# Low-density lipoprotein retention: implications for atherosclerosis development.

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## Summary

Atherosclerosis is a leading cause of death and it is distinguished by the accumulation of lipoprotein lipid within the arterial wall. An ionic interaction of positively charged regions of apolipoprotein B with matrix proteins, including proteoglycans, is thought to initiate atherosclerosis. Local biological responses to these retained lipoproteins, including the lipoprotein oxidation by reactive oxygen species and a chronic macrophage and T-cell-dominated inflammatory response, promote subsequent lesion development. Potential future therapeutic approaches include attempts to block the interaction of apolipoprotein B lipoproteins with the specific subendothelial matrix molecules that mediate retention and to interfere with accessory molecules within the arterial wall that promote retention such as lipoprotein lipase, secretory sphingomyelinase, and secretory phospholipase A2. The aim of the present work is to review the state of the art concerning to lipoprotein-proteoglycans interaction and the subsequent atherogenic development.

## Introduction

Atherosclerosis is the leading cause of death in the Western world and the incidence is increasing globally. The retention (1-3) and oxidation of low-density lipoproteins (LDL) within the arterial wall (4,5) initiates a complex series of biochemical and inflammatory/immunomodulatory reactions involving multiple cell types ultimately leading to the development of unstable atherosclerotic plaques (6,7).
In the past few years, substantial evidence has been obtained for interactions between lipoproteins and proteoglycans (PGs) in the pathogenesis of atherosclerosis (8). In vivo, apoprotein B (apoB) and chondroitin sulfate (CS) have been shown to colocalize in the injured intima of rabbits (9), and apoB/CS PGs complexes have been eluted from human atherosclerotic lesions (10).

Development and progression of atherosclerosis is dependent on the level and duration of exposure to circulating ApoB lipoproteins, with higher levels and longer duration leading to more advanced disease (11). The majority of ApoB found in blood is associated with LDL. Historically, serum concentrations of total cholesterol and LDL levels have served as good surrogates for atherosclerosis, because they have correlated consistently with atherosclerotic burden and cardiovascular events in a large number of epidemiological, preclinical, and clinical studies. Numerous randomized clinical outcomes trials studying a variety of lipid-lowering therapies have provided convincing evidence of a direct relationship between reduction of LDL and lowering of cardiovascular disease risk (reviewed in 12).

It is believed that LDL oxidation does not take place in the circulation, and must occur in the arterial wall because serum lipids of lipoproteins are well protected from oxidation by the robust antioxidant defenses and LDL itself contains most of alpha-tocopherol, as a major transport vehicle (13). LDL may be exposed on PG to cell-derived oxidants in the subendothelial space of artery and then oxidized (14). Due to the importance of the binding between LDL and arterial PGs in the present paper, we summarize the recent criteria on LDL-PGs interactions and its relationship with atherogenesis.

**Arterial proteoglycans**

PGs are macromolecules composed of a core protein and complex, linear, long-chain carbohydrates, called glycosaminoglycans (GAGs). GAGs consist of repeating disaccharide units bearing negatively charged sulfate and carboxy groups. GAGs are covalently bound to the core protein via a tetrasaccharide linkage [GlcA-Gal-Gal-Xyl]. There are distinct types of GAGs: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin, keratin sulfate, and hyaluronan. Several classes of CS and DS containing PGs are found in the artery. These include versican, a large CS containing PGs, and biglycan and decorin, 2 small leucine-rich PGs containing DS and CS/DS, respectively. HS containing PGs, the syndecans and glypicans, are associated with the cell membrane of smooth muscle cells (SMCs) and endothelial cells. Perlecan, a >500 kDa molecule that is the major HS in subendothelial matrix, is also an important component of the artery wall (15).

The PGs of the pericellular environment are anchored to the cell plasma membrane, whereas those secreted by the cells are part of the extracellular space and usually do not physically connect with cells. The PGs of the extracellular space are versican, decorin, and biglycan. The pericellular environment includes the subendothelial matrix perlecan and the cell surface proteoglycans, syndecan and glypican. Plasma lipoproteins, therefore, encounter a variety of types of PGs throughout the artery wall (16).

Vascular SMCs, endothelial cells, and macrophages are responsible for the synthesis of arterial wall PGs. Pathological states such as atherosclerosis, hypertension, and diabetes are characterized by altered patterns of PGs synthesis by these cells (17). The composition of PGs synthesized by vascular cells fluctuates with the physiological state of the cells. Numerous factors, including extracellular matrix components, transforming growth factor-1 (TGF-$\beta$1), platelet-derived growth factor (PDGF), oxidized LDL (ox-LDL), and nonesterified fatty acids modulate SMC PGs synthesis (18,19).

**Plaque Initiation and Progression**

Atherogenesis begins with the retention of atherogenic lipoproteins in the subendothelium of susceptible areas of the arterial tree as. In response to these retained lipoproteins, particularly those that undergo atherogenic modifications such as oxidation and aggregation, a series of biological and maladaptive inflammatory responses ensue: i) monocytes and other inflammatory cells enter the intima; ii) monocytes differentiate into macrophages, which then ingest retained and modified lipoproteins and become cholesteryl ester loaded foam cells; iii) macrophages and other inflammatory cells contribute to a state of inflammation that fails to properly resolve; and iiii) smooth muscle cells populate the intima, leading to collagen synthesis (20).

At this stage, the plaques are usually asymptomatic because of outward remodeling of the artery to preserve lumenal blood flow and a fibrous cap that protects the lesion from disruption (21). However, some of these plaques, unrelated to plaque size per se, may undergo necrotic breakdown, thinning of the fibrous cap, a heightened state of inflammation, and an accumulation of unesterified cholesterol (22). Many of the hallmarks of impaired inflammation resolution are evident in these plaques, including continued entry and poor egress of inflammatory cells, defective clearance of apoptotic cells, and a suppressed fibrotic “scarring” response (23). These so-called “vulnerable plaques” are at risk for plaque disruption through fibrous cap rupture or endothelial erosion, which in turn can trigger acute thrombosis. If the thrombosis is extensive and not quickly resolved, acute vascular occlusion and tissue infarction occurs, leading to acute myocardial infarction, unstable angina, sudden cardiac death, or stroke.

**Determinants for endothelium permeability to LDL**

Possible determinants of lipoprotein retention within the arterial wall include lipoprotein size (24), other lipoprotein properties (eg, electrical charge and cholesterol enrichment), and endothelial permeability (25). The influence of these determinants on lipoprotein retention and atherosclerotic disease in humans is much less certain than plasma lipoprotein concentration and the onset and duration of lipoprotein elevation. With regard to size, extremely large lipoproteins, such as >500-nm nonhydrolyzed chylomicrons, are too big to enter the arterial wall and thus do not directly promote atherogenesis (26). Although the entry of ~100-nm chylomicrons remnant lipoproteins may not be as great as that of smaller LDL particles, the fact that they deliver ~40 times more cholesterol per particle after retention can explain their atherogenicity (27).

Although so-called small, dense LDL (~20 nm) may be more atherogenic than larger LDL (~30 nm), it is probably unlikely that the mechanism arises from size-related effects on endothelial permeability. Rather, the presence of small, dense LDL is associated with increased lipoprotein binding to arterial PGs in vitro, and conversion of apoB lipoproteins into a small, dense form by treatment with phospholipase A2 in vitro increases their affinity to PGs (27).

Assessing the role of endothelial permeability in lipoprotein retention is less understood. Experiments conducted in rabbits suggest that differences in lipoprotein permeation into susceptible versus resistant segments of the arterial wall are not important in lipoprotein retention in the earliest stages of lesion initiation (28). However, other investigators have argued that lipoprotein permeation becomes an increasingly important variable as lesions progress and may be a relevant factor in human atherosclerosis (29). On the other hand, previous work has suggested that blood pressure lowering, an important

clinical intervention, reduced endothelial permeability to LDL (reviewed in 3). Another determinant of lipoprotein permeation may be endothelial cell turnover and apoptosis, (30) although no evidence whatsoever exists for frank endothelial "injury" or denudation in common forms of atherogenesis (1).

**Lipoprotein-proteoglycans binding in early atherogenesis**

The interaction between LDL and PGs could involve positively charged amino acids in apoB100. A search for multiple positive amino acid-rich regions of apoB led to the description of LDL-derived peptides that associated with heparin affinity gel. Studies by Camejo identified 8 clusters of positively charged amino acids in apoB100 (31). However, these clusters were identified in delipidated fragments of apoB100 in the presence of urea and binding studies were performed in low-ionic-strength buffers. So, these results might not illustrate the GAG-binding sites that are functional when apoB is associated with lipids or in physiological conditions.

Different types of PGs differ in their core protein and in the type, number, and sulfation of sugar groups. The most likely retention reaction involves the interaction of positively charged domains of the protein component of lipoproteins, notably apoB, with negatively charged sulfate groups on the PGs sugars (32). However, participation of lipoprotein lipids and proteoglycan core proteins also has been reported (8).

PGs that contain side chains of CS appear to play a particularly key role in lipoprotein retention, especially in early atherogenesis (32).More specifically, biglycan and, to a lesser extent, versican may be the most important of the CS-containing proteoglycans in apoB lipoprotein retention within human arteries (33). Of interest, a recent study comparing intimal proteoglycans in atherosclerosis-susceptible versus -resistant regions of the human arterial tree showed enhanced deposition of a CS-containing proteoglycan called lumican in the susceptible regions (34).

Of note, hyperlipidemic mice deficient in the CS-PGs decorin developed larger arterial lesions, whereas those overexpressing decorin exhibited smaller lesions (35). These data, which point to an overall antiatherogenic role for decorin, illustrate that different species of arterial wall PGs play different roles in atherogenesis, some of which appear unrelated to their ability to retain lipoproteins per se. For example, decorin inhibits transforming growth factor-$\beta$1, a cytokine in the arterial wall that stimulates the synthesis of versican and biglycan CS-PGs with increased LDL affinity (36). Finally, a recent study showed that partial deficiency of the proteoglycan perlecan, which is expressed in the murine arterial wall, also was associated with a decrease in early atherogenesis (37).

## Conclusions

Despite the complexity of advanced atherosclerosis, a clear root cause exists- subendothelial retention of apoB containing lipoproteins- that has been and should continue to be a major focus of interventions to combat atherothrombotic vascular disease. Other targets suggested directly by the Response-to-Retention model of atherogenesis offer promising opportunities in this regard. In particular, increasing knowledge of how atherogenic lipoproteins enter the arterial wall and are retained will likely suggest new therapeutic approaches.

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