

**Low Density Lipoprotein and its
effect on human blood platelets**

**Ingrid A.M. Relou ^{1,3}, Christian M. Hackeng ²,
Jan-Willem N. Akkerman ^{1,3}, Ernst Malle ⁴**

¹ Laboratory for Thrombosis and Haemostasis, Department of Haematology, University Medical Center Utrecht, Utrecht, The Netherlands

² Laboratory for Clinical Chemistry, University Hospital Maastricht, Maastricht, The Netherlands

³ Institute for Biomembranes, Utrecht University, Utrecht, The Netherlands

⁴ Institute of Medical Biochemistry and Molecular Biology, Karl-Franzens University Graz, Graz, Austria

Summary

Events leading to hyperreactivity of human blood platelets are accompanied with an enhanced risk for atherosclerosis and arterial thrombosis. Lipoprotein disorders affect platelet functions, and hypersensitive platelets are observed in various stages of hyperlipidemia. Low Density Lipoprotein (LDL), a circulating complex of lipids and proteins that is increased in hypercholesterolemia, enhances platelet function and increases sensitivity of platelets to several naturally occurring agonists. LDL sensitizes platelets via binding of apoB100 to a receptor on the platelet membrane and via transfer of lipids to the platelet membrane. The receptor that mediates binding of LDL to the platelet and initiates subsequent intracellular signaling cascades has not yet been identified. Modification of native LDL generates a platelet-activating particle and this interaction might contribute to the development of the atherosclerotic plaque. Lysophosphatidic acid is formed upon mild oxidation of LDL and is responsible for subsequent platelet activation induced by the modified LDL-particle. Thus, LDL changes the functions of platelets via a broad spectrum of interactions.

Introduction

Lipoprotein-related disorders are caused by abnormalities in the synthesis or processing of plasma lipoprotein particles. Cells may bind Low Density Lipoprotein (LDL) particles, endocytose and transfer them to lysosomes where the receptor releases the particle. Subsequently, the receptor is recycled back to the cell surface. Components of LDL particles are hydrolyzed and free cholesterol is used for cell function and stability or deposited intracellularly as cholesterol ester. After sufficient cell loading with cholesterol, the rate of LDL-uptake becomes downregulated which is apparent as a lower number of cell surface receptors. This process is mediated by receptors that belong to the family of the LDL-receptors. The LDL-receptor family consists of a growing number of members that share structural similarity and function in endocytosis ¹.

K_D (mol/L)	B_{max} molecules/platelet	Reference
$1.6 \pm 0.5 \times 10^{-8}$	1470 ± 640	20
$4.0 \pm 1.8 \times 10^{-8}$	7075 ± 4800	21
2.0×10^{-8}	1965 ± 177	19
$5.0 \pm 0.9 \times 10^{-8}$	1348 ± 126	17
$6.0 \pm 2.1 \times 10^{-8}$	2940 ± 860	18

Table 1. Binding properties of native LDL to human blood platelets

The classical LDL-receptor detected on fibroblasts is also termed the apolipoprotein (apo) B/E receptor. Defects in receptor-ligand interaction correlate with high plasma LDL concentrations and an increased risk for cardiovascular disease ². An example of a defect in the LDL pathway is the R3500Q mutation in the apoB100 protein, which impairs the binding of LDL to the receptor in patients with familial defective apoB100 ³. An example of a defect in LDL-processing is a mutation in the *LDL-receptor* gene in patients with familial hypercholesterolemia, again resulting in diminished LDL-binding.

Human blood platelets play an important role in the pathogenesis of atherosclerosis and acute coronary syndromes. The increased hypersensitivity and hyperaggregability of platelets from hypercholesterolemic patients detected in vivo and in vitro suggest that high levels of atherogenic lipoproteins, e.g. LDL, may alter platelet function ⁴⁻⁶. Indeed, LDL sensitizes platelets in vitro to a variety of stimulating agents such as ADP, collagen and thrombin ⁷⁻¹⁵.

Binding of native LDL to platelets

Binding of cholesterol-rich LDL to the membrane is the first step in the initiation of alterations in platelets that make them more responsive to activating agents⁵. The question whether this step involves a specific receptor or merely includes perturbations of the membrane bilayer is still unanswered. Binding studies using ¹²⁵I-labeled LDL revealed the presence of specific and saturable binding sites on non-stimulated platelets, ranging from 1000 to 8000 sites per platelet (Table 1)¹⁶⁻²¹.

The fact that the binding capacity, i.e. K_d- and B_{max}-values for LDL to the platelets was similar to that reported for LDL-binding to other cells suggested the presence of similar binding proteins/receptors for LDL on platelets⁵. However, reports on how different lipoprotein classes interfered with each other were controversial with some reports showing inhibition of LDL-binding by anti-atherogenic High Density Lipoprotein (HDL) and vice-versa^{20,21}, while others were showing minimal interference^{8,16}. Curtiss et al²¹ reported competition between HDL and LDL but suggested the presence of two binding sites for lipoproteins; an HDL binding site that interacts poorly with LDL and an LDL binding site that reacts with or is altered by HDL binding. The capacity of HDL to inhibit LDL binding was not mediated by apoE. Koller et al²⁰ also proposed distinct binding sites for LDL and HDL as the nature of inhibition seemed not simple competition based on analysis of binding kinetics. However, after ligand blotting, they concluded that LDL and HDL bound to a single class of lipoprotein-binding proteins in the platelet membrane²². The lipoprotein-binding sites showed higher affinity for HDL than LDL and the lipoproteins interfered with binding of each other in a non-competitive manner.

LDL-binding to platelets was independent of divalent ions, as EDTA treatment did not alter LDL-binding²¹. This, however, differs from the binding characteristics of the classical LDL-receptor through which LDL is removed from the circulation via holoparticle uptake². Furthermore, an antibody directed against the ligand binding domain of the classical LDL-receptor did not alter the binding of LDL to platelets¹⁷. Taken together, these findings supported the concept that LDL-binding sites on platelets differ from the apoB/E-receptors on nucleated cells. Studies of LDL-platelet interaction after enzymatic treatment revealed a different pattern of proteolytic susceptibility between the 'platelet LDL-receptor' and the classical LDL-receptor; protease-treatment abolished LDL-binding to fibroblasts but not to platelets²³. Hence, a platelet receptor that is resistant to proteolytic digestion might be involved in LDL-binding to platelets. Pedreno et al²⁴ reported that LDL binds to a receptor which is coupled to a pertussis toxin sensitive G-protein. Ligand-binding assays demonstrated that high levels of LDL (1.5 g/L) downregulate the number of binding sites suggesting a conformational change of the receptor or even the possibility of internalization of receptors. This downregulation was reversible and time- and dose-dependent and inhibited by protein kinase C inhibitors.

Attempts to identify candidate LDL-binding proteins by ligand blotting experiments revealed that LDL binds to platelet membrane proteins with molecular masses in between 115 and 156 kDa^{19,22}. Purification and immunochemical characterization identified these proteins as glycoprotein IIb (CD41) and glycoprotein IIIa (CD61). Both are constituents of the glycoprotein IIb-IIIa complex, also known as integrin α IIb β 3, which is the receptor for fibrinogen and a few other adhesive proteins²⁵. Immunoelectron microscopy revealed colocalization of gold-labeled LDL with fibrinogen on platelets²⁶, confirming the concept that the fibrinogen receptor could also act as a possible LDL-receptor on the platelets. These observations appear in agreement with findings that LDL increases the exposure of fibrinogen binding sites on platelets^{7,26} and that platelets from familial hypercholesterolemia patients - compared with platelets from normolipidemic subjects - bind more fibrinogen²⁷.

In divergence from the concept that integrin α IIb β 3 serves as an LDL-receptor on platelets, Hackeng et al²⁸ showed that specific antibodies directed against integrin α IIb β 3 failed to inhibit LDL-binding to the platelets. Furthermore, similar binding characteristics were found with platelets from controls and patients suffering from type I and II Glanzmann's thrombasthenia that are deficient in integrin α IIb β 3. Together, these observations argue against a role for integrin α IIb β 3 as the putative LDL-receptor on the platelets^{28,29}. Also the fact that a single platelet contains about 50 000 surface-exposed copies of this integrin, which is about 10 fold more than the number of LDL-receptors identified in ligand binding studies appears in conflict with such a role. Although these studies propose that α IIb β 3 might not act as LDL-receptor on the platelets, they did demonstrate that this integrin contributes to LDL-induced platelet sensitization via ligand-induced signal generation in a process known as outside-in signaling²⁸.

Recently, Riddell et al³⁰ demonstrated that platelets and the megakaryocytic cell lines MEG-01 and HEL express LRP8, a new member of the LDL-receptor family. This receptor differs from the LRP receptor which is absent in platelets³¹. Platelet LRP8 is a variant of the previously identified LDL-receptor family member ApoER2 that is predominantly expressed in the brain. The extracellular domain of ApoER2 contains 7 - 8 ligand binding domains of which domains 4-6 are absent in platelet LRP8. LDL-receptor family members function in receptor-mediated endocytosis and signal transduction. ApoER2 is known to bind apoE, but not the major apolipoprotein of LDL, apoB100. The cytoplasmic tail of ApoER2 contains only a single NPxY motif, which is known as an endocytosis motif. However, compared to other members of the LDL-receptor family, the rate of endocytosis mediated by ApoER2 is low, suggesting that this domain plays a role in intracellular signal transduction^{30,32}.

The fact that antibodies directed against the binding domain of apoB100 for the apoB/E-receptor, the so called B-site, impaired binding of LDL to the platelets³³

supports the hypothesis that a member of the LDL-receptor family mediates binding of LDL to the platelet via specific domains in the apolipoprotein moiety. This is confirmed by the fact that the LDL-receptor binding site for apoB100 induced platelet signal transduction via the platelet-LDL binding site. This might point towards the presence of a yet unidentified member of the LDL-receptor on the outer leaflet of human platelets. Alternatively, the platelet LRP8 receptor might be capable of binding apoB100 and be responsible for LDL-induced platelet sensitization. After prolonged platelet-LDL contact the signaling pathway induced by LDL-binding to the platelet LDL-receptor becomes desensitized³³. This is in agreement with the previously reported reversible downregulation of binding sites for LDL and the observation that the LDL signaling pathway via a pertussis toxin sensitive G-protein coupled receptor becomes desensitized²⁴.

Lipid transfer between LDL and platelets

Apart from apoB100, LDL consists of a lipid portion containing free and esterified cholesterol, as well as triglycerides and phospholipids. Each of these constituents have the potential to change the composition of platelet membranes thereby altering platelet dynamics and function. The combined pools of sphingomyelin, phosphatidylcholine and phosphatidylethanolamine comprise more than two-thirds of the total phospholipid content of lipoproteins. A similar composition is found in platelets³⁴. Phospholipids play a role in signal transduction events in platelets. Cytosolic phospholipase A₂ cleaves fatty acids from phospholipids e.g. phosphatidylethanolamine and phosphatidylcholine leading to the generation of bioactive eicosanoids. Thus, for proper signal transduction, phospholipids have to be generated. This can be accomplished by resynthesis or remodeling of phospholipids at the level of the cell membrane or by incorporation of phospholipids from circulating lipoproteins. Thus a second mechanism by which LDL alters platelet functions is by changing the composition of phospholipids, ether-phospholipids in particular, which are preferential targets for dietary ω -3 polyunsaturated fatty acids³⁵⁻³⁷. Platelets trap diacyl-, alkylacyl-, and alkenylacyl-glycerophospholipids from vesicles by simple diffusion³⁸ and a similar mechanism might occur when lipoproteins act as lipid donors. Engelmann et al³⁹ have shown that LDL rapidly donates sphingomyelin, phosphatidylcholine and phosphatidylethanolamine to platelets³⁹. Platelet agonists increase the specific transfer of ethanolamine phospholipids from LDL to the platelet membrane via a mechanism that is partially prevented by inhibitors of protein kinase C⁴⁰. Furthermore, LDL directly contributes to the formation of platelet eicosanoids by supplying both phospholipid-bound or free arachidonic acid^{41,42}.

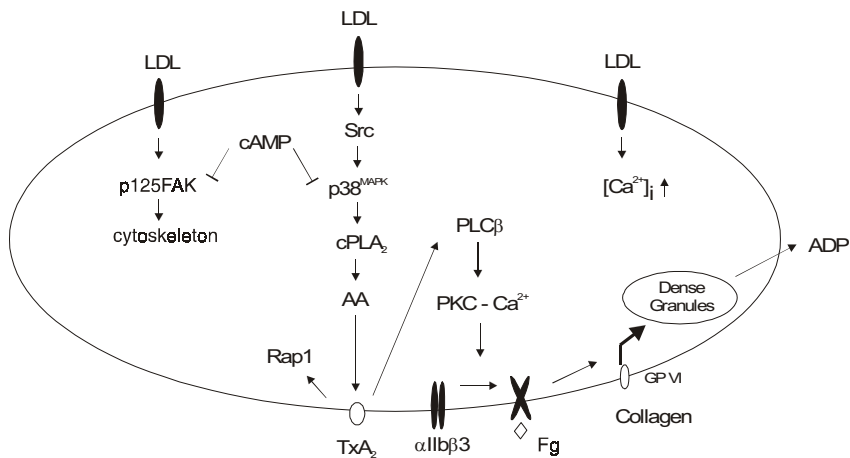


Figure 1. Signaling pathways in human platelets induced by LDL

LDL interacts with its receptor on the platelet membrane and initiates phosphorylation of Focal Adhesion Kinase (FAK). A second pathway is mediated by p38^{MAPK} which results in enhanced fibrinogen binding to integrin α IIb β 3 via phospholipase A₂ (PLA₂), arachidonic acid (AA), thromboxane A₂ (TxA₂), phospholipase C β (PLC β) and protein kinase C (PKC). In addition, LDL induces an increase in intracellular Ca²⁺. These signals lead to exposure of α IIb β 3 and fibrinogen (Fg) binding and secretion via a mechanism enhanced by outside-in signaling via α IIb β 3.

Signaling by LDL

The underlying mechanisms of enhanced platelet activation by LDL are multifactorial and inhibition of the Na⁺/H⁺ antiporter that regulates the cytosolic pH and activation of the phosphatidylinositol cycle by LDL have been addressed⁴³⁻⁴⁵. Enhanced activation of protein kinase C, phosphorylation of the protein kinase C substrate pleckstrin (47 kDa), Ca²⁺ influx and mobilization from the dense tubular system and subsequent stimulation of phospholipase A₂ by LDL have been reported^{43, 44, 46-48}.

Hackeng et al⁴⁹ reported that a first step in platelet sensitization by LDL is the release of arachidonic acid via activation of p38 mitogen activated protein kinase (p38^{MAPK}) and subsequent activation of cytosolic phospholipase A₂. P38^{MAPK} is a member of the family of proline directed serine/threonine kinases, which is activated by the simultaneous phosphorylation of Thr¹⁸⁰ and Tyr¹⁸² (Figure 1)⁵⁰. This activation was not inhibited by a wide variety of inhibitors of platelet signaling, including Ca²⁺ mobilization, phospholipase C-activation, thromboxane A₂-formation, and extracellular signal-regulated kinase 1/2-activation indicating that p38^{MAPK} is upstream of several platelet signal transduction pathways and therefore an early step in the signaling cascade initiated by LDL. Inhibition of protein kinase C slightly reduced LDL-induced p38^{MAPK} phosphorylation, suggesting a modulating role for this kinase.

The LDL-induced p38^{MAPK} phosphorylation is under control of cAMP as addition of agents that raise intracellular cAMP levels (prostaglandin I₂ and dibutyryl cAMP) prevented LDL-induced p38^{MAPK} phosphorylation. The transduction of signals generated by LDL into the cell was neither mediated by integrin $\alpha 2\beta 1$ nor by Fc γ R1a which are both known to signal towards p38^{MAPK} after receptor activation. Candidate platelet LDL-receptors such as integrin α IIb β 3, CD36 (platelet glyco-protein IV), and CD68 (gp110) were not implicated in LDL-induced p38^{MAPK}-activation either.

P125FAK is a focal adhesion kinase implicated in signaling pathways mediated by integrins, G-protein coupled receptors, tyrosine kinase receptors, and the v-Src and v-Crk oncoproteins. Phosphorylated p125FAK is the docking site for signaling proteins such as the p85 regulatory subunit of phosphoinositol 3-kinase and phospholipase C γ ^{51, 52}. LDL induced p125FAK phosphorylation in a dose- and time-dependent manner independent of integrin α IIb β 3 since platelets from patients with Glanzmann's thrombastenia showed the same phosphorylation pattern of p125FAK as did control platelets ⁵³. Similar to p38^{MAPK}, LDL-signaling to p125FAK was independent of integrin $\alpha 2\beta 1$, the Fc γ R1a receptor, and the lysophosphatidic acid (LPA) receptor and not affected by inhibitors of cyclooxygenase-1, protein kinase C, extracellular signal-regulated kinase 1/2 or p38^{MAPK}. Furthermore, precipitation of activated small GTPases revealed that LDL activated Rap1 and Ral but not Ras ⁵⁴.

In summary, the mechanism by which LDL affects platelets is likely to be based on two processes. First, there is binding and activation of an LDL receptor on the platelets that appears to differ from the classical LDL-receptor on nucleated cells. Second, there is exchange of lipids between LDL particles and the plasma membrane. Both processes appear to alter signal transduction cascades in the platelet resulting in an increased sensitivity to platelet activating agents.

Lipid lowering treatment

Drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase – the rate-limiting enzyme in endogenous cholesterol biosynthesis pathway - have proven useful in secondary prevention in patients with myocardial infarction or coronary heart disease ⁵⁵. In agreement with this observation, platelet reactivity and arachidonic acid metabolism in hyperlipoproteinemia are sensitive to lipid-lowering intervention ^{56, 57}. Apart from reducing platelet aggregation via lowering of platelet cholesterol, lovastatin, simvastatin and fluvastatin reduced platelet aggregation via a second effect of the drug binding on the platelets since the inhibition did not correlate with the change in platelet cholesterol. A third possibility is that statins alter the LDL particle thereby interfering with LDL-binding to platelets ⁵⁸⁻⁶⁰. Fluvastatin also decreased the membrane activation markers P-selectin (CD62P) and CD63 re-

flecting a reduced platelet activity in type II hypercholesterolemic patients *ex vivo*⁶¹. Schrör et al⁶² reported that reduction of total and LDL serum cholesterol by simvastatin normalized the platelet functions in patients who suffered from familial hypercholesterolemia. Platelets from untreated patients who suffered from familial hypercholesterolemia showed enhanced aggregation responses, secretion of ATP and release of thromboxane after stimulation by collagen and ADP⁶. Simvastatin treatment significantly decreased these responses to the range found in normocholesterolemic subjects^{62, 63}. After 8- and 12-weeks of intake of pravastatin, both ADP-induced platelet aggregation, thromboxane B₂ and expression of P-selectin were reduced⁶⁴.

CD40L, which is upregulated in hypercholesterolemia and expressed on activated platelets, is also downregulated by extensive pravastatin and cerivastatin treatment⁶⁵. CD40L is a member of the tumor necrosis factor family of ligands and appears to be an α IIb β 3 ligand and necessary for stability of arterial thrombi⁶⁶. Pravastatin treatment increased the activity of the Na⁺/K⁺ pump in hypercholesterolemic patients via cholesterol depletion of the platelet membrane⁶⁷. Platelet-procoagulant activity was reduced by administration of simvastatin, atorvastatin, cerivastatin and prolonged treatment with pravastatin and fluvastatin. However, there was no correlation with LDL-cholesterol changes which might indicate that there was a direct effect on the platelets⁶⁸. Duration and dose of statin administration appeared critical for the influence on platelets as short-term pravastatin therapy (4 weeks) had no effect on thromboxane B₂ production or even enhanced platelet aggregability *ex vivo* and increased plasma serotonin levels^{69, 70}. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors may prevent signaling via the Ras and Rho pathways in platelets that are dependent on isoprenylation, thereby preventing signaling via these pathways. In platelets, Ras is activated by the G-protein receptor agonist thrombin, the glycoprotein VI agonist convulxin and the cytokine receptor Mpl agonist thrombopoietin⁷¹. In many cell types Ras activation starts signaling to the p42^{MAPK} (ERK2) and further signal transduction to the nucleus but in the anucleated platelet the role of Ras is still unknown⁷². Possibly, active, GTP-bound Ras contributes to the regulation of glycoprotein IIb-IIIa (integrin α IIb β 3), which is the binding site for fibrinogen and mediates platelet aggregation.

A second small GTPase that might be affected by cholesterol lowering therapy is Rho, which signals to myosin light chain phosphatase, cytoskeletal rearrangements and platelet shape change⁷³. At present it is uncertain whether these platelet functions are disturbed when cholesterol synthesis is attenuated by administration of statins. In summary, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors lower plasma cholesterol levels. Current evidence indicates that this is not the only explanation for a reduction in platelet function and other mechanisms are likely to contribute to this inhibition.

Platelet interaction with modified LDL

Modification of LDL and subsequent uptake by scavenger receptors on monocyte-derived macrophages results in lipid-laden foam cells and is thought to initiate and accelerate the development of an arterial lesion. A characteristic of scavenger receptors is that the binding of modified LDL does not trigger downregulation of receptor expression and that uptake proceeds without downregulation until the cells transform to foam cells. The first example of a modified LDL-particle that bound to scavenger receptors on macrophages was acetylated LDL, a form of LDL that is not observed under physiological conditions. Acetylated LDL was taken up by macrophages without inducing downregulating receptor numbers, indicating that the binding was independent of the LDL receptor⁷⁴. There are several modifications of LDL such as glycation and aggregation of LDL particles that can enhance uptake by macrophages *in vitro* and *in vivo* but most studies have focused on the oxidation of LDL.

Several scavenger receptor subtypes have been identified such as scavenger receptor class A (AI and AIII) and class B (B-I and CD36). Scavenger receptors on macrophages bind acetylated LDL and different forms of oxidized LDL (ox-LDL). Platelets contain an ox-LDL receptor that does not bind acetylated LDL⁷⁵. CD36 has been identified as a scavenger receptor on platelets. It consists of two membrane-spanning domains with both the N- and C-terminal domains located at the cytosolic side⁷⁶. Monocyte-derived macrophages from CD36-deficient patients showed decreased binding and uptake of ox-LDL illustrating that CD36 functions in the binding of ox-LDL. Antibodies directed against the ox-LDL-binding domain of CD36 (155-183) reduced ox-LDL binding to platelets thereby identifying CD36 as an ox-LDL receptor on platelets⁷⁵.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) - a novel ox-LDL receptor - has also been recently identified on platelets and megakaryocytic cell lines⁷⁷. LOX-1 is a type-II membrane protein that belongs to the C-type lectin family and is translocated to the membrane upon thrombin stimulation by fusion of alpha granule membranes with the plasma membrane. Analysis of binding proteins for ox-LDL revealed that CD36 and LOX-1 served as major binding proteins in platelets. As resting platelets exposed little LOX-1, CD36 might be the ox-LDL receptor on resting platelets. In contrast, LOX-1 might be the dominant receptor involved in ox-LDL binding to activated platelets⁷⁷.

During oxidation of LDL, heterogeneous modification occurs as diverse radical-mediated chemical changes occur both in the lipid and the apoB100 moiety. Hydroperoxides, primary products of lipid peroxidation, oxysterols as well as a variety of secondary products are generated. Many of these are aldehyde-containing products e.g. malondialdehyde and hydroxynonenals that modify apoB100 side chains which may alter the binding properties of LDL and thereby the reactivity of platelets^{78, 79}.

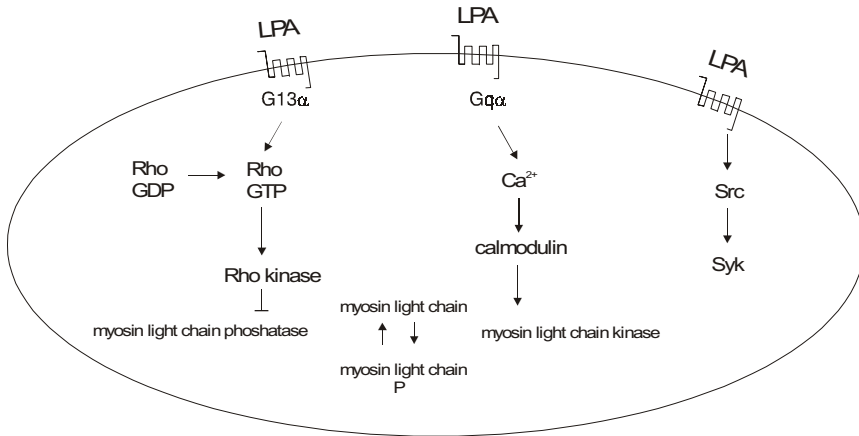


Figure 2. Signaling pathways involved in platelet shape change induced by moxLDL

The platelet-activating component of mox-LDL, lysophosphatidic acid (LPA) binds to its receptor which initiates signaling via G₁₃ and G_q signaling. Signaling via G₁₃ leads to activation of Rho kinase and inhibits myosin light chain phosphatase. Signaling via G_q leads to an increase in Ca²⁺ and together with calmodulin to activation of myosin light chain kinase. The result of both routes is phosphorylation of myosin light chain, actin-myosin interaction and cytoskeleton reorganization during shape change. The third route involves activation of Src and Syk (modified from ⁷²).

In addition, oxidation of apoB-100 leads to modification of amino acid side chains, cleavage of amide linkages and crosslinking between amino acids.

In vivo, different reactive oxygen and nitrogen species (such as superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid, peroxynitrite), lipid hydroperoxides and the products of enzymatic pathways including lipoxygenases and myeloperoxidase, contribute to the development of atherosclerosis. Oxygen free radicals that are generated by platelets may enhance or initiate platelet aggregation and secretion ^{80,81}. The involvement of lipid peroxidation in platelet signaling is illustrated by the peroxidation of arachidonate by cyclooxygenase 1 leading to endoperoxide and thromboxane formation ^{82,83}. In particular the effect of isoprostanes, eicosanoids that are non-enzymatic products of peroxidation of arachidonyl-containing phospholipids catalyzed by free radicals, have been shown to enhance platelet aggregation and secretion ^{84,85}.

In in vitro studies, copper-oxidized LDL (ox-LDL) is frequently used as an experimental tool to study the effect of oxidation of LDL on platelet functions (reviewed in ⁵)⁸⁶⁻⁹⁰. Ox-LDL inhibited the plasma membrane Ca²⁺-ATPase resulting in an increase in cytoplasmic Ca²⁺ and increased platelet sensitivity to agonists ⁸⁷. Furthermore, incubation of platelets with ox-LDL induced shape change and pseudopodia formation ⁹¹. Ox-LDL activated platelets and accelerated adhesion. This raises the possibility that oxidation of LDL contributes to thrombosis and arteriosclerosis ⁹¹.

However, in other studies ox-LDL failed to affect platelet function⁹². During oxidation of LDL, both the lipid and the protein moiety undergo chemical changes via radical-mediated reactions. During the modification chemically-active lipid and apolipoprotein degradation products are formed in ox-LDL. This extensive damage of LDL inflicted by copper-induced oxidation leads to an unreproducible pattern of platelet-ox-LDL interactions. In particular, oxidized derivatives of cholesterol generated by different modes of oxidation such as specific enzymatic oxidation, chemical oxidation, autooxidation and lipid peroxidation may act either as inhibitors or potentiators of platelet aggregation^{93, 94}. The degree of oxidation as well as consumption of antioxidants has not always been characterized by appropriate analytical techniques. Mildly oxidized/minimally modified LDL (mox-LDL) enhanced platelet aggregability and release reaction to a greater extent than heavily ox-LDL^{89, 92}. Weidtmann et al⁹² reported that activation of platelets by mox-LDL occurred through a phospholipase A₂ and cyclooxygenase-dependent pathway. Native LDL contains minimal amounts of LPA and upon mild oxidation, the LPA content increases 8-fold⁹⁵. In vivo, mild oxidation of LDL is caused by radical formation by activated macrophages and endothelial cells. LPA is present in extracellular lipid deposits and foam cells of human atherosclerotic lesions where modified lipoproteins may accumulate and is therefore considered a biologically relevant component. LPA is exposed on the surface of mildly oxidized LDL particles and interacts with LPA-receptors on the platelet membrane thereby initiating platelet activation. Platelets contain the LPA receptors LPA₁, LPA₂, LPA₃⁹⁶. LPA-receptors are seven transmembrane receptors coupled to the G-proteins G_i, which inhibits adenylate cyclase and thereby formation of cAMP, G₁₃, which signals to the small GTPase Rho and regulates platelet shape change and G_q, an activator of phospholipase C_{βIII}, which signals to Ca²⁺ mobilization and protein kinase C (reviewed in⁹⁶). Antagonists of the LPA-receptor prevented platelet activation induced by mox-LDL⁹⁵. However, LPA was not involved in sensitization of platelets induced by native LDL as the LPA-receptor blocker N-palmitoyl-L-serine-phosphoric acid failed to affect LDL-enhanced fibrinogen binding to platelets⁹⁷. LPA induced platelet activation and induced phosphatidylinositol-4-phosphate formation via phosphatidylinositol-4-kinase activation, through processes that were independent of protein kinase C and thromboxane A₂^{98, 99}. Retzer and coworkers⁷³ reported that mox-LDL induced platelet shape change via the GTPase Rho and Rho-kinase-dependent phosphorylation of myosin light chain and via moesin which are both steps required for platelet shape change (Fig. 2). Low concentration of mox-LDL did not change the intracellular Ca²⁺ concentration during shape change. This observation makes it unlikely that the calcium/calmodulin-dependent activation of myosin light chain kinase is involved during shape change. However, Maschberger et al¹⁰⁰ published that mox-LDL stimulated two additional signal transduction pathways in human platelets: the Src family kinase-mediated stimulation of protein tyrosine

phosphorylation and the Syk induced by low concentrations of mox-LDL as well as the stimulation of the Ca^{2+} influx induced by higher concentrations. Activation of platelets by mox-LDL was inhibited by LPA-receptor antagonists as well as lovastatin which could have implications in the prevention and therapy of cardiovascular disease ^{100, 101}.

Platelet aggregation was enhanced to a greater extent by LDL from diabetic patients than by LDL from healthy donors, suggesting that glycation may act as an agent that alters platelet function ¹⁰². However, both the reactivity of platelets to various aggregating agents and the production of thromboxane B_2 were similar for the various LDL-preparations, although their degree of glycosylation varied according to the concentration of glucose in the incubation media ¹⁰². Phospholipids react directly with glucose to form advanced glycosylation end products that initiate lipid oxidation ¹⁰³. In vitro glycated LDL caused a significant increase in platelet aggregation and enhancement of thromboxane B_2 synthesis, increase of intracellular calcium concentrations and inhibition of Na^+/K^+ -ATPase activity ^{102, 104, 105}. Hence, glycation induces compositional and structural changes in LDL. Glycated LDL may interact with platelets differently and may play a role in the vascular complications of diabetes. Another highly reactive oxidant generated enzymatically via the myeloperoxidase- H_2O_2 -halide system of activated phagocytes is hypochlorous acid/hypochlorite (HOCl/OCl⁻). Unlike free radical oxidants, HOCl preferentially modifies the apolipoprotein moiety of LDL. HOCl-modified LDL induced a dose-dependent increase of thrombin- and ADP-induced platelet aggregation ¹⁰⁶. Also irreversible platelet aggregation and secretion from the dense granules have been reported ^{107, 108}. HOCl-LDL binds to similar binding sites on platelets as does native LDL ¹⁰⁸. However, HOCl-LDL stimulated platelet plasma membrane Ca^{2+} -ATPase which resulted in decreased $[\text{Ca}^{2+}]_i$ ¹⁰⁹. HOCl-modified epitopes are present in vivo and HOCl-modified apoB100 has been extracted from advanced human atherosclerotic lesions ¹¹⁰. To date, it is not clear whether highly reactive chloramines and/or methionine sulfoxides or secondary radicals derived from chloramines of apoB100 contributing to HOCl-induced lipid peroxidation of LDL are responsible for effects on platelets. In addition, myeloperoxidase uses nitrite, a major endproduct of nitric oxide metabolism, as a substrate to nitrate protein tyrosine residues and lipid peroxidation ¹¹¹. These observations support the fact that in addition to reactive oxygen species, also reactive nitrogen species may alter platelet function. Peroxynitrite generated from nitric oxide radicals and superoxide anion radicals is present in human atherosclerotic lesions (as indicated by the presence of nitrotyrosine) and nitration was observed in early subintimal fatty streaks ¹¹². Peroxynitrite rapidly induces tyrosine nitration of platelet membrane proteins and may even prevent phosphorylation of signaling proteins involved in platelet activation apparently by cGMP-independent pathways leading to decreased platelet aggregability ¹¹³⁻¹¹⁵.

In conclusion, native LDL sensitizes platelets via a receptor-mediated signaling cascade and via lipid exchange. The result is an increase in sensitivity to platelet activating agents and enhanced aggregation and secretion responses. Modification of native LDL makes LDL an independent platelet activator. Oxidation leads to formation of LPA which activates platelets via the LPA-receptor. Modification of lipoproteins resulting in HOCl-LDL and glycated generates platelet-sensitizing particles. These mechanisms make LDL a potentially prothrombotic factor and explain the increased risk for atherosclerosis in patients with hypercholesterolemia.

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References

1. Howell BW, Herz J. The LDL receptor gene family: signaling functions during development. *Curr Opin Neurobiol.* 2001; 11: 74-81
2. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 1986; 232: 34-47
3. Hansen PS. Familial defective apolipoprotein B-100. *Dan Med Bull.* 1998; 45: 370-382
4. Carvalho AC, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. *N Engl J Med.* 1974; 290: 434-438
5. Malle E, Sattler W. Platelets and the lipoproteins: Native, modified and platelet modified lipoproteins. *Platelets.* 1994; 5: 70-83
6. 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
7. van Willigen G, Gorter G, Akkerman JWN. LDLs increase the exposure of fibrinogen binding sites on platelets and secretion of dense granules. *Arterioscler Thromb.* 1994; 14: 41-46
8. Aviram M, Brook JG. Platelet activation by plasma lipoproteins. *Prog Cardiovasc Dis.* 1987; 30: 61-72
9. Bruckdorfer KR. The effects of plasma lipoproteins on platelet responsiveness and on platelet and vascular prostanoid synthesis. *Prostaglandins Leukot Essent Fatty Acids.* 1989; 38: 247-254
10. Hassall DG, Forrest LA, Bruckdorfer KR, Marenah CB, Turner P, Cortese C, Miller NE, Lewis B. Influence of plasma lipoproteins on platelet aggregation in a normal male population. *Arteriosclerosis.* 1983; 3: 332-338
11. Beitz J, Panse M, Forster W. Low density lipoprotein (LDL) from male volunteers stimulated the thromboxane formation by human platelets. *Prostaglandins Leukot Med.* 1983; 10: 443-448
12. Colli S, Maderna P, Tremoli E, Baraldi A, Rovati GE, Gianfranceschi G, Nicosia S. Prostacyclin-lipoprotein interactions. Studies on human platelet aggregation and adenylate cyclase. *Biochem Pharmacol.* 1985; 34: 2451-2457
13. Beitz J, Mest HJ. Thromboxane A2 (TXA2) formation by washed human platelets under the influence of low and high density lipoproteins from healthy donors. *Prostaglandins Leukot Med.* 1986; 23: 303-309
14. Beitz J, Block HU, Beitz A, Muller G, Winkler L, Dargel R, Mest HJ. Endogenous lipoproteins modify the thromboxane formation capacity of platelets. *Atherosclerosis.* 1986; 60: 95-99
15. Shmulewitz A, Brook JG, Aviram M. Native and modified low-density-lipoprotein interaction with human platelets in normal and homozygous familial-hypercholesterolaemic subjects. *Biochem J.* 1984; 224: 13-20
16. Aviram M, Brook JG. Platelet interaction with high and low density lipoproteins. *Atherosclerosis.* 1983; 46: 259-268
17. Pedreno J, de Castellarnau C, Cullare C, Sanchez J, Gomez Gerique J, Ordonez Llanos J, Gonzalez Sastre F. LDL binding sites on platelets differ from the "classical" receptor of nucleated cells. *Arterioscler Thromb.* 1992; 12: 1353-1362
18. Malle E, Ibovnik A, Steinmetz A, Kostner GM, Sattler W. Identification of glycoprotein IIb as the lipoprotein(a)-binding protein on platelets. Lipoprotein(a) binding is independent of an arginyl-glycyl-aspartate tripeptide located in apolipoprotein(a). *Arterioscler Thromb.* 1994; 14: 345-352
19. Hassall DG, Desai K, Owen JS, Bruckdorfer KR. Detection of a protein in human platelet membranes which binds low-density lipoproteins. *Platelets* 1990; 1:29-35. *Platelets.* 1990; 1: 29-35
20. Koller E, Koller F, Doleschel W. Specific binding sites on human blood platelets for plasma lipoproteins. *Hoppe Seylers Z Physiol Chem.* 1982; 363: 395-405

21. Curtiss LK, Plow EF. Interaction of plasma lipoproteins with human platelets. *Blood*. 1984; 64: 365-374
22. Koller E. Lipoprotein-binding proteins in the human platelet plasma membrane. *FEBS Lett*. 1986; 200: 97-102
23. Pedreno J, Fernandez R. Proteolytic susceptibility of platelet low density lipoprotein receptor. *Lipids*. 1995; 30: 927-933
24. Pedreno J, Hurt-Camejo E, Wiklund O, Badimon L, Masana L. Low-density lipoprotein (LDL) binds to a G-protein coupled receptor in human platelets. Evidence that the proaggregatory effect induced by LDL is modulated by down-regulation of binding sites and desensitization of its mediated signaling. *Atherosclerosis*. 2001; 155: 99-112
25. Koller E, Koller F, Binder BR. Purification and identification of the lipoprotein-binding proteins from human blood platelet membrane. *J Biol Chem*. 1989; 264: 12412-12418
26. Volf I, Koller E, Bielek E, Koller F. Colocalization of gold-labeled LDL and fibrinogen on platelets: enhanced fibrinogen binding induced by LDL. *Am J Physiol*. 1997; 273: C118-C129
27. DiMinno G, Silver MJ, Cerbone AM, Rainone A, Postiglione A, Mancini M. Increased fibrinogen binding to platelets from patients with familial hypercholesterolemia. *Arteriosclerosis*. 1986; 6: 203-211
28. Hackeng CM, Huigsloot M, Pladet MW, Nieuwenhuis HK, Rijn HJMv, Akkerman JWN. Low-density lipoprotein enhances platelet secretion via integrin- α IIb β 3-mediated signaling. *Arterioscler Thromb Vasc Biol*. 1999; 19: 239-247
29. Pedreno J, Fernandez R, Cullare C, Barcelo A, Elorza MA, de Castellarnau C. Platelet integrin α IIb β 3 (GPIIb-IIIa) is not implicated in the binding of LDL to intact resting platelets. *Arterioscler Thromb Vasc Biol*. 1997; 17: 156-163
30. Riddell DR, Vinogradov DV, Stannard AK, Chadwick N, Owen JS. Identification and characterization of LRP8 (apoER2) in human blood platelets. *J Lipid Res*. 1999; 40: 1925-1930
31. Riddell DR, Siripurapu V, Vinogradov DV, Gliemann J, Owen JS. Blood platelets do not contain the low-density receptor-related protein (LRP). *Biochem Soc Trans*. 1998; 26: S244
32. Li Y, Lu W, Marzolo MP, Bu G. Differential functions of members of the low density lipoprotein receptor family suggested by their distinct endocytosis rates. *J Biol Chem*. 2001; 276: 18000-18006
33. Relou IAM, Gorter G, van Rijn HJ, Akkerman JW. Platelet activation by the apoB/E receptor-binding domain of LDL. *Thromb Haemost*. 2002; 87: 880-887
34. Broekman MJ, Handin RI, Derksen A, Cohen P. Distribution of phospholipids, fatty acids, and platelet factor 3 activity among subcellular fractions of human platelets. *Blood*. 1976; 47: 963-971
35. Turini ME, Holub BJ. Eicosanoid/thromboxane A₂-independent and -dependent generation of lysoplasmenylethanolamine via phospholipase A₂ in collagen-stimulated human platelets. *Biochem J*. 1993; 289: 641-646
36. Aukema HM, Holub BJ. Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanolamine phospholipid in human platelets. *J Lipid Res*. 1989; 30: 59-64
37. Malle E, Kostner GM. Effects of fish oils on lipid variables and platelet function indices. *Prostaglandins Leukot Essent Fatty Acids*. 1993; 49: 645-663
38. Malle E, Schwengerer E, Paltauf F, Hermetter A. Transfer of pyrene-labelled diacyl-, alkylacyl-, and alkenylacyl-glycerophospholipids from vesicles to human blood platelets. *Biochim Biophys Acta*. 1994; 1189: 61-64
39. Engelmann B, Kogl C, Kulschar R, Schaipp B. Transfer of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin from low- and high-density lipoprotein to human platelets. *Biochem J*. 1996; 315: 781-789
40. Engelmann B, Schaipp B, Dobner P, Stoeckelhuber M, Kogl C, Siess W, Hermetter A. Platelet agonists enhance the import of phosphatidylethanolamine into human platelets. *J Biol Chem*. 1998; 273: 27800-27808

41. Dobner P, Engelmann B. Low-density lipoproteins supply phospholipid-bound arachidonic acid for platelet eicosanoid production. *Am J Physiol.* 1998; 275: E777-E784
42. Surya II, Gorter G, Akkerman JWN. Arachidonate transfer between platelets and lipoproteins. *Thromb Haemost.* 1992; 68: 719-726
43. Nofer JR, Tepel M, Kehrel B, Wierwille S, Walter M, Seedorf U, Zidek W, Assmann G. Low-density lipoproteins inhibit the Na⁺/H⁺ antiport in human platelets. A novel mechanism enhancing platelet activity in hypercholesterolemia. *Circulation.* 1997; 95: 1370-1377
44. 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: 753-759
45. Block LH, Knorr M, Vogt E, Locher R, Vetter W, Groscurth P, Qiao BY, Pometta D, James R, Regenass M, Pletscher A. Low density lipoprotein causes general cellular activation with increased phosphatidylinositol turnover and lipoprotein catabolism. *Proc Natl Acad Sci U S A.* 1988; 85: 885-889
46. Dunn RC, Schachter M, Miles CM, Feher MD, Tranter PR, Bruckdorfer KR, Sever PS. Low-density lipoproteins increase intracellular calcium in aequorin-loaded platelets. *FEBS Lett.* 1988; 238: 357-360
47. Andrews HE, Aitken JW, Hassall DG, Skinner VO, Bruckdorfer KR. Intracellular mechanisms in the activation of human platelets by low-density lipoproteins. *Biochem J.* 1987; 242: 559-564
48. Fetkovska N. Platelet activation by low-density lipoprotein and serotonin: effects of calcium antagonists. *J Cardiovasc Pharmacol.* 1992; 19 Suppl 3: S25-8
49. Hackeng CM, Relou IA, Pladet MW, Gorter G, van Rijn HJ, Akkerman JW. Early platelet activation by low density lipoprotein via p38MAP kinase. *Thromb Haemost.* 1999; 82: 1749-1756
50. Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med.* 1996; 74: 589-607
51. Zhang X, Chattopadhyay A, Ji QS, Owen JD, Ruest PJ, Carpenter G, Hanks SK. Focal adhesion kinase promotes phospholipase C-gamma1 activity. *Proc Natl Acad Sci U S A.* 1999; 96: 9021-9026
52. Chen HC, guan JL. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A.* 1994; 91: 10148-10152
53. Hackeng CM, Pladet MW, Akkerman JW, van Rijn HJ. Low density lipoprotein phosphorylates the focal adhesion-associated kinase p125(FAK) in human platelets independent of integrin alphaIIb beta3. *J Biol Chem.* 1999; 274: 384-388
54. Hackeng CM, Franke B, Relou IA, Gorter G, Bos JL, van Rijn HJ, Akkerman JW. Low-density lipoprotein activates the small GTPases Rap1 and Ral in human platelets. *Biochem J.* 2000; 349: 231-238
55. Teo KK, Burton JR. Who Should Receive HMG CoA Reductase Inhibitors? *Drugs.* 2002; 62: 1707-1715
56. Schror K. Platelet reactivity and arachidonic acid metabolism in type II hyperlipoproteinaemia and its modification by cholesterol-lowering agents. *Eicosanoids.* 1990; 3: 67-73
57. Milionis HJ, Elisaf MS, Mikhailidis DP. Platelet function and lipid-lowering interventions. *Platelets.* 1999; 10: 357-367
58. Osamah H, Mira R, Sorina S, Shlomo K, Michael A. Reduced platelet aggregation after fluvastatin therapy is associated with altered platelet lipid composition and drug binding to the platelets. *Br J Clin Pharmacol.* 1997; 44: 77-83
59. Tsakiris DA, Keller U, Zulewski H, Miserez AR, Wolf F, Marbet GA. Simvastatin reduces activation of normal platelets by LDL isolated from patients with familial hypercholesterolaemia and familial defective apolipoprotein B. *Eur J Clin Pharmacol.* 1997; 53: 277-279
60. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S. Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: antiatherogenic effects of HMG-CoA reductase inhibitor therapy. *J Cardiovasc Pharmacol.* 1998; 31: 39-45

61. Huhle G, Abletshauer C, Mayer N, Weidinger G, Harenberg J, Heene DL. Reduction of platelet activity markers in type II hypercholesterolemic patients by a HMG-CoA-reductase inhibitor. *Thromb Res.* 1999; 95: 229-234
62. Schror K, Lobel P, Steinhagen-Thiessen E. Simvastatin reduces platelet thromboxane formation and restores normal platelet sensitivity against prostacyclin in type IIa hypercholesterolemia. *Eicosanoids.* 1989; 2: 39-45
63. Notarbartolo A, Davi G, Averna M, Barbagallo CM, Ganci A, Giammarresi C, La Placa FP, Patrono C. Inhibition of thromboxane biosynthesis and platelet function by simvastatin in type IIa hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 1995; 15: 247-251
64. Ma LP, Nie DN, Hsu SX, Yin SM, Xu LZ, Nunes JV. Inhibition of platelet aggregation and expression of alpha granule membrane protein 140 and thromboxane B2 with pravastatin therapy for hypercholesterolemia. *J Assoc Acad Minor Phys.* 2002; 13: 23-26
65. Cipollone F, Mezzetti A, Porreca E, Di Febbo C, Nutini M, Fazia M, Falco A, Cuccurullo F, Davi G. Association between enhanced soluble CD40L and prothrombotic state in hypercholesterolemia: effects of statin therapy. *Circulation.* 2002; 106: 399-402
66. Andre P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR, Wagner DD. CD40L stabilizes arterial thrombi by a beta3 integrin—dependent mechanism. *Nat Med.* 2002; 8: 247-252
67. Lijnen P, Echevaria-Vazquez D, Petrov V. Influence of cholesterol-lowering on plasma membrane lipids and function. *Methods Find Exp Clin Pharmacol.* 1996; 18: 123-136
68. Puccetti L, Bruni F, Bova G, Cercignani M, Palazzuoli A, Console E, Auteri A, Pasqui AL. Effect of diet and treatment with statins on platelet-dependent thrombin generation in hypercholesterolemic subjects. *Nutr Metab Cardiovasc Dis.* 2001; 11: 378-387
69. Broijersen A, Eriksson M, Larsson PT, Beck O, Berglund L, Angelin B, Hjerdahl P. Effects of selective LDL-apheresis and pravastatin therapy on platelet function in familial hypercholesterolaemia. *Eur J Clin Invest.* 1994; 24: 488-498
70. Milani M, Cimminiello C, Lorena M, Arpaia G, Soncini M, Bonfardeci G. Effects of two different HMG-CoA reductase inhibitors on thromboxane production in type IIA hypercholesterolemia. *Biomed Pharmacother.* 1996; 50: 269-274
71. Tulasne D, Bori T, Watson SP. Regulation of RAS in human platelets. Evidence that activation of RAS is not sufficient to lead to ERK1-2 phosphorylation. *Eur J Biochem.* 2002; 269: 1511-1517
72. Akkerman JW: Platelet signalling: GTP-binding proteins, in Gresele P, Page CP, Fuster V, Vermynen J (eds): *Platelets in thrombotic and non-thrombotic disorders.* ed1. Cambridge University Press, 2002, pp 204-220
73. Retzer M, Siess W, Essler M. Mildly oxidised low density lipoprotein induces platelet shape change via Rho-kinase-dependent phosphorylation of myosin light chain and moesin. *FEBS Lett.* 2000; 466: 70-74
74. Brown MS, Goldstein JL. Atherosclerosis. Scavenging for receptors. *Nature.* 1990; 343: 508-509
75. Volf I, Moeslinger T, Cooper J, Schmid W, Koller E. Human platelets exclusively bind oxidized low density lipoprotein showing no specificity for acetylated low density lipoprotein. *FEBS Lett.* 1999; 449: 141-145
76. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem.* 1993; 268: 11811-11816
77. Chen M, Kakutani M, Naruko T, Ueda M, Narumiya S, Masaki T, Sawamura T. Activation-dependent surface expression of LOX-1 in human platelets. *Biochem Biophys Res Commun.* 2001; 282: 153-158
78. Katzman PL, Bose R, Henry S, McLean DL, Walker S, Fyfe C, Perry Y, Mymin D, Bolli P. Serum lipid profile determines platelet reactivity to native and modified LDL-cholesterol in humans. *Thromb Haemost.* 1994; 71: 627-632

79. Malle E, Ibovnik A, Leis HJ, Kostner GM, Verhallen PF, Sattler W. Lysine modification of LDL or lipoprotein(a) by 4-hydroxynonenal or malondialdehyde decreases platelet serotonin secretion without affecting platelet aggregability and eicosanoid formation. *Arterioscler Thromb Vasc Biol.* 1995; 15: 377-384
80. Gorog P, Kovacs IB. Lipid peroxidation by activated platelets: a possible link between thrombosis and atherogenesis. *Atherosclerosis.* 1995; 115: 121-128
81. Iuliano L, Colavita AR, Leo R, Pratico D, Violi F. Oxygen free radicals and platelet activation. *Free Radic Biol Med.* 1997; 22: 999-1006
82. Lagarde M, Lemaitre D, Calzada C, Vericel E. Involvement of lipid peroxidation in platelet signalling. *Prostaglandins Leukot Essent Fatty Acids.* 1997; 57: 489-491
83. Calzada C, Vericel E, Lagarde M. Low concentrations of lipid hydroperoxides prime human platelet aggregation specifically via cyclo-oxygenase activation. *Biochem J.* 1997; 325: 495-500
84. Leitinger N, Blazek I, Sinzinger H. The influence of isoprostanes on ADP-induced platelet aggregation and cyclic AMP-generation in human platelets. *Thromb Res.* 1997; 86: 337-342
85. Smith CCT, Betteridge DJ, Nourooz-Zadeh J. The generation of the F₂-isoprostane 8-epi-PGF₂ by human platelets on collagen stimulation. *Platelets.* 1999; 10: 253-256
86. Chen LY, Mehta P, Mehta JL. Oxidized LDL decreases L-arginine uptake and nitric oxide synthase protein expression in human platelets: relevance of the effect of oxidized LDL on platelet function. *Circulation.* 1996; 93: 1740-1746
87. Zhao B, Dierichs R, Miller FN, Dean WL. Oxidized low density lipoprotein inhibits platelet plasma membrane Ca(2+)-ATPase. *Cell Calcium.* 1996; 19: 453-458
88. Naseem KM, Goodhall AH, Bruckdorfer KR. Differential effects of native and oxidatively modified low-density lipoproteins on platelet function. *Platelets.* 1997; 8: 163-173
89. Vlasova II. The effect of oxidatively modified low-density lipoproteins on platelet aggregability and membrane fluidity. *Platelets.* 2000; 11: 406-414
90. Dardik R, Varon D, Tamarin I, Zivelin A, Salomon O, Shenkman B, Savion N. Homocysteine and oxidized low density lipoprotein enhanced platelet adhesion to endothelial cells under flow conditions: distinct mechanisms of thrombogenic modulation. *Thromb Haemost.* 2000; 83: 338-344
91. Zhao B, Rickert CH, Filler TJ, Liu B, Verhallen PF, Dierichs R. Adhesion of washed blood platelets in vitro is advanced, accelerated, and enlarged by oxidized low-density lipoprotein. *Am J Hematol.* 1995; 49: 177-182
92. Weidtmann A, Scheithe R, Hrboticky N, Pietsch A, Lorenz R, Siess W. Mildly oxidized LDL induces platelet aggregation through activation of phospholipase A2. *Arterioscler Thromb Vasc Biol.* 1995; 15: 1131-1138
93. Mahfouz MM, Kummerow FA. Oxysterols and TBARS are among the LDL oxidation products which enhance thromboxane A2 synthesis by platelets. *Prostaglandins Other Lipid Mediat.* 1998; 56: 197-217
94. Blache D, Bontoux G. Biological effects of oxysterols on platelet function. *Thromb Res.* 1988; 50: 221-230
95. Siess W, Zangl KJ, Essler M, Bauer M, Brandl R, Corrinth C, Bittman R, Tigyi G, Aepfelbacher M. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc Natl Acad Sci U S A.* 1999; 96: 6931-6936
96. Siess W. Athero- and thrombogenic actions of lysophosphatidic acid and sphingosine-1-phosphate. *Biochim Biophys.* 2002; 1582: 204-215
97. Korporaal SJ, Relou IA, van Rijn HJ, Akkerman JW. Lysophosphatidic acid-independent platelet activation by low-density lipoprotein. *FEBS Lett.* 2001; 494: 121-124
98. Tori M, Tolnai Festetics E, Bertoni A, Moratti R, Balduini C, Sinigaglia F. Lysophosphatidic acid induces protein tyrosine phosphorylation in the absence of phospholipase C activation in human platelets. *Platelets.* 1997; 8: 181-187

99. Mani I, Gaudette DC, Holub BJ. Increased formation of phosphatidylinositol-4-phosphate in human platelets stimulated with lysophosphatidic acid. *Lipids*. 1996; 31: 1265-1268
100. Maschberger P, Bauer M, Baumann-Siemons J, Zangl KJ, Negrescu EV, Reininger AJ, Siess W. Mildly oxidized low density lipoprotein rapidly stimulates via activation of the lysophosphatidic acid receptor Src family and Syk tyrosine kinases and Ca²⁺ influx in human platelets. *J Biol Chem*. 2000; 275: 19159-19166
101. Essler M, Retzer M, Bauer M, Zangl KJ, Tigyi G, Siess W. Stimulation of platelets and endothelial cells by mildly oxidized LDL proceeds through activation of lysophosphatidic acid receptors and the Rho/Rho-kinase pathway. Inhibition by lovastatin. *Ann N Y Acad Sci*. 2000; 905:282-6.: 282-286
102. Watanabe J, Wohltmann HJ, Klein RL, Colwell JA, Lopes-Virella MF. Enhancement of platelet aggregation by low-density lipoproteins from IDDM patients. *Diabetes*. 1988; 37: 1652-1657
103. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci U S A*. 1993; 90: 6434-6438
104. Ferretti G, Rabini RA, Bacchetti T, Vignini A, Salvolini E, Ravaglia F, Curatola G, Mazzanti L. Glycated low density lipoproteins modify platelet properties: a compositional and functional study. *J Clin Endocrinol Metab*. 2002; 87: 2180-2184
105. Takeda H, Yano T, Kishikawa H, Miyata T, Shinohara M, Yamaguchi E, Kobori S, Fan JL, Tokunaga O, Shichiri M. Abnormalities in platelets and vascular endothelial cells induced by glycated lipoproteins. *Intern Med*. 1992; 31: 746-751
106. Opper C, Schlusler G, Sattler W, Malle E. Effects of hypochlorite-modified low density lipoproteins and high density lipoproteins on platelet function. *Platelets*. 1998; 9: 339-341
107. Volf I, Roth A, Cooper J, Moeslinger T, Koller E. Hypochlorite modified LDL are a stronger agonist for platelets than copper oxidized LDL. *FEBS Lett*. 2000; 483: 155-159
108. Volf I, Bielek E, Moeslinger T, Koller F, Koller E. Modification of protein moiety of human low density lipoprotein by hypochlorite generates strong platelet agonist. *Arterioscler Thromb Vasc Biol*. 2000; 20: 2011-2018
109. Zabe M, Feltzer RE, Malle E, Sattler W, Dean WL. Effects of hypochlorite-modified low-density and high-density lipoproteins on intracellular Ca²⁺ and plasma membrane Ca(2+)-ATPase activity of human platelets. *Cell Calcium*. 1999; 26: 281-287
110. Hazell LJ, Arnold L, Flowers D, Waeg G, Malle E, Stocker R. Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J Clin Invest*. 1996; 97: 1535-1544
111. Podrez EA, Abu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic Biol Med*. 2000; 28: 1717-1725
112. Beckmann JS, Ye YZ, Anderson PG, Chen J, Accavitti MA, Tarpey MM, White CR. Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. *Biol Chem Hoppe Seyler*. 1994; 375: 81-88
113. Mondoro TH, Shafer BC, Vostal JG. Peroxynitrite-induced tyrosine nitration and phosphorylation in human platelets. *Free Radic Biol Med*. 1997; 22: 1055-1063
114. Naseem KM, Low SY, Sabetkar M, Bradley NJ, Khan J, Jacobs M, Bruckdorfer KR. The nitration of platelet cytosolic proteins during agonist-induced activation of platelets. *FEBS Lett*. 2000; 473: 119-122
115. Low SY, Sabetkar M, Bruckdorfer KR, Naseem KM. The role of protein nitration in the inhibition of platelet activation by peroxynitrite. *FEBS Lett*. 2002; 511: 59-64

