CHAPTER 9

SUMMARY, GENERAL DISCUSSION
AND FUTURE PERSPECTIVES
I SUMMARY

The studies described in this thesis focus on the alterations in the ability of plasma to stimulate cellular cholesterol efflux and on changes in lipid transfer protein-mediated reactions in type 1 and type 2 diabetes mellitus. Possible consequences of such changes for cardiovascular risk are also assessed.

Chapter 1 consists of a general introduction on HDL metabolism, lipid transfer proteins and peripheral cholesterol efflux under normal conditions and in type 1 and type 2 diabetes mellitus. The purpose and hypotheses of the studies described in this thesis are outlined in chapter 2.

In chapter 3 the ability of plasma to generate pre β-HDL and to promote cellular cholesterol efflux was determined in 14 sufficiently controlled, moderately hypercholesterolaemic type 1 diabetic patients and 13 healthy subjects. Pre β-HDL formation (both expressed in apo A-I concentration and in % of apo A-I) was not different in diabetic patients compared to healthy subjects. HDL phospholipids, plasma apo A-I, EST, CET, CETP activity and PLTP activity were higher in type 1 diabetic patients compared to control subjects. Cholesterol efflux from Fu5AH hepatoma cells, which express SR-BI but no ABCA1, to plasma from type 1 diabetic patients was higher compared to plasma from healthy subjects. Also with fibroblasts, which express ABCA1 after cholesterol loading but no SR-BI, the ability of plasma to promote cholesterol efflux was increased with plasma from diabetic patients compared to plasma from healthy subjects. Simvastatin therapy decreased plasma total cholesterol, VLDL+LDL cholesterol, LDL cholesterol, triglycerides and apo B levels. This treatment also decreased plasma CETP activity, CET and EST, but no significant changes were seen in plasma LCAT and PLTP activity. Simvastatin treatment resulted in a modest increase in HDL cholesterol levels, whereas pre β-HDL formation tended to decrease. No changes in the ability of plasma to promote cellular cholesterol efflux were observed after simvastatin treatment, neither with Fu5AH cells nor with human fibroblasts. The relative decreases in plasma CET in response to various doses of simvastatin were positively correlated with the relative decreases in VLDL+LDL cholesterol, plasma triglycerides and plasma CETP activity. In turn, the relative changes in HDL cholesterol after treatment with simvastatin were correlated negatively with the relative changes in plasma CET. Furthermore, the relative changes in pre β-HDL formation were positively correlated with the relative changes in plasma triglycerides and negatively with the relative changes in HDL cholesterol.

From this study we concluded that the ability of plasma from moderately hypercholesterolaemic type 1 diabetic patients to stimulate cholesterol efflux out of
cultured Fu5AH cells and fibroblasts is enhanced, probably as a consequence of higher apo A-I, HDL phospholipids and PLTP activity. It therefore appears that, as far as the ability of plasma to stimulate cholesterol efflux is concerned, there is no defect in this early step in the RCT pathway in type 1 diabetic patients. The increase in HDL cholesterol in response to simvastatin was related to the drop in plasma CET, which in turn was attributable to a decrease in apo B containing lipoproteins and in CETP activity. The simvastatin-induced changes in the HDL cholesterol concentration are not associated with a further increase in cholesterol efflux out of fibroblasts and Fu5AH cells.

Chapter 4 describes the effect of a frequently recommended low saturated fat, low cholesterol diet on the ability of plasma to generate pre β-HDL and to promote cellular cholesterol efflux in moderately hypercholesterolaemic type 1 diabetic patients. After diet intervention no changes were seen in VLDL+LDL cholesterol, HDL cholesterol and HDL phospholipids, triglycerides, as well as in plasma apo A-I and apo B levels. However, pre β-HDL formation decreased in response to the diet. Plasma CET activity also decreased, but plasma PLTP and LCAT activity levels did not change. The ability of plasma to promote cholesterol efflux from both Fu5AH cells and human skin fibroblasts remained unaffected after diet intervention. Therefore, it was concluded that, as far as the ability of plasma to stimulate cell-derived cholesterol removal is concerned, the reverse cholesterol transport pathway is unchanged, despite the drop in pre β-HDL formation.

Cholesterol efflux from cultured fibroblasts to plasma from hypertriglyceridaemic type 2 diabetic patients was evaluated in chapter 5. In this study, 56 normotriglyceridaemic healthy subjects, 56 normotriglyceridaemic type 2 diabetic patients and 28 hypertriglyceridaemic (fasting plasma triglycerides > 2.0 mmol/l) type 2 diabetic patients participated. Pre β-HDL and pre β-HDL formation (expressed in apo A-I concentration) were unchanged in both diabetic groups, in spite of lower plasma apo A-I. Accordingly, when expressed as percentage of plasma apo A-I, pre β-HDL as well as plasma pre β-HDL formation were higher in hypertriglyceridaemic type 2 diabetic patients compared to normotriglyceridaemic control subjects and normotriglyceridaemic type 2 diabetic patients. Plasma EST, CET and PLTP activity were also higher in hypertriglyceridaemic type 2 diabetic patients than in normotriglyceridaemic type 2 diabetic patients and healthy subjects. The ability of plasma to stimulate cholesterol efflux out of fibroblasts was elevated in hypertriglyceridaemic compared to normotriglyceridaemic diabetic patients and control subjects. Univariate regression analysis showed that cholesterol efflux from fibroblasts to plasma from diabetic patients was positively correlated with PLTP activity, pre β-HDL formation (expressed in apo A-I concentration), HDL phospholipids and
HDL triglycerides, but not with HDL cholesterol. In the combined subjects, multiple linear regression analysis revealed that cellular cholesterol efflux to plasma was positively and independently determined by pre-β-HDL formation, plasma PLTP activity and EST without an independent effect of the presence of diabetes. Therefore, it was concluded that the ability of hypertriglyceridaemic plasma from type 2 diabetic patients to remove excess cholesterol from cultured human fibroblasts is enhanced, possibly consequent to high plasma PLTP activity and EST. Pre-β-HDL formation in diabetic hypertriglyceridaemia may be involved in cellular cholesterol efflux.

Chapter 6 describes the relationship between plasma CET and IMT. Eighty-seven type 2 diabetic patients and 82 control subjects participated in this study. In the diabetic patients, plasma total cholesterol was slightly lower than in the healthy control subjects, but non-HDL cholesterol and apo B levels were similar. Plasma triglycerides were higher, whereas apo A-I and HDL cholesterol levels were lower in type 2 diabetic patients than in control subjects. Both plasma CETP mass and CET were higher in diabetic patients. In type 2 diabetes mellitus, plasma CET was positively determined by plasma triglycerides, non-HDL cholesterol, CETP and, interestingly, the interaction between plasma CETP and triglycerides. This illustrates that the effect of the plasma CETP level on CET becomes more important with higher triglycerides. To evaluate subclinical atherosclerosis, IMT of the carotid artery was measured by ultrasonography. Carotid IMT was higher in type 2 diabetic patients compared to control subjects, as expected. Besides age, gender and pulse pressure, CET was an independent determinant of IMT in both diabetic patients and control subjects. This suggests that an increased transfer rate of cholesteryl esters from HDL to apo-B containing lipoproteins may contribute to the development of atherosclerosis. Since the effect of the CETP level on CET becomes more important with higher triglycerides, lowering plasma levels of active CETP, e.g. by pharmacological CETP inhibition, may be expected to be beneficial in hypertriglyceridaemic diabetic patients.

Chapter 7 determines the possible influence of an elevated plasma PLTP activity level, as seen in type 2 diabetes mellitus, on carotid IMT. Eighty-seven type 2 diabetic patients and 83 healthy subjects were compared. Plasma PLTP activity was higher in type 2 diabetic patients compared to the control subjects. In type 2 diabetic patients, multiple stepwise regression analysis revealed that plasma PLTP activity level was an independent determinant of carotid IMT, in addition to age, gender, pulse pressure and HDL cholesterol. The magnitude of the contribution of plasma PLTP activity on carotid IMT was comparable to that of HDL cholesterol. In control subjects, there was no significant effect of plasma PLTP activity on IMT. From these results we concluded that elevated plasma PLTP activity may be involved in accelerated atherosclerosis in type
2 diabetes mellitus.

In chapter 8 the relationships between the plasma activities and concentrations of the lipid transfer proteins CETP and PLTP were examined in 16 type 2 diabetic patients and 16 healthy control subjects. Plasma CETP and PLTP mass were measured by ELISA using monoclonal antibodies. In type 2 diabetic patients, HDL cholesterol levels were lower and plasma triglycerides higher compared to healthy subjects. Plasma CETP activity and plasma CETP concentration were not significantly different between diabetic patients and healthy subjects, but CETP activity/mass ratio was lower in diabetic patients compared to healthy subjects. Plasma PLTP activity was higher in type 2 diabetic patients, but the concentration of this lipid transfer protein was similar in both groups. This resulted in a higher plasma mass-adjusted PLTP activity. Multiple stepwise regression analysis showed that plasma CETP activity was positively related to plasma CETP concentration and negatively to the diabetic state. Plasma PLTP concentration was positively associated with HDL cholesterol and negatively with the waist-hip ratio in multiple stepwise regression analysis. Plasma PLTP activity did correlate with plasma PLTP concentration, but only after adjustment for plasma triglycerides and the waist-hip ratio. It was concluded that plasma CETP specific activity is lower in type 2 diabetes mellitus compared to healthy subjects. Mass-adjusted plasma PLTP activity is higher in type 2 diabetic patients, but this increase is associated with features of the metabolic syndrome rather than with diabetes mellitus per se.

II General Discussion and Future Perspectives

Cellular cholesterol efflux and pre β-HDL

In the first part of this thesis, we tested the hypothesis that the ability of plasma to promote cellular cholesterol efflux is altered in type 1 and type 2 diabetic patients. It is increasingly recognized that cholesterol transport from cells to extra-cellular acceptors is a complex process in which various complementary mechanisms play a role. In addition to passive aqueous diffusion, energy-dependent cholesterol efflux by transmembrane transporter proteins represents an important pathway, although the relative contributions of the various pathways involved in cellular cholesterol removal are still unknown. ABCA1 and ABCG1 are ATP binding cassette transporters involved in cellular cholesterol efflux [1,2]. ABCA1 facilitates efflux of cholesterol and phospholipids to lipid-free and lipiddoor apo A-I, commonly designated as pre β-HDL [2-4]. It has been proposed that by transferring phospholipids to apo A-I, ABCA1 results in the formation of particles which are subsequently able to accept cell-derived unesterified cholesterol [4]. The
importance of the ABCA1 system for the development of atherosclerosis can be deduced from observations showing that macrophages present in atheromas express ABCA1, that overexpression of ABCA1 decreases atherosclerosis in animal studies, and that human ABCA1 deficiency, known as Tangier’s disease, results in a four-fold increased risk of cardiovascular disease [5-7]. ABCG1 has been discovered more recently and is currently subject of intensive research. This ATP-binding cassette transporter is thought to act in conjunction with ABCA1, by facilitating net cellular cholesterol mass efflux, resulting in further cholesterol enrichment of HDL particles. SR-BI, or its human counterpart CLA1, is a member of the CD36 superfamily, and facilitates bi-directional transmembrane cholesterol transport. The overall direction of cholesterol transport depends on the cholesterol concentration gradient between the intra- and extracellular compartment. SR-BI not only exchanges cellularly-derived unesterified cholesterol with cholesterol in mature HDL particles, but also mediates the cellular uptake of HDL cholesteryl esters. By this latter action it may even inhibit the net cholesterol efflux from cells to plasma HDL [8]. Its expression is unaffected by cholesterol loading of the cell, in contrast to the expression of ABCA1 which is enhanced by cellular cholesterol loading [9].

In our studies, we were interested in the properties of diluted whole plasma, as the acceptor medium, to stimulate cell-derived cholesterol efflux. Under these experimental conditions, the cell system was held constant thus allowing us to determine the extent to which cellular cholesterol efflux is affected by variations in the plasma to be tested. We used two cell systems to investigate the capacity of plasma to promote cellular cholesterol efflux. Human skin fibroblasts express ABCA1 abundantly after cholesterol loading but no SR-BI, while Fu5AH rat hepatoma cells express SR-BI, but no ABCA1. It is likely that ABCG1 expression is low or absent in both cell systems, which is in contrast with various lines of macrophages that have been recently used to discern the mechanisms involved in cellular cholesterol transport [10,11]. With human cultured fibroblasts validation experiments were carried out demonstrating that influx of cholesterol was far less than efflux, thus supporting that predominantly net cholesterol efflux was measured under the experimental conditions employed.

Looking at the capacity of plasma to stimulate cholesterol efflux from human skin fibroblasts and Fu5AH model cells, it was found in chapter 3 that an increased cellular cholesterol efflux to moderately hypercholesterolaemic plasma from type 1 diabetic patients is likely to be in part accounted for by higher plasma PLTP activity and elevated plasma HDL levels. Cholesterol efflux remained elevated after simvastatin treatment, which in part normalized the high plasma EST, CET and CETP activities and further increased HDL levels. In chapter 4 it was found that plasma pre β-HDL formation and CETP activity in type 1 patients are lowered by a low saturated fat, low cholesterol
diet, but this intervention did not affect the capacity of plasma to stimulate cellular cholesterol efflux. As described in chapter 5, an increased cellular cholesterol efflux was observed in hypertriglyceridaemic plasma from type 2 diabetic patients, in association with higher plasma PLTP activity, and elevated plasma EST, whereas pre β-HDL levels were unaltered.

Summarizing these studies, it is plausible that elevated plasma PLTP activity contributes to an increased cellular cholesterol efflux. The influence of plasma PLTP activity on promoting cellular cholesterol efflux seems to be direct and not via its effect to increase pre β-HDL generation, since lowering of these particles by statin treatment or diet intervention in type 1 patients did not affect the capacity of plasma to stimulate cholesterol efflux, whereas cholesterol efflux to hypertriglyceridaemic type 2 diabetic plasma was increased despite unchanged absolute pre β-HDL levels. Next to high plasma PLTP activity, an increase in EST independently contributed to increased cellular cholesterol efflux. This increased rate of cholesterol esterification may be one mechanism explaining the unaltered plasma pre β-HDL concentration in type 2 patients, despite increased PLTP activity. Taken together, it is possible that LCAT-mediated cholesterol esterification is involved in fibroblast cholesterol efflux to diluted whole plasma. Furthermore, the independent relationship of pre β-HDL formation with fibroblast cholesterol efflux is in keeping with the possibility that these particles are able to promote cholesterol efflux via ABCA1. Despite this relationship, a drop in pre β-HDL after diet intervention, probably consequent to a decrease in CETP activity, did not result in a change in cholesterol efflux. Also, diabetic patients had a higher cholesterol efflux capacity than healthy control subjects, despite a comparable pre β-HDL formation. Thus although it is likely that pre β-HDL is important for cellular cholesterol efflux it, appears that this process is to a relevant extent determined by other plasma components and yet incompletely understood pathways, including passive diffusion.

Concerning the diabetic state, it seemed conceivable beforehand that the capacity of plasma to promote cellular cholesterol efflux would be impaired, at least in type 2 diabetic patients with low HDL. However, in our experiments it turned out that the ability of plasma to stimulate cholesterol efflux from human skin fibroblasts was increased using plasma from both type 1 diabetic and hypertriglyceridaemic type 2 diabetic patients, as described in chapter 3 and chapter 5. Moreover, we recently found that fibroblast cholesterol efflux to plasma from subjects with the metabolic syndrome is maintained in spite of low HDL cholesterol and plasma apo A-I [12]. Apart from a relation with high plasma PLTP activity, the increased efflux out of Fu5AH cells and fibroblasts to plasma from type 1 diabetic patients coincided with increases in plasma apo A-I, EST, CET, CETP activity and HDL phospholipids. Cellular cholesterol efflux as measured with
both cells remained increased after HMG-CoA reductase inhibition. In this respect, an increased ability of plasma in type 1 diabetes mellitus to stimulate cellular cholesterol efflux both before and after cholesterol lowering may be interpreted as a protecting mechanism against the development of atherosclerosis. In sufficiently controlled type 1 diabetic patients, there appears to be no major influence of glycation of plasma proteins on the ability to remove cellular cholesterol. In type 2 diabetes mellitus, the increased ability of plasma to stimulate cellular cholesterol efflux is predominantly governed by an elevated plasma PLTP activity and EST. Collectively, we hypothesize that, as far as the ability of extracellular cholesterol acceptors to affect early steps in the reverse cholesterol transport pathway is concerned, a defence mechanism that protects against atherosclerosis may be operative in the diabetic state.

Preβ-HDL formation, expressed in apo A-I concentration, was found to be unaltered in type 1 and in type 2 diabetic patients as demonstrated in chapter 3 and in chapter 5. Of note, preβ-HDL concentration as well as formation was shown to be higher in hypertriglyceridaemic type 2 diabetic patients when expressed as a percentage of plasma apo A-I (chapter 5). This relative increase in preβ-HDL coincided with elevated plasma PLTP activity and CET. Although considerable differences in preβ-HDL concentrations have been reported in the literature, our findings agree with a recent report demonstrating that the relative proportion of apo A-I in pre HDL is increased in type 2 diabetes as a result of increased conversion of α-HDL to preβ-HDL [13]. This observation may be explained by elevated PLTP activity together with increased rates of cholesteryl ester transfer. It is, therefore, concluded that there is no defect in the ability of diabetic plasma to generate preβ-HDL particles.

**Lipid transfer proteins and development of atherosclerosis**

In the second part of this thesis we tested the relationships of carotid IMT, representing a marker of atherosclerosis, with plasma cholesteryl ester transfer (chapter 6) and PLTP activity (chapter 7) in type 2 diabetic patients and non-diabetic subjects. Our findings that IMT is positively associated with plasma cholesteryl ester transfer, and that both plasma cholesteryl ester transfer and IMT are higher in type 2 diabetic patients, are in agreement with the possibility that a high plasma transport rate of cholesteryl esters from HDL towards apo B-containing lipoproteins confers a proatherogenic phenomenon. In accord with these results, it has been shown that plasma CETP mass is a determinant of increased cardiovascular risk, but only in subjects with relatively high plasma triglycerides [14]. Of note, our group has recently shown that in subjects with low triglycerides, a high plasma CETP mass is a determinant of low rather than of high cardiovascular risk [15]. It seems, therefore, possible that the relationship
of cardiovascular disease with the plasma CETP level is dependent on the metabolic context, with the combination of low HDL cholesterol and hypertriglyceridaemia, as frequently found in type 2 diabetes mellitus, representing a potentially important target for intervention aimed at lowering plasma cholesteryl ester transfer. As our study (chapter 6) also shows that the effect of plasma CETP on cholesteryl ester transfer increases with higher triglycerides, it appears to be logical that CETP inhibition is particularly beneficial in hypertriglyceridaemic circumstances. This hypothesis is confirmed in a recent study showing that treatment with torcetrapib on top of atorvastatin markedly attenuates the postprandial increase in triglyceride-rich lipoproteins in type IIB hyperlipidaemic subjects [16]. Against this background it is important to briefly discuss the effects of CETP inhibitor therapy that has been published during the last year. Three studies have become available assessing the effect of torcetrapib in combination with atorvastatin compared to atorvastatin alone on an ultrasonographic marker of atherosclerosis. In a study in patients with coronary stenosis, 24 months treatment with atorvastatin+torcetrapib 60 mg resulted in a relative increase in HDL cholesterol of 59% as well as a decrease in LDL cholesterol and plasma triglycerides. No significant effect was seen on atheroma volume. However, on average systolic and diastolic blood pressure increased by 6.5 and 2.8 mm Hg, respectively in the torcetrapib group [17]. In another study in patients with a mixed dyslipidaemia (triglycerides > 1.7 mmol/l), HDL cholesterol was raised by 65% and plasma triglycerides decreased by 12%. No significant change on the maximum carotid IMT was seen, and systolic and diastolic blood pressure increased by 6.6 and 2.5 mmHg respectively [18]. In a comparable study in patients with familial hypercholesterolaemia, plasma HDL cholesterol increased by 54% and plasma triglycerides decreased by 8%. No effect was seen on carotid IMT progression. In this study, systolic and diastolic blood pressure increased by 4.1 and 1.8 mmHg, respectively [19]. The ILLUMINATE trial, the first study using torcetrapib with a cardiovascular event as primary outcome measure, was terminated prematurely because of an increased risk of death and cardiovascular events in the group receiving torcetrapib. Type 2 diabetic patients and patients with a cardiovascular event less than five years previously were included. After 12 months treatment plasma HDL had increased by 72% and plasma triglyceride levels had decreased by 9% in the torcetrapib+atorvastatin group, whereas systolic and diastolic blood pressure were raised by 5.4 and 2.0 mmHg respectively. The trial was terminated, since interim analysis showed a significantly higher number of events in the torcetrapib+atorvastatin compared to atorvastatin alone. The increased morbidity in the torcetrapib treated group could be related to the increase in blood pressure. Torcetrapib treatment was accompanied by a decrease in serum plasma potassium and an increase in serum sodium, bicarbonate and aldosterone. Post-hoc analysis showed an increased risk
of death in patients treated with torcetrapib whose reduction in potassium or increased in bicarbonate was greater than the median range [20]. The effect on blood pressure of this CETP inhibiting agent was also described in phase II studies [21,22], although to a lesser extent. A key question that still has to be resolved is whether the increase in blood pressure after torcetrapib should be considered to be an off-target effect of the drug itself or related to a previously unrecognized association between CETP regulation and activation of the renin-angiotensin-aldosterone (RAAS) system. In this respect it seems re-assuring that another CETP inhibitor, anacetrapib does not affect blood pressure and RAAS activation in a phase I-II study in healthy subjects [23]. In conclusion, it is still unsettled at present whether CETP inhibitor treatment may result in cardiovascular protection under certain circumstances.

Phospholipid transfer protein

In agreement with other studies [24,25], we described in chapters 3 and 4 that plasma PLTP activity is increased in type 1 diabetes mellitus. Also, in type 2 diabetes mellitus plasma PLTP activity is elevated (chapters 5 and 7). We previously found that an increase in plasma PLTP activity is related to elevated plasma triglycerides and indexes of obesity [26-28]. Recently, we showed that elevated plasma PLTP is determined by the metabolic syndrome and by type 2 diabetes mellitus per se [29]. One group however, reported that insulin resistance is an independent predictor of low plasma PLTP activity [30]. The plasma PLTP activity levels in that study were extremely low compared those reported by various other groups. Therefore the validity of this finding can be questioned [31].

PLTP is a lipid transfer protein with multifaceted properties. Initially, PLTP was regarded as anti-atherogenic considering its role in HDL remodelling, resulting in the formation of pre \( \beta \)-HDL [32-35]. PLTP exerts also a direct interaction with ABCA1 at the peripheral cell surface, resulting in stimulated cellular cholesterol efflux [36,37]. However, when the issue of atherosclerosis susceptibility was directly addressed in PLTP over-expression mouse models this lipid transfer protein appeared to be pro-atherogenic [38]. Importantly, over-expression of human PLTP in transgenic mice induced an almost 50% increase in hepatic VLDL secretion [39]. Conversely, PLTP deficiency in apo B-transgenic and apo E-deficient mice showed reduced production of apo B-containing lipoproteins, which resulted in less atherosclerosis [38]. Furthermore, PLTP deficiency in mice was shown to ameliorate diet-induced hypercholesterolaemia and inflammation [40]. However, in models without increased levels of apo B-containing lipoproteins, systemic PLTP over-expression also resulted in increased atherosclerosis [41,42]. In human atherosclerotic lesions PLTP is highly expressed, mostly in macrophages [43,44]. Bone marrow transplantation studies were conducted to induce PLTP over-expression
or deficiency in these cells specifically. In PLTP deficient macrophage models a decreased atherosclerotic lesion area was observed [45], whereas LDL-receptor knockout mice receiving bone marrow from transgenic mice with over-expression of human PLTP showed a 2-fold induction of plasma PLTP activity, increased PLTP-protein expression in the atherosclerotic lesions, as well as increased atherosclerotic lesion size [46]. On the other hand, PLTP-deficient macrophages were reported to increase atherogenicity in LDL-receptor knockout/PLTP-deficient mice [45]. A recent study showed atheroprotective qualities of elevated macrophage PLTP activity in a background of low systemic PLTP activity [47]. All together, animal experimental data concerning atherogenicity of macrophage-derived PLTP have yielded partly conflicting results, and it is conceivable that the effects of PLTP on atherosclerosis development may be site specific.

Data addressing the relationship between plasma PLTP activity and atherosclerosis in humans are limited. Schlitt et al. found that plasma PLTP activity is significantly elevated in patients with coronary artery disease compared to control subjects. Multivariate regression analysis showed that plasma PLTP activity had an independent predictive value for coronary artery disease [48]. This correlation may be a reflection of the association of plasma PLTP activity with inflammatory markers in patients with cardiovascular disease [49]. Plasma PLTP activity is reported to be elevated [50] as well as diminished [51] in patients with peripheral atherosclerosis. Several causes can be an explanation for these conflicting outcomes. Clinical characteristics of the populations studied with respect to the incidence of smoking, diabetes mellitus, obesity, and lipid lowering treatment all having an influence on plasma PLTP activity, may vary between studies. There also may be a different distribution between highly active and low active plasma PLTP due to differences in plasma factors modulating this distribution, like apo A1 and apo E [52,53]. Furthermore, it was proposed that plasma PLTP activity may be increased during the acute phase of a coronary event because of a systemic inflammation response [51]. In chapter 7 it is clearly demonstrated that plasma PLTP activity is positively associated with subclinical atherosclerosis in non-smoking, event free humans.

Future perspectives

Our results with respect to cellular cholesterol efflux, as described in this thesis, may prompt to develop new experimental strategies enabling us to determine the impact of variations in the acceptor medium in the ability of plasma to promote
cellular cholesterol efflux, as compared to cellular alterations in the cholesterol efflux potential itself. For example, novel cell systems, such as individual subjects’ peripheral blood macrophages, can be employed, and the effect of cellular changes as opposed to alterations in extra-cellular acceptors can be tested. Recently, this approach was used to study efflux defects occurring in subjects with isolated low HDL [54]. Together with the insights provided in this thesis, such a strategy will contribute to an integrated view on alterations in the various pathways involved in the reverse cholesterol transport process in type 1 and type 2 diabetes mellitus. Ultimately, the importance of altered cellular cholesterol efflux on the development of cardiovascular disease should be evaluated in a prospective study with special attention to differences between type 1 and type 2 diabetic patients.

The impact of plasma lipid transfer reactions on atherosclerosis development will continue to be an important area of research. After the disappointing results from the ILLUMINATE trial, there remains a believe in the possible beneficial effects of CETP inhibiting agents [20]. From our research, it can be concluded that treatment with CETP inhibitors is expected to be most promising in conditions with high triglycerides. Obviously, it will be essential to determine whether the adverse cardiovascular consequences associated with the use of torcetrapib is solely attributable to an off-target effect of this particular drug or class of drugs or for example to a hitherto unrecognized interaction of CETP action and the RAAS system. An increased plasma PLTP activity, as seen in type 2 diabetes mellitus, could have a pro-atherogenic effect. When this effect is confirmed in a prospective study, agents targeting plasma PLTP activity may be developed, thereby creating a new pharmacological strategy. Finally, the identification of genetically determined (partial) PLTP deficiency or substantially elevated PLTP activity would certainly help to unravel the role of PLTP on atherosclerosis development in man.
REFERENCES

4 Vaughan AM, Oram JF. ABCA1 and ABCG1 or ABCG4 act sequentially to remove cellular cholesterol and generate cholesterol-rich HDL. J Lipid Res 2006;47:2433-43.


Summary, general discussion and future perspectives


