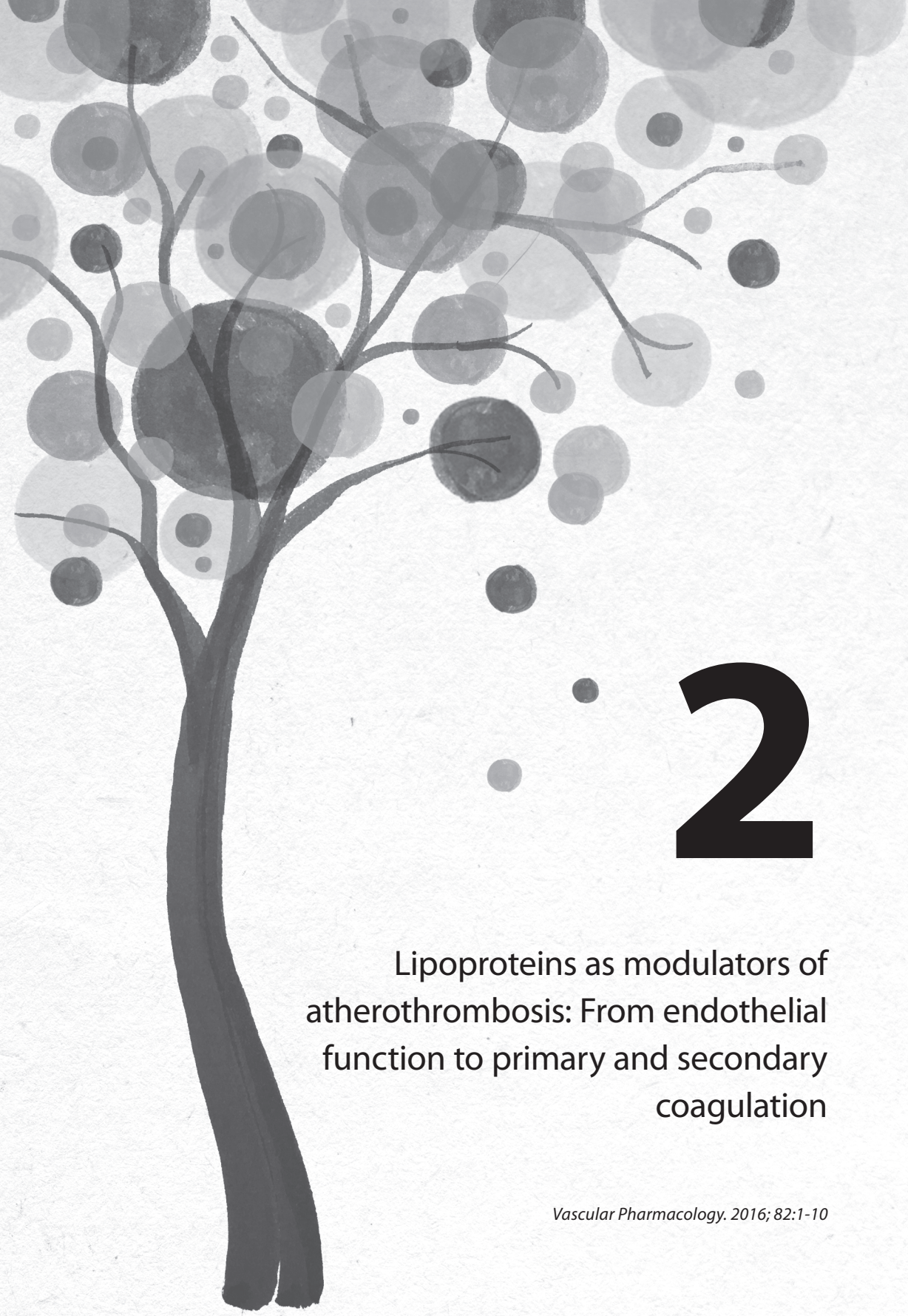
Cholesterol metabolism and hematopoiesis interaction in atherothrombosis

Ouweneel, A.B.

Amber B. Ouweneel & Miranda van Eck

Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands



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Lipoproteins as modulators of atherothrombosis: From endothelial function to primary and secondary coagulation

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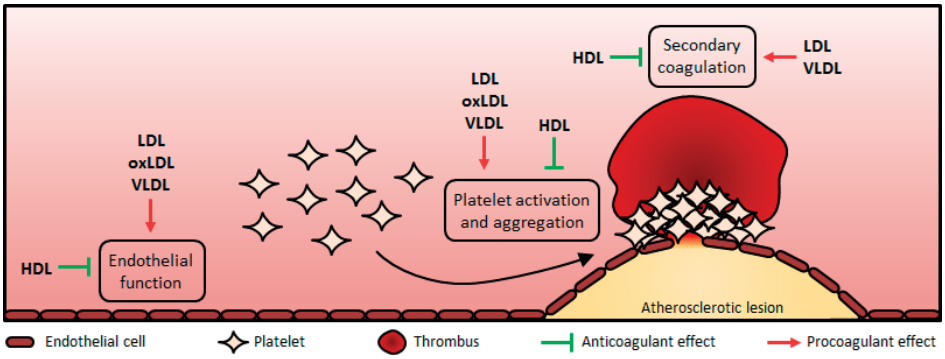
ABSTRACT

Atherothrombosis is a complication of atherosclerosis that causes acute cardiovascular events such as myocardial infarction and stroke. Circulating lipid levels are highly correlated with atherosclerotic plaque development. In addition, experimental evidence suggests that lipids also directly influence thrombosis and influence the risk and the outcome of acute cardiovascular events. Plasma lipoproteins influence three aspects important to atherothrombosis: endothelial function, platelet aggregation (primary coagulation) and secondary coagulation. Overall, VLDL, LDL and oxLDL promote thrombus formation, whereas HDL shows antithrombotic actions. In this review we will address the current knowledge about modulation of atherothrombosis by lipoproteins, summarizing findings from *in vitro* and *in vivo* animal studies, as well as from observational and interventional studies in humans. We will conclude with future perspectives for lipid modulation in the prevention of atherothrombosis.



Graphical abstract. Schematic overview of the modulation of atherothrombosis by (modified) lipoproteins.

INTRODUCTION

Pathophysiology of atherosclerosis and atherothrombosis

Atherosclerosis is a lipid-driven progressive inflammatory disease, characterized by the accumulation of lipids and fibrous elements in medium and large sized arteries [1]. Atherosclerosis develops largely asymptomatic over a lifetime. However, as lesion development progresses, atherosclerosis can become complicated by atherothrombosis. This can be caused by either plaque rupture or superficial erosion of the plaque [2]. Upon rupture or erosion, subendothelial collagen and thrombogenic plaque material, such as macrophage tissue factor (TF), are exposed to the arterial circulation. This leads to thrombus formation on top of the ruptured or eroded plaque [3]. Within one minute after rupture, platelets adhere and aggregate on collagenous plaque components. After three minutes, the thrombus is characterized by thrombin and fibrin formation, and by the activation of coagulation, a process entirely triggered by plaque-derived TF [2]. This thrombus formation can lead to rapid occlusion of the vessel, a cause myocardial infarction, ischemic stroke and sudden death. This deadly nature of atherothrombosis has made it a critical target for investigation.

Plaque rupture versus plaque erosion

Autopsy studies done several decades ago showed that plaque rupture most commonly led to fatal coronary atherothrombosis [4,5] , whereas a minority of the fatal events was caused by superficial erosion of the plaque. These studies also demonstrated that plaques prone to rupture, so-called vulnerable plaques, are characterized by a thin fibrous cap, and a large lipid core with a relative abundance of inflammatory leukocytes [5]. Although the concept of the vulnerable plaque has been largely accepted and widely used in research, lately it has been subject of debate. Questions have been raised about the dominant mechanisms implicated in atherothrombosis. Recent evidence suggests that plaques with thin fibrous caps and large lipid pools seldomly rupture and cause clinical events [6,7]. Often multiple presumed vulnerable plaques reside in coronary and other arteries. However, these do not inevitably rupture.

As opposed to lesions associated with plaque rupture, vulnerable plaques underlying areas of superficial erosion do not have thin fibrous caps. Furthermore, they harbor fewer inflammatory cells and lack large lipid pools [8]. Interestingly, from studying specimens from the Athero-express biobank we know that there has been a shift in human atherosclerotic plaques morphology over approximately the last 12 years. Plaques obtained from more recent patients with symptomatic carotid artery disease show significantly more fibrous, non-inflammatory characteristics. Moreover, this trend is also visible in asymptomatic patients [9–11]. This shift is possibly due to altered disease demographics and changes in risk factor profiles, such as (passive) smoking and lipid lowering treatment [9].

Lipid lowering reinforces the fibrous cap, decreases the lipid pool and reduces inflammation in both animals and humans [10–12]. Possibly, this shift in plaque characteristics could lead to a subsequent shift in plaque rupture versus erosion occurrence [13,14]. The consequences of this possible shift are under investigation.

Lipoproteins

Lipoproteins are macromolecular complexes of lipids and proteins that are essential for the transport of cholesterol, triglycerides and fat-soluble vitamins in the blood. Based on their relative densities, five major classes of lipoproteins can be distinguished, being chylomicrons (CM), very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). CM, VLDL, IDL and LDL serve to deliver dietary and hepatic triglycerides and cholesterol to peripheral tissues. In humans, the main structural apolipoprotein (apo) on CM is the apoB48 molecule, while VLDL, IDL and LDL are identified by an apoB100 protein. Moreover, a specific subtype of LDL can be distinguished, lipoprotein (a) (Lp(a)), an LDL-like particle with an apolipoprotein (apo(a)) moiety attached to it.

Native HDL is primarily formed by the liver and the intestine and serves as a cholesterol acceptor from peripheral tissues. In that way HDL mediates reverse cholesterol transport from the periphery to the liver, where it can be excreted via bile or repackaged as VLDL for delivery to tissues or used for the generation of native HDL particles. HDL is heterogenous in terms of its density, size, shape, surface charge and composition [15]. Based on shape, HDL can be divided in spherical and non-spherical particles, which are often referred to as pre-β HDL based on their surface charge. Pre-β HDL can be divided in lipid-poor apoA-I molecules, single apoA-I molecules complexed with a small number of phospholipids, or discoidal particles which contain two or three apoA-I molecules complexed with multiple phospholipid molecules and a small amount of unesterified cholesterol. Upon esterification of free cholesterol to cholesterol esters by the enzyme lecithin cholesterol acyltransferase (LCAT), discoidal HDL can mature into spherical HDL particles. Spherical HDL particles can be divided into two major subclasses based on density: small dense HDL_3, and larger, less dense HDL_2. HDL particles can contain over 80 different proteins, more than 200 lipid species, and several microRNAs. Among these proteins, apoA-I is the most abundant on HDL particles, followed by apoA-II. A minor subpopulation of HDL carries apoE as their main apolipoprotein [16].

High levels of cholesterol are strongly correlated with the incidence of cardiovascular disease. In healthy individuals, cholesterol levels are below 5 mmol/L. A rise of 2 mmol/L cholesterol increases the risk of death by cardiovascular disease by 50% [17]. This is most likely attributable to LDL, the main carrier of cholesterol in human plasma. In contrast, large population studies have consistently shown that low HDL cholesterol, as well as apoA-I levels are independent, inverse predictors of cardiovascular disease

risk [18–22]. There is also ample experimental evidence for a causative role for LDL in the development of atherosclerosis. At places in the arterial tree with turbulent blood flow, LDL can accumulate in the arterial intima, where it is prone to oxidative modification. Oxidized LDL (oxLDL) is taken up by macrophages, which, upon excess cholesterol loading, become foam cells. Macrophage foam cell formation in the arterial intima is the start of an atherosclerotic plaque [23]. Epidemiological studies have shown that low HDL is associated with an increased risk for CVD as a results of atherosclerotic plaque development. Although a causal role for HDL is still under debate, many studies have shown protective effects of HDL on the artery wall [24]. An important mechanism by which HDL is protective lies in their function as cholesterol acceptor. Macrophages are able to efflux excess cholesterol by transporting this to HDL particles via ATP binding cassette (ABC) transporters, which reduces foam cell formation.

Due to these pivotal roles of LDL and HDL in the initiation and progression of atherosclerotic lesions, it is reasoned that this is their main role in the pathogenesis of cardiovascular disease. However, lipoproteins are being more and more recognized as multi-purpose players in cardiovascular disease. VLDL, LDL and HDL all carry a variety of proteins, aside their lipid constituents and apolipoproteins that influence their functional characteristics. On HDL, for example a large number of the proteins present are involved in the acute-phase response [25]. Furthermore, an analysis of the proteins found on human VLDL and LDL revealed that for both particles, 25% of all functional pathways in which the proteins were active, are related to coagulation and hemostasis [26,27]. Elevated plasma cholesterol and elevated lp(a) are identified as risk factors in venous thrombosis. Treatment of hypercholesterolemia by statin therapy reduces the risk of both venous and arterial thrombosis [28–32]. The protective effects of statins on arterial thrombosis may be partly explained by cholesterol-independent mechanisms [33]. Nonetheless, it is tempting to speculate that the reduction in risk of thrombosis caused by statin treatment is at least partially due to statin-induced reduction of procoagulant lipoproteins and/or enhancement of anticoagulant lipoprotein-mediated reactions.

ENDOTHELIAL FUNCTION

The endothelium is an important regulator of vascular homeostasis. Among its functions are the regulation of vasomotor tone, platelet activity, thrombosis and fibrinolysis, and leukocyte adhesion. Atherosclerosis and hypercholesterolemia are associated with endothelial dysfunction [34]. Furthermore, there is growing recognition that endothelial dysfunction, next to its effects on the development of atherosclerosis, also affects the development of atherothrombotic complications. In humans, there are several studies that show a predictive effect of endothelial dysfunction on cardiovascular events [35–41].

Furthermore, cardiovascular disease risk reduction therapies improve endothelial function, whereas the cardiovascular disease risk is increased in subjects in which the endothelium fails to respond to the treatment [42].

eNOS and nitric oxide production

A signaling molecule implicated in the regulation of endothelial function is Nitric Oxide (NO). NO is generated by endothelial nitric oxide synthase (eNOS) in response to physical stimulators such as sheer stress, and is a potent platelet inhibitor, as well as a regulator of vasodilation [43]. In endothelial cells, eNOS activity is strongly correlated with its localization in the caveolae [44]. Caveolae are flask-shaped invaginations of the plasma membrane, enriched in cholesterol, glycosphingolipids, sphingomyelin, and lipid-anchored membrane proteins, which is essential for normal caveolae function, and eNOS activation and regulation [45].

In vitro studies with endothelial cells showed that exposure to oxLDL, but not native LDL or HDL, causes a fall in the sterol content in the caveolae. Experiments with radiolabeled sterols, showed that these sterols are transferred to oxLDL particles [46]. The changes in lipid environment in the caveolae caused by oxLDL induces eNOS expression and stimulates the movement of eNOS from the caveolae to other cellular compartments [46]. As a consequence, oxLDL strongly attenuates the activation of eNOS upon stimulation with acetylcholine, an important vasodilator [46]. This reduced activation was solely due to the change in subcellular eNOS localization, rather than eNOS phosphorylation, which is known to regulate eNOS activity. The effects of oxLDL on eNOS localization and activation are mediated by CD36, as was shown by antibody blockade of this receptor [47]. In line with these *in vitro* studies, apoE deficient mice, which have high levels of VLDL and LDL and develop spontaneous atherosclerosis, display no blood pressure change when stimulated with acetylcholine. Furthermore, eNOS is not present in caveolae of these mice [47].

The effects of oxLDL are counteracted by HDL [47]. The addition of HDL to medium containing oxLDL prevented the changes in caveolae lipid environment, and the oxLDL-mediated changes in subcellular localization of eNOS. This effect of HDL is not caused by inhibition of oxLDL-induced cholesterol export from caveolae, but rather by supplying cholesterol esters that were depleted. Furthermore, HDL restored the acetylcholine-induced stimulation of the enzyme. The ability of HDL to reverse the oxLDL-induced alteration in eNOS localization is mediated by the HDL receptor Scavenger receptor BI (SR-BI), which is highly present in caveolae and colocalized with eNOS [48]. Antibody blocking of SR-BI prevents the HDL-mediated restoration of eNOS localization and activation [47].

Besides its effect on eNOS localization, HDL can also directly stimulate eNOS activity in endothelial cells. HDL added to cultured endothelial cells stimulates eNOS activity in

a concentration dependent manner [48]. LDL and lipoprotein deficient control serum did not have this effect. Stimulation with normal serum yields a similar response as HDL. However, when endothelial cells were simultaneously stimulated with HDL and excess LDL, the activation of eNOS was attenuated. Importantly, eNOS was not activated by purified forms of apoA-I or apoA-II [48]. Furthermore, anti-apoA-I antibodies block eNOS activation by HDL, but lipid-free apoA-I fails to stimulate eNOS activation. These findings suggest that apoA-I is necessary but not sufficient for eNOS stimulation [48].

In a recent study by Chiesa and colleagues published in this issue of Vascular Pharmacology, evidence is provided that LCAT deficient mice, which display a pronounced reduction in HDL levels [49], show a lower acetylcholine-induced NO dependent relaxation in absence of changes in eNOS expression. Moreover, the aortas of LCAT deficient mice showed a reduced contractility when stimulated with noradrenalin. The results, however, are attributed to an increase in b2-adrenergic receptor mediated relaxation and not due to the reduced HDL levels in these animals. In line, the authors show that in apoA-I knockout mice the responses are unaltered.

Like its effects on eNOS localization, the capability of HDL to activate eNOS is mediated by SR-BI. HDL enhances eNOS activation and NO-dependent aortic relaxation of aortic rings of wild-type but not SR-BI deficient mice [48]. Furthermore, infusion of apoA-I protected wild-type, but not SR-BI or eNOS deficient mice, from deep vein thrombosis in a platelet independent fashion [50].

All together, these studies suggest an important role for lipoproteins in the modulation of eNOS localization and bioavailability. Furthermore, studies with apoE, apoA-I, and LCAT deficient mice show that these effects also have functional consequences.

Prostacyclin

In addition to the production of NO, endothelial cells also produce prostacyclin (PGI_2), which can modify thrombosis by inhibiting platelet aggregation [51]. PGI_2 is synthesized from arachidonic acid in a pathway that involves the enzyme cyclooxygenase (COX), which exists in two isoforms: COX-1, which is constitutively expressed, and COX-2, which is inducible [52].

HDL stimulates endothelial PGI_2 synthesis by the provision of arachidonic acid, as well as by inducing COX-2 expression [53–57]. Furthermore, HDL was shown to enhance the release of prostaglandins, the precursors for PGI_2, in isolated hearts from rabbits and rats [58,59]. Although to a lesser extent than intact HDL, delipidated HDL also enhances PGI_2 synthesis [54] suggesting that both HDL-associated lipids as well as apolipoproteins on the HDL particle are involved.

Von Willebrand Factor

Von Willebrand Factor (vWF) is produced by endothelial cells and stored in Weibel-Palade bodies [60]. Upon vascular injury in high sheer stress vessels, such as is the case in atherothrombosis, vWF mediates platelet adherence to the endothelium at sites of damage. Importantly, patients with hypercholesterolemia have higher plasma levels of vWF [61]. Levels of circulating vWF are inversely associated with HDL in patients with peripheral vascular disease[62]. Furthermore, LDL and oxLDL induce the release of von Willebrand factor from human endothelial cells *in vitro* [62].

Coagulation modulation

Besides the modulation of platelet reactivity, the vascular endothelium also influences the coagulation cascade. For example, TF pathway inhibitor (TFPI) is secreted by endothelial cells to inhibit the extrinsic pathway of coagulation. Epidemiological studies in humans have shown correlations between hyperlipidemia and circulating TFPI. The correlations were highly dependent on the type of hyperlipidemia [63]. TFPI was increased in patients with familial hypercholesterolemia (FH), whereas it was slightly decreased in patients with familial hypertriglyceridemia. TFPI activity was positively correlated with lipid and protein components of LDL (LDL-C and apoB) and of HDL (HDL-C and apoA-I). TFPI was negatively correlated with the triglyceride level [63]. However, in a study performed in Japanese coronary artery patients, HDL was negatively correlated with TFPI levels [64].

In addition to TFPI, coagulation is also modulated via heparin sulfate proteoglycans (HSPG), which are proteoglycans with covalently bound heparin sulfate, and are expressed on the endothelial cell surface. HSPGs can bind a wide variety of ligands including apoE and lipoprotein lipase, two key molecules in lipoprotein metabolism, as well as antithrombin III, an inhibitor of several factors of the coagulation cascade [65–67]. Endothelial HSPGs are decreased by oxLDL, an effect abolished by the presence of HDL [68]. Moreover, apoE-containing HDL increases endothelial production of HSPGs rich in biologically active heparin-binding domains [65]. Pre-incubation of endothelial cells with HDL therefore led to significantly higher binding of antihrombin III as compared to controls.

Apoptosis

An intact endothelial layer is critical for hemostasis in the vascular wall. Induction of endothelial apoptosis *in vivo* drives endothelial denudation. This can lead to superficial erosion of atherosclerotic plaques, and subsequent thrombus formation [69–72]. In addition, disturbances in vascular function and acute coronary events may be induced by thrombogenic membrance microparticles released from apoptotic endothelial cells [73,74].

OxLDL promotes apoptosis of human coronary endothelial cells by causing a sustained increase in intracellular Ca2+, resulting in the death of endothelial cells [75]. This effect is reversed by HDL, which prevents the increase in intracellular Ca2+. Purified apoA-I mimics this effect [76]. HDL also inhibits endothelial cell apoptosis induced by TNF-alpha and growth factor deprivation [76,77]. ApoA-I partially mimics the effect of HDL, whereas apoA-II has no effect on apoptosis. HDL preserves mitochondrial integrity and inhibits the release of cytochrome C into the cytoplasm. These effects are mediated by the protein kinase Akt, an ubiquitous tranducer of antiapoptotic signals [77]. Two HDL-associated lysosphingolipids, sphingophosphorylcholine and lysosulfatide, also stimulate Akt and inhibit apoptosis. Therefore, it is believed that the protective function of HDL is caused by the combined activation by lysospingolipids and apoA-I [77].

PLATELETS

Platelets are small anucleate cells that play a key role in homeostasis and respond rapidly to changes in the endothelial integrity and exposure of subendothelial structures. Platelet activation results in cytoskeletal rearrangement and the secretion of storage granule content, and as such, platelets are a key player in atherothrombosis [78]. An analysis of the relative contribution of platelets and other implicated factors (including plaque rupture, inflammation, coagulation factors, and cholesterol) in the etiology of acute coronary syndromes led to the conclusion that platelet changes are more important than plaque rupture in the etiology of acute myocardial infarction [79]. Hence, drugs that modify platelet behavior have become the cornerstone of therapy for acute coronary syndromes. Platelet COX-1 inhibitors (aspirin), platelet ADP receptor antagonists (*e.g.* clopidogrel) and glycoprotein IIb/IIIa antagonists (e.g. tirofiban) are used as part of standard care and have proven morbidity and mortality rate benefits [80]. Importantly, lipoproteins have been shown to affect platelet function at various levels.

Platelet density and volume

Platelets are produced from megakaryocytes in the bone marrow, a process called thrombopoiesis. Dyslipidemia leads to altered characteristics of megakaryocytes, which can influence platelet count and function, and in this way modulate the risk of atherothrombosis via the megakaryocyte-platelet hemostatic axis [81].

Hypercholesterolemic humans, as well as rabbits and guinea pigs, are found to have larger megakaryocytes with a higher mean ploidy [82–84]. Megakaryocytes with these characteristics are generally considered to produce larger and more active platelets [81]. A substantial body of clinical evidence demonstrates increased platelet density and volume in the setting of acute coronary syndromes, and implies mean platelet volume as both

a causal and prognostic factor. Multiple studies have shown that patients with acute myocardial infarction or unstable angina display an increased mean platelet volume and/or platelet density compared to patients with stable coronary disease [85–88]. Given that the lifespan of a platelet is 10 days, and that 90% of platelets measured shortly after acute myocardial infarction would have been circulating before the occlusive event, a causal relationship between acute coronary syndromes and platelet density and mean platelet volume has been suggested. Platelet density and volume after acute myocardial infarction predicted outcome in a study involving 1716 patients, in whom mean platelet volume was measured after acute myocardial infarction [89]. Mean platelet volume was found to be an independent predictor of both recurrent acute myocardial infarction and death for up to two years after the first event. Notably, mean platelet volume was independently and more powerfully predictive than other variables, such as blood pressure, cholesterol, or smoking.

Membrane-cholesterol mediated platelet reactivity

LDL and VLDL increase platelet cholesterol content, and stimulate platelet activation *in vitro* [90,91]. Previous studies in animal models have shown that increased platelet cholesterol is due to the uptake of circulating lipoproteins by megakaryocytes, which subsequently passed on the cholesterol into future platelets [82,83]. Both increased plasma cholesterol and an increase in platelet membrane cholesterol enhances the sensitivity of human platelets to aggregating agents [90–93]. Similar results are found in patients with FH, whose cells lack or have defective LDL receptors, resulting in elevated plasma LDL levels. Platelets of FH patients have increased α-granule secretion [94], increased superoxide anion production [95], increased fibrinogen binding [96], and subsequent enhanced platelet aggregation after stimulation [94,97,98]. In addition, platelets of FH patients and hyperlipidemic apoE deficient mice circulate in an activated state [99–101]. In contrast to the elevated plasma LDL levels in FH patients, plasma from abetalipoproteinemia patients lack all apoB-containing lipoproteins. In accordance, platelets from these patients aggregate poorly and show impaired arachidonic acid release and thromboxane A_2 (TxA_2) generation [102]. Purified apoE-containing phospholipid vesicles inhibit platelet aggregation in response to ADP, epinephrine, thrombin and collagen [103,104]. This is probably the consequence of its cholesterol depleting effects on the cell membrane. In line, cholesterol-depleted platelets poorly respond to agonists [90].

Receptor mediated platelet activity

In addition to the effects on membrane cholesterol incorporation, LDL and HDL also affect platelet function via a direct interaction. By binding to platelet receptors, lipoproteins induce rapid activation of signal transduction pathways that enhance or inhibit platelet activation [105].

Native LDL-induced signaling in platelets is mediated by a splice variant of the apoE receptor-2 (apoER2), apoER2'. LDL binds to this receptor via the so-called B-site of apoB100 [106], and binding of LDL to platelets via apoER2' results in the formation of platelet-activating TxA_2 [107]. Consequently, binding of LDL to platelets leads to enhanced platelet aggregation [106]. In contrast to LDL, oxLDL enhances the platelet response to agonists via an interaction with the scavenger receptors CD36 and scavenger receptor A (SR-A) [108–110].

Most studies to date support a direct inhibitory effect of HDL or its major fraction, HDL_3, on platelet activation and the subsequent formation of venous and arterial thrombi [111–114]. However, the receptor through which different HDL particles exert their function has long been disputable. In early studies, integrin $\alpha_{IIb}\beta_3$ has been studied as a receptor for HDL_3 signaling. However, its role remains controversial until this time [105]. In 2011, SR-BI was identified as the primary binding site for HDL_3 [115]. Binding of HDL_3 to SR-BI on platelets inhibits agonist-induced activation and aggregation. These effects were mediated by protein kinase C. SR-BI deficient platelets were not affected by HDL_3 [115].

The binding affinity of a receptor for HDL is defined by the apolipoprotein moiety on the HDL particle. Since HDL_3 only contains trace amounts of apoE and apoC, receptor binding by HDL_3 is presumably mediated by apoA-I [116]. ApoA-I has been found to inhibit platelet function [90,103]. Furthermore, SR-BI is known to bind apoA-I in other cell types, so it is likely that a similar mechanism occurs in platelets. Inhibition of platelet activation is observed by HDL_2 and apoE-rich HDL [117–122]. These particles inhibit platelet function such as shape change, inositol phospholipid production, and reduce LDL-induced NO synthase expression [122,123]. Chemical modification of apoE residues in HDL abolishes binding to the platelets and prevents its anti-aggregatory effects [118]. Binding of HDL_3 to platelets is inhibited by HDL_2, suggesting that HDL_3 and HDL_2 bind to the same receptor [124]. However, as the apoER2' receptor mediates apoE signaling in platelets, apoE-rich HDL_2 particles possibly also bind to platelets via this receptor [125]. Binding of apoE-containing lipoproteins to apoER2' impairs platelet signaling by increasing cGMP through NO production [125]. As mentioned earlier, LDL binds to apoER2' via apoB100, which enhances platelet activation. ApoER2' is thus capable of facilitating either activating or inhibitory signals initiated through the binding of apoB100- or apoE-containing lipoproteins, respectively. These differential effects may be explained by the fact that multiple apoE molecules on apoE-bearing lipoproteins can induce clustering of apoER2' receptors, while this is not possible for the binding of apoB100, from which there is only a single molecule found on an LDL particle [125]. A complex of clustered receptors may initiate different signaling pathways compared to a single receptor. Further studies are required to elucidate how apoER2' is capable of mediating both activating and inhibitory signals.

SECONDARY COAGULATION

Secondary coagulation is the clotting of blood through activation of the coagulation cascade. The conversion of the soluble plasma protein fibrinogen into insoluble fibrin fibers, mediated by thrombin, is the central step of the coagulation cascade, and, after platelet adhesion, the second step in atherothrombosis. It starts with the exposure of blood to TF (extrinsic pathway) or negatively charged surfaces (intrinsic pathway), which causes a waterfall effect. The waterfall eventually culminates in a common pathway in which the prothrombinase complex (FXa and FVa) converts prothrombin into thrombin [126,127]. Within the coagulation cascade, both negative and positive feedback reactions are important for maintaining homeostasis and, when necessary, a fast, massive coagulation response, respectively.

Hypercholesterolemia is associated with hypercoagulability and an increase in venous thrombosis risk [128,129]. In venous thrombosis, especially the pathological activation of the coagulation cascade plays a key role, suggesting a direct effect of hypercholesterolemia on secondary coagulation.

Prothrombin activation and thrombin formation

Almost all clotting factors bind lipids, although with varying affinities. Hypertriglyceridemia is associated with increased levels of all vitamin K-dependent procoagulant factors. Furthermore, binding of clotting factors to lipids alters their activity. Triglyceride-rich lipoproteins bind vitamin K-dependent clotting factors and promote the procoagulant reaction [130–136]. For example, studies using purified lipoproteins and clotting factors showed that VLDL enhances prothrombin activation by FXa in the presence of Va. LDL and HDL are substantially less capable of inducing prothrombin activation [133,134]. On the contrary, HDL is inversely correlated with plasma thrombin activation markers such as prothrombin fractions F1+2, the peptides cleaved from prothrombin during its conversion to thrombin [137].

Tissue factor and factor VII activation

TF is the primary initiator of the extrinsic coagulation pathway. It is a surface-bound protein found on many cells in the subendothelial tissue. Furthermore, it is highly present in atherosclerotic plaques [138,139]. TF expression by endothelial cells and macrophages is stimulated by minimally oxidized and acetyl-modified LDL respectively [30,140]. In contrast, HDL and apoA-I suppress TF activity [141].

Factor VII is the first enzyme encountered in the extrinsic pathway, and is directly activated by TF. Lipoproteins enhance the activation of factor VII. Each lipoprotein species supports factor VII activation by factor Xa, but not by factor IXa, in the absence of

TF. ApoA-II has been shown to inhibit the activation of factor X by the TF-factor VIIa complex, inhibiting the first step in of the extrinsic coagulation pathway [142].

Fibrinogen

The step from fibrinogen to fibrin is the last step in the coagulation cascade, an is a crucial step for stabilizing the blood clot. Fibrinogen levels are elevated in FH subjects [143,144]. In multiple population studies it was found that fibrinogen is positively associated with LDL cholesterol, Lp(a), and triglycerides. Furthermore, it was inversely associated with HDL cholesterol [145–148].

Activated Protein C pathway

The protein C pathway provides a major physiological anticoagulant mechanism to downregulate thrombin formation by proteolytically inactivating factors Va and VIIIa in plasma. HDL enhances anticoagulation via activated protein C (APC). There is a positive correlation between plasma apoA-I levels and *in vitro* inactivation of factor Va by APC and protein S [149]. However, it has been described that this APC-enhancing activity of HDL is only present in the HDL_2, but not HDL_3 fraction [150].

In 2010, there was a report by Oslakovic *et al*. that stated that the anticoagulant properties attributed to HDL were actually caused by contaminating negatively charged phospholipid membranes, and not by HDL itself [151]. However, later research by Fernandez et al. showed that HDL loses its anticoagulant properties after a freeze-thaw cycle [152]. Furthermore, it has also been shown that anti-apoA-I antibodies remove most of the HDL ability to enhance APC:protein S activity [152]. Antibodies against apoC-III also block the ability of HDL to enhance protein C anticoagulant activity, suggesting that apolipoproteins on HDL fractions are responsible for this anticoagulant trait of HDL [150].

LIPID MODULATION AS A POTENTIAL TREATMENT FOR ACUTE CARDIOVASCULAR DISEASE AND ATHEROTHROMBOSIS

Currently, the standard of care for patients at risk for acute coronary artery disease are statins, which are 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Numerous long-term, placebo-controlled clinical trials have conclusively demonstrated that statins reduce the risk of morbidity and mortality from cardiovascular disease. Furthermore, as mentioned previously, statin therapy also decreases the risk of both venous thrombosis and arterial thrombosis [29–32,153,154]. Although there are several types of statins available today, the typical reduction in relative risk of cardiovascular disease ranges from 24–37% [155]. These percentages highlight the need for improvement by novel therapeutic approaches.

PCSK9 inhibitors

Pro-protein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein that binds to the surface LDL receptor (LDLR) and targets it towards lysosomal degradation. As a consequence, the number of LDLRs at the cell surface is decreased, and LDL clearance is reduced. Inhibition or loss-of-function mutations of PCSK9 result in increased surface LDLR and improved LDL clearance [156]. Two recent reports describe the results of studies with monoclonal antibodies against PCSK9 and their potential effects on CVD events. The administration of both alirocumab and evolucumab were associated with a reduced rate of major CVD events [157,158]. Numerous compounds that inhibit PCSK9 are currently under development and tested in clinical trials [159]. One small study reported that plasma PCSK9 levels are positively associated with platelet counts in stable coronary artery disease patients [160]. Platelet counts are, in turn, positively associated with cardiovascular death [161]. However, the implications of these findings remain to be elucidated. Unfortunately, to date, no effects of PCSK9 or PCSK9 inhibition, on endothelial function, blood coagulation or thrombosis have been reported. Possibly, the ongoing trials will elucidate potential role of this nature.

CETP inhibitors

Cholesteryl ester transfer protein (CETP) is a plasma protein that facilitates the transport of cholesteryl esters and triglycerides between VLDL, LDL and HDL. When CETP is inhibited, cholesterol accumulates in the HDL lipoprotein fraction, as opposed to LDL and VLDL lipoproteins, thus improving the overall plasma lipoprotein profile. Trials in humans have shown that CETP inhibitors effectively raise HDL cholesterol levels. Unfortunately however, in 2007, the CETP inhibitor torcetrapib unexpectedly showed increased fatality and cardiovascular events. This was most likely due to increased blood pressure and aldosterone levels as trials with novel CETP inhibitors later showed that the negative effects of torcetrapib were the consequence of off target effects of the compound, and not of CETP inhibition in general [162]. The novel CETP inhibitors dalcetrapib, evacetrapib and anacetrapib did not show harmful effects on blood pressure or aldosterone levels [162]. Dalcetrapib increased HDL cholesterol levels but did not reduce the risk of recurrent cardiovascular events in patients who had had a recent acute coronary syndrome [163]. However, this may have been due to polymorphisms in the adenylyl cyclase 9 gene (ADCY9) [164]. ADCY9 is a membrane-bound protein affected by changes in caveolae. The finding that ADCY9 is important in the therapeutic outcome of increasing HDL cholesterol has renewed interest in a potential role of HDL in modulating signal transduction by changing cholesterol concentration in cellular membrane substructures [165]. Unfortunately, to date, there is no data available of a potential role of CETP, or inhibition thereof, on platelets, blood coagulation or thrombosis.

rHDL

A promising strategy for lipid modulation in thrombosis is the use of synthetic reconstituted HDL (rHDL) [166]. rHDL are HDL-like particles containing phospholipids and human apoA-I or one of its variants such as apoA-I_{Milano}, which are functionally more effective. In a study by Lerch et al, rHDL was shown to dose-dependently inhibit *in vitro* platelet reactivity after stimulation [167]. Moreover, experiments with platelet-rich plasma from volunteers who had been infused with rHDL were performed. In these experiments, both arachidonic acid- and collagen-induced platelet aggregation were reduced. The extent of inhibition negatively correlated with plasma concentrations of apoA-I, HDL-C and the dose of rHDL infused. These data correlated with studies in rats, in which administration of recombinant apoA-I_{Milano} inhibited platelet aggregation and $FeCl_3$-induced arterial thrombus formation [168].

In patients with Type 2 Diabetes Mellitus, who exhibit enhanced platelet reactivity and an increased risk of cardiovascular disease, rHDL infusion significantly reduced *ex vivo* platelet aggregation and thrombus formation under flow [169]. However, in this study, the effects were mainly ascribed to the isolated phospholipid component of rHDL, and not to apoA-I. rHDL also reduces coagulation responses in LPS-induced endotoxemia, in which it lowers plasma levels of prothrombin and tPA [170].

At least four different formulations of rHDL have been tested in clinical trials [166]. Although the underlying mechanisms remain to be elucidated, rHDL infusions are a promising therapeutic strategy to reduce thrombosis risk in a variety of conditions where platelet hyperreactivity and hypercoagulability pose a threat. As such rHDL therapy may thus possibly also be of benefit for patients at risk for atherothrombotic complications.

LXR targeting

Liver X receptors (LXRs) are nuclear transcription factors that regulate the expression of genes involved in cholesterol catabolism to bile acids and cholesterol efflux. Their natural ligands are oxysterols, which are cholesterol derivatives [171]. Ligand-stimulated LXR activation yields anti-inflammatory and athero-protective effects [172–174]. The LXR family consist of two members: LXRα and LXRβ, each with distinct expression patterns. LXRβ is ubiquitously expressed, while LXRα expression is restricted to tissues active in lipid metabolism [171]. Recently, it was shown that LXRβ is present in human platelets [175]. LXR ligands inhibit platelet function stimulated through a range of physiologic agonists. Furthermore, ligand stimulation inhibits the ability of platelets to form thrombi *in vivo*, affecting both the size and the stability of growing thrombi [175]. Importantly, LXR stimulation allowed initial thrombi to form, crucial for tissue repair after vascular injury, but prevented occlusion of the vessel through decreased thrombus stability. These properties render LXR agonism a promising method for inhibiting pathological thrombus formation without disturbing physiological homeostasis and wound healing.

Because of their key roles in cholesterol metabolism, LXRs have since long been a target for drug discovery. However, thus fat therapeutic application of LXR agonists has been hampered due to the fact that LXR also modulates fatty acid and carbohydrate metabolism in tissues such as liver, adipose and skeletal muscle [176]. Mice treated with a synthetic LXR agonist demonstrate marked hypertriglyceridemia, a condition mainly attributed to LXRα expression in hepatocytes [177]. In order to eliminate this side effect, the use of LXRβ-specific agonists might be beneficial, as the function of both family members does not seem to overlap [178]. Compounds that selectively target LXRβ are currently under development [179]. However, no data on the effect of LXRβ agonism on platelet function or thrombosis have been reported so far.

CONCLUSIONS AND PERSPECTIVES

There is great interest in the development of novel pharmacological intervention strategies to reduce atherothrombotic complications of atherosclerosis. From experimental studies, it is clear that lipoproteins influence the thrombotic capacity of the blood and in that way may alter atherothrombosis. Overall, VLDL, LDL and oxLDL promote thrombus formation, whereas HDL shows antithrombotic actions.

Therapies aimed at lowering (V)LDL cholesterol levels are already standard practice in patients with high risk for atherothrombotic complications, and will most likely not only influence plaque integrity but also thrombosis. In addition to lowering (V)LDL, raising HDL levels may be an attractive therapeutic strategy to improve the outcome of atherothrombotic complications. However, although low HDL levels are predictive of coronary artery disease, increasing HDL has yet to prove its therapeutic value.

In the quest for HDL-raising drugs, it is important to keep in mind that not only the circulating levels of HDL cholesterol matter. The particle composition, HDL subclass, surface apolipoproteins and phospholipids, are of the utmost importance for its antithrombotic function. Hence, detailed structure-function analysis are needed to identify clinically relevant, antithrombotic HDL subpopulations in order to develop effective therapeutic approaches to reduce atherothrombotic risk.

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REFERENCES

1. Ross R. Atherosclerosis - An inflammatory disease. *N Engl J Med*. 1999;340(2):115-126. doi:10.1056/nejm199901143400207.
2. Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. *Nat Rev Cardiol*. 2011;8(9):502-512. doi:10.1038/nrcardio.2011.91.
3. Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med*. 2008;359(9):938-949. doi:10.1056/NEJMra0801082.
4. Falk E, Shah PK, Fuster V. Coronary Plaque Disruption. *Circulation*. 1995;92:657-671. http://circ.ahajournals.org/content/92/3/657.long.
5. Davies MJ. Stability and Instability: Two Faces of Coronary Atherosclerosis The Paul Dudley White Lecture 1995. *Circulation*. 1996;94:2013-2020. http://circ.ahajournals.org/content/94/8/2013.long.
6. Buffon A, Biasucci LM, Liuzzo G, D'Onofrio G, Crea F, Maseri A. Widespread coronary inflammation in unstable angina. *N Engl J Med*. 2002;346(24):1845-1853.
7. Crea F, Liuzzo G. Pathogenesis of Acute Coronary Syndromes. *J Am Coll Cardiol*. 2013;61(1):1-11. doi:10.1016/j.jacc.2012.07.064.
8. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111(25):3481-3488. doi:10.1161/CIRCULATIONAHA.105.537878.
9. Libby P, Pasterkamp G. Requiem for the 'vulnerable plaque.' *Eur Heart J*. 2015:ehv349. doi:10.1093/eurheartj/ehv349.
10. Underhill HR, Yuan C, Zhao X-Q, Kraiss LW, Parker DL, Saam T, Chu B, Takaya N, Liu F, Polissar NL, Neradilek B, Raichlen JS, Cain V a., Waterton JC, Hamar W, Hatsukami TS. Effect of rosuvastatin therapy on carotid plaque morphology and composition in moderately hypercholesterolemic patients: A high-resolution magnetic resonance imaging trial. *Am Heart J*. 2008;155(3):584.e1-584.e8. doi:10.1016/j.ahj.2007.11.018.
11. Libby P. How does lipid lowering prevent coronary events? New insights from human imaging trials. *Eur Heart J*. 2015;36(8):472-474. doi:10.1093/eurheartj/ehu510.
12. Libby P. Mechanisms of Acute Coronary Syndromes and Their Implications for Therapy — NEJM. *N Engl J Med*. 2013;368(21):2004-2013. doi:10.1056/NEJMra1216063.
13. Hu S, Jia H, Vergallo R, Abtahian F, Tian J, Soeda T, Rosenfield K, Jang I-K. Plaque Erosion : In Vivo Diagnosis and Treatment Guided by Optical Coherence Tomography. *JACC Cardiovasc Interv*. 2014;7(6):e63-e64. doi:10.1016/j.jcin.2013.10.024.
14. Braunwald E. Coronary Plaque Erosion : Recognition and Management. *JACC Cardiovasc Imaging*. 2013;6(3):288-289. doi:10.1016/j.jcmg.2013.01.003.
15. Rye KA, Clay M a., Barter PJ. Remodelling of high density lipoproteins by plasma factors. *Atherosclerosis*. 1999;145(2):227-238. doi:10.1016/S0021-9150(99)00150-1.
16. Annema W, von Eckardstein A. High-density lipoproteins. Multifunctional but vulnerable protections from atherosclerosis. *Circ J*. 2013;77(10):2432-2448. doi:10.1253/circj.CJ-13-1025.
17. Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat Med*. 2002;8(11):1257-1262. doi:10.1038/nm1102-1257.
18. Gordon DJ, Knoke J, Probstfield JL, Superko R, Tyroler HA. High-density lipoprotein cholesterol and coronary heart disease in hypercholesterolemic men: the Lipid Research Clinics Coronary Primary Prevention Trial. *Circulation*. 1986;74(6):1217-1225.
19. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. *Lancet (London, England)*. 1977;1(8019):965-968.

20. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med*. 1977;62(5):707-714.
21. Miller M, Seidler A, Kwiterovich PO, Pearson TA. Long-term predictors of subsequent cardiovascular events with coronary artery disease and "desirable" levels of plasma total cholesterol. *Circulation*. 1992;86(4):1165-1170.
22. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302(18):1993-2000. doi:10.1001/jama.2009.1619.
23. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: A dynamic balance. *Nat Rev Immunol*. 2013;13(10):709-721. doi:10.1038/nri3520.
24. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR, Bangdiwala S, Tyroler H a. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*. 1989;79(1):8-15. doi:10.1161/01.CIR.79.1.8.
25. Vaisar T, Pennathur S, Green PS, Gharib S a, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao X, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *J Clin Invest*. 2007;117(3). doi:10.1172/JCI26206DS1.
26. Dashty M, Motazacker MM, Levels J, de Vries M, Mahmoudi M, Peppelenbosch MP, Rezaee F. Proteome of human plasma very low-density lipoprotein and low-density lipoprotein exhibits a link with coagulation and lipid metabolism. *Thromb Haemost*. 2014;111(3):518-530. doi:10.1160/TH13-02-0178.
27. Rezaee F, Casetta B, Levels JHM, Speijer D, Meijers JCM. Proteomic analysis of high-density lipoprotein. *Proteomics*. 2006;6(2):721-730. doi:10.1002/pmic.200500191.
28. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. *JAMA*. 1998;279(20):1643-1650.
29. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213.
30. Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins Inhibit Tissue Factor in Cultured Human Macrophages: A Novel Mechanism of Protection Against Atherothrombosis. *Arterioscler Thromb Vasc Biol*. 1997;17(2):265-272. doi:10.1161/01.ATV.17.2.265.
31. Dangas G, Smith DA, Unger AH, Shao JH, Meraj P, Fier C, Cohen AM, Fallon JT, Badimon JJ, Ambrose JA. Pravastatin: an antithrombotic effect independent of the cholesterol-lowering effect. *Thromb Haemost*. 2000;83(5):688-692.
32. Fenton JW 2nd, Shen GX, Minnear FL, Brezniak D V, Jeske WP, Walenga JM, Bognacki JJ, Ofosu FA, Hassouna HI. Statin drugs and dietary isoprenoids as antithrombotic agents. *Hematol Oncol Clin North Am*. 2000;14(2):483-90, xi.
33. Takemoto M, Liao JK. Pleiotropic Effects of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors. *Arterioscler Thromb Vasc Biol*. 2001;21(11):1712-1719. doi:10.1161/hq1101.098486.
34. Levine GN, Keaney JFJ, Vita JA. Cholesterol reduction in cardiovascular disease. Clinical benefits and possible mechanisms. *N Engl J Med*. 1995;332(8):512-521. doi:10.1056/NEJM199502233320807.
35. Vita J a. Endothelial function. *Circulation*. 2011;124(25):906-913. doi:10.1161/CIRCULATIONAHA.111.078824.

36. Suwaidi J a, Hamasaki S, Higano ST, Nishimura R a, Holmes DR, Lerman a. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101(9):948-954. doi:10.1161/01.CIR.101.9.948.
37. Schächinger V, Britten MB, Zeiher a M. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101(16):1899-1906. doi:10.1161/01.CIR.101.16.1899.
38. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad a., Waclawiw M a., Nour KR a., Quyyumi a. a. Prognostic Value of Coronary Vascular Endothelial Dysfunction. *Circulation*. 2002;106(6):653-658. doi:10.1161/01.CIR.0000025404.78001.D8.
39. Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial Flow-Mediated Dilation Predicts Incident Cardiovascular Events in Older Adults: The Cardiovascular Health Study. *Circulation*. 2007;115(18):2390-2397. doi:10.1161/CIRCULATIONAHA.106.678276.
40. Gokce N. Risk Stratification for Postoperative Cardiovascular Events via Noninvasive Assessment of Endothelial Function: A Prospective Study. *Circulation*. 2002;105(13):1567-1572. doi:10.1161/01.CIR.0000012543.55874.47.
41. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med*. 1995;332(8):481-487. doi:10.1056/NEJM199502233320801.
42. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol*. 2002;40(3):505-510. doi:10.1016/S0735-1097(02)01976-9.
43. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Annu Rev Physiol*. 2002;64:749-774. doi:10.1146/annurev.physiol.64.081501.155952.
44. Shaul PW. Endothelial nitric oxide synthase, caveolae and the development of atherosclerosis. *J Physiol*. 2003;547(1):21-33. doi:10.1113/jphysiol.2002.031534.
45. Chang WJ, Rothberg KG, Kamen B a., Anderson RGW. Lowering the cholesterol content of MA104 cells inhibits receptor-mediated transport of folate. *J Cell Biol*. 1992;118(l):63-69. doi:10.1083/jcb.118.1.63.
46. Blair A, Shaul PW, Yuhanna IS, Conrad P a., Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. *J Biol Chem*. 1999;274(45):32512-32519. doi:10.1074/jbc.274.45.32512.
47. Uittenbogaard a, Shaul PW, Yuhanna IS, Blair a, Smart EJ. High density lipoprotein prevents oxidized low density lipoprotein-induced inhibition of endothelial nitric-oxide synthase localization and activation in caveolae. *J Biol Chem*. 2000;275(15):11278-11283. doi:10.1074/jbc.275.15.11278.
48. Yuhanna IS, Zhu Y, Cox BE, Hahner LD, Osborne-Lawrence S, Lu P, Marcel YL, Anderson RG, Mendelsohn ME, Hobbs HH, Shaul PW. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med*. 2001;7(7):853-857. doi:10.1038/89986.
49. Lambert G, Sakai N, Vaisman BL, Neufeld EB, Marteyn B, Chan C-C, Paigen B, Lupia E, Thomas a., Striker LJ, Blanchette-Mackie J, Csako G, Brady JN, Costello R, Striker GE, Remaley a. T, Brewer HB, Santamarina-Fojo S. Analysis of Glomerulosclerosis and Atherosclerosis in Lecithin Cholesterol Acyltransferase-deficient Mice. *J Biol Chem*. 2001;276(18):15090-15098. doi:10.1074/jbc.M008466200.

50. Brill A, Yesilaltay A, De Meyer SF, Kisucka J, Fuchs T a., Kocher O, Krieger M, Wagner DD. Extrahepatic high-density lipoprotein receptor SR-BI and ApoA-I protect against deep vein thrombosis in mice. *Arterioscler Thromb Vasc Biol*. 2012;32(8):1841-1847. doi:10.1161/ATVBAHA.112.252130.
51. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*. 1976;263(5579):663-665. doi:10.1038/263663a0.
52. Caughey GE, Cleland LG, Penglis PS, Gamble JR, James MJ. Roles of Cyclooxygenase (COX)-1 and COX-2 in Prostanoid Production by Human Endothelial Cells: Selective Up-Regulation of Prostacyclin Synthesis by COX-2. *J Immunol*. 2001;167(5):2831-2838. doi:10.4049/jimmunol.167.5.2831.
53. Fleisher LN, Tall a R, Witte LD, Miller RW, Cannon PJ. Stimulation of arterial endothelial cell prostacyclin synthesis by high density lipoproteins. *J Biol Chem*. 1982;257:6653-6655.
54. Pomerantz KB, Fleisher LN, Tall a R, Cannon PJ. Enrichment of endothelial cell arachidonate by lipid transfer from high density lipoproteins: relationship to prostaglandin I2 synthesis. *J Lipid Res*. 1985;26(10):1269-1276. http://www.ncbi.nlm.nih.gov/pubmed/3934304.
55. Myers DE, Huang WN, Larkins RG. Lipoprotein-induced prostacyclin production in endothelial cells and effects of lipoprotein modification. *Am J Physiol*. 1996;271(5 Pt 1):C1504-C1511.
56. Cockerill GW, Saklatvala J, Ridley SH, Yarwood H, Miller NE, Oral B, Nithyanathan S, Taylor G, Haskard DO. High-Density Lipoproteins Differentially Modulate Cytokine-Induced Expression of E-Selectin and Cyclooxygenase-2. *Arterioscler Thromb Vasc Biol*. 1999;19(4):910-917. doi:10.1161/01.ATV.19.4.910.
57. Norata GD. HDL3 Induces Cyclooxygenase-2 Expression and Prostacyclin Release in Human Endothelial Cells Via a p38 MAPK/CRE-Dependent Pathway: Effects on COX-2/PGI-Synthase Coupling. *Arterioscler Thromb Vasc Biol*. 2004;24(5):871-877. doi:10.1161/01.ATV.zhq0504.1403.
58. Calabresi L. High-Density Lipoproteins Protect Isolated Rat Hearts From Ischemia-Reperfusion Injury by Reducing Cardiac Tumor Necrosis Factor-alpha Content and Enhancing Prostaglandin Release. *Circ Res*. 2003;92(3):330-337. doi:10.1161/01.RES.0000054201.60308.1A.
59. Van Sickle W a, Wilcox HG, Malik KU, Nasjletti a. High density lipoprotein-induced cardiac prostacyclin synthesis in vitro: relationship to cardiac arachidonate mobilization. *J Lipid Res*. 1986;27(5):517-522. http://www.ncbi.nlm.nih.gov/pubmed/3090180.
60. Reininger AJ, Heijnen HFG, Schumann H, Specht HM, Schramm W, Ruggeri ZM. Mechanism of platelet adhesion to von Willebrand factor and microparticle formation under high shear stress. 2006;107(9):3537-3545. doi:10.1182/blood-2005-02-0618. Supported.
61. Davi G, Romano M, Mezzetti a, Procopio a, Iacobelli S, Antidormi T, Bucciarelli T, Alessandrini P, Cuccurullo F, Bittolo BG. Increased levels of soluble P-selectin in hypercholesterolemic patients. *Circulation*. 1998;97(10):953-957. doi:10.1161/01.CIR.97.10.953.
62. Blann AD, Dobrotova M, Kubisz P, N MC. von Willebrand factor, soluble P-selectin, tissue plasminogen activator and plasminogen activator inhibitor in atherosclerosis. *Thromb Haemost*. 1995;74(2):626-630. http://www.ncbi.nlm.nih.gov/pubmed/8584997.
63. Zitoun D, Bara L, Basdevant A, Samama MM. Levels of Factor VIIc Associated With Decreased Tissue Factor Pathway Inhibitor and Increased Plasminogen Activator

Inhibitor-1 in Dyslipidemias. *Arterioscler Thromb Vasc Biol*. 1996;16:77-81.

64. Kawaguchi a, Miyao Y, Noguchi T, Nonogi H, Yamagishi M, Miyatake K, Kamikubo Y, Kumeda K, Tsushima M, Yamamoto a, Kato H. Intravascular free tissue factor pathway inhibitor is inversely correlated with HDL cholesterol and postheparin lipoprotein lipase but proportional to apolipoprotein A-II. *Arterioscler Thromb Vasc Biol*. 2000;20:251-258. doi:10.1161/01.ATV.20.1.251.
65. Paka L, Kako Y, Obunike JC, Pillarisetti S. Endothelial Production of Heparan Sulfate Rich in Biologically Active Heparin-like Domains. *Biochemistry*. 1999;274(8):4816-4823.
66. de Agostini a I, Watkins SC, Slayter HS, Youssoufian H, Rosenberg RD. Localization of anticoagulantly active heparan sulfate proteoglycans in vascular endothelium: antithrombin binding on cultured endothelial cells and perfused rat aorta. *J Cell Biol*. 1990;111(3):1293-1304. doi:10.1083/jcb.111.3.1293.
67. Pillarisetti S, Paka L, Sasaki A, Vanni-reyes T, Yin B, Parthasarathy N, Wagner WD, Goldberg IJ. Endothelial Cell Heparanase Modulation of Lipoprotein. *Biochemistry*. 1997;272(25):15753-15759.
68. Pillarisetti S, Paka L, Obunike JC, Berglund L, Goldberg IJ. Subendothelial retention of lipoprotein (a). Evidence that reduced heparan sulfate promotes lipoprotein binding to subendothelial matrix. *J Clin Invest*. 1997;100(4):867-874. doi:10.1172/JCI119602.
69. Durand E, Scoazec a., Lafont a., Boddaert J, Al Hajzen a., Addad F, Mirshahi M, Desnos M, Tedgui a., Mallat Z. In vivo induction of endothelial apoptosis leads to vessel thrombosis and endothelial denudation: A clue to the understanding of the mechanisms of thrombotic plaque erosion. *Circulation*. 2004;109(21):2503-2506. doi:10.1161/01.CIR.0000130172.62481.90.
70. Bombeli T, Schwartz BR, Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood*. 1999;93(11):3831-3838.
71. Bombeli T, Karsan a, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. *Blood*. 1997;89(7):2429-2442. doi:10.1016/S0887-7963(97)80117-4.
72. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996;93:1354–1363.
73. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui a. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*. 2000;101(8):841-843. doi:10.1161/01.CIR.101.8.841.
74. Martínez MC, Tesse A, Zobairi F, Andriantsitohaina R. Shed membrane microparticles from circulating and vascular cells in regulating vascular function. *Am J Physiol Heart Circ Physiol*. 2005:1004-1009. doi:10.1152/ajpheart.00842.2004.
75. Li D, Yang B, Mehta JL. Ox-LDL induces apoptosis in human coronary artery endothelial cells: role of PKC, PTK, bcl-2, and Fas. *Am J Physiol*. 1998;275(2 Pt 2):H568-H576.
76. Sugano M, Tsuchida K, Makino N. High-Density Lipoproteins Protect Endothelial Cells from Tumor Necrosis Factor-α-Induced Apoptosis. *Biochem Biophys Res Commun*. 2000;272(3):872-876. doi:10.1006/bbrc.2000.2877.
77. Nofer J-R, Levkau B, Wolinska I, Junker R, Fobker M, von Eckardstein a., Seedorf U, Assmann G. Suppression of Endothelial Cell Apoptosis by High Density Lipoproteins (HDL) and HDL-associated Lysosphingolipids. *J Biol Chem*. 2001;276(37):34480-34485. doi:10.1074/jbc.M103782200.

78. Davì G, Patrono C. Mechanisms of disease: Platelet activation and atherothrombosis. *N Engl J Med*. 2007;357(24):2482-2494. doi:10.1056/NEJMra071014.
79. Monaco C, Mathur A, Martin JF. What causes acute coronary syndromes? Applying Koch's postulates. *Atherosclerosis*. 2005;179(1):1-15. doi:10.1016/j.atherosclerosis.2004.10.022.
80. Bonaca MP, Steg PG, Feldman LJ, Canales JF, Ferguson JJ, Wallentin L, Califf RM, Harrington R a., Giugliano RP. Antithrombotics in Acute Coronary Syndromes. *J Am Coll Cardiol*. 2009;54(11):969-984. doi:10.1016/j.jacc.2009.03.083.
81. Martin JF, Kristensen SD, Mathur A, Grove EL, Choudry FA. The causal role of megakaryocyte-platelet hyperactivity in acute coronary syndromes. *Nat Rev Cardiol*. 2012;9(11):658-670. http://dx.doi.org/10.1038/nrcardio.2012.131.
82. Martin JF, Slater DN, Kishk YT, Trowbridge E a. Platelet and megakaryocyte changes in cholesterol-induced experimental atherosclerosis. *Arteriosclerosis*. 2015;5(6):604-612. doi:10.1161/01.ATV.5.6.604.
83. Schick BP, Schick PK. The effect of hypercholesterolemia on guinea pig platelets, erythrocytes and megakaryocytes. *Biochim Biophys Acta*. 1985;833(2):291-302. http://www.ncbi.nlm.nih.gov/pubmed/3970955.
84. Pathansali R, Smith N, Bath P. Altered megakaryocyte–platelet haemostatic axis in hypercholesterolaemia. *Platelets*. 2001;12(5):292-297. doi:10.1080/09537100120058810.
85. Martin JF, Plumb J, Kilbey RS, Kishk YT. Changes in volume and density of platelets in myocardial infarction. *Br Med J (Clin Res Ed)*. 1983;287(6390):456-459. doi:10.1136/bmj.287.6390.456.
86. Cameron HA, Phillips R, Ibbotson RM, Carson PH. Platelet size in myocardial infarction. *Br Med J (Clin Res Ed)*. 1983;287(6390):449-451. doi:10.1136/bmj.287.6390.449.
87. Pizzulli L, Yang A, Martin JF, Lüderitz B. Changes in platelet size and count in unstable angina compared to stable angina or non-cardiac chest pain. *Eur Heart J*. 1998;19(1):80-84. doi:10.1053/euhj.1997.0747.
88. Endler G, Klimesch A, Sunder-Plassmann H, Schillinger M, Exner M, Mannhalter C, Jordanova N, Christ G, Thalhammer R, Huber K, Sunder-Plassmann R. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. *Br J Haematol*. 2002;117(2):399-404.
89. Martin J, Bath P, Burr ML. Influence of Platelet Size on Outcome after Myocardial Infarction. *Lancet*. 1991;338(8780):1409-1411. doi:0140-6736(91)92719-I [pii].
90. Shattil SJ, Anaya-Galindo R, Bennett J, Colman RW, Cooper RA. Platelet hypersensitivity induced by cholesterol incorporation. *J Clin Invest*. 1975;55(3):636-643. doi:10.1172/JCI107971.
91. Hochgraf E, Levy Y, Aviram M, Brook JG, Cogan U. Lovastatin decreases plasma and platelet cholesterol levels and normalizes elevated platelet fluidity and aggregation in hypercholesterolemic patients. 1994;1994/01/01(1):11-17.
92. Ravindran R, Krishnan LK. Increased Platelet Cholesterol and Decreased Percentage Volume of Platelets as a Secondary Risk Factor for Coronary Artery Disease. *Pathophysiol Haemost Thromb*. 2007;36(1):45-51. doi:10.1159/000112639.
93. Opper C, Clement C, Schwarz H, Krappe J, Steinmetz a, Schneider J, Wesemann W. Increased number of high sensitive platelets in hypercholesterolemia, cardiovascular diseases, and after incubation with cholesterol. *Atherosclerosis*. 1995;113(2):211-217. doi:002191509405448R [pii].
94. Betteridge DJ, Cooper MB, Saggerson ED, Prichard BN, Tan KC, Ling E, Barbera G, McCarthy S, Smith CC. Platelet function in

patients with hypercholesterolaemia. *Eur J Clin Invest*. 1994;24 Suppl 1:30-33.

95. Sanguigni V, Pignatelli P, Caccese D, Pulcinelli FM, Lenti L, Magnaterra R, Martini F, Lauro R, Violi F. Increased superoxide anion production by platelets in hypercholesterolemic patients. *Thromb Haemost*. 2002;87(5):796-801.
96. Pawlowska Z, Swiatkowska M, Krzeslowska J, Pawlicki L, Cierniewski CS. Increased platelet-fibrinogen interaction in patients with hypercholesterolemia and hypertriglyceridemia. *Atherosclerosis*. 1993;103(1):13-20. http://www.ncbi.nlm.nih.gov/pubmed/8280181.
97. Garlichs CD, John S, Schmeisser A, Eskafi S, Stumpf C, Karl M, Goppelt-Struebe M, Schmieder R, Daniel WG. Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia. *Circulation*. 2001;104(20):2395-2400.
98. Cooper MB, Tan KCB. Platelet transmembrane signalling responses t o collagen in familial hypercholesterolaemia. 1994:737-743.
99. Bröijersén a, Hamsten a, Eriksson M, Angelin B, Hjemdahl P. Platelet activity in vivo in hyperlipoproteinemia - Importance of combined hyperlipidemia. *Thromb Haemost*. 1998;79(2):268-275. http://www.scopus.com/inward/record.url?eid=2-s2.0-2642640424&partnerID=40&md5=fac322b92ff03a0b644134e516b7c996.
100. Davì G, Gresele P, Violi F, Basili S, Catalano M, Giammarresi C, Volpato R, Nenci GG, Ciabattoni G, Patrono C. Diabetes Mellitus, Hypercholesterolemia, and Hypertension but Not Vascular Disease Per Se Are Associated With Persistent Platelet Activation In Vivo: Evidence Derived From the Study of Peripheral Arterial Disease. *Circ*. 1997;96(1):69-75. doi:10.1161/01.CIR.96.1.69.
101. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med*. 2002;9(1):61-67. doi:10.1038/nm810.
102. Surya II, Mommersteeg M, Gorter G, Erkelens DW, Akkerman JW. Abnormal platelet functions in a patient with abetalipoproteinemia. *Thromb Haemost*. 1991;65(3):306-311. http://europepmc.org/abstract/MED/1904656.
103. Riddell DR, Graham A, Owen JS. Apolipoprotein E Inhibits Platelet Aggregation through the L-Arginine:Nitric Oxide Pathway: IMPLICATIONS FOR VASCULAR DISEASE. *J Biol Chem*. 1997;272(1):89-95. doi:10.1074/jbc.272.1.89.
104. Higashihara M, Kinoshita M, Teramoto T, Kume S, Kurokawa K. The role of apoE in inhibitory effects of apoE-rich HDL on platelet function. *FEBS Lett*. 1991;282(1):82-86. doi:http://dx.doi.org/10.1016/0014-5793(91)80449-D.
105. Korporaal SJA, Akkerman J-WN. Platelet activation by low density lipoprotein and high density lipoprotein. *Pathophysiol Haemost Thromb*. 2006;35(3-4):270-280. doi:10.1159/000093220.
106. Korporaal SJA, Relou IAM, van Eck M, Strasser V, Bezemer M, Gorter G, van Berkel TJC, Nimpf J, Akkerman J-WN, Lenting PJ. Binding of Low Density Lipoprotein to Platelet Apolipoprotein E Receptor 2′ Results in Phosphorylation of p38 MAPK. *J Biol Chem*. 2004;279(50):52526-52534. doi:10.1074/jbc.M407407200.
107. Akkerman JWN. From low-density lipoprotein to platelet activation. *Int J Biochem Cell Biol*. 2008;40(11):2374-2378. doi:10.1016/j.biocel.2008.04.002.
108. Assinger A, Schmid W, Eder S, Schmid D, Koller E, Volf I. Oxidation by hypochlorite converts protective HDL into a potent platelet agonist. *FEBS Lett*. 2008;582(5):778-784. doi:10.1016/j.febslet.2008.02.001.
109. Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, Poliakov E, Sun M, Finton PJ, Curtis BR, Chen J,

Zhang R, Silverstein RL, Hazen SL. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat Med*. 2007;13(9):1086-1095. doi:10.1038/nm1626.

110. Korporaal SJA, Van Eck M, Adelmeijer J, Ijsseldijk M, Out R, Lisman T, Lenting PJ, Van Berkel TJC, Akkerman J-WN. Platelet Activation by Oxidized Low Density Lipoprotein Is Mediated by Cd36 and Scavenger Receptor-A. *Arterioscler Thromb Vasc Biol*. 2007;27(11):2476-2483. doi:10.1161/ATVBAHA.107.150698.
111. Nofer JR, Walter M, Kehrel B, Seedorf U, Assmann G. HDL3 Activates Phospholipase D in Normal but Not in Glycoprotein IIb/IIIa-Deficient Platelets. *Biochem Biophys Res Commun*. 1995;207(1):148-154. doi:http://dx.doi.org/10.1006/bbrc.1995.1165.
112. Nazih H, Nazih-Sanderson F, Magret V, Caron B, Goudemand J, Fruchart JC, Delbart C. Protein kinase C-dependent desensitization of HDL3-activated phospholipase C in human platelets. *Arterioscler Thromb Vasc Biol*. 1994;14(8):1321-1326. doi:10.1161/01.ATV.14.8.1321.
113. Vergani CG, Plancher AC, Zuin M, Cattaneo M, Tramaloni C, Maccari S, Roma P, Catapano AL. Bile lipid composition and haemostatic variables in a case of high density lipoprotein deficiency (Tangier disease). *Eur J Clin Invest*. 1984;14(1):49-54.
114. Naqvi TZ, Shah PK, Ivey P a, Molloy MD, Thomas a M, Panicker S, Ahmed a, Cercek B, Kaul S. Evidence that high-density lipoprotein cholesterol is an independent predictor of acute platelet-dependent thrombus formation. *Am J Cardiol*. 1999;84(9):1011-1017. http://www.ncbi.nlm.nih.gov/pubmed/10569655.
115. Brodde MF, Korporaal SJA, Herminghaus G, Fobker M, Van Berkel TJC, Tietge UJF, Robenek H, Van Eck M, Kehrel BE, Nofer J-R. Native high-density lipoproteins inhibit platelet activation via scavenger receptor BI. *Atherosclerosis*. 2011;215(2):374-382. doi:10.1016/j.atherosclerosis.2010.12.026.
116. Weisgraber KH, Mahley RW. Subfractionation of human high density lipoproteins by heparin-Sepharose affinity chromatography. *J Lipid Res*. 1980;21(3):316-325.
117. Ardlie NG, Selley ML, Simons L a. Platelet activation by oxidatively modified low density lipoproteins. *Atherosclerosis*. 1989;76:117-124. doi:10.1016/0021-9150(89)90094-4.
118. Desai K, Bruckdorfer KR, Hutton R a, Owen JS. Binding of apoE-rich high density lipoprotein particles by saturable sites on human blood platelets inhibits agonist-induced platelet aggregation. *J Lipid Res*. 1989;30(6):831-840.
119. Aviram M, Brook JG. The effect of blood constituents on platelet function: role of blood cells and plasma lipoproteins. *Artery*. 1983;11(4):297-305.
120. Aviram M, Brook JG. Platelet interaction with high and low density lipoproteins. *Atherosclerosis*. 1983;46(3):259-268. doi:http://dx.doi.org/10.1016/0021-9150(83)90176-4.
121. Aviram M, Sirtori CR, Colli S, Maderna P, Morazzoni G, Tremoli E. Plasma lipoproteins affect platelet malondialdehyde and thromboxane B2 production. *Biochem Med*. 1985;34(1):29-36. doi:http://dx.doi.org/10.1016/0006-2944(85)90059-6.
122. Knorr M, Locher R, Vogt E, Vetter W, Block LH, Ferracin F, Lefkovits H, Pletscher A. Rapid activation of human platelets by low concentrations of low-density lipoprotein via phosphatidylinositol cycle. *Eur J Biochem*. 1988;172(3):753-759. doi:10.1111/j.1432-1033.1988.tb13953.x.
123. Mehta JL, Chen LY. Reversal by high-density lipoprotein of the effect of oxidized low-density lipoprotein on nitric oxide synthase protein expression in human platelets. *J Lab Clin Med*. 1996;127(3):287-295. doi:http://dx.doi.org/10.1016/S0022-2143(96)90097-9.

124. Pedreno J, Vila M, Masana L. Mechanisms for regulating platelet high density lipoprotein type3 binding sites: evidence that binding sites are downregulated by a protein kinase C-dependent mechanism. *Thromb Res*. 1999;94(1):33-44. doi:S0049-3848(98)00196-0 [pii].

125. Riddell DR, Vinogradov D V, Stannard AK, Chadwick N, Owen JS. Identification and characterization of LRP8 (apoER2) in human blood platelets. *J Lipid Res*. 1999;40:1925-1930.

126. MacFarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*. 1964;202:498-499.

127. Davie EW, Ratnoff OD. Waterfall Sequence for Intrinsic Blood Clotting. *Science*. 1964;145(3638):1310-1312. doi:10.1126/science.145.3638.1310.

128. Kawasaki T, Kambayashi J, Ariyoshi H, Sakon M, Suehisa E, Monden M. Hypercholesterolemia as a risk factor for deep-vein thrombosis. *Thromb Res*. 1997;88(1):67-73.

129. Kawasaki T, Kambayashi J, Sakon M. Hyperlipidemia: a novel etiologic factor in deep vein thrombosis. *Thromb Res*. 1995;79(2):147-151.

130. Bradley WA, Gianturco SH. Vitamin K-dependent proteins bind to very low-density lipoproteins. *Semin Thromb Hemost*. 1988;14(3):253-257. doi:10.1055/s-2007-1002786.

131. de Sousa JC, Soria C, Ayrault-Jarrier M, Pastier D, Bruckert E, Amiral J, Bereziat G, Caen JP. Association between coagulation factors VII and X with triglyceride rich lipoproteins. *J Clin Pathol*. 1988;41(9):940-944.

132. Silveira A, Karpe F, Blomback M, Steiner G, Walldius G, Hamsten A. Activation of coagulation factor VII during alimentary lipemia. *Arterioscler Thromb a J Vasc Biol / Am Hear Assoc*. 1994;14(1):60-69.

133. Moyer MP, Tracy RP, Tracy PB, van't Veer C, Sparks CE, Mann KG. Plasma lipoproteins support prothrombinase and other procoagulant enzymatic complexes. *Arterioscler Thromb Vasc Biol*. 1998;18(3):458-465.

134. Rota S, McWilliam NA, Baglin TP, Byrne CD. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. *Blood*. 1998;91(2):508-515.

135. Xu N, Ohlin AK, Zhou L, Nilsson A. Binding of prothrombin to chyle chylomicrons: effects of temperature and calcium ions, and role of surface phospholipids. *Thromb Res*. 1995;80(1):35-46.

136. Xu N, Dahlback B, Ohlin AK, Nilsson A. Association of vitamin K-dependent coagulation proteins and C4b binding protein with triglyceride-rich lipoproteins of human plasma. *Arterioscler Thromb Vasc Biol*. 1998;18(1):33-39.

137. MacCallum PK, Cooper JA, Martin J, Howarth DJ, Meade TW, Miller GJ. Haemostatic and lipid determinants of prothrombin fragment F1.2 and D-dimer in plasma. *Thromb Haemost*. 2000;83(3):421-426.

138. Mackman N, Tilley RE, Key NS. Role of the Extrinsic Pathway of Blood Coagulation in Hemostasis and Thrombosis. *Arterioscler Thromb Vasc Biol*. 2007;27(8):1687-1693. doi:10.1161/ATVBAHA.107.141911.

139. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A*. 1989;86(8):2839-2843.

140. Drake TA, Hannani K, Fei HH, Lavi S, Berliner JA. Minimally oxidized low-density lipoprotein induces tissue factor expression in cultured human endothelial cells. *Am J Pathol*. 1991;138(3):601-607.

141. Carson SD. Plasma high density lipoproteins inhibit the activation of coagulation factor X by factor VIIa and tissue factor. *FEBS Lett*. 1981;132(1):37-40. doi:http://dx.doi.org/10.1016/0014-5793(81)80422-X.

142. Carson SD, Ross SE. Effects of lipid-binding proteins apo A-I, apo A-IL, beta 2-glycoprotein I, and C-reactive protein on activation of factor X by tissue factor--factor VIIa. *Thromb Res*. 1988;50(5):669-678.
143. Lowe GD, Drummond MM, Third JL, Bremner WF, Forbes CD, Prentice CR, Lawrie TD. Increased plasma fibrinogen and platelet-aggregates in type II hyperlipoproteinaemia. *Thromb Haemost*. 1980;42(5):1503-1507.
144. DiMinno G, Silver MJ, Cerbone AM, Rainone A, Postiglione A, Mancini M. Increased fibrinogen binding to platelets from patients with familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 1986;6(2):203-211. doi:10.1161/01.ATV.6.2.203.
145. Cremer P, Nagel D, Labrot B, Mann H, Muche R, Elster H, Seidel D. Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Göttingen Risk Incidence and Prevalence Study (GRIPS). *Eur J Clin Invest*. 1994;24(7):444-453. doi:10.1111/j.1365-2362.1994.tb02373.x.
146. Koenig W, Sund M, Ernst E, Mraz W, Hombach V, Keil U. Association between rheology and components of lipoproteins in human blood. Results from the MONICA project. *Circ*. 1992;85(6):2197-2204. doi:10.1161/01.CIR.85.6.2197.
147. Halle M, Berg A, Keul J, Baumstark MW. Association Between Serum Fibrinogen Concentrations and HDL and LDL Subfraction Phenotypes in Healthy Men. *Arterioscler Thromb Vasc Biol*. 1996;16(1):144-148. doi:10.1161/01.ATV.16.1.144.
148. Møller L, Kristensen TS. Plasma fibrinogen and ischemic heart disease risk factors. *Arterioscler Thromb Vasc Biol*. 1991;11(2):344-350. doi:10.1161/01.ATV.11.2.344.
149. Griffin JH, Kojima K, Banka CL, Curtiss LK, Fernandez JA. High-density lipoprotein enhancement of anticoagulant activities of plasma protein S and activated protein C. *J Clin Invest*. 1999;103(2):219-227. doi:10.1172/JCI5006.
150. Griffin JH, Fernández JA, Deguchi H. Plasma Lipoproteins, Hemostasis and Thrombosis. *Thromb Haemost*. 2001;86(7):386-394. http://www.schattauer.de/t3page/1214.html?manuscript=2840&L=1.
151. Oslakovic C, Norstrøm E, Dahlbäck B. Reevaluation of the role of HDL in the anticoagulant activated protein C system in humans. *J Clin Invest*. 2010;120(5):1396-1399. doi:10.1172/JCI42260.
152. Fernandez JA, Deguchi H, Banka CL, Witztum JL, Griffin JH. Re-Evaluation of the Anticoagulant Properties of High-Density Lipoprotein—Brief Report. *Arterioscler Thromb Vasc Biol*. 2015;35(3):570-572. doi:10.1161/ATVBAHA.114.304938.
153. Rosenson RS, Lowe GDO. Effects of lipids and lipoproteins on thrombosis and rheology. *Atherosclerosis*. 1998;140(2):271-280. doi:http://dx.doi.org/10.1016/S0021-9150(98)00144-0.
154. Szczeklik A, Musial J, Undas A, Gajewski P, Gora P, Swadzba J, Jankowski M. Inhibition of thrombin generation by simvastatin and lack of additive effects of aspirin in patients with marked hypercholesterolemia. *J Am Coll Cardiol*. 1999;33(5):1286-1293.
155. Stroes E. Statins and LDL-cholesterol lowering: an overview. *Curr Med Res Opin*. 2005;21(sup6):S9-S16. doi:10.1185/030079905X59102.
156. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A*. 2003;100(3):928-933. doi:10.1073/pnas.0335507100.
157. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein E a. Efficacy and

Safety of Evolocumab in Reducing Lipids and Cardiovascular Events. *N Engl J Med*. 2015;372(16):1500-1509. doi:10.1056/NEJMoa1500858.

158. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJP, ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372(16):1489-1499. doi:10.1056/NEJMoa1501031.
159. Shimada YJ, Cannon CP. PCSK9 (Proprotein convertase subtilisin/kexin type 9) inhibitors: past, present, and the future. *Eur Heart J*. 2015;36(36):2415-2424. doi:10.1093/eurheartj/ehv174.
160. Li S, Zhu C, Guo Y, Xu R, Zhang Y, Sun J, Li J. The Relationship between the Plasma PCSK9 Levels and Platelet. 2015:76-84.
161. Thaulow E, Erikssen J, Sandvik L, Stormorken H, Cohn PF. Blood platelet count and function are related to total and cardiovascular death in apparently healthy men. *Circulation*. 1991;84(2):613-617. doi:10.1161/01.CIR.84.2.613.
162. Duivenvoorden R, Fayad ZA. Safety of CETP inhibition. *Curr Opin Lipidol*. 2012;23(6):518-524. doi:10.1097/MOL.0b013e32835916b3.
163. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter L a., Leitersdorf E, McMurray JJV, Mundl H, Nicholls SJ, Shah PK, Tardif J-C, Wright RS. Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome. *N Engl J Med*. 2012;367(22):2089-2099. doi:10.1056/NEJMoa1206797.
164. Tardif J-C, Rheaume E, Lemieux Perreault L-P, Gregoire JC, Feroz Zada Y, Asselin G, Provost S, Barhdadi a., Rhainds D, L'Allier PL, Ibrahim R, Upmanyu R, Niesor EJ, Benghozi R, Suchankova G, Laghrissi-Thode F, Guertin M-C, Olsson a. G, Mongrain I, Schwartz GG, Dube M-P. Pharmacogenomic Determinants of the Cardiovascular Effects of Dalcetrapib. *Circ Cardiovasc Genet*. 2015;8(2):372-382. doi:10.1161/CIRCGENETICS.114.000663.
165. Niesor EJ, Benghozi R. Potential Signal Transduction Regulation by HDL of the β2-Adrenergic Receptor Pathway. Implications in Selected Pathological Situations. *Arch Med Res*. 2015;46(5):361-371. doi:10.1016/j.arcmed.2015.05.008.
166. Krause BR, Remaley AT. Reconstituted HDL for the acute treatment of acute coronary syndrome. *Curr Opin Lipidol*. 2013;24(6):480-486. doi:10.1097/MOL.0000000000000020.
167. Lerch PG, Spycher MO, Doran JE. Reconstituted high density lipoprotein (rHDL) modulates platelet activity in vitro and ex vivo. *Thromb Haemost*. 1998;80(2):316-320. doi:98080316 [pii].
168. Li D, Weng S, Yang B, Zander DS, Saldeen T, Nichols WW, Khan S, Mehta JL. Inhibition of arterial thrombus formation by ApoA1 Milano. *Arterioscler Thromb Vasc Biol*. 1999;19(2):378-383. doi:10.1161/01.ATV.19.2.378.
169. Calkin AC, Drew BG, Ono A, Duffy SJ, Gordon M V, Schoenwaelder SM, Sviridov D, Cooper ME, Kingwell BA, Jackson SP. Reconstituted High-Density Lipoprotein Attenuates Platelet Function in Individuals With Type 2 Diabetes Mellitus by Promoting Cholesterol Efflux. *Circ*. 2009;120(21):2095-2104. doi:10.1161/CIRCULATIONAHA.109.870709.
170. Pajkrt D, Lerch PG, van der Poll T, Levi M, Illi M, Doran JE, Arnet B, van den Ende A, ten Cate JW, van Deventer SJ. Differential effects of reconstituted high-density lipoprotein on coagulation, fibrinolysis and platelet activation during human endotoxemia. *Thromb Haemost*. 1997;77(2):303-307.

171. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear Receptors and Lipid Physiology: Opening the X-Files. *Sci*. 2001;294(5548):1866-1870. doi:10.1126/science.294.5548.1866.

172. Joseph SB, Castrillo A, Laffitte B a., Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med*. 2003;9(2):213-219. doi:10.1038/nm820.

173. Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, Tran J, Tippin TK, Wang X, Lusis AJ, Hsueh WA, Law RE, Collins JL, Willson TM, Tontonoz P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci U S A*. 2002;99(11):7604-7609. doi:10.1073/pnas.112059299.

174. Tangirala RK, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, Daige CL, Thomas D, Heyman RA, Mangelsdorf DJ, Wang X, Lusis AJ, Tontonoz P, Schulman IG. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci*. 2002;99(18):11896-11901. doi:10.1073/pnas.182199799.

175. Spyridon M, Moraes LA, Jones CI, Sage T, Sasikumar P, Bucci G, Gibbins JM. LXR as a novel antithrombotic target. *Blood*. 2011;117(21):5751-5761. doi:10.1182/blood-2010-09-306142.

176. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat Rev Mol Cell Biol*. 2012;13(4):213-224. doi:10.1038/nrm3312.

177. Peet DJ, Turley SD, Ma W, Janowski B a., Lobaccaro JM a, Hammer RE, Mangelsdorf DJ. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα. *Cell*. 1998;93(5):693-704. doi:10.1016/S0092-8674(00)81432-4.

178. Alberti S, Schuster G, Parini P, Feltkamp D, Diczfalusy U, Rudling M, Angelin B, Björkhem I, Pettersson S, Gustafsson J-Å. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRβ-deficient mice. *J Clin Invest*. 2001;107(5):565-573. doi:10.1172/JCI9794.

179. McGeehan GM, Lala DS, Zhao Y, Noto PB, Zhuang L, Claremon DA, Meng S, Bukhtiyarov Y, Gregg RR. Abstract 334: The LXRβ Selective Agonist, VTP-38443, Significantly Decreases Plaque Cholesterol Ester Content and Inflammation in a Murine Model of Accelerated Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2015;35(Suppl 1):A334-A334. http://atvb.ahajournals.org/content/35/Suppl_1/A334.abstract.