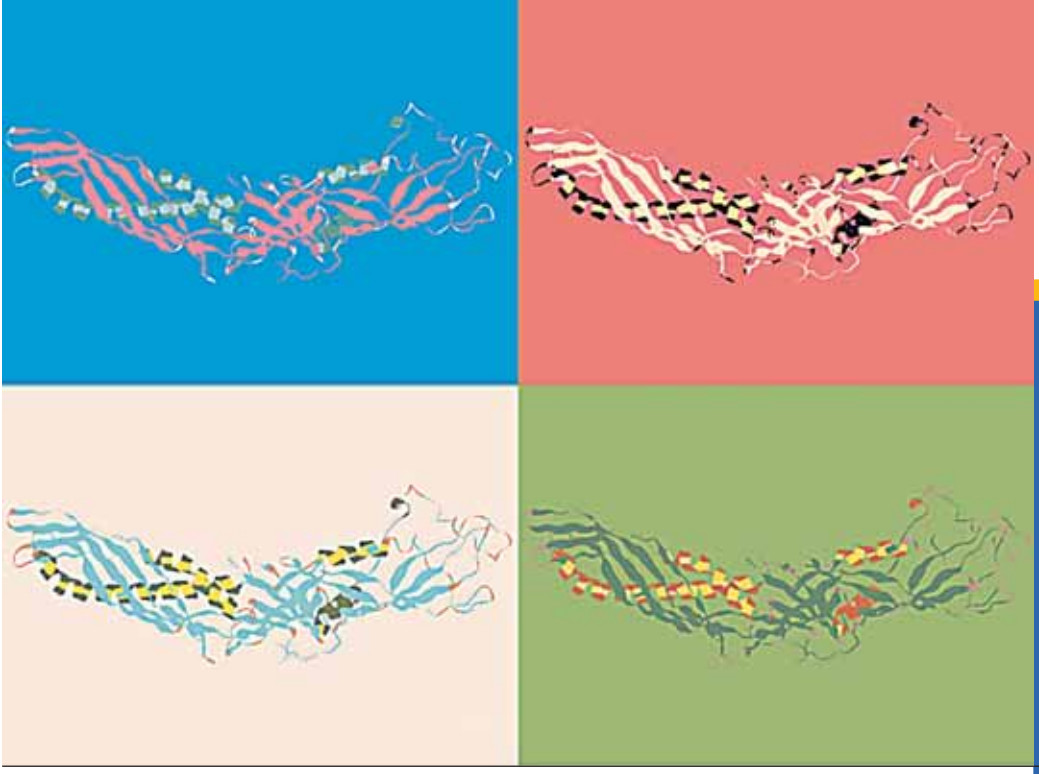

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THE HIGH- AND LOW-ACTIVITY FORMS OF HUMAN PLASMA PHOSPHOLIPID TRANSFER PROTEIN (PLTP)

A C A D E M I C D I S S E R T A T I O N

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Cover: The structural model of human PLTP. Modeling was performed using the human PLTP sequence in the Swiss Prot database (accession number P55058) and the GENO3D modeling program based on the X-ray structure of human BPI. Artistic adaptation of the image by Pekka Jänis.

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ABSTRACT

Plasma phospholipid transfer protein (PLTP) plays a crucial role in high-density lipoprotein (HDL) metabolism and reverse cholesterol transport (RCT). It mediates the generation of preβ-HDL particles, enhances the cholesterol efflux from peripheral cells to preβ-HDL, and metabolically maintains the plasma HDL levels by facilitating the transfer of post-lipolytic surface remnants of triglyceride-rich lipoproteins to HDL. In addition to the antiatherogenic properties, recent findings indicate that PLTP has also proatherogenic characteristics, and that these opposite characteristics of PLTP are dependent on the site of PLTP expression and action. In human plasma, PLTP exists in a high-activity (HA-PLTP) and a low-activity form (LA-PLTP), which are associated with macromolecular complexes of different size and composition.

The aims of this thesis were to isolate the two PLTP forms from human plasma, to characterize the molecular complexes in which the HA- and LA-PLTP reside, and to study the interactions of the PLTP forms with apolipoproteins (apo) and the ability of apolipoproteins to regulate PLTP activity. In addition, we aimed to study the distribution of the two PLTP forms in a Finnish population sample as well as to find possible regulatory factors for PLTP by investigating the influence of lipid and glucose metabolism on the balance between the HA- and LA-PLTP. For these purposes, an enzyme-linked immunosorbent assay (ELISA) capable of determining the serum total PLTP concentration and quantitating the two PLTP forms separately was developed.

In this thesis, it was demonstrated that the HA-PLTP isolated from human plasma copurified with apoE, whereas the LA-PLTP formed a complex with apoA-I. The separation of these two PLTP forms was carried out by a dextran sulfate ($DxSO_4$)-$CaCl_2$ precipitation of plasma samples before the mass determination. A similar immunoreactivity of the two PLTP forms in the ELISA could be reached after a partial sample denaturation by SDS. Among normolipidemic Finnish individuals, the mean PLTP mass was 6.6 ± 1.5 mg/l and the mean PLTP activity 6.6 ± 1.7 μmol/ml/h. Of the serum PLTP concentration, almost 50% represented HA-PLTP. The results indicate that plasma HDL levels could regulate PLTP concentration, while PLTP activity could be regulated by plasma triglyceride-rich very low-density lipoprotein (VLDL) concentration. Furthermore, new evidence is presented that

PLTP could also play a role in glucose metabolism. Finally, both PLTP forms were found to interact with apoA-I, apoA-IV, and apoE. In addition, both apoE and apoA-IV, but not apoA-I, were capable of activating the LA-PLTP. These findings suggest that the distribution of the HA- and LA-PLTP in human plasma is subject to dynamic regulation by apolipoproteins.

Keywords: atherosclerosis, HDL metabolism, reverse cholesterol transport, apolipoprotein, phospholipid transfer protein

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TIIVISTELMÄ

Plasman fosfolipidin siirtäjäproteiinilla (PLTP) on tärkeä merkitys high-density-lipoproteiinien (HDL) aineenvaihdunnassa ja kolesterolin takaisinkuljetus-järjestelmässä. PLTP kykenee muodostamaan preβ-HDL partikkeleita, lisäämään kolesterolin ulosvirtausta soluista näille partikkeleille, sekä ylläpitämään plasman HDL-tasoja kuljettamalla lipolyysin yhteydessä muodostuvia triglyseridi-pitoisten partikkeleiden fosfolipidijäämiä HDL-partikkeleille. Ateroskleroosin kehittymiseltä suojaavien ominaisuuksien lisäksi tutkimukset ovat osoittaneet, että PLTP:lla on myös ateroskleroosia edistäviä ominaisuuksia, ja että nämä vastakkaiset ominaisuudet ovat riippuvaisia PLTP:n ilmentymis- ja toimintaympäristöstä elimistössä. Ihmisen verenkierrossa PLTP esiintyy aktiivisessa (high-activity, HA-PLTP) ja matala-aktiivisessa muodossa (low-activity, LA-PLTP), jotka molemmat ovat liittyneinä kooltaan ja koostumukseltaan toisistaan eroaviin makromolekulaarisiin rakenteisiin.

Tämän väitöskirjatyön tavoitteena oli eristää nämä kaksi PLTP-muotoa ihmisen verenkierrosta, karakterisoida molekyylirakenteet, joihin HA- ja LA-PLTP ovat liittyneinä, sekä tutkia PLTP-muotojen vuorovaikutusta apolipoproteiinien (apo) kanssa ja näiden kykyä säädellä PLTP:n aktiivisuutta. Lisäksi tavoitteena oli tutkia PLTP-muotojen esiintymistä suomalaisessa näyteaineistossa, sekä löytää mahdollisia PLTP:n säätelytekijöitä tutkimalla sekä lipidi- että glukoosiaineenvaihdunnan vaikutusta HA- ja LA-PLTP:n väliseen tasapainoon. Tähän tarkoitukseen kehitettiin entsyymi-immunokemiallinen menetelmä (ELISA) PLTP:n kokonaispitoisuuden ja kahden eri PLTP-muodon pitoisuuksien määrittämiseksi.

Tässä väitöskirjatyössä osoitettiin, että ihmisen verenkierrosta eristetty HA-PLTP -molekyylikompleksi sisältää myös apoE:tä, kun taas LA-PLTP muodostaa kompleksin apoA-I:n kanssa. Nämä kaksi PLTP-muotoa erotettiin toisistaan saostamalla plasmanäytteet dekstraanisulfaatti ($DxSO_4$)-$CaCl_2$:lla ennen pitoisuuksien määrittämistä. PLTP:n eri muotojen havaittiin reagoivan yhteneväisesti ELISA-menetelmässä, jos seeruminäytteet ensin osittain denaturoitiin SDS:lla. Keskimääräinen PLTP-pitoisuus suomalaisessa näyteaineistossa oli 6.6 ± 1.5 mg/l ja keskimääräinen PLTP-aktiivisuus 6.6 ± 1.7 μmol/ml/h. HA-PLTP:n osuus kokonaispitoisuudesta oli lähes 50 %. Tämän tutkimuksen mukaan verenkierron HDL-tasot saat-

tavat säädellä PLTP:n pitoisuutta, kun taas PLTP-aktiivisuus näyttäisi liittyvän verenkierron triglyseridi-rikkaiden very low-density-lipoproteiinien (VLDL) pitoisuuteen. Lisäksi havaittiin, että PLTP saattaa vaikuttaa myös glukoosiaineenvaihduntaan. Lopuksi osoitettiin, että molemmat PLTP-muodot vuorovaikuttavat apoA-I:n, apoA-IV:n ja apoE:n kanssa, ja sekä apoE että apoA-IV, muttei apoA-I, pystyvät aktivoimaan LA-PLTP:n. Perustuen tämän väitöskirjatyön tuloksiin apolipoproteiinit säätelevät dynaamisesti HA- ja LA-PLTP:n jakaumaa ihmisen verenkierrossa.

Avainsanat: ateroskleroosi, HDL:n aineenvaihdunta, käänteinen kolesterolin kuljetusjärjestelmä, apolipoproteiini, fosfolipidin siirtäjäproteiini

CONTENTS

ABBREVIATIONS

ABC	adenosine triphosphate-binding cassette transporter
AD	Alzheimer's disease
Apo	apolipoprotein
BMI	body mass index
BPI	bactericidal permeability-increasing protein
C	cholesterol
C/EBP	CCAAT/enhancer-binding protein
CAD	coronary artery disease
cDNA	complementary deoxyribonucleic acid
CE	cholesteryl ester
CETP	cholesteryl ester transfer protein
CM	chylomicron
CMC	critical micellar concentration
CRP	C-reactive protein
CSF	cerebrospinal fluid
$DxSO_4$	dextran sulfate
ELISA	enzyme-linked immunosorbent assay
FXR	farnesoid X-activated receptor
HA-PLTP	high-activity form of phospholipid transfer protein
HDL	high-density lipoprotein
HL	hepatic lipase
HOMA IR	homeostasis model assessment for insulin resistance
H-S	heparin-sepharose
IDL	intermediate-density lipoprotein
kDa	kilodalton

LA-PLTP	low-activity form of phospholipid transfer protein
LBP	lipopolysaccharide-binding protein
LCAT	lecithin:cholesterol acyltransferase
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
LPL	lipoprotein lipase
LPS	lipopolysaccharide
LRP	low-density lipoprotein receptor-related protein
LT/LBP	lipid transfer/lipopolysaccharide-binding protein family
LXR	liver X receptor
mAb	monoclonal antibody
mRNA	messenger ribonucleic acid
NF-kB	nuclear factor kappa immunoglobulin enhancer-binding protein
pAb	polyclonal antibody
PAF	platelet-activating factor
PAF-AH	platelet-activating factor acetyl hydrolase
PL	phospholipid
PLA_2	phospholipase A_2
PLTP	phospholipid transfer protein
$PLTP_{endo}$	endogenous PLTP activity assay
$PLTP_{exo}$	exogenous PLTP activity assay
PON1	paraoxonase-1
PPAR	peroxisome proliferator activated receptor
RCT	reverse cholesterol transport
R-PLTP	recombinant phospholipid transfer protein
SAA	serum amyloid A
SDS-PAGE	sodium dodecylsulfate-polyacrylamide gel electrophoresis

SR-BI	scavenger receptor class B type I
SREBP	sterol regulatory element binding protein
TC	total cholesterol
TG	triglyceride
TGRL	triglyceride-rich lipoprotein
UC	unesterified cholesterol
VLDL	very low-density lipoprotein
WHR	waist-to-hip circumference ratio

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals. Please note that the maiden name Kärkkäinen has been used in the articles I and II.

I Kärkkäinen, M., Oka, T., Olkkonen, V.M., Metso, J., Hattori, H., Jauhiainen, M. & Ehnholm, C. (2002). Isolation and partial characterization of the inactive and active forms of human plasma phospholipid transfer protein (PLTP). *J Biol Chem,* 277:15413-15418.

II Siggins, S.*, Kärkkäinen, M.*, Tenhunen, J., Metso, J., Tahvanainen, E., Olkkonen, V.M., Jauhiainen, M. & Ehnholm, C. (2004). Quantitation of the active and low-active forms of human plasma phospholipid transfer protein by ELISA. *J Lipid Res,* 45**:**387-395.

III Jänis, M.T., Siggins, S., Tahvanainen, E., Vikstedt, R., Silander, K., Metso, J., Aromaa, A., Taskinen, M.R., Olkkonen, V.M., Jauhiainen, M. & Ehnholm, C. (2004). Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample. *J Lipid Res,* 45:2303-2309.

IV Jänis, M.T., Metso, J., Lankinen, H., Strandin, T., Olkkonen, V.M., Rye, K.A., Jauhiainen, M. & Ehnholm, C. (2005). Apolipoprotein E activates the low-activity form of human phospholipid transfer protein. *Biochem Biophys Res Commun,* 331:333-340.

*the authors contributed equally to the study

1 INTRODUCTION

Atherosclerotic cardiovascular diseases are the major causes of death in western societies. The prevalence of coronary artery diseases (CAD), however, increases rapidly also in developing countries and formerly socialist economies of Europe. Thus, within the next 15 years, CAD are expected to be the main cause of death globally (Murray & Lopez, 1997).

Also in Finland, despite a considerable decrease in serum cholesterol levels since the 1980's (Puska et al., 1995), a large part of the population has an increased risk of cardiovascular diseases. This is mainly due to a rising incidence of diabetes, obesity, and metabolic syndrome. Although the serum high-density lipoprotein (HDL) cholesterol levels have increased among a part of the Finnish population, about 30% of the population still has too high (≥6.5 mmol/l) serum total cholesterol concentration (Aromaa & Koskinen, 2002).

Lipid-modifying drugs, especially the statins, which inhibit an important rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, have successfully been used for lowering plasma low-density lipoprotein (LDL) cholesterol levels in several different large-scale clinical trials (Heart Protection Study Collaborative, 2002; Ong, 2002). In addition to cholesterol lowering effect, statins exert many other beneficial effects such as enhancing the stability of atherosclerotic plaques, decreasing oxidative stress, and reducing vascular inflammation (Takemoto & Liao, 2001). Statin monotherapy, however, may have its limits in the inhibition of the development of CAD (Waters et al., 2004). Therefore, the treatment of CAD has recently been extended and targeted to other risk factors, like low serum HDL cholesterol level (Linsel-Nitschke & Tall, 2005).

The protective role of HDL is well established and was reported for the first time already in the 1950's (Barr et al., 1951; Nikkilä, 1953). Several epidemiological and clinical studies have shown the inverse relationship between serum HDL cholesterol and prevalence of CAD (Assmann & Schulte, 1992; Castelli et al., 1986; Jacobs et al., 1990; Kannel, 1983; Kannel et al., 1979). This protective effect of HDL is independent of other risk factors and linked to a process called reverse cholesterol transport (RCT), i.e., the ability of HDL to transport cholesterol from peripheral tissues to the liver for excretion (Fielding & Fielding, 1995).

One important regulator of HDL metabolism is phospholipid transfer protein, PLTP, which exists in high- and low-activity forms in the circulation. In this study, one major focus has been to investigate the distribution of these two PLTP forms in a Finnish population sample. For this purpose methods were developed to isolate these

two PLTP forms and determine their concentrations in human plasma. Regulation mechanisms of PLTP activity and mass distribution were assessed by studying the interactions of PLTP with different apolipoproteins (apo) and correlating PLTP concentrations with different lipid and carbohydrate parameters. Finally, activation of the low-activity form of PLTP was studied to elucidate the possible metabolic roles of the two PLTP forms.

Both human and animal studies indicate that in the future, PLTP could serve as a target molecule in the prevention and treatment of atherosclerotic cardiovascular diseases. Understanding the regulatory mechanisms of PLTP activity and concentration may lead to new therapeutic strategies selectively targeted to either of the two PLTP forms.

2 REVIEW OF THE LITERATURE

2.1 Atherosclerosis

Atherosclerosis is a multifactorial, systemic disease of the large and middle-sized arteries that begins typically in later childhood yet being asymptomatic. It is characterized by the accumulation of cholesterol, calcium, cell-waste products, and other substances into focal areas of the innermost layer of an arterial wall, leading to a local inflammation (Stary et al., 1995).

The foamy core region of a lesion consists of extracellular lipid droplets and lipid-laden macrophages, also known as foam cells, described by the Greek word 'athero' meaning porridge. This core region is surrounded by a protective fibrous cap consisting of smooth muscle cells, collagen, and calcium that increases the arterial wall stiffness. Immune cells like T cells, monocytes, and mast cells infiltrate the lesion and produce inflammatory cytokines and proteolytic enzymes further accelerating the inflammatory process and causing weakening of the fibrous cap (Frostegård et al., 1999; Kovanen et al., 1995; Lindstedt & Kovanen, 2004). A gradual lesion development can finally lead to a slowly progressive occlusion of the vessel lumen and to acute vascular events like strokes, heart attacks or peripheral vascular diseases. The conversion of a stable disease to a life-threatening condition is usually due to a plaque disruption concurrent with thrombosis (Davies, 1996; Falk et al., 1995).

2.1.1 Risk factors for atherosclerosis

Several retrospective and prospective autopsy studies have shown the impact of both genetic and environmental risk factors on the development of atherosclerotic cardiovascular diseases. Among the genetic risk factors, including family history of CAD, male gender, age, hypertension, obesity, and diabetes, elevated levels of serum total and LDL cholesterol as well as decreased levels of HDL cholesterol are probably the most important ones. Personal attributes like physical inactivity, high fat diet, and cigarette smoking have also been shown to associate with increased risk of cardiovascular diseases in several epidemiologic studies as reviewed by Solberg and Strong (1983). In addition, infection and chronic inflammation caused both by bacteria and viruses play a role in the pathogenesis of atherosclerosis (Leinonen & Saikku, 2002). Recently, the impact of air pollution on vascular inflammation and atherogenesis has been shown in an animal model (Sun et al., 2005).

2.1.2 Development and progression of the disease

Atherosclerotic vascular diseases have been studied for decades but only in recent years a remarkable progress in the research has been reached with the help of modern molecular biology approaches, research in animal models, and epidemiological studies of the disese. The mechanisms underlying the origin and development of the disease have not been fully clarified but an injury to the endothelium and the retention of atherogenic lipoproteins into subendothelial space play a central role in disease progression (Ross, 1993; Williams & Tabas, 1995). During recent years, atherosclerosis is generally considered as an inflammatory disease (Hansson, 2005).

Increased total serum cholesterol in patients with hypercholesterolemia leads to infiltration and retention of LDL particles into the arterial intima, the innermost layer of an artery. Proteoglycans in the subendothelium play a critical role in the retention of apoB-containing lipoproteins (Skalen et al., 2002; Williams & Tabas, 1995). Aggregation, fusion, and oxidative and enzymatic modifications of LDL particles lead to release of inflammatory phospholipids (Leitinger, 2003) and activation of endothelial cells causing increased expression of several leukocyte adhesion molecules (Cybulsky & Gimbrone, 1991; Nakashima et al., 1998). Adhesion of platelets to the endothelium may also stimulate the expression of adhesion molecules and secretion of chemokines promoting the interaction of leukocytes with the activated endothelial cells (Massberg et al., 2002). Monocytes, lymphocytes, and other blood cells attach and migrate through the endothelial cell junctions into the subendothelial space where monocytes eventually differentiate into macrophages (Smith et al., 1995). Macrophages express a broad range of scavenger receptors and Toll-like receptors, which are involved in pathogen recognition, phagocytosis of apoptotic cells, and induction of adaptive immunity (Janeway & Medzhitov, 2002; Peiser et al., 2002). Scavenger receptors bind, among other polyanionic ligands, modified LDL particles, which results in the accumulation of excess intracellular cholesterol and formation of foam cells, which are the early signs of the developing lesion. Scavenger receptors class A and CD36 are the principal mediators of modified LDL uptake (Kunjathoor et al., 2002; Suzuki et al., 1997). Toll-like receptors, for their part, can induce cell activation leading to the secretion of chemokines and inflammatory cytokines as well as the release of proteases, reactive oxygen species, and nitrogen radicals. Ultimately, this leads to enhanced inflammation and tissue damage mediated in addition to macrophages by several other immune cells (Hansson, 2001).

Chronic inflammation in an arterial wall causes changes in the concentrations of several plasma proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, and albumin, accompanied by the plasma lipoproteins (Gabay &

Kushner, 1999; Khovidhunkit et al., 2004). The plasma triglyceride (TG) and very low-density lipoprotein (VLDL) levels increase owing to elevated hepatic fatty acid synthesis (Hardardottir et al., 1995), accelerated adipose tissue lipolysis (Greenberg et al., 2001; Zhang et al., 2002), decreased VLDL clearance (Feingold et al., 1992; Tripp et al., 1993), and suppressed fatty acid β-oxidation and ketogenesis (Beylot et al., 1989; Takeyama et al., 1990). Increasing evidence suggests a direct role for triglyceride-rich lipoproteins in atherogenesis (Havel, 2000; Malloy & Kane, 2001).

Unlike the triglyceride metabolism, there are marked species-specific differences in the cholesterol metabolism during infection and inflammation. The total serum cholesterol concentration decreases in humans and other primates, whereas in rodents the opposite has been reported (Feingold et al., 1995; Sammalkorpi et al., 1988). Although the LDL cholesterol levels decrease in humans during inflammation, the relative portion of small dense LDL particles increases (Feingold et al., 1993). These particles can rapidly enter the arterial wall intima and are more susceptible to oxidative modifications leading to enhanced particle uptake by macrophages and other immune cells, thus promoting atherogenesis (Chait et al., 1993; Hurt-Camejo et al., 1990). Like LDL fraction, the structure and composition of HDL particles change during inflammation. Various acute phase reactants, such as SAA and ceruloplasmin, can displace apoA-I, paraoxonase-1 (PON1), and other proteins in HDL changing the particle composition to a proatherogenic direction. In addition, the concentrations of several enzymes and proteins regulating HDL metabolism decrease during inflammation leading to decreased HDL levels and suppressed reverse cholesterol transport (Van Lenten et al., 2001). Even though an immune response may begin as a protective process it may become excessive if prolonged and the consequenses can be injurious contributing to the progression of atherosclerosis.

2.2 Lipoprotein metabolism

Lipoproteins are water-soluble particles consisting of a hydrophobic core of triglycerides and cholesteryl esters (CE), and a hydrophilic surface of a monolayer of phospholipids (PL), unesterified cholesterol (UC), and apolipoproteins. Lipoproteins are needed to transport lipids and other hydrophobic compounds from one tissue site to another. They are continuously modified in the circulation by numerous proteins and enzymes, which alter their structure and functions. In consequence, each lipoprotein class covers a broad range of particle sizes, densities, and compositions (Betteridge, 1999). Lipoproteins can be classified into five main categories depending on their size, lipid and protein composition, electrophoretic mobility, and hydrated density as presented in Table 1 (Havel et al., 1955). Intestinal chylomicrons (CM) and hepatic very low-density lipoproteins (VLDL) are secreted to the circula-

tion where the hydrolysis of the triglycerides of these particles yields smaller remnant particles. VLDL hydrolysis generates intermediate-density lipoproteins (IDL) and low density-lipoproteins (LDL), which transport triglycerides, cholesterol, and other substances to the peripheral tissues where they are utilized for energy production or synthesis of various lipid-derived compounds such as hormones. High density-lipoproteins (HDL) function in the opposite manner transporting excess cholesterol from non-hepatic tissues to the liver for recycling or excretion in the bile. An additional lipoprotein class, lipoprotein(a), is a genetically determined, very heterogenous group of particles, which resemble in structure the LDL and are thought to play a role in atherogenesis (Berglund & Ramakrishnan, 2004). The major routes for extracellular lipid transport, mediated by the lipoproteins, are the exogenous and endogenous pathways, and the process called reverse cholesterol transport, which are discussed below in more detail (Figure 1).

Table 1. Properties of the main lipoprotein classes in human plasma

Class	Diameter (nm)	Density (g/ml)	Electrophoretic mobility	Chemical composition (% of dry mass)				
				Core		Surface		
				TG	CE	UC	PL	Prot[a]
CM	25-500	0.93	at origin	86	3	2	7	2
VLDL	30-80	0.95-1.006	pre-β	55	12	7	18	8
IDL	25-35	1.006-1.019	slow pre-β	23	29	9	19	19
LDL	18-25	1.019-1.063	β	6	42	8	22	22
HDL_2	9-12	1.063-1.125	α	5	17	5	33	40
HDL_3	7-9	1.125-1.210	α	3	13	4	25	55
Lp(a)	30[b]	1.055-1.085	slow pre-β	3	33	9	22	33

TG, triglyceride; CE, cholesteryl ester; UC, unesterified cholesterol; PL, phospholipid; Prot, proteins; Lp(a), lipoprotein(a).
[a]Protein does not include bound carbohydrate.
[b]The size of Lp(a) varies a lot owing to the heterogeneity in apo(a) gene and apo(a) protein.
Adapted from Betteridge et al., 1999.

2.2.1 Exogenous lipid transport

Chylomicrons

Dietary lipids and fat-soluble vitamins are absorbed from the gut and released to the lymphatic system and to the circulation by enterocytes in the form of chylomicrons (CM). Chylomicrons are heterogenous, triglyceride-rich particles ranging in diameter from 25 to 500 nm. The size of the CM is dependent on the absorption rate and amount of free fatty acids and cholesterol, which are first esterified within the enterocytes and then packaged into CM. The fatty acids of chylomicron triglycerides, but not of phospholipids, reflect the fatty acid composition of dietary lipids (Hussain et al., 2001; Kayden et al., 1963). Lipid absorption promotes the release of apolipoproteins, which are needed for the assembly and secretion of CM. ApoB-48 is a prerequisite for the formation of chylomicrons. It is translated and post-transscriptionally edited from apoB messenger ribonucleic acid (mRNA) (Powell et al., 1987), and in humans it is only synthesized in the gut. In addition to apoB-48, the intestine generates significant amounts of apoA-I and apoA-II, and all of the apoA-IV, which is a constituent apolipoprotein of triglyceride-rich lipoproteins as well as high density lipoproteins (Green & Glickman, 1981).

Once in the circulation, chylomicrons further increase their apolipoprotein content by transfer of apoC and apoE from HDL particles (Havel et al., 1973). Via apoC-II, chylomicrons bind to the capillary endothelium-bound lipoprotein lipase (LPL) that hydrolyses the CM triglycerides (Fielding & Fielding, 1977). This results in the release of fatty acids to the surrounding tissue, principally adipose tissue and skeletal muscle. In addition to LPL, chylomicrons are also modified by cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). These two proteins mediate the transfer of cholesteryl esters, phospholipids, and triglycerides between different lipoproteins in the circulation (Tall, 1995). The hydrolysis and exchange of CM core lipids and the loss of surface phospholipids lead to the formation of chylomicron remnant particles, which are depleted of triglyceride and enriched with cholesteryl esters, apoB-48, and apoE. CM remnants are rapidly and effectively cleared from the circulation through binding of apoE either to the LDL receptor (LDLR) or the LDL receptor-related protein (LRP) expressed on the surface of hepatocytes (Herz et al., 1988; Hussain et al., 1991; Windler et al., 1980a; Windler et al., 1980b). Thus, chylomicrons serve to transfer dietary TG and fatty acids to the peripheral cells for storage or oxidation and dietary cholesterol to the liver for recycling or catabolism.

2.2.2 Endogenous lipid transport

In addition to the lipids obtained from the diet, cholesterol and triglycerides are also made endogenously by the liver, which is the main organ regulating the lipid flow in the blood as well as in the enterohepatic circulation (Dietschy et al., 1993). Concomitantly with lipid synthesis, human hepatocytes synthesize a broad range of apolipoproteins including apoA-I, apoA-II, apoB-100, apoC-II, apoC-III, and apoE, which are needed for the lipoprotein assembly and secretion (Dixon & Ginsberg, 1993; Rash et al., 1981; Zannis et al., 1981).

Very low density lipoproteins

ApoB-100 is an important structural protein of VLDL and LDL particles. It encompasses 30-40% of the protein of plasma VLDL and more than 95% of the protein content of plasma LDL. ApoB-100 contains both hydrophobic and hydrophilic sequences, which interact with lipid and water interfaces, respectively (Olofsson et al., 1987), and a LDL receptor-binding domain needed for LDL particle uptake by both the peripheral and the liver cells (Knott et al., 1986; Yang et al., 1986). The assembly and secretion of apoB-containing lipoproteins are regulated primarily at a post-translational level mainly by newly synthesized lipids (Dixon & Ginsberg, 1993). Especially, the availability of the core lipids, cholesteryl esters and triglycerides, has been proposed to play a critical role in the regulation of hepatic apoB secretion rate and the density of the secreted lipoproteins (Cianflone et al., 1990; Dixon et al., 1991; Fuki et al., 1989; Ginsberg et al., 1987). Moreover, studies in cultured primary rat hepatocytes indicate that a large proportion of newly synthesized apoB is degraded intracellularly (Borchardt & Davis, 1987). The presence of oleate has been demonstrated to inhibit the intracellular degradation of apoB in HepG2 cells (Dixon et al., 1991), whereas fatty acids of the ω-3 series, such as eicosapentaenoic acid and docosahexaenoic acid (Wang et al., 1993), as well as insulin (Sparks & Sparks, 1993), have been reported to promote the intracellular degradation of apoB.

The synthesis and secretion of large TG-rich VLDL particles, 40-70 nm in diameter, occur mainly in the postabsorptive state when the concentrations of CM and their remnants have decreased in the plasma. The core lipids of VLDL are either newly

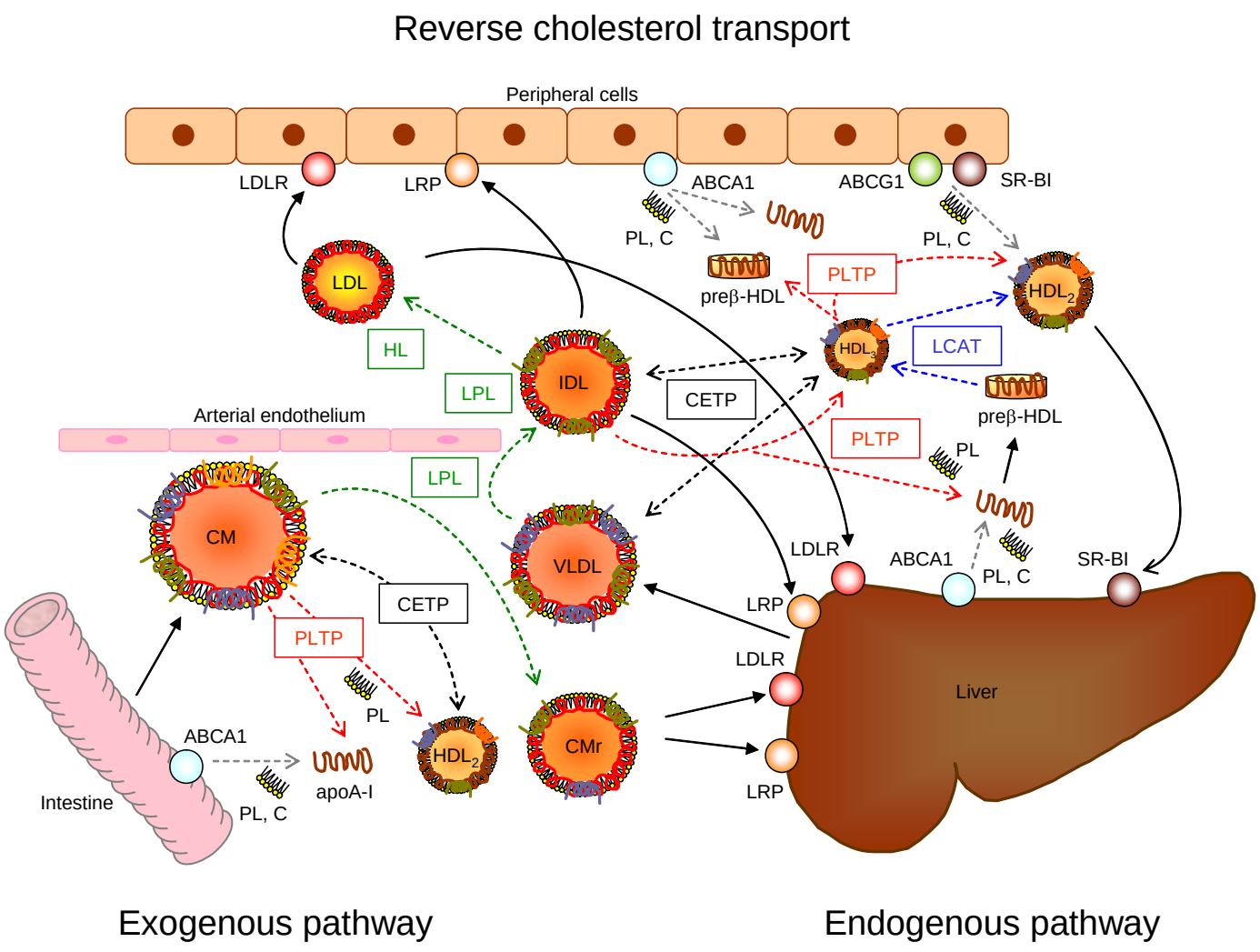

Figure 1. Lipoprotein transport and metabolism. Solid arrows represent protein transfer processes and dashed arrows actions exerted by specific proteins and enzymes. See text for further details.

synthesized or taken up from plasma in a form of CM or other lipoprotein remnants. Similarly to CM, the size and density of VLDL seem to be regulated mainly by the cellular triglyceride content (Olofsson et al., 1987). Large $VLDL_1$ particles are secreted when excess TG are available while smaller $VLDL_2$ particles, and to some extent also IDL and LDL, are secreted when cellular TG concentration is reduced but cholesterol is present for particle assembly (Chait et al., 1980; Ginsberg et al., 1985; Kissebah et al., 1981). VLDL particles share a common delipidation pathway with CM regulated by numerous enzymes and cell-surface receptors. In addition to apoB-100, the other apolipoproteins of VLDL, including the C apolipoproteins and apoE, appear to have significant roles in VLDL catabolism. ApoC-III has been proposed to interfere with the interaction of apoE with the liver receptors inhibiting VLDL particle uptake (Windler & Havel, 1985), whereas apoC-II is an obligatory cofactor for LPL-mediated TG hydrolysis. As lipolysis proceeds, VLDL particles become smaller and denser resulting in the formation of VLDL remnants known as IDL. It appears that smaller $VLDL_2$ particles are more effectively converted to IDL and LDL than large $VLDL_1$ particles (Packard et al., 1984).

Intermediate density lipoproteins

Hydrolysis of VLDL triglycerides by LPL generates particles that lie between VLDL and LDL with respect to size and density. Loss of the core lipids alters the surface structure of VLDL leading to reduced affinity of the C apolipoproteins to the surface lipids and the transfer of apoC to HDL and newly secreted VLDL and chylomicrons. In consequence, intermediate density lipoproteins contain only apoB-100 and apoE on their surface. The excess surface phospholipids are transferred to HDL by PLTP while CETP increases the cholesteryl ester content of VLDL and IDL (Eisenberg, 1985; Tall, 1995; Tall & Small, 1978). IDL are cleared from the circulation by cell membrane receptors mediated by apoE or converted to LDL through the actions of LPL and hepatic lipase (HL) (Demant et al., 1991; Demant et al., 1988; Mahley, 1988; Nicoll & Lewis, 1980).

Low density lipoproteins

The lipolytic cascade continues with the hydrolysis of the core triglycerides of IDL by HL inducing the transfer of apoE to HDL (Nicoll & Lewis, 1980). This results in the formation of LDL particles which have apoB-100 as their sole apolipoprotein. LDL is the principal lipoprotein fraction transporting cholesterol to the peripheral cells. Both the peripheral and the liver cells express LDL receptors on their surface but the catabolism of LDL particles is principally mediated by hepatic LDLR (Carew et al., 1982). Unlike macrophage scavenger receptors, the expression of

LDL receptors is regulated by the intracellular cholesterol content (Brown & Goldstein, 1986; Kunjathoor et al., 2002; Steinberg et al., 1989).

2.2.3 Reverse cholesterol transport

Cells require only minor amounts of cholesterol to their essential needs like membrane synthesis or hormone production. Most of them control the cholesterol homeostasis by regulating the expression of plasma membrane receptors and transporter proteins although cholesterol efflux by passive diffusion occurs to some extent in all cells (Phillips et al., 1987). Cholesterol efflux from peripheral macrophages is the first step in reverse cholesterol transport (RCT) that is defined as a process in which excess cholesterol of peripheral tissues is transported to the liver for recycling or for excretion into the bile (Glomset, 1968). Several proteins can mediate phospholipid and cholesterol efflux to various extracellular acceptors, especially to plasma HDL or its apolipoproteins, which play crucial roles in this process as illustrated in Figure 2 (von Eckardstein et al., 2001).

High density lipoproteins

HDL particles are produced in both the liver and the intestine. Besides the *de novo* synthesis, nascent HDL particles are also formed during lipolysis as lipid-poor apoA-I becomes associated with the excess surface components of triglyceride-rich lipoproteins (Patsch et al., 1978; Tall et al., 1982). High-density lipoproteins are the smallest, densest, and the most protein-rich particles among the serum lipoproteins. They carry numerous proteins embedded in a phospholipid monolayer. ApoA-I and apoA-II are the most abundant apolipoproteins of HDL but they also contain a wide range of other apolipoproteins like apoE, apoA-IV, apoC, apoD, and apoJ (Eisenberg, 1984). Besides apolipoproteins, small amounts of various plasma enzymes and other proteins as well as smaller molecules are transported by HDL. These include lecithin:cholesterol acyl transferase (LCAT), platelet-activating factor acetyl hydrolase (PAF-AH), serum paraoxonase-1 (PON1), serum amyloid A (SAA), lipopolysaccharide (LPS), and vitamins such as beta-carotene (Van Lenten et al., 2001).

In the circulation HDL undergo multiple modifications by several plasma enzymes and lipid transfer proteins yielding a very heterogenous group of particles. The two major HDL subclasses are HDL_2 and HDL_3, which have a globular shape and α-mobility (von Eckardstein et al., 1994). The antiatherogenic property of HDL has been connected to increased concentration of HDL_2 subpopulation (Ballantyne et al., 1982; Miller et al., 1981). The smallest HDL particles, preβ-HDL, are discoidal in

shape and have an important function as acceptors of excess cholesterol from peripheral tissue cells (Castro & Fielding, 1988). The unesterified cholesterol of preβ-HDL is esterified by LCAT and transferred to the core of the particle generating spherical HDL_3 particles. These particles can take up more unesterified cholesterol from the surrounding cells giving rise to larger HDL_2 particles. In addition to passive diffusion (Phillips et al., 1998), specific transporter proteins like ATP-binding cassette transporters (ABC) of the sub-family A (Brewer et al., 2004; Wang et al., 2003b) and the sub-family G (Klucken et al., 2000; Wang et al., 2004) as well as scavenger receptor class B type I (SR-BI) (Trigatti et al., 2003) have been shown to mediate the cholesterol efflux from cell membranes to HDL. The actions of CETP, HL, and PLTP have a great impact on the size, composition, and level of HDL in the circulation (Huuskonen et al., 2001; Wang & Briggs, 2004). CETP transports cholesteryl esters from the core of HDL to less dense lipoproteins, CM, VLDL, IDL, and LDL, in exchange for triglycerides. Subsequently, HDL particles become enriched in TG and larger in size. Larger HDL_2 particles are then converted into smaller HDL_3 particles on HL-mediated hydrolysis of phospholipids and triglycerides. PLTP, in turn, transfers phospholipid-rich surface remnants released during VLDL and CM hydrolysis to HDL. CETP, HL, and PLTP are all capable of generating preβ-HDL, thus sustaining the RCT process (Barrans et al., 1994; Lie et al., 2001). Furthermore, PLTP has been reported to be able to modulate the activities of LCAT and CETP (Tollefson et al., 1988; Tu et al., 1993). The final step in RCT is the selective uptake of HDL-associated cholesteryl esters mediated not only by SR-BI but also by other cell surface proteins such as HL (Brundert et al., 2003). In addition, the β-chain of ATP synthase has been shown to mediate hepatic endocytosis of holo-HDL particles, i.e., both apolipoproteins and lipids (Martinez et al., 2003). Lipid-poor apoA-I and holo-HDL particles have been found to be cleared from the circulation by cubilin, an endocytic receptor that is co-expressed with megalin in the kidney (Hammad et al., 2000; Hammad et al., 1999; Kozyraki et al., 1999).

In addition to the important role of HDL particles in RCT, they also have other potentially antiatherogenic functions (Nofer et al., 2002; Van Lenten et al., 2001). High-density lipoproteins are able to prevent monocyte binding to the endothelium and penetration into the arterial intima by inhibiting the expression of several adhesion molecules (Cockerill et al., 1995; Navab et al., 1991). HDL, and in particular apoA-I, are capable of preventing the accumulation of oxidized lipids into the arterial intima, thus protecting LDL particles from oxidation and reducing the endocytosis of LDL by macrophages (Hayek et al., 1995; Navab et al., 2000). Most of these protective activities are exerted by the enzymes associated with HDL such as LCAT (Liu & Subbaiah, 1994), PON1 (Watson et al., 1995a), and PAF-AH (Watson et al., 1995b).

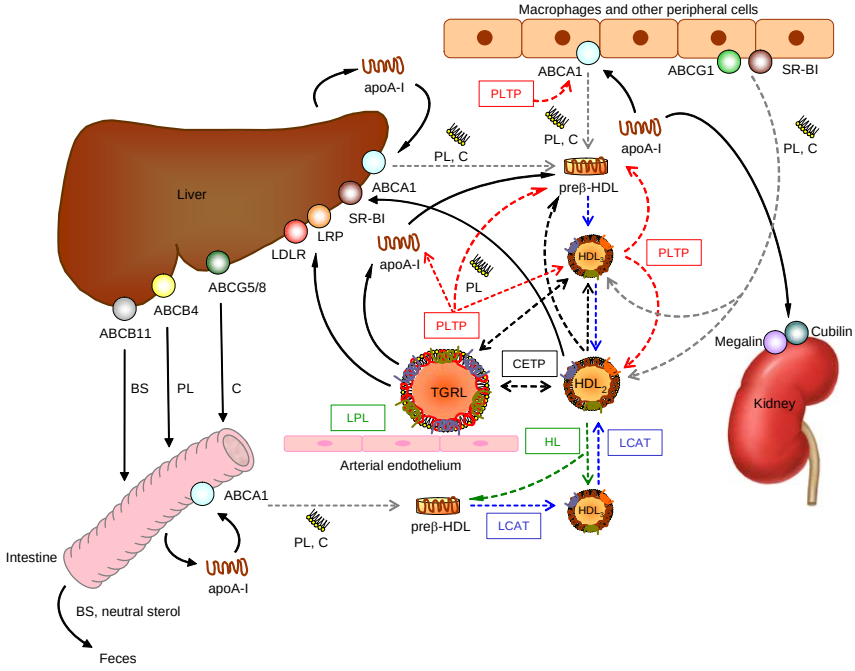

Figure 2. Schematic model of the major pathways involved in RCT. ApoA-I, secreted by the liver and the intestine, released from lipolysed triglyceride-rich lipoproteins (TGRL) or generated by interconversion of HDL_3 and HDL_2, is loaded with phospholipids (PL) and cholesterol (C) by ABCA1. Besides apoA-I, the thus formed preβ-HDL particles serve as acceptors of PL and C from macrophages and other peripheral tissue cells. Interaction of PLTP with ABCA1 enhances the flux of excess C to preβ-HDL particles, which are converted to spherical HDL_3 and HDL_2 particles through the action of LCAT. Spherical HDL particles can be further loaded with PL and C by ABCG1 and SR-BI in macrophages. CETP and HL convert larger HDL_2 particles into smaller HDL_3 particles with a concomitant generation of preβ-HDL. Contrary to CETP and HL, PLTP generates preβ-HDL from HDL_3. The endocytic receptors, cubilin and megalin, act in conjunction to mediate the endocytosis of lipid-poor apoA-I by the kidney. Cholesteryl esters of HDL are transported to TGRL by CETP in exchange for triglycerides or selectively taken up by hepatic SR-BI. TGRL are taken up by the hepatic LDL receptor (LDLR) or the LDL receptor-related protein (LRP). Subsequently, C can be secreted into the bile either in the free form or after conversion as bile salt (BS). After transport via the bile into the intestine, C and BS are reabsorbed or excreted in the feces. Solid arrows represent protein transfer processes and dashed arrows actions exerted by specific proteins and enzymes. Adapted from van der Velde & Groen, 2005.

ABC transporters

ABCA1 is a member of the ABC transporter superfamily of membrane bound proteins that use ATP to mediate the transfer of several molecules across the cell membranes. Currently, 48 ABC transporters are known to exist in humans. They can be divided into seven sub-families (A-G) of which the A, B, C, D, and G sub-families are suggested to be involved in lipid trafficking (Tannert et al., 2003). ABCA1 is a full-transporter that comprises of two similar halves, each containing six transmembrane domains and an intracellular domain with an ATP-binding cassette. ABCA1 plays an important role in the removal of phospholipids and cholesterol from peripheral cells to lipid-poor apoA-I or other helical apolipoproteins of HDL (Hara & Yokoyama, 1991; Oram et al., 2000). Mutations in the ABCA1 gene cause Tangier disease and hypoalphalipoproteinemia indicating the important role of ABCA1 in nascent HDL particle assembly (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Rust et al., 1999). In a recent study, the lipidation of newly synthesized apoA-I in Golgi compartments and at the plasma membrane was shown to be dependent on ABCA1 (Maric et al., 2005).

ABCA1 is expressed in several human tissues and cell types. The hepatic and intestinal ABCA1 contribute to the production of plasma HDL while macrophage ABCA1 protects arteries from lipid deposition and development of atherosclerotic plaques (Brunham et al., 2006; Oram et al., 2000; Timmins et al., 2005). The ABCA1 mRNA expression has been shown to increase during human adipocyte and monocyte differentiation, being strongest in lipid-loaded macrophages (Kielar et al., 2001; Langmann et al., 1999). Interaction of ABCA1 with apoA-I or other helical apolipoproteins has been shown to protect it from the degradation mediated by calpain protease, thereby increasing the cellular ABCA1 concentration (Arakawa & Yokoyama, 2002; Wang et al., 2003a). Besides the helical apolipoproteins, phospholipid transfer protein (PLTP) has been shown to interact with ABCA1 and protect it from proteolysis (Oram et al., 2003).

Similarly to ABCA1, phospholipid efflux and HDL assembly is also mediated by ABCA7 (Wang et al., 2003b), whereas ABCB4 and ABCB11 have been shown to transport mainly phosphatidylcholine and bile salts, respectively, from the liver into the bile (Gerloff et al., 1998; van Helvoort et al., 1996). In addition, some members of a sub-family of half-size ABC transporters including ABCG1, ABCG5, and ABCG8, have been shown to have critical roles in cholesterol transport (Figure 2). ABCG1 participates in the RCT process by mediating cholesterol and phospholipid efflux from macrophages to α-HDL (Klucken et al., 2000). It appears that the activity of ABCG1 is distinct from the activity of ABCA1 which performs the initial lipidation of apoA-I. The nascent preβ-HDL particles generated by ABCA1 serve as acceptors for ABCG1-mediated cellular cholesterol efflux (Gelissen et al., 2006).

Like ABCG1, ABCG4 has been shown to mediate cholesterol efflux from macrophages to α-migrating HDL but not to lipid-poor apoA-I (Wang et al., 2004). ABCG5 and ABCG8, which function as heterodimers, have been identified to promote cholesterol excretion from the liver to the bile duct and the export of absorbed dietary sterols back to the gut lumen (Berge et al., 2000; Lee et al., 2001).

Scavenger receptor class B type I

Scavenger receptor class B type I (SR-BI) mediates cholesterol efflux from macrophages to HDL_3 (Acton et al., 1996; Ji et al., 1997; van der Velde & Groen, 2005) as well as selective uptake of HDL-associated cholesteryl esters and unesterified cholesterol by the liver (Trigatti et al., 2003). It has been shown *in vivo* that modulation of hepatic SR-BI expression regulates the rate of cholesterol efflux from macrophages and the sterol excretion in the feces (Zhang et al., 2005). Mice overexpressing SR-BI in the liver had reduced plasma HDL cholesterol levels and increased macrophage-derived sterol excretion in the feces, whereas mice deficient in SR-BI had increased plasma HDL cholesterol levels and markedly reduced macrophage-derived sterol excretion in the feces, suggesting that hepatic SR-BI expression regulates the rate of RCT inversely to its effect on plasma HDL cholesterol levels and that the rate of RCT cannot be predicted based only on measurement of plasma HDL cholesterol. In addition to the important role of SR-BI in RCT, it has also been identified to mediate cholesterol transport to steroidogenic tissues for steroid hormone synthesis. Besides SR-BI, several other cell surface receptors and HDL-binding proteins on hepatocytes as well as on intestinal mucosal cells, fibroblasts, and adipocytes have been shown to bind HDL (Fidge, 1999).

2.3 Interactions of apolipoproteins with lipid transfer proteins and lipolytic enzymes

Besides the important role as lipid transporters, exchangeable apolipoproteins also play many other roles within the body. They mediate lipoprotein uptake in the liver and other tissues and function as co-factors for plasma enzymes and lipid transfer proteins. Recently, apoE has been shown to mediate lipid antigen presentation to T cells (van den Elzen et al., 2005). The ability of apolipoproteins to interact with lipids plays a central role in these functions. All soluble apolipoproteins contain amphipathic helixes of 11 or more residues that associate with lipid surfaces. The polar side of the helix faces the aqueous phase and the non-polar side the lipid surface of lipoproteins (Ibdah et al., 1989; Segrest et al., 1974). The association rate of helical apolipoproteins with the lipid surface is dependent on the concentration ratio of cholesterol and phospholipids and usually occurs spontaneously at the transition

temperature of phospholipids (Jonas & Mason, 1981; Pownall et al., 1979; Pownall et al., 1978). It has been established that the α-helical repeats are the key structural elements for the proper function of the apolipoproteins (Saito et al., 2004).

The exchangeable apolipoproteins are part of a multigene family, and members of the A, C, and E classes of apolipoproteins share a similar genomic structure (Li et al., 1988). The tertiary structures of apoA-I and apoE have been shown to be related and share a common two-domain conformation. The amino-terminal end of both proteins has been shown to contain a series of amphipathic α-helical repeats, most of which are punctuated with prolines, while the carboxy-terminal region has been shown to be primarily responsible for lipid binding. The helical repeats of the N-terminal region have been demonstrated to form a helix bundle conformation in the lipid-free state. Upon lipid binding the helix bundle opens, which has been shown to result in enhanced binding activity of apoE to its receptors such as LDLR or SR-BI. Respectively, the N-terminal helix of apoA-I has been shown to be critical for activation of LCAT (Saito et al., 2004).

ApoA-I is the main protein component of plasma HDL fraction. It plays a critical role in reverse cholesterol transport through several actions. First, it has been shown to interact with the cell membrane transporter protein, ABCA1, which facilitates the efflux of PL and UC leading to the formation of nascent preβ-HDL particles (Oram, 2003). Second, apoA-I has been found to serve as the most effective co-factor for LCAT (Fielding et al., 1972), and to adopt a belt-like arrangement in discoidal preβ-HDL particles (Segrest et al., 1999), which has been suggested to be optimal for the interaction of apoA-I with LCAT (Jonas, 1991). Third, apoA-I has been reported to mediate the hepatic uptake of HDL cholesteryl esters by acting as a ligand for the cell membrane receptor SR-BI (Williams et al., 2000).

Human apoE exists in three major isoforms, $apoE_2$, $apoE_3$, and $apoE_4$, coded by three common apoE alleles (ε2, ε3, and ε4) of the apoE gene. The most common isoform, $apoE_3$, contains a cysteine and arginine at positions 112 and 158, respectively, while $apoE_2$ contains cysteine and $apoE_4$ arginine at both positions (Weisgraber et al., 1981). Similarly to apoA-I, apoE is thought to be an antiatherogenic molecule due to its profound effect on plasma lipoprotein levels. ApoE has been shown to serve as a ligand not only for the LDLR (Mahley, 1988) but also to the LRP, the VLDL receptor, and the apoE receptor 2 (Herz & Bock, 2002). On the other hand, although the ε2 allele has been shown to associate with lower LDL cholesterol (Sing & Davignon, 1985; Volcik et al., 2006) it has also been shown to be related to type III hyperlipoproteinemia (Gregg et al., 1981; Mahley et al., 1999; Utermann et al., 1977), whereas the ε4 allele has been shown to associate with both hypercholesterolemia and type V hyperlipoproteinemia (Gregg et al., 1986; Srinivasan et al., 1999). In addition, $apoE_4$ isoform of apoE has been reported to be over-

represented among the patients with Alzheimer's disease (Mahley & Huang, 1999; Weisgraber & Mahley, 1996).

At present, the structure-function relationships of the other members of the multigene family, apoA-II, apoA-IV, apoA-V, and apoC are not so well known. Like apoA-I, apoA-II is a major protein component of HDL. Probably due to the hydrophobic nature of apoA-II, it binds lipids with high affinity and is able to displace apoA-I from HDL particles (Ibdah et al., 1989). Similarly to apoA-I and apoE, the amino acid sequence of apoA-IV is dominated by multiple α-helical repeats of 22 residues (Li et al., 1988). However, apoA-IV lacks the hydrophobic C-terminal lipid-binding domain, which has been suggested to explain the low affinity of apoA-IV for lipoproteins. Despite this, apoA-IV has been demonstrated to mimic certain roles of apoA-I such as activation of LCAT (Steinmetz & Utermann, 1985) and promotion of cholesterol efflux (Remaley et al., 2001). ApoA-V, the newest member of the exhangeable apolipoprotein family, has recently been identified as an important regulator of plasma triglyceride concentration (Pennacchio et al., 2001), while the members of class C apolipoproteins have been reported to display both inhibitory and stimulatory effects on several plasma enzymes and lipid transfer proteins (Saito et al., 2004).

Besides the members of the multigene family discussed above, there are two other major apolipoprotein genes in humans: apoD and apoB. The genomic structures of apoD and apoB have been shown to differ substantially from each other as well as from the genomic organizations of the apolipoproteins of the multigene family (Blackhart et al., 1986; Drayna et al., 1987). ApoD is a member of the lipocalin superfamily and has been shown to associate with preβ-migrating lipoprotein species *in vitro* (Francone et al., 1989). It has been speculated that apoD might favor heme-related compounds over cholesterol or cholesteryl esters. The specific function of apoD, however, awaits further definition (Peitsch & Boguski, 1990). ApoB-100, the main protein constituent of VLDL, IDL, LDL, and Lp(a), is the largest apolipoprotein and shares a common amino-terminal sequence with apoB-48, the apolipoprotein of CM (Chen et al., 1987; Powell et al., 1987). Among all apolipoproteins, apoB-100 has been most clearly associated with the progression of atherosclerotic diseases (Sniderman et al., 1980).

2.3.1 Lipid transfer/lipopolysaccharide-binding protein family

Several HDL apolipoproteins are able to enhance the transfer reaction of cholesteryl ester transfer protein (CETP). The interactions between different apolipoproteins and CETP have been studied intensively but only a little are known about the asso-

ciations of apolipoproteins with the other members of the protein family: lipopolysaccharide-binding protein (LBP), bactericidal permeability-increasing protein (BPI), and phospholipid transfer protein (PLTP), which is discussed in detail in the following chapter.

Cholesteryl ester transfer protein

Cholesteryl ester transfer protein (CETP) is a 74 kilodalton (kDa) glycoprotein, which plays a crucial role in lipoprotein metabolism. The C-terminal region of CETP contains a highly hydrophobic neutral lipid-binding domain (Albers et al., 1984; Tall et al., 1983). CETP mediates the transfer and exchange of cholesteryl esters, triglycerides, and phospholipids but unlike PLTP, it is incapable of transferring phospholipids from phosphatidylcholine-containing liposomes to HDL_3 (Lagrost et al., 1994a). Furthermore, CETP and PLTP have been reported to show no overlap in their functions *in vivo* (Kawano et al., 2000). It has been shown that stabilization of substrate lipid particles with either apoA-I, apoA-II, apoC-II, apoC-III, apoE (Nishikawa et al., 1988) or apoA-IV (Main et al., 1996) considerably increases the CETP-mediated lipid transfer activity. ApoA-I, apoA-II, and apoE have been demonstrated to be more potent activators of CETP than apoC-III (Ohnishi & Yokoyama, 1993). Furthermore, increased apoA-II concentration in HDL particles has been shown to result in reduced CETP-mediated transfer rate of cholesteryl esters between LDL and HDL (Lagrost et al., 1994b), while the phospholipid transfer activity of CETP has been reported to be inhibited by a lipid transfer inhibitor protein identified as apoF (Wang et al., 1999).

Lipopolysaccharide-binding protein

Lipopolysaccharide-binding protein (LBP) promotes the binding of lipopolysaccharides (LPS) to lipoprotein particles, resulting in the neutralization of the bioactivity of LPS (Wurfel et al., 1994). On the other hand, LBP is also able to transfer LPS to CD14, a protein found both as a soluble form in the circulation and as a membrane bound protein on monocytes, macrophages, and neutrophils, which has been shown to result in enhanced cellular defense responses (Hailman et al., 1994). LBP has been demonstrated to interact with HDL (Ulevitch et al., 1979) and purified apoA-I (Vreugdenhil et al., 2001) as well as to transfer phospholipids *in vitro* (Yu et al., 1997). LBP has also been reported to circulate in association with LDL and VLDL both in healthy and septic subjects. The association of LBP with apoB-containing lipoproteins was shown to enhance the capacity of LDL and VLDL to bind LPS. ApoB have been found to mediate the interaction between LBP and lipoproteins at least in part (Vreugdenhil et al., 2001).

Bactericidal permeability-increasing protein

Unlike the other members of the lipid transfer/lipopolysaccharide-binding protein (LT/LBP) family, bactericidal permeability-increasing protein (BPI) is a cellular, membrane-anchored protein. It can be found mainly in polymorphonuclear leukocytes and to a lesser extent in monocytes and macrophages. BPI binds specifically and with high affinity to Gram-negative bacteria. It kills bacteria by increasing the permeability of their outer membrane as well as by activating the enzymes that degrade bacterial cell wall (Elsbach et al., 1979; Weiss et al., 1978). LPS has been shown to be the factor that mediates the binding of BPI to bacteria. Thus far, BPI is the sole member of the LT/LBP family to have its crystal structure determined (Beamer et al., 1997). The association of BPI with apolipoproteins is currently unknown.

2.3.2 Lipases

Lipoprotein lipase

Lipoprotein lipase (LPL) plays a crucial role in lipid transport (Wang et al., 1992). It is anchored to the vascular endothelium in extra-hepatic tissues via the interaction with heparan sulphate proteoglycans (Spillmann et al., 2006). LPL hydrolyses TG and PL of CM and VLDL particles. To be catalytically active, LPL requires a specific co-factor, apoC-II (Havel et al., 1970; LaRosa et al., 1970). Consistent with this requirement, a significant accumulation of TG has been observed in patients with an inherited defect of the apoC-II gene (Breckenridge et al., 1978). In contrast to apoC-II, *in vitro* studies have shown that apoC-III (Jackson et al., 1986; Liu et al., 2000), apoE (McConathy & Wang, 1989), and apoA-I (Yamamoto et al., 2003) can inhibit the activity of LPL.

Hepatic lipase

Hepatic lipase (HL), associated mainly with plasma membranes of liver endothelial cells (Kuusi et al., 1979), hydrolyses the triglycerides of IDL leading to the formation of LDL particles (Nicoll & Lewis, 1980). In addition, HL hydrolyses the core TG of HDL_2 particles resulting in the formation of smaller HDL_3 particles as well as preβ-HDL particles (Barrans et al., 1994). It has no obligatory need for apoC-II or any other apolipoprotein to be catalytically active. However, it has been demonstrated that both apoA-I and apoA-II inhibit the HL-mediated lipolysis of triglycerides (Kubo et al., 1981). Contrary to these findings, Mowri and coworkers (1992) have reported that HL-mediated hydrolysis of both PL and TG was more efficient when apoA-II was present in HDL particles. In addition, it has been reported that apoE is able to enhance the HL-mediated hydrolysis of HDL phospholipids (Thuren et al., 1992).

Endothelial lipase

Endothelial lipase (EL) is a newly identified member of the triglyceride lipase gene family (Hirata et al., 1999; Jaye et al., 1999). EL has been shown to display high phospholipase and low triglyceride lipase activity and to hydrolyze mainly phospholipids of HDL particles (McCoy et al., 2002). The two major apolipoproteins of HDL, apoA-I and apoA-II, have been shown to regulate the binding affinity of EL to HDL. In addition, apoA-I and apoA-II have been reported to have a significant influence on the hydrolysis rate of HDL phospholipids. Hydrolysis has been shown to be greater in HDL particles containing both apoA-I and apoA-II than in particles containing only apoA-I. However, only minimal hydrolysis of phospholipids could be detected among particles containing only apoA-II. Furthermore, EL was found to have higher affinity for apoA-I -containing particles than particles containing both apoA-I and apoA-II (Caiazza et al., 2004; Jahangiri et al., 2005).

2.3.3 Other enzymes

Lecithin:cholesterol acyl transferase

Lecithin:cholesterol acyl transferase (LCAT) is a plasma enzyme which catalyzes the formation of cholesteryl esters and lysolecithin by transferring the fatty acyl group at the sn-2 position of phosphatidylcholine to the hydroxyl group of cholesterol (Glomset, 1968). LCAT is known to be activated by several plasma apolipoproteins, the most important being apoA-I (Fielding et al., 1972; Furukawa & Nishida, 1979; Sorci-Thomas et al., 1998; Soutar et al., 1975; Yokoyama et al., 1978). ApoE (Chen & Albers, 1985; Steinmetz et al., 1985; Zorich et al., 1985), apoA-IV (Chen & Albers, 1985; Steinmetz & Utermann, 1985) and apoA-II in the presence of apoA-I (Chen & Albers, 1986) as well as apoC-I (Soutar et al., 1975) and apoD (Kostner, 1974; Steyrer & Kostner, 1988) can also act as cofactors for LCAT although they are not as efficient as apoA-I in this function. Recently, the ability of different apoE isoforms to activate LCAT was studied and $apoE_2$ appeared to be the most efficient co-factor for LCAT among the apoE isoforms (Rye et al., 2006). On the other hand, apoA-II, apoC-II, and apoC-III have been shown to inhibit the LCAT reaction by displacing apoA-I, the enzyme itself, or both from the lipid surface of substrate vesicles *in vitro* (Chung et al., 1979; Nishida et al., 1986).

Platelet-activating factor acetyl hydrolase

Platelet-activating factor acetyl hydrolase (PAF-AH) is a plasma enzyme secreted primarily by macrophages. It regulates plasma platelet-activating factor (PAF) levels by removing the acetyl group from the sn-2 position of PAF resulting in the forma-

tion of a biologically inactive lyso-PAF. Other anti-inflammatory properties of PAF-AH include its ability to metabolize oxidized phospholipids (Stafforini et al., 1996; Tjoelker et al., 1995). PAF-AH has been shown to protect LDL from oxidation *in vitro* (Subramanian et al. 1999). Most of the serum PAF-AH is associated with LDL and the remainder with HDL. The direct interaction between PAF-AH and apoB or other apolipoproteins is currently unknown.

Paraoxonase-1

Paraoxonase-1 (PON1) belongs to a family of A-esterases and is secreted mainly by the liver. It is able to destroy oxidized lipids present in modified LDL particles, thus playing an important role as an antiatherogenic molecule. In addition, it hydrolyses active metabolites of organophosphate toxins such as paraoxon. PON1 protects HDL and LDL particles from oxidation by hydrolysing lipid peroxides, cholesteryl linoleate hydroperoxides, and hydrogen peroxides, which are the major reactive oxygen species produced during inflammation (Shih et al. 1998). PON1 activity (Boman, 1980) as well as concentration (Blatter Garin et al., 1994) has been shown to correlate positively with serum HDL cholesterol, apoA-I, and apoA-II concentration. In addition, PON1 activity has also been found to correlate positively with serum triglyceride and apoB concentration (Saha et al., 1991) and to associate with HDL_3 particles containing apoE and apoJ (Bergmeier et al., 2004).

Phospholipase A_2

Phospholipase A_2 (PLA_2) enzymes hydrolyze phospholipids at the sn-2 position generating free fatty acids and lysolipids. PLA_2 are widely distributed and exist in many isoforms (Kougias et al., 2006). Among these, secretory PLA_2–II favors the formation of bioactive lipids such as lysophosphatidylcholine, and it has also been identified as an independent risk factor for coronary artery disease (Rader, 2000). ApoC-I has been reported to inhibit the PLA_2-mediated hydrolysis of phospholipids (Poensgen, 1990).

2.4 Phospholipid transfer protein (PLTP)

Specialized lipid transfer proteins mediate the extracellular transfer of neutral lipids and phospholipids between different lipoproteins as well as lipoproteins and cell membranes. Phospholipid transfer protein (PLTP, formerly named lipid transfer protein II, LTP-II) was isolated from plasma in the early 1980s during the purification of CETP (also referred to as LTP-I), the first known lipid transfer protein in plasma (Tall et al., 1983; Tollefson et al., 1988). The determination of the PLTP complementary deoxyribonucleic acid (cDNA) sequence and detailed characteriza-

tion of the gene structure revealed that PLTP shares significant sequence homology with human CETP, LBP, and BPI (Day et al., 1994; Tu et al., 1995a). PLTP is a multifunctional player in lipoprotein metabolism and reverse cholesterol transport known to promote the exchange and transfer of phospholipids between TG-rich lipoproteins and HDL during lipolysis (Tall et al., 1985; Tollefson et al., 1988). As a consequence, HDL particles become rich in phospholipids and can accommodate more cholesterol for esterification by LCAT (Jonas, 1991). PLTP is also found to interact with a cell membrane transporter protein, ABCA1, and enhance the cholesterol efflux from peripheral cells (Oram et al., 2003). In addition, PLTP has been shown to modulate HDL particle size and composition, thus generating preβ-HDL particles, a process known as HDL conversion (Jauhiainen et al., 1993; Tu et al., 1993). These functions are suggested to represent antiatherogenic properties of PLTP. On the other hand, PLTP may be proatherogenic as it has been found to be present in human atherosclerotic lesions (Desrumaux et al., 2003; Laffitte et al., 2003; O'Brien et al., 2003) as well as to facilitate the secretion of apoB-containing lipoproteins from hepatocytes (Jiang et al., 2001; Lie et al., 2002). As PLTP in human plasma exists in two different forms (Oka et al., 2000a), the challenge for future is to reveal whether the inactive form, encompassing over 50% of the total protein mass and incapable of transferring phospholipids, has a physiological role in lipid and lipoprotein metabolism.

2.4.1 Molecular characteristics of PLTP

PLTP protein structure

Plasma phospholipid transfer protein is a serum glycoprotein that has over 40% hydrophobic amino acids. Its apparent molecular mass is 80 kDa. Human PLTP shares 93% sequence homology with pig (Pussinen et al., 1997b) and 83% homology with mouse PLTP (Albers et al., 1995; Jiang & Bruce, 1995). PLTP cDNA sequence, 1750 base pairs in length, contains an open reading frame of 1518 nucleotides followed by a 3'-untranslated region of 184 nucleotides (Day et al., 1994). The cDNA encodes a mature protein of 476 amino acids and a preceding signal sequence of 17 amino acids. Most of the mature protein is coded by the exons 3 to 15. A putative heparin binding domain is located within exon 12 (Tu et al., 1995a). The calculated molecular mass of the mature protein is 54719 Da. The discrepancy between the predicted molecular mass (55 kDa) and the 80 kDa protein mass estimated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is mainly due to glycosylation. Besides numerous potential O-glycosylation sites, six potential N-glycosylation sites at amino acids 47, 77, 100, 126, 228, and 381 can be predicted

from the PLTP sequence of the mature protein (Day et al., 1994). The presence of abundant N-glycans and importance of N-glycosylation for PLTP secretion have been shown in stably transfected inducible HeLa cells (Huuskonen et al., 1998). Similar results have been obtained in COS-7 cells also demonstrating that no single glycosylation site is required for PLTP secretion (Qu et al., 2006).

PLTP has also the potential to form two intramolecular disulfide bonds due to the four cysteines at residues 5, 129, 168, and 318. The Cys_{129} and Cys_{168} are essential for the stability and proper function of PLTP (Huuskonen et al., 1999). These two cysteines, which correspond to Cys_{135} and Cys_{175} in BPI, are conserved among all known members of the LT/LBP family (Day et al., 1994; Qu et al., 1999), while the two other cysteines of PLTP at residues 5 and 318 are not conserved within the gene family (Beamer et al., 1997). However, the importance of the Cys_5 for correct folding and secretion of pig PLTP has been demonstrated (Pussinen et al., 1997b).

The structure of PLTP has been modeled based on the three-dimensional structure of BPI (Beamer et al., 1997) and sequence alignments of the entire protein family (Huuskonen et al., 1999). According to this model, PLTP is suggested to have a boomerang-shaped two-domain structure (Figure 3). Each domain contains a hydrophobic lipid-binding pocket, which was shown to be essential for the phospholipid transfer activity of PLTP. The N-terminal pocket is suggested to have a more central role in phospholipid transfer, while the C-terminal pocket is suggested to play a significant role in the interaction between PLTP and HDL (Huuskonen et al., 1999). In accordance with this implication, it has been revealed by site-directed mutagenesis of charged amino acids that mutations in the N-terminal pocket significantly decrease the lipid transfer activity of PLTP without affecting HDL binding, whereas amino acid substitutions in C-terminal pocket result both in reduced HDL binding and decreased lipid transfer activity of PLTP (Ponsin et al., 2003). Two distinct basic amino acid residues of PLTP, Arg_{218} and Arg_{245}, might mediate the electrostatic interaction of PLTP with negatively charged groups of HDL and other lipoproteins. This is supported by the finding that these two basic amino acid residues at the N-terminal pocket are conserved among the entire protein family and correspond to Lys_{233} and Arg_{259} of CETP (Jiang et al., 1995), which was first demonstrated to bind lipoproteins via electrostatic and hydrophobic interactions (Masson et al., 1996; Nishida et al., 1993). More recently, it has been reported that a cluster of hydrophobic residues exposed at the N-terminal tip of the PLTP molecule substitutes for the positively charged LPS-binding region found on the surface of LBP and BPI, and is critical for the interaction of PLTP with HDL. These data indicate that at least two distinct HDL-binding sites exist on PLTP and the binding may involve both electrostatic and hydrophobic interactions (Desrumaux et al., 2001).

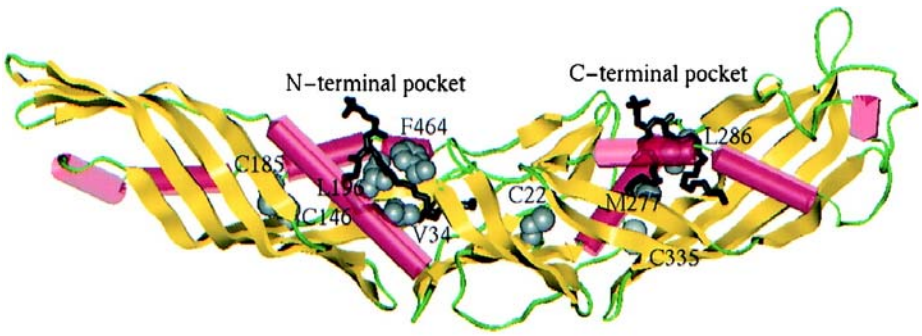

Figure 3. The structural model of human PLTP. The α helices are shown as red cylinders, the β strands as yellow arrows, and the phosphatidylcholine molecules as black sticks. The amino acid F464 is suggested to have a significant role in PLTP-mediated phospholipid transfer as well as in maintaining the proper conformation of the C-terminal tail of the protein (Huuskonen et al., 1999). Reproduced with the permission of The American Society for Biochemistry and Molecular Biology.

PLTP gene structure and regulation

PLTP belongs to the LT/LBP family together with LBP, BPI, and CETP (Day et al., 1994). PLTP shares 24% sequence homology with human LBP and 26% homology with human BPI (Day et al., 1994). The PLTP gene has been mapped to chromosome 20q12-q13.1 (Whitmore et al., 1995) and both the LBP and BPI genes to chromosome 20q11.23-q12 (Gray et al., 1993), whereas the CETP gene has been mapped to chromosome 16q12-q21 (Lusis et al., 1987). The exon-intron organizations of the genes for human PLTP, LBP, BPI, and CETP are highly conserved (Hubacek et al., 1997; Tu et al., 1995a). In addition, PLTP has identical number of amino acids as CETP and shares 20% sequence homology with it. Both PLTP and CETP genes have 16 exons, and the corresponding exons are similar in size. In addition, eight out of fifteen exon-intron junctions are located at the same amino acid residues. Although PLTP shares somewhat higher sequence homology with LBP and BPI, it is functionally more similar to CETP as both of these proteins facilitate the transfer of lipids between lipoproteins. This and the similar genomic organization suggest that the PLTP and CETP genes evolved from a common ancestral gene (Tu et al., 1995a).

The transcription process for the human PLTP gene may be initiated at several locations as four transcriptional initiation sites have been mapped at positions -75, -76, -77, and -80 with respect to the translational starting codon. The functional promoter region of the gene coding for human PLTP consists of a TATA box, a high

GC region, and multiple consensus sequences for the potential binding of several transcription factors, which appear to be of the ubiquitous type. These include Sp1, AP-2, AP-3, CCAAT/enhancer-binding protein (C/EBP), and nuclear factor kappa immunoglobulin enhancer-binding protein (NF-kB) (Tu et al., 1995b). In addition, several potential binding sites for peroxisome proliferator activated receptor (PPAR) and sterol regulatory element binding protein (SREBP) have been found (Tu & Albers, 1999). The response elements for NF-kB suggest that lipopolysaccharide could regulate the expression of the human PLTP gene (Jiang & Bruce, 1995; Sen & Baltimore, 1986). On the other hand, the presence of both SREBP and SP-1 recognition motifs within DNA sequences between -769 and -719 in the human PLTP gene implies that cholesterol or lipoproteins via SREBP may regulate PLTP transcription. In addition, bile acids and the farnesoid X-activated receptor (FXR) have been reported to enhance the expression of PLTP gene (Urizar et al., 2000), while the down-regulation of the human PLTP gene by fibrate is suggested to occur via PPAR regulatory pathway (Tu & Albers, 1999). More recently, the liver X receptors (LXR) and the heterodimer of LXR and retinoid X receptor have been shown to induce PLTP expression in macrophages (Cao et al., 2002; Desrumaux et al., 2003; Laffitte et al., 2003). In mice, the apoF gene and the gene encoding for FXR, Nr1h4, have been suggested to be the candidate genes located within one of the five identified loci involved in the regulation of PLTP activity (Korstanje et al., 2004).

The DNA sequence between -230 and -159 relative to the first transcriptional initiation site is suggested to be responsible for the full promoter activity. The potential binding sites for glucocorticoid receptor, Sp1, and AP-2 are located within this region (Tu et al., 1997b). However, mutagenesis of the response elements for PPAR and C/EBP at -322 to -299 caused reduced promoter activity of the PLTP gene, which may indicate the importance of these transcription factors for the regulation of the human PLTP gene transcription (Tu & Albers, 1999). In addition, no single region within the gene promoter is fully responsible for the promoter activity but several DNA sequences seem to be crucial for the basal transcription of the human PLTP gene (Tu et al., 1995b; Tu et al., 1997b).

Genetic variation of the human PLTP gene has been reported. In a Finnish population study, nucleotide changes at four sites were observed in the promoter region of the PLTP gene. In addition, two other polymorphisms causing amino acid changes Phe_2Leu and $Arg_{121}Trp$ were identified. However, none of the polymorphisms affected the serum phospholipid transfer activity (Tahvanainen et al., 1999). In a population study of French Canadians, intronic variants of the PLTP gene have been found to associate with obesity-related phenotypes. However, no polymorphism was found within the promoter or the coding regions of the PLTP gene (Bosse et al., 2005). On the other hand, a number of sequence anomalies within the transcribed

region of PLTP gene have been identified in a population study carried out in USA, and variation in the PLTP gene has been demonstrated to impact disorders of HDL metabolism. A polymorphism in exon 1 of PLTP, the c.-34G>C minor allele, has been shown to associate with antiatherogenic changes in HDL cholesterol, TG, and their ratio. Furthermore, a missense mutation of the Arg_{235} has been observed to result in decreased PLTP activity (Aouizerat et al., 2006).

PLTP tissue expression

Numerous human cell types and tissues express PLTP mRNA. The highest expression levels have been detected in the ovary, thymus, and placenta. Moderate levels of PLTP mRNA have been found in the pancreas, small intestine, testes, lung, and prostate. In the kidney, liver, and spleen the mRNA expression was found to be low and even lower in the heart, colon, skeletal muscle, leukocytes, and brain (Albers et al., 1995). This indicates that PLTP expression is locally regulated within each tissue type. Relatively high concentrations of PLTP mRNA detected in the ovary, placenta, testes, and prostate may suggest that PLTP plays a role in reproduction and fetal development. It has also been speculated that expression of PLTP in the lung might have an important role in maintaining normal function of this organ. In a mouse model for emphysema as well as in emphysematous patients, PLTP mRNA expression has been reported to be 3- to 4-fold higher than in the control subjects indicating that a hypoxic stimulus occurring in emphysema may contribute to the enhanced PLTP gene expression (Jiang et al., 1998). Recently, PLTP has been shown to play a key role in sperm motility and male fertility in a mouse model (Drouineaud et al., 2006). The expression of PLTP mRNA in small intestine and enhanced secretion of the protein during postprandial conditions may have a significant role as PLTP facilitates the transfer of phospholipids from triglyceride-rich lipoproteins to HDL during lipolysis. In spite of low expression level of PLTP mRNA in the liver, it could still be a major contributor to the plasma PLTP concentration due to its large organ mass (Albers et al., 1995). In human plasma, PLTP circulates mainly together with larger HDL_2 particles (Speijer et al., 1991), and its concentration has been estimated to be 4-15 mg/l (Desrumaux et al., 1999a; Huuskonen et al., 2000a; Oka et al., 2000b). Recently, PLTP-mediated PL transfer activity has also been detected in human tear fluid (Jauhiainen et al., 2005).

The tissue expression of PLTP mRNA varies between species. The tissue distribution of mouse and pig PLTP mRNA has been reported to be similar and to differ to some extent from the human mRNA distribution. Unlike the human brain tissue, relatively high levels of PLTP mRNA have been detected both in the mouse and pig brain. In addition, relatively high expression levels of PLTP mRNA have been detected in human placenta, intestine, and kidney, while in the corresponding mouse or

pig tissues the expression levels were low (Albers et al., 1995; Day et al., 1994; Pussinen et al., 1997b).

High- and low-activity forms of PLTP

The development of the methods for determining PLTP concentration (Desrumaux et al., 1999a; Huuskonen et al., 2000a; Oka et al., 2000b) revealed that PLTP exists in human plasma in two different forms, one with high activity (HA-PLTP) and the other with low activity (LA-PLTP) (Oka et al., 2000a). However, the estimates of the relative concentrations of the HA- and LA-PLTP in human plasma vary. This has been suggested to be due to different reactivities of the antibodies used in mass assays against the two PLTP forms (Murdoch et al., 2002b).

Only one gene has been reported to code for both forms of the protein indicating that the regulation of PLTP activity occurs at the post-translational level. The two PLTP forms in human plasma have been shown to associate with distinct macromolecular populations. In size-exclusion chromatography, PLTP activity has been demonstrated to elute with an average mass of 160 kDa, whereas the low-activity form of PLTP has been shown to elute in a position corresponding to a molecular size of 520 kDa (Oka et al., 2000a). In plasma of mice expressing human PLTP, two populations of PLTP have also been observed. One population has been shown to have low specific activity, whereas the other population displayed high specific activity (Jaari et al., 2001). Furthermore, PLTP secreted by human hepatoma cell line, HepG2, has been shown to resemble the high-activity form of PLTP found in human plasma. Similarly to HA-PLTP in human plasma, PLTP purified from HepG2 cell medium has been found to elute in size-exclusion chromatography at the position corresponding to a molecular size of 160 kDa. In addition, PLTP has been demonstrated to co-elute with apoE but not with apoA-I or apoB-100, and the activity could be inhibited only with antibodies against PLTP or apoE (Siggins et al., 2003). Contrary to earlier findings, in the study of Cheung and Albers (2006), active PLTP was found to associate primarily with apoA-I but not with apoE-containing lipoproteins when detected directly from the plasma samples without a prior purification of the two PLTP forms.

2.4.2 Functions of PLTP

Lipid transfer

PLTP is a non-specific lipid transfer protein that preferentially transfers phospholipids between different lipoproteins (Massey et al., 1985). The bulk of PLTP-mediated phospholipid transfer most probably occurs between HDL particles as 89 mol % of lipoproteins in men and 94 mol % of lipoproteins in women are HDL (Smith et al.,

1983). Unlike CETP, PLTP is unable to transfer neutral lipids although the C-terminal end of the protein is somewhat hydrophobic. It has been shown, however, that PLTP is able to enhance the CETP-mediated transfer of cholesteryl esters between HDL_3 and LDL or VLDL (Lagrost et al., 1994a; Tollefson et al., 1988). PLTP-mediated PL transfer is suggested to occur through protein and lipoprotein bi- or trimolecular collisions (Rao et al., 1997) and without formation of a tight complex with phospholipids (Huuskonen et al., 1996). In addition, the electric charge of lipoproteins has been shown to influence the activity of the human plasma PLTP suggesting that the interaction of PLTP with lipoproteins is electrostatic. The effect of lipoprotein surface potential on the activity of PLTP was found to be similar to that of CETP. The optimal surface potential value of HDL particles for maximal transfer rate of PLTP was observed to be -11.6 mV (Desrumaux et al., 1998).

PLTP has been shown to transfer a variety of lipids with two acyl chains and a polar head group (Huuskonen et al., 1996; Rao et al., 1997). The most preferred substrate was found to be diacylglycerol followed by phosphatidic acid, sphingomyelin, phosphatidylcholine, phosphatidylglycerol, cerebroside, and phosphatidyl-ethanolamine. Increase in acyl chain length from 16 or 18 to 20 methylenes or decrease from 10 to 6 methylenes has been shown to associate with slower PLTP-mediated transfer rates, whereas unsaturation of one acyl chain of phosphatidylcholine molecule greatly enhanced the transfer by PLTP. The exchange of unsaturated fatty acids between sn-1/sn-2 positions was observed to have only small effects on PLTP transfer rate. In addition, PLTP was shown to transfer phosphatidylcholine less efficiently from reassembled HDL that were small in size or with high UC content (Rao et al., 1997). The latter may be explained by a competition reaction as it has been reported that PLTP is also capable of transferring unesterified cholesterol (Nishida & Nishida, 1997).

Varying levels of PLTP activity can be detected in plasmas of several vertebrate species. The activity levels in mouse and rat have been reported to be higher than in humans, while in rabbit and guinea pig the activity was significantly lower than in humans. In hamster, dog, pig, and monkey PLTP activity was comparable to the levels detected in human plasma (Cheung et al., 1996). On the other hand, in the study of Speijer and coworkers (1991), rat was reported to have considerably lower activity levels as compared to humans. Furthermore, it has been reported that animals such as chicken, pig, rabbit, and man, which are relatively susceptible for atherogenesis, display lower mean PLTP activity than animals such as cat, dog, mouse, and rat, which in turn can resist the progression of atherosclerotic diseases more effectively (Guyard-Dangremont et al., 1998).

HDL conversion

PLTP is an important mediator of HDL metabolism, having a considerable impact on the heterogeneity of circulating HDL and especially on the generation of preβ-HDL particles simultaneously with large-sized HDL. PLTP and CETP have been shown to have opposite effects on HDL size distribution as PLTP promotes the formation of HDL_{2b} particles at the expense of HDL_{3a}, whereas CETP increases the relative proportion of both HDL_{3b} and HDL_{3c} at the expense of HDL_{2a} (Lagrost et al., 1996). On the other hand, PLTP has also been shown to be capable of promoting the formation of preβ-HDL particles from HDL_2 (Marques-Vidal et al., 1997). In fluorescence spectroscopic measurements, PLTP has been shown to promote mixing of cholesteryl esters in the core of reassembled HDL particles (Lusa et al., 1996) indicating that the remodeling of HDL particles occurs through a particle fusion process (Albers et al., 1995; Jauhiainen et al., 1993; Lusa et al., 1996; Settasatian et al., 2001; Tu et al., 1993; von Eckardstein et al., 1996), which has also been demonstrated by ^{1}H NMR spectroscopy (Korhonen et al., 1998). According to the hypothesis of Settasatian and coworkers (2001), the initial event in HDL_3 particle conversion involves interaction of PLTP with the phospholipid surface of HDL particles, which induces an increase in the surface pressure either due to increased lipid concentration on the particle surface or penetration of PLTP into the lipid monolayer. This, in turn, causes fusion of HDL particles and formation of an unstable large particle that rearranges into small particles each containing two apoA-I molecules (Figure 4). Alternatively, the unstable fusion particle could rearrange into a more stable, large fusion product containing four apoA-I molecules with a concomitant dissociation of lipid-free or lipid-poor apoA-I molecules. In accordance with this hypothesis, it has been shown that PLTP-mediated HDL conversion is dependent on efficient phospholipid transfer (Huuskonen et al., 2000b).

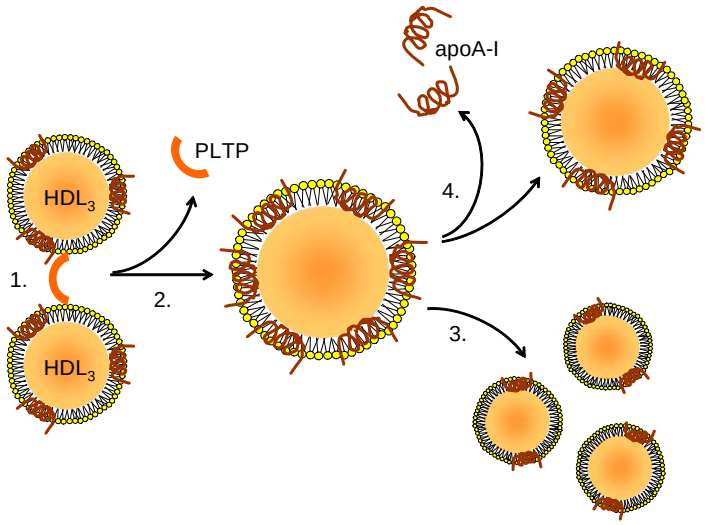

Figure 4. A model for PLTP-mediated remodeling of HDL. The formation of a ternary collision complex of two HDL_3 particles, each of which contain three molecules of apoA-I, and of one boomerang-shaped PLTP molecule (1) leads to a fusion of HDL_3 particles and subsequent formation of a large unstable particle with six molecules of apoA-I (2). The fusion product either rearranges into three small particles each containing two apoA-I molecules (3) or is converted into a large, more stable fusion particle with a concomitant dissociation of two lipid-free or lipid-poor apoA-I molecules (4). Adapted from Settasatian et al., 2001.

Triglyceride enrichment of HDL has been reported to enhance HDL remodeling by PLTP (Rye et al., 1998), while oxidative modifications of HDL impair the PLTP-mediated HDL conversion (Pussinen et al., 2003). Enhanced conversion of TG-rich HDL particles has been suggested to be due to the destabilization of apoA-I in TG-rich HDL (Settasatian et al., 2001). In addition, a hydrophobic cluster in the N-terminal end of PLTP has been shown to be critical for the conversion activity of the protein (Desrumaux et al., 2001). The molar ratio of apoA-I and apoA-II in HDL particles has been reported to have an influence on PLTP-mediated HDL conversion (Albers et al., 1995). PLTP has been reported to have a distinctly different effect on the HDL subpopulations containing only apoA-I or both apoA-I and apoA-II. The difference could be observed in the size distribution of these subpopulations after the interconversion by PLTP. Larger average size of fusion particles were observed

among a subpopulation containing only apoA-I. Also, increasing concentrations of apoA-II in HDL have been shown to result in impaired remodeling of HDL by PLTP, and the conversion was found to be totally inhibited when HDL particles contained only apoA-II. This suggests a significant role for apoA-I in the HDL conversion process (Lusa et al., 1996; Pussinen et al., 1997a).

Besides human recombinant or plasma-derived PLTP, also mouse and pig recombinant or plasma-derived PLTP, respectively, has been shown to promote HDL interconversion *in vitro* (Albers et al., 1995; Pussinen et al., 1995). This suggests that PLTP may function similarly in these species. Furthermore, the results from experimental studies with mice transgenic for the human apoA-I gene and overexpressing human PLTP demonstrate that PLTP facilitates HDL conversion also *in vivo* (Ehnholm et al., 1998).

Other functions

PLTP has been found to enchance cholesterol efflux from peripheral cells and the uptake of cholesterol by preβ-HDL (von Eckardstein et al., 1996) as well as by HDL particles with electrophoretic mobility between alpha and preβ (Wolfbauer et al., 1999). This has been shown to occur through an interaction of PLTP with a cell membrane transporter protein, ABCA1 (Oram et al., 2003). In accordance with this, endogenous PLTP synthesized by macrophage foam cells has been shown to have a significant contribution to the ABCA1-mediated cholesterol efflux in these cells (Lee-Rueckert et al., 2006). In addition, macrophage-derived PLTP has been observed to inhibit the development of atherosclerotic lesions in hyperlipidemic mice, whereas PLTP deficiency was observed to result in significantly smaller concentrations of vitamin E and increased oxidative stress of macrophages, thus suggesting an antiatherogenic role for PLTP within the vessel wall (Valenta et al., 2006).

In addition, it has been suggested that PLTP could display inherent protease activity on apoA-I. The cleaveage has been demonstrated to occur in the C-terminal end of apoA-I, between the residues Ala_{196} and Thr_{197} (Jauhiainen et al., 1999). However, the effect of PLTP-mediated proteolysis of apoA-I on RCT has not been elucidated. On the other hand, mast cell chymase has been shown to degrade both PLTP and preβ-HDL particles generated by PLTP *in vitro* (Lee et al., 2003). Furthermore, mast cell chymase has also been shown to degrade apoA-II and apoE in mouse plasma deficient for apoA-I, which has been shown to result in impaired cholesterol efflux from macrophage foam cells (Lee et al., 2002).

Besides lipoprotein metabolism, PLTP may also play a role in antimicrobial defense and maintenance of the antioxidant status of cells. In addition to lipids, plasma PLTP facilitates the transfer of some other amphipathic molecules, such as lipopoly-

saccharides (Hailman et al., 1996) and α-tocopherol (Kostner et al., 1995), between lipoproteins as well as between lipoproteins and cells. HDL and other plasma lipoproteins are important neutralizers of the bioactivity of LPS, a membrane lipid of Gram-negative bacteria. PLTP has been shown to release LPS from Gram-negative bacterial membranes when HDL particles are present, thus reducing the host immunological responses to LPS (Vesy et al., 2000). Unlike LBP or soluble CD14, PLTP is incapable of enhancing the release of LPS from monocyte cell surface (Kitchens et al., 1999). It has been reported, however, that PLTP is able to neutralize the biological activity of LPS simply by binding it, thus preventing its recognition by soluble or membrane bound CD14 or other cellular receptors. Also, PLTP was found to inhibit interleukin-6 production in response to LPS (Hailman et al., 1996). On the other hand, injection of LPS into mice has been shown to cause a significant decrease both in PLTP activity and mRNA expression in the lung, adipose tissue, and liver (Jiang & Bruce, 1995).

The most potent antioxidant form of vitamin E, α-tocopherol, is secreted from the liver into the bloodstream and transported by lipoproteins in the plasma compartment. It has been demonstrated that PLTP promotes the transfer of α-tocopherol from HDL to oxidized LDL, suggesting an important role for PLTP in restoring the antioxidant profile of plasma LDL particles, hence hindering the development of atherosclerosis (Desrumaux et al., 1999b). In addition, PLTP was shown to sustain the endothelium-dependent arterial relaxation by supplying endothelial cells with α-tocopherol. Furthermore, PLTP deficiency has been shown to result in significant depletion of brain α-tocopherol producing a chronic and moderate oxidative stress and increased anxiety in both homozygous and heterozygous PLTP deficient mice (Desrumaux et al., 2005). On the other hand, PLTP deficiency (PLTP-/-) has been demonstrated to result in increased vitamin E content of VLDL and LDL, thereby protecting them from oxidation (Jiang et al., 2002). PLTP has also been proposed to facilitate the transfer of amphotericin B, an antibiotic used in the treatment of systemic fungal infections, to HDL (Patankar & Wasan, 2006).

2.4.3 Factors affecting PLTP activity and concentration

Diet-derived and personal attributes

In obese individuals, PLTP activity has been observed to be elevated. PLTP activity has been shown to correlate positively both with body mass index (BMI) and waist-to-hip circumference ratio (WHR) (Cheung et al., 2002; Dullaart et al., 1994b; Kaser et al., 2001; Murdoch et al., 2000; Tahvanainen et al., 1999). Decreased PLTP activity upon diet-induced weight loss has been shown to associate with changes in sub-

cutaneous fat and free fatty acids (Murdoch et al., 2003). In obese women, a short-term weight loss has been shown to result in a concomitant and parallel reduction in PLTP and CETP concentrations (Tzotzas et al., 2006). Furthermore, weight loss after gastric banding surgery has been demonstrated to result in a significant decrease in PLTP activity as well as increased HDL_2 particle size (Kaser et al., 2004).

The influence of dietary lipids on PLTP activity has been studied quite intensively. When compared with a dairy fat-based diet, PLTP activity and HDL cholesterol concentration have been shown to reduce significantly during a stearic acid diet, whereas diet enriched in unsaturated fatty acids with *trans* double bonds had no influence on PLTP activity despite reduced HDL cholesterol levels (Aro et al., 1997). In normolipidemic men and women consuming a lauric acid diet, PLTP activity was found to be higher than in subjects consuming diet enriched in palmitic acid. This indicates that also the chain length of saturated fatty acids modulates serum PLTP activity. The influence of oleic acid diet was reported not to differ significantly from the influences of saturated diets on PLTP activity (Lagrost et al., 1999b). Cholesterol feeding has been shown to result in significant rises in both PLTP and CETP activities in rabbits (Meijer et al., 1993). As in rabbits, high fat and high cholesterol diet increased serum phospholipid transfer activity in mice, which was also associated with increased PLTP mRNA levels in the lung, suggesting that elevated plasma lipid concentration regulates PLTP expression (Jiang & Bruce, 1995; Tu et al., 1999). Consistent with these findings, a large intravenous fat load has been shown to stimulate plasma PLTP activity in healthy men (Riemens et al., 1999a). However, after administration of a single fatty meal containing moderate amounts of cholesterol, PLTP activity has been reported to remain unchanged in normolipidemic men (Syeda et al., 2003). In addition, PLTP activity has been shown to correlate positively with serum triglyceride concentration (Tahvanainen et al., 1999). High amounts of the cholesterol-raising diterpenes, cafestol and kahweol, which are present in unfiltered coffee, have been shown to significantly increase serum PLTP activity (van Tol et al., 1997).

Moderate alcohol consumption has been shown to correlate negatively with CAD (Rimm et al., 1991) and positively with plasma HDL levels (Castelli et al., 1977). Among alcohol abusers, plasma PLTP activity has been observed to be significantly higher than in the control subjects (Liinamaa et al., 1997). In addition, in alcoholic patients, PLTP activity has been reported to reduce significantly after alcohol withdrawal (Lagrost et al., 1996) with a concomitant shift of HDL particle size towards smaller particles (Taskinen et al., 1982; Välimäki et al., 1993). Furthermore, elevated HDL cholesterol associated with moderate alcohol consumption has been reported not to be related to altered plasma PLTP, CETP or LCAT activity levels (Riemens et al., 1997).

Besides dietary lipids and alcohol consumption, smoking has been reported to influence PLTP activity. Among normolipidemic men, smoking has been shown to impair several steps in the RCT process. In the fasting (Zaratin et al., 2004) or postprandial state (Mero et al., 1998), smoking has been shown to result in reduced activities of several plasma enzymes and lipid transfer proteins including PLTP, CETP, and HL. Concerning the plasma lipid transfer protein activities, opposite results have been reported by Dullaart and colleagues (1994a) although unfavorable changes in plasma lipoprotein profile were also observed in this study.

Inflammation

PLTP activity has been reported to be elevated in patients with a systemic inflammation (Barlage et al., 2001) or a severe acute-phase response (Pussinen et al., 2001b). PLTP mass, on the other hand, has been found to be significantly lower in patients with acute-phase response than in the control subjects (Pussinen et al., 2001b) despite the finding that during the acute phase response, the synthesis of several plasma glycoproteins is increased in the liver (Mookerjea et al., 1983). Also, in an experimental study, PLTP-mediated remodeling of acute-phase HDL_3 particles has been demonstrated to be more efficient than the conversion of native HDL_3 particles despite the displacement of apoA-I by SAA. This could be explained in part by elevated triglyceride content of HDL during acute phase response (Pussinen et al., 2001a; Rye et al., 1998). Furthermore, the apoA-I of acute-phase HDL was found to be more prone to PLTP-mediated degradation (Jauhiainen et al., 1999), which may lead to accelerated catabolism of degraded apoA-I and result in decreased HDL levels during inflammation (Brinton et al., 1994; Brinton et al., 1991). Elevated PLTP activity has also been shown to be related to chronic subclinical inflammation in patients with type 2 diabetes (Tan et al., 2005).

Diabetes

Patients with type 1 or type 2 (non-insulin-dependent) diabetes display elevated serum PLTP activity (Colhoun et al., 2002; Riemens et al., 1998b) and mass (Desrumaux et al., 1999a). PLTP activity has been shown to correlate with concentrations of small, dense LDL particles and apoE in patients with type 2 diabetes (Tan et al., 2003; Tan et al., 2006). Furthermore, treatment with atorvastatin has been shown to result in decreased PLTP activity with a concomitant decrease in apoE concentration (Dallinga-Thie et al., 2006). In type 2 diabetes, plasma cholesteryl ester transfer has been found to be elevated and associated with higher plasma PLTP activity levels as well as with triglyceride concentration (Riemens et al., 1998a). Furthermore, in subjects with type 2 diabetes or CAD, PLTP activity and HDL particles containing both apoA-I and apoA-II have been reported to be the main deter-

minants inducing the plasma cholesterol efflux potential from Fu5HA rat hepatoma cells (Syvänne et al., 1996). On the other hand, lowered plasma phospholipid transfer activity and increased diacylglycerol concentration among plasma lipoproteins have been demonstrated among type 2 diabetic patients (Elchebly et al., 1996). The discrepancy between different studies has been speculated to result from the substrate specificity of PLTP and favoring of diacylglycerols over phospholipids leading to a bias in PLTP activity assay measuring only transferred PL (Lalanne et al., 1999). In non-diabetic, insulin resistant subjects, plasma PLTP activity has also been reported to be elevated (Riemens et al., 1999c; Van Tol et al., 1997) resulting in enhanced preβ-HDL formation and cellular cholesterol efflux despite lower total HDL levels (Dullaart & van Tol, 2001). In addition, insulin infusion has been shown to result in decreased PLTP activity (Riemens et al., 1999b; Riemens et al., 1999c).

Alzheimer's disease

Increased PLTP mRNA and protein expression in central nervous system has been demonstrated in patients with Alzheimer's disease (AD) indicating a role for PLTP in lipid metabolism in the brain (Vuletic et al., 2003). In cell culture experiments, neurons, microglia, and astrocytes have been shown to synthesize and secrete active PLTP. In addition, the presence of PLTP also in oligodendroglia has been demonstrated by immunohistochemistry indicating that all classes of brain cells synthesize PLTP. PLTP activity has been detected in cerebrospinal fluid (CSF) of neurologically healthy subjects representing approximately 15% of the plasma activity (Vuletic et al., 2003). However, in patients with probable AD or with other neurological diseases, PLTP activity in CSF was found to be significantly lower than in control subjects (Vuletic et al., 2005). In the study of Demeester and colleagues (2000), PLTP mass and activity in CSF of both healthy and AD subjects have been reported to represent only 1% of the values detected in human plasma. The differences in observed activity levels in CSF between the two studies might result from different methods used for determining PLTP activity. The activity in CSF was also shown to correlate positively with apoE concentration in both AD and control subjects and to associate with apoE-containing lipoprotein particles to some extent. In addition, PLTP was shown to significantly enhance the secretion of apoE in primary human astrocytes *in vitro* (Vuletic et al., 2005). On the other hand, statins with differing blood-brain barrier penetrability, simvastatin and pravastatin, have been reported to have different effects on CSF PLTP activity and apoE concentration in modestly hypercholesterolemic, cognitively intact individuals (Vuletic et al., 2006). Simvastatin, which penetrates blood-brain barrier, was reported to significantly increase PLTP activity in CSF, while pravastatin, which does not penetrate blood-brain barrier, had no such effect. In addition, treatment with both simvastatin and

pravastatin was shown to result in lowered CSF apoE concentration, indicating that the statin treatment might have affected the relationship between PLTP and apoE.

2.4.4 PLTP and atherosclerosis

Clinical studies

The role of PLTP activity in human diseases and other physiological states has been studied quite intensively as discussed above, while the evaluation of PLTP concentration still awaits further investigation. Several studies have demonstrated a positive correlation between PLTP activity and HDL cholesterol (Cheung et al., 2002; Murdoch et al., 2000; Murdoch et al., 2002a) although opposite results have also been reported (Huuskonen et al., 2000a; Schlitt et al., 2003). In a prospective study of Japanese men, PLTP concentration was found to correlate positively with serum HDL cholesterol and negatively with TG, LDL cholesterol, and BMI. In addition, a significant, independent, and inverse relationship was reported between serum PLTP concentration and the risk of cardiovascular diseases (Yatsuya et al., 2004). However, PLTP activity has been shown to be positively related to the metabolism of the apoB-containing lipoproteins (Cheung et al., 2002; Murdoch et al., 2000; Murdoch et al., 2002a; Riemens et al., 1998a). Both gender and obesity-related factors have been suggested to modulate the effect of PLTP activity on the apoB-containing lipoproteins (Cheung et al., 2002). In healthy obese men or in patients with type 1 or type 2 diabetes, PLTP activity has been observed to be increased from 15 to 50% (Colhoun et al., 2002; Dullaart et al., 1994b; Riemens et al., 1998b). Moreover, in contrast to PLTP concentration, PLTP activity has been shown to be an independent risk factor for CAD (Schlitt et al., 2003).

Results from immunohistochemistry studies have demonstrated that PLTP is highly expressed by macrophages and smooth muscle cells within human atherosclerotic lesions. Cholesterol loading of macrophages has been demonstrated to result in 2- to 3-fold increase in both PLTP mRNA and protein expression as well as in elevated activity (Desrumaux et al., 2003; Laffitte et al., 2003; O'Brien et al., 2003). Hypoxia has been shown to increase PLTP mRNA expression in the lung (Jiang et al., 1998) and to result in the formation of TG-loaded macrophage foam cells within the atherosclerotic lesions (Bostrom et al., 2006), which may in part explain the enhanced expression of PLTP within the lesions. Furthermore, PLTP has been shown to accumulate on extracellular matrix, colocalizing with apoA-I, apoE, apoB, and the vascular proteoglycan biglycan, to which PLTP has been observed to mediate HDL binding *in vitro*. This function of PLTP has been reported to be independent of its phospholipid transfer

activity, suggesting that both the active and inactive forms of PLTP can facilitate HDL retention on vascular proteoglycans (O'Brien et al., 2003).

In treatment of atherosclerosis, the most effective and commonly used agents for lowering LDL cholesterol and raising HDL cholesterol are statins and niacin, respectively. Treatment of patients with low HDL and CAD with simvastatin-niacin has been shown to result in increased concentration of HDL particles containing apoA-I as the sole apolipoprotein. However, despite the positive relationship between PLTP activity and HDL particles containing apoA-I at baseline (Cheung et al., 1999), no consistent change in PLTP activity could be detected after treatment with simvastatin-niacin, suggesting that the drug therapy might have affected this relationship (Cheung et al., 2001). Furthermore, it has been suggested that simvastatin treatment of patients with type IIb hyperlipidemia does not affect PLTP mass although a significant increase in PLTP activity has been detected in an endogenous lipoprotein-dependent assay. However, no change in PLTP activity could be observed in an exogenous lipoprotein-independent assay among these patients (Lagrost et al., 1999a). Hypertriglyceridemia has been shown to result in decreased HDL cholesterol and PLTP mass values. Bezafibrate-induced increase in HDL cholesterol, however, has been reported not to affect PLTP concentration. The bezafibrate therapy has been suggested to improve insulin resistance, and thereby lead to a decrease in PLTP activity (Jonkers et al., 2003).

Animal models

Genetically altered mouse models have played a crucial role in elucidating the impact of PLTP on lipoprotein metabolism and progression of atherosclerosis. The mouse gene encoding phospholipid transfer protein has been mapped to chromosome 2 (LeBoeuf et al., 1996), and the genomic organizations of both the mouse and human PLTP genes have been found to be similar (Tu et al., 1997a). Revealing of the mouse PLTP gene structure has enabled the modulation of PLTP gene expression.

A human PLTP deficiency state has not been described, whereas in mice, PLTP deficiency has been shown to result in decreased plasma HDL levels independent of diet. In addition, in PLTP deficient (PLTP-/-) mice on a high fat diet, an increase in VLDL and LDL phospholipids, unesterified cholesterol, and cholesteryl esters without a change in apoB concentration has also been observed. This has been reported to result from accumulation of IDL/LDL size vesicular lipoproteins enriched in UC and PL, indicating impaired transfer of surface remnants from TG-rich lipoproteins to HDL (Jiang et al., 1999). These accumulating lamellar lipoproteins have been reported to contain apoA-IV and apoE as major apolipoproteins (Qin et al., 2000). Hepatic lipase and SR-BI have been demonstrated to have a major role in the clearance of PL and UC of these surface remnants in PLTP-/- mice (Kawano et al.,

2002). Experimental studies with hypercholesterolemic mice deficient of the LDL receptor (LDLR-/-) and with normal plasma apoA-I levels have demonstrated the atheroprotective role of macrophage-derived PLTP within the blood vessel wall (Valenta et al., 2006). In these mice, PLTP was found to inhibit lesion development, suggesting that pro- or antiatherogenic properties of PLTP are dependent on the site of protein expression and action. In addition, PLTP deficiency was demonstrated to result in decreased α-tocopherol content and increased oxidative stress of bone marrow macrophages *in vitro*.

Mice expressing human PLTP have significantly higher serum HDL cholesterol and lower non-HDL cholesterol than wildtype mice despite similar plasma PLTP activity levels (Albers et al., 1996). Overexpression of human PLTP in mice, wildtype or transgenic for the human apoA-I gene, has been shown to result in increased formation of preβ-HDL particles (Ehnholm et al., 1998; Föger et al., 1997; Jiang et al., 1996). Consistent with this finding, plasma of mice overexpressing human PLTP has been demonstrated to be more efficient in preventing the cholesterol accumulation in cultured macrophages than plasma of wildtype mice (van Haperen et al., 2000), indicating an important role for PLTP in modulating RCT *in vivo*. As mice by nature do not express CETP, the relative contribution of PLTP and CETP to the generation of preβ-HDL particles has been studied in mice transgenic either for human PLTP or human CETP or both. The results from this study have demonstrated that PLTP rather than CETP is responsible for the formation of preβ-HDL (Lie et al., 2001). Furthermore, a dramatic reduction in total HDL cholesterol and enhanced hepatic uptake of HDL phospholipids and cholesteryl esters as well as increased fecal bile acid excretion have been reported in mice with elevated PLTP activity (Ehnholm et al., 1998; Föger et al., 1997; Jiang et al., 1996; Post et al., 2003).

Several experimental studies have identified plasma PLTP as a risk factor for atherosclerosis. It has been reported that a 2-fold increase in PLTP activity either in heterozygous LDL receptor-deficient (LDLR+/-) mice or in mice with apoE deficiency (apoE-/-), results in moderately increased VLDL secretion, increased atherosclerotic lesions, decreased plasma content of vitamin E, increased lipoprotein oxidation, and increased auto-antibodies against oxidized LDL (van Haperen et al., 2002; Yang et al., 2003). However, a moderate 1.3-fold increase in PLTP activity in these mice has been shown not to associate with atherogenesis (Yang et al., 2003). In mice transgenic for human CETP, a 2.8-fold overexpression of PLTP has been shown to result in a 1.5-fold increase in VLDL secretion as compared to mice expressing only human CETP (Lie et al., 2002). In heterozygous LDL receptor-deficient (LDLR+/-) mice expressing both human PLTP and human CETP genes, expression of PLTP has been shown to result in a dose-dependent decrease in VLDL and LDL cholesterol as well as in HDL cholesterol (Lie et al., 2004). However, de-

spite the reduction of apoB-containing lipoproteins, these mice have been found to display increased atherosclerotic lesion areas as well as increased number of sections containing cholesterol clefts, thus demonstrating the atheroprotective role of HDL cholesterol and HDL-associated antioxidative enzymes, PON1 and PAF-AH.

On the other hand, PLTP deficiency (PLTP-/-) has been shown to associate with decreased secretion and plasma levels of apoB-containing lipoproteins in human apoB transgenic mice and in apoE-deficient (apoE-/-) mice (Jiang et al., 2001). PLTP deficiency has been shown to be related to decreased liver content of vitamin E as well as increased hepatic oxidant concentration. Moreover, PLTP deficiency has been reported to stimulate the intracellular oxidant-dependent proteolysis of newly synthesized apoB (Jiang et al., 2005). In PLTP-/- and LDLR-/- mice, accumulation of vitamin E has been reported to protect lipoproteins from oxidation and to result in lower atherosclerosis susceptibility despite a lack of reduction in apoB-containing lipoproteins (Jiang et al., 2002). Furthermore, PLTP deficiency (PLTP-/-) both in mice expressing human apoB and in LDLR-/- mice has been reported to result in higher affinity of plasma HDL for oxidized phospholipids, thereby reducing the ability of LDL to induce monocyte chemotactic activity *in vitro* (Yan et al., 2004).

PLTP has a vital role in the maintenance of plasma HDL levels. In addition, the ability of PLTP to generate preβ-HDL and to promote cell-surface binding of HDL has been shown to play an important role in enhancing flux of excess cholesterol from peripheral tissue cells and retarding atherogenesis. However, growing body of evidence suggests that inhibition of PLTP could serve as a target in the treatment of atherosclerosis. As PLTP has been shown to display both pro- and antiatherogenic properties depending on the site of protein expression and action, the overall effects of pharmacological modulation or inhibition of the PLTP gene on lipid and glucose metabolism are difficult to predict and remain a challenge for future studies. As the effect of CETP inhibitors on atherogenesis is now under clinical investigations (Okamoto et al., 2000), it is interesting to speculate, whether a phenotype of decreased serum apoB-lipoproteins and of normal HDL levels would result from the combined inhibition of CETP and PLTP in humans.

3 AIMS OF THE STUDY

1. To isolate and characterize the high-activity (HA) and low-activity (LA) forms of human plasma PLTP.
2. To develop an enzyme-linked immunosorbent assay (ELISA) to quantitatively determine the HA- and LA-PLTP concentrations in human plasma.
3. To study the distribution of the HA- and LA-PLTP and their relationship with lipid and carbohydrate parameters in a population sample.
4. To investigate the regulation mechanisms of PLTP activation by studying the interactions of PLTP with apoA-I, apoE, and apoA-IV.

4 MATERIALS AND METHODS

4.1 List of published methods

The methods used in the original articles and in this thesis are listed in the table below in alphabetical order. The Roman numerals indicate the original publication, in which the corresponding method is described.

Method	Original publication
Activation experiments of LA-PLTP	IV
Baculovirus expression of PLTP	II, IV
Biosensor analysis of PLTP interactions	IV
Detergent treatment of PLTP	II
Dextran-sulfate-$CaCl_2$ precipitation of LA-PLTP	II
Enhanced Chemiluminescence detection	I, II, IV
Enzymatic lipid analysis	I, III
Genotyping of apoE	III
Heparin-Sepharose Affinity Chromatography	I, II
Hydrophobic Chromatography	I
Hydroxylapatite Chromatography	I
Immunoaffinity Chromatography	I, II
Immunoprecipitation of PLTP	I
Lowry protein determination	I, II, III, IV
Non-denaturing gradient gel electrophoresis	IV
Plasma apolipoprotein and lipoprotein analysis	I, III, IV
Plasma CRP, glucose, and insulin analysis	III
PLTP activity assay	I, II, III, IV
PLTP mass determination by an ELISA	I, II, III, IV
Preparation of antibodies	I, II
Preparation of apolipoproteins	IV
Preparation of proteoliposomes and reconstituted HDL	IV
Purification of HA- and LA-PLTP	I, II, IV
SDS-PAGE and Western blotting	I, II, IV
Size-exclusion chromatography	I, II, IV
Statistical analyses	II, III

5 RESULTS AND DISCUSSION

5.1 The high-activity form of human plasma PLTP interacts with apoE and the low-activity form with apoA-I *in vitro*

Human plasma contains two forms of PLTP. One form has been demonstrated to display high specific activity (high-activity form, HA-PLTP), while the other form has been found to display no detectable PL transfer activity (low-activity form, LA-PLTP). Furthermore, these two PLTP forms in native human plasma have been shown to associate with distinct lipoprotein populations. In size-exclusion chromatography, active PLTP has been demonstrated to elute in the position of HDL with an average mass of 160 kDa, whereas the LA-PLTP has been shown to elute between LDL and large HDL, corresponding to an average molecular size of 520 kDa (Oka et al., 2000a). As previous reports have only described the purification of active PLTP (Barter et al., 1988; Jauhiainen et al., 1993; Pussinen et al., 1995; Tollefson et al., 1988) it was important to develop a method for the purification of the LA-PLTP as well.

5.1.1 Isolation and partial purification of the HA- and LA-PLTP

Heparin-Sepharose Affinity Chromatography

PLTP has been suggested to contain a heparin binding domain (Day et al., 1994). In this thesis, we separated the two forms of PLTP by Heparin-Sepharose (H-S) affinity chromatography. When fresh human plasma was subjected to a large-scale H-S column and recycled on the column overnight, 93 ± 5% (n=3) of the PLTP activity and 70 ± 15% (n=3) of the PLTP mass applied were bound to the matrix. The bound PLTP activity and mass were eluted with a linear 0-0.5 M NaCl gradient. The LA-PLTP displayed lower affinity for heparin and eluted first at a NaCl concentration of 0.15-0.2 M, while the HA-PLTP eluted at a 0.3-0.4 M concentration of NaCl (I, Fig. 1). In this first step of purification, the total recoveries of PLTP activity and mass were 110 ± 16% (n=3) and 90 ± 21% (n=3), respectively. HA-PLTP fractions represented less than 5% of the total PLTP mass recovered. The specific activity of recovered HA-PLTP was 3.5 μmol/h/μg, and that of the LA-PLTP 0.04 μmol/h/μg. For a comparison, the specific activity of PLTP in whole human plasma was 0.36 ± 0.22 μmol/h/μg as measured by the ELISA (Huuskonen et al., 2000a). The higher

affinity of the HA-PLTP for heparin could be due to the contribution of other proteins present in the protein complex, such as apoE. Alternatively, conformational differences between the LA- and HA-PLTP could also cause the difference in heparin binding affinity.

To verify that the sizes of the isolated PLTP forms correspond to those present in human plasma, the two PLTP fractions were applied to size-exclusion chromatography (I, Fig. 2). Both the LA- and HA-PLTP eluted in positions similar to those described by Oka and coworkers (2000a). The LA-PLTP eluted in a position corresponding to an average molecular mass of 520 kDa, while the HA-PLTP eluted with an average molecular mass of 160 kDa, demonstrating that H-S affinity chromatography does not disturb the integrity of the native macromolecular complexes of the LA- and HA-PLTP.

To further enrich the HA-PLTP isolated by a large-scale H-S affinity chromatography column, the fractions containing PLTP activity were subjected to a small-scale H-S column (I, Fig. 7). The HA-PLTP retained by the column was eluted with a linear 0.1-1.0 M NaCl gradient. After the second step of purification, 73% of the PLTP activity applied to the column was recovered. Analysis of the eluted fractions revealed that apoA-I eluted immediately before PLTP, whereas apoE was found to co-elute with the HA-PLTP. The eluted fractions containing the HA-PLTP were subjected to a size-exclusion chromatography column to analyze the size of the HA-PLTP complex (I, Fig. 4). The results indicated an elution position for the HA-PLTP corresponding to a molecular mass of 160 kDa.

Hydroxylapatite Chromatography

In the final step of the HA-PLTP purification, the fractions enriched with PLTP activity were combined and subjected to a hydroxylapatite [$Ca_5(PO_4)_3(OH)$] chromatography column, which binds proteins through Ca^{2+} and OH^- ions (I, Fig. 8). The bound material was eluted with a linear 1-50 mM sodium phosphate gradient followed by 100 mM sodium phosphate. PLTP activity eluted at a sodium phosphate concentration of approximately 20 mM. Of the HA-PLTP applied, the eluted fractions contained 91% of PLTP mass, 36% of PLTP activity, and some apoE but no apoA-I. The majority of bound apoE and apoA-I was eluted with 100 mM sodium phosphate. Size-exclusion chromatography demonstrated that after the final step of the HA-PLTP purification, both PLTP activity and mass as well as apoE co-eluted in the position of 160 kDa corresponding to the elution position of the HA-PLTP of human plasma (I, Fig. 9). Thus, the methods used for the purification of the HA-PLTP did not dissociate the molecular complex in which the HA-PLTP resides. In each purification step, the elution position of the HA-PLTP in size-exclusion chromatography remained unchanged in the presence or absence of high concentration (8

M) of urea indicating that the HA-PLTP complex might represent i) a PLTP dimer, ii) a urea-resistant protein complex, or iii) a PLTP molecule associated with lipids.

Hydrophobic Chromatography

To further enrich the LA-PLTP isolated by a large-scale H-S affinity chromatography, the fractions containing the majority of PLTP mass and only very little activity were subjected to hydrophobic chromatography on a butyl-Sepharose column (I, Fig. 3). The column was eluted with 50% ethanol, and 79% of the LA-PLTP mass could be recovered. The elution position of the LA-PLTP coincided with that of apoA-I, which comprised more than 90% of the total protein amount eluted. To analyze the size of the LA-PLTP complex after this purification step, the eluted fractions were applied to a size-exclusion chromatography column (I, Fig. 4). The LA-PLTP was found to elute with an approximated molecular mass of 520 kDa corresponding to the size of the LA-PLTP complex isolated from human plasma. However, when gel filtration analysis was performed in the presence of 8 M urea, the LA-PLTP was found to elute in a position corresponding to a molecular mass of 160 kDa, indicating that high concentration of urea can partly dissociate the LA-PLTP complex (I, Fig. 4). In principle, the LA-PLTP complex could represent a homomultimer of PLTP or PLTP associated with other plasma proteins and lipids.

Immunoaffinity Chromatography

The last step in the purification of the LA-PLTP comprised of an anti-PLTP immunoaffinity chromatography column prepared from the monoclonal antibody (mAb) against human PLTP (I, Fig. 5). Of the PLTP mass recovered from the hydrophobic chromatography column, 85% was retained by the anti-PLTP column and could be eluted by 0.1 M glycine, pH 2.5, containing 0.2% Tween 20. Most of the apoA-I did not bind to the matrix although a significant amount of apoA-I could be co-eluted with the LA-PLTP. Size-exclusion chromatography of the eluted LA-PLTP fractions revealed that the size of the LA-PLTP complex changed significantly in this purification step (I, Fig. 4). The molecular mass of the LA-PLTP was found to be 160 kDa corresponding to that of the HA-PLTP. However, no phospholipid transfer activity could be detected in the eluted LA-PLTP fractions. Binding of PLTP to its antibody might have dissociated a PLTP-apoA-I subcomplex, which may have originally been associated with large, HDL-like structures. High concentration of urea did not have any further influence on the size of the LA-PLTP complex (I, Fig. 4).

5.1.2 Characterization of the partially purified HA- and LA-PLTP

SDS-PAGE analysis

Analysis of the HA-PLTP fraction after the last purification step by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Coomassie staining, and Western blotting revealed a strong protein band in the region of 110-120 kDa and a band of 80 kDa, which was identified as PLTP by Western blotting (I, Fig. 6A). In addition, three groups of bands in the regions of 65-75 kDa, 55-65 kDa, and 30-35 kDa could be detected. Of these, the protein with a molecular mass of 34 kDa was identified as apoE by Western blotting. The identity and possible association of the other observed proteins with PLTP remains to be resolved. In the characterized HA-PLTP fraction no apoA-I could be detected.

Similar analysis of the LA-PLTP fraction after the last purification step revealed several protein bands with the apparent molecular masses of 80 kDa, 48 kDa, 40 kDa, and 28 kDa (I, Fig. 6B). Of these, the protein with a molecular mass of 80 kDa was identified as PLTP and the protein with a molecular mass of 28.1 kDa as apoA-I by Western blotting. No apoE could be detected in the LA-PLTP fraction.

Immunoprecipitation of PLTP

Previous *in vitro* and *in vivo* studies have suggested that PLTP is capable of binding to apolipoproteins (Barlage et al., 2001; Pussinen et al., 1998). Immunoprecipitation of human plasma as well as the LA- and HA-PLTP fractions obtained by size-exclusion chromatography was performed to investigate the association of PLTP with apoA-I, the major apolipoprotein of HDL (I, Fig. 10). About 95% of the PLTP mass was removed by anti-PLTP polyclonal antibody (pAb) from plasma as well as from both the LA- and HA-PLTP fractions. Similarly, the anti-PLTP pAb efficiently removed most of the PLTP activity from plasma and the HA-PLTP fraction. The LA-PLTP was omitted in this analysis due to its non-detectable PL transfer activity.

Immunoprecipitations were also performed using a pAb against apoA-I. This antibody precipitated about 80% of plasma PLTP protein, whereas only a minor 5% decrease was detected in plasma PLTP activity. Immunoprecipitation of the LA- and HA-PLTP fractions caused coprecipitation of 90% and 70% of the PLTP protein, respectively. However, only a marginal 3% decrease in the PLTP activity of the HA-PLTP fraction could be observed. Similar results were obtained when the LA-PLTP fraction obtained from immunoaffinity chromatography and the HA-PLTP fraction obtained from hydroxylapatite chromatography were immunoprecipitated with the same antibodies against PLTP and apoA-I.

These results indicate that the LA-PLTP and apoA-I form a physical complex, whereas the HA-PLTP does not form a tight complex with apoA-I. However, we observed that the HA-PLTP copurified with apoE, which is in agreement with the finding that during an inflammatory state PLTP activity increases significantly, and under these conditions the PLTP protein comigrates with apoE in two-dimensional gel electrophoresis (Barlage et al., 2001). In addition, active PLTP secreted from HepG2 cells has been shown to resemble the HA-PLTP in human plasma and coelute with apoE in size-exclusion chromatography (Siggins et al., 2003). Recently, higher PLTP activity among type 2 diabetic patients has been demonstrated to be associated with increased apoE concentration (Tan et al., 2006). All these findings support the hypothesis that apoE may be involved in maintaining PLTP in its active form. Contrary to our findings, Cheung and Albers (2006) have reported that immunoprecipitation of human plasma with an anti-apoA-I antibody resulted in 98% coprecipitation of plasma PLTP activity, indicating that PLTP activity in human plasma associates with apoA-I containing lipoproteins. On the other hand, it has been reported that HDL isolated by ultracentrifugation contains up to 40% of the total plasma PLTP mass but only a minor portion of plasma PLTP activity (Oka et al., 2000a). Moreover, Huuskonen and coworkers (2000a) have shown that PLTP activity in human plasma correlates negatively with HDL cholesterol. The latter findings are in agreement with our observation that the LA-PLTP associated with apoA-I, the main apolipoprotein of HDL, is of low specific activity.

5.2 The human plasma PLTP concentration is unevenly distributed between the high- and low-activity forms of PLTP

Although the role of PLTP activity in different physiological conditions has been studied quite intensively, the significance of PLTP concentration is far from resolved. Besides the ELISA used to assay PLTP concentration in cell media and lysates (Qu et al., 1999), three different methods to determine PLTP concentration in human plasma have been reported (Desrumaux et al., 1999a; Huuskonen et al., 2000a; Oka et al., 2000b). However, the results of these reports are conflicting probably due to the presence of two forms of PLTP in human plasma (Oka et al., 2000a) and different immunoreactivities of the antibodies used in mass assays against the two PLTP forms, as reported by Murdoch and colleagues (2002b). It has been suggested that PLTP adopts a different conformation when associated with lipoprotein particles of different size and surface curvature, which may result in either exposure or masking of epitopes on the surface of PLTP. Alternatively, interaction of PLTP with lipids and other proteins might lead to masking of epitopes and impaired immunoreactivity. The observation that PLTP mass and activity in human

plasma do not correlate (Huuskonen et al., 2000a; Oka et al., 2000b) supports the finding of Murdoch and coworkers about differences in the reactivity of antibodies against the HA- and LA-PLTP. Only in one study a significant correlation between PLTP mass and activity has been reported (Desrumaux et al., 1999a). To resolve these discrepancies and to provide a tool for future analyses to study the physiological implications of the distribution of PLTP between the two forms, we undertook to develop an ELISA method capable of determining the concentrations of both the HA- and LA-PLTP in human plasma.

5.2.1 Effect of various detergents on the immunoreactivity of PLTP

To investigate whether the HA- and LA-PLTP forms react differently against antibodies, 40 ng of each PLTP form as determined by ELISA (Huuskonen et al., 2000a) were subjected to SDS-PAGE under reducing conditions. Western blot analysis and semiquantitative scanning of the protein bands revealed that the intensity of the HA-PLTP was approximately 3-fold stronger than the intensity of the LA-PLTP (II, Fig. 1A). This result indicated that, as compared to the LA-PLTP, the concentration of the HA-PLTP was underestimated by the ELISA due to weak immunoreactivity. Therefore, we decided to evaluate the impact of a sample pretreatment with a denaturant on the immunoreactivity of PLTP in ELISA.

The effects of five detergents including anionic sodium dodecyl sulphate (SDS), cationic cetyltrimethylammonium bromide (CTAB), zwitterionic CHAPS, nonionic n-octyl-β-D-glucopyranoside (OG), and nonionic polyoxyethylene sorbitan monolaurate (Tween 20) on the immunoreactivity of human plasma PLTP were investigated using the ELISA method (Huuskonen et al., 2000a). All detergents were assayed at 0.1%, 0.5%, and at the critical micellar concentration (CMC), which varied from 0.007% for Tween 20 to 0.5% for CHAPS. Of the detergents tested, the anionic SDS was observed to cause the highest increase in the immunoreactivity of plasma PLTP at all assayed detergent concentrations (II, Table 1). Compared with plasma assayed without any detergent pretreatment, the immunoreactivity of PLTP increased 4-fold when plasma was treated with 0.5% SDS before the assay. No further increase in immunoreactivity was obtained with higher concentrations of SDS. The other detergents caused only a modest increase (Tween 20), no increase at all (OG and CHAPS) or a decrease in PLTP immunoreactivity (CTAB). Hence, we decided to recalibrate the ELISA method by incorporating a sample pretreatment with 0.5% SDS, which most likely either disrupts interactions between PLTP and other proteins or lipids masking the antibody epitope or causes a conformational change leading to an exposure of the epitope. The other steps in the ELISA were performed as described by Huuskonen and coworkers (2000a).

5.2.2 Calibration of the ELISA

For the primary standardization, highly purified recombinant human PLTP (r-PLTP) produced in the baculovirus protein expression system (Huuskonen et al., 2000b) as well as active PLTP purified from human plasma were treated with 0.5% SDS for 30 minutes at +22°C, and thereafter used to create standard curves in the range of 25-100 ng PLTP/ml. The ELISA was found to be linear over the entire range used and suitable for the quantification of PLTP concentration as low as 12.5 μg/l (II, Fig. 2). The primary standard curves obtained either with r-PLTP or human plasma PLTP did not differ significantly from each other. Each primary standard point differed by an average of 4.3% from the mean value (n=3).

To investigate the effect of serum components on the immunoreactivities of the HA- and LA-PLTP that were isolated as described (I), increasing amounts of each PLTP form was added to foetal bovine serum before the determination of their concentration with the ELISA. The mean recoveries of added HA- and LA-PLTP were 90% and 109%, respectively, indicating that serum does not interfere with the measurement of either PLTP form. After exclusion of the possible interfering effect of serum on the ELISA, we established a secondary standard using a plasma sample from a healthy, normolipidemic male volunteer. When analyzed after SDS pretreatment, the mean PLTP mass in the secondary standard was 5.9 mg/l. The secondary standard was pretreated with 0.5% SDS and diluted to cover the PLTP mass range from 25 to 200 ng PLTP/ml. The slope of the secondary standard curve was found to be similar to those obtained with the primary calibrators (II, Fig. 2 and Fig. 3). Each secondary standard value differed by an average of 8.3% from the mean value (n=8). The intra-assay variation was 8% and the inter-assay variation 13%.

5.2.3 Measurement of the PLTP concentrations in human serum

Total PLTP concentration in serum

Total PLTP concentrations in human serum samples of 80 randomly selected individuals, representing a subsample of the Health 2000 Health Examination Survey (Aromaa & Koskinen, 2002), were determined by the recalibrated ELISA. In these subjects, the mean serum PLTP mass was 5.81 ± 1.33 mg/l (mean ± SD) covering the range of 2.78-10.06 mg/l, and the mean serum PLTP activity was 5.84 ± 1.39 μmol/ml/h covering the range of 3.21-11.15 μmol/ml/h. This yields the mean specific activity of about 1.0 μmol/μg/h for total serum PLTP. A weak, yet significant correlation ($r=0.345$, $p<0.01$) between total serum PLTP mass and activity was observed (II, Fig. 7A). In this study, the mean serum PLTP mass obtained is consid-

erably lower than reported previously with similar assays (Huuskonen et al., 2000a; Oka et al., 2000b) but close to that reported with a competitive immunoassay (Desrumaux et al., 1999a). The reason for these discrepancies is obviously the weak immunoreactivity of the active PLTP in its native conformation that was used as a primary calibrator in these assays (Huuskonen et al., 2000a; Oka et al., 2000b). This results in low absorbance values obtained with the active primary calibrator and, consequently, too high values are obtained for serum samples containing abundant LA-PLTP, whose epitopes are apparently fully exposed for the antibodies also in the native conformation of the protein.

HA- and LA-PLTP concentrations in serum

To determine the proportions of the two PLTP forms in human plasma, separation of the HA- and LA-PLTP was necessary. For this purpose, we first applied size-exclusion chromatography (II, Fig. 4.). When the fractions were analyzed for PLTP mass and activity without SDS pretreatment, almost all of the detectable PLTP mass eluted in the position of the LA-PLTP. However, when the mass assay was carried out with SDS pretreatment of the fractions, the distribution of PLTP mass between the LA- and HA-PLTP was evident. This is in agreement with the finding that antibodies against PLTP display different reactivities to the HA- and LA-PLTP when they are in their native conformation (Murdoch et al., 2002b). The size-exclusion chromatography revealed that the LA-PLTP comprises about 65% of the total PLTP mass, whereas 35% of the total PLTP mass resides in the HA-PLTP fraction.

To further investigate the proportions of the HA- and LA-PLTP in human plasma and reproducibility of the separation method, we performed repeated plasma runs on Heparin-Sepharose affinity chromatography (n=6) and determined PLTP mass both from the unbound and bound fractions (II, Fig. 5). The bound fractions were found to comprise about 40% of the total PLTP mass, and more than 95% of the PLTP activity applied bound to the column matrix, indicating that the bound fractions represented the HA-PLTP. The unbound fractions encompassed about 50% of the total PLTP mass with no detectable PLTP activity, thus representing the LA-PLTP form. The separated HA- and LA-PLTP, 40 ng of each protein form, were then subjected to SDS-PAGE. Western blot analysis and scanning of the PLTP bands revealed that the immunoreactivities of both the HA- and LA-PLTP were similar, suggesting that the partial denaturation of PLTP by SDS apparently increases the accessibility of epitopes on the HA-PLTP resulting in similar immunoreactivities of the two PLTP forms in the ELISA and more reliable values for PLTP concentrations (II, Fig. 1B).

The mAb JH66, used as the capture antibody in the ELISA, was then used in immunoaffinity chromatography of human plasma to study the immunoreactivity of the

mAb JH66 to the HA- and LA-PLTP forms (II, Fig. 6). We observed that of the PLTP activity applied, none was retained by the column and 35% of the total PLTP mass could be recovered from the unbound fractions as analyzed by the ELISA and denaturing SDS pretreatment of the samples. After elution of the column with 0.1 M glycine, pH 2.5, it was revealed that 67% of the total PLTP mass bound to the mAb JH66. In addition, if plasma was treated with 0.5% SDS and thereafter applied to the immunoaffinity chromatography column, all of the PLTP protein was retained by the column. These results further support the conclusion that after SDS pretreatment, the ELISA method is capable of measuring both PLTP forms of human plasma.

Development of the ELISA for clinical use

Although the two forms of PLTP can be separated by size-exclusion, Heparin-Sepharose, and immunoaffinity chromatography methods, they are too cumbersome to be used in the analysis of a large number of serum samples. To overcome this problem, we decided to use dextran sulfate ($DxSO_4$)-$CaCl_2$ precipitation, which has been used in the purification of PLTP (Tu et al., 1993). We observed that after $DxSO_4$-$CaCl_2$ precipitation of human plasma, almost all of the PLTP activity could be recovered from the supernatant, while only trace amounts of PLTP protein could be measured unless the sample was pretreated with 0.5% SDS. This suggested that most of the LA-PLTP was precipitated, which was also confirmed by Western blotting. Similarly, only negligible PLTP mass was detected for the HA-PLTP fraction separated by the three different chromatographic methods if the mass determination was performed without a denaturing sample pretreatment.

To evaluate whether $DxSO_4$-$CaCl_2$ precipitation could be used to separate the HA- and LA-PLTP in plasma samples, we performed the analysis of PLTP activity and mass from precipitated normolipidemic plasma samples (n=8). By subtracting the HA-PLTP mass measured in the supernatant of the precipitated plasma sample from the total plasma PLTP mass, a good estimate of the LA-PLTP mass was obtained. The analysis revealed that on average 84% of the PLTP activity and 37% of the PLTP mass could be recovered in the supernatant, suggesting that 63% of PLTP mass, representing the LA-PLTP, had been precipitated. The distribution of PLTP mass obtained with $DxSO_4$-$CaCl_2$ precipitation was found to be similar to those obtained with the three different chromatographic methods, indicating that the HA-PLTP represents approximately 40% and the LA-PLTP 60% of the total PLTP mass. Thus, $DxSO_4$-$CaCl_2$ precipitation is a preferable method for separation of the two PLTP forms especially when assaying a large number of samples.

Having established the assay method suitable also for clinical use, we analyzed the mass of the HA- and LA-PLTP from the serum of 80 randomly selected Finnish subjects, representing a subsample of the Health 2000 Health Examination Survey

(Aromaa & Koskinen, 2002). In this population, the total PLTP concentration was 5.81 ± 1.33 mg/l (mean ± SD) and the mean HA-PLTP concentration 1.87 ± 0.85 mg/l (mean ± SD), leading to an estimate LA-PLTP concentration of 3.94 ± 1.40 mg/l in the precipitate. Thus, the HA-PLTP was found to represent 32% and the LA-PLTP 68% of the total serum PLTP mass. The mean PLTP activity recovered in the supernatant represented 88% of the activity measured in serum before precipitation. This yields a specific activity of 2.7 μmol/μg/h for the HA-PLTP, which was found to be similar to that of active PLTP secreted by human hepatoma cells (Siggins et al., 2003). For a comparison, if our method had resulted in complete separation of the two PLTP forms, the specific activity of the HA-PLTP would have been 3.1 μmol/μg/h demonstrating that $DxSO_4$-$CaCl_2$ precipitation, as a separation method, is close to optimal. Moreover, the HA-PLTP concentration displayed a stronger positive correlation with serum PLTP activity ($r=0.536$, $p<0.01$) than did total PLTP mass in serum ($r=0.345$, $p<0.01$), further demonstrating successful separation of the HA-PLTP from the LA-PLTP. No correlation between the LA-PLTP mass and serum PLTP activity could be observed, which also illustrates the specificity of $DxSO_4$-$CaCl_2$ precipitation in the isolation of the two PLTP forms (II, Fig. 7).

5.3 The high- and low-activity forms of PLTP correlate differently with lipid and carbohydrate parameters in a Finnish population sample

The development of the ELISA method for quantitation of both PLTP forms in human plasma provides an accurate and useful tool for establishing the relationship of the HA- and LA-PLTP with associated lipoproteins and other plasma factors, as well as for detecting possible changes in the distribution of human PLTP concentration in clinical serum samples originating from different physiological conditions. In this thesis, the influence of plasma lipoprotein profile and lipid composition, as well as some parameters of glucose metabolism, on the balance between the LA- and HA-PLTP populations in Finnish individuals participating in the Health 2000 Health Examination Survey was investigated. The study was approved by The Working Group for Cardiovascular Diseases and Diabetes and by The Planning and Steering Group of the Research for the Health 2000 Health Examination Survey.

5.3.1 Characteristics of the study population

For this study, 125 men and 125 women were randomly chosen as a subsample of the Health 2000 Health Examination Survey carried out in Finland (Aromaa &

Koskinen, 2002). The mean age of the study population was 55 years, and the body mass index (BMI) 26.1 ± 4.0 (mean ± SD). A significant gender difference was observed in total cholesterol (TC), HDL cholesterol (HDL-C), and apoA-I, which were higher among women, whereas the waist-to-hip circumference ratio (WHR) and glucose were higher among men (III, Table 1). The other parameters studied did not differ significantly between genders.

5.3.2 Measurement of the PLTP activities and concentrations in serum

The analysis of PLTP activity was performed by two different radiometric methods. The exogenous assay ($PLTP_{exo}$) measures PL transfer to exogenously added HDL (Damen et al., 1982), while the endogenous assay ($PLTP_{endo}$) measures PL transfer to endogenous serum HDL (Lagrost et al., 1999a). We observed that the two assays resulted in quite different values for serum PLTP activity. The mean serum $PLTP_{exo}$ activity was 6.59 ± 1.66 μmol/ml/h (mean ± SD), whereas the serum $PLTP_{endo}$ activity was 1.37 ± 0.29 μmol/ml/h (III, Table 2). No significant difference between genders was observed in the PLTP activity.

The serum HA- and LA-PLTP concentrations were quantitated by the ELISA method as described (II). The mean total PLTP concentration in sera of 250 subjects was 6.56 ± 1.45 mg/l, covering the range of 2.78-10.89 mg/l. Similar values for PLTP concentration have been reported earlier with a competitive ELISA (Desrumaux et al., 1999a). The mean HA-PLTP concentration was 3.00 ± 1.21 mg/l and the mean LA-PLTP concentration 3.56 ± 1.14 mg/l. However, a large individual variation existed between the relative amounts of the two PLTP forms as the concentration ranges were 0.92-7.63 mg/l for the HA-PLTP and 1.20-8.43 mg/l for the LA-PLTP (III, Table 2). Like serum PLTP activity, PLTP concentrations did not differ significantly between genders.

Serum $PLTP_{exo}$ activity showed a significant positive correlation with total serum PLTP concentration ($r=0.45$, $p<0.001$) and HA-PLTP concentration ($r=0.38$, $p<0.001$). Furthermore, a weak positive correlation ($r=0.17$, $p<0.01$) was observed between $PLTP_{exo}$ activity and LA-PLTP concentration (III, Fig. 1), whereas serum $PLTP_{endo}$ activity demonstrated a weak positive correlation only with HA-PLTP concentration (III, Table 3). A significant negative correlation between serum HA- and LA-PLTP was detected (III, Fig.2).

5.3.3 Correlation of PLTP with clinical and biochemical parameters

To investigate how the two PLTP forms relate to lipid and carbohydrate metabolism and to find possible regulatory factors for the two PLTP forms, we performed a statistical analysis between the PLTP parameters and selected clinical as well as biochemical parameters (III, Table 4). Total serum PLTP concentration correlated positively with age, and $PLTP_{exo}$ activity with BMI and WHR, which is in accordance with previous findings (Huuskonen et al., 2000a; Jonkers et al., 2003). In addition, $PLTP_{endo}$ activity showed a negative correlation with age. We observed that none of the PLTP parameters correlated with gender or CRP in the studied population sample, which was composed of normolipidemic individuals. It has been reported, however, that in patients with a severe acute phase response, PLTP activity correlates positively and PLTP mass negatively with CRP. Furthermore, in these patients, PLTP mass was found to correlate negatively with SAA (Pussinen et al., 2001b).

In this study sample, total serum PLTP concentration and HA-PLTP concentration were found to display a similar positive correlation with HDL-C. In addition, HA-PLTP mass correlated positively with apoA-I. Similar relationship between PLTP mass and HDL-C has also been reported earlier (Oka et al., 2000b; Oka et al., 2002). A weak negative correlation was observed between total PLTP mass and TG, while no measured lipid parameter correlated with LA-PLTP concentration. Serum $PLTP_{endo}$ activity demonstrated a significant positive correlation with all studied lipid and lipoprotein parameters but apoB. By definition, the assay is dependent on the serum HDL-C and will therefore correlate with the HDL-C and apoA-I (Lagrost et al., 1999a). The correlation of $PLTP_{endo}$ activity also with TC, TG, and LDL cholesterol (LDL-C) suggests that other lipoproteins affect the outcome of the assay as well. Furthermore, $PLTP_{endo}$ activity showed a weak positive correlation only with HA-PLTP mass, suggesting that the assay rather reflects the activity of PLTP as modulated by the composition and concentration of endogenous lipoproteins than the amount of active PLTP in serum. As $PLTP_{exo}$ activity showed a positive correlation with total, HA- and LA-PLTP concentrations, it is a preferable method for determining the serum PLTP activity levels. In addition, positive correlations between $PLTP_{exo}$ activity and serum TC, TG, apoB, and apoE were observed. However, unlike previous reports (Cheung et al., 2002; Murdoch et al., 2000), no correlation between $PLTP_{exo}$ activity and LDL-C was found in this study population. This indicates that PLTP activity might correlate with VLDL, which is in agreement with the hypothesis of Murdoch and colleagues (2002a) who suggested that the increase of apoB associated with VLDL particles results in increased PLTP activity to facilitate the transfer of post-lipolytic PL-rich surface remnants to HDL.

As a weak positive correlation between $PLTP_{exo}$ activity and apoE was observed, and it has been shown that serum apoE concentration is dependent on the apoE genotype (Smit et al., 1988), apoE allele distribution in the study population was analyzed. ApoE allele frequencies in the study sample were observed to be similar to those reported earlier for Finns (Ehnholm et al., 1986). ApoE genotypes were divided in three subgroups. Group 1 included the genotypes E_3/E_3 (n=137), group 2 the genotypes E_3/E_4 and E_4/E_4 (n=78), and group 3 the genotypes E_2/E_2, E_2/E_3, and E_2/E_4 (n=17). The carriers of ε2 allele in group 3 showed significantly increased apoE concentration ($p<0.001$), TG concentration ($p<0.05$) as well as $PLTP_{endo}$ activity ($p<0.001$) as presented in Table 2. However, we did not detect any differences in $PLTP_{exo}$ activity or PLTP concentrations between the groups. Therefore, we consider that the increased $PLTP_{endo}$ activity in group 3 is attributable to a difference in serum lipoprotein profile that is reflected in the outcome of the assay.

In previous studies, PLTP concentration (Desrumaux et al., 1999a) and activity (Riemens et al., 1998b) have been associated with glucose metabolism. Therefore, the relationship of PLTP parameters with glucose and insulin was investigated. In addition, the homeostasis model assessment for insulin resistance (HOMA IR) calculated from the fasting plasma glucose and serum insulin concentrations (Matthews et al., 1985) was used to investigate how the PLTP parameters relate with insulin resistance. Serum total and HA-PLTP concentrations as well as $PLTP_{exo}$ activity demonstrated a similar positive correlation with serum glucose concentration, whereas the LA-PLTP concentration tended to associate with serum insulin. Furthermore, in this population sample, $PLTP_{exo}$ activity was observed to correlate positively with insulin resistance determined as HOMA IR, which is in agreement with previous findings (Jonkers et al., 2003; Riemens et al., 1998b).

The present study suggests that both lipid and glucose metabolism affect the distribution of the two PLTP forms and the level of PLTP activity in serum. The finding

Table 2. Characteristics of the subgroups of apoE isoforms (mean ± SD)

	ApoE (mg/l)	**TG (mmol/l)**	**$PLTP_{endo}$ activity (μmol/ml/h)**
Group 1 (n=137)	28.1 ± 14.5	1.45 ± 0.84	1.35 ± 0.18
Group 2 (n=78)	20.7 ± 15.8	1.42 ± 0.87	1.35 ± 0.19
Group 3 (n=17)	39.9 ± 18.6[a]	2.12 ± 2.55[b]	1.64 ± 0.88[a]

[a]$p<0.001$, [b]$p<0.05$

that the expression of the PLTP gene is regulated by glucose via a PPAR-mediated pathway further supports the relationship between PLTP and glucose metabolism (Tu & Albers, 2001) although the detailed mechanisms by which glucose and insulin could modulate the distribution of the HA- and LA-PLTP remain to be resolved.

5.4 ApoE and apoA-IV activate the low-activity form of human PLTP

Exchangeable apolipoproteins play an essential role in HDL assembly and metabolism. Numerous apolipoproteins have been found to function as co-factors for different plasma enzymes and lipid transfer proteins regulating HDL metabolism and to enhance their activities. The factors regulating PLTP activity as well as the distribution of PLTP between the two forms are currently unknown and may involve interactions of PLTP with apolipoproteins. PLTP has been reported to interact both with apoA-I and apoA-II (Pussinen et al., 1998). In addition, we observed that the HA-PLTP copurifies with apoE, while the LA-PLTP is associated with apoA-I (I), and that PLTP activity correlates positively with apoE (III). Therefore, interactions of the two PLTP forms with apolipoproteins as well as the ability of apolipoproteins to transform the low-activity form of PLTP into an active form were investigated.

5.4.1 Interactions of apolipoproteins with PLTP

In this thesis, the binding of recombinant PLTP (r-PLTP), representing an active PLTP form, and the low-activity form of human plasma PLTP (LA-PLTP) to apoA-I, apoA-IV, and apoE using surface plasmon resonance analysis on a Biacore 2000 instrument was investigated in more detail. To minimize the effects of contaminating serum proteins on the interactions, r-PLTP was primarily chosen to be used as an analyte and recombinant apoE and apoA-IV, or highly purified apoA-I were used as immobilized ligands on the sensor chip surface. The sensorgrams obtained with the Biacore instrument clearly demonstrated a concentration-dependent binding of both analytes, r-PLTP and LA-PLTP, to apoA-I and apoE within a nanomolar range (IV, Fig. 1). Both analytes also showed binding to apoA-IV. A polyclonal anti-PLTP antibody was used as an additional analyte after injection of PLTP to demonstrate that a stable association of PLTP with apolipoproteins and not only a donation of phospholipids was taking place on the sensor chip surface. The relatively low R_{max} values, presented as resonance units (RU), indicate that the interactions were fast and varied in their stability. Regarding the binding of PLTP, this may in part be due to unfavorable orientations of the apolipoproteins on the surface of a flow cell. The

effect of each PLTP concentration on the binding response at a steady-state phase of each interaction was evaluated. These equilibrium-binding responses (IV, Fig. 2) demonstrate that in the interactions where the saturation level was reached, the estimated K_D values were comparable with the K_D values derived from the reaction rates obtained from the sensorgram data (IV, Table 2).

The binding affinities of r-PLTP and LA-PLTP to apoA-I, apoA-IV, and apoE were determined from rate equations by simultaneous k_a/k_d simulated kinetics using a Langmuir model (IV, Table 2). Several other models, including a two-state reaction, bivalent analyte, and parallel reactions, supported the K_D values obtained by using the Langmuir model. Data obtained using the different kinetic models suggest that the interactions of PLTP with apolipoproteins involve more complex binding dynamics, which is possibly influenced by phospholipids associated with PLTP, than a simple 1:1 -binding stoichiometry. We observed that both PLTP forms had different affinities for the three apoE isoforms, $apoE_2$, $apoE_3$, and $apoE_4$. Due to the slow dissociation rates (k_d) of the analytes, $apoE_2$ was found to be the most stable binding partner, whereas binding of both r-PLTP and LA-PLTP to the other apolipoproteins was driven by fast association kinetics. The binding affinities and interaction rates of r-PLTP with the apoE isoforms were similar to those of LA-PLTP. However, the association rate (k_a) of active r-PLTP with apoA-I was 10-fold slower than that of LA-PLTP. Due to the fast dissociation rate of LA-PLTP from apoA-I, the corresponding K_D value for this interaction remained relatively high. In contrast to apoA-I, the affinity of apoA-IV was lower for r-PLTP than for LA-PLTP. This was observed to be due to the fast dissociation rate of r-PLTP from apoA-IV. The mean χ^2 values, 2.65 for r-PLTP and 0.50 for LA-PLTP, illustrate the reliability of the results. Thus, both the active r-PLTP and the LA-PLTP interact with apoA-I, apoA-IV, and apoE, albeit with different affinities and interaction rates. The physiological importance of the different affinities of PLTP for the apoE isoforms remains a topic for future studies.

5.4.2 Activation of the LA-PLTP with apoE and apoA-IV

To examine the possible physiological roles of apolipoproteins in the regulation of PLTP activity, proteoliposomes containing apoA-I, apoA-IV, or apoE as protein constituent, as well as phosphatidylcholine and unesterified cholesterol, were prepared by the cholate dialysis technique (IV, Table 1). The incubations of proteoliposomes with the LA-PLTP, which had no detectable PL transfer activity, were carried out at +37°C for 30 minutes, after which the preincubated samples were assayed for PLTP activity. The incubation of the apoE proteoliposomes with the LA-PLTP at different molar ratios resulted in a concentration-dependent activation of the LA-

PLTP (IV, Fig. 3). The mean PLTP activity of 264 nmol/ml/h was reached after the incubation with the highest concentration of apoE proteoliposomes. No differences between the proteoliposomes containing $apoE_2$, $apoE_3$, or $apoE_4$ in their ability to activate the LA-PLTP were observed. This is in line with the observation that in a Finnish population sample apoE genotype had no influence on the PLTP activity (III). The proteoliposomes containing apoA-IV were also capable of activating the LA-PLTP to a similar extent as the apoE proteoliposomes. No significant differences in LA-PLTP activation were detected whether native HDL_3 or reconstituted HDL particles were used as acceptors in the PLTP-activity assays. However, no activation of the LA-PLTP could be detected when incubations were performed with proteoliposomes containing apoA-I, which supports the previous finding that apoA-I is a major component of the LA-PLTP complex (I). These results suggest that apoE and apoA-IV, and possibly some other apolipoproteins, play a role in the regulation of PLTP activity. In agreement with this, PLTP activity has been shown to correlate positively with apoE, and PLTP has been shown to colocalize exclusively with apoE-containing HDL particles in patients with a chronic inflammation (Barlage et al., 2001). Furthermore, it has been suggested that apoA-IV could act as an activator for the HDL conversion factor, which was later revealed to be PLTP. On the other hand, neither apoA-I, apoA-II, apoC, nor apoE could enhance the remodeling of HDL_3 particles by the conversion factor (Barter et al., 1988). However, infusion of apoA-I/PL discs into human subjects has been shown to result in increased formation of preβ-HDL particles (Nanjee et al., 1999) and elevated PLTP activity with a concomitant decrease in PLTP concentration (Kujiraoka et al., 2004). Contrary to these findings, we could not observe any enhancement of PLTP-mediated phospholipid transfer by apoA-I, apoE, or apoA-IV, which were incubated with the HA-PLTP (data not shown). Consistent with our finding that the LA-PLTP associates with apoA-I, the low plasma PLTP concentration in patients with hypoalphalipoproteinemia and the increased PLTP concentration in subjects with CETP deficiency have been demonstrated to be due to differences in the concentration of inactive PLTP, while the levels of active PLTP remained comparable to those of the control subjects. Moreover, the changes in PLTP concentrations were accompanied by alterations in the relative proportions of large HDL particles containing inactive PLTP and smaller particles containing active PLTP, suggesting that plasma lipoprotein profile regulates the abundance of the HA- and LA-PLTP forms (Oka et al., 2002).

6 SUMMARY AND CONCLUSIONS

The presence of two different forms of PLTP, associated with macromolecular complexes of different size, has been demonstrated in human plasma (Oka et al., 2000a). In the first part of this thesis, a method to isolate these two PLTP forms from human plasma was developed and the molecular complexes in which the HA- and LA-PLTP reside were partially characterized. It was observed that the HA-PLTP copurified with apoE, whereas the LA-PLTP formed a complex with apoA-I.

The second part of this thesis aimed at developing an ELISA method that enables quantitation of the two PLTP forms in human plasma. Partial denaturation of PLTP by SDS was found to apparently increase the accessibility of epitopes on the surface of the HA-PLTP and to result in similar immunoreactivity of both PLTP forms. The separation of the two PLTP forms is necessary before their mass determination. For the analysis of a large number of serum samples, $DxSO_4$-$CaCl_2$ precipitation is a preferable approach to separate the HA- and LA-PLTP. The ELISA method developed for quantitation of both PLTP forms in human plasma provides an accurate tool for studing the relationship of the HA- and LA-PLTP with lipoproteins and other plasma factors, as well as for detecting possible changes in the distribution of human PLTP in different physiological conditions and disease states.

In the third part of this thesis, the influence of serum parameters related to lipid and glucose metabolism on the balance between the LA- and HA-PLTP as well as the distribution of the two PLTP forms in a population sample were investigated. Among Finnish individuals participating in the Health 2000 Health Examination Survey, the mean PLTP mass was 6.6 ± 1.5 mg/l and the mean $PLTP_{exo}$ activity 6.6 ± 1.7 μmol/ml/h. Of the serum PLTP concentration, almost 50% represents HA-PLTP. A large individual variation, however, was detected in the distribution of the two PLTP forms. Both total PLTP and HA-PLTP concentrations were found to correlate positively with HDL-C. In addition, correlation of PLTP activity with TC, TG, apoB, and apoE suggests that PLTP activity could be regulated by plasma VLDL concentration. Of the studied parameters of glucose metabolism, glucose correlated positively with serum total PLTP and HA-PLTP concentration, whereas LA-PLTP concentration showed a positive correlation with serum insulin. Furthermore, PLTP activity was found to be related to insulin resistance. These results support the previous findings that in addition to lipoprotein metabolism, PLTP could also play a role in glucose metabolism.

In this thesis, it was observed that the HA-PLTP of human plasma copurifies with apoE and serum PLTP activity correlates positively with apoE concentration. There-

fore, interaction of PLTP with apolipoproteins and the ability of apolipoproteins to regulate PLTP activity were studied. Using surface plasmon resonance analysis, we observed that both r-PLTP, representing an active PLTP form, and the LA-PLTP interact with apoA-I, apoA-IV, and apoE. In addition, apoE and apoA-IV, but not apoA-I, are capable of activating the LA-PLTP. These findings suggest that the distribution of the HA- and LA-PLTP in human plasma is subject to a dynamic regulation by apolipoproteins.

Based on the findings reported in this thesis, a model is suggested (Fig. 5) in which nascent PLTP of high specific activity enters the circulation possibly associated with the secreted nascent VLDL particles. After the transfer of post-lipolytic, PL-rich surface remnants of TG-rich lipoproteins to HDL, a portion of PLTP may become associated with apoA-I in large HDL-like particles, and thereby be sequestered into the LA-PLTP complex. Whether this is a reversible process also *in vivo* or whether the LA-PLTP is just an intermediate directed to the catabolic route remains to be resolved.

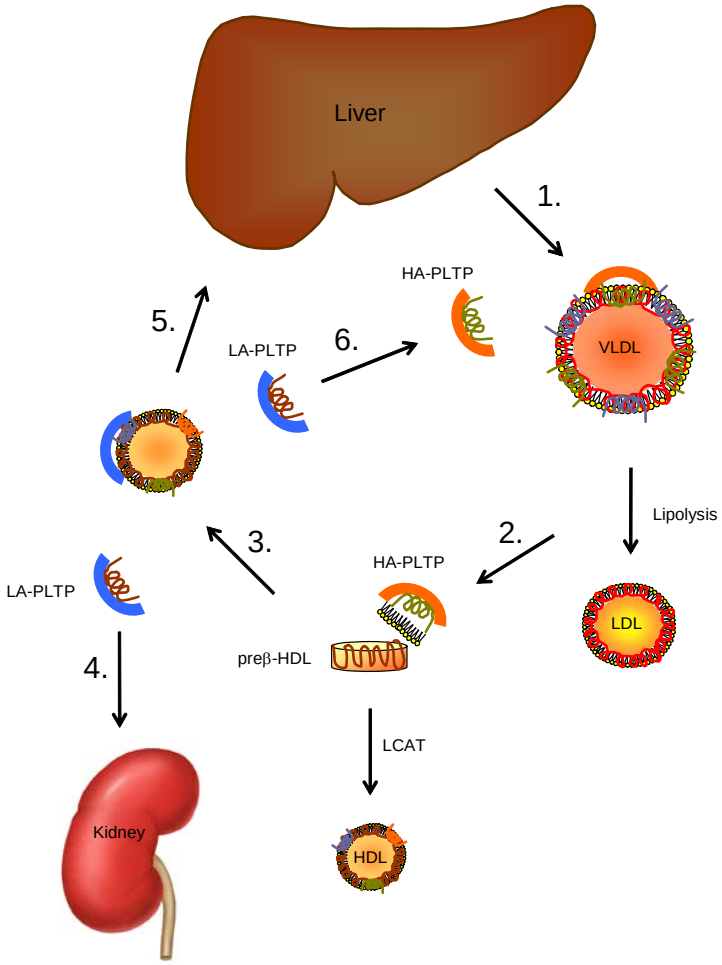

Figure 5. A schematic model for the conversion of the HA-PLTP to the LA-PLTP. The boomerang-shaped PLTP molecule is secreted from the liver as a high-activity form probably associated with apoE on the surface of VLDL (1). During or after the transfer of lipolytic surface remnants of TG-rich lipoproteins to lipid-poor HDL (2), PLTP becomes associated with apoA-I in large HDL-like particles and loses measurable PL transfer activity (3). The thus formed LA-PLTP can be catabolized together with apoA-I through the kidney (4) or taken up by the liver for degradation (5). Alternatively, changes in the plasma lipoprotein profile and in the associations with apolipoproteins can re-activate LA-PLTP (6).

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