The puzzle of high-density lipoprotein in cardiovascular prevention
El-Harchaoui, Abdelkarim

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
chapter 11

Summary and future perspectives
In the present thesis, different aspects of coronary artery disease risk reduction in relation to lipid modifying interventions are addressed. The first part focuses on improvements of classical risk assessment strategies by evaluating the additive predictive value of 'novel' lipid markers. The second part discusses the anti-atherogenic part of cholesterol metabolism, specifically the role of CETP and HDL cholesterol in prevention of coronary artery disease (CAD).

**PART I**

In **chapter 2** we evaluated the associations between LDL particle number and LDL size compared to the traditional lipid moieties LDL cholesterol and non-high density lipoprotein cholesterol (non-HDL cholesterol) and risk of future CAD. We addressed this hypothesis in a large prospective case-control study nested in the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) population study. We showed that LDL particle number and non-HDL cholesterol were more closely associated with the occurrence of future CAD than levels of LDL cholesterol. However, following adjustment for HDL cholesterol and triglyceride levels, the predictive capacity of LDL particle number was comparable to that of LDL cholesterol. Nonetheless, both LDL particle number and non-HDL cholesterol had incremental value on top of the Framingham Risk Score in multivariate analyses. Based on these data, we conclude that routine use LDL of particle number and or LDL particle size are not to be recommended for CAD risk assessment in the general population.

The same EPIC-Norfolk study was used in **chapter 3** to explore the relation between HDL size, HDL particle number and cardiovascular risk. In this study, we measured HDL size and HDL particle number and observed that these parameters were differentially associated with other cardiovascular risk factors, and were independently associated with the risk of future CAD. HDL size was strongly associated with well-known components of the metabolic syndrome (waist-to-hip ratio, apolipoprotein B and triglycerides), whereas HDL particle number was not. As a consequence, the relationship between HDL size and risk of future CAD risk was virtually abolished upon adjustment for metabolic parameters. The relationship between HDL particle number and CAD risk was independent of metabolic parameters. These findings indicate that HDL is a heterogeneous lipid fraction and that various HDL subpopulations are differentially associated with other cardiovascular risk factors and with cardiovascular risk. These findings have important implications for our current understanding and use of HDL in cardiovascular prevention:

First, the abolishment of a ‘protective’ effect of HDL-size following correction for metabolic parameters feeds the hot debate ‘if HDL protection is truly causal or merely associative in nature’. The close relation between HDL and metabolic derangements implies that merely increasing HDL in the light of the metabolic syndrome does not automatically correct the atherogenic
state. In addition, it lends further support to the concept that ‘the capacity of an HDL-increase to protect against cardiovascular disease should be tested in randomized clinical trials, since the epidemiological association does not necessarily prove causality’.

Second, these findings may bear direct consequences for the development of therapeutic strategies that modify HDL cholesterol. HDL-raising therapies that primarily affect HDL-size may have different effects on cardiovascular risk than those increasing HDL particle number.

In chapter 4 we directly compared the accuracy of apolipoprotein B/A-I ratio to standard lipid measurements to predict future CAD. The apolipoprotein B/A-I ratio was associated with future CAD events, independent of traditional lipid values (adjusted odds ratio, 1.85 [95% CI, 1.15 to 2.98]), including the total cholesterol–HDL cholesterol ratio, and independent of the Framingham risk score (adjusted odds ratio, 1.77 [CI, 1.31 to 2.39]). However, the overall impact did not outweigh that of traditional lipid values in predicting future CAD events (area under the receiver-operating characteristic curve, 0.670 for total cholesterol/HDL cholesterol ratio vs. 0.673 for apolipoprotein B/A-I ratio [P = 0.38]) and did not have an incremental predictive value over and above the Framingham risk score (area under the receiver-operating characteristic curve, 0.594 for Framingham risk score alone versus 0.617 for Framingham risk score plus apolipoprotein B/A-I ratio [P < 0.001]). These findings indicate that in a cohort of apparently healthy persons not receiving lipid-lowering therapy, apolipoprotein B/A-I ratio was more closely associated with future CAD events than the total cholesterol–HDL cholesterol ratio, but that the 2 measures were equivalent in their ability to discriminate between persons with and those without cardiovascular events. Thus, replacement of traditional lipid values with the apolipoprotein B/A-I ratio adds little to CAD risk assessment in the general population. However, it should be taken into account that other characteristics of the test (see future perspectives) already offer distinct advantages as compared to traditional lipid testing.

Moreover, the use of apolipoprotein B and apoAI also allows for more careful categorisation of lipid abnormalities (e.g. high triglycerides associated with higher apolipoprotein B indicates a pro-atherogenic tendency) allowing for more ‘personalized’ medicine particularly in subjects with ‘metabolic’ dyslipidemia.

FUTURE PERSPECTIVES

The search for additional markers besides LDL cholesterol to serve as the best lipoprotein to target CAD risk reduction is based on several hypotheses. First, expanding lipid measurements with LDL particle number and/or apolipoprotein B/A-I may have the potential to improve coronary disease risk assessment as well as decisions about LDL-treatment intensity, since they account for aspects of lipid-atherogeneity that are incompletely reflected by LDL cholesterol levels. Second, the prevalence of the metabolic syndrome is increasing worldwide. In these
patients the discrepancies between LDL cholesterol and other lipoprotein measures such as apolipoprotein B, LDL particle number or non-HDL cholesterol are accentuated resulting in attenuation of the accuracy of LDL cholesterol-based risk prediction in this population. Third, from a therapeutic point of view, large trials have shown that for patients on statin treatment non-HDL cholesterol, apolipoprotein B and LDL particle number perform superior at predicting CAD outcomes compared to LDL cholesterol.

What are the clinical consequences in the near future? In primary prevention the addition of LDL particle number has no clear additional value. If any, there may be a role in monitoring patients who are on statin therapy. Further studies are required to resolve this issue.

The implementation of the apolipoprotein B/A-I ratio is a matter of intense debate. Several large studies have shown that the apolipoprotein B/A-I is a good predictor for the occurrence of cardiovascular disease in the general population (INTERHEART, AMORIS, Quebec Prospective Cardiovascular Study). Yet, the actual level of improvement may be limited since it only becomes significant in case of a very large sample size (INTERHEART). At the same time, the apolipoprotein B/A-I index has several practical advantages. First, both apolipoprotein B/A-I and Apolipoprotein A-I are measured directly by standardized and internationally well validated techniques with excellent laboratory reproducibility. Second, the 2 components of the ratio reflect the two sides of the risk equation, namely the atherogenic apolipoprotein B and the anti-atherogenic apolipoprotein A-I. Third, the possibility to reliably assess lipoprotein values also in non-fasting blood samples offers a large practical advantage for both patients as well as laboratories. Finally, the additional ‘diagnostic’ value in a metabolic context (diabetes, metabolic syndrome) allows for more careful choices in potential medication. Based on these considerations, routine implementation of apolipoprotein B and A-I, either replacing the traditional lipid spectrum or, alternatively, added on top of the traditional lipid spectrum, has been advocated.

What about HDL as a therapeutic target? The current vision is that HDL is a potential target in the fight against atherosclerosis. However the HDL particle is also a complex lipoprotein that has a variety of manifestations and properties which complicates effective pharmacological interventions. Moreover in the study described in Chapter 3 we show that different aspects of HDL are differently associated with cardiovascular risk and cardiovascular risk factors. As already described above the close relation between HDL and metabolic parameters supports to the theory that ‘the capacity of HDL-increase to protect against cardiovascular disease should be tested in randomized clinical trials, since the epidemiological association does not necessarily prove causality’. 
PART II

In the first chapter (chapter 5) of the second part we measured fecal sterol excretion, as the end product of the reverse cholesterol pathway, in patients with genetically low HDL cholesterol levels. We observed that subjects with familial hypoalphalipoproteinemia (FHA) had significantly lower fecal sterol excretion compared to healthy controls. Moreover we found a strong positive correlation between HDL cholesterol and fecal sterol excretion. The correlation between HDL cholesterol levels and excretion parameters suggests that endogenous HDL cholesterol levels are a marker for the rate of cholesterol excretion from the body, at least in subjects with profoundly lowered HDL cholesterol levels. Although definite conclusions on the relation between HDL and RCT cannot be drawn from this study, because of the small sample size, it stresses the need for further research on the ‘rate-limiting’ impact of circulating HDL on whole body reverse cholesterol in humans.

Cholesteryl ester transfer protein (CETP) plays a key role in lipid metabolism and potentially also in reverse cholesterol transport by transferring triglycerides and cholesterol between lipoproteins. In this study we evaluated whether the levels of CETP affect the postprandial changes in HDL cholesterol levels (chapter 6). We observed that plasma CETP levels contribute to the HDL cholesterol decrease following consumption of a fat rich meal. A similar but less pronounced decrease in HDL cholesterol was seen following two carbohydrate-rich meals. The change in HDL cholesterol concentration following fat-rich meals was inversely and independently related to fasting CETP concentrations, fasting HDL cholesterol and postprandial changes in triglycerides. This findings may have consequences for CETP inhibitors that are currently in development in a way that they may be more effective in the postprandial state.

In chapter 7 we evaluated the relation of genetic CETP deficiency to CAD risk in the general population. The CETP-629C→A polymorphism is a common polymorphism in the general population and is associated with decreased CETP activity and increased HDL cholesterol levels. Given the storm surrounding CETP as a potential target for therapeutic intervention, we tried to obtain more insight into the relation between CETP and cardiovascular risk. Although this polymorphism was associated with life-long increased plasma HDL cholesterol levels mediated by genetically determined lower CETP levels, CETP polymorphisms were not associated with cardiovascular protection. Based on these findings, one could postulate that CETP inhibition does not protect against cardiovascular disease progression. However, a recent meta-analysis of 132 studies with approximately 200,000 subjects showed that the common CETP variants Taq1B, 1405V and -629C/A were associated with modest decrease in CETP activity, modest increase in HDL cholesterol levels, and were associated with a weakly reduction in coronary heart disease risk of ~5% (1). In contrast to the meta-analysis and in line with our results, the PREVEND study demonstrated that -629A and Taq1B carriers had increased risk of CHD despite
higher HDL cholesterol levels(2). Although lack of statistical power in our study may explain the absence of a relation between the CETP -629C→A polymorphism and CAD, the possibility of publication bias in the meta-analysis cannot be ruled out. Since the controversy around this issue has not been definitely terminated, a careful follow-up of CETP inhibitors currently in clinical development is warranted.

In chapter 8 we provide an overview of the role of CETP as a novel approach to prevent CAD. CETP-inhibitors are currently being investigated because of their ability to increase high-density lipoprotein cholesterol levels. It was concluded that the relationship between CETP and lipoprotein metabolism is complex, and may depend largely on the metabolic context. During the preparation of this manuscript interim results of a large ongoing end point-trial trial with the CETP-inhibitor torcetrapib at that time became clear (ILLUMINATE)(3). The negative findings resulted in premature discontinuation of this trial due to increased mortality in torcetrapib-treated subjects. Shortly after the termination of this trial data from surrogate endpoint trials with torcetrapib in patients with mixed dyslipidemia (RADIANCE I)(4) and hypercholesterolemia (RADIANCE II)(5) were published. Despite a considerable increase of plasma HDL cholesterol in all these trials, torcetrapib did not slow down the progression of atherosclerosis as estimated by measurements of the carotid IMT. Since the termination of the ILLUMINATE trial, new data became available that demonstrated that torcetrapib treatment was associated with a substantial increase in aldosterone levels, changes in serum electrolytes indicative of mineral corticoid excess, and elevated blood pressure. Posthoc analysis of the two IMT trials showed that those with the greatest increase in systolic blood pressure had the largest progression in their carotid imaging–based assessment of atherosclerotic burden (6). Moreover it was recently shown in rats (normotensive and hypertensive models) that torcetrapib increased blood pressure in a dose dependent way with a concomitant increase in gene expression of renin angiotensin system in the adrenal glands and aorta. In contrast, dalcetrapib another CETP inhibitor, had no effect on blood pressure and on RAAS-related gene expression (7). In line with these observations, no clinically relevant effects on blood pressure have been observed in phase II trials with dalcetrapib (8) and anacetrapib (9). These findings suggest that the off-target effects seen with torcetrapib are compound-specific and not common to other agents that act on CETP.

Chapter 9 discusses the mechanisms of how CETP-inhibition might affect the process of atherosclerosis. In this chapter we discuss the role of CETP inhibition further than increasing HDL cholesterol. We discuss how CETP inhibition affects circulating lipids and lipoproteins, and focus on the manner in which this may influence cholesterol exchange processes between the periphery, the circulation and the liver. Finally the possible effects of CETP inhibition on fecal sterol excretion, on the anti-inflammatory and anti-oxidative properties of HDL are briefly discussed.
Finally chapter 10 describes the results of CETP inhibition in patients with familial hypoalphalipoproteinemia. In this study modest CETP inhibition resulted in beneficial effects beyond its HDL increasing action, including a reduced number of small LDL particles as well as augmentation of the plasma anti-oxidant capacity (reduced ox-LDL-autoantibodies and enhanced serum paraoxonase-1 activity). These findings suggest an anti-atherogenic effect of CETP-inhibition in patients with genetically low HDL cholesterol levels.

**FUTURE PERSPECTIVES**

Similar to increased LDL cholesterol, low levels of HDL cholesterol constitute a major, independent cardiovascular risk factor at least in epidemiological studies. Given this fact, unravelling the mechanisms leading to low HDL cholesterol levels are of substantial and biological importance. Before any new treatment can be used in practice it is important to look at these mechanisms. In this respect the role of CETP in cardiovascular disease reduction is still undefined. Consequently the role of CETP inhibitors is currently at equipoise. Without exception, all CETP inhibitors have positive effects on HDL cholesterol levels. Despite the tremendous increases in HDL cholesterol levels, the impact of CETP inhibition with torcetrapib on atherosclerosis is disappointing. The off target-toxicity of this specific drug played undoubtedly a role in the negative outcomes. However, despite these off target effects, it is still unclear whether the mechanism of CETP inhibition is beneficial or on the contrary harmful irrespective of increasing HDL cholesterol levels. Ongoing phase III programs for dalcetrapib and Anacetrapib will have to provide proof of concept. Within these programs it will be imperative to closely monitor safety (focused at blood pressure, RAAS and aldosteron), although currently available data underscore the lack of these toxicities until now.

It is possible that CETP inhibition might impair RCT by generating HDL particles that are incapable at promoting cholesterol efflux and/or by reducing the return of HDL derived cholesterol to the liver for excretion from the body. Torcetrapib did not change fecal cholesterol excretion, however this was a small study (10). The effect of CETP inhibition on RCT in humans has yet to be definitively tested.

The unexpected failure of torcetrapib highlights unresolved questions in the HDL field. Given the functional heterogeneity of HDL particles, simply increasing low HDL cholesterol levels appears inadequate. In view of the potential pitfalls regarding the measurements of solely HDL cholesterol, these measurement should be combined with the evaluation of HDL function when assessing the therapeutic efficacy of HDL-targeted therapies. Promotion of reverse cholesterol transport is an important mechanisms by which HDL is thought to protect against atherosclerosis, and methods to assess the key functions in this pathway gain attraction. Evaluation of the potential effects of HDL-targeted interventions on RCT and atherosclerosis requires reliable
assays of HDL function and surrogate markers of efficacy. Such assays may involve assessments of whole-body efflux with 13C-cholesterol infusion and measurements of the capacity of HDL to induce cellular cholesterol efflux through the ABCA1, ABCG1 and SR-BI pathways. Evaluation of such potential RCT targeted interventions requires validation and subsequently evaluation in HDL increasing interventions, ideally in a head to head comparison with cholesterol efflux generators such as LXR agonist en ABCA1 agonists. With such an approach, pharmacologically-induced HDL-cholesterol elevation can be anticipated to finally translate into diminished cardiovascular risk and clinical benefit.
REFERENCES