HDL cholesterol: atherosclerosis and beyond
Bochem, A.E.

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Summary, Interpretation and Future Perspectives

A. E. Bochem
This thesis addresses the metabolism of high density lipoprotein (HDL) and its role in atherogenesis, steroidogenesis, hematopoiesis and inflammation. Chapter 1 gives an overview of the evidence from genetic studies for a role of HDL-c in atherogenesis as an introduction to this thesis. Whereas epidemiological studies have unequivocally shown that HDL-c is inversely associated with risk for CVD, the failure of HDL-c increasing therapies cast doubt on the causal role of HDL-c in atheroprotection. Studies in monogenic disorders causing low HDL-c such as ABCA1, APOA-1 and LCAT could not provide a definite answer to the causality issue, nor could studies of disorders causing high HDL-c such as CETP, SR-BI and APOC3 deficiency. Genome Wide Association Studies (GWAS) have not shown an effect of SNPs affecting only HDL-c levels on CVD risk. The debate whether HDL-c is an actor in atherogenesis or a mere biomarker, is at the center of current cardiovascular research.

In Part 1 of this thesis, we focus on the correlation between HDL metabolism, ABC transporters and atherosclerosis.

In Chapter 2, we show that plasma levels of apoA-I do not offer additional predictive value over plasma levels of HDL-c in the Epic-Norfolk Prospective Population Study. Interestingly, within the highest HDL-c quartiles, higher apoA-I levels associate with a higher prevalence of CVD, possibly due to the higher prevalence of cardiovascular risk factors in association with higher apoA-I levels. This could be a relevant finding in light of the current development of apoA-I increasing strategies.

In Chapter 3, the evidence for suppression and prevention of atherosclerosis by ABC transporters is reviewed. The atheroprotective effects of ABC transporters have long been attributed to their capacity to transport cholesterol from lipid-laden macrophages in the vessel wall to HDL for subsequent delivery to the liver for biliary excretion. However, by promoting cholesterol efflux, ABC transporters also control the proliferation and mobilization of hematopoietic stem and multipotential progenitor cells in the bone marrow and spleen. Furthermore, ABC transporters suppress inflammatory responses in the arterial wall and control platelet production and thrombosis. These processes likely play an important role in the development of CVD in humans.

Chapter 4 describes the prevalence of ABCA1 mutations in 78 subjects with HDL-c below the 10th percentile. Sixteen subjects were found to carry 19 variants in ABCA1. Functionality of the mutations was assessed by means of cellular cholesterol efflux potential. Seven out of eight missense mutations resulted in a significant loss of cholesterol efflux capacity. In Chapter 5 we subsequently assessed whether the cholesterol efflux impairment of these mutations are associated with increased atherosclerosis as assessed by carotid ultrasound and the highly sensitive method of carotid 3T MRI. ABCA1 mutation carriers were shown to have more atherosclerosis compared to controls. These data support the development of strategies to upregulate ABCA1 in patients with established cardiovascular disease. However, further studies are needed to elucidate which of ABCA1’s properties is responsible for these findings.

In murine models, Abca1 deficiency has been shown to lead to endothelial dysfunction. Pulse wave velocity is an independent risk factor for cardiovascular disease and is
associated with endothelial function. In Chapter 6 we show that ABCA1 mutation carriers not only have more atherosclerosis, but also display increased arterial stiffness as assessed by pulse wave velocity, independent of HDL cholesterol levels. Furthermore, we conclude that PWV may be a valuable, cost-effective, non-invasive addition to current CVD monitoring practice, given its strong correlations with vessel wall thickness parameters. Chapter 7 describes a unique case of combined deficiency of ABCA1 and APOAI in a 36-year old patient who experienced a myocardial infarction. This combined molecular defect is consistent with the observed near absence of HDL-c in plasma. Since specific HDL-c increasing therapy is not available yet, treatment is limited to the modulation of other risk factors, including LDL-c lowering.

After studying the consequences of genetically determined low HDL-c for atherosclerosis, we addressed the consequences of genetically determined high HDL-c. In Chapter 8, we report the prevalence and clinical consequences of APOC3 mutation carriership in individuals with plasma HDL-c levels above the 95th percentile. In five out of 80 individuals, an APOC3 mutation was found. We found two novel mutations and one previously reported mutation. Two of these three mutations show a clear effect on lipid profiles, accompanied by large decreases of plasma apoCIII, whereas the third mutation is associated with less pronounced changes in the lipid profile and a smaller decrease in plasma apoCIII. This study confirms an association between loss of apoCIII and favourable lipid profiles and lends support to apoCIII lowering therapy which is currently tested in a clinical trial. In Chapter 9, we sequenced the gene encoding SR-BI in 120 unrelated individuals with HDL-c above the 90th percentile. We found two novel mutations in the gene encoding SR-BI. Our data expand the number of documented human mutations in this gene to three. The novel mutations segregate with high HDL-c within the family, indicating that the SR-BI receptor is a physiologically relevant HDL receptor in humans.

ApoA-I is the structural protein of the HDL particle and it plays a crucial role in many of the beneficial effects of HDL. Having investigated consequences of both low and high HDL cholesterol, we subsequently review the potential applicability of pharmacologically increasing apoA-I by means of apoA-I mimetic peptides in Chapter 10. While genetic studies show conflicting results on correlations between HDL-c and CVD, experimental studies have yielded sufficient encouraging data to proceed with the development of HDL-c raising strategies. There is evidence that ApoA-I mimetic peptides may be a promising supplement to current strategies. However, results from human studies are sparse and more research in the human setting is needed. In Chapter 11, we review the promise of another class of HDL-c increasing agents in CVD prevention: CETP inhibitors. CETP inhibition has been shown to increase HDL-c levels in man. However, torcetrapib, the first CETP inhibitor tested in phase III trials resulted in increased mortality possibly due to apparent compound-specific vasopressor effects. More recently, dalcetrapib, another CETP inhibitor, did not show an effect on CVD outcome while raising HDL-c by 30%, thereby refuting the HDL-c hypothesis. Anacetrapib and evacetrapib are currently tested in phase III clinical trials and have not shown adverse effects thus far. Both compounds not only increase HDL-c
by 29-51%, they also decrease LDL-c (36-41%) while anacetrapib lowers lipoprotein (a) by 17%. Combined, these effects are anticipated to decrease CVD risk and the results will be revealed in 2017. However, these compounds will not aid us in answering the question whether pharmacological increase of HDL-c leads to decreased CVD risk.

In part II of this thesis, we focus on the role of HDL in adrenal function, hematopoiesis and inflammation. In Chapter 12 we assessed adrenal function in Lcat knockout mice. In vitro studies have suggested that HDL and LDL can provide cholesterol for steroid hormone synthesis by the adrenal gland. We report that HDL deficiency in Lcat knockout mice, characterized by dramatically decreased HDL-c levels, is associated with a 40–50% lower adrenal steroid output. These findings highlight the important novel role for HDL as a cholesterol donor for the adrenal steroidogenesis in mice. In Chapter 13, we subsequently assessed adrenal function in male subjects with low HDL-c due to mutations in ABCA1 or LCAT, as well as in subjects with low HDL-c without underlying genetic defect. We show that basal adrenal steroidogenesis is decreased in male subjects with low plasma HDL-c, irrespective of its origin. These findings support a role for HDL as a cholesterol donor for basal adrenal steroidogenesis in humans. In contrast, Chapter 14 describes that adrenal steroidogenesis is not lower in female ABCA1 or LCAT mutation carriers. This gender-related discrepancy underscores the importance of gender specific analyses in cholesterol-related research.

Chapters 12 and 13 provide evidence for a role for HDL derived cholesterol in adrenal steroidogenesis. However, in homozygous subjects with extremely low HDL-c, substantial adrenal steroidogenesis was present. Furthermore, basal adrenal steroidogenesis but not adrenal response to ACTH was impaired in low HDL-c subjects, indicating that other pathways may supply the adrenal gland with cholesterol in acute settings. We therefore hypothesized that LDL provides the human adrenal gland with substrate for hormone synthesis. In Chapter 15 we therefore assessed cortisol response to ACTH in patients with impaired uptake of LDL derived cholesterol due to defective LDL receptor function or defective apolipoprotein B (the ligand for LDL binding to its receptor) and controls. Response to ACTH was lower in patients with impaired uptake of LDL derived cholesterol compared to controls. Our data support a role for LDL derived cholesterol in the adrenal response to stress in humans. Current therapeutics, aiming to lower plasma LDL cholesterol to below 1 mmol/L may jeopardize adrenal function.

In recent years, it has become clear that atherosclerosis is an inflammatory process. Previous studies have shown that mice with defects in cellular cholesterol efflux are characterized by hematopoietic stem cell (HSPC) and myeloid proliferation, leading to more circulating inflammatory cells, likely contributing to atherogenesis. In Chapter 16, we hypothesized that the combination of hypercholesterolemia and defective cholesterol efflux would promote HSPC expansion and leukocytosis more prominently than either alone. Our data suggest that in mice, a balance of cholesterol uptake and efflux mechanisms may be driving HSPC proliferation and monocytosis. Higher monocyte counts in children with FH and low HDL-cholesterol suggest a similar pattern in humans, possibly contributing to the atherosclerotic phenotype in hypercholesterolemic subjects.
Given recent findings in mice that defective cellular cholesterol efflux pathways increased inflammatory gene expression in monocytes and macrophages, as well as production of inflammatory cells, we hypothesized in Chapter 17 that human ABCA1 mutation carriers would be characterized by increased inflammation. We show that ABCA1 mutation carriers are characterized by pro-inflammatory changes of the arterial wall as assessed by PET-CT as well as a systemic pro-inflammatory state. In vitro experiments were performed to determine whether the pro-inflammatory phenotype originates from plasma or a cellular component using plasma isolated from ABCA1 mutation carriers. Our data confirm a pro-inflammatory state in ABCA1 mutation carriers as reflected by increased circulating cytokines, most likely secondary to a cellular effect of ABCA1 deficiency. Statins seem to exert beneficial anti-inflammatory effects, as shown by normalization of PET-CT and plasma cytokine levels.

**Interpretation and future perspectives**

Although epidemiological studies show that HDL-c is inversely associated with CVD risk, HDL-c increasing therapies have not resulted in lower incidence of CVD. This has cast doubt on the atheroprotective capacities of HDL. However, it has also increased interest in the roles of HDL in processes unrelated or indirectly related to atherosclerosis. This thesis does not answer the question whether HDL is an atheroprotective entity or not, but it provides pieces to the puzzle of HDL metabolism.

Studies in ApoAI (chapter 7), ApoCIII (chapter 8), SR-BI (chapter 9) and LCAT (chapter 12, 13 and 14) mutation carriers give us an insight into their specific effects on HDL metabolism. ABC transporter deficiency is a particularly interesting condition since it leads to both cholesterol accumulation and low plasma HDL-c (chapter 3, 4, 5, 6, 7, 13, 14, 17).

We show that ABCA1 mutation carriership is associated with increased atherosclerosis and increased arterial stiffness. The finding that ABCA1 mutation carriership does not uniformly result in an increased CVD event rate, underscores the complexity of HDL metabolism. Although we show that novel mutations in the genes encoding SR-BI and APOC3 are associated with favourable lipid profiles, the sample size and cross-sectional design of the study do not allow us to draw conclusions regarding CVD risk.

Despite setbacks, considerable effort is still put into HDL-c increasing strategies as reviewed in chapters 10 and 11. In order to predict their efficacy and side effects, it is important to oversee the whole range of effects that are mediated by HDL. In that regard, more insight is needed on the effects of HDL beyond atherosclerosis. The novel finding that low HDL-c is associated with decreased steroidogenesis in males is intriguing and is an example of how crosstalk between research fields can lead to important findings and an increased understanding of human physiology. Other examples include the importance of the lymphatic vessel route for reverse cholesterol transport and atherosclerosis, the role of cholesterol accumulation in cancer and macular degeneration, and the role of cholesterol efflux in haematopoiesis.

The roles of HDL and ABC transporters in inflammation form a particularly fascinating field. The paradigm that atherosclerosis is an inflammatory process, has been widely
acknowledged. Our findings in chapter 17 constitute the first evidence for increased inflammation in *ABCA1* deficiency in humans. The role of inflammation in atherosclerosis, as well as its importance in a range of other diseases, secures a strong research interest where crosstalk between researchers from different areas may turn out crucial.

In conclusion, HDL and ABC transporters are definitely actors of importance in multiple physiological processes in humans. The notion that HDL may not be the atheroprotective particle it was long assumed to be, elicited a queste to explain the inverse correlation between HDL-c and CVD risk by taking the broad spectrum of effects of HDL-c on various organ systems into consideration. This enabled a shift of interest and resources towards exploration of effects of HDL-c beyond reverse cholesterol transport and advanced the insight in human physiology. ABC transporters aid us in shedding light on the importance of cholesterol homeostasis in human physiology. The full range of effects of ABC transporters has not been elucidated to date, and more research is needed on their function in different tissues and organ systems.

Andrea Bochem
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Reference List


