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Chapter 2

High-density lipoprotein and atherogenesis: from fatty streak to clinical event

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Introduction

Today there is substantial evidence to support a strong inverse and independent relationship between circulating levels of high-density lipoprotein (HDL) levels and the risk of cardiovascular disease\textsuperscript{1-2}. Trials assessing approaches to raise HDL have demonstrated that elevation of this lipoprotein decreases the incidence of cardiovascular events\textsuperscript{3-4}. Moreover, studies in patients with hereditary forms of HDL-deficiency as well as genetically engineered animals underscore causality between low HDL levels and atherosclerosis progression\textsuperscript{5-7}. Over the past few years, HDL has become a major component of risk stratification algorithms to assess individual cardiovascular risk.

HDL constitutes a heterogeneous family with a predominance of spherical over discoidal particles with a density and size between 1.06-1.21 and 5-17 nm, respectively. The physiological relevance of different types of particles is poorly understood, however, spherical HDL particles in particular have multiple atheroprotective activities\textsuperscript{8}. HDL's most abundant protein apolipoprotein A-I (apoA-I) contains 10 amphiphatic helices (mostly class A), allowing it to bind lipids on the one hand and to associate with the aqueous environment on the other\textsuperscript{9}. These features facilitate the ability of apoA-I to promote cellular cholesterol efflux and to activate lecithin:cholesterol acyltransferase (LCAT)\textsuperscript{10}. Noticeably, the molecular structure of apoA-I can be affected by the HDL core composition, particularly with reference to discoidal particles. By using reconstituted HDL, adding triglycerides (TG) to a discoidal particle was found to reduce the helix content with ensuing reduced particle stability, whereas cholesterol esters (CE) rendered the opposite. Also in spherical HDL, increasing the CE/TG ratio in particles increased the $\alpha$-helix content and stability\textsuperscript{11}. Alternative HDL constituents include the poorly understood apoA-II and a variety of other proteins, including apoA-IV, apoC-I, -II and -III, apoJ, apoM, serum amyloid A, ceruloplasmin, transferrin, and enzymes such as LCAT and PON1. All these proteins may affect HDL function in a qualitative manner.

HDL and apoA-I have been implicated in many atheroprotective mechanisms. Classically, HDL and apoA-I are considered to play a principal role in reverse cholesterol transport (RCT), a process that regulates intracellular cholesterol homeostasis. In fact, RCT comprises three main steps involving the adenosine triphosphate binding cassette protein A1 (ABCA1)-mediated efflux of excessive cholesterol from peripheral cells (e.g. macrophages) to lipid-poor apoA-I, the subsequent esterification by LACT of HDL-associated cholesterol, and finally receptor-mediated delivery of cholesterol ester to the liver for biliary excretion\textsuperscript{12}. Doing so, HDL may prevent intracellular cholesterol accumulation with ensuing elevation of oxysterols and enhanced generation of reactive oxygen species (ROS)\textsuperscript{13} Otherwise, HDL-mediated RCT has been associated with the mobilization of excessive amounts of tissue cholesterol in atheromatous plaques\textsuperscript{14}. Although it is rather difficult to measure integrated RCT \textit{in vivo}, Zhang et al. demonstrated enhanced macrophage-specific RCT in mice, dictated by apo-A-I\textsuperscript{15}. In addition, pro-apoA-I infusion in 4 hypercholesterolemic subjects increased faecal sterol excretion by 39\%\textsuperscript{16}. Notwithstanding, the rate of RCT may not be reliably reflected by levels of circulating HDL/apoA-I, as reported by studies of whole body RCT\textsuperscript{17-19}.
HDL and endothelial function

HDL has recently emerged as a clinically significant agonist of eNOS either via direct mechanisms (ie. modulating its activity, expression and subcellular distribution) or through its ability to inhibit LDL-oxidation with ensuing uncoupling of eNOS, producing superoxide anions rather than NO\textsuperscript{20-21}. Nitric oxide (NO) that is important for responsiveness to receptor dependent stimuli, and controlling neutrophil adherence, VSMC proliferation and platelet aggregation/adhesion\textsuperscript{22}, is synthesized by eNOS through the conversion of L-arginine to L-citrulline. Its activity is regulated by complex signaling transduction pathways including activation of the kinases that alter the phosphorylation of this enzyme, MAPK signaling, the intracellular Ca\textsuperscript{2+} content, and calcium-calmodulin complexes \textsuperscript{22}. It has become evident that HDL may activate eNOS by targeting its phosphorylation and increasing intracellular Ca\textsuperscript{2+}, and that apo-A-I is required but not sufficient for eNOS activation \textsuperscript{23-25}. HDL-associated components, involving ceramide and estradiol have also been implicated in HDL’s vasodilatory effects\textsuperscript{26,27}. Nofer et al. reported that HDL-induced vasodilation in part could be attributed to 3 major lysophospholipids (SPC, S1P, and LSF) present in HDL, while deficiency of the lysophospholipid receptor S1P\textsubscript{3} abolished Ca\textsuperscript{2+}-mobilization and Akt-mediated eNOS phosphorylation in response to HDL\textsuperscript{28}. Further, HDL may upregulate the abundance of eNOS by preserving eNOS protein stability via ERK1/2 and Akt\textsuperscript{29}. HDL may also alter eNOS localization and activity by controlling the cholesterol homeostasis of caveolae, which are specialized membrane domains that harbor and coordinate many signal transduction pathways \textsuperscript{30,31}. Both N-terminal myristoylation and palmitoylation targets eNOS to endothelial caveolae, whereas the lability of NO and the complex regulatory mechanisms of eNOS emphasizes the impact of eNOS’s intracellular localization on its enzyme activity \textsuperscript{32,33}. Oxidized LDL (oxLDL) causes CD36-dependent cholesterol depletion of caveolae with subsequent redistribution of eNOS, compromising the ability to activate the enzyme. HDL may preserve the unique lipid environment within caveolae by donating cholesterol esters through the receptor SR-B1, highly expressed in endothelial caveolae\textsuperscript{31,34}. In addition, acute stimulatory effects of apoA-I have been reported on intracellular cholesterol trafficking from the Golgi apparatus to the cell-surface in human fibroblasts \textsuperscript{35}, which implies an increased number of membrane caveolae with subsequent reshuttling of eNOS to caveolar regions in endothelial cells. Other mechanisms to preserve NO bioavailability, involve HDL carrying antioxidant enzymes (eg. PON and PAF-AH) that reverses the cytotoxic effects of oxLDL\textsuperscript{36}, bolster the antioxidant status of endothelial cells and reduces the susceptibility to oxidation of LDL\textsuperscript{37,38}. Recently, apo-A-I mimetics have been shown to protect endothelial cell function by preventing LDL from uncoupling eNOS activity to favor O2 anion production over NO production \textsuperscript{39,40}. Last, HDL has been shown to maintain endothelial cell integrity by attenuating apoptosis \textsuperscript{41,42} and by promoting endothelial cell proliferation\textsuperscript{43}

HDL and atherothrombosis

HDL has been implicated in the regulation of thrombosis, both arterial and venous.
With reference to the arterial, anti-thrombotic abilities, HDL has been demonstrated to promote endothelial synthesis of prostacyclin, which synergistically interact with NO. Underlying mechanisms include provision of arachidonate and upregulation of COX-2 expression. Further, HDL attenuates important prothrombotic factors on blood cell surfaces including selectins and tissue factor (TF), as well as cell-derived, microparticles to decrease thrombus formation. Indirectly, HDL has been found to reduce TF-induction on endothelial cells by stimulating NO synthesis (see abovementioned mechanisms). HDL may further counterbalance TF by enhancing the anti-coagulant activity of TFPI and the protein C-pathway. The latter has been demonstrated in vitro, where large HDL functions as an anticoagulant cofactor when it enhances the inactivation of purified factor Va by activated protein C (APC) and protein S. Moreover, infusion of HDL in cholesterol fed rabbits upregulated endothelium-derived thrombomodulin, which supports APC-generation and suppresses thrombin synthesis. Further, HDL may promote fibrinolysis by downregulating PAI-1 and upregulating tPA. With regard to platelets, administration of exogenous HDL in humans have been shown to inhibit platelet activation in vivo. In detail, HDL has been found able to inhibit platelet activation directly or indirectly via downregulating the release of platelet activating factor or by upregulating NO. In addition, HDL may beneficially affect the prostacyclin/thromboxane A2 balance by upregulating prostacyclin production and downregulating biosynthesis of TxA2.

In summary

The strong, inverse relationship between plasma HDL concentration and the risk of cardiovascular disease (CVD) has been a consistent finding throughout numerous epidemiological studies. It has been recognized that the anti-atherogenic effect of HDL is not only confined to its established role in the reverse cholesterol transport pathway, but also includes anti-inflammatory, anti-oxidative and direct vascular effects. Strategies aimed at increasing HDL-C therefore hold great promise.

Outline of this thesis

In this thesis entitled "High-density lipoprotein and C-reactive protein, friend and foe in cardiovascular disease" we set out to examine two novel cardiovascular risk factors for their potential role in mediating atherogenesis. In the following section we will briefly address the contents of the individual chapters included in this thesis.

In Chapter 1 & 2 we review the current literature with reference to CRP and HDL and focus on their causal role during the various stages of atherogenesis. The profile of each of these
mediators has been postulated from the available, mostly in vitro data, and served as background for designing the experiments, described in chapter 4-10. With emerging therapies targeting various pro-atherogenic pathways, there is a great need for the development of reliable surrogate markers to assess the beneficial ‘therapeutical profile’ of novel interventions. End point studies are expensive, labour-intensive and time-consuming, where intermediate endpoints are much easier to evaluate the impact of novel mediators and/or therapeutic interventions on cardiovascular disease progression.

Chapter 3 How good are our current available methods to assess these surrogate markers? Anticipating our studies evaluating the in vivo effects of HDL and CRP, chapter 3 deals with the ins and outs of emerging, noninvasive imaging modalities to assess early cardiovascular disease and discusses whether they may serve as suitable intermediate endpoints in future trials.

Chapter 4-6 Circulating HDL levels have been inversely related to cardiovascular risk by numerous epidemiological studies. The fact that low HDL is often associated with other risk-factors such as enhanced triglyceride levels and insulin resistance might suggest that those, instead of low HDL per se, might be predominantly responsible for the observed relationship with cardiovascular events. In chapter 4 & 5, we sought to determine the clinical consequences of an isolated low-HDL state for cardiovascular risk and its derivative or surrogate biomarkers such as endothelial vasoreactivity and intima-media thickness. Doing so, we provide evidence in both chapters for a pro-atherosclerotic state associated with low-HDL per se. In chapter 5 & 6, we questioned whether the adverse effects coupled to low-HDL are reversible? Convincing evidence is provided to show the beneficial properties of HDL elevation to correct endothelial dysfunction and lipid abnormalities. Taken together, these data suggest that therapeutically targeting HDL is a proper strategy to temper atherosclerosis progression.

Chapter 7-9 The concept of CRP as a risk factor over life is fully compliant with the data from large epidemiological surveys, in which CRP correlates with both atherosclerosis progression as well as with acute cardiovascular events. Numerous experimental studies implicate CRP as a direct partaker in atherosclerosis progression. In absence of solid evidence related to these data in man, we infused recombinant human CRP in human volunteers and sought to reproduce the reported activities in vivo in chapter 7. We provide convincing evidence that CRP is capable of activating pathways involved in atherosclerosis progression. We conclude from these findings that CRP constitutes a mediator of atherothrombotic disease. But, how can CRP be linked to acute cardiovascular events? Since leukocytes are thought to play a major role in destabilization of the ‘culprit lesion’, we sought to determine whether CRP may adversely affect the inflammatory profile of circulating leukocytes and thereby may contribute to the occurrence of an acute event (chapter 8). We provide evidence that this pathway of activating peripheral leukocytes may serve well for CRP to mechanistically accelerate the process of
plaque evolution to culminate eventually in an event. Since cardiovascular factors are considered to have greater impact in humans, already characterized by early vascular disease, we evaluated the effects of CRP in asymptomatic subjects with familial hypercholesterolemia in comparison with normolipidemic controls. In chapter 9, we provide evidence, that CRP collaborates with present traditional risk factors to exacerbate early cardiovascular disease.

Chapter 10 Weighing the evidence gathered in chapter 4-9 in combination with data from recent experimental studies, we wondered whether pretreatment with HDL may prevent the adverse effects of CRP on established pathway of atherosclerosis progression. For that matter, HDL and CRP may represent ‘classical’ opponents in the development and complications of cardiovascular disease. In chapter 10, we describe the counter-regulatory capacity of HDL to attenuate CRP-mediated adverse effects.

Reference List

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