and adipocytes.

2.3.1.3.1 Acylation-stimulating protein – *in vitro* studies

Soon after the discovery of ASP it was shown that fibroblasts from subjects with hyperapoB are resistant to the effects of ASP (Cianflone et al. 1990a). This was confirmed by Kwiterovich et al. (1990, 1994), who showed that incorporation of oleate into cellular TG, cholesteryl esters and phospholipids is reduced in fibroblasts from subjects with hyperapoB as compared with control cells. Adipocytes from obese subjects responded normally to ASP (Walsh et al. 1989). Maslowska et al. (1997a) showed that chylomicrons contain a component that stimulates ASP and C3 production from adipocytes. This component was later suggested to be transthyretin, a plasma protein associated with retinol-binding protein (Scantlebury et al. 1998).

2.3.1.3.2 Acylation-stimulating protein – *in vivo* studies

Cianflone and co-workers (1997) have found significantly higher fasting plasma ASP levels in 208 CHD patients than in 59 age-matched control subjects. There was a positive relation between plasma ASP and serum TG and apoB in CHD patients. In linear regression analysis TG, VLDL cholesterol and VLDL apoB contributed significantly to the variation in plasma ASP (Cianflone et al. 1997). Maslowska et al. (1999) determined plasma ASP concentrations in 53 morbidly obese (BMI > 35 kg/m^2) and 183 non-obese control subjects. The median plasma ASP was significantly higher in obese than in non-obese subjects. In the non-obese subjects, ASP correlated significantly with TG and FFA, but not with BMI or apoB. In obese subjects, none of the variables studied correlated significantly with plasma ASP levels (Maslowska et al. 1999). Weyer et al. (2000) observed that plasma ASP was associated with percent body fat in 33 non-diabetic Pima Indians. No correlations between ASP and BMI, glucose, or insulin values were found, but there was a significant correlation between plasma ASP and serum C3. In a fat load study, fasting plasma ASP was associated with percent body fat, TG and FFA (Weyer et al. 1999). When 29 type 2 diabetic patients were treated with pioglitazone, glibenclamide or placebo for 6 months, the decrease in ASP correlated significantly with the decrease in HbA_{1c} (Ebeling et al. 2001).

Most *in vivo* studies on humans have focused on examining the postprandial ASP response. The first study was performed by Cianflone et al. (1989a) on seven healthy, normolipidemic subjects. They reported a sustained and significant increase in plasma ASP postprandially. It has since become apparent that the polyclonal antibody used by Cianflone and colleagues in the competitive enzyme-linked immunosorbent assay (ELISA) detected not only ASP but also C3. The serum concentration of C3 is much higher than that of ASP, ranging from 0.7 to 1.4 g/l in normal subjects, which explains why the reported plasma ASP concentrations

were some 1000 times higher than reported in later studies by Cianflone and Sniderman or other authors. Charlesworth et al. (1998) aimed to confirm the results of the first fat load study using radioimmunoassay (Amersham) for ASP measurements. They measured plasma ASP and C3 levels in eight healthy volunteers after a fatty meal. Six subjects were challenged with another fat meal. A sustained rise in plasma ASP was observed in one subject after the first meal, and in two subjects after the second meal. The fasting ASP concentrations averaged 147±33 ng/ml, and those at 4 h 147±40 ng/ml. The authors concluded that despite substantial individual variation in postprandial ASP levels, no uniform, significant response in plasma ASP could be observed after the fat meals. Weyer and Pratley (1999) tested whether fasting and postprandial plasma ASP levels were increased in seven lean and eight obese Pima Indians compared to an age, sex and BMI matched group of Caucasians. Plasma ASP decreased in response to the fatty meal in all four groups with no differences between the groups.

Instead of measuring ASP concentrations in peripheral plasma, Saleh et al. (1998) measured the veno-arterial gradient of ASP across abdominal subcutaneous adipose tissue in 12 subjects after an oral fat load. Sandwich ELISA was used to measure plasma ASP concentrations. It was demonstrated that ASP production by human adipose tissue *in vivo* is accentuated postprandially. Furthermore, the changes in TG clearance and fatty acid incorporation into adipose tissue followed a similar time course as ASP. Kalant and co-workers (2000) have more recently reported an increase in ASP release postprandially from subcutaneous adipose tissue of eight lean women, whereas in the eight obese subjects no significant response in ASP production was detected.

C3 knockout mice, which are also necessarily ASP deficient, have provided an interesting tool with which to examine the role of ASP in lipid metabolism. Wetsel and co-workers (1999) were the first to examine the lipid profile of C3(-/-) and wild-type mice. The average TG, cholesterol, apoB and FFA concentrations in C3(-/-) mice were no different from those in C3(+/+) mice. No differences in lipoprotein levels were observed either. Furthermore, there was no difference in postprandial TG and FFA levels. Contrasting results were subsequently reported by Murray et al. (1999) from the group of Sniderman and Cianflone. Even though no differences in fasting plasma lipid levels were found in C3(-/-) and C3(+/+) mice, TG clearance was delayed in male ASP knockout mice. Interestingly, an intraperitoneal injection of ASP accelerated TG clearance in male ASP-deficient mice. With regard to other consequences of defective FFA trapping in ASP-deficient mice, it has been reported that adipose tissue mass is greater in wild-type (C3+/+) than in C3(-/-) mice (Murray et al. 2000).

2.3.1.4 Complement C3

The complement system, which comprises several enzymes and regulator proteins, plays an essential role in infection defence and tissue injury. The protein cascade can be activated in two ways: the classical pathway, which involves binding of an antibody to complement component C1, or the alternative pathway, which is triggered by binding of C3 to surfaces such as microbes or cholesterol aggregates. An intriguing concept is the link between the complement system and adipose tissue biology. As discussed in chapter 2.3.1.3, complement C3 is a precursor

protein of ASP, and is synthesised and secreted by adipocytes (Choy et al. 1992, White et al. 1992). Activation of the alternative complement pathway and generation of ASP is depicted in **Figure 3**.

The link between C3 and lipid metabolism is implied by the association of serum C3 concentration with serum lipid levels. Uza et al. (1982) have reported higher serum C3 levels in hypertriglyceridemic subjects as compared to normolipidemic control subjects. Correlations between serum C3 and TC, TG, LDL cholesterol, BMI, systolic blood pressure (SBP), blood glucose and insulin have been detected (Muscari et al. 1990, Muscari et al. 1995b, Muscari et al. 1998, Muscari et al. 2000, Weyer et al. 2000). Serum C3 also seems to be elevated in obesity and type 2 diabetes (Koistinen et al. 1998, Ebeling et al. 1999).

The serum concentration of C3 is increased in inflammatory states such as systemic lupus and rheumatoid arthritis. Atherosclerosis has been suggested to be an inflammatory disorder, which provides an interesting link between atherosclerosis and C3, an acute phase protein. Complement components, including C3 and complement inhibitory proteins, have been isolated from human arterial wall (Hollander et al. 1979, Hansson et al. 1984, Vlaicu et al. 1985b, Niculescu et al. 1987b, Niculescu et al. 1989). As evidence that complement activation takes place in arterial intima, terminal complement complexes (C5b-9) have been localised in fibrous plaques, fatty streaks, and also in normal arterial intima (Vlaicu et al. 1985a, Niculescu et al. 1987a, Niculescu et al. 1987b). The level of complement deposition was related to the degree of atherosclerosis: more C5b-9 complexes were found in fibrous plaques than in fatty streaks. Seifert and co-workers (1990) have shown that there is a lipid component in human atherosclerotic lesions that can activate complement. This lipid component proved not to be native or oxidised LDL (Seifert et al. 1990, Wieland et al. 1999). However, enzymatically modified LDL has been shown to activate the alternative complement pathway (Bhakdi 1998).

Elevated serum C3 levels have been observed in subjects with peripheral atherosclerotic disease (Muscari et al. 1988). An association between elevated serum C3 and myocardial infarction has been reported both retrospectively (Muscari et al. 1995b) and prospectively in men (Muscari et al. 1995a). However, serum C3a (corresponding to C3a-desArg) levels were not increased in subjects with previous myocardial infarction (Muscari et al. 1995b).

Two major electrophoretic variants of C3 protein, the ”fast” $C3^F$ and the ”slow” $C3^S$, have been identified. These variants are inherited in an autosomal dominant fashion, indicating that they represent two allelic variants of the C3 gene (Barnum et al. 1989). The $C3^F$ allele has been found to be associated with atherosclerotic disease (Sörensen et al. 1975, Kristensen et al. 1978).

2.3.2 Release of fatty acids from adipose tissue

The primary function of adipose tissue is to store energy as triglycerides, and to release these energy stores as fatty acids when dietary energy substrates are lacking. The rate-limiting enzyme in releasing fatty acids from adipose tissue is hormone-sensitive lipase. Besides adipose tissue, HSL is to a lesser extent

expressed in muscle tissue, mammary gland, and testis in humans (Holm et al. 2000). In rodents HSL is also expressed in pancreatic β-cells (Mulder et al. 1999). Alternative splicing of the HSL gene in exon 6 results in formation of a short, catalytically inactive human HSL protein (Laurell et al. 1997). HSL catalyses hydrolysis of triglycerides to diglycerides and monoglycerides. Monoglyceride lipase is needed to finally catalyse the breakdown of monoglycerides to glycerol and fatty acids. Monoglyceride lipase is not hormonally regulated, and is abundant in adipose tissue.

2.3.2.1 Regulation of hormone-sensitive lipase activity

Hormone-sensitive lipase is so called because it is regulated by hormones: catecholamines and insulin (**Figure 4**). Catecholamines are the most important stimulators of lipolysis. Adipocytes express three different β-adrenoreceptors: β_1, β_2, and β_3, and α_2-adrenoreceptors. Catecholamines stimulate lipolysis by binding to β-receptors that are coupled to adenylyl cyclase through the GTP-sensitive G_s proteins. Adenylyl cyclase catalyses the formation of cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A, which finally phosphorylates the serine residues in HSL (Anthonsen et al. 1998). The main consequence of HSL phosphorylation has been proposed to involve translocation of HSL from cytosol to the surface of lipid droplets (Egan et al. 1992, Brasaemle et al. 2000). In addition to catecholamines, thyroid-stimulating hormone, glucagon, cholecystokinin and parathyroid hormone can stimulate lipolysis (Arner 1996), but these are of minor importance. The main physiological factors that increase lipolysis are starvation and exercise, as reviewed by Coppack et al. (1994).

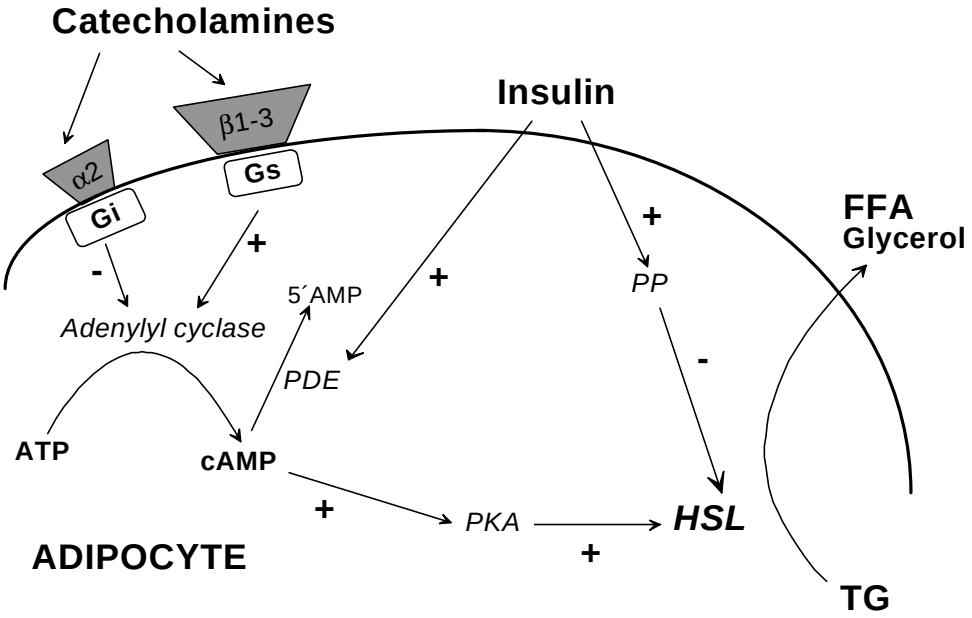

Figure 4. *Schematic illustration of regulation of HSL activity in adipose tissue. See text (chapter 2.3.2.1) for explanation. α2 denotes α2-adrenergic receptor; β1-3, β-adrenergic receptors 1, 2, and 3; PDE, phosphodiesterase; PKA, protein kinase A; PP, protein phosphatase. + = stimulation, - = inhibition.*

Insulin is the major antilipolytic hormone. The mechanisms by which insulin inhibits lipolysis are not completely understood, but several mechanisms exist. Insulin stimulates activation of phosphodiesterase 3, which enhances cAMP degradation in adipocytes (Manganiello et al. 1973, Hagström-Toft et al. 1995). Exposure of human fat cells to insulin is followed by translocation of β-receptors to intracellular space, which reduces lipolytic sensitivity to β-agonists (Engfeldt et al. 1992). Catecholamines can also inhibit lipolysis *via* α_2-adrenoreceptors, which inhibit adenylyl cyclase through the GTP-sensitive G_i proteins (Arner 1996). Dephosphorylation of HSL is enhanced by protein phosphatases, which can be stimulated by insulin (Olsson et al. 1987, Strålfors et al. 1989, Wood et al. 1993).

Holm et al. (2000) recently reviewed alternative, possibly tissue-specific mechanisms for HSL regulation. Perilipins are a family of phosphoproteins that form a protective barrier on the surface of intracellular lipid droplets. A novel cytokine produced by adipocytes - tumour necrosis factor alpha (TNF-α) - increases lipolysis by lowering the level of perilipins on the surface of lipid droplets (Souza et al. 1998). Leptin (Wang et al. 1999) and nitric oxide (Andersson et al. 1999) have also been implicated in regulation of lipolysis.

2.3.2.2 Regulation of hormone-sensitive lipase gene expression

Glucose deprivation reduces HSL gene expression in adipocytes *in vitro* (Raclot et al. 1998). Unexpectedly, β-receptor agonists and cAMP also decrease HSL mRNA levels in adipocytes, which may represent a long-term counter-regulatory effect (Plée-Gautier et al. 1996). Neither insulin (Plée-Gautier et al. 1996) nor oleate (Raclot et al. 1998) influences HSL expression. TNF-α treatment has been shown to down-regulate HSL gene expression in 3T3-L1 cells (Sumida et al. 1990). Whether TNF-α also reduces HSL protein expression *in vitro* is not clear (Green et al. 1994, Rosenstock et al. 2001). To summarise, the transcriptional control of HSL is not well understood. HSL gene expression affects HSL activity, but it seems to have a less important role in the control of lipolysis than post-translational mechanisms (Hellström et al. 1996, Reynisdottir et al. 1998, Large et al. 1999).

2.3.2.3 Effects of anatomical location and gender on lipolysis

Lipolytic response to catecholamines depends on the anatomical location of the fat depot. Visceral adipocytes exhibit higher catecholamine-induced lipolysis than subcutaneous adipocytes (Östman et al. 1979, Mauriege et al. 1987, Richelsen et al. 1991). The clinical importance of this regional difference lies in the fact that FFA released from omental fat are drained by the portal vein directly to the liver. The different rate of lipolysis in subcutaneous and visceral adipocytes is thought to result from a higher number of inhibitory α2-adrenergic receptors in subcutaneous as compared to visceral fat (Östman et al. 1979, Mauriege et al. 1987, Richelsen et al. 1991), whereas the number of β-receptors is increased in omental vs. subcutaneous adipocytes (Rebuffé-Scrive et al. 1989, Hellmer et al. 1992). Increased β_3-receptor function, increased ability of cAMP to induce lipolysis in visceral fat cells (Arner 1999), and resistance to insulin-induced suppression of lipolysis also contribute to increased FFA release from visceral fat (Roust et al. 1993, Meek et al. 1999).

2.3.2.4 Lipolysis in familial combined hyperlipidemia and insulin resistance

Reynisdottir et al. (1995) examined catecholamine-activated lipolysis and HSL activity in 10 non-obese male FCHL patients and 22 healthy, unrelated, age and BMI-matched control subjects. The lipolytic response of FCHL patients compared with the control subjects was reduced by about 65% regardless of the level at which lipolysis was stimulated: catecholamine receptors, adenylyl cyclase or cAMP. HSL enzyme activity was 40% lower in FCHL patients than in controls. When the same ten FCHL subjects were later compared with another ten control subjects, a 45% reduction in HSL activity was observed in FCHL subjects (Reynisdottir et al. 1997). In another study of 15 FCHL patients and 15 control subjects, the maximum lipolytic capacity and HSL activity were again found to be reduced in FCHL subjects (Reynisdottir et al. 1998). This reduction was attributed to a 70% reduction in HSL protein expression, whereas there was no difference in HSL mRNA levels between the two groups.

The effect of insulin resistance on lipolysis has been a target for several studies. A reduction in the number of β_2-receptors, and therefore reduction in catecholamine-stimulated lipolysis, was found in subcutaneous fat cells of elderly male subjects with insulin resistance (Reynisdottir et al. 1994). In another group of similar subjects examined by the same authors, HSL activity was not impaired in subcutaneous fat samples, whereas LPL activity was reduced by 43% (Reynisdottir et al. 1997). Recently van der Kallen et al. (2000) studied lipolysis by incubating adipocytes with isoprenaline or insulin. Insulin did not suppress FFA release from adipocytes derived from ten FCHL subjects when compared with adipocytes from subjects with type 2 diabetes or healthy control subjects.

2.3.3 Peroxisome proliferator-activated receptor γ (PPARγ)

PPARs are transcription factors belonging to a family of nuclear hormone receptors. Upon activation PPARs heterodimerize with the retinoid X receptor, bind to specific PPAR response elements (PPREs) in the promoters of their target genes, and enhance or suppress the transcription of these genes (**Figure 5**). PPARs have also been shown to affect gene transcription by interfering with certain signalling pathways in a fashion independent of DNA binding, as reviewed by Pineda Torra et al. (1999). The three different PPAR genes, α, γ and δ (NUC 1, FAAR, β) have distinct tissue distributions. PPARα is mainly expressed in tissues with a high level of fatty acid oxidation: liver, muscle, kidney, heart and intestinal mucosa (Braissant et al. 1996, Auboeuf et al. 1997). PPARδ is ubiquitously expressed, but its physiological function is unknown so far (Auboeuf et al. 1997), though preliminary data on its influence on lipid homeostasis and reverse cholesterol transport in monkeys (Oliver et al. 2001) and foam cell formation *in vitro* (Vosper et al. 2001) have been presented. PPARγ comprises three different isoforms: PPARγ1, γ2 (Fajas et al. 1997) and γ3 (Fajas et al. 1998), which are derived from the same gene by alternate promoter usage and different splicing. PPARγ2 protein contains 28 additional amino acids encoded by PPARγ2 specific exon B. PPARγ1 is predominantly expressed in adipose tissue and the large intestine, and to a lesser extent in kidney, liver, small intestine, heart, spleen, monocyte/macrophages and other hematopoietic cells, endothelial cells and vascular smooth muscle cells (Greene et al. 1995, Auboeuf et al. 1997, Fajas et al. 1997, Vidal-Puig et al. 1997,

Marx et al. 1998a, Marx et al. 1998b, Tontonoz et al. 1998, Marx et al. 1999). Minute amounts (10-30 fold less than in adipose tissue) of PPARγ mRNA have been detected also in muscle (Fajas et al. 1997). The expression of PPARγ2 is more strictly confined to adipose tissue, where it represents approximately 20% of total PPARγ (Auboeuf et al. 1997). One study has reported the presence of PPARγ2 in muscle (Vidal-Puig et al. 1997). The expression of PPARγ3 transcript is directed by an independent promoter, and is confined to adipose tissue, macrophages and colon epithelium (Fajas et al. 1998, Ricote et al. 1998). PPARγ3 protein is identical to PPARγ1 (Fajas et al. 1998).

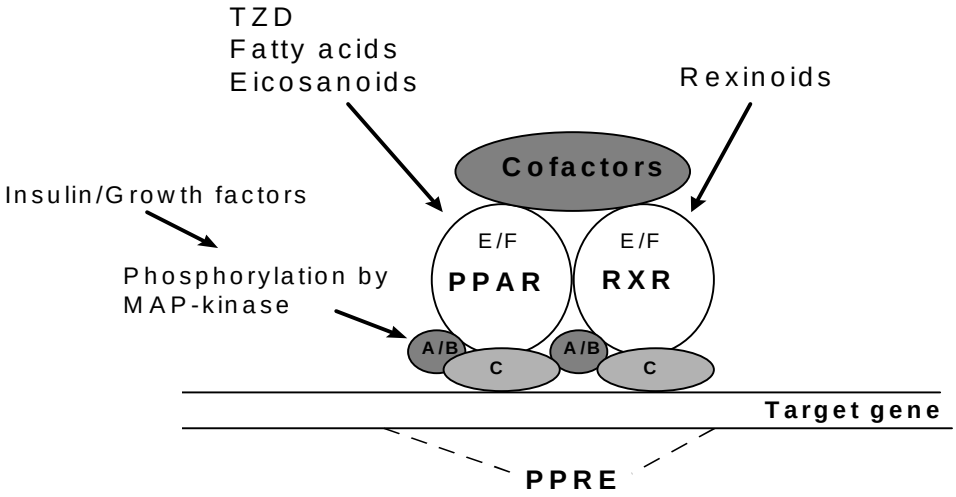

Figure 5. *Mechanisms of PPAR regulation. Ligand binding to E/F domain leads to binding of the PPAR/RXR receptor heterodimer to PPAR-response element (PPRE) of the target gene through the C-domain. Phosphorylation of the A/B domain by mitogen-activated protein (MAP) kinase down-regulates the receptor activity. The activated receptor dimer associates with co-factors. This involves release of co-repressor complexes and association with co-activator complexes that contain proteins that modulate histone acetylation state, and thereby open chromatin for more efficient transcription. This in turn facilitates target gene transcription (Spiegelman 1998, Auwerx 1999, Escher and Wahli 2000).*

PPARγ plays a pivotal role in adipocyte differentiation. It controls the transcription of several central genes of energy and lipid homeostasis in adipose tissue. Recently PPARγ has also been reported to influence atherogenesis through its effects on gene expression in endothelium, smooth muscle cells and monocyte/macrophages. Only those functions of PPARγ that are directly related to insulin resistance and fatty acid and lipid metabolism will be discussed below.

2.3.3.1 PPARγ in insulin resistance and fatty acid metabolism

The huge interest in PPARs in recent years is due not least to the discovery that antidiabetic drugs of the thiazolidinedione (TZD) group are synthetic ligands of PPARγ (Forman et al. 1995, Lehmann et al. 1995), and that it is PPARγ that mediates the antidiabetic effect of TZDs (Berger et al. 1996). There are several

mechanisms by which PPARγ activation may improve insulin sensitivity. Adipocyte differentiation involves induction of several genes that are necessary for insulin action: insulin receptor, insulin receptor substrate-1, and glucose transporter GLUT-4 (Cornelius et al. 1994). However, most glucose disposal takes place in muscle, where PPARγ is expressed only in trace amounts. It is possible that the minute amount of PPARγ in muscle is enough to exert its effects. The other possibility is that PPARγ in adipose tissue induces production of signalling molecules that affect insulin sensitivity in muscle and liver (Schoonjans et al. 1997). The latter option is supported by the observation of Chao et al. (2000) on transgenic A-ZIP mice that have virtually no white adipose tissue. These A-ZIP mice exhibit insulin resistance, hyperlipidemia and fatty liver. Treatment with rosiglitazone or troglitazone did not lower the glucose or insulin levels in the A-ZIP mice, suggesting that it is adipose tissue that is necessary for the insulin-sensitising effect of TZDs. Okuno et al. (1998) reported that activation of PPARγ by troglitazone induced apoptosis of large adipocytes in Zucker rats, potentially promoting the appearance of smaller, more insulin-sensitive adipocytes.

Two groups of signalling molecules are generated in adipose tissue: firstly, protein cytokines such as leptin, TNF-α, resistin and adiponectin, and secondly, fatty acid derivatives. PPARγ activation reduces the expression of leptin in rat and 3T3-L1 adipocytes (De Vos et al. 1996, Kallen et al. 1996) resulting in increased food intake and availability of substrates to be stored in adipocytes. This may seem illogical, but clinical observations in lipoatrophy and animal models have proved that a ”normal” amount of adipose tissue is necessary for normal glucose homeostasis (Moller and Flier 1991, Chao et al. 2000). There is also evidence that leptin may directly disturb the action of insulin in human hepatic and rat adipose cells (Cohen et al. 1996, Müller et al. 1997). TNF-α is known to induce insulin resistance (Hotamisligil et al. 1994) and TZDs reduce TNF-α expression in rodents (Hofmann et al. 1994, Okuno et al. 1998), which possibly contributes to their insulin-sensitising effect in humans. Resistin is a recently discovered, adipocyte-derived signalling molecule (Steppan et al. 2001). Resistin expression *in vivo* is specific to adipose tissue, and resistin can be found in the serum of normal mice (Steppan et al. 2001). Steppan and co-workers (2001) reported that serum resistin levels are elevated in animal models of obesity and insulin resistance, and that TZDs markedly reduce resistin gene expression and protein secretion (Steppan et al. 2001). However, contradictory results were published by Way et al. (2001). Adiponectin (AdipoQ, Acrp30 or apM-1) is another novel adipocytokine (Scherer et al. 1995, Hu et al. 1996b, Maeda et al. 1996). It may provide an interesting link between obesity and vascular disease, as adiponectin suppresses TNF-α induced expression of adhesion molecules in human aortic endothelial cells (Ouchi et al. 2000). Plasma levels of adiponectin are decreased in subjects with obesity (Arita et al. 1999) or CHD (Ouchi et al. 1999), but can be increased by TZDs (Maeda et al. 2001).

PPREs have been identified in several genes that are implicated in FFA metabolism. These include adipocyte fatty acid binding protein (aP2) (Pelton et al. 1999), ACS (Schoonjans et al. 1995, Martin et al. 1997), FATP-1 (Martin et al. 1997, Motojima et al. 1998), FAT (Motojima et al. 1998) and LPL (Schoonjans et al. 1996, Lefebvre et al. 1997), which are all up-regulated by PPARγ activation. Increased expression of these genes in adipose tissue may enhance FFA trapping, lower FFA flux to muscle, and thus improve glucose uptake in muscle. Recently it was shown

that PPARγ expression in human skeletal muscle is correlated with the expression of FABP and LPL in the same muscle samples (Lapsys et al. 2000).

2.3.3.2 Effect of PPARγ activation on serum lipid levels

PPARγ affects plasma levels of TG-rich lipoproteins mainly by influencing TG clearance (Lefebvre et al. 1997). As mentioned above, PPARγ activation may enhance FFA trapping in adipose tissue by inducing several central genes involved in fatty acid uptake. PPARγ activation by TZDs induces LPL expression (Schoonjans et al. 1996, Lefebvre et al. 1997), and thus enhances lipolysis and FFA uptake. Interestingly, in the A-ZIP mice that completely lack white adipose tissue, serum TG level decreased and fatty acid oxidation increased upon TZD treatment. Concomitantly, the PPARγ mRNA level in the liver of the A-ZIP mice was markedly increased, and rosiglitazone treatment further increased the triglyceride content of the fatty livers (Chao et al. 2000).

2.3.3.3 Regulation of PPARγ activity and expression

The mechanisms that regulate PPAR activity are depicted in **Figure 5**. TZDs are synthetic ligands of PPARγ. With regard to the natural ligands/activators, a number of eicosanoids and fatty acids have been reported to bind to, and activate, PPARγ (Kliewer et al. 1997, Krey et al. 1997). The most potent naturally occurring ligand of PPARγ is prostaglandin J2 (Forman et al. 1995, Kliewer et al. 1995). Nisoli et al. (2000) infused Intralipid and heparin in nine non-obese male volunteers. The increased availability of FFA induced marked increases in mRNA levels of FAT, PPARγ2, leptin and TNF-α in subcutaneous adipose tissue from gluteal region. As PPARγ activation is known to suppress TNF-α expression, the authors speculated that the increment in TNF-α gene expression by FFA may present a mechanism that aims to compensate for excessive lipid accumulation (Nisoli et al. 2000). Another example of a molecule that is both a target gene and a regulator of PPARγ is leptin. Leptin treatment of rats increased adipose tissue PPARγ expression (Qian et al. 1998), whereas PPARγ activation reduced the expression of leptin in rat adipocytes (De Vos et al. 1996, Kallen and Lazar 1996).

Rieusset et al. (1999) have shown that insulin acutely increases PPARγ1 and PPARγ2 expression in human adipose tissue both *in vitro* and *in vivo*. On the other hand, insulin-stimulated phosphorylation of PPARγ lowers receptor activity (Hu et al. 1996a, Adams et al. 1997). TZDs seemed, paradoxically, to lower PPARγ mRNA levels in 3T3-L1 adipocytes, but the expression of genes that are positively regulated by PPARγ such as aP2 or LPL was not reduced (Perrey et al. 2001). In another study on 3T3-L1 adipocytes there was no reduction in either PPARγ or aP2 expression by TZDs (Kallen and Lazar 1996).

A recent observation showed that selective retinoid X receptor agonists (rexinoids) can activate retinoid X receptor in the receptor heterodimer, and thus rexinoids, too, are involved in the transcriptional control of genes implicated in lipid metabolism (Mukherjee et al. 1998, Martin et al. 2000).

Phosphorylation of the N-terminal domain of PPARγ2 by mitogen-activated protein kinase reduces the ability of PPARγ2 to bind to PPREs (Hu et al. 1996a) (**Figure 5**).

Mutations affecting this phosphorylation site may augment receptor activity leading to obesity (Ristow et al. 1998). Ligand binding induces conformational changes in PPARs that allow interaction with co-activators and release of co-repressors, as reviewed by Escher and Wahli (2000). Several putative cofactors of PPARγ have been shown to interact with PPARγ, but their importance has not been established, and no PPARγ specific co-activators have been identified (Auwerx 1999).

2.3.4 The causes and consequences of abnormal fatty acid metabolism in familial combined hyperlipidemia

The elevated plasma concentration of FFA in FCHL can result from either reduced uptake of FFA in peripheral tissues or increased release of FFA from either circulating TG-rich lipoproteins or adipose tissue (**Figure 1**).

LPL is the key enzyme that hydrolyses TG in chylomicrons and VLDL. As the activity of LPL in FCHL has been shown to be either normal (Reynisdottir et al. 1997) or reduced (Babirak et al. 1992), enhanced liberation of FFA from plasma lipoproteins is not a likely explanation for elevated plasma FFA in FCHL. As regards increased mobilisation of fatty acids from adipocytes, the evidence is opposite. Lipolysis and HSL activity have been reported to be decreased in FCHL (Reynisdottir et al. 1995, Reynisdottir et al. 1997, Reynisdottir et al. 1998). Thus the most likely explanation is impaired fatty acid trapping by adipocytes, although this has not been directly verified so far.

Elevated serum FFA can have several harmful consequences (**Figure 6**). The idea that elevated serum FFA levels play a major role in the development of insulin resistance was first introduced in 1963 by Randle et al. (1963). The key point of the hypothesis was that increased plasma FFA causes increased β-oxidation of fatty acids in muscle. An increase in intracellular acetyl-CoA and citrate concentrations results in decreased glucose oxidation and finally in reduced glucose uptake. Since then, it has been confirmed that physiological elevations of plasma FFA reduce peripheral insulin sensitivity both in healthy subjects and in subjects with type 2 diabetes (Ferrannini et al. 1983, Boden et al. 1994, Boden 1996a). The effect of reduced insulin sensitivity is further reinforced by the stimulation of hepatic glucose output by FFA (Ferrannini et al. 1983, Fanelli et al. 1993, Boden et al. 1994). The connection between hyperinsulinemia and elevated FFA can be also explained by reduced hepatic clearance of insulin caused by FFA (Peiris et al. 1986, Svedberg et al. 1990).

The effects of FFA on insulin secretion have been summarised by Boden (1996b). The *in vitro* effects of FFA on insulin secretion observed in animals proved different from the *in vivo* effects in normal human subjects, as high plasma FFA increased insulin secretion rate *in vivo*. Obese individuals who may have constantly elevated FFA levels may eventually lose the ability to raise insulin secretion in response to FFA. The suppressive effect of insulin on hepatic glucose production is then abolished, resulting in hyperglycemia.

An elevated serum FFA level not only affects glucose and insulin homeostasis, but also has important consequences in lipid metabolism. As discussed in chapter 2.2.1.1, the availability of FFA determines to a major extent the rate of hepatic VLDL

apoB production. In subjects exhibiting insulin resistance and elevated FFA levels, VLDL apoB production may not be suppressed postprandially. This leads to competition for LPL between chylomicrons and VLDL particles, which further enhances postprandial lipemia. If now FFA uptake into adipocytes is also impaired, LPL activity can be inhibited by locally elevated FFA levels (Peterson et al. 1990). Circulating FFA are directed to the liver, where they can further increase VLDL production. Concomitantly, FFA impair insulin sensitivity in peripheral tissues, which in turn hampers peripheral FFA uptake.

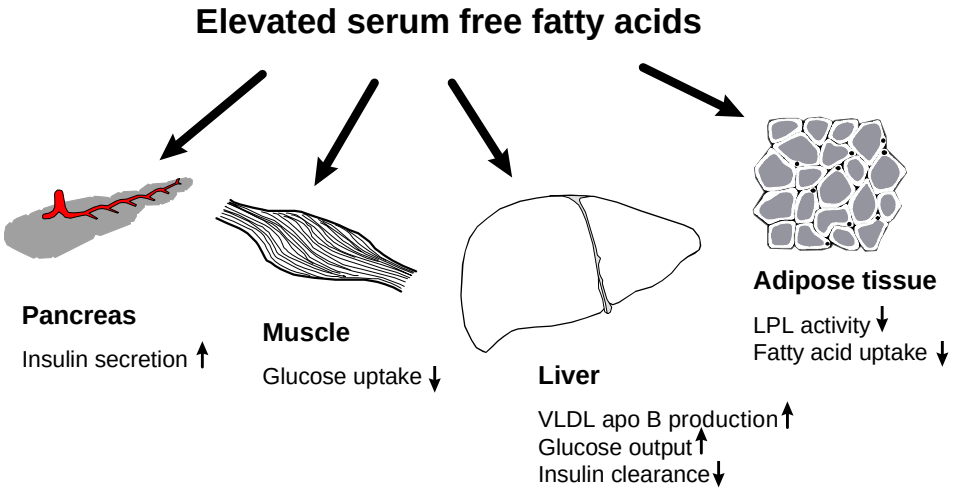

Figure 6. *The influence of elevated serum free fatty acids on metabolism in different organs. See text (chapter 2.3.4) for details.*

2.4 Carotid artery atherosclerosis and B-mode ultrasonography

The central role of total and LDL cholesterol as risk factors for CHD has been clearly established (Martin et al. 1986, The Scandinavian Simvastatin Survival Study Group 1994, Shepherd et al. 1995). Recent evidence shows that a low level of serum HDL cholesterol (Gordon et al. 1989, Rubins et al. 1999) and high level of serum TG are also independently associated with an increased risk of CHD (Manninen et al. 1992, The BIP Study Group 2000).

The initial atherosclerotic thickening of the arterial wall is compensated for by enlargement of the arterial lumen. The potential lumen area may diminish up to 40% until the lumen diameter starts to decrease (Glagov et al. 1987). Angiography provides information only on the lumen diameter, not changes in the arterial wall. Likewise, Doppler ultrasound gives information on the flow in the vessel. Both methods are highly relevant in clinical practice, but can only detect the late stages of atherosclerosis. Nowadays arterial walls can also be examined by intravascular ultrasound or techniques based on computerised tomography or magnetic resonance imaging, but all these methods are either invasive, inconvenient for the patient, and/or expensive, which makes them unsuitable for population studies or

extensive intervention trials. B-mode ultrasonography is an inexpensive, non-invasive method for assessing early atherosclerotic changes of the arterial wall.

The method relies on visualisation of specific double-line echo patterns that have been shown to correspond to the intima and media layers of the arterial wall. B-mode ultrasonography has become widely used as a surrogate end point of cardiovascular disease. Boissel et al. (1992) have suggested the following criteria for a valid surrogate end point: (1) it must be convenient (e.g. it occurs more often, and is easier to assess, than the clinical end point) and non-invasive, (2) the causality between the surrogate end point and the clinical end point should be established, and (3) in intervention studies an estimate of the anticipated clinical benefit should be deducible from the changes in the surrogate end point. As will be discussed below, all these conditions are met by B-mode ultrasonography with IMT measurement.

2.4.1 Carotid artery intima-media thickness as a surrogate end point for cardiovascular disease

The validity of the far-wall (FW) IMT measurements has been established by comparing the IMT obtained by ultrasound imaging with the IMT determined by microscopy in pathologic evaluation (Pignoli et al. 1986, Wendelhag et al. 1991, Wong et al. 1993). However, the validity of near-wall (NW) measurements can be debated for the following reasons. It is the tissue interfaces with a sufficient difference in acoustic impedance that produce an echo. The anatomical location of a structure is defined by the leading edge (the upper edge) of the echo. Thus, thickness of an anatomical structure is defined as the distance between the leading edges of two different echoes (**Figure 7**). Similar double-line patterns can usually be seen in both the FW and the NW.

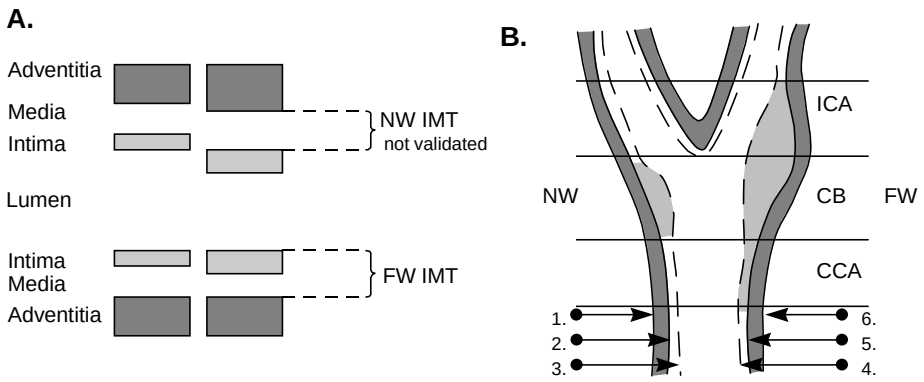

Figure 7. *A. Schematic illustration of anatomical (left) correlates of the echoes seen in B-mode ultrasound images (right). See text (chapter 2.4.1) for explanation. B. Anatomy of the carotid artery. NW is the near-wall, FW the far-wall. Numbers indicate the acoustic interfaces as follows: 1. periadventitia-adventitia, 2. adventitia-media, 3. intima-lumen, 4. lumen-intima, 5. media-adventitia, and 6. adventitia-periadventitia (Bond et al. 1989, Rubba and Faccenda 1993). The distance between points 4 and 5 corresponds to the far-wall IMT.*

To measure the intima-media complex in the FW, the leading edges of the lumen-intima interface and the media-adventitia interface are visualised. In the NW, the intima-lumen interface is usually well defined. However, the bright echoes produced by the adventitia overlap the echo originating from the adventitia-media interface, which cannot therefore be accurately visualised (Wendelhag et al. 1991).

Regardless of the physical facts mentioned above, there is data to support the use of NW measurements. Furberg et al. (Furberg et al. 1994a) have stated that even if there is a small systematic difference between NW and FW measurements, the progression rate of atherosclerosis does not differ between the NW and the FW. Furthermore, the inclusion of NW measurements markedly reduces the variability of progression. This in turn reduces the sample size required.

The intima and media layers of the arterial wall cannot be distinguished from each other by ultrasound. It can therefore be argued that thickening of the intima-media complex does not necessarily reflect only atherosclerosis, but also fibromuscular hypertrophy of the arterial media.

Atherosclerosis is a process that usually affects all parts of the arterial tree to some extent. The degree of carotid atherosclerosis has been shown to correlate with atherosclerosis in coronary arteries in autopsy studies (Young et al. 1960, Mitchell et al. 1962). Furthermore, superficial peripheral arteries, such as carotid arteries, are easier to image than coronary arteries. The causality between carotid artery IMT and clinical cardiovascular disease has been established in several prospective studies. The studies show that thickening of carotid artery walls predicts CHD or cerebrovascular disease (Salonen JT and Salonen R 1991, Bots et al. 1997, Chambless et al. 1997, Hodis et al. 1998, O´Leary et al. 1999). Cross-sectional studies have also shown a relationship between carotid artery IMT and clinically prevalent CHD and cerebrovascular and peripheral arterial disease (O´Leary et al. 1992, Bots et al. 1994a, Burke et al. 1995, Allan et al. 1997). A strong relationship between carotid IMT and angiographic presence of CHD has been shown by a number of authors (Crouse et al. 1987, Craven et al. 1990, Wofford et al. 1991, Adams et al. 1995).

Further evidence of the relationship between carotid artery IMT and cardiovascular disease can be obtained from studies that have shown an association between carotid IMT and the well-known risk factors for CHD, such as smoking, elevated serum TC, LDL cholesterol, TG, decreased HDL cholesterol, hypertension and age (Crouse et al. 1987, Tell et al. 1989, Salonen R and Salonen JT 1990, Salonen R and Salonen JT 1991b, Dempsey et al. 1992, O´Leary et al. 1992, Ryu et al. 1992, Wendelhag et al. 1992, Zanchetti et al. 1998).

Both overt type 2 diabetes (Geroulakos et al. 1994b, Pujia et al. 1994, Bonora et al. 1997a, el-Barghouti et al. 1997), and also asymptomatic hyperglycemia in non-diabetic subjects and impaired glucose tolerance have been associated with thickening of carotid IMT (Yamasaki et al. 1995, Hanefeld et al. 1999a). In non-diabetic subjects, the relationship between fasting plasma glucose and IMT has been weak or non-existent (Temelkova-Kurktschiev et al. 1998, Zanchetti et al. 1998, Hanefeld et al. 1999b).

With regard to the CHD risk factors typical of FCHL, postprandial triglycerides and remnant lipoproteins have been shown to be related to carotid IMT in healthy subjects, independent of fasting TG (Ryu et al. 1992, Sharrett et al. 1995, Karpe et al. 1998, Boquist et al. 1999, Karpe et al. 2001). Decreased insulin sensitivity/hyperinsulinemia has also been associated with carotid artery wall thickness (Folsom et al. 1994, Agewall et al. 1995, Howard et al. 1996, Bonora et al. 1997b, Hedblad et al. 2000, Bokemark et al. 2001). However, as Hedblad et al. (2000) speculated, this association may be due to covariance with other, established risk factors for CHD. LDL particle size was associated with the occurrence of moderate to large carotid artery plaques in subjects with insulin resistance (Hulthe et al. 1999) and with common carotid artery (CCA) IMT in healthy middle-aged men (Skoglund-Andersson et al. 1999), but there was no association between carotid artery IMT and LDL particle size in hypercholesterolemic subjects (Hulthe et al. 2000).

Associations between carotid IMT and some of the more novel CHD risk factors have also been reported. Carotid IMT has been shown to correlate with plasminogen activator inhibitor in CHD patients and healthy subjects (Salomaa et al. 1995, Vrtovec et al. 1999), and weakly with C-reactive protein in healthy, middle-aged women (Hak et al. 1999).

The first randomised, placebo-controlled lipid-lowering study to demonstrate treatment benefit using carotid IMT as an end point was the Cholesterol Lowering Atherosclerosis Study (CLAS) (Blankenhorn et al. 1993). The rate of progression of carotid IMT in asymptomatic individuals was either reduced or stopped in three years by pravastatin treatment in the Carotid Atherosclerosis Italian Ultrasound Study (CAIUS) and the Kuopio Atherosclerosis Prevention Study (KAPS) (Salonen et al. 1995, Mercuri et al. 1996). The Asymptomatic Carotid Artery Progression Study (ACAPS) and the Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries Study (PLAC-II) showed that statin treatment reduces both the progression of carotid IMT and the risk of cardiovascular events in asymptomatic subjects and CHD patients (Furberg et al. 1994b, Crouse et al. 1995).

3 AIMS OF THE STUDY

FCHL is a complex disorder most probably caused by the combined effect of several genes and environmental factors. According to one major hypothesis, most metabolic abnormalities observed in FCHL can be explained by disturbances in adipose tissue fatty acid metabolism.

This work aimed to test this hypothesis by determining whether regulators of adipose tissue metabolism do differ between affected FCHL family members and their unaffected relatives or unrelated control subjects. The studies were focused on selected key regulators of adipose tissue fatty acid and TG metabolism.

1. The serum concentration of complement C3 is elevated in subjects with atherosclerotic disease or dyslipidemia. The aim of *Studies I* and *II* was to examine whether serum C3 concentration is elevated in affected members of FCHL families as compared with their unaffected relatives. If it is, is there evidence that this is due to increased C3 production from adipocytes?

2. Acylation-stimulating protein is *in vitro* a potent stimulator of TG synthesis. *Study II* aimed to answer the question whether plasma ASP concentration is increased in affected members of FCHL families as compared with their unaffected relatives, and whether plasma ASP concentration changes postprandially in FCHL subjects.

3. Hormone-sensitive lipase is the key enzyme in hydrolysis of triglycerides in adipocytes. *Study III* was performed to determine whether the activity of adipose tissue hormone-sensitive lipase is reduced in Finnish FCHL patients, and whether HSL activity affects the lipid phenotype of FCHL patients.

4. Peroxisome proliferator-activated receptor γ is a transcription factor that regulates expression of several important genes controlling fatty acid metabolism in adipose tissue. The aim of *Study IV* was to investigate whether there are differences in the adipose tissue gene expression of PPARγ between FCHL patients and normolipidemic control subjects.

Should disturbances in adipose tissue metabolism prove critical for the development of FCHL, these alterations should be reflected in the degree of atherosclerosis in FCHL family members.

5. *Study V* aimed to assess whether disturbances in adipose tissue fatty acid and TG metabolism, or consequently the elevated fatty acid or TG levels, are reflected in the degree of atherosclerosis in FCHL family members. Additionally the study aimed to assess how the currently used lipid criteria can identify FCHL family members with atherosclerotic lesions from those without.

4 STUDY SUBJECTS

4.1 EUFAM Study

The European multicenter study on familial dyslipidaemias in patients with premature coronary heart disease (EUFAM Study) was a European Commission supported project aimed at resolving the metabolic and genetic abnormalities that cause FCHL and other familial dyslipidemias. The purpose of the study was to collect and carefully characterise a large number of FCHL families in four European countries. All results presented in this thesis are based on the FCHL families identified at the Helsinki (Helsinki University Central Hospital) and Turku (Turku University Central Hospital) centres in Finland during the EUFAM Study that began in 1995, and in the preceding pilot study. Local ethics committees approved the study protocols of the EUFAM Study and the substudies presented in this thesis. All subjects gave an informed consent before participating in the study.

4.2 Family collection

A flow-chart on family collection for the EUFAM Study is shown in **Figure 8**. FCHL probands were selected from patients undergoing elective coronary angiography or from angiography registers. The inclusion criteria for the probands were: (1) age 30-60 years, (2) at least 50% stenosis in at least one coronary artery as assessed by standard coronary angiography, or clinically verified CHD, (3) serum TC and/or TG $\geq 90^{th}$ age and gender-specific Finnish population percentile, (4) at least three accessible first-degree relatives, and (5) no history of type 1 diabetes mellitus, hepatic or renal disease, and no hypothyroidism. Familial hypercholesterolemia was excluded in each proband using the lymphocyte culture method (Cuthbert et al. 1986).

If the proband met the inclusion criteria and no exclusion criteria were present, all first-degree relatives had their serum lipid concentrations determined in the second phase of the study. Families with at least two affected (serum TC and/or TG $\geq 90^{th}$ percentile) family members (excluding families presenting lipid phenotype IIA only) were included in the final phase of the study, in which all accessible relatives were examined.

From each family member over 5 years of age, venous blood samples were collected after an overnight fast for determination of serum lipids, other biochemical parameters, and isolation of DNA. Study subjects older than 15 years underwent a 2-hour OGTT. Subjects completed standard questionnaires to provide data on previous medical history, medication, smoking and alcohol consumption. Waist and hip circumferences, weight and height were recorded, and BMI calculated as kg/m^2. Blood pressure was measured with a mercury sphygmomanometer with the subject in the supine position.

The TC and TG percentiles utilised in the EUFAM Study for subjects older than 25 years were derived from the FINRISK study performed in Finland in 1992 to assess the levels of CHD risk factors (Vartiainen et al. 1994). For subjects younger than 25 years, the Cardiovascular Risk Factors in Young Finns study was utilised to obtain

corresponding fractiles (Porkka et al. 1994). Calculation of the lipid cut-off points is described in detail by Porkka et al. (Porkka et al. 1997).

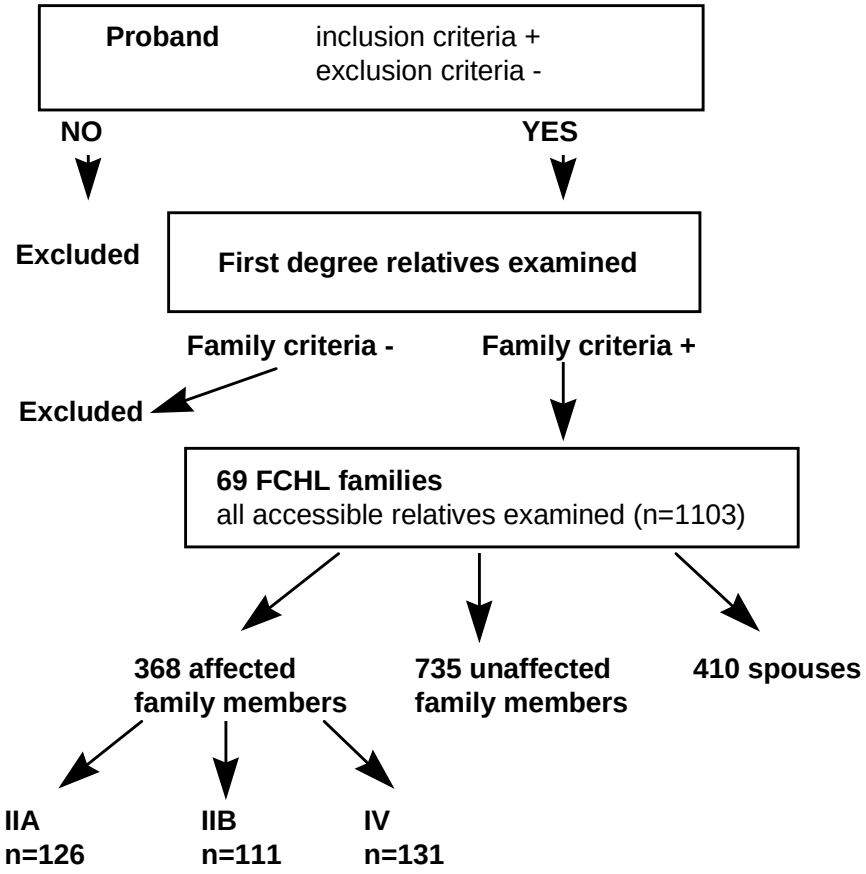

Figure 8. *Flow-chart on family collection for the EUFAM Study in Helsinki and Turku. See text (chapter 4.2) for details.*

4.3 Study subjects

All FCHL family members in these studies originally participated in the EUFAM Study. Subjects from a total of 48 FCHL families were included in the present studies. In each family (except for a family in which all first-degree relatives had lipid phenotype IV and other affected family members represented phenotype IIA, and a family in which first-degree relatives had phenotype IIA but phenotype IIB was seen in the second degree relatives) two different lipid phenotypes were represented among first-degree relatives or the subjects had lipid phenotype IIB. There were only four families in which no family member had lipid phenotype IIB. If the subject´s serum TC and/or TG exceeded, or was equal to the 90th age and gender-specific population percentile, the subject was categorised as affected (FCHL patient).

In *Study II* only serum TG, instead of both TG and TC, was used to determine the affection status. This was done because ASP is expected primarily to affect FFA and triglyceride metabolism, and only secondarily to influence serum cholesterol levels. None of the study subjects used lipid lowering medication, or had interrupted it for four weeks before blood sample collection or fat biopsy.

Table 1. *Subject characteristics.*

	M/F (n)	**Age** (years)	**BMI** (kg/m^2)	**TC** (mmol/l)	**TG** (mmol/l)	**ApoB** (mg/dl)
Study I						
Affected	10/17	39±19	25.7±4.7	6.59±1.32	2.17±1.43	126±40
Unaffected	15/11	38±21	23.3±3.7	4.98±0.99	1.14±0.44	88±22
Study II						
Affected	27/39	41±11	27.8±3.7	6.39±1.20	2.76±1.55	125±34
Unaffected	33/51	40±13	25.1±3.6	5.76±1.21	1.31±0.55	94±26
Fat load study						
FCHL	5/5	42±7	27.4±2.0	6.46±1.03	3.08±1.45	128±21
Controls	5/5	41±8	27.4±2.8	4.94±0.82	1.09±0.20	85±15
Study III						
FCHL	15/25	43±12	26.4±3.6	6.48±1.04	2.00±1.09	120±25
Controls	4/8	46±7	25.1±2.2	5.36±0.41	0.95±0.21	87±13
Study IV						
FCHL	16/25	41±12	27.2±4.3	6.39±0.97	1.75±0.88	111±22
Controls	5/9	43±9	26.2±2.8	5.18±0.57	1.11±0.48	89±18
Study V						
Affected	31/46	40±11	26.2±4.0	6.36±1.15	2.14±1.80	119±31
Unaffected	26/45	41±10	24.8±3.7	5.23±0.76	1.13±0.43	84±20

M/F, males/females.

Study I included 53 FCHL family members from 11 FCHL families for whom data on serum lipid and C3 levels were available. Serum complement levels of the 27 affected family members were compared with those of their 26 unaffected relatives. The affected subjects represented equally the three different lipid phenotypes IIA (*n*=10), IIB (*n*=8), and IV (*n*=9). Three unaffected and six affected subjects had type 2 diabetes.

One hundred and fifty non-diabetic members of 35 FCHL families provided data on fasting plasma ASP concentration and were included in *Study II*. Sixty-six study subjects had a serum TG level that exceeded the 90^{th} age and gender-specific percentile, and were categorised as affected in this study. The other 84 family members were coded as unaffected, and were compared with the affected family

members. Eighteen subjects had already participated in *Study I*, but serum lipids and C3 were measured on two different occasions.

Ten healthy, normoglycemic FCHL subjects (5 males and 5 females) with serum TG exceeding the age and gender-specific 90th percentile (lipid phenotypes IIB (*n*=6) or IV (*n*=4)) were included in the oral fat load study. Ten unrelated, normolipidemic control subjects were carefully matched for age, sex, and BMI. One female patient and her control subject smoked 15 cigarettes per day. One patient and her corresponding control subject were postmenopausal.

For *Study III*, subcutaneous adipose tissue biopsy specimens were obtained from 45 FCHL patients who belonged to 13 FCHL families. In addition, fat biopsy specimens were taken from three family members whose serum apoB exceeded the 90th age and gender-specific percentile. These three subjects were coded affected and included only in the linkage analysis when serum apoB, instead of TC or TG, was used as a trait. Twelve unrelated, normolipidemic subjects were also biopsied, and these served as a control group. These 12 subjects were either spouses of FCHL family members or spouses from families previously excluded from the EUFAM Study because of the low number of affected family members. Five affected subjects included in linkage analysis had type 2 diabetes. The subjects with diabetes were excluded from other analyses, which consequently comprised 40 non-diabetic affected FCHL family members (11 with phenotype IIA, 22 with phenotype IIB, and 7 with phenotype IV) and 12 control subjects.

For *Study IV*, an adipose tissue sample was available from 41 non-diabetic FCHL patients from 22 families. Fourteen unrelated, normolipidemic control subjects served as a control group. Seventeen FCHL patients and eight control subjects had already been previously biopsied for *Study III*.

Study V. In principle, all non-diabetic members of the families collected for the EUFAM Study in Helsinki and Turku centres who were over 18 years of age and lived a reasonable distance from Helsinki were invited for carotid ultrasonography examination. Family members who currently used, or had regularly used, lipid lowering medication were excluded because the treatment might have affected their IMTs, and more importantly, their serum lipid levels. Moreover, most family members who use lipid lowering medication have CHD, and interrupting medication another time was considered unethical. Thus, 39 FCHL probands and 5 relatives with both CHD and lipid lowering medication and 11 relatives with lipid lowering medication but no CHD, were excluded. A total of 148 members from 39 FCHL families (~ 4 subjects/family) participated in *Study V*.

5 METHODS

5.1 Lipid, lipoprotein, and other analytical methods

Serum TC and TG concentrations were determined with an automated Cobas Mira analyser (Hoffman-La Roche, Basel, Switzerland) by enzymatic methods (kits 07 3664 3 and 07 3680 5, respectively). Serum HDL cholesterol was quantified enzymatically after precipitation with phosphotungstic acid and magnesium chloride (kit 07 2067 4, Hoffman-La Roche, Basel, Switzerland). In *Studies I* and *III* serum LDL cholesterol concentration was calculated using the Friedewald formula irrespective of serum TG concentration (serum TG exceeded 4.52 mmol/l in five subjects in *Studies I* and *III*). For *Studies II* and *V* LDL was separated by sequential flotation in an ultracentrifuge (Taskinen et al. 1988). Commercial quality control samples were used to standardise the measurement of lipids and apolipoproteins. Interassay coefficients of variation (CV) for lipid and apolipoprotein measurements were studied over a 12-month observation period. CVs for lipid measurements were 2% for TC and TG and 3% for HDL cholesterol. As for lipid measurements, the laboratory participates in an international quality assessment scheme organised by Labquality (Helsinki, Finland).

Serum total apoB, serum apoA-I, and apoA-II concentrations were measured by immunoturbidimetry (Orion Diagnostica, Espoo, Finland; kit 67249 and Boehringer-Mannheim, Mannheim, Germany; kits 726 478 and 726 486, respectively). Interassay CV was 4% for apoB, apoA-I and apoA-II determinations. Commercial standards used for apolipoprotein assays had been calibrated against the controls from the Centers for Disease Control and Prevention.

The OGTT was performed with a 75 g dose of glucose. Blood was drawn at 0, 30, 60 and 120 minutes for measurement of blood glucose, serum free insulin and FFA. Blood glucose concentrations were measured using glucose dehydrogenase method (Gluc-DH, Merck Oy, Darmstadt, Germany). Serum free insulin concentrations were determined by radioimmunoassay using the Phadeseph Insulin RIA kit (Pharmacia, Uppsala, Sweden). The interassay CV of insulin measurements varied between 6% and 7% in low and high controls, respectively. Concentrations of FFA in serum were measured using the microfluorometric method of Miles et al. (1983). CVs over a 12-month period were 8% for the low control and 4% for the high control.

5.2 Acylation-stimulating protein, C3, and C4 measurements

ASP concentrations were measured from EDTA-plasma using ELISA (Quidel, San Diego, USA), which uses monoclonal human anti-C3a-desArg as a detecting antibody. Each measurement was performed in triplicate and all samples from a fat load test (patient and control subject) were measured in the same assay if possible. The inter- and intra-assay CVs were 12% and 2%, respectively.

The ASP results obtained with Quidel´s ELISA kit were compared with the results derived using sandwich ELISA, which is used by Sniderman and Cianflone (Saleh et al. 1998). ASP concentrations of 73 samples (not included in the present study)

were determined by both methods. The Spearman´s correlation coefficient for the two methods was 0.59, $P<0.001$.

Serum concentrations of C3 and C4 were determined at the Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, by nephelometry, using antibodies against C3c and C4 (Behringwerke AG, Marburg, Germany) and a BN-100 nephelometer (Hoechst Fennica, Helsinki, Finland). The interassay CVs for C3 and C4 determinations were 8% and 4%, respectively.

5.3 Oral fat load test and density gradient ultracentrifugation

The test meal was a 1000 kcal mixed meal containing 72 g of fat, 50 g of carbohydrates and 38 g of protein. The total amount of cholesterol was 500 mg. The test was started in the morning after a 12-hour fast by inserting a plastic cannula into the patient´s antecubital vein and by drawing the fasting blood samples. After consumption of the meal, blood samples were collected at 2, 3, 4, 6, 8, and 9 hours postprandially. The study subjects were fasted during the test and were only allowed to drink water until the last blood samples were taken.

The density of plasma samples obtained at the various time points during the oral fat load test was adjusted to d=1.10 kg/l with saline. Aprotinin (50 IU/ml) and phenylmethylsulphonyl fluoride (1mmol/l) were added as preservatives. Four ml of plasma were placed in a 13.4 ml tube (Ultra-Clear, Beckman, Palo Alto, USA) and carefully overlayered with 3.0 ml of d=1.065 kg/l and d=1.020 kg/l, and 2.8 ml of d=1.006 kg/l NaCl solutions. A Beckman Optima LC ultracentrifuge with an SW40 Ti swinging bucket rotor was used for ultracentrifugation at 40 000 rpm at +15°C. Chylomicrons, representing Svedberg flotation units [S_f] >400 fraction were isolated and collected by aspirating the top 1.0 ml fraction after a 32-minute run. The tube was refilled with d=1.006 kg/l saline, and ultracentrifugation was continued under the same conditions for 3 h 28 min. Thereafter, large VLDL particles (VLDL1, [S_f] 60-400) were collected as previously. Other lipid fractions were isolated as described elsewhere (Karpe and Hamsten 1994, Mero et al. 1998).

5.4 Hormone-sensitive lipase activity

The subjects were studied in the morning after a 12-hour fast. A subcutaneous fat biopsy specimen of 300-1000 mg was obtained under local anaesthesia from the paraumbilical region using a 14G needle and a syringe. The adipose tissue specimen obtained was immediately placed in saline, frozen in liquid nitrogen, and stored at -80°C.

The assay was performed essentially as described by Fredrikson (1981) and Frayn (1993). Adipose tissue samples of approximately 300 mg were homogenised in 600 µl (300 µl if less than 200 mg of adipose tissue) of a buffer containing 0.25 mol/l sucrose, 1 mmol/l EDTA, 1 mmol/l dithioerythritol and the protease inhibitors leupeptin (20 µg/ml), antipain (20 µg/ml) and pepstatine (1 µg/ml). The samples were then centrifuged at 12 000 rpm for 3 hours at 4°C in an Eppendorf 5403 centrifuge. Thereafter, the fat-free infranatant was recovered for analysis of HSL

activity using 1(3)-mono-[^{3}H]-oleoyl-2-*O*-oleylglycerol as substrate (Tornqvist et al. 1978). The substrate was mixed with phospholipid as stabiliser, and emulsified by sonication within one hour before the assay. The substrate was obtained from the Department of Medical and Physiological Chemistry at Lund University in Sweden. All samples were incubated in triplicate at 37°C for 30 min. Methanol-chloroform-heptane was used to stop the reaction. The labelled FFA were isolated by addition of 0.1 mmol/l potassium carbonate buffer (pH 10.5). After centrifugation for 20 min at 2000 rpm, an aliquot of the supernatant was collected for scintillation counting. One unit of enzyme activity is defined as 1 µmol of fatty acid released/min. The HSL activity was related to the total protein concentration of the sample, which was measured using BCA protein assay (Pierce, Rockford, USA). The within-run CV for the HSL activity assay is 7% (Reynisdottir et al. 1995). All samples of *Study III* were analysed on a single occasion.

5.5 Quantification of adipose tissue gene expression

Adipose tissue samples were obtained as described for HSL. An RNeasy total RNA kit (Qiagen, Courtaboeuf, France) was used to prepare total RNA from fat samples. RNA samples were quantified by spectrophotometry, and absorbance ratios at 260/280 nm were between 1.7 and 2.0. The average yield of total RNA was 1.8±0.7 µg of total RNA/100 mg adipose tissue. Total RNA samples were stored at -80°C in water dilution until analysed. The different mRNAs were quantified using a reverse transcription reaction followed by a competitive polymerase chain reaction (RT-cPCR) (Auboeuf and Vidal 1997). The assay consists of a specific first strand cDNA synthesis followed by co-amplification of the reverse transcription product with known amounts of a DNA competitor molecule. The construction of competitor molecules for C3 (Dusserre et al. 2000), HSL (Laville et al. 1996), and PPARγ1 and PPARγ2 (Auboeuf et al. 1997) is described elsewhere. The reverse transcription reaction was performed from 0.1 µg of total RNA in the presence of 2.5 U of thermostable reverse transcriptase (*Tth* DNA polymerase, Promega, Charbonieres, France) and 15 pmol of one of the designed antisense primers, in conditions that ensure that all target mRNA molecules are transformed into single strand cDNA (Auboeuf and Vidal 1997). The sequences of the primers used are presented in **Table 2**.

Table 2. *Sequences of the primers used for the RT-cPCR in Study IV.*

Gene	Sense primers	Antisense primers
C3	5´-TCCTGGACTGCTGCAACTAC-3´	5`-AATCTCCCACGTGGTGATGG-3´
HSL	5´-TCTTCTGCACCAGCCACAAC-3´	5´-AGATGGTCTGCAGGAATGGC-3´
PPARγ	5´-TCTCTCCGTAATGGAAGACC-3´	5´-GCATTATGAGACATCCCCAC-3´
PPARγ2	5´-GCGATTCCTTCACTGATAC-3´	5´-TCCTGGACTGCTGCAACTAC-3´

For the competitive PCR reaction, the reverse transcription product was added to a PCR mix. The sense primers were 5´-labelled with the CY-5 fluorescent dye (Eurogentec, Seraing, Belgium). Four aliquots of 20 µl were transferred into 0.5 ml microtubes containing a different, but known, amount of the competitor in a volume

of 5 µl. The PCR mixture and the competitive PCR reaction have been described in detail (Dusserre et al. 2000). The fluorescent-labelled PCR products were separated in 4% polyacrylamide gel with an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Uppsala, Sweden), and analysed using Fragment Manager software (Pharmacia, Sweden) (Auboeuf et al. 1997). The target mRNAs and the competitors generate different sized PCR products, which enables their separation by polyacrylamide gel electrophoresis. To determine the concentration of the target mRNA, the logarithm of the ratio of the competitor to the target cDNA peak surface was plotted versus the logarithm of the amount of competitor added in the PCR mix. However, to correct for differences in nucleotide number, the ratio competitor/target was first multiplied by a correction factor obtained by dividing the number of base pairs in target cDNA by the number of base pairs in the competitor (244/229 for C3, 252/306 for HSL, 474/400 for PPARγ, and 580/506 for PPARγ2). At the competition equivalence point (log ratio=0) the initial concentration of the target corresponds to the initial concentration of competitor added (Gilliland et al. 1990). Finally, the value obtained was multiplied by two since the competitor is double-stranded as opposed to single-stranded target RNA. The PPARγ1 mRNA expression level was obtained by subtracting the amount of PPARγ 2 mRNA from the total PPARγ mRNA level. Contamination with genomic DNA was excluded by performing the reverse transcription step without reverse transcriptase in the control samples. The interassay CV in studies performed identically to *Study IV* has been 4% to 12% (Auboeuf et al. 1997, Auboeuf and Vidal 1997).

5.6 B-mode ultrasonography and intima-media thickness measurement

Ultrasound scannings were performed with a Hewlett-Packard Image Point M2410A ultrasound system (Hewlett-Packard, Andover, USA) equipped with a 10 MHz linear array transducer. Scannings were videotaped with a Panasonic AG-MD830E PAL S-VHS VCR (Matsushita Electric Industrial Co., Ltd., Osaka, Japan). One physician (K.Y.) carried out all ultrasound examinations. During scanning, the patient´s head was rotated 45 degrees away (if possible) from the side being scanned in the supine position. Longitudinal images from three distinct projections (anterolateral, lateral and posterolateral) were displayed for CCA, carotid bulb (CB), and internal carotid artery (ICA). Scannings were focused and measurements carried out at a total of 28 sites: the FW and the NW of six arterial segments: right and left distal 1 cm of CCA, CB, and proximal 1 cm of ICA. In CCA and CB all three projections were used, and in ICA a single angle with the best visibility was used.

IMT measurements were made by a single reader at Oy Jurilab Ltd (www.jurilab.com). Computer analysis of ultrasound images to measure IMT was performed with a PC equipped with a video frame grabber interfaced to an S-VHS VCR. The Prosound software, developed by Robert Selzer (Caltech, Pasadena, USA), was used to measure the IMTs (Selzer et al. 1994). This software digitises the video ultrasound image, locates the interfaces, and computes the IMTs. Three variables are derived from each measurement: the minimum, mean and maximum IMT. IMT measurements from videotapes were made at a total of 28 sites corresponding to the 28 sites where the scanning was focused. No measurements were done at the sites of highly echogenic structures, which were shown as acoustic shadowing. All measurements were made in the diastole, assessed as the

phase when the lumen diameter is at its smallest and IMT at its largest. All outcome variables were calculated on a per subject basis. The average of all mean IMT measurements (mean-IMT) over 28 sites (or fewer, if measurement could not be performed at all 28 sites) was chosen as the primary outcome variable. Likewise, the average of maximum IMTs (max-IMT), the average of mean far-wall IMTs (FW-IMT), the average of mean near-wall IMTs (NW-IMT) and the average of maximum IMTs for each segment (CCA-IMT, CB-IMT, ICA-IMT), were calculated as secondary outcome variables.

Intra-sonographer variability in the scannings was estimated by scanning 16 subjects (10% of all study subjects) twice on two different occasions within three weeks. The Spearman´s correlation coefficient between the mean-IMTs of the paired scannings was 0.927 (P<0.001), CV was 2.4%, and the absolute difference (mean±SD) 0.028±0.024 mm. In the three carotid artery segments the correlations between two max-IMT measurements ranged between 0.71 (ICA) and 0.96 (CB), CV was 2.8% (CCA) - 7.2% (ICA), and absolute difference (mean±SD) varied from 0.039±0.036 mm (CCA) to 0.091±0.092 mm (ICA). To assess the intra-reader variability, the reader measured the scannings of 10 study subjects twice. The Spearman´s correlation coefficient between the mean-IMTs of the paired scannings was 0.997 (P<0.001), CV was 0.29%, and the absolute difference 0.003±0.005 mm.

5.7 Statistical analyses

Data are expressed as mean±SD, frequencies, or percentages. The characteristics of fat load study subjects are reported as median and interquartile range (*Study II*). Area under the curve (AUC) and incremental AUC calculations were performed using the trapezoid rule (Matthews et al. 1990).

For statistical comparisons of study groups, Chi-square or Fisher´s exact test was used for categorical variables. The non-parametric Mann-Whitney U test was used for continuous variables in *Study I* and to compare FCHL patients and controls in the fat load study. Because the study subjects were obtained from a family material, and were related to each other, they did not fulfill the assumption of independence. To correct, at least partly, for the non-independence of study subjects, two-way analysis of variance (ANOVA) was used for statistical comparison of the groups in *Studies II-V*. Family number (which indicates belonging to a certain family and is identical for all members of a family) was entered as a random factor in two-way ANOVA.

To examine correlations between variables, Spearman´s correlation coefficients were calculated in *Study I*. In *Studies II, IV,* and *V* multivariate analysis was used to calculate correlations and partial correlations. This enabled the use of family number as an independent variable, again to correct for the non-independence of study subjects. In the fat load study, within-group changes from baseline to postprandial values, and differences in the postprandial responses between the groups were assessed by repeated-measures ANOVA with the Greenhouse-Geisser adjustment (Ludbrook 1994).

Multivariate analysis in *Study V* was performed with the $\log_{10}$-transformed mean-IMT as the dependent variable. All variables showing a correlation (adjusted only for family number) with mean-IMT that was at least moderate (P<0.20), but not necessarily statistically significant, were chosen for the multivariate analysis. Variables with only a moderate association with mean-IMT were also selected, because although not important alone, they may be important predictors when examined together with other parameters (Hosmer and Lemeshow 1989). Also in this analysis, family number was forced into the model. A backward variable selection method was used. Variables were removed from the model until the best fitting model with the maximum adjusted multiple R^2 was achieved.

To assess familial correlations the FCOR program of S.A.G.E. package v. 3.1 (S.A.G.E. 1997) was used in *Studies II* and *III*. The FCOR program performs correlation analyses in families taking into account the structure of the pedigrees. A new version of the FCOR program, FCOR2 (S.A.G.E. release 4.0 Beta 6) was available for *Study III*. The FCOR2 program is also able to assess the significance of the correlations. Familial correlation analyses were performed using the weight method ”uniform weight to pedigrees”.

Linkage analysis was used in *Study III* to investigate whether low HSL activity is linked to FCHL or FCHL-related phenotype. We used the MLINK program of the Linkage package (Lathrop et al. 1984, Ott 1991). This program can be used to compute the overall likelihood of the data on two alternative assumptions: that the two loci or traits are linked with the given recombination fraction (θ) and that they are not linked. The lod score is the logarithm to the base 10 of the ratio of these two likelihoods. This method can also be utilised to study whether two phenotypic traits are linked. The penetrances of the traits studied were not known and therefore, to avoid problems of unknown or incomplete penetrances, an affecteds-only strategy was used. The affecteds-only strategy uses phenotypic information only from the affected individuals. The unaffected individuals are coded as unknown. Also, because the mode of inheritance is unknown, both dominant and recessive modes of inheritance for both FCHL and low HSL activity were tested. For linkage analysis, the prevalence of FCHL was estimated as 1-2% (Grundy et al. 1987), and a gene frequency of 0.6% was used for the dominant mode of inheritance and 10.95% for the recessive model.

6 RESULTS

6.1 Complement C3

6.1.1 Serum C3

Serum C3 concentrations were measured in *Studies I, II, and V*. The data from the three studies are combined below. If the subject had participated in more than one study, the measurement of *Study II* was preferred. Only non-diabetic subjects were included in the current analyses. The 221 family members were divided into three groups according to their serum TG and TC levels. The high TG group (*n*=83) comprised subjects representing lipid phenotypes IIB and IV, while the high TC group (*n*=47) contained subjects with phenotype IIA. Family members whose TC and TG were both below the 90th percentile, were coded as unaffected (*n*=91).

Mean (±SD) serum C3 levels of the family members are shown in **Table 3**. The three phenotype groups had significantly different serum C3 levels (*P*<0.001) (**Table 3, Figure 9**). When adjusted for BMI, the difference was still significant (*P*=0.05). In post-hoc analyses the high TG group differed from the high TC group (*P*=0.004), and the unaffected group (*P*<0.001). There was also a significant difference in mean serum C3 level between the subjects with high TC and the unaffected relatives (*P*=0.001). Men had a slightly higher average serum C3 than women, but the difference did not reach statistical significance (1.5±0.5 g/l vs. 1.4± 0.3 g/l, *P*=0.06), unless adjusted for BMI (*P*=0.043).

Table 3. *Subject characteristics combined from Studies I, II, and V.*

	Lipid phenotype						
	n	**High TG**	n	**High TC**	n	**Unaffected**	*P*-value [a]
Age (years)	83	40.0±13.6	47	42.4±14.0	91	39.7±14.3	0.45
Males/females, (% M)	83	33/50 (40)	47	20/27 (43)	91	38/53 (42)	0.94
BMI (kg/m^2)	83	27.4±4.3 [b]	47	24.8±3.2	91	24.7±3.8	0.02
Waist-hip ratio	82	0.88±0.09 [c]	41	0.84±0.09	81	0.83±0.08	0.03
TG (mmol/l)	83	2.64±1.52	47	1.44±0.54 [d]	91	1.19±0.45	
TG-max (mmol/l)	83	3.33±1.92	47	1.71±0.57	91	1.24±0.45	
TC (mmol/l)	83	6.26±1.23	47	6.72±1.10	91	5.17±0.83	
TC-max (mmol/l)	83	6.70±1.23	47	7.30±0.92	91	5.33±0.81	
ApoB (mg/dl)	83	124±34	47	114±23	89	85±20 [e]	<0.001
HDL-C (mmol/l)	83	1.21±0.36 [b]	47	1.61±0.47	91	1.53±0.42	<0.001
Glucose (mmol/l)	82	4.6±0.6	41	4.6±0.6	85	4.3±0.5	0.17
Insulin (mU/l)	83	10.1±4.7 [b]	44	6.8±3.3	89	7.0±3.4	<0.001
C3 (g/l)	83	1.6±0.4 [b]	47	1.4±0.4	91	1.2±0.2 [e]	<0.001
ASP (ng/ml)	77	151±53	39	133±63	75	137±55	0.66

TG-max and TC-max are the highest TG and TC values, respectively, measured for each subject at any visit during the EUFAM Study. HDL-C is HDL cholesterol. [a] Assuming all study subjects are independent. [b] P<0.01 vs. high TC group and the unaffected. [c] P<0.001 vs. the unaffected, [d] P<0.05 vs. the unaffected, [e] P<0.01 vs. the high TG and high TC groups.

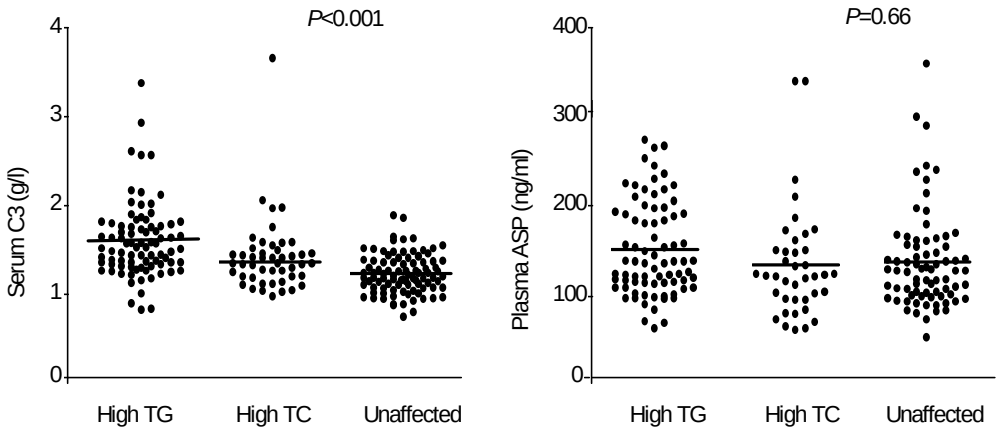

Figure 9. *Individual serum C3 and plasma ASP concentrations shown separately for the three phenotype groups. P-values denote differences between the three groups. Horizontal lines represent the means of the groups*

Gender and family number-adjusted correlations between serum C3 and selected clinical and biochemical variables are shown in **Table 4**. Serum C3 values are plotted against serum TG and insulin-AUC values in **Figure 10**. Correlations with serum C3 were not significantly affected by adjustment for BMI (data not shown).

Table 4. *Gender and family number-adjusted correlations of serum C3 concentration (g/l), plasma ASP concentration (ng/ml), and adipose tissue C3 mRNA expression (amol/µg of total RNA) with selected clinical and biochemical variables.*

	Serum C3		**Plasma ASP**		**C3 mRNA**	
	n	r	n	r	n	r
Age (years)	221	0.11	191	0.01	40	0.24
BMI (kg/m^2)	221	0.28	191	0.11	40	0.36 [a]
Waist-hip ratio	204	0.38	190	0.16	39	0.31
TG (mmol/l)	221	0.62	191	0.21 [b]	40	0.25
TC (mmol/l)	221	0.48	191	0.10	40	0.31
ApoB (mg/dl)	219	0.62	190	0.12	36	0.31
HDL-C (mmol/l)	221	-0.42	191	-0.12	40	-0.07
Glucose-AUC (mmol/l·h^{-1})	203	0.32	182	0.03	39	0.34 [a]
Insulin-AUC (mU/l·h^{-1})	202	0.39	181	0.12	38	0.36 [a]
FFA-AUC (µmol/l·h^{-1})	195	0.35	175	0.06	35	0.22
C3 (g/l)					39	0.20
ASP (ng/ml)	191	0.30			34	-0.02

HDL-C denotes HDL cholesterol. All correlations with serum C3, except for age, were significant (P<0.001). [a] P<0.05, [b] P<0.01. Significance levels are reported assuming all study subjects are independent.

Familial correlations of serum C3 were calculated in *Study II* (see *Table 4* in *Study II*). Age and gender-adjusted values obtained by calculating residuals for the traits were used in the analyses. A statistically significant sibling-sibling correlation for C3 (r=0.26, P<0.01) was found in 103 sibling pairs, suggesting that serum C3 level may be familial. The parent-offspring correlation (r=0.15 in 54 pairs) did not reach statistical significance. Serum C3 correlated with TG (r=0.22, P<0.01), HDL cholesterol (r=-0.20, P<0.05), and FFA-AUC (r=0.24, P<0.01) in siblings. The parent-offspring correlations showed significant positive values between C3 and insulin (r=0.21, P<0.05), and BMI (r=0.21, P<0.05), and a negative correlation between C3 and HDL cholesterol (r=-0.32, P<0.01). These correlations imply that C3 may share a common genetic background with TG, HDL cholesterol, insulin, FFA and BMI.

6.1.2 C3 gene expression in adipose tissue

In *Study IV*, the mRNA expression level of C3 was quantified in subcutaneous adipose tissue of 41 affected FCHL family members and 14 normolipidemic control subjects. The C3 mRNA level of one FCHL subject was 86.0 amol/µg of total RNA, which is much more than the maximum for the other subjects (46.3 amol/µg total RNA). The value obtained in this sample remained identical when the measurement was repeated. This subject was excluded from further analyses. C3 mRNA expression was similar in FCHL subjects and the normolipidemic control subjects (20.0±9.8 vs. 19.7±9.4 amol/µg total RNA, P=1.00). No gender difference was observed in adipose tissue C3 mRNA expression (P=0.40).

The gender and family number-adjusted correlations between adipose tissue C3 mRNA levels and selected FCHL-related variables in FCHL patients are shown in **Table 4**. C3 mRNA levels are plotted against serum TG and insulin-AUC values in **Figure 10**.

The number of control subjects in the present study was limited, but significant gender-adjusted correlations between C3 mRNA expression and glucose-AUC (r=0.80, P=0.004) and insulin-AUC (r=0.68, P=0.02) were also seen in the control group. The adipose tissue C3 mRNA expression correlated relatively strongly with serum C3 (r=0.53) and plasma ASP (r=0.59) in controls when adjusted for gender but the correlations did not reach statistical significance. Adjustment for age or BMI did not significantly influence any of the above results.

6.2 Acylation-stimulating protein

6.2.1 Fasting plasma acylation-stimulating protein

Fasting plasma ASP concentrations were measured in *Studies II and V*. The data from these two studies have been combined below, as for C3 above. Mean (±SD) plasma ASP levels are shown in **Table 3**. There were no statistically significant differences in mean plasma ASP levels between the three groups (P=0.66) (**Figure 9**). Adjustment for BMI did not affect the results. Unlike with C3, plasma ASP level (mean±SD) was higher in females (150±57 ng/ml) than in males (130±53 ng/ml, P=0.006), regardless of BMI.

Gender and family number-adjusted correlations between plasma ASP and selected clinical and biochemical variables are shown in **Table 4** (for gender, age and family number-adjusted correlations see *Study II*). The correlation between plasma ASP and serum TG was no longer significant if adjusted for BMI (data not shown).

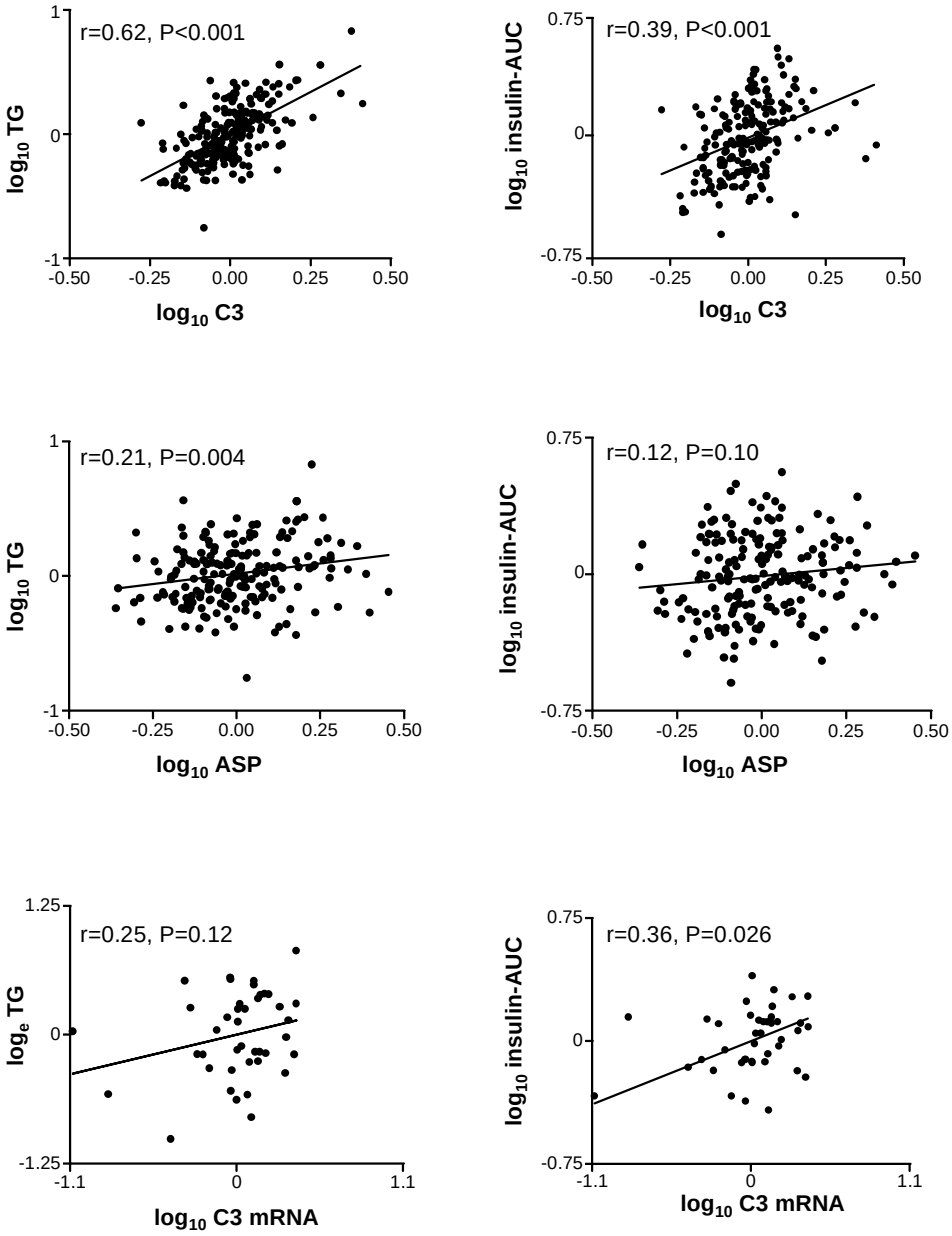

Figure 10. *Scatter plots of serum C3, plasma ASP, and adipose tissue C3 mRNA levels vs. serum TG and insulin-AUC values in FCHL family members. All variables were logarithmically transformed and adjusted for gender and family number. Adjusted numbers were obtained by calculating residuals using multivariate analysis. Therefore, the scales of the axes do not have clinical significance.*

Age and gender-adjusted values were used in the familial correlation analyses. A significant parent-offspring correlation for ASP was found (r=0.24, P<0.05). However, the sibling-sibling correlation for ASP was not significant (r=0.06) even though the number of pairs studied was higher for siblings (103 pairs) than for parents and offspring (55 pairs). Caution should therefore be exercised in any interpretation of the parent-offspring correlation. No significant familial correlations were observed between plasma ASP and the other FCHL-related traits.

6.2.2 Postprandial plasma acylation-stimulating protein

Postprandial plasma ASP concentrations were measured in 10 hypertriglyceridemic, unrelated FCHL patients and in 10 age, gender and BMI-matched normolipidemic control subjects (for subject characteristics see *Table 2* in *Study II*). The fasting ASP level was higher in FCHL patients (122±25 ng/ml, range 75-164 ng/ml) than in control subjects (92±18 ng/ml, range 65-119 ng/ml, P=0.009). There was no significant postprandial change in plasma ASP in either FCHL patients (P=0.31) or control subjects (P=0.25), and the responses did not differ between the two groups (P=0.47) (**Figure 11**). No clear peaks were observed in postprandial ASP levels. Accordingly, although higher, the average incremental AUC of ASP in FCHL patients was not significantly different from that in control subjects (117±42 vs. 46± 51 ng/ml·h^{-1}, P=0.10).

TG concentration of chylomicrons increased significantly in both groups postprandially (P<0.001 for both groups) (**Figure 11**), and the response was more marked in FCHL patients than in control subjects (P=0.002). Serum FFA concentration increased significantly during the test (P<0.001 for both groups), but the response did not differ between the groups (P=0.29). Postprandial insulin response was higher in FCHL patients than in control subjects (P=0.05).

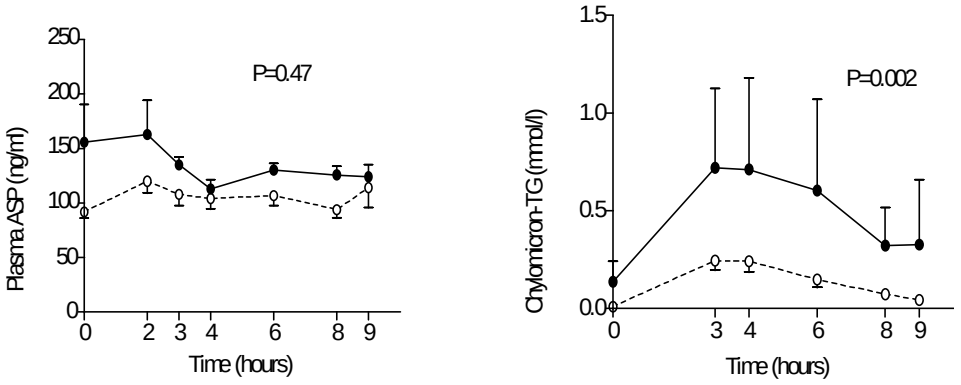

Figure 11. *Postprandial plasma ASP and chylomicron-TG concentrations in FCHL patients (black circles) and control subjects (open circles) after an oral fat load. Data points are means, error bars indicate standard error of mean. P-values denote differences between the two groups.*

6.3 Hormone-sensitive lipase

6.3.1 Hormone-sensitive lipase activity in adipose tissue

Subcutaneous adipose tissue HSL activity was measured in 40 FCHL patients and 12 normolipidemic control subjects (see *Study III* for subject characteristics). The average HSL activity (mU/mg protein) of all FCHL subjects (22±8) did not differ from that of the control subjects (22±7, *P*=0.97). HSL activity in the three phenotype groups (IIA, IIB and IV) averaged 23±6, 22±8 and 22±10 mU/mg protein, respectively, and did not differ from HSL activity in the 12 normolipidemic spouses (*P*=0.75). Adjustment for age or BMI did not significantly affect the results (data not shown). The individual HSL activities are shown in *Figure 1* in *Study III*. The enzyme activity (mU/mg protein) in male subjects averaged 21±8 and in female subjects 23±8 (*P*=0.242).

Familial correlations of adipose tissue HSL activity were calculated for 25 sibling pairs. A significant sibling-sibling correlation (*r*=0.51, *P*<0.01) was found, indicating that HSL activity may be familial. With regard to other FCHL related traits, significant correlations between HSL activity and TC (*r*=0.39, *P*<0.05) and waist-hip ratio (*r*=0.44, *P*<0.05) were found in 28 sibling pairs. No significant familial correlations were observed for TG, apoB, glucose, insulin or BMI. The above *P*-values in familial correlation analyses were reported assuming that all pairs were independent.

No linkage between HSL activity and FCHL was found using any of the inheritance patterns (see chapter 5.7 on statistical analyses). The best lod score of 1.53 (θ =0.00) was obtained using recessive inheritance mode for both traits. No significant linkage could be found when linkage between HSL activity and serum TG (lod score 1.56, θ=0.00, recessive inheritance mode used for both traits), apoB (1.27), TC (0.88), FFA-AUC (0.70) or lipid phenotype IIB (0.91) were examined.

6.3.2 Hormone-sensitive lipase gene expression in adipose tissue

Subcutaneous adipose tissue HSL mRNA expression was quantified in 40 FCHL patients and 14 normolipidemic control subjects. The adipose tissue HSL mRNA level in the FCHL patients (183±121 amol/µg total RNA) did not differ from that of the control subjects (213±163 amol/µg total RNA, *P*=0.86). There was no difference between HSL gene expression between men (191±120 amol/µg total RNA) and women (191±141 amol/µg total RNA, *P*=0.94).

In FCHL patients, the only significant gender-adjusted correlations of HSL mRNA expression were observed with BMI (*r*=0.42, *P*=0.009) and waist-hip ratio (*r*=0.36, *P*=0.029). Seventeen FCHL subjects had data on both adipose tissue HSL activity and gene expression. No significant correlation was observed between these two parameters (*r*=0.11, *P*=0.69) when adjusted for gender.

In the control subjects, HSL mRNA expression showed a significant inverse correlation with waist-hip ratio (*r*=-0.64, *P*=0.020) when adjusted for gender. A relatively high, though statistically insignificant, correlation between HSL gene expression and insulin-AUC was observed in control subjects when adjusted for

gender (r=-0.53, P=0.10). Adjustment for age or BMI did not significantly affect the above correlations except for the correlation between HSL mRNA level and waist-hip ratio in control subjects, which weakened when adjusted for BMI (r=-0.44, P=0.14).

6.4 PPARγ gene expression in adipose tissue

In *Study IV*, PPARγ1 and PPARγ2 gene expression were measured from subcutaneous fat samples of 41 FCHL subjects and 14 normolipidemic control subjects. The amount of PPARγ1 mRNA was derived by subtracting the amount of PPARγ2 mRNA from total PPARγ mRNA.

No statistically significant differences were detected between the affected FCHL family members and the control subjects with regard to mRNA levels of PPARγ1 (17 ±8 vs. 17±6 amol/µg total RNA, P=0.63) or PPARγ2 (2.0±1.7 vs. 2.1±1.1 amol/µg total RNA, P=0.39). PPARγ1 gene expression was comparable in males and females (14±8 vs. 19±7 amol/µg total RNA, P=0.42). Women had significantly higher PPARγ2 gene expression in adipose tissue than men (2.4±1.6 vs. 1.4±1.3 amol/µg total RNA, P=0.001). Adjustment for age or BMI had no significant effect on the differences between the groups or genders (data not shown).

In FCHL patients, PPARγ1, which represents about 85% of total PPARγ, correlated significantly with FFA-AUC when adjusted for gender (r=0.37, P=0.03). The gender-adjusted correlation between FFA-AUC and PPARγ2 was stronger (r=0.46, P=0.006) (see *Figure 2* in *Study IV*). In control subjects, no significant correlation with FFA-AUC was observed for PPARγ1 or PPARγ2 mRNA (r=0.02 and r=0.06, respectively). Instead, PPARγ1 mRNA showed a strong negative correlation with TG when adjusted for gender (r=-0.87, P<0.001) (*Figure 3* in *Study IV*), irrespective of age or BMI (data not shown). The correlation between PPARγ2 and TG was no longer significant when adjusted for gender (r=-0.28, P=0.38).

6.5 Carotid artery intima-media thickness in familial combined hyperlipidemia family members

For subject characteristics, see *Tables 1* and *2* in *Study V*. The average mean-IMT of all subjects was 0.74±0.14 mm. The average mean-IMT of the affected family members (0.75±0.15 mm) did not differ from that of the unaffected subjects (0.73± 0.13 mm, P=0.90). The result was not significantly influenced by adjustment for BMI or SBP (data not shown). No significant differences between the affected and unaffected subjects were observed in the other IMT outcome variables either (**Table 5**).

Because age had a significant effect on IMT, the mean-IMT was also examined in three age groups, each spanning approximately 15 years: 19-35, 36-50, and over 50 years. The number of the affected and unaffected individuals was comparable in each age group. No significant differences in mean-IMT were observed between the affected and unaffected in any age group (*Figure 1* in *Study V*).

Table 5. *Intima-media thicknesses (mm) for the study subjects.*

	Affected (n=77)		**Unaffected** (n=71)		
	mean±SD	range	mean±SD	range	*P*-value [a]
Mean-IMT	0.75±0.15	0.53-1.23	0.73±0.13	0.53-1.24	0.95
FW-IMT	0.74±0.16	0.50-1.25	0.73±0.15	0.51-1.33	0.84
NW-IMT	0.76±0.15	0.52-1.21	0.73±0.12	0.51-1.16	0.71
Max-IMT	0.95±0.19	0.64-1.51	0.93±0.17	0.67-1.49	0.89
CCA-IMT	0.89±0.15	0.63-1.49	0.87±0.13	0.70-1.21	0.91
CB-IMT	1.04±0.28	0.63-1.94	1.02±0.24	0.62-1.97	0.76
ICA-IMT	0.87±0.22	0.50-1.66	0.81±0.15	0.55-1.30	0.47

Mean-IMT is the average of all mean IMT measurements over the 28 sites. FW-IMT is the average of all mean far-wall IMT measurements; NW-IMT, the average of all mean near-wall IMT measurements. Max-IMT denotes the average of maximum IMTs over the 28 sites. CCA-IMT, CB-IMT, and ICA-IMT represent the average of maximum IMTs of the respective segments. [a] *Assuming all study subjects are independent.*

There were no significant differences in mean-IMT between males (0.75±0.12 mm) and females (0.73±0.15 mm, P=0.26), or between smokers (current and ex-smokers) (0.74±0.14 mm) and non-smokers (0.74±0.14 mm, P=0.19). Hypertensive (SBP ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or antihypertensive medication) subjects had higher mean-IMT (0.84±0.14 mm) than normotensive subjects (0.71±0.12 mm, P<0.001).

For the results of the correlation analyses, see *Study V*. The strongest correlation for mean-IMT was found with age (r=0.81, P<0.001). Of the lipid and lipoprotein variables, mean-IMT correlated statistically significantly with TC, LDL cholesterol, TG and apoB. Significant correlations were also observed between mean-IMT and glucose-AUC, but not with insulin-AUC or FFA-AUC. Mean-IMT correlated significantly with the following clinical variables: BMI, waist-hip ratio, SBP, DBP, pulse pressure and cigarette years. When adjusted for age, most of the above mentioned correlations tended to decrease (see *Study V*). Adjustment for gender did not significantly influence any of the correlations presented (data not shown). The only significant correlation between mean-IMT and C3 or ASP was found with C3 when adjusted for age (r=0.19, P=0.02).

In multivariate analysis the final model explained 75.4% of the variation in mean-IMT (adjusted multiple R^2=0.754). Age had by far the highest standardised coefficient (0.747, P<0.001), followed by log-transformed pulse pressure (0.212, P<0.001) and gender (-0.193, P=0.002, indicating that men had higher average mean-IMT than women). ApoB and waist-hip ratio did not reach statistical significance in the final model. ApoB correlated significantly with LDL cholesterol. When multivariate analysis was performed without including apoB in the original model, LDL cholesterol remained in the final model, but still did not reach statistical significance. In this model, cigarette years also contributed significantly to the variation in mean-IMT (data not shown).

7 DISCUSSION

7.1 Study subjects

A unique, carefully characterised FCHL family material was collected in the EUFAM Study. Population-derived age and gender-specific lipid percentiles were used to categorise FCHL family members as affected or unaffected. The maximum TC and TG values measured for the individual at any visit during the EUFAM Study were used to determine the subject´s phenotype. Lipid values at each study visit were used for the analyses involving other data obtained at that particular visit. It was therefore possible that subjects presenting lipid phenotype IIA at the first visit could exhibit phenotype IIB at a later visit. If the lipid values were below the 90th percentiles at a later visit, the subject was still considered affected. During the course of the EUFAM Study it was found that the study subjects tended to have lower lipid values at later, optional visits, than at the first study visit. This is in line with a recent observation in a Dutch FCHL cohort that only 74% of affected family members showed a constant diagnosis of FCHL over a 5-year period (Veerkamp et al. 2001). This change of phenotype may have resulted from the subjects becoming aware of their dyslipidemia, and subsequent changes in the lifestyle. It also raises the question of whether subjects, who, after changing their diet and losing some weight have significantly lower lipid values than before, are truly genetically affected. The combined data on subjects in *Studies I, II,* and *V* (**Table 3**) shows, however, that the affected subjects were clearly hyperlipidemic as compared with the unaffected relatives, and that the lipid values are comparable with those in other FCHL cohorts (Castro Cabezas et al. 1993b, Reymer et al. 1995, Bredie et al. 1996), though the average TC was lower than that in the Eastern Finnish FCHL families (Pihlajamäki et al. 2000a).

In this thesis the affected FCHL family members were compared with two different subject groups. The primary principle was to examine whole FCHL families, and in studies *I, II,* and *V* the FCHL patients were compared with their unaffected relatives. However, when invasive or otherwise laborious study procedures such as fat biopsies and density gradient ultracentrifugation were involved, this was not possible. This is why in Studies *III* and *IV* unrelated, normolipidemic subjects served as a control group.

With regard to the unaffected family members, it must be noted that these subjects were not necessarily normolipidemic but their TC and TG levels were below the respective age and gender-specific 90th percentiles. If the parameters studied are considered representative of the corresponding metabolic abnormalities, these parameters should be different in the affected and the unaffected relatives. However, overlapping in phenotypes makes it more difficult to detect these differences between the affected and unaffected family members. The unrelated, normolipidemic control subjects were required to have lipid values not only below the 90th percentile, but their TC had to be below 6.0 mmol/l and TG below 1.7 mmol/l.

7.2 Methods

7.2.1 Quantification of adipose tissue gene expression

RT-cPCR was used to quantify specific mRNAs in small samples of adipose tissue. This method has become widely used to detect mRNAs of relatively low expression levels. Northern blot and *in situ* hybridisation are less sensitive methods than RT-cPCR, and they are at best only roughly quantitative. Ribonuclease protection assay is considered to lie between Northern blotting and RT-cPCR in sensitivity. Whether the RT-cPCR method really is quantitative has been debated. The major requirement for obtaining accurate results is that the target and the competitor are amplified with the same efficiency. As the amplification process is exponential, variations in the amplification efficiency may result in large differences in the quantities of the end products. Use of homologous DNA competitors and thus identical primers for both the target and the competitor, should minimise the difference in amplification efficiency. Conflicting results have been reported on the amplification efficiencies of target and competitor fragments. It has been shown that shorter fragments are amplified more efficiently than longer ones (McCulloch et al. 1995, Hiltunen et al. 1998), whereas Auboeuf et al. (1997), Auboeuf and Vidal (1997), and Gilliland et al. (1990) have shown that the ratio of PCR products of the target and the competitor remains constant through the amplification. Thus the method can be regarded as at least semi-quantitative. In *Study IV* mRNA expression was not corrected for house keeping gene expression, because the classical reference genes are expressed at extremely high levels as compared with the mRNAs of interest in *Study IV*. Moreover, the present studies did not aim at comparing expression of different genes or the same genes in different tissues, and clear differences between the samples can be reliably detected.

The efficiency of the reverse transcription step under conditions similar to those in *Study IV* has been assessed by Auboeuf and Vidal (1997). They demonstrated that close to 100% of RNA was transformed into cDNA during the reverse transcription step. The problem of reproducibility in quantifying mRNA expression depends significantly on the reproducibility of RNA isolation and correct measurement of the initial concentration of the total RNA preparation. In *Study IV*, RNA was isolated and the amount of total RNA measured from all samples on a few occasions in similar conditions. In assays identical to *Study IV*, the CV varied between 9 to 18% when mRNA was measured several times in certain tissue samples.

The other analytical methods will be discussed under their respective chapters below.

7.2.2 Statistical analyses

Family materials pose a major challenge to statistical analyses, because the subjects who are related to each other do not meet the assumption of independent observations. However, there are no uniform rules or recommendations as to how to analyse this kind of data. Statistical tools developed for genetics have proved to be of some help, but were primarily developed for analysing genotype data.

In *Study I* the data were analysed as if the subjects were independent, since the problem of non-independent study subjects was only recognised as a major problem at around this time. In later studies family number (identical in all members of a certain family) was included in both the variance and multivariate analyses. This partly corrects for the non-independence of study subjects, but also reduces the power of the analyses, and cannot take into account the pedigree structure.

Linkage analysis can be used to study whether two phenotypic traits are linked. The unknown mode of inheritance of FCHL required using both possible inheritance models: the recessive and the dominant (*Study III*). To avoid problems of unknown penetrance, an affecteds-only strategy was used in linkage analysis. The affected individuals were coded as affected with a 100% penetrance and the unaffected individuals as unknown. Thus, no-one in the families was coded as "healthy" and there was no need to estimate the penetrance in the unaffected individuals. The gene frequencies used in *Study III* were based on the report by Grundy et al. (Grundy et al. 1987) and were also utilised in the characterisation of the novel FCHL locus (Pajukanta et al. 1998).

7.3 Serum C3

Serum C3 concentrations observed in the present studies are comparable with those determined in other studies that have investigated the relationship between serum C3 and hyperlipidemia or atherosclerosis (Uza et al. 1982, Muscari et al. 2000, Weyer et al. 2000). Age has not had a significant influence on serum C3 levels in earlier studies either (Muscari et al. 1990, Muscari et al. 2000). No significant gender difference in serum C3 was found in the combined data from *Studies I, II,* and *V* unless adjusted for BMI. Muscari and co-workers (1990) found higher serum C3 levels in men than in women, whereas the difference was apparent only after age adjustment in a study by Weyer et al. (2000).

The present data show correlations between serum C3 concentration and various factors related to CHD risk and/or metabolic syndrome: TG, TC, apoB, HDL cholesterol, glucose-AUC, insulin-AUC, FFA-AUC, BMI and waist-hip ratio. These results are in line with previous reports (Uza et al. 1982, Muscari et al. 1990, Muscari et al. 1995b, Muscari et al. 1998, Muscari et al. 2000, Weyer et al. 2000). The basis for these correlations is not known. Increased production of C3 may take place in atherosclerotic plaques, liver or adipose tissue.

Atherosclerotic plaques contain complement components (Hollander et al. 1979, Hansson et al. 1984, Vlaicu et al. 1985b, Niculescu et al. 1987b, Niculescu et al. 1989), complement activation takes place in arterial intima (Vlaicu et al. 1985a, Niculescu et al. 1987a, Niculescu et al. 1987b), and activated monocyte/macrophages situated in atherosclerotic vessel walls are able to secrete C3 (Niculescu and Rus 1999). The relationship between serum C3 and various CHD risk factors may therefore reflect the degree of atherosclerosis, which is enhanced by the CHD risk factors. Serum C3 can be compared with C-reactive protein, also an acute phase protein that increases in response to tissue injury and infection (Pepys 1981), is localised in atherosclerotic lesions (Hatanaka et al. 1995), and correlates with CHD mortality (Kuller et al. 1996). The lack of a correlation

between serum C3 and carotid artery IMT in *Study V* may be explained by uncomplicated atherosclerosis in asymptomatic study subjects as opposed to ”vulnerable” plaques with more pronounced inflammatory processes in subjects with CHD.

Cucuianu has shown that C3 is not the only hepatic protein that is increased in hyperlipidemia. Elevated serum levels of lecithin:cholesterol acyltranferase (Cucuainu et al. 1975), plasminogen activator inhibitor (Cucuianu et al. 1991) and fibronectin (Cucuianu et al. 1985) have been observed in hypertriglyceridemic and obese subjects. Cucuianu suggested that the factors that enhance VLDL apoB production may also enhance the synthesis of other proteins in the liver (Cucuianu 1998).

Muscari et al. (2000) have focused attention on insulin as a possible mediator of the above correlations between serum C3 and variables related to the metabolic syndrome. There may be common mechanisms mediated by, say, cytokines that enhance secretion of C3 and induce insulin resistance, which in turn is associated with several CHD risk factors. Results of the familial correlation analyses (*Study II*) suggest that a common set of genes may affect the serum levels of C3, TG, HDL cholesterol, insulin, FFA and BMI, although the effects of a common environment could not be estimated. The nature of the common mechanisms that putatively affect both serum C3 levels and lipid profiles and/or insulin resistance is not known. Yudkin et al. (1999) proposed that adipose tissue is a determinant of a low-level, chronic inflammatory state that is reflected by elevated systemic concentrations of cytokines such as interleukin-6, TNF-α and C-reactive protein. TNF-α is known to impair insulin sensitivity (Hotamisligil et al. 1994) and to modulate the acute-phase response (including C3 production) in the liver (Perlmutter et al. 1986, Ramadori et al. 1988). Whether increased C3 production by adipocytes or atherosclerotic plaques would be reflected in serum C3 levels is not known. Several reports have shown correlations between serum C3 and BMI, which supports the role of adipose tissue in this respect. However, most of C3 is produced in the liver.

In *Study IV*, adipose tissue C3 mRNA expression correlated with insulin levels and BMI. Adipose tissue C3 mRNA expression did not differ between FCHL patients and control subjects. Although a 40% difference between the groups would have been required to detect the difference at a significance level of 0.05, there was not even a trend towards a difference in C3 gene expression. The effect of C3 on lipid metabolism is not expected to be a major one. Therefore very subtle differences in C3 gene expression are not likely to be reflected significantly in serum lipid levels.

In summary, the origin of increased serum C3 concentration in dyslipidemia is not known. No compelling evidence for an increased C3 production by adipocytes was found, but the results of the familial correlation analysis (*Study II*) and adipose tissue C3 gene expression (*Study IV*) do not allow a complete exclusion of this hypothesis.

7.4 Plasma acylation-stimulating protein

Several methods have been used to determine plasma concentrations of ASP. The ELISA assay (Quidel) used in *Study II* uses monoclonal human anti-C3a-desArg as detecting antibody. The average ASP level in our studies (combined from *Studies II* and *V*) was 142±56 ng/ml (range 52-360 ng/ml), which seems to be comparable with the values in studies by Weyer et al. (2000) and Charlesworth et al (1998), in which radioimmunoassay (Amersham) was used to detect human C3a-desArg after precipitation of C3. The plasma ASP concentrations reported recently by the group of Sniderman and Cianflone using the sandwich ELISA (Maslowska et al. 1999) are clearly higher than those in our study subjects. The direct comparison of the ELISA assay used in *Studies II* and *V* and the sandwich ELISA used by Sniderman and Cianflone resulted in a correlation coefficient of 0.59 (see chapter 5.2).

Complement activation and rapid cleavage of C3a into C3a-desArg by plasma carboxypeptidases occur easily during the collection of blood samples. On some occasions it is therefore difficult to know whether the high ASP concentrations truly represent a high ASP level or only complement activation during the sample collection. In the present studies, an arbitrary limit of 400 ng/ml was set to exclude falsely high ASP concentrations.

No differences in plasma ASP levels were found between the three phenotype groups when data from *Studies II* and *V* were combined, although the subjects with high TG had numerically higher plasma ASP levels than their relatives with only high TC or the unaffected relatives. In the literature, higher plasma ASP concentrations have been reported in subjects with CHD than in control subjects (Cianflone et al. 1997), and in obese vs. non-obese subjects (Maslowska et al. 1999).

When data from *Studies II* and *V* were combined, the only significant gender-adjusted correlation with plasma ASP was found for serum TG. Correlations observed in *Study II* between ASP and apoB, HDL cholesterol, insulin, BMI and waist-hip ratio were not significant in the combined material. These results are in line with previous studies in which a positive relationship between plasma ASP and TG has been found (Cianflone et al. 1997, Maslowska et al. 1999, Weyer and Pratley 1999). Correlations between plasma ASP and FFA or body fat content have also been observed, though not as consistently as with TG (Maslowska et al. 1999, Weyer and Pratley 1999).

There was no correlation between fasting plasma ASP and carotid artery IMT in *Study V*. As with C3, this can be explained by the lack of complicated atherosclerotic lesions and thus the relatively low degree of inflammation in vessel walls. Other explanations will be discussed below.

The fat load study was the first to assess the postprandial response of peripheral plasma ASP in hyperlipidemic subjects. The results of the fat load study reported by Cianflone et al. (1989a) are not comparable with the present results because of the aberrantly high plasma ASP levels due to methodological problems. More recent reports on plasma ASP concentrations after a fatty meal have detected virtually no

response (Charlesworth et al. 1998) or plasma ASP levels have decreased postprandially (Weyer and Pratley 1999).

Sniderman et al. (2000) have speculated that the partly contradictory results obtained on ASP deficient mice may be due to differences in insulin sensitivity: both ASP and insulin are required for effective FFA trapping. Insulin can in part compensate for the lack of ASP/impaired ASP function. Better insulin sensitivity may explain why ASP deficiency does not alter FFA trapping as much in female as in male mice (Sniderman et al. 2000). In *Study II*, the postprandial insulin response was greater in FCHL patients than in control subjects. This did not, however, cause a difference in postprandial ASP responses.

The evidence for the existence of the ASP receptor is indirect (Cianflone et al. 1990a, Zhang et al. 1998). The most serious criticism against the ASP pathway hypothesis is based on the fact that the ASP receptor has not been identified. This hampers the determination of the importance of the ASP pathway on lipid metabolism *in vivo*.

The conclusion drawn from *Study II* is that even if there were changes in adipose tissue ASP production postprandially, they can not be detected in peripheral plasma. The same may be true for fasting plasma ASP levels. The opposite is true of C3. Serum concentrations of C3 show strong correlations with several lipid and related biochemical parameters, although it is not clear whether the increased serum C3 originates from adipose tissue, or has any relevance as regards the function of the ASP pathway. It is also of note that C3 is a direct gene product, whereas generation of ASP involves several other proteins. Peake et al. (1997) have actually suggested that it is the synthesis of factor B, not C3, that is the rate-limiting step in ASP production of adipocytes. The data in this work do not contain information with regard to ASP function or ASP concentrations at the level of adipose tissue. Therefore, the hypothesis of ASP pathway as a regulator of adipose tissue metabolism, cannot be ruled out.

7.5 Hormone-sensitive lipase

Adipose tissue HSL activity has been reported to be reduced in two Swedish FCHL cohorts (Reynisdottir et al. 1995, Reynisdottir et al. 1997, Reynisdottir et al. 1998). As FCHL is a heterogeneous disorder, it is quite possible that different defects contribute to development of FCHL in different populations. *Study III* was performed to investigate whether HSL activity is reduced in subcutaneous adipose tissue of Finnish FCHL patients. The HSL activity of 45 Finnish FCHL patients did not differ from that of the 12 normolipidemic control subjects. Neither were there differences between the groups representing different lipid phenotypes.

There are several explanations as for the contradictory results between the Finnish and Swedish FCHL families. Firstly, it may be that HSL activity plays a more marked role in FCHL in Swedish than in Finnish FCHL families, and that HSL defect may influence the FCHL phenotype significantly only in some families. The Swedish FCHL families were originally collected using the 95^{th} percentiles of TC and TG (Ericsson et al. 1992), and thus the Swedish subjects may have represented a more

serious dyslipidemia than the Finnish subjects. The slight differences in abdominal obesity may also have modified the results. Methodological differences can, however, be ruled out, as the assays were performed using the same method in the same laboratory in all studies. It is the differences between the substrate batches that prevent comparison of absolute enzyme activities between the studies.

It is known that post-translational mechanisms have a more significant effect on HSL activity than the transcriptional control of the HSL gene (Hellström et al. 1996, Reynisdottir et al. 1998, Large et al. 1999). However, in obese subjects low HSL activity was associated with a reduction in HSL gene expression (Large et al. 1999). Therefore, in order also to assess this level of HSL regulation, HSL gene expression was quantified in subcutaneous adipose tissue of 40 FCHL patients and 12 control subjects in *Study IV*. No differences in HSL mRNA expression between the FCHL and control subjects were found. There was no significant correlation between HSL activity and HSL mRNA expression in the 17 subjects who had data on both parameters.

Pajukanta and co-workers (1997) have previously excluded HSL as a major gene in the Finnish FCHL families, as no linkage between the HSL locus and FCHL could be found. The results of the linkage analysis in *Study III* could not completely rule out the linkage between HSL activity and FCHL trait, even if the result was not positively significant either. It is therefore possible that HSL activity has a modifying role in FCHL. This concept is supported by the results of the familial correlation analysis.

The significant sibling-sibling correlation of HSL, as well as familial correlations with TC and waist-hip ratio, suggest that HSL activity may be familial and segregate with the lipid phenotype in some families. In the study by Reynisdottir et al. (Reynisdottir et al. 1995) HSL activity did not correlate with serum lipid levels, whereas the lipolysis rate was correlated with serum TG, HDL cholesterol and apoB. On the other hand, the function of all three β-receptors was normal in FCHL. In this work the lipolytic cascade was not investigated at levels other than HSL. HSL is the rate-limiting step in lipolysis, but defects at other levels of the lipolytic cascade may affect FCHL phenotype. It must also be remembered that the current method measures only the amount of total activatable enzyme, as the phosphorylated and dephosphorylated forms of HSL have equal activity towards the monoacylmonoalkylglycerol substrate (Frayn et al. 1993). As discussed in chapter 2.3.2.3, visceral adipocytes are more sensitive to catecholamine-induced lipolysis than subcutaneous adipocytes. HSL activity in visceral adipose tissue has not been investigated so far.

7.6 PPARγ

The relationship between PPARγ mRNA expression level in subcutaneous adipose tissue and serum lipid and lipoprotein levels was investigated in *Study IV*. No obvious correlations between PPARγ mRNA expression and serum lipid levels were observed in FCHL patients. However, a positive correlation between FFA-AUC and both PPARγ1 and PPARγ2 mRNAs was found in FCHL patients but not in control subjects. PPARγ mRNA showed a strong inverse correlation with serum TG in

control subjects, suggesting that effective trapping of fatty acids by adipose tissue may enhance maintenance of normal serum TG levels in normolipidemic subjects.

Observational clinical studies on PPARγ have focused on examining the effect of PPARγ gene polymorphisms on serum lipids, obesity and diabetes. The Pro12Ala variant of exon B of PPARγ2 gene has reduced transactivation capacity *in vitro*, which may lead to less efficient transcription of the target genes (Deeb et al. 1998). Deeb et al. (1998) originally speculated that this would result in reduced fat mass accumulation and improved insulin sensitivity. The effects of the Pro12Ala variant on obesity and serum lipids in humans have been variable (Beamer et al. 1998, Deeb et al. 1998, Mori et al. 1998, Mancini et al. 1999, Ringel et al. 1999, Valve et al. 1999, Meirhaeghe et al. 2000, Pihlajamäki et al. 2000c, Hasstedt et al. 2001). Most, but not all studies have shown that the Ala-allele is associated with improved insulin sensitivity (Deeb et al. 1998, Mori et al. 1998, Koch et al. 1999, Pihlajamäki et al. 2000c, Stumvoll et al. 2001) and decreased risk of type 2 diabetes (Deeb et al. 1998, Altshuler et al. 2000, Douglas et al. 2001).

Clinical studies on the effect of PPARγ gene expression on serum lipids are few. Correlations between PPARγ mRNA expression and CHD risk factors have been reported: the association has been positive with HDL cholesterol and apoA-I, and negative with LDL cholesterol and apoB (Bastard et al. 1999, Krempler et al. 2000, Zeghari et al. 2000). Some, but not all studies have shown negative correlations between PPARγ gene expression and insulin resistance or fasting insulin (Auboeuf et al. 1997, Krempler et al. 2000, Zeghari et al. 2000). Results from studies investigating the relationship with obesity have been inconsistent (Auboeuf et al. 1997, Vidal-Puig et al. 1997, Montague et al. 1998, Bastard et al. 1999, Rieusset et al. 1999, Krempler et al. 2000), and adipose tissue PPARγ mRNA levels have not been associated with type 2 diabetes (Auboeuf et al. 1997, Rieusset et al. 1999).

Considering the effect of TZDs and the function of LPL, FATP-1 and ACS, an inverse correlation between PPARγ gene expression and FFA-AUC would have been expected. The positive correlation seen in FCHL patients may indicate a disturbance of this metabolic pathway. The reason for the lack of a correlation between PPARγ mRNA expression and FFA-AUC in normal subjects may lie in the limited number or the control subjects, and the known wide variation in serum FFA levels.

That PPARγ mRNA expression was not strongly associated with serum lipids in FCHL patients is not surprising, despite the central role PPARγ plays in FFA metabolism. It is not known to which extent PPAR expression regulates PPAR activity. Furthermore, relatively little is known about the regulation of PPAR activity, and new mechanisms emerge continuously. It may also be that other factors contributing to FCHL may override the effects of PPARγ in FCHL patients. This was also suggested in the study by Pihlajamäki and co-workers, who reported no difference in the frequency of Pro12Ala variants between subjects with and without dyslipidemia, but this PPARγ polymorphism still seemed to modify the insulin and lipid levels (Pihlajamäki et al. 2000c).

In summary, *Study IV* was of limited size, and the conclusions drawn must be cautious. However, even if it leaves the relationships between PPARγ mRNA levels

and serum lipids open, it still suggests that PPARγ may play a modifying role in FCHL. Whether the effect of PPARγ is mediated by its effects on energy homeostasis, fatty acid metabolism, or insulin resistance, requires further studies.

7.7 Carotid artery intima-media thickness

7.7.1 Scanning protocol

Atherosclerotic lesions tend to occur later in the CCA than in the ICA or CB (Solberg et al. 1971, Salonen JT and Salonen R 1993). Combined data from ACAPS, PLAC-II and the Multicenter Isradipine/Diuretic Atherosclerosis Study (MIDAS) showed that IMT was thickest in CB, followed by ICA and CCA (Furberg et al. 1994a). Quantitative differences in associations between cardiovascular risk factors and atherosclerosis at different sites in the carotid artery have been reported (Tell et al. 1989). Salonen and Salonen have observed that in middle-aged hypercholesterolemic men, serum LDL cholesterol is associated more strongly with the CCA than with CB IMT, whereas SBP is associated more strongly with IMT in CB than CCA (Salonen JT and Salonen R 1994). In *Study V*, data from all segments were included in the primary outcome variable mean-IMT. This was done to avoid problems of multiple testing, because the number of study subjects would not have allowed separate analyses of the segments.

CCA IMT measurement can usually be obtained from approximately 99% of subjects (O´Leary et al. 1992, Espeland et al. 1996), but the percentage is markedly lower, 67-98%, in CB or ICA (O´Leary et al. 1992, Probstfield et al. 1993, Espeland et al. 1996). Variability is also greater in the ICA than CB, and lowest in the CCA (Furberg et al. 1994a), which has raised the question of whether ICA should be excluded at least from progression studies (Furberg al. 1994a). As *Study V* was cross-sectional, the possibly larger variation in ICA and CB IMT measurements was not considered a major problem. Furthermore, when the primary outcome variable mean-IMT was calculated excluding ICA measurements, there was no obvious change in variation (0.75±0.15 mm in affected vs. 0.74±0.14 mm in unaffected subjects). The validity of NW measurements has already been discussed in chapter 2.4.1. In *Study V*, NW measurements were included in the analysis to optimise the power of the study.

The intra-sonographer and intra-reader variabilities in *Study V* are reported in chapter 5.6. Even though an exact comparison is hampered by differences in scanning protocols, the reproducibility data compare well with those reported by Kanters et al. (1997) in a review article: intra-sonographer variability varied between a mean±SD absolute difference of 0.02±0.02 and 0.66±1.13 mm, CV of 2.4% and 10.6%, and correlation coefficient of 0.62 and 0.97. The respective figures for intra-reader variability were 0.01±0.04 and 0.65±0.69, 3.1% and 18.3%, and 0.58 and 1.00. The comparably low variation in repeated scannings in *Study V* may in part be due to relatively thin intima-media complexes, as the variation is known to grow with increasing thickness of the carotid FW IMT (Bots et al. 1994b). Furthermore, the study lacked between-observer variability, as there was only a single person carrying out the scannings and another performing the measurements.

7.7.2 Carotid artery intima-media thickness in familial combined hyperlipidemia

The aim of *Study V* was to assess whether alterations in regulators affecting fatty acid and TG metabolism are reflected in the degree of atherosclerosis in FCHL family members. However, when the study was planned it was anticipated that some of the adipose tissue key regulators studied would turn out as significant determinants of serum lipid levels in FCHL family members. As no such major determinants were found, *Study V* was finally focused to assess the validity of the currently used lipid criteria as predictors of atherosclerosis. The degree of atherosclerosis of asymptomatic FCHL family members has not been previously investigated.

Family members with a history of CHD or stroke were excluded from the study for several reasons. First, subjects with CHD were mostly the probands of the families, and thus had been included in the study because they, by definition, had both CHD and serum lipids levels that met the EUFAM Study criteria. Secondly, subjects with clinical cardiovascular disease are known to represent a population with a high degree of atherosclerosis and thickening of carotid artery walls (Craven et al. 1990, Wofford et al. 1991, Burke et al. 1995). Thirdly, subjects with lipid lowering medication had already at least once paused their medication for the purposes of the EUFAM Study. It was considered unethical to pause their medication another time, as most of these subjects also had cardiovascular disease.

The fact that there was no difference in carotid artery IMT between the affected and unaffected family members may naturally be due to the size of the study population. However, it is difficult to estimate how big a difference in IMT would be of true clinical significance. Also, comparison of absolute IMT values with other studies is difficult because of the differences in study subjects and in scanning and reading protocols. Two articles have reported that a common carotid artery IMT $\geq$ 0.85 mm has a 83-85% predictive value of CHD (Geroulakos et al. 1994a, Hodis et al. 1996), which could be an indication of a relatively low degree of atherosclerosis in the asymptomatic FCHL family members studied here. The lack of a difference in carotid IMT between the two groups could also reflect a greater CHD risk in all FCHL relatives compared with a normal population. However, this does not seem likely considering the IMT values observed in this study.

The association between carotid IMT and serum lipids has been established in several earlier reports (Crouse et al. 1987, Salonen R and Salonen JT 1991b, O´Leary et al. 1992, Ryu et al. 1992). In *Study V*, all lipid variables studied (except for HDL cholesterol and apoA-I) correlated significantly with mean-IMT. One explanation for the lack of a correlation between carotid IMT and serum HDL cholesterol is that the FCHL family members did not have particularly low HDL cholesterol levels, and thus the effect may not have been detectable. The association between lipid variables and age was confirmed by attenuation of these correlations when adjusted for age. Age as such is not likely to cause thickening of the arterial wall, but its strong relationship with IMT is more probably explained by lifelong exposure to other CHD risk factors, such as hyperlipidemia, hypertension and smoking.

In *Study V*, all blood pressure variables (SBP, DBP and pulse pressure) correlated with mean-IMT. Again, adjustment for age weakened the correlations between blood pressure and carotid IMT. In the literature, SBP has been consistently related to carotid atherosclerosis, whereas DBP has not, or the relationship has been negative or J-shaped (Salonen R and Salonen JT 1991a, O´Leary et al. 1992, Arnett et al. 1996, Zanchetti et al. 1998, Lakka et al. 1999). Pulse pressure was chosen to represent the blood pressure variables in the multivariate analysis, because there is evidence that the pulsatile components of blood pressure are important risk factors for CHD, and pulse pressure may be an even better predictor of CHD than SBP (Franklin et al. 1997, Zanchetti et al. 1998, Lakka et al. 1999). A single measurement of blood pressure was performed in *Study V*. This may in some cases have led to an incorrect diagnosis of hypertension. Further bias may have resulted from the antihypertensive medication used by eight study subjects. However, it is more likely that the use of antihypertensive medication weakened the relationship observed between blood pressure and carotid IMT

There was no significant difference in mean-IMT between smokers (current or ex-smokers) and non-smokers. Current and ex-smokers were combined because all these subjects were currently or had been exposed to cigarette smoking. Association between smoking and carotid artery IMT has been well documented in literature (Crouse et al. 1987, Tell et al. 1989, Salonen R and Salonen JT 1990, Salonen R and Salonen JT 1991b, Dempsey and Moore 1992, O´Leary et al. 1992). The fact that there was no difference in mean-IMT between the two groups divided by smoking history may be due to the design of the study, which was not planned primarily to examine the effect of smoking on carotid IMT. The smoking data were collected through questionnaires during study visits. Cigarette years were calculated using the number of cigarettes smoked daily at the time of the study visit. However, the subjects´ smoking habits may have changed over the years. Furthermore, as is known to be the case for alcohol consumption, the subjects may have under-reported the number of cigarettes they smoke daily. Even so, the correlation between cigarette years and mean-IMT was significant.

In *Study V*, fasting glucose and glucose-AUC correlated significantly with mean-IMT. When adjusted for age, only the correlation with glucose-AUC remained significant. This is in line with the results of Temelkova-Kurktschiev et al. (1998) who reported, in non-diabetic subjects, only a weak correlation between fasting plasma glucose and carotid IMT, which disappeared when adjusted for age and gender. An association between atherosclerosis and hyperinsulinemia or reduced insulin sensitivity has been established (Folsom et al. 1994, Agewall et al. 1995, Howard et al. 1996, Bonora et al. 1997b). Whether this relationship is totally independent of the other related risk factors such as lipids, hypertension and obesity, has been debated (Hedblad et al. 2000, Bokemark et al. 2001). It is possible that FCHL family members with impaired glucose tolerance use lipid lowering medication (and consequently were excluded from the study) more frequently than their otherwise healthy relatives. Thus, the present study may underestimate the association of glucose and insulin with early atherosclerotic changes in FCHL.

It was interesting that in the multivariate analysis no lipid parameter contributed significantly to the variation in carotid IMT, whereas age, sex and pulse pressure were the most important predictors of the mean-IMT. The minor influence of lipids on IMT in FCHL family members raises the question of whether the carotid IMT is an indicator of atherosclerosis, a disorder of arterial intima. The intima and media layers of the arterial wall cannot be distinguished from each other by ultrasound, and therefore it is possible that the strong association between carotid IMT and pulse pressure observed in this study is not solely a result of increased atherosclerosis, but also of fibromuscular hypertrophy of the arterial media.

The only lipid/lipoprotein parameter that remained in the final model in the multivariate analysis was apoB, but its effect on IMT variation was not statistically significant. Even if apoB was excluded from the model, LDL cholesterol still did not contribute significantly to the variation in mean-IMT. The effect of apoB also seemed to override the effect of smoking history on IMT, since with apoB in the model, cigarette years did not remain in the best model, as opposed to a model with no apoB. The effect of LDL cholesterol on atherosclerosis is well established, and is also shown in *Study V* by the strong correlation between LDL cholesterol and IMT. That apoB seemed to affect the variation in IMT even more than LDL cholesterol may reflect the fact that apoB is a better measure of the number of LDL particles than is LDL cholesterol.

In summary, the IMT findings in FCHL family members indicate that the current lipid criteria alone are of limited value in predicting the long-term risk of cardiovascular disease in asymptomatic members of FCHL families. It is unlikely that identification of the major genes of FCHL would resolve this problem either, because FCHL is a heterogeneous disorder. Furthermore, the results of *Study V* underline the importance of other CHD risk factors, in addition to lipids, in the risk assessment and treatment of FCHL family members.

8 SUMMARY AND CONCLUSIONS

This work examined whether selected key regulators of adipose tissue fatty acid and triglyceride metabolism play a central role in the pathogenesis of FCHL and thus can separate affected FCHL family members from their unaffected relatives or unrelated control subjects. Carotid artery ultrasonographic examination was carried out to assess the validity of the currently used lipid criteria for categorising the FCHL family members as affected or unaffected.

Serum C3 concentration was found to be higher in FCHL family members with elevated serum TG than in the relatives with elevation of serum TC only, or the unaffected relatives. Serum C3 level correlated significantly with serum lipids and lipoproteins. The results of the familial correlation analyses imply that serum C3 levels may be familial and that C3 may share a common genetic background with TG, HDL cholesterol, insulin, FFA and BMI. Subcutaneous adipose tissue C3 gene expression of FCHL patients did not differ from that of the control subjects. The results do not provide convincing evidence for the hypothesis that the increased serum C3 levels observed in FCHL are due to increased production by adipocytes, which would try to increase ASP formation and compensate for the potential defects in the ASP/C3 pathway in adipose tissue.

Serum C3 concentration was not associated with carotid artery IMT in FCHL family members, and thus does not reflect the degree of atherosclerosis in these subjects. Serum C3 may be a surrogate marker of atherosclerosis through its relationship with various CHD risk factors.

Plasma ASP concentration was no higher in affected than in unaffected FCHL family members. There was no significant postprandial response in plasma ASP after an oral fat load in FCHL patients or in normolipidemic control subjects. Correlations between plasma ASP and serum lipids were markedly weaker than those for serum C3, and in the combined data from *Studies II* and *V* there was no significant correlation between serum C3 and plasma ASP. This further supports the conclusion drawn from *Study II*: even if there were abnormalities in ASP production/function in the adipose tissue of FCHL patients, they cannot be detected by measuring ASP concentrations in peripheral plasma.

Subcutaneous adipose tissue HSL activity or HSL gene expression was not reduced in Finnish FCHL patients. HSL does not have a major role in the pathogenesis of FCHL. However, the significant sibling-sibling correlation of HSL activity, as well as familial correlations with TC and waist-hip ratio, suggest that HSL activity may be familial and may modify the lipid phenotype in some FCHL families.

PPARγ gene expression in the subcutaneous adipose tissue of FCHL patients did not differ from that of normolipidemic control subjects. Surprisingly, PPARγ gene expression correlated positively with FFA-AUC in FCHL patients. In control subjects, there was a strong inverse correlation between PPARγ1 expression and serum TG. The lack of this correlation in FCHL patients may indicate that dysfunction of PPARγ-regulated TG metabolism could modify serum TG levels in FCHL.

There was no difference in carotid artery IMT between affected and unaffected FCHL family members. In multivariate analysis, age, gender and pulse pressure, but not lipid variables, were significant predictors of carotid artery IMT in FCHL family members.

In conclusion, the key regulators of adipose tissue fatty acid metabolism studied in this thesis were found not to be of major importance in the pathogenesis of FCHL. Future studies with novel methodology will perhaps offer an opportunity to investigate adipose tissue fatty acid trapping directly. The currently used lipid criteria alone are of limited value in predicting the long-term risk of cardiovascular disease in asymptomatic members of FCHL families. The results encourage the search for new diagnostic and prognostic criteria for FCHL.

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10 REFERENCES

Aalto-Setälä K, Benlian P, Bowyer D, Hofker M, James RW, Poledne R and Steinmetz A. 1998. European Lipoprotein Club: Report of the 20th annual conference, Tutzing, 8-11 September 1997. *Atherosclerosis* 137:223-229.

Aarsland A, Chinkes D and Wolfe RR. 1996. Contributions of de novo synthesis of fatty acids to total VLDL-triglyceride secretion during prolonged hyperglycemia/hyperinsulinemia in normal man. *J Clin Invest* 98:2008-2017.

Abumrad NA, El-Maghrabi MR, Ez-Zoubir A, Lopez E and Grimaldi PA. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. *J Biol Chem* 268:17665-17668.

Adams MR, Nakagomi A, Keech A, Robinson J, McCredie R, Bailey BP, Freedman SB and Celermajer DS. 1995. Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. *Circulation* 92:2127-2134.

Adams M, Reginato MJ, Shao D, Lazar MA and Chatterjee VK. 1997. Transcriptional activation by peroxisome proliferator-activated receptor γ is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. *J Biol Chem* 272:5128-5132.

Agewall S, Fagerberg B, Attvall S, Wendelhag I, Urbanavicius V and Wikstrand J. 1995. Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk. *Stroke* 26:956-960.

Aguilar-Salinas CA, Barrett PHR, Pulai J, Zhu XL and Schonfeld G. 1997. A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism. *Arterioscler Thromb Vasc Biol* 17:72-82.

Aitman TJ, Godsland IF, Farren B, Crook D, Wong HJ and Scott J. 1997. Defects of insulin action on fatty acid and carbohydrate metabolism in familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 17:748-754.

Allan PL, Mowbray PI, Lee AJ and Fowkes GR. 1997. Relationship between carotid intima-media thickness and symptomatic and asymptomatic peripheral arterial disease. The Edinburgh Artery Study. *Stroke* 28:348-353.

Allayee H, Aouizerat BE, Cantor RM, Dallinga-Thie GM, Krauss RM, Lanning CD, Rotter JI, Lusis AJ and de Bruin TW. 1998. Families with familial combined hyperlipidemia and families enriched for coronary artery disease share genetic determinants for the atherogenic lipoprotein phenotype. *Am J Hum Genet* 63:577-585.

Allayee H, Dominguez KM, Aouizerat BE, Krauss RM, Rotter JI, Lu J, Cantor RM, de Bruin TW and Lusis AJ. 2000. Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia. *J Lipid Res* 41:245-252.

Altshuler D, Hirschhorn J, Klannemark M, Lindgren C, Vohl M, Nemesh J, Lane C, Schaffner S, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson T, Daly M, Groop L and Lander E. 2000. The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76-80.

Anber V, Griffin BA, McConnell M, Packard CJ and Shepherd J. 1996. Influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. *Atherosclerosis* 124:261-271.

Andersson K, Gaudiot N, Ribiere C, Elizalde M, Giudicelli Y and Arner P. 1999. A nitric oxide-mediated mechanism regulates lipolysis in human adipose tissue in vivo. *Br J Pharmacol* 126:1639-1645.

Anthonsen MW, Rönnstrand L, Wernstedt C, Degerman E and Holm C. 1998. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties *in vitro*. *J Biol Chem* 273:215-221.

Aouizerat BE, Allayee H, Cantor RM, Dallinga-Thie GM, Lanning CD, de Bruin TWA, Lusis AJ and Rotter JI. 1999a. Linkage of a candidate locus to familial combined hyperlipidemia. Lecithin:cholesterol acyltransferase on 16q. *Arterioscler Thromb Vasc Biol* 19:2730-2736.

Aouizerat BE, Allayee H, Cantor RM, Davis RC, Lanning CD, Wen PZ, Dallinga-Thie GM, de Bruin TW, Rotter JI and Lusis AJ. 1999b. A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. *Am J Hum Genet* 65:397-412.

Arad Y, Ramakrishnan R and Ginsberg HN. 1990. Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J Lipid Res* 31:567-582.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T and Matsuzawa Y. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79-83.

Arner P. 1996. Regulation of lipolysis in fat cells. *Diabetes Rev* 4:450-463.

Arner P. 1999. Catecholamine-induced lipolysis in obesity. *Int J Obes* 23 (Suppl. 1):10-13.

Arner P, Engfeldt P and Östman J. 1982. Changes in the metabolism of fatty acids in adipose tissue in obese patients with primary hypertriacylglycerolemia. *J Lipid Res* 23:422-427.

Arnett DK, Tyroler HA, Burke G, Hutchinson R, Howard G and Heiss G. 1996. Hypertension and subclinical carotid artery atherosclerosis in blacks and whites. The Atherosclerosis Risk in Communities Study. *Arch Intern Med* 156:1983-1989.

Ascaso JF, Merchante A, Lorente RI, Real JT, Martinez-Valls J and Carmena R. 1998. A study of insulin resistance using the minimal model in nondiabetic familial combined hyperlipidemia patients. *Metabolism* 47:508-513.

Auboeuf D and Vidal H. 1997. The use of the reverse transcription-competitive polymerase chain reaction to investigate the *in vivo* regulation of gene expression in small tissue samples. *Anal Biochem* 245:141-148.

Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M and Vidal H. 1997. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-α in humans. No alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 46:1319-1327.

Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC and Krauss RM. 1988. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 260:1917-1921.

Austin MA, Brunzell JD, Fitch WL and Krauss RM. 1990a. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arterioscler* 10:520-530.

Austin MA, King M-C, Vranizan KM and Krauss RM. 1990b. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 82:495-506.

Auwerx J. 1999. PPARγ, the ultimate thrifty gene. *Diabetologia* 42:1033-1049.

Babirak SP, Iverius P-H, Fujimoto WY and Brunzell JD. 1989. Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. *Arterioscler* 9:326-334.

Babirak SP, Brown BG and Brunzell JD. 1992. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscler Thromb* 12:1176-1183.

Baldo A, Sniderman AD, St-Luce S, Kohen Avramoglu R, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S and Cianflone K. 1993. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J Clin Invest* 92:1543-1547.

Baldo A, Sniderman AD, St. Luce S, Zhang X-J and Cianflone K. 1995. Signal transduction pathway of acylation stimulating protein: involvement of protein kinase C. *J Lipid Res* 36:1415-1426.

Barnum S, Fey G and Tack B. 1989. Biosynthesis and genetics of C3. *Curr Top Microbiol* 153:23-43.

Bastard J, Hainque B, Dusserre E, Bruckert E, Robin D, Vallier P, Perche S, Robin P, Turpin G, Jardel C, Laville M, Forest C and Vidal H. 1999. Peroxisome proliferator activated receptor-γ, leptin and tumor necrosis factor-α mRNA expression during very low calorie diet in subcutaneous adipose tissue in obese women. *Diabetes-Metab Res Rev* 15:92-98.

Beamer BA, Yen CJ, Andersen RE, Muller D, Elahi D, Cheskin LJ, Andres R, Roth J and Shuldiner AR. 1998. Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-γ2 gene with obesity in two Caucasian populations. *Diabetes* 47:1806-1808.

Beisiegel U, Weber W, Ihrke G, Herz J and Stanley KK. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341:162-164.

Beisiegel U, Weber W and Bengtsson-Olivecrona G. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci USA* 88:8342-8346.

Berger J, Bailey P, Biswas C, Cullinan C, Doebber T, Hayes N, Saperstein R, Smith R and Leibowitz M. 1996. Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ: binding and activation correlate with antidiabetic actions in db/db mice. *Endocrinology* 137:4189-4195.

Berk PD, Zhou S-L, Kiang C-L, Stump D, Bradbury M and Isola LM. 1997. Uptake of long chain free fatty acids is selectively up-regulated in adipocytes of Zucker rats with genetic obesity and non-insulin-dependent diabetes mellitus. *J Biol Chem* 272:8830-8835.

Bhakdi S. 1998. Complement and atherosclerosis: the unknown connection. *Ann Med* 6:503-507.

Blankenhorn DH, Selzer RH, Crawford DW, Barth JD, Liu C, Liu C, Mack WJ and Alaupovic P. 1993. Beneficial effects of colestipol-niacin therapy on the common carotid artery. Two-

and four-year reduction of intima-media thickness measured by ultrasound. *Circulation* 88:20-28.

Boden G. 1996a. Fatty acids and insulin resistance. *Diabetes Care* 19:394-395.

Boden G. 1996b. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 45:3-10.

Boden G, Chen X, Ruiz J, White JV and Rossetti L. 1994. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438-2446.

Boissel J-P, Collet J-P, Moleur P and Haugh M. 1992. Surrogate end points: a basis for a rational approach. *Eur J Clin Pharmacol* 43:235-244.

Bokemark L, Wikstrand J, Attvall S, Hulthe J, Wedel H and Fagerberg B. 2001. Insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). *J Int Med* 249:59-67.

Bond MG, Wilmoth SK, Enevold GL, Strickland HL. 1989. Detection and monitoring of asymptomatic atherosclerosis in clinical trials. *Am J Med* 86 (Suppl. 4):33-36.

Bonora E, Tessari R, Micciolo R, Zenere M, Targher G, Padovani R, Falezza G and Muggeo M. 1997a. Intimal-medial thickness of the carotid artery in nondiabetic and NIDDM patients. Relationship with insulin resistance. *Diabetes Care* 20:627-631.

Bonora E, Willeit J, Kiechl S, Oberhollenzer F, Egger G, Bonadonna R and Muggeo M. 1997b. Relationship between insulin and carotid atherosclerosis in the general population. The Bruneck Study. *Stroke* 28:1147-1152.

Boquist S, Ruotolo G, Tang R, Björkegren J, Bond MG, de Faire U, Karpe F and Hamsten A. 1999. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. *Circulation* 100:723-728.

Boren J, Rustaeus S, Wettesten M, Andersson M, Wiklund A and Olofsson S-O. 1993a. Influence of triacylglycerol biosynthesis rate on the assembly of apoB-100-containing lipoproteins in Hep G2 cells. *Arterioscler Thromb* 13:1743-1754.

Boren J, Wettesten M, Rustaeus S, Andersson M and Olofsson S-O. 1993b. The assembly and secretion of apoB-100-containing lipoproteins. *Biochem Soc T* 21:487-493.

Bots M, Hofman A and Grobbee DE. 1994a. Common carotid intima-media thickness and lower extremity arterial atherosclerosis. The Rotterdam Study. *Arterioscler Thromb* 14:1885-1891.

Bots M, Mulder P, Hofman A, van Es G and Grobbee DE. 1994b. Reproducibility of carotid vessel wall thickness measurements. The Rotterdam Study. *J Clin Epidemiol* 47:921-930.

Bots ML, Hoes AW, Koudstaal PJ, Hofman A and Grobbee DE. 1997. Common carotid intima-media thickness and risk of stroke and myocardial infarction. *Circulation* 96:1432-1437.

Braissant O, Foufelle F, Scotto C, Dauca M and Wahli W. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* 137:354-366.

Brasaemle DL, Levin DM, Adler-Wailes DC and Londos C. 2000. The lipolytic stimulation of 3T3-L1 adipocytes promotes the translocation of hormone-sensitive lipase to the surfaces of lipid storage droplets. *Biochim Biophys Acta* 1483:251-262.

Bredie SJH, Kiemeney LA, de Haan AFJ, Demacker PNM and Stalenhoef AFH. 1996. Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. *Am J Hum Genet* 58:812-822.

Bredie SJ, van Drongelen J, Kiemeney LA, Demacker PN, Beaty TH and Stalenhoef AF. 1997a. Segregation analysis of plasma apolipoprotein B levels in familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 17:834-840.

Bredie SJH, Tack CJJ, Smits P and Stalenhoef AFH. 1997b. Nonobese patients with familial combined hyperlipidemia are insulin resistant compared with their nonaffected relatives. *Arterioscler Thromb Vasc Biol* 17:1465-1471.

Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E and McDonald GB. 1983. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. *J Lipid Res* 24:147-155.

Burke G, Evans G, Riley W, Sharrett A, Howard G, Barnes R, Rosamond W, Crow R, Rautaharju P and Heiss G. 1995. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. *Stroke* 26:386-391.

Byrne CD, Brindle NP, Wang TW and Hales CN. 1991. Interaction of non-esterified fatty acid and insulin in control of triacylglycerol secretion by Hep G2 cells. *Biochem J* 280:99-104.

Byrne CD, Wang TW and Hales CN. 1992. Control of Hep G2-cell triacylglycerol and apolipoprotein B synthesis and secretion by polyunsaturated non-esterified fatty acids and insulin. *Biochem J* 288:101-107.

Byrne C, Wareham N, Brown D, Clark P, Cox L, Day N, Palmer C, Wang T, Williams D and Hales C. 1994. Hypertriglyceridaemia in subjects with normal and abnormal glucose tolerance: relative contributions of insulin secretion, insulin resistance and suppression of plasma non-esterified fatty acids. *Diabetologia* 37:889-896.

Campbell PJ, Carlson MG, Hill JO and Nurjhan N. 1992. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol* 263:E1063-E1069.

Carlson L and Walldius G. 1976. Fatty acid incorporation into human adipose tissue in hypertriglyceridemia. *Eur J Clin Invest* 6:195-211.

Castro Cabezas M, de Bruin TWA, Jansen H, Kock LAW, Kortlandt W and Erkelens DW. 1993a. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler Thromb* 13:804-814.

Castro Cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H and Erkelens DW. 1993b. Impaired fatty acid metabolism in familial combined hyperlipidemia. *J Clin Invest* 92:160-168.

Castro Cabezas M, de Bruin TWA, Kock LAW, Kortlandt W, van Linde-Sibenius Trip M, Jansen H and Erkelens DW. 1993c. Simvastatin improves chylomicron remnant removal in familial combined hyperlipidemia without changing chylomicron conversion. *Metabolism* 42:497-503.

Castro Cabezas M, Erkelens D, Kock L and de Bruin T. 1994. Postprandial apolipoprotein B100 and B48 metabolism in familial combined hyperlipidaemia before and after reduction of fasting plasma triglycerides. *Eur J Clin Invest* 24:669-678.

Chait A, Albers JJ and Brunzell JD. 1980. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridaemia. *Eur J Clin Invest* 10:17-22.

Chait A, Brazg RL and Tribble DL. 1993. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 94:350-356.

Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR and Clegg LX. 1997. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol* 146:483-494.

Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O and Reitman ML. 2000. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest* 106:1221-1228.

Charlesworth J, Peake P, Campbell L, Pussel B, O´Grady S and Tzilopoulos T. 1998. The influence of oral lipid loads on acylation stimulating protein (ASP) in healthy volunteers. *Int J Obes* 122:1096-1102.

Choy LN, Rosen BS and Spiegelman BM. 1992. Adipsin and an endogenous pathway of complement from adipose cells. *J Biol Chem* 267:12736-12741.

Cianflone K, Vu H, Walsh M, Baldo A and Sniderman A. 1989a. Metabolic response of acylation stimulating protein to an oral fat load. *J Lipid Res* 30:1727-1733.

Cianflone KM, Sniderman AD, Walsh MJ, Vu HT, Gagnon J and Rodriguez MA. 1989b. Purification and characterization of acylation stimulating protein. *J Biol Chem* 264:426-430.

Cianflone KM, Maslowska MH and Sniderman AD. 1990a. Impaired response of fibroblasts from patients with hyperapobetalipoproteinemia to acylation-stimulating protein. *J Clin Invest* 85:722-730.

Cianflone KM, Yasruel Z, Rodriguez MA, Vas D and Sniderman AD. 1990b. Regulation of apoB secretion from HepG2 cells: evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. *J Lipid Res* 31:2045-2055.

Cianflone K, Dahan S, Monge JC and Sniderman AD. 1992. Pathogenesis of carbohydrate-induced hypertriglyceridemia using HepG2 cells as a model system. *Arterioscler Thromb* 12:271-277.

Cianflone K, Zhang X-J, Genest JJ and Sniderman A. 1997. Plasma acylation-stimulating protein in coronary artery disease. *Arterioscler Thromb Vasc Biol* 17:1239-1244.

Cohen B, Novick D and Rubinstein M. 1996. Modulation of insulin activities by leptin. *Science* 274:1185-1188.

Coon H, Myers RH, Borecki IB, Arnett DK, Hunt SC, Province MA, Djousse L and Leppert MF. 2000. Replication of linkage of familial combined hyperlipidemia to chromosome 1q with additional heterogeneous effect of apolipoprotein A-I/C-III/A-IV locus. The NHLBI Family Heart Study. *Arterioscler Thromb Vasc Biol* 20:2275-2280.

Coppack SW, Jensen MD and Miles JM. 1994. In vivo regulation of lipolysis in humans. *J Lipid Res* 35:177-193.

Coresh J, Beaty TH and Kwiterovich PO Jr. 1993. Inheritance of plasma apolipoprotein B levels in families of patients undergoing coronary arteriography at an early age. *Genet Epidemiol* 10:159-176.

Cornelius P, MacDougald O and Lane M. 1994. Regulation of adipocyte development. *Annu Rev Nutr* 14:99-129.

Cortner JA, Coates PM, Bennett MJ, Cryer DR and Le N-A. 1991. Familial combined hyperlipidaemia: use of stable isotopes to demonstrate overproduction of very low-density lipoprotein apolipoprotein B by the liver. *J Inherit Metab Dis* 14:915-922.

Craven T, Ryu J and Espeland M. 1990. Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis. A case control study. *Circulation* 82:1230-1242.

Crouse JR, Toole JF, McKinney WM, Dignan MB, Howard G, Kahl FR, McMahan MR and Harpold GH. 1987. Risk factors for extracranial carotid atherosclerosis. *Stroke* 18:990-996.

Crouse JR, Byington RP, Bond MG, Espeland MA, Craven TE, Sprinkle JW, McGovern ME and Furberg CD. 1995. Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLAC-II). *Am J Cardiol* 75:455-459.

Cuchel M, Schaefer EJ, Millar JS, Jones PJ, Dolnikowski GG, Vergani C and Lichtenstein AH. 1997. Lovastatin decreases de novo cholesterol synthesis and LDL apo B-100 production rates in combined-hyperlipidemic males. *Arterioscler Thromb Vasc Biol* 17:1910-1917.

Cucuianu M. 1998. Hyperlipidemia and serum complement. *Atherosclerosis* 137:441-442.

Cucuianu M, Opincaru A and Tapalaga D. 1975. Similar behaviour of lecithin:cholesterol acyltransferase and pseudocholinesterase in liver disease and hyperlipoproteinemia. *Clin Chim Acta* 59:73-95.

Cucuianu M, Rus H, Cristea A, Niculescu F, Bedeleanu D, Porutiu D and Roman S. 1985. Clinical studies on plasma fibronectin and factor XIII; with special reference to hyperlipoproteinemia. *Clin Chim Acta* 147:273-281.

Cucuianu M, Knauer O and Roman S. 1991. α2-antiplasmin, plasminogen activator inhibitor (PAI) and dilute blood clot lysis time in selected disease states. *Thromb Haemostasis* 66:586-591.

Cullen P, Bernadette F, Scott J and Farrall M. 1994. Complex segregation analysis provides evidence for a major gene acting on serum triglyceride levels in 55 British families with familial combined hyperlipidemia. *Arterioscler Thromb* 14:1233-1249.

Cuthbert J, East C, Bilheimer D and Lipsky P. 1986. Detection of familial hypercholesterolemia by assaying functional low-density lipoprotein receptors on lymphocytes. *N Engl J Med* 314:879-883.

Dallinga-Thie G, Bu X-D, van Linde-Sibenius Trip M, Rotter JI, Lusis AJ and de Bruin TW. 1996. Apolipoprotein A-I/C-III/A-IV gene cluster in familial combined hyperlipidemia: effects on LDL-cholesterol and apolipoproteins B and C-III. *J Lipid Res* 37:136-147.

Dallinga-Thie GM, van Linde-Sibenius Trip M, Rotter JI, Cantor RM, Bu X, Lusis AJ and de Bruin TWA. 1997. Complex genetic contribution of the apo AI-CIII-AIV gene cluster to familial combined hyperlipidemia. Identification of different susceptibility haplotypes. *J Clin Invest* 99:953-961.

Dashti N. 1992. The effect of low density lipoproteins, cholesterol, and 25-hydroxycholesterol on apolipoprotein B gene expression in HepG2 cells. *J Biol Chem* 267:7160-7169.

De Bruin TW, Mailly F, van Barlingen HH, Fisher R, Castro Cabezas M, Talmud P, Dallinga-Thie GM and Humphries SE. 1996. Lipoprotein lipase gene mutations D9N and N291S in four pedigrees with familial combined hyperlipidaemia. *Eur J Clin Invest* 26:631-639.

De Rijke YB, Bredie SJ, Demacker PN, Vogelaar JM, Hak-Lemmers HL and Stalenhoef AF. 1997. The redox status of coenzyme Q10 in total LDL as an indicator of in vivo oxidative modification. Studies on subjects with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 17:127-133.

De Vos P, Lefebvre A-M, Miller SG, Guerre-Millo M, Wong K, Saladin R, Hamann LG, Staels B, Briggs MR and Auwerx J. 1996. Thiazolidinediones repress *ob* gene expression in rodents via activation of peroxisome proliferator-activated receptor γ. *J Clin Invest* 98:1004-1009.

Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, Laakso M, Fujimoto W and Auwerx J. 1998. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284-287.

Dejager S, Bruckert E and Chapman J. 1993. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. *J Lipid Res* 34:295-308.

Demant T, Carlson LA, Holmquist L, Karpe F, Nilsson-Ehle P, Packard CJ and Shepherd J. 1988. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J Lipid Res* 29:1603-1611.

Dempsey RJ and Moore RW. 1992. Amount of smoking independently predicts carotid artery atherosclerosis severity. *Stroke* 23:693-696.

Dixon JL, Furukawa S and Ginsberg HN. 1991. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from Hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B. *J Biol Chem* 266:5080-5086.

Douglas JA, Erdos MR, Watanabe RM, Braun A, Johnston CL, Oeth P, Mohlke KL, Valle TT, Ehnholm C, Buchanan TA, Bergman RN, Collins FS, Boehnke M and Tuomilehto J. 2001. The peroxisome proliferator-activated receptor-γ2 Pro12Ala variant. Association with type 2 diabetes and trait differences. *Diabetes* 50:886-890.

Du EZ, Wang SL, Kayden HJ, Sokol R, Curtiss LK and Davis RA. 1996. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in abetalipoproteinemia. *J Lipid Res* 37:1309-1315.

Durrington PN, Newton RS, Weinstein DB and Steinberg D. 1982. Effects of insulin and glucose on very low density lipoprotein triglyceride secretion by cultured rat hepatocytes. *J Clin Invest* 70:63-73.

Dusserre E, Moulin P and Vidal H. 2000. Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. *Biochim Biophys Acta* 1500:88-96.

Ebeling P, Teppo A-M, Koistinen H, Viikari J, Rönnemaa T, Nissen M, Bergkulla S, Salmela P, Saltevo J and Koivisto V. 1999. Troglitazone reduces hyperglycemia and selectively acute-phase serum proteins in patients with Type II diabetes. *Diabetologia* 42:1433-1438.

Ebeling P, Teppo A-M, Koistinen HA and Koivisto VA. 2001. Concentration of the complement activation product, acylation-stimulating protein, is related to C-reactive protein in patients with type 2 diabetes. *Metabolism* 50:283-287.

Eckel RH. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic disorders. *N Engl J Med* 320:1060-1068.

Egan JJ, Greenberg AS, Chang MK, Wek SA, Moos MCJ and Londos C. 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc Natl Acad Sci USA* 89:8537-8541.

El-Barghouti N, Elkeles R, Nicolaides A, Geroulakos G, Dhanjil S and Diamond J. 1997. The ultrasonic evaluation of the carotid intima-media thickness and its relation to risk factors of atherosclerosis in normal and diabetic population. *Int Angiol* 16:50-54.

Engfeldt P, Hellmer J, Wahrenberg H and Arner P. 1992. Effects of insulin on adrenoceptor binding and the rate of catecholamine-induced lipolysis in isolated human fat cells. *J Biol Chem* 263:15553-15560.

Ericsson S, Eriksson M, Berglund L and Angelin B. 1992. Metabolism of plasma low density lipoproteins in familial combined hyperlipidaemia: effect of acipimox therapy. *J Int Med* 232:313-320.

Escher P and Wahli W. 2000. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res* 448:121-138.

Espeland MA, Craven TE, Riley WA, Corson J, Romont A and Furberg CD. 1996. Reliability of longitudinal ultrasonographic measurements of carotid intimal-medial thickness. *Stroke* 27:480-485.

Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre A-M, Saladin R, Najib J, Laville M, Fruchart J-C, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H and Auwerx J. 1997. The organization, promoter analysis, and expression of the human PPARγ gene. *J Biol Chem* 272:18779-18789.

Fajas L, Fruchart J and Auwerx J. 1998. PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter. *FEBS Lett* 438:55-60.

Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelli F, Pampanelli S, Perriello G, De Feo P, Brunetti P, Gerich JE, Bolli GB. 1993. Demonstration of a critical role for free fatty acids in mediating counterregulatory stimulation of gluconeogenesis and suppression of glucose utilization in humans. *J Clin Invest* 92:1617-1622.

Ferrannini E, Barrett EJ, Bevilacqua S and DeFronzo RA. 1983. Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 72:1737-1747.

Fielding CJ and Fielding PE. 1995. Molecular physiology of reverse cholesterol transport. *J Lipid Res* 36:211-228.

Fisher WR, Zech LA, Kilgore LL and Stacpoole PW. 1991. Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [^{3}H]leucine tracer. *J Lipid Res* 32:1823-1836.

Flier JS, Cook KS, Usher P and Spiegelman BM. 1987. Severely impaired adipsin expression in genetic and acquired obesity. *Science* 237:405-408.

Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB and Hutchinson RG for the Atherosclerosis Risk in Communities (ARIC) Study Investigators. 1994. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. *Stroke* 25:66-73.

Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM and Evans RM. 1995. 15-deoxy-12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83:803-812.

Franklin SS, Sutton-Tyrrell K, Belle SH, Weber MA and Kuller LH. 1997. The importance of pulsatile components of hypertension in predicting carotid stenosis in older adults. *J Hypertens* 15:1143-1150.

Frayn KN, Langin D, Holm C and Belfrage P. 1993. Hormone-sensitive lipase: quantitation of enzyme activity and mRNA level in small biopsies of human adipose tissue. *Clin Chim Acta* 216:183-189.

Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA, Boland O and Coppack SW. 1994. Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 266:E308-E317.

Fredrikson G, Strålfors P, Östen Nilsson P and Belfrage P. 1981. Hormone-sensitive lipase of rat adipose tissue. *J Biol Chem* 256:6311-6320.

Frykman PK, Brown MS, Yamamoto T, Goldstein JL and Herz J. 1995. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc Natl Acad Sci USA* 92:8453-8457.

Fungwe TV, Cagen L, Wilcox HG and Heimberg M. 1992. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. *J Lipid Res* 33:179-191.

Furberg C, Byington R and Craven T. 1994a. Lessons learned from clinical trials with ultrasound end-points. *J Int Med* 236:575-580.

Furberg CD, Adams HP Jr, Applegate WB, Byington RP, Espeland MA, Hartwell T, Hunninghake DB, Lefkowitz DS, Probstfield J, Riley WA and Young B. 1994b. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. *Circulation* 90:1679-1687.

Gagné E, Genest J Jr, Zhang H, Clarke L and Hayden M. 1994. Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. *Arterioscler Thromb* 14:1250-1257.

Galeano NF, Milne R, Marcel YL, Walsh MT, Levy E, Ngu'yen T-D, Gleeson A, Arad Y, Witte L and Al-Haideri M. 1994. Apoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride-rich LDL of normal size. *J Biol Chem* 269:511-519.

Gehrisch S, Kostka H, Tiebel M, Patzak A, Paetzold A, Julius U, Schroeder M and Jaross W. 1999. Mutations of the human hepatic lipase gene in patients with familial combined hyperlipidemia. *J Mol Med* 77:728-734.

Genest J, Sniderman A, Cianflone K, Teng BB, Wacholder S, Marcel Y and Kwiterovich P Jr. 1986. Hyperapobetalipoproteinemia. Plasma lipoprotein responses to oral fat load. *Arterioscler* 6:297-304.

Genest JJ Jr, Martin-Munley SS, McNamara JR, Ordovas JM, Jenner J, Myers RH, Silberman SR, Wilson PW, Salem DN and Schaefer EJ. 1992. Familial lipoprotein disorders in patients with premature coronary artery disease. *Circulation* 85:2025-2033.

Germinario R, Sniderman A, Manuel S, Lefebvre S, Baldo A and Cianflone K. 1993. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. *Metabolism* 42:574-580.

Geroulakos G, O'Gorman DJ, Kalodiki E, Sheridan DJ and Nicolaides AN. 1994a. The carotid intima-media thickness as a marker of the presence of severe symptomatic coronary artery disease. *Eur Heart J* 15:781-785.

Geroulakos G, Ramaswami G, Veller M, Fisher G, Renton S, Nicolaides A, Waldron H, Diamond J and Elkeles R. 1994b. Arterial wall changes in type 2 diabetic subjects. *Diabetic Med* 11:692-695.

Gibbons G and Wiggins D. 1995. The enzymology of hepatic very-low-density lipoprotein assembly. *Biochem Soc T* 23:495-500.

Gilliland G, Perrin S, Blanchard K and Bunn HF. 1990. Analysis of cytokine mRNA and DNA: detection and quantitation by competitive polymerase chain reaction. *Proc Natl Acad Sci USA* 87:2725-2729.

Ginsberg HN. 1995. Synthesis and secretion of apolipoprotein B from cultured liver cells. *Curr Opin Lipidol* 6:275-280.

Ginsberg HN. 1996. Diabetic dyslipidemia. Basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. *Diabetes* 45:S27-S30.

Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R and Brown WV. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J Clin Invest* 78:1287-1295.

Glagov S, Weisenberg E, Zarins CK, Stankunavicius R and Kolettis GJ. 1987. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 316:1371-1375.

Goldstein JL, Schrott HG, Hazzard WR, Bierman EL and Motulsky AG. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest* 52:1544-1568.

Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DRJ, Bangdiwala S and Tyroler HA. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 79:8-15.

Gotto AMJ, Pownall HJ and Havel RJ. 1986. Introduction to the plasma lipoproteins. *Methods Enzymol* 128:3-41.

Green A, Dobias SB, Walters DJ and Brasier AR. 1994. Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. *Endocrinology* 134:2581-2588.

Greene M, Blumberg B, McBride O, Yi H, Kronquist K, Kwan K, Hsieh L, Greene G and Nimer S. 1995. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr* 4:281-299.

Groenendijk M, Cantor RM, Blom NH, Rotter JI, de Bruin TW and Dallinga-Thie GM. 1999. Association of plasma lipids and apolipoproteins with the insulin response element in the apoC-III promoter region in familial combined hyperlipidemia. *J Lipid Res* 40:1036-1044.

Groenendijk M, Cantor RM, de Bruin TW and Dallinga-Thie GM. 2001. New genetic variants in the apoA-I and apoC-III genes and familial combined hyperlipidemia. *J Lipid Res* 42:188-194.

Grundy SM, Chait A and Brunzell JD. 1987. Familial combined hyperlipidemia workshop. *Arterioscler* 7:203-207.

Guérin M, Bruckert É, Dolphin P and Chapman M. 1996. Absence of cholesteryl ester transfer protein-mediated cholesteryl ester mass transfer from high-density lipoprotein to low-density lipoprotein particles is a major feature of combined hyperlipidaemia. *Eur J Clin Invest* 26:485-494.

Hagström-Toft E, Bolinder J, Eriksson S and Arner P. 1995. Role of phosphodiesterase III in the antilipolytic effect of insulin in vivo. *Diabetes* 44:1170-1175.

Hak AE, Stehouwer CDA, Bots ML, Polderman KH, Schalkwijk CG, Westendorp ICD, Hofman A and Witteman JCM. 1999. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. *Arterioscler Thromb Vasc Biol* 19:1986-1991.

Hanefeld M, Koehler C, Schaper F, Fuecker K, Henkel E and Temelkova-Kurktschiev T. 1999a. Postprandial plasma glucose is an independent risk factor for increased carotid intima-media thickness in non-diabetic individuals. *Atherosclerosis* 144:229-235.

Hanefeld M, Temelkova-Kurktschiev T, Schaper F, Henkel E, Siegert G and Koehler C. 1999b. Impaired fasting glucose is not a risk factor for atherosclerosis. *Diabetic Med* 16:212-218.

Hansson GK, Holm J and Kral JG. 1984. Accumulation of IgG and complement factor C3 in human arterial endothelium and atherosclerotic lesions. *Acta Pathol Microbiol Immunol Scand [A]* 92:429-435.

Hasstedt SJ, Ren QF, Teng K and Elbein SC. 2001. Effect of the peroxisome proliferator-activated receptor-γ2 $Pro^{12}Ala$ variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds. *J Clin Endocrinol Metab* 86:536-541.

Hatanaka K, Li XA, Masuda K, Yutani C and Yamamoto A. 1995. Immunohistochemical localization of C-reactive protein-binding sites in human atherosclerotic aortic lesions by a modified streptavidin-biotin-staining method. *Pathol Int* 45:635-641.

Hayden MR, Kirk H, Clark C, Frohlich J, Rabkin S, McLeod R and Hewitt J. 1987. DNA polymorphisms in and around the Apo-A1-CIII genes and genetic hyperlipidemias. *Am J Hum Genet* 40:421-430.

Hedblad B, Nilsson P, Janzon L and Berglund G. 2000. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmö, Sweden. *Diabetic Med* 17:299-307.

Hellerstein MK, Christiansen M, Kaempfer S, Kletke C, Wu K, Reid JS, Mulligan K, Hellerstein NS and Shackleton CH. 1991. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* 87:1841-1852.

Hellmer J, Marcus C, Sonnenfeld T and Arner P. 1992. Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J Clin Endocrinol Metab* 75:15-20.

Hellström L, Langin D, Reynisdottir S, Dauzats M and Arner P. 1996. Adipocyte lipolysis in normal weight subjects with obesity among first-degree relatives. *Diabetologia* 39:921-928.

Hiltunen TP, Luoma JS, Nikkari T and Ylä-Herttuala S. 1998. Expression of LDL receptor, VLDL receptor, LDL receptor-related protein, and scavenger receptor expression during lesion development. *Circulation* 97:1079-1086.

Hodis HN, Mack WJ and Barth J. 1996. Carotid intima-media thickness as a surrogate end point for coronary artery disease. *Circulation* 94:2311-2312.

Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Liu C and Azen SP. 1998. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med* 128:262-269.

Hoffer MJ, Bredie SJ, Boomsma DI, Reymer PW, Kastelein JJ, de Knijff P, Demacker PN, Stalenhoef AF, Havekes LM and Frants RR. 1996. The lipoprotein lipase (Asn291→Ser) mutation is associated with elevated lipid levels in families with familial combined hyperlipidemia. *Atherosclerosis* 119:159-167.

Hoffer MJ, Bredie SJ, Snieder H, Reymer PW, Demacker PN, Havekes LM, Boomsma DI, Stalenhoef AF, Frants RR and Kastelein JJ. 1998. Gender-related association between the -93T→G/D9N haplotype of the lipoprotein lipase gene and elevated lipid levels in familial combined hyperlipidemia. *Atherosclerosis* 138:91-99.

Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil GS and Spiegelman BM. 1994. Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* 134:264-270.

Hokanson JE, Krauss RM, Albers JJ, Austin MA and Brunzell JD. 1995. LDL physical and chemical properties in familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 15:452-459.

Hollander W, Colombo MA, Kirkpatrick B and Paddock J. 1979. Soluble proteins in the human atherosclerotic plaque. With spectral reference to immunoglobulins, C3-complement component, alpha 1-antitrypsin and alpha 2-macroglobulin. *Atherosclerosis* 34:391-405.

Holm C, Österlund T, Laurell H and Contreras JA. 2000. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu Rev Nutr* 20:365-393.

Hosmer DW and Lemeshow S. 1989. Applied logistic regression. NewYork: John Wiley & sons.

Hotamisligil GS, Budavari A, Murray D and Spiegelman BM. 1994. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. *J Clin Invest* 94:1543-1549.

Howard G, O´Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P and Bergman R for the IRAS Investigators. 1996. Insulin sensitivity and atherosclerosis. *Circulation* 93:1809-1817.

Hu E, Kim JB, Sarraf P and Spiegelman BM. 1996a. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science* 274:2100-2103.

Hu E, Liang P and Spiegelman BM. 1996b. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697-10703.

Hugli T. 1989. Structure and function of C3a anaphylatoxin. *Curr Top Microbiol* 153:23-43.

Hulthe J, Wiklund O, Olsson G, Fagerberg B, Bokemark L, Nivall S and Wikstrand J. 1999. Computerized measurement of LDL particle size in human serum. Reproducibility studies and evaluation of LDL particle size in relation to metabolic variables and the occurrence of atherosclerosis. *Scand J Clin Lab Invest* 59:649-661.

Hulthe J, Wiklund O, Bondjers G and Wikstrand J. 2000. LDL particle size in relation to intima-media thickness and plaque occurrence in the carotid and femoral arteries in patients with hypercholesterolaemia. *J Int Med* 248:45-52.

Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel J-M and Williams RG. 1989. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. *Arterioscler* 9:335-344.

Isola L, Zhou S, Kiang C-L, Stump D, Bradbury M and Berk P. 1995. 3T3 fibroblasts transfected with a cDNA for mitochondrial aspartate aminotransferase express plasma membrane fatty acid-binding protein and saturable fatty acid uptake. *Proc Natl Acad Sci USA* 92:9866-9870.

Janus ED, Nicoll AM, Turner PR, Magill P and Lewis B. 1980. Kinetic bases of the primary hyperlipidaemias: studies of apolipoprotein B turnover in genetically defined subjects. *Eur J Clin Invest* 10:161-172.

Jarvik GP, Brunzell JD, Austin MA, Krauss RM, Motulsky AG and Wijsman E. 1994. Genetic predictors of FCHL in four large pedigrees. Influence of apoB level major locus predicted genotype and LDL subclass phenotype. *Arterioscler Thromb* 14:1687-1694.

Juo S-HH, Bredie SJ, Kiemeney LA, Demacker PN and Stalenhoef AF. 1998. A common genetic mechanism determines plasma apolipoprotein B levels and dense LDL subfraction distribution in familial combined hyperlipidemia. *Am J Hum Genet* 63:586-594.

Kalant D, Phelis S, Fielding BA, Frayn KN, Cianflone K and Sniderman AD. 2000. Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J Lipid Res* 41:1963-1968.

Kallen CB and Lazar MA. 1996. Antidiabetic thiazolidinediones inhibit leptin (*ob*) gene expression in 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 93:5793-5796.

Kanters SDJM, Algra A, van Leeuwen MS and Banga J-D. 1997. Reproducibility of in vivo carotid intima-media thickness measurements. *Stroke* 28:665-671.

Karjalainen L, Pihlajamäki J, Karhapää P and Laakso M. 1998. Impaired insulin-stimulated glucose oxidation and free fatty acid suppression in patients with familial combined hyperlipidemia: a precursor defect for dyslipidemia? *Arterioscler Thromb Vasc Biol* 18:1548-1553.

Karpe F and Hamsten A. 1994. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J Lipid Res* 35:1311-1317.

Karpe F and Hamsten A. 1995. Postprandial lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol* 6:123-129.

Karpe F, Steiner G, Olivecrona T, Carlson LA and Hamsten A. 1993. Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J Clin Invest* 91:748-758.

Karpe F, de Faire U, Mercuri M, Bond MG, Hellénius ML and Hamsten A. 1998. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis* 141:307-314.

Karpe F, Boquist S, Tang R, Bond GM, de Faire U and Hamsten A. 2001. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. *J Lipid Res* 42:17-21.

Kildsgaard J, Zsigmond E, Chan L and Wetsel RA. 1999. A critical evaluation of the putative role of C3adesArg (ASP) in lipid metabolism and hyperapobetalipoproteinemia. *Mol Immunol* 36:869-876.

Kissebah AH, Alfarsi S and Adams PW. 1981. Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in man: normolipemic subjects, familial hypertriglyceridemia and familial combined hyperlipidemia. *Metabolism* 30:856-868.

Kissebah AH, Alfarsi S and Evans DJ. 1984. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanisms of the multiple lipoprotein phenotypic expression. *Arterioscler* 4:614-624.

Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC and Lehmann JM. 1995. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor and promotes adipocyte differentiation. *Cell* 83:813-819.

Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM and Lehmann JM. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc Natl Acad Sci USA* 94:4318-4323.

Koch M, Rett K, Maerker E, Volk A, Haist K, Deninger M, Renn W and Häring HU. 1999. The PPARγ2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of Type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 42:758-762.

Koistinen HA, Koivisto VA and Ebeling P. 1998. Serum complement protein C3 is a marker of insulin resistance, which is related to obesity, but not to hyperglycemia. *Diabetes* 47(Suppl. 1):A311.

Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, Gliemann J and Beisiegel U. 1996. Hepatic lipase mediates the uptake of chylomicrons and β-VLDL into cells via the LDL receptor-related protein (LRP). *J Lipid Res* 37:926-936.

Krempler F, Breban D, Oberkofler H, Esterbauer H, Hell E, Paulweber B and Patsch W. 2000. Leptin, peroxisome proliferator-activated receptor-γ, and CCAAT/enhancer binding protein-α mRNA expression in adipose tissue of humans and their relation to cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 20:443-449.

Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG and Wahli W. 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* 11:779-791.

Kristensen BO and Petersen GB. 1978. Association between coronary heart disease and the $C3^F$ -gene in essential hypertension. *Circulation* 58:622-625.

Kukita H, Hamada M, Hiwada K and Kokubu T. 1985. Clinical significance of measurements of serum apolipoprotein A-I, A-II and B in hypertriglyceridemic male patients with and without coronary artery disease. *Atherosclerosis* 55:143-149.

Kuller LH, Tracy RP, Shaten J and Meilahn EN. 1996. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. *Am J Epidemiol* 144:537-547.

Kwiterovich PO Jr, White S, Forte T, Bachorik PS, Smith H and Sniderman A. 1987. Hyperapobetalipoproteinemia in a kindred with familial combined hyperlipidemia and familial hypercholesterolemia. *Arterioscler* 7:211-225.

Kwiterovich P Jr, Motevalli M and Miller M. 1990. Acylation-stimulatory activity in hyperapobetalipoproteinemic fibroblasts: enhanced cholesterol esterification with another serum basic protein, BP II. *Proc Natl Acad Sci USA* 87:8980-8984.

Kwiterovich PO Jr, Coresh J and Bachorik PS. 1993. Prevalence of hyperapobetalipoproteinemia and other lipoprotein phenotypes in men (aged <50 years) and women (<60 years) with coronary artery disease. *Am J Cardiol* 71:631-639.

Kwiterovich PO Jr, Motevalli M and Miller M. 1994. The effect of three serum basic proteins on the mass of lipids in normal and hyperapoB fibroblasts. *Arterioscler Thromb* 14:1-7.

Lagrost L, Desrumaux C, Masson D, Deckert V and Gambert P. 1998. Structure and function of the plasma phospholipid transfer protein. *Curr Opin Lipidol* 9:203-209.

Lakka TA, Salonen R, Kaplan GA and Salonen JT. 1999. Blood pressure and the progression of carotid atherosclerosis in middle-aged men. *Hypertension* 34:51-56.

Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ and Després J-P. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. *Circulation* 95:69-75.

Lapsys NM, Kriketos AD, Lim-Fraser M, Poynten AM, Lowy A, Furler S, Chisholm D and Cooney G. 2000. Expression of genes involved in lipid metabolism correlate with peroxisome proliferator-activated receptor γ expression in human skeletal muscle. *J Clin Endocrinol Metab* 85:4293-4297.

Large V, Reynisdottir S, Langin D, Fredby K, Klannemark M, Holm C and Arner P. 1999. Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects. *J Lipid Res* 40:2059-2065.

Lathrop GM, Lalouel J-M, Julier CA and Ott J. 1984. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443-3446.

Laurell H, Grober J, Vindis C, Lacombe T, Dauzats M, Holm C and Langin D. 1997. Species-specific alternative splicing generates a catalytically inactive form of human hormone-sensitive lipase. *Biochem J* 328:137-143.

Laville M, Auboeuf D, Khalfallah Y, Vega N, Riou J and Vidal H. 1996. Acute regulation by insulin of phosphatidylinositol-3-kinase, rad, glut 4, and lipoprotein lipase mRNA levels in human muscle. *J Clin Invest* 98:43-49.

Lefebvre A-M, Peinado-Onsurbe J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J and Staels B. 1997. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler Thromb Vasc Biol* 17:1756-1764.

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM and Kliewer SA. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR γ). *J Biol Chem* 270:12953-12956.

Lewis GF. 1997. Fatty acid regulation of very low density lipoprotein production. *Curr Opin Lipidol* 8:146-153.

Lewis GF, Uffelman KD, Szeto LW and Steiner G. 1993. Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes* 42:833-842.

Lewis GF, Zinman B, Uffelman KD, Szeto L, Weller B and Steiner G. 1994. VLDL production is decreased to a similar extent by acute portal vs. peripheral venous insulin. *Am J Physiol* 267:E566-E572.

Lewis GF, Uffelman KD, Szeto LW, Weller B and Steiner G. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J Clin Invest* 95:158-166.

Ludbrook J. 1994. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc Res* 28:303-311.

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y and Matsubara K. 1996. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most Abundant Gene Transcript 1). *Biochem Biophys Res Commun* 221:286-289.

Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I and Matsuzawa Y. 2001. PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094-2099.

Malmström R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Järvinen H, Shepherd J and Taskinen M-R. 1997. Defective regulation of triglyceride metabolism by insulin in the liver in non-insulin-dependent diabetes mellitus. *Diabetologia* 40:454-462.

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

Mancini FP, Vaccaro O, Sabatino L, Tufano A, Rivellese AA, Riccardi G and Colantuoni V. 1999. Pro12Ala substitution in the peroxisome proliferator-activated receptor-γ2 is not associated with type 2 diabetes. *Diabetes* 48:1466-1468.

Manganiello V and Vaughan M. 1973. An effect of insulin on cyclic adenosine 3´:5´-monophosphate phosphodiesterase activity in fat cells. *J Biol Chem* 248:7164-7170.

Mann CJ, Yen FT, Grant AM and Bihain BE. 1991. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. *J Clin Invest* 88:2059-2066.

Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Mänttäri M, Heinonen OP and Frick MH. 1992. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* 85:37-45.

Marcil M, Boucher B, Gagné E, Davignon J, Hayden M and Genest J Jr. 1996. Lack of association of the apolipoprotein A-I-C-III-A-IV gene XmnI and SstI polymorphisms and of the lipoprotein lipase gene mutations in familial combined hyperlipoproteinemia in French Canadian subjects. *J Lipid Res* 37:309-319.

Martin G, Schoonjans K, Lefebvre A-M, Staels B and Auwerx J. 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARγ activators. *J Biol Chem* 272:28210-28217.

Martin G, Poirier H, Hennuyer N, Crombie D, Fruchart JC, Heyman RA, Besnard P and Auwerx J. 2000. Induction of the fatty acid transport protein 1 and acyl-CoA synthase genes by dimer-selective rexinoids suggests that the peroxisome proliferator-activated receptor-retinoid X receptor heterodimer is their molecular target. *J Biol Chem* 275:12612-12618.

Martin MJ, Hulley SB, Browner WS, Kuller LH and Wentworth D. 1986. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361 662 men. *Lancet* 2:933-936.

Marx N, Schönbeck U, Lazar M, Libby P and Plutzky J. 1998a. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res* 83:1097-1103.

Marx N, Sukhova G, Murphy C, Libby P and Plutzky J. 1998b. Macrophages in human atheroma contain PPARγ. Differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes in vitro. *Am J Pathol* 153:17-23.

Marx N, Bourcier T, Sukhova G, Libby P and Plutzky J. 1999. PPARγ activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARγ as a potential mediator in vascular disease. *Arterioscler Thromb Vasc Biol* 19:546-551.

Maslowska M, Scantlebury T, Germinario R and Cianflone K. 1997a. Acute in vitro production of acylation stimulating protein in differentiated human adipocytes. *J Lipid Res* 38:1-11.

Maslowska M, Sniderman AD, Germinario R and Cianflone K. 1997b. ASP stimulates glucose transport in cultured human adipocytes. *Int J Obes* 21:261-266.

Maslowska M, Vu H, Phelis S, Sniderman A, Rhode B, Blank D and Cianflone K. 1999. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. *Eur J Clin Invest* 29:679-686.

Matthews J, Altman DG, Campbell M and Royston P. 1990. Analysis of serial measurements in medical research. *Br Med J* 300:230-235.

Mauriege P, Galitzky J, Berlan M and Lafontan M. 1987. Heterogeneous distribution of beta and alpha-2 adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *Eur J Clin Invest* 17:156-165.

McCulloch R, Choong C and Hurley D. 1995. An evaluation of competitor type and size for use in the determination of mRNA by competitive PCR. *PCR Methods Appl* 4:219-226.

McNamara J, Campos H, Ordovas JM, Peterson J, Wilson PW and Schaefer EJ. 1987. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results from the Framingham Offspring Study. *Arterioscler* 7:483-490.

McPherson R, Mann CJ, Tall AR, Hogue M, Martin L, Milne RW and Marcel YL. 1991. Plasma concentrations of cholesteryl ester transfer protein in hyperlipoproteinemia. Relation to cholesteryl ester protein activity and other lipoprotein variables. *Arterioscler Thromb* 11:797-804.

Medh JD, Bowen SL, Fry GL, Ruben S, Hill J, Wong H and Chappell DA. 1999. Hepatic triglyceride lipase promotes low density lipoprotein receptor-mediated catabolism of very low density lipoproteins in vitro. *J Lipid Res* 40:1263-1275.

Meek SE, Nair KS and Jensen MD. 1999. Insulin regulation of regional free fatty acid metabolism. *Diabetes* 48:10-14.

Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Auwerx J, Deeb S and Amouyel P. 2000. Impact of the peroxisome proliferator activated receptor γ2 Pro12Ala polymorphism on adiposity, lipids and non-insulin-dependent diabetes mellitus. *Int J Obes* 24:195-199.

Memon RA, Fuller J, Moser AH, Smith PJ, Grunfeld C and Feingold KR. 1999. Regulation of putative fatty acid transporters and acyl-CoA synthetase in liver and adipose tissue in *ob*/*ob* mice. *Diabetes* 48:121-127.

Mercuri M, Bond MG, Sirtori CR, Veglia F, Crepaldi G and Feruglio FS. 1996. Pravastatin reduces carotid intima-media thickness progression in an asymptomatic hypercholesterolemic Mediterranean population: the Carotid Atherosclerosis Italian Ultrasound Study. *Am J Med* 101:627-634.

Mero N, Syvänne M, Rosseneu M, Labeur C, Hilden H and Taskinen M-R. 1998. Comparison of three fatty meals in healthy normolipidaemic men: high post-prandial retinyl ester response to soybean oil. *Eur J Clin Invest* 28:407-415.

Miles J, Glasscock R, Aikens J, Gerich J and Haymond M. 1983. A microfluorometric method for the determination of free fatty acids in plasma. *J Lipid Res* 24:96-99.

Mitchell J and Schwartz C. 1962. Relationship between arterial disease in different sites. A study of the aorta and coronary, carotid, and iliac arteries. *Br Med J* 5288:1293-1301.

Moller DE and Flier JS. 1991. Insulin resistance - mechanisms, syndromes, and implications. *N Engl J Med* 325:938-948.

Montague CT, Prins JB, Sanders L, Zhang J, Sewter CP, Digby J, Byrne CD and O'Rahilly S. 1998. Depot-related gene expression in human subcutaneous and omental adipocytes. *Diabetes* 47:1384-1391.

Mori Y, Kim-Motoyama H, Katakura T, Yasuda K, Kadowaki H, Beamer BA, Shuldiner AR, Akanuma Y, Yazaki Y and Kadowaki T. 1998. Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men. *Biochem Biophys Res Commun* 251:195-198.

Motojima K, Passilly P, Peters JM, Gonzalez FJ and Latruffe N. 1998. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor α and γ activators in a tissue- and inducer specific manner. *J Biol Chem* 273:16710-16714.

Mukherjee R, Strasser J, Jow L, Hoener P, Paterniti JRJ and Heyman RA. 1998. RXR agonists activate PPARα-inducible genes, lower triglycerides, and raise HDL levels in vivo. *Arterioscler Thromb Vasc Biol* 18:272-276.

Mulder H, Stenson Holst L, Svensson H, Degerman E, Sundler F, Ahrén B, Rorsman P and Holm C. 1999. Hormone-sensitive lipase, the rate-limiting enzyme in triglyceride hydrolysis, is expressed and active in β-cells. *Diabetes* 48:228-232.

Müller G, Ertl J, Gerl M and Preibisch G. 1997. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J Biol Chem* 272:10585-10593.

Murray I, Sniderman A and Cianflone K. 1999. Mice lacking acylation stimulating protein (ASP) have delayed postprandial triglyceride clearance. *J Lipid Res* 40:1671-1676.

Murray I, Havel PJ, Sniderman AD and Cianflone K. 2000. Reduced body weight, adipose tissue, and leptin levels despite increased energy intake in female mice lacking acylation-stimulating protein. *Endocrinology* 141:1041-1049.

Muscari A, Bozzoli C, Gerratana C, Zaca F, Rovinetti C, Zauli D, La Placa M and Puddu P. 1988. Association of serum IgA and C4 with severe atherosclerosis. *Atherosclerosis* 74:179-186.

Muscari A, Bozzoli C, Puddu GM, Rovinetti C, Fiorentini GP, Roversi RA and Puddu P. 1990. Correlations between serum lipids and complement components in adults without demonstrated atherosclerotic disease. *Atherosclerosis* 81:111-118.

Muscari A, Bozzoli C, Puddu GM, Sangiorgi Z, Dormi A, Rovinetti C, Descovich GC and Puddu P. 1995a. Association of serum C3 levels with the risk of myocardial infarction. *Am J Med* 98:357-364.

Muscari A, Bozzoli C, Massarelli G, Puddu GM, Palareti G, Legnani C, D´Atena T, Mazzuca A, Miniero R, Toscano V, Conte R and Puddu P. 1995b. Complement components and fibrinogen: correlations with previous myocardial infarction. *Cardiology* 86:232-237.

Muscari A, Massarelli G, Bastagli L, Poggiopollini G, Tomassetti V, Volta U, Puddu GM and Puddu P. 1998. Relationship between serum C3 levels and traditional risk factors for myocardial infarction. *Acta Cardiol* 56:345-354.

Muscari A, Massarelli G, Bastagli L, Poggiopollini G, Tomassetti V, Drago G, Martignani C, Pacilli P, Boni P and Puddu P. 2000. Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men. *Eur Heart J* 21:1081-1090.

Nevin DN, Brunzell JD and Deeb SS. 1994. The LPL gene in individuals with familial combined hyperlipidemia and decreased LPL activity. *Arterioscler Thromb* 14:869-873.

Niculescu F and Rus H. 1999. Complement activation and atherosclerosis. *Mol Immunol* 36:949-955.

Niculescu F, Hugo F, Rus HG, Vlaicu R and Bhakdi S. 1987a. Quantitative evaluation of the terminal C5b-9 complement complex by ELISA in human atherosclerotic arteries. *Clin Exp Immunol* 69:477-483.

Niculescu F, Rus HG and Vlaicu R. 1987b. Immunohistochemical localization of C5b-9, S-protein, C3d and apolipoprotein B in human arterial tissues with atherosclerosis. *Atherosclerosis* 65:1-11.

Niculescu F, Rus HG, Porutiu D, Ghiurca V and Vlaicu R. 1989. Immunoelectron-microscopic localization of S-protein/vitronectin in human atherosclerotic wall. *Atherosclerosis* 78:197-203.

Nikkilä EA and Aro A. 1973. Family study of serum lipids and lipoproteins in coronary heart disease. *Lancet* 1:954-959.

Nisoli E, Carruba MO, Tonello C, Macor C, Federspil G and Vettor R. 2000. Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-γ2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-α gene expression in human subcutaneous fat by lipid infusion. *Diabetes* 49:319-324.

O´Leary DH, Polak JF, Kronmal RA, Kittner S, Bond MG, Wolfson SJ, Bommer W, Price T, Gardin J and Savage P. 1992. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. *Stroke* 23:1752-1760.

O´Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL and Wolfson SK. 1999. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med* 340:14-22.

Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y and Kadowaki T. 1998. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest* 101:1354-1361.

Olivecrona G and Olivecrona T. 1995. Triglyceride lipases and atherosclerosis. *Curr Opin Lipidol* 6:291-305.

Olivecrona T and Bengtsson-Olivecrona G. 1990. Lipoprotein lipase and hepatic lipase. *Curr Opin Lipidol* 1:222-239.

Oliver WR Jr, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM. 2001. A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci USA* 98:5306-5311.

Olsson H and Belfrage P. 1987. The regulatory and basal phosphorylation sites of hormone-sensitive lipase are dephosphorylated by protein phosphatase-1, 2A and 2C but not by protein phosphatase-2B. *Eur J Biochem* 168:399-405.

Ott J. 1991. Analysis of Human Genetic Linkage. Baltimore: Johns Hopkins University Press**.**

Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T and Matsuzawa Y. 1999. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100:2473-2476.

Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T and Matsuzawa Y. 2000. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-κB signaling through a cAMP-dependent pathway. *Circulation* 102:1296-1301.

Packard CJ and Shepherd J. 1997. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler Thromb Vasc Biol* 17:3542-3556.

Pajukanta P, Nuotio I, Terwilliger JD, Porkka KVK, Ylitalo K, Pihlajamäki J, Suomalainen AJ, Syvänen A-C, Lehtimäki T, Viikari JSA, Laakso M, Taskinen M-R, Ehnholm C and Peltonen L. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat Genet* 18:369-373.

Pajukanta P, Porkka KVK, Antikainen M, Taskinen M-R, Perola M, Murtomäki-Repo S, Ehnholm S, Nuotio I, Suurinkeroinen L, Lahdenkari A-T, Syvänen A-C, Viikari JSA, Ehnholm C and Peltonen L. 1997. No evidence of linkage between familial combined hyperlipidemia and genes encoding lipolytic enzymes in Finnish families. *Arterioscler Thromb Vasc Biol* 17:841-850.

Pajukanta P, Terwilliger JD, Perola M, Hiekkalinna T, Nuotio I, Ellonen P, Parkkonen M, Hartiala J, Ylitalo K, Pihlajamäki J, Porkka K, Laakso M, Viikari J, Ehnholm C, Taskinen M-R and Peltonen L. 1999. Genomewide scan for familial combined hyperlipidemia genes in

Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol and apolipoprotein B levels. *Am J Hum Genet* 64:1453-1463.

Pan M, Liang J, Fisher EA and Ginsberg HN. 2000. Inhibition of translocation of nascent apolipoprotein B across the endoplasmic reticulum membrane is associated with selective inhibition of the synthesis of apolipoprotein B. *J Biol Chem* 35:27399-27405.

Patsch J. 1998. Influence of lipolysis on chylomicron clearance and HDL cholesterol levels. *Eur Heart J* 19(Suppl. H):H2-H6.

Peake PW, O'Grady S, Pussell BA and Charlesworth JA. 1997. Detection and quantification of the control proteins of the alternative pathway of complement in 3T3-L1 adipocytes. *Eur J Clin Invest* 27:922-927.

Pei W, Baron H, Müller-Myhsok B, Knoblauch H, Al-Yahyaee SA, Hui R, Wu X, Liu L, Busjahn A, Luft FC and Schuster H. 2000. Support for linkage of familial combined hyperlipidemia to chromosome 1q21-q23 in Chinese and German families. *Clin Genet* 57:29-34.

Peiris AN, Mueller RA, Smith GA, Struve MF and Kissebah AH. 1986. Splanchnic insulin metabolism in obesity. Influence of body fat distribution. *J Clin Invest* 78:1648-1657.

Pelton PD, Zhou L, Demarest KT and Burris TP. 1999. PPARγ activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. *Biochem Biophys Res Commun* 261:456-458.

Pepys M. 1981. C-reactive protein fifty years on. *Lancet* 1:653-657.

Perlmutter DH, Dinarello CA, Punsal PI and Colten HR. 1986. Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. *J Clin Invest* 78:1349-1354.

Perrey S, Ishibashi S, Yahagi N, Osuga J, Tozawa R, Yagyu H, Ohashi K, Gotoda T, Harada K, Chen Z, Iizuka Y, Shionoiri F and Yamada N. 2001. Thiazolidinedione- and tumor necrosis factor alpha-induced downregulation of peroxisome proliferator-activated receptor gamma mRNA in differentiated 3T3-L1 adipocytes. *Metabolism* 50:36-40.

Peterson J, Bihain BE and Bengtsson-Olivecrona G. 1990. Fatty acid control of lipoprotein lipase: a link between energy metabolism and lipid transport. *Proc Natl Acad Sci USA* 87:909-913.

Pignoli P, Tremoli E, Poli A, Oreste P and Paoletti R. 1986. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 74:1399-1406.

Pihlajamäki J, Rissanen J, Heikkinen S, Karjalainen L and Laakso M. 1997. Codon 54 polymorphism of the human intestinal fatty acid binding protein 2 gene is associated with dyslipidemias but not with insulin resistance in patients with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 17:1039-1044.

Pihlajamäki J, Rissanen J, Valve R, Heikkinen S, Karjalainen L and Laakso M. 1998. Different regulation of free fatty acid levels and glucose oxidation by the Trp64Arg polymorphism of the β3-adrenergic receptor gene and the promoter variant (A-3826G) of the uncoupling protein 1 gene in familial combined hyperlipidemia. *Metabolism* 47:1397-1402.

Pihlajamäki J, Karjalainen L, Karhapää P, Vauhkonen I and Laakso M. 2000a. Impaired free fatty acid suppression during hyperinsulinemia is a characteristic finding in familial combined hyperlipidemia but insulin resistance is observed only in hypertriglyceridemic patients. *Arterioscler Thromb Vasc Biol* 20:164-170.

Pihlajamäki J, Karjalainen L, Karhapää P, Vauhkonen I, Taskinen M-R, Deeb SS and Laakso M. 2000b. G-250A substitution in promoter of hepatic lipase gene is associated with dyslipidemia and insulin resistance in healthy control subjects and in members of families with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 20:1789-1795.

Pihlajamäki J, Miettinen R, Valve R, Karjalainen L, Mykkänen L, Kuusisto J, Deeb S, Auwerx J and Laakso M. 2000c. The Pro12Ala substitution in the peroxisome proliferator activated receptor gamma 2 is associated with an insulin-sensitive phenotype in families with familial combined hyperlipidemia and in nondiabetic elderly subjects with dyslipidemia. *Atherosclerosis* 151:567-574.

Pihlajamäki J, Valve R, Karjalainen L, Karhapää P, Vauhkonen I and Laakso M. 2001. The hormone-sensitive lipase gene in familial combined hyperlipidemia and insulin resistance. *Eur J Clin Invest* 31:302-308.

Pineda Torra I, Gervois P and Staels B. 1999. Peroxisome proliferator-activated receptor alpha in metabolic disease, inflammation, atherosclerosis and aging. *Curr Opin Lipidol* 10:151-159.

Plée-Gautier E, Grober J, Duolus E, Langin D and Forest C. 1996. Inhibition of hormone-sensitive lipase gene expression by cAMP phorbol esters in 3T3-F442A and BFC-1 adipocytes. *Biochem J* 318:1057-1063.

Porkka KVK, Viikari J, Rönnemaa T, Marniemi J and Åkerblom HK. 1994. Age and gender specific serum lipid and apolipoprotein fractiles of Finnish children and young adults. The Cardiovascular Risk in Young Finns Study. *Acta Paediatr* 83:838-848.

Porkka KVK, Nuotio I, Pajukanta P, Ehnholm C, Suurinkeroinen L, Syvänne M, Lehtimäki T, Lahdenkari A-T, Lahdenperä S, Ylitalo K, Antikainen M, Perola M, Raitakari OT, Kovanen P, Viikari JSA, Peltonen L and Taskinen M-R. 1997. Phenotype expression in familial combined hyperlipidemia. *Atherosclerosis* 133:245-253.

Probstfield JL, Byington RP, Egan DA, Espeland MA, Margitic SE, Riley WAJ and Furberg CD. 1993. Methodological issues facing studies of atherosclerotic change. *Circulation* 87(Suppl. II):II-74-II-81.

Pujia A, Gnasso A, Irace C, Colonna A and Mattioli PL. 1994. Common carotid arterial wall thickness in NIDDM subjects. *Diabetes Care* 17:1330-1336.

Pullinger CR, North JD, Teng BB, Rifici VA, Ronhild de Brito AE and Scott J. 1989. The apolipoprotein B gene is constitutively expressed in HepG2 cells: regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J Lipid Res* 30:1065-1077.

Qian H, Hausman GJ, Compton MM, Azain MJ, Hartzell DL and Baile CA. 1998. Leptin regulation of peroxisome proliferator-activated receptor-γ, tumor necrosis factor, and uncoupling protein-2 expression in adipose tissues. *Biochem Biophys Res Commun* 246:660-667.

Raclot T, Dauzats M and Langin D. 1998. Regulation of hormone-sensitive lipase expression by glucose in 3T3-F442A adipocytes. *Biochem Biophys Res Commun* 245:510-513.

Ramadori G, Van Damme J, Rieder H and Meyer zum Buschenfelde KH. 1988. Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 β and tumor necrosis factor-α. *Eur J Immunol* 18:1259-1264.

Randle P, Hales C, Garland P and Newsholme E. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* i:785-789.

Reaven GM. 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595-1607.

Reaven GM and Greenfield MS. 1981. Diabetic hypertriglyceridemia: evidence for three clinical syndromes. *Diabetes* 30:66-75.

Rebuffé-Scrive M, Andersson B, Olbe L and Björntorp P. 1989. Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metabolism* 38:453-458.

Reymer PWA, Gronemeyer BE, Gagné E, Miao L, Appelman EEG, Seidel JC, Kromhout D, Bijvoet SM, van de Oever K, Bruin T, Hayden MR and Kastelein JJP. 1995. A frequently occurring mutation in the lipoprotein lipase gene (Asn291Ser) contributes to the expression of familial combined hyperlipidemia. *Hum Mol Genet* 4:1543-1549.

Reynisdottir S, Ellerfeldt K, Wahrenberg H, Lithell H and Arner P. 1994. Multiple lipolysis defects in the insulin resistance (metabolic) syndrome. *J Clin Invest* 93:2590-2599.

Reynisdottir S, Eriksson M, Angelin B and Arner P. 1995. Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *J Clin Invest* 95:2161-2169.

Reynisdottir S, Angelin B, Langin D, Lithell H, Eriksson M, Holm C and Arner P. 1997. Adipose tissue lipoprotein lipase and hormone-sensitive lipase: contrasting findings in familial combined hyperlipidemia and the insulin resistance syndrome. *Arterioscler Thromb Vasc Biol* 17:2287-2292.

Reynisdottir S, Eriksson M, Angelin B and Arner P. 1998. Hormone-sensitive lipase expression in adipose tissue in FCHL. *Atherosclerosis* 138:S6.

Ribalta J, La Ville AE, Vallvé JC, Humphries S, Turner PR and Masana L. 1997. A variation in the apolipoprotein C-III gene is associated with an increased number of circulating VLDL and IDL particles in familial combined hyperlipidemia. *J Lipid Res* 38:1061-1069.

Richelsen B, Pedersen SB, Moeller-Pedersen T and Bak JF. 1991. Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E2. *Metabolism* 40:990-996.

Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W and Glass CK. 1998. Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 95:7614-7619.

Rieusset J, Andreelli F, Auboeuf D, Roques M, Vallier P, Riou J, Auwerx J, Laville M and Vidal H. 1999. Insulin acutely regulates the expression of the peroxisome proliferator-activated receptor-γ in human adipocytes. *Diabetes* 48:699-705.

Ringel J, Engeli S, Distler A and Sharma AM. 1999. Pro12Ala missense mutation of the peroxisome proliferator activated receptor γ and diabetes mellitus. *Biochem Biophys Res Commun* 254:450-453.

Ristow M, Müller-Wieland D, Pfeiffer A, Krone W and Kahn CR. 1998. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N Engl J Med* 339:953-959.

Rose HG, Kranz P, Weinstock M, Juliano J and Haft JI. 1973. Inheritance of combined hyperlipoproteinemia: evidence for a new lipoprotein phenotype. *Am J Med* 54:148-160.

Rosenstock M, Greenberg A and Rudich A. 2001. Distinct long-term regulation of glycerol and non-esterified fatty acid release by insulin and TNF-α in 3T3-L1 adipocytes. *Diabetologia* 44:55-62.

Roust LR and Jensen MD. 1993. Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes* 42:1567-1573.

Rubba P. 1978. Fractional fatty acid incorporation into human adipose tissue (FIAT) in hypertriglyceridemia. *Atherosclerosis* 29:39-42.

Rubba P and Faccenda F. 1993. Noninvasive ultrasound techniques versus angiography for monitoring drug-induced changes of the arterial walls. *Faseb J* 7:1491-1498.

Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, Faas FH, Linares E, Schaefer EJ, Schectman G, Wilt TJ and Wittes J. 1999. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 341:410-418.

Ryu JE, Howard G, Craven TE, Bond MG, Hagaman AP and Crouse JR III. 1992. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. *Stroke* 23:823-828.

S.A.G.E. 1997. Statistical Analysis for Genetic Epidemiology, Release 3.1. Computer program package available from the Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western University, Cleveland, USA.

Saleh J, Summers LK, Cianflone K, Fielding BA, Sniderman AD and Frayn KN. 1998. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. *J Lipid Res* 39:884-891.

Salomaa V, Stinson V, Kark J, Folsom A, Davis C and Wu K. 1995. Association of fibrinolytic parameters with early atherosclerosis. *Circulation* 91:284-290.

Salonen R and Salonen JT. 1990. Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. *Atherosclerosis* 81:33-40.

Salonen R and Salonen JT. 1991a. Carotid atherosclerosis in relation to systolic and diastolic blood pressure: Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann Med* 23:23-27.

Salonen R and Salonen JT. 1991b. Determinants of carotid intima-media thickness: a population-based ultrasonography study in Eastern Finnish men. *J Int Med* 229:225-231.

Salonen R, Nyyssönen K, Porkkala E, Rummukainen J, Belder R, Park J-S and Salonen JT. 1995. Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. *Circulation* 92:1758-1764.

Salonen JT and Salonen R. 1993. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation* 87(Suppl. II):II-56-II-65.

Salonen JT and Salonen R. 1994. Risk factors for carotid and femoral atherosclerosis in hypercholesterolaemic men. *J Int Med* 236:561-566.

Salonen JT and Salonen R. 1991. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. *Arterioscler Thromb* 11:1245-1249.

Saxena U, Witte LD and Goldberg IJ. 1989. Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *J Biol Chem* 264:4349-4355.

Scantlebury T, Maslowska M and Cianflone K. 1998. Chylomicron-specific enhancement of acylation stimulating protein and precursor protein C3 production in differentiated human adipocytes. *J Biol Chem* 273:20903-20909.

Schaffer J and Lodish H. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79:427-436.

Scherer PE, Williams S, Fogliano M, Baldini G and Lodish HF. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746-26749.

Schneeman BO, Kotite L, Todd KM and Havel RJ. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc Natl Acad Sci USA* 90:2069-2073.

Schoonjans K, Martin G, Staels B and Auwerx J. 1997. Peroxisome proliferator activated receptors, orphans with ligands and functions. *Curr Opin Lipidol* 8:159-166.

Schoonjans K, Peinado-Onsurbe J, Lefebvre A-M, Heyman RA, Briggs M, Deeb S, Staels B and Auwerx J. 1996b. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 15:5336-5348.

Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T and Auwerx J. 1995. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J Biol Chem* 270:19269-19276.

Seifert PS, Hugo F, Tranum-Jensen J, Zahringer U, Muhly M and Bhakdi S. 1990. Isolation and characterization of a complement-activating lipid extracted from human atherosclerotic lesions. *J Exp Med* 172:547-557.

Selzer RH, Hodis HN, Kwong-Fu H, Mack WJ, Lee PL, Liu C and Liu C. 1994. Evaluation of computerized edge tracking for quantifying intima-media thickness of the common carotid artery from B-mode ultrasound images. *Atherosclerosis* 111:1-11.

Sharrett AR, Chambless LE, Heiss G, Paton CC and Patsch W. 1995. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. *Arterioscler Thromb Vasc Biol* 15:2122-2129.

Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH and Packard CJ for the West of Scotland Coronary Prevention Study Group. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 333:1301-1307.

Skoglund-Andersson C, Tang R, Bond MG, de Faire U, Hamsten A and Karpe F. 1999. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. *Arterioscler Thromb Vasc Biol* 19:2422-2430.

Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B and Kwiterovich PO Jr. 1980. Association of coronary atherosclerosis with hyperapobetalipoproteinemia [increased protein but normal cholesterol levels in human plasma low density (β) lipoproteins]. *Proc Natl Acad Sci USA* 77:604-608.

Sniderman AD, Wolfson C, Teng B, Franklin FA, Bachorik PS and Kwiterovich PO Jr. 1982. Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. *Ann Intern Med* 97:833-839.

Sniderman A, Teng B, Genest J, Cianflone K, Wacholder S and Kwiterovich P Jr. 1985. Familial aggregation and early expression of hyperapobetalipoproteinemia. *Am J Cardiol* 55:291-295.

Sniderman A, Brown BG, Stewart BF and Cianflone K. 1992. From familial combined hyperlipidemia to hyperapoB: unravelling the overproduction of hepatic apolipoprotein B. *Curr Opin Lipidol* 3:137-142.

Sniderman AD, Maslowska M and Cianflone K. 2000. Of mice and men (and women) and the acylation-stimulating protein pathway. *Curr Opin Lipidol* 11:291-296.

Solberg L and Eggen D. 1971. Localization and sequence of development of atherosclerotic lesions in the carotid and vertebral arteries. *Circulation* 43:711-724.

Souza SC, de Vargas LM, Yamamoto MT, Lien P, Franciosa MD, Moss LG and Greenberg AS. 1998. Overexpression of perilipin A and B blocks the ability of tumor necrosis factor-α to increase lipolysis in 3T3-L1 adipocytes. *J Biol Chem* 273:24665-24669.

Spiegelman BM. 1998. PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507-514.

Spiegelman BM and Flier JS. 1996. Adipogenesis and obesity: rounding out the big picture. *Cell* 87:377-389.

Steiner G, Haynes FJ, Yoshino G and Vranic M. 1984. Hyperinsulinemia and in vivo very-low-density lipoprotein-triglyceride kinetics. *Am J Physiol* 246:E187-E192.

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS and Lazar MA. 2001. The hormone resistin links obesity to diabetes. *Nature* 409:307-312.

Stremmel W, Strohmeyer G, Borchard F, Kochwa S and Berk PD. 1985. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc Natl Acad Sci USA* 82:4-8.

Strålfors P and Honnor RC. 1989. Insulin-induced dephosphorylation of hormone-sensitive lipase. Correlation with lipolysis and cAMP-dependent protein kinase activity. *Eur J Biochem* 182:379-385.

Stumvoll M, Wahl HG, Loblein K, Becker R, Machicao F, Jacob S and Haring H. 2001. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes* 50:876-881.

Sumida M, Sekiya K, Okuda H, Tanaka Y and Shiosaka T. 1990. Inhibitory effect of tumor necrosis factor on gene expression of hormone-sensitive lipase in 3T3-L1 adipocytes. *J Biochem* 107:1-2.

Suzuki H, Kawarabayasi Y, Kondo J, Abe T, Nishikawa K, Kimura S, Hashimoto T and Yamamoto T. 1990. Structure and regulation of rat long-chain acyl-CoA synthetase. *J Biol Chem* 265:8681-8685.

Svedberg J, Björntorp P, Smith U and Lönnroth P. 1990. Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes* 39:570-574.

Sörensen H and Dissing J. 1975. Association between the $C3^F$ gene and atherosclerotic vascular diseases. *Hum Hered* 25:279-283.

Tahvanainen E, Jauhiainen M, Funke H, Vartiainen E, Sundvall J and Ehnholm C. 1999. Serum phospholipid transfer protein activity and genetic variation of the PLTP gene. *Atherosclerosis* 146:107-115.

Tahvanainen E, Pajukanta P, Porkka K, Nieminen S, Ikävalko L, Nuotio I, Taskinen M-R, Peltonen L and Ehnholm C. 1998. Haplotypes of the ApoA-I/C-III/A-IV gene cluster and familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 18:1810-1817.

Takahashi S, Kawarabayasi Y, Nakai T, Sakai J and Yamamoto T. 1992. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc Natl Acad Sci USA* 89:9252-9256.

Tao Y, Cianflone K, Sniderman AD, Colby-Germinario SP and Germinario RJ. 1997. Acylation-stimulating protein (ASP) regulates glucose transport in the rat L6 muscle cell line. *Biochim Biophys Acta* 1344:221-229.

Taskinen M-R. 1995. Insulin resistance and lipoprotein metabolism. *Curr Opin Lipidol* 6:153-160.

Taskinen M-R, Kuusi T, Helve E, Nikkilä E and Yki-Järvinen H. 1988. Insulin therapy induces antiatherogenic changes of serum lipoproteins in noninsulin-dependent diabetes. *Arterioscler* 8:168-177.

Tato F, Vega GL, Tall AR and Grundy SM. 1995. Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 15:112-120.

Tell GS, Howard G and McKinney WM. 1989. Risk factors for site specific extracranial carotid artery plaque distribution as measured by B-mode ultrasound. *J Clin Epidemiol* 42:551-559.

Temelkova-Kurktschiev T, Koehler C, Schaper F, Henkel E, Hahnefeld A, Fuecker K, Siegert G and Hanefeld M. 1998. Relationship between fasting plasma glucose, atherosclerosis risk factors and carotid intima media thickness in non-diabetic individuals. *Diabetologia* 41:706-712.

Teng B, Sniderman AD, Soutar AK and Thompson GR. 1986. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J Clin Invest* 77:663-672

Teng B, Forse A, Rodriguez A and Sniderman A. 1988. Adipose tissue glyceride synthesis in patients with hyperapobetalipoproteinemia. *Can J Physiol Pharmacol* 66:239-242.

The BIP Study Group. 2000. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. The Bezafibrate Infarction Prevention (BIP) Study. *Circulation* 102:21-27.

The Scandinavian Simvastatin Survival Study Group. 1994. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 344:1383-1389.

Tobey TA, Greenfield M, Kraemer F and Reaven GM. 1981. Relationship between insulin resistance, insulin secretion, very low density lipoprotein kinetics, and plasma triglyceride levels in normotriglyceridemic man. *Metabolism* 30:165-171.

Tontonoz P, Nagy L, Alvarez J, Thomazy V and Evans R. 1998. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 93:241-252.

Tornqvist H, Björgell P, Krabisch L and Belfrage P. 1978. Monoacylmonoalkylglycerol as a substrate for diacylglycerol hydrolase activity in adipose tissue. *J Lipid Res* 19:654-656.

Toyota Y, Yamamura T, Miyake Y and Yamamoto A. 1999. Low density lipoprotein (LDL) binding affinity for the LDL receptor in hyperlipoproteinemia. *Atherosclerosis* 147:77-86.

Tybjærg-Hansen A, Nordestgaard BG, Gerdes LU, Færgeman O and Humphries SE. 1993. Genetic markers in the apo AI-CIII-AIV gene cluster for combined hyperlipidemia, hypertriglyceridemia, and predisposition to atherosclerosis. *Atherosclerosis* 100:157-169.

Uza G, Cristea A and Cucuianu M. 1982. Increased level of the complement C3 protein in endogenous hypertriglyceridemia. *J Clin Lab Immunol* 8:101-105.

Vakkilainen J, Porkka KVK, Nuotio I, Pajukanta P, Suurinkeroinen L, Ylitalo K, Viikari JSA, Ehnholm C and Taskinen M-R. 1998. Glucose intolerance in familial combined hyperlipidaemia. *Eur J Clin Invest* 28:24-32.

Valve R, Sivenius K, Miettinen R, Pihlajamäki J, Rissanen A, Deeb SS, Auwerx J, Uusitupa M and Laakso M. 1999. Two polymorphisms in the peroxisome proliferator-activated receptor-γ gene are associated with severe overweight among obese women. *J Clin Endocrinol Metab* 84:3708-3712.

Van Harmelen V, Reynisdottir S, Cianflone K, Degerman E, Hoffstedt J, Nilsell K, Sniderman A and Arner P. 1999. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J Biol Chem* 274:18243-18251.

Van der Kallen CJH, Bouwman FG, van de Hulst RWJ, Boeckx WD and de Bruin TWA. 2000. Adipose tissue insulin resistance in familial combined hyperlipidemia (FCH), but not type 2 diabetes mellitus (DM2). Paper presented at the the XIIth International Symposium on Atherosclerosis, Stockholm, Sweden.

Vartiainen E, Puska P, Jousilahti P, Korhonen HJ, Tuomilehto J and Nissinen A. 1994. Twenty-year trends in coronary risk factors in North Karelia and in other areas of Finland. *Int J Epidemiol* 23:495-504.

Veerkamp M, de Graaf J, Bredie S, Hendriks J and Stalenhoef A. 2001. Diagnosis of familial combined hyperlipidaemia (FCH) based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. *Eur J Clin Invest* 31:20.

Venkatesan S, Cullen P, Pacy P, Halliday D and Scott J. 1993. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler Thromb* 13:1110-1118.

Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF and Flier JS. 1997. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99:2416-2422.

Vlaicu R, Niculescu F, Rus HG and Cristea A. 1985a. Immunohistochemical localization of the terminal C5b-9 complement complex in human aortic fibrous plaque. *Atherosclerosis* 57:163-177.

Vlaicu R, Rus HG, Niculescu F and Cristea A. 1985b. Immunoglobulins and complement components in human aortic atherosclerotic intima. *Atherosclerosis* 55:35-50.

Vogelberg KH, Gries FA and Moschinski D. 1980. Hepatic production of VLDL-triglycerides. Dependence of portal substrate and insulin concentration. *Horm Metab Res* 12:688-694.

Vosper H, Patel L, Khoudoli GA, Hill A, Macphee CH, Pinto I, Smith SA, Suckling KE, Wolf CR and Palmer CNA. 2001. PPAR-delta promotes lipid accumulation in macrophages. Paper presented at the meeting "PPARs: from basic science to clinical applications", Florence, Italy.

Vrtovec B, Keber I, Gadzijev A, Bardorfer I and Keber D. 1999. Carotid intima-media thickness of young coronary patients. *Coronary Artery Dis* 10:407-411.

Walsh M, Sniderman A, Cianflone K, Vu H, Rodriguez M and Forse R. 1989. The effects of ASP on the adipocyte of the morbidly obese. *J Surg Res* 46:470-473.

Wang M-Y, Lee Y and Unger RH. 1999. Novel form of lipolysis induced by leptin. *J Biol Chem* 274:17541-17544.

Way JM, Görgün CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver WR Jr, Willson TM, Kliewer SA, Hotamisligil GS. 2001. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor γ agonists. *J Biol Chem* 276:25651-25653.

Wendelhag I, Gustavsson T, Suurkula M, Berglund G and Wikstrand J. 1991. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol* 11:567-577.

Wendelhag I, Wiklund O and Wikstrand J. 1992. Arterial wall thickness in familial hypercholesterolemia. Ultrasound measurement of intima-media thickness in the common carotid artery. *Arterioscler Thromb* 12:70-77.

Wetsel RA, Kildsgaard J, Zsigmond E, Liao W and Chan L. 1999. Genetic deficiency of acylation stimulating protein (ASP(C3ades-Arg)) does not cause hyperapobetalipoproteinemia in mice. *J Biol Chem* 274:19429-19433.

Weyer C and Pratley RE. 1999. Fasting and postprandial plasma concentrations of acylation-stimulating protein (ASP) in lean and obese Pima Indians compared to Caucasians. *Obes Res* 7:444-452.

Weyer C, Tataranni PA and Pratley RE. 2000. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care* 23:779-785.

White RTD, Damm D, Hancock N, Rosen BS, Lowell BB, Usher P, Flier JS and Spiegelman BS. 1992. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. *J Biol Chem* 267:9210-9213.

Wieland E, Dorweiler B, Bonitz U, Lieser S, Walev I and Bhakdi S. 1999. Complement activation by oxidatively modified low-density lipoproteins. *Eur J Clin Invest* 29:835-841.

Wijsman EM, Brunzell JD, Jarvik GP, Austin MA, Motulsky AG and Deeb SS. 1998. Evidence against linkage of familial combined hyperlipidemia to the apolipoprotein AI-CIII-AIV gene complex. *Arterioscler Thromb Vasc Biol* 18:215-226.

Willnow T. 1997. Mechanisms of hepatic chylomicron remnant clearance. *Diabetic Med* 14:S75-S80.

Wofford JL, Kahl FR, Howard GR, McKinney WM, Toole JF and Crouse JRI. 1991. Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. *Arterioscler Thromb* 11:1786-1794.

Wojciechowski AP, Farrall M, Cullen P, Wilson TM, Bayliss JD, Farren B, Griffin BA, Caslake MJ, Packard CJ, Shepherd J, Thakker R and Scott J. 1991. Familial combined hyperlipidemia linked to the apolipoprotein AI-CIII-AIV gene cluster on chromosome 11q23-q24. *Nature* 349:161-164.

Wong M, Edelstein J, Wollman J and Bond MG. 1993. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. *Arterioscler Thromb* 13:482-486.

Wood SL, Emmison N, Borthwick AC and Yeaman SJ. 1993. The protein phosphatases responsible for dephosphorylation of hormone-sensitive lipase in isolated rat adipocytes. *Biochem J* 295:531-535.

Wu X, Sakata N, Dixon J and Ginsberg HN. 1994. Exogenous VLDL stimulates apolipoprotein B secretion from HepG2 cells by both pre- and posttranslational mechanisms. *J Lipid Res* 35:1200-1210.

Xu CF, Talmud P, Schuster H, Houlston R, Miller G and Humphries S. 1994. Association between genetic variation at the APO AI-CIII-AIV gene cluster and familial combined hyperlipidaemia. *Clin Genet* 46:385-397.

Yamasaki Y, Kawamori R, Matsushima H, Nishizawa H, Kodama M, Kubota M, Kajimoto Y and Kamada T. 1995. Asymptomatic hyperglycaemia is associated with increased intimal plus medial thickness of the carotid artery. *Diabetologia* 38:585-591.

Yang W-S, Nevin DN, Peng R, Brunzell JD and Deeb SS. 1995. A mutation in the promoter of the lipoprotein lipase (LPL) gene in a patient with familial combined hyperlipidemia and low LPL activity. *Proc Natl Acad Sci USA* 92:4462-4466.

Yasruel Z, Cianflone K, Sniderman A, Rosenbloom M, Walsh M and Rodriguez M. 1991. Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids* 26:495-499.

Young W, Gofman JW and Tandy R. 1960. The quantitation of atherosclerosis. III. The extent of correlation of degrees of atherosclerosis within and between the coronary and cerebral vascular beds. *Am J Cardiol* 6:300-308.

Yudkin JS, Stehouwer C, Emeis J and Coppack S. 1999. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction. A potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19:972-978.

Zanchetti A, Bond MG, Hennig M, Neiss A, Mancia G, Dal Palù C, Hansson L, Magnani B, Rahn K, Reid J, Rodicio J, Safar M, Eckes L and Ravinetto R on behalf of the ELSA investigators. 1998. Risk factors associated with alterations in carotid intima-media thickness in hypertension: baseline data from the European Lacidipine Study on Atherosclerosis. *J Hypertens* 16:949-961.

Zeghari N, Vidal H, Younsi M, Ziegler O, Drouin P and Donner M. 2000. Adipocyte membrane phospholipids and PPAR-γ expression in obese women: relationship to hyperinsulinemia. *Am J Physiol Endocrinol Metab* 279:E736-E743.

Zhang X-J, Cianflone K, Genest J and Sniderman A. 1998. Plasma acylation stimulating protein (ASP) as a predictor of impaired cellular biological response to ASP in patients with hyperapoB. *Eur J Clin Invest* 28:730-739.

Östman J, Arner P, Engfeldt P and Kager L. 1979. Regional differences in the control of lipolysis in human adipose tissue. *Metabolism* 28:1198-1205.