High-density lipoprotein as a modulator of platelet and coagulation responses

Marco van der Stoep¹,², Suzanne J.A. Korporaal²†, and Miranda Van Eck¹*†

¹Division of Biopharmaceutics, Cluster BioTherapeutics, Leiden Academic Centre for Drug Research, Gorlaeus Laboratories, PO Box 9502, 2300RA Leiden, The Netherlands; and ²Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

Received 15 March 2014; revised 8 May 2014; accepted 23 May 2014; online publish-ahead-of-print 1 June 2014

Platelets and coagulation factors are involved in the process of haemostasis, which ensures undisturbed blood flow upon vessel wall damage. However, excessive platelet aggregation and/or coagulation may lead to arterial or venous thrombosis. Pro-atherogenic lipoproteins, including native and oxidized low-density lipoprotein (LDL), are associated with an increased susceptibility to thrombosis. In contrast, numerous epidemiological studies have established an inverse correlation between high-density lipoprotein (HDL) levels and the risk for thrombosis. In addition to its role in reverse cholesterol transport, HDL also interacts with platelets, the coagulation cascade, and the vascular endothelium. Native HDL prevents platelet hyperreactivity by limiting intraplatelet cholesterol overload, as well as by modulating platelet signalling pathways after binding platelet HDL receptors such as scavenger receptor class B type I (SR-BI) and apoER2. The antithrombotic properties of native HDL are also related to the suppression of the coagulation cascade and stimulation of clot fibrinolysis. Furthermore, HDL stimulates the endothelial production of nitric oxide and prostacyclin, which are potent inhibitors of platelet activation. Thus, HDL’s antithrombotic actions are multiple and therefore, raising HDL may be an important therapeutic strategy to reduce the risk of arterial and venous thrombosis.

Keywords
High-density lipoprotein • Arterial thrombosis • Venous thrombosis • Platelets • Coagulation

This article is part of the Spotlight Issue on HDL biology: new insights in metabolism, function, and translation.

1. Introduction

Low levels of circulating high-density lipoprotein (HDL) particles are generally associated with a higher incidence of atherosclerotic cardiovascular disease.¹–⁴ Much of the atheroprotective effect of HDL is ascribed to its role in reverse cholesterol transport, removing excess cholesterol from peripheral tissues and delivering it to the liver for biliary secretion.⁵–⁷ However, HDL is more and more recognized as a multipurpose player in cardiovascular health and disease, exhibiting anti-inflammatory, antioxidative, immunomodulatory, anti-apoptotic, and antithrombotic effects.⁸ For example, HDL has been shown to be an independent predictor of platelet-dependent thrombosis formation.⁹ The antithrombotic actions of HDL, which include the modulation of platelet reactivity, coagulation, and endothelial function, will be addressed in this review, summarizing recent findings from in vitro and in vivo animal studies, as well as from observational and interventional studies in humans.

1.1 HDL heterogeneity

Human HDL (1.063–1.21 g/mL) is heterogeneous in terms of its density, size, shape, surface charge, and composition (Table 1).¹⁰ Based on their size and density, HDL particles can be classified into two major subclasses: (i) small, dense (1.125–1.21 g/mL) HDL₃ and (ii) larger, less dense (1.063–1.125 g/mL) HDL₂.¹¹ When separated by gradient gel electrophoresis, further subspecies can be identified: HDL₂a (largest) > HDL₂b > HDL₂c > HDL₃b > HDL₃a > HDL₃c. Distinct HDL subpopulations have also been identified on the basis of their apolipoprotein composition. Some particles contain only apolipoprotein A-I (apoA-I), whereas others contain both apoA-I and apoA-II, or apoA-I and apoE. In general, HDL₂ particles are apoE- and apoA-I-rich, whereas HDL₃ particles are apoE-poor and apoA-I- and apoA-II-rich. It has been suggested that apoA-I-containing HDL may be more cardioprotective than apoA-II-containing HDL,¹² and that HDL₂ might be superior to HDL₃ in this respect.¹³ A high level of heterogeneity is also found in the lipid content across HDL subpopulations. HDL consists predominantly of phospholipids and sphingomyelin (40–60% of total weight), while cholesteryl esters (30–40%), triglycerides (5–12%), and unesterified cholesterol (5–10%) make up the rest of the particle. A recent structure–function analysis revealed that several phospholipid classes, and primarily negatively charged phosphatidylserine and phosphatidic acid, are enriched in HDL₃, which was positively associated with its cholesterol efflux capacity, as well as its antioxidative, anti-inflammatory,
anti-apoptotic, and antithrombotic activities. Antithrombotic activity of HDL subfractions was evaluated as their ability to inhibit H2O2-induced platelet activation, by measuring phosphorylation of p38 mitogen-activated protein kinase (p38MAPK) and the production of thromboxane B2 (TxB2), the inactivate metabolite of TxA2, which is produced by activated platelets. Small, dense HDL3 was superior over larger and less dense HDL2 in the prevention of H2O2-induced p38MAPK phosphorylation and TxB2 production. These differences potentially reflect the distinct proteome and lipidome of HDL3 compared with HDL2, as HDL3 is enriched in bioactive lipids and proteins. The exact contribution of the different constituents of HDL on its antithrombotic activity will be discussed throughout this review.

1.2 Aetiology of thrombosis

Undisturbed blood flow upon vessel wall damage is ensured by the process of haemostasis that involves platelets and coagulation factors. However, excessive platelet aggregation or coagulation may lead to two different forms of thrombosis, i.e. arterial and venous thrombosis, respectively. Arterial ‘white’ thrombi develop in areas of high shear stress, are platelet-rich, and manifest as myocardial infarction, unstable angina, ischaemic stroke, or peripheral artery disease. In contrast, venous ‘red’ thrombi usually develop in low flow vessels, are cell-rich, and their formation is highly dependent on hypercoagulability and venous stasis. Increasing evidence suggests an association between arterial and venous thrombosis. Prandoni et al. were the first to demonstrate a higher prevalence of asymptomatic atherosclerosis in patients with previous idiopathic venous thromboembolism (VTE), also after adjustment for risk factors of atherosclerosis. Later studies also observed an increased incidence of atherosclerotic cardiovascular events in VTE patients. However, other studies found no such correlation. Correspondingly, two large cohort studies found that hypertension, dyslipidaemia, diabetes mellitus, smoking, and physical activity were significantly associated with the risk of myocardial infarction due to arterial thrombosis, but not with VTE. Therefore, it was suggested that the link between venous and arterial thrombosis may be largely explained by mechanisms of thrombus formation and hypercoagulability rather than the development of atherosclerosis. For example, recent evidence suggests that procoagulant microparticles and neutrophil extracellular traps might be prothrombotic mechanisms shared by both arterial and venous thrombosis.

2. HDL and the incidence of arterial thrombosis

2.1 Platelets and the development of arterial thrombosis

The primary role of platelets is to seal the damaged vessel wall upon injury and to stimulate repair of the endothelium by the release of cytokines, chemokines, and growth factors. Upon endothelial damage, von Willebrand factor (vWF), released into the circulation from endothelial Weibel–Palade bodies, adheres to collagen in the subendothelium exposed to the flowing blood in the injured area. Platelets can bind to the immobilized vWF via the glycoprotein Ib-V-IX (GPIb-V-IX) complex on the platelet membrane. However, the interaction of vWF with GPIb-V-IX is transient and does not allow stable adhesion, but it initiates the tethering of platelets over the endothelium and induces a weak intracellular signalling pathway. This allows subsequent interaction of additional receptors such as integrin αIIbβ3, αIβ1, and GPVI with vWF and collagen, respectively, resulting in firm adhesion. The activated platelets release its alpha and dense granule contents, and synthesize and release platelet-activating factor (TxA2). Further stabilization is ensured by platelet spreading, which is primarily mediated by integrin αIIbβ3. The spread platelet provides a new surface for the next platelet to adhere to via fibrinogen that is bound by integrin αIIbβ3 of two adjacent platelets, thereby inducing platelet aggregation (Figure 1).

Platelets are also involved in the development of acute coronary syndromes and cerebrovascular diseases. Rupture of an atherosclerotic plaque leads to activation of platelets through the exposure of

Table 1: Heterogeneity in the physiochemical properties of HDL particles in healthy normolipidaemic subjects

<table>
<thead>
<tr>
<th></th>
<th>HDL1b</th>
<th>HDL1a</th>
<th>HDL2a</th>
<th>HDL3a</th>
<th>HDL3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (nm)</td>
<td>10.4</td>
<td>10.3</td>
<td>9.9</td>
<td>8.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Density (g/mL)</td>
<td>1.099</td>
<td>1.107</td>
<td>1.123</td>
<td>1.155</td>
<td>1.186</td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
<td>410</td>
<td>400</td>
<td>360</td>
<td>200</td>
<td>160</td>
</tr>
<tr>
<td>ApoA-I (%)</td>
<td>4.5</td>
<td>4</td>
<td>3–4</td>
<td>3</td>
<td>2–3</td>
</tr>
<tr>
<td>ApoE (%)</td>
<td>≤2</td>
<td>≤2</td>
<td>≤2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Others (%)</td>
<td>apoE-rich</td>
<td>apoA-I-rich</td>
<td>apoE-rich</td>
<td>apoA-I-rich</td>
<td>apoA-II-rich</td>
</tr>
<tr>
<td>Surface lipids (mol/mol HDL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td>130</td>
<td>140</td>
<td>120</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Unesterified cholesterol</td>
<td>70</td>
<td>40</td>
<td>25</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Core lipids (mol/mol HDL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>180</td>
<td>160</td>
<td>140</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>30</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Relative lipid content (%)</td>
<td>65</td>
<td>60</td>
<td>55</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>Relative protein content (%)</td>
<td>35</td>
<td>40</td>
<td>45</td>
<td>55</td>
<td>65</td>
</tr>
</tbody>
</table>

Based on their size and density, HDL particles can be classified into six classes, which differ in lipid and apolipoprotein content. Table adopted from Kontush and Chapman.
subendothelial collagen and plaque material and subsequent thrombus formation on top of the ruptured plaque, which might cause ischaemic complications. Besides their role in the end stage of plaque formation, platelets are also implicated in the onset and development of atherosclerosis. For example, platelets have been shown to adhere to the vascular endothelium of apoE-deficient (apoE−/−) mice before atherosclerotic lesions become visible. In addition, activated platelets are an important source of adhesion molecules and chemokines, and they mediate leucocyte recruitment towards the vascular wall and the extravasation of circulating mononuclear cells.

2.2 HDL protects against arterial thrombosis

Pro-atherogenic lipoproteins, including native and oxidized (very) low-density lipoprotein (V)LDL, are associated with an increased susceptibility to thrombosis. In contrast, HDL has anticoagulant effects and protects against cardiovascular disease. Numerous studies have shown that low levels of HDL-cholesterol (HDL-C) are an important risk factor for coronary artery disease (CAD). The Atherosclerosis Risk in Communities (ARIC) study found lower HDL-C levels in men (1.07 vs. 1.18 mmol/L) and women (1.30 vs. 1.51 mmol/L) with CAD. Similarly, the Emerging Risk Factors Collaboration, involving 68 prospective studies of cardiovascular risk factors, found 6.4 cases of CAD per 1000 subjects in the lowest tertile (0–33%) of plasma HDL-C levels, whereas the incidence was much lower (2.4 per 1000) in the highest tertile. In the Ludwigshafen RIsk and Cardiovascular health (LURIC) study, HDL-C was strongly associated with cardiovascular death in people without CAD, but not in patients with stable and unstable CAD, indicating that the inverse relationship of HDL-C with cardiovascular death is weakened in these patients. Even in patients treated with intense statin therapy and having target levels of LDL-C, HDL-C remains a predictor of outcome for major adverse cardiovascular events. Limiting the build-up of cholesterol in atherosclerotic plaques is an important atheroprotective function of HDL, but the platelet-modulatory actions of HDL, summarized in Figure 2, also play a central role.

2.3 HDL and platelet formation and clearance

Lipoproteins can influence platelet count and characteristics, and modulate the risk of atherothrombosis via the megakaryocyte–platelet haemostatic axis. Dyslipidaemia leads to altered characteristics of megakaryocytes, precursor cells residing in the bone marrow responsible for platelet production. This is illustrated by the observation that megakaryocytes from hypercholesterolaemic rabbits, guinea pigs, and humans are significantly larger and have a higher mean ploidy than those from matched control subjects. High-ploidy megakaryocytes are generally considered to produce larger and more active platelets, as observed in experimentally induced thrombocytopenia. Not only high plasma lipid levels, but also processes implicated in the export of lipids from megakaryocytes, influence the characteristics of megakaryocytes. In line with this, Murphy et al. observed increased megakaryocyte proliferation and platelet production in LDL receptor-deficient (LDLr−/−) mice with bone marrow-specific ATP-binding cassette transporter G4 (ABCG4) deficiency, which display defective cholesterol efflux from megakaryocytes to HDL. Conversely, infusions with reconstituted HDL (rHDL) significantly reduced platelet counts in LDLr−/− mice, but not in LDLr−/−/ABCG4−/− mice, indicating an essential role for ABCG4 in mediating the ability of rHDL to reduce megakaryocyte proliferation and platelet production.

The effect of modulation of HDL metabolism on platelet count and morphology was also studied in mice that are deficient for the HDL receptor scavenger receptor class B type I (SR-BI−/−). SR-BI−/− mice have an unusually high plasma unesterified-to-total cholesterol (UC: TC) ratio, and the cholesterol is carried by abnormally large HDL particles, reflecting impaired delivery of cholesterol to the liver. SR-BI deficiency was shown to cause thrombocytopenia, while the circulating platelets

---

**Figure 1** Schematic overview of platelet adhesion and aggregation upon vascular damage. (1) Under normal conditions, platelets circulate in a resting state at high velocity. (2) After damage of the endothelium, plasma vWF will bind to the exposed subendothelial collagen at the site of injury. Collagen-bound vWF captures circulating platelets by binding to the GP Ib-V-IX complex on the platelet membrane. (3) This interaction is transient and will result in the slowing down and rolling of platelets over the damaged vessel wall. Firm adhesion of the platelet to the exposed collagen is mediated via integrin α2β1. The interaction of GPVI with collagen leads to further platelet activation. (4) Activated platelets will spread and form a surface to which other platelets can bind. (5) Upon activation, platelets release their alpha and dense granule contents (e.g. ADP), and synthesize and release platelet-activating TxA2. Integrin αIIbβ3 in its active conformation on activated platelets binds fibrinogen, which forms a bridge between two activated platelets.
displayed abnormal cell morphology and were abnormally large due to an increased UC content. A high-fat/high-cholesterol diet further enhanced the effects of SR-BI deficiency on platelet count and morphology. Increased platelet clearance is believed to be the driving force behind the observed thrombocytopenia, since the rate of platelet production was unaffected. The increased UC content and reduced lifespan of these platelets appeared to be primarily dependent on the external environment, and not to be an intrinsic feature of these platelets, as wild-type platelets adopted the same characteristics as resident platelets when infused into SR-BI−/− mice. Furthermore, the UC content and clearance rate of SR-BI−/− platelets was at least partially normalized after infusion into wild-type mice. Noteworthy, hypomorphic liver-specific SR-BI−/− mice with similarly elevated HDL-C levels as total body SR-BI−/− mice also displayed a dramatic reduction in circulating platelet count. In contrast, platelet-specific deletion of SR-BI by specifically disrupting SR-BI in bone marrow-derived cells from wild-type mice by a bone marrow transplantation approach did not affect platelet count, size, or UC content. Platelet characteristics were also studied in SR-BI−/− mice expressing cholesteryl ester transfer protein (CETP), which normalized HDL-C levels. In these mice, the plasma UC:TC ratio was partially restored but remained elevated. Probably as a result of this, SR-BI−/−/CETP mice still displayed thrombocytopenia.

The functional consequence of SR-BI dysfunction in humans has been studied in people carrying a heterozygous SR-BI variant, P297S. The mutation carriers had increased HDL-C levels (1.8 vs. 1.4 mmol/L), but their plasma UC:TC ratio was unchanged. Mean platelet counts did not differ significantly between P297S carriers and non-carriers, but platelets from carriers were UC-rich, as observed in SR-BI−/− mice.

### 2.4 Impact of high HDL-C due to SR-BI deficiency on platelet reactivity and thrombosis

We, and others, also studied the effect of SR-BI deficiency on platelet function and thrombosis susceptibility. Platelets from SR-BI−/− mice displayed a lower aggregation response ex vivo, although their basal activation state was higher, as judged by the presence of both active integrin αIIbβ3 and surface-expressed P-selectin (Figure 2). In line with this, SR-BI−/− mice displayed increased adhesion of platelets to immobilized fibrinogen in vitro and an increased susceptibility to FeCl3-induced arterial thrombosis in the left carotid artery in vivo. Platelet-specific SR-BI deficiency, induced by transplantation of SR-BI−/− bone marrow into wild-type mice, did not change platelet characteristics of the recipient mice. Likewise, platelets isolated from hyperlipidaemic SR-BI−/− mice after reconstitution with wild-type bone marrow were phenotypically similar to the resident platelets from SR-BI−/− mice. Thus, dyslipidaemia and, as a result, platelet cholesterol overload, rather than SR-BI deficiency in platelets, are responsible for the platelet hyperreactivity observed in SR-BI−/− mice. Similarly, platelets from human heterozygous SR-BI P297S carriers displayed a reduced ex vivo aggregation response, but a higher basal activation state, and increased adhesion to and spreading on immobilized fibrinogen, showing for the first time the link between SR-BI function and platelet response in humans.

### 2.5 Receptor-mediated modulation of platelet activity by HDL

HDL is also able to modulate platelet reactivity by binding specific receptors on the platelet surface, leading to activation of platelet signalling pathways. Initially, the fibrinogen receptor integrin αIIbβ3 was described to mediate HDL-induced signalling, since (i) small, dense HDL3 bound to proteins with a molecular weight of 115 and 156 kDa, corresponding to CD41 and CD61, the two subunits of αIIbβ3, (ii) an antibody directed against CD61 inhibited the binding of HDL3 to integrin αIIbβ3, and (iii) HDL3-induced signalling was impaired in αIIbβ3-deficient platelets. However, other studies refuted these findings, since αIIbβ3 ligands did not interfere with HDL3 binding to platelets, and vice versa. Correspondingly, HDL3-mediated signalling was not impaired in platelets from patients with Type I and II Glanzmann’s thrombasthenia, which lack...
apoE-rich HDL2 to apoER2 4–6, both in megakaryocytic cell lines and in platelets. Binding of (LRP8)/apoE receptor 2 (apoER2) (apoER2 family member low-density lipoprotein receptor-related protein 8 containing HDL. They identified a splice variant of the LDL receptor leagues68 reported another possible platelet receptor for apoE-ovation, respectively (Fig. 2).64 Importantly, SR-BI−/− platelets were not affected by HDL1 or other SR-BI ligands, which further pointed to SR-BI as a functional receptor for HDL attenuating agonist-activated platelet activation. In addition, platelets express CD36, another member of the class B scavenger receptor superfamily that binds HDL.62 However, deficiency of platelet CD36 did not affect the inhibitory effect of HDL2 on platelet reactivity.

Compared with native HDL, CuSO4 or myeloperoxidase-oxidized HDL (oxHDL) is a more potent inhibitor of agonist-activated platelet ac-
tivation, and this effect is also mediated by SR-BI.63 Calzada et al. studied platelet function in patients with abetalipoproteinemia (ALBP) lacking apoB-containing lipoproteins in plasma. These patients primarily transport cholesterol via HDL, which is oxidized due to impaired protection against oxidative stress, as a result of extremely low plasma levels of the antioxidant vitamin E.65 Platelets isolated from plasma of ALBP patients are hyperreactive to collagen, arachidonic acid, and thrombin compared with those isolated from control plasma upon resuspension in a buffer solution. However, in platelet-rich plasma, no differences in the extent of aggregation between control and ALBP platelets were observed, indicating that ALBP plasma contains a protective factor preventing the augmented platelet aggregation. Lipoprotein removal from ALBP plasma abolished this protective effect. Since oxHDL is the sole lipoprotein in ALBP plasma, it is most likely this oxidized HDL that is responsible for the inhibitory effect. Here too, SR-BI was identified as the receptor for oxHDL on platelets. The results from these studies are in seeming contrast to others that show that, upon (heavy) oxidation by hypochlorite, HDL enhances platelet aggregation.65,66 In these studies, oxHDL was found to bind to platelet CD36. Hence, the degree and type of HDL oxidation seem to determine the affinity for either SR-BI or CD36 and thereby its capacity to inhibit or stimulate agonist-induced platelet activation, respectively (Fig. 2).67

In addition to SR-BI as a functional receptor for HDL, Owen and col-
leagues68 reported another possible platelet receptor for apoE-containing HDL. They identified a splice variant of the LDL receptor family member low-density lipoprotein receptor-related protein 8 (LRP8)/apoE receptor 2 (apoER2) (apoER2′), lacking binding repeats 4–6, both in megakaryocytic cell lines and in platelets. Binding of apoE-rich HDL2 to apoER2′ was found to reduce adenosine diphosphate (ADP)-, epinephrine-, and collagen-induced platelet aggregation, whereas the apoE-poor fraction only had a minor inhibitory effect.69 Similarly, phospholipid vesicles containing apoE suppressed platelet aggregation.70 Platelet inhibition may be due to apoE-mediated stimulation of the release of nitric oxide (NO) from platelets, which acts on soluble guanylate cyclase to elevate levels of anti-aggregatory cGMP.71

Interestingly, apoER2′ also mediates signalling induced by LDL, which leads to enhanced platelet aggregation.71 LDL binds platelet apoER2′ via the so-called B-site of apoB100.72 Binding of LDL to platelets results in phosphorylation and activation of intracellular p38MAPK.73 Subsequently, this Ser/Thr kinase phosphorylates and activates cytosolic phospholipase A2, which leads to the formation of platelet-activating TxA2.74,75 HDL is also able to induce p38MAPK activation, although the response is weaker than observed for LDL.76 Residues 142–152 of apoE closely resemble the B-site of apoB100, and a peptide mimicking this domain also induces phosphorylation of p38MAPK. Hence, the platelet-activating property of apoE-containing HDL2 probably lies within this part of apoE and may also be responsible for the platelet-activating effects of HDL observed in some studies.76

3. HDL and venous thrombosis

While the inverse relationship between plasma HDL-C levels and the risk of arterial thrombosis has been well described, the effect of HDL-C levels on the risk of venous thrombosis is less clear. Deguchi et al.77 found significantly lower levels of (large) HDL2 particles, HDL-C and apoA-I in patients with deep venous thrombosis (DVT) with or without pulmonary embolism. This was paralleled by significantly higher levels of LDL particles. In a later study of VTE cases, they calculated a relative risk of recurrence of 0.87 (95% CI 0.80–0.94) for each increase of plasma apoA-I by 0.1 mg/mL.78 Patients with VTE had a lower frequency of the Taq B2 allele, a polymorphism which is associated with decreased CETP levels and activity, and thus higher HDL-C levels.79 Correspondingly, in a meta-analysis of 21 case–control and cohort studies, Ageno et al.80 observed significantly lower levels of HDL-C in VTE patients. However, others found no such correlation,81–83 or even an association of high HDL-C levels with an increased risk of VTE in women.82 These latter observations were likely the result of prothrombotic effects of hormone therapy, which concomitantly increases HDL-C levels.86

3.1 HDL as a modulator of the coagulation cascade

The conversion of the soluble plasma protein fibrinogen into insoluble fibrin fibres, mediated by thrombin (factor Ila, FIIa), is the central step of the coagulation cascade (Fig. 3). The cascade starts with the exposure of blood to tissue factor (TF) via the extrinsic pathway, or with negatively charged surfaces via the intrinsic pathway. In the extrinsic pathway, after exposure to blood, TF binds to circulating active FVII (FVIIa). This complex, known as the extrinsic tenase complex, activates FX forming FXa. The intrinsic pathway starts with the activation of coagulation factor XII, which will lead to the activation of factor XI. Activated FXI (FXIa) will then activate factor IX, which forms a complex with FVIIa. The resulting intrinsic tenase complex then converts FX to its active form. Both pathways culminate in the common pathway of coagulation, in which the prothrombinase complex (FXa and FVα) converts prothrombin into thrombin.

HDL modulates the coagulation cascade at different levels (see Fig. 3 for overview). For example, a positive correlation between plasma apoA-I levels and the in vitro anticoagulant response was found, as well as an inverse relation between HDL and prothrombin fragments F1 + 2, the peptide cleaved from prothrombin during its conversion to thrombin.87 ApoA-I also neutralizes the procoagulant properties of anionic phospholipids.88 Incorporation of apoA-I into anionic vesicles prevents the formation of the prothrombinase complex due to the inability of FVa to bind to the vesicles, while binding of prothrombin and FXa is unaffected. Similarly, addition of serum neutralizes the procoagulant effect of anionic liposomes, by mediating the transfer of phospholipids to either apoA-I- or apoB-containing lipoproteins.89
Another way by which HDL exerts anticoagulant functions is via the protein C pathway. This pathway comprises the generation of the proteolytically active form of the vitamin K-dependent zymogen protein C (activated protein C, APC), which is able to inactivate FVa and FVIIIa.\textsuperscript{90,91} The anticoagulant activity of APC is enhanced by vitamin K-dependent protein S, which serves as a cofactor for APC. Purified HDL, but not LDL, enhances protein S-dependent cleavage of FVa at Arg206 by APC.\textsuperscript{92} Moreover, infusion of rHDL in an experimental rabbit model of atherosclerosis increased the expression of thrombomodulin in lesions, which is an additional coagulant factor that supports activation of protein C and suppresses thrombin generation.\textsuperscript{93}

3.2 HDL is positively associated with fibrinolysis

Clot formation is counteracted by the process of fibrinolysis, inducing the degradation of a blood clot. The stability of a blood clot is determined by the rate of coagulation and fibrinolysis. Dense and poorly lysable clot formation is observed in cardiovascular disease.\textsuperscript{94} During fibrinolysis, fibrin is cleaved by plasmin, which is formed from plasminogen by tissue- and urokinase-type plasminogen activator (tPA and uPA), both secreted by endothelial cells. Elevated HDL-C levels are associated with increased fibrin clot permeability and reduced clot lysis time.\textsuperscript{95} Moreover, both HDL-C and HDL size are inversely related to the levels of PA inhibitor-I (PAI-I), an inhibitor of tPA and uPA, implying that HDL particles might help reduce the thrombotic risk by promoting plasminogen generation and thus fibrinolysis.\textsuperscript{96,97} Correspondingly, both HDL-C and HDL size correlate with the levels of D-dimer, a fibrin degradation product. In contrast, oxHDL, which has been detected in atherosclerotic plaques, has been found to promote PAI-I expression in endothelial cells, implying that oxHDL reduces fibrinolysis and contributes to clot stability.\textsuperscript{98}

4. HDL and the vascular endothelium

The vascular endothelium is very important in the regulation of platelet and coagulation responses. For example, endothelial cells produce NO, which is a potent platelet inhibitor. NO is generated by endothelial NO synthase (eNOS), in response to agonists of various cell surface receptors and physical stimuli such as shear stress.\textsuperscript{99} NO acts by directly activating platelet guanylyl cyclase, resulting in an increase of intraplatelet cGMP, which represses platelet activation.\textsuperscript{100} Pro-atherogenic lipids such as oxLDL inhibit eNOS activation by changing its subcellular localization.\textsuperscript{101} This effect is counteracted by HDL, thereby retaining eNOS localization and NO production.\textsuperscript{102} HDL is also able to directly stimulate eNOS in endothelial cells in a process that requires apoA-I binding to SR-BI.\textsuperscript{103} In a murine flow restriction model in the inferior vena cava, infusion of apoA-I protected wild-type mice from DVT, but not SR-BI\textsuperscript{-/-} or eNOS\textsuperscript{-/-} mice.\textsuperscript{104} As apoA-I did not affect platelet aggregation, this effect was platelet-independent.

In addition to promoting NO production, HDL can also induce the synthesis of prostacyclin (PGI\textsubscript{2}), which inhibits platelet aggregation by increasing intraplatelet cAMP. PGI\textsubscript{2} is synthesized from arachidonate in a pathway that involves cyclooxygenase (COX).\textsuperscript{105} There are two isoforms of this enzyme: COX-1, which is constitutively expressed, and COX-2, which is inducible. Endothelial PGI\textsubscript{2} synthesis is stimulated by HDL, by the provision of arachidonic acid, and by inducing COX-2 expression.\textsuperscript{106–110} Moreover, in isolated rabbit and rat hearts, HDL was shown to enhance the release of prostaglandins, which are precursors of PGI\textsubscript{2} synthesis.\textsuperscript{111,112} Little is known about the effect of HDL on PGI\textsubscript{2} synthesis in humans, except for the positive correlation of plasma HDL-C levels with the plasma concentration of the PGI\textsubscript{2} metabolite 6-keto PGF\textsubscript{1α}.\textsuperscript{113,114}

Upon vascular injury, platelets are recruited to the damaged vascular wall and under high shear stress, platelet adherence is mediated by vWF, a constituent of endothelial Weibel–Palade bodies.\textsuperscript{115} In patients
with peripheral vascular disease, levels of circulating vWF are inversely associated with HDL. Further evidence for an effect of HDL on endothelial vWF secretion was found in a mouse model of DVT, where apoA-I inhibited platelet recruitment by the venous endothelium, presumably by suppressing the release from Weibel-Palade bodies.

The vascular endothelium not only modulates platelet reactivity, but also the coagulation response. For example, tissue factor pathway inhibitor (TFPI) is secreted by endothelial cells to inhibit the extrinsic pathway of coagulation. So far, the existence of epidemiological associations between circulating TFPI and HDL in humans is still unclear, as one study suggested a negative correlation, whereas another showed a positive correlation. The endothelium also modulates coagulation by expressing heparin-like proteoglycans on the endothelial cell surface, enabling the endothelium to bind and activate antithrombin III, which subsequently inhibits the coagulation cascade by suppressing various coagulation factors, mainly FIIa, FXa, and FIXa. The apoE moiety of HDL was shown to increase endothelial production of heparan sulfate rich in biologically active heparin-like domains, leading to a significantly higher binding of antithrombin III to the matrix of endothelial cells pre-incubated with HDL compared with the matrix of non-treated control cells.

In addition to its suppressive effects on primary and secondary haemostasis, HDL may also reduce thrombosis by inhibiting apoptosis of endothelial cells, which would otherwise turn the endothelium into a procoagulant surface. HDL suppresses the mitochondrial pathway of apoptosis by preserving mitochondrial integrity and inhibiting the release of cytochrome c into the cytoplasm. These anti-apoptotic effects are mediated by the protein kinase Akt, an ubiquitous transducer of anti-apoptotic signals. Since two isolated lysosphingolipids, sphingophosphorylcholine and lysosulfatide, also stimulate Akt and inhibit apoptosis, it is believed that these components of native HDL are responsible for the endothelium-protective effects of HDL.

5. rHDL as the treatment for arterial and venous thrombosis

Therapeutic agents that raise HDL-C are currently being tested for the prevention and treatment of cardiovascular diseases. One promising strategy is the use of synthetic rHDL, containing phospholipids and human apoA-I or one of its variants, such as apoA-IIMilano, which is functionally more effective. Lerch et al. tested the effects of rHDL on platelet reactivity, and found a dose-dependent inhibition of in vitro platelet aggregation by rHDL after stimulation with arachidonic acid, collagen, epinephrine, or ADP. Subsequently, they performed ex vivo experiments with platelet-rich plasma isolated from volunteers who had been infused with rHDL. Both arachidonic acid- and collagen-induced platelet aggregation were reduced, and the extent of inhibition negatively correlated with plasma concentration of apoA-I. rHDL-C, and the dose of rHDL infused. Consistent with these findings, administration of recombinant apoA-IIMilano inhibited platelet aggregation and FeCl3-induced arterial thrombus formation in rats. These effects were platelet-dependent, as no effects on vasocostriction or endothelium-dependent relaxation were observed.

Calkin et al. studied the benefit of rHDL therapy in patients with Type 2 diabetes mellitus, who have an increased risk of cardiovascular disease and exhibit enhanced platelet reactivity. rHDL infusion significantly reduced ex vivo platelet aggregation and thrombus formation under flow. These effects were mainly ascribed to the isolated phospholipid component of rHDL, and not to apoA-I. Moreover, rHDL enhanced efflux of cholesterol from platelets and interfered with lipid raft assembly in the platelet membrane, as did other unspecific cholesterol acceptors such as cyclodextrin or phosphatidylcholine-containing vesicles, which also reduced the platelet response. In contrast, native HDL particles were much less effective in removing cholesterol and did not affect platelet aggregation, although it must be noted that sub-physiological concentrations were used. These observations suggest that rHDL affects platelets mainly by promoting cholesterol efflux and changing lipid raft organization, rather than by inducing platelet signalling via specific receptors on the platelet membrane.

rHDL has also been shown to reduce coagulation responses. For example, in LPS-induced endotoxaemia, rHDL reduces plasma levels of prothrombin fragment F1 + 2 and induces plasma levels of tPA. Although the underlying mechanisms remain to be elucidated, rHDL infusions are a promising therapeutic strategy to reduce thrombosis risk, not only in patients with cardiovascular disease, but also under other conditions where platelet hyperreactivity and hypercoagulability pose a threat.

6. Conclusion

In addition to its role in reverse cholesterol transport, the vascular protective effects of HDL can at least be partially explained by its antithrombotic actions. Native HDL prevents platelet hyperreactivity by limiting intraplatelet cholesterol overload, as well as by modulating platelet signalling pathways. The antithrombotic properties of native HDL are also related to suppression of the coagulation cascade and stimulation of clot fibrinolysis. Furthermore, HDL stimulates the endothelial production of NO and PGJ2, which are potent inhibitors of platelet activation. Raising HDL levels may therefore be an important therapeutic strategy to reduce the risk of arterial and venous thrombosis. However, not only the level of circulating HDL is an important determinant for its antithrombotic effects, but its composition is also an important determinant. The subclass, apolipoprotein and lipid composition, and the degree of oxidation determine to which receptor the HDL particle will bind, and thereby the capacity to inhibit or stimulate agonist-induced platelet activation. Hence, more detailed structure–function analyses are needed to identify clinically relevant, antithrombotic HDL subpopulations, in order to diagnose patients at risk and to develop effective therapeutic approaches to reduce thrombotic risk.

Conflict of interest: none declared.

Funding

This work was supported by the Landsteiner Foundation for Blood Transfusion Research (grant 0912F to M.v.d.S. and S.J.A.K.); the Netherlands Heart Foundation (Established Investigator grant 2007T056 to M.V.E); and the Netherlands Organization for Scientific Research (grant 91813603 to M.V.E).

References


