# Subendothelial Lipoprotein Retention as the Initiating Process in Atherosclerosis: Update and Therapeutic Implications

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# Subendothelial Lipoprotein Retention as the Initiating Process in Atherosclerosis

## Update and Therapeutic Implications

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***Abstract***—The key initiating process in atherogenesis is the subendothelial retention of apolipoprotein B–containing lipoproteins. Local biological responses to these retained lipoproteins, including a chronic and maladaptive macrophage- and T-cell–dominated inflammatory response, promote subsequent lesion development. The most effective therapy against atherothrombotic cardiovascular disease to date—low density lipoprotein–lowering drugs—is based on the principle that decreasing circulating apolipoprotein B lipoproteins decreases the probability that they will enter and be retained in the subendothelium. Ongoing improvements in this area include more aggressive lowering of low-density lipoprotein and other atherogenic lipoproteins in the plasma and initiation of low-density lipoprotein–lowering therapy at an earlier age in at-risk individuals. 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 $A_2$. Although not the primary focus of this review, therapeutic strategies that target the proatherogenic responses to retained lipoproteins and that promote the removal of atherogenic components of retained lipoproteins also hold promise. The finding that certain human populations of individuals who maintain lifelong low plasma levels of apolipoprotein B lipoproteins have an ≈90% decreased risk of coronary artery disease gives hope that our further understanding of the pathogenesis of this leading killer could lead to its eradication. **(*Circulation*. 2007;116:1832-1844.)**

**Key Words:** atherosclerosis ■ cardiovascular diseases ■ extracellular matrix ■ lipoproteins ■ prevention ■ proteoglycans ■ statins

Twelve years ago, inspired by the pioneering work of others, we outlined a straightforward theory of atherogenesis to integrate the most reliable data at the time on how atherosclerotic lesions develop.[1] Called the Response-to-Retention model of atherogenesis, it emphasizes what we concluded was the root cause and necessary initiating event of atherogenesis: the subendothelial retention of apolipoprotein (apo) B–containing lipoproteins in susceptible but still prelesional areas of the arterial wall.[1,2] Biological responses to retained and subsequently modified lipoproteins, notably a chronic and maladaptive macrophage- and T-cell–dominated inflammatory response and changes in smooth muscle cell localization and phenotype, could explain virtually all of the features known to exist during the initiation and progression of atherosclerosis (Figure 1).

As will be evident below, data over the last 12 years have provided critical support for this hypothesis. For example, our understanding of the molecular basis of lipoprotein retention has expanded greatly, particular with regard to the roles of specific subendothelial chondroitin sulfate (CS) proteoglycans and accessory molecules within the arterial wall.[3–8] Of major significance, studies in genetically engineered mice have established a causal relationship between lipoprotein-matrix interactions and early atherogenesis (see section below on lipoprotein retention).[9,10] Moreover, a recent autopsy study of children and young adults who died of noncardiac causes showed a spectrum of changes in the subendothelium of susceptible areas within the right coronary artery, ranging from no lipid to very small amounts of lipoprotein-derived lipid with no inflammatory cells to larger amounts lipoprotein lipids associated with the first signs of macrophage infiltration and then finally to conversion of these macrophages into frank foam cells (Figure 2).[11] These changes take place within a common—and initially normal—structure of the human vascular wall called diffuse intimal thickening (see Figures 1 and 2) that is unfortunately rich in proretentive molecules.[12]

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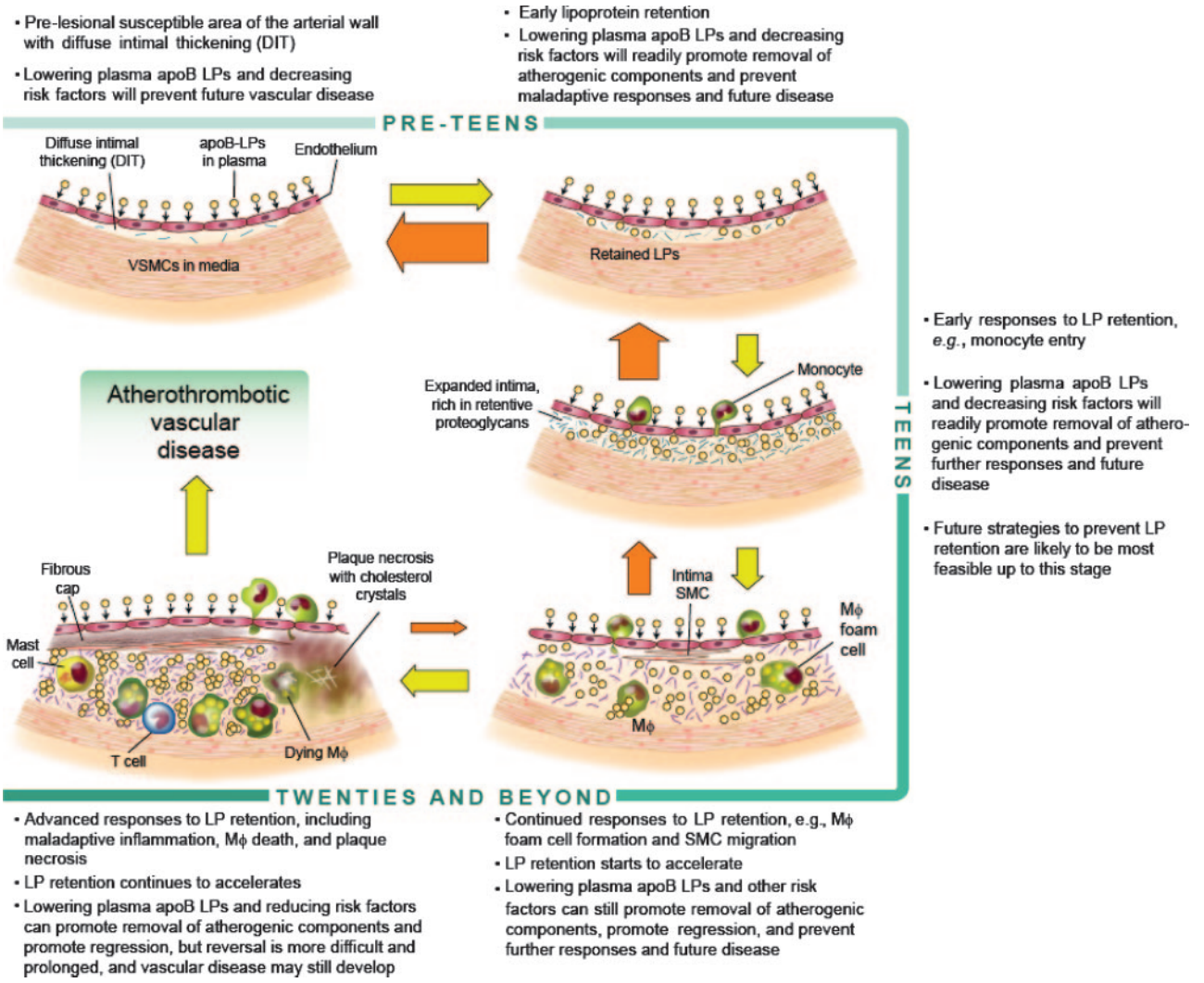


**Figure 1.** Therapeutic implications of the Response-to-Retention model of atherogenesis. As described in the text and previous reviews, atherogenesis is initiated by the focal retention of apoB lipoproteins (LPs) on subendothelial extracellular matrix molecules, particularly proteoglycans. Retention is likely facilitated by accessory molecules like LpL, S-SMase, and $sPLA_2$. These retained LPs become modified (eg, aggregated and oxidized), and elicit a series of biological responses that develop into a maladaptive inflammatory response. In particular, monocytes enter the subendothelium, differentiate into macrophages (M$\varphi$s), and ingest the retained and modified LPs to become cholesterol-laden foam cells. Eventually, T cells, mast cells, and other inflammatory cells enter the lesions and, along with macrophages, contribute to the aforementioned maladaptive inflammatory response. The process is accelerated by amplified LP retention in established lesions. Smooth muscle cells (SMCs) migrate into the intima and promote formation of a collagenous fibrous cap, probably representing a scar-like response to wall off the lesion. However, as the lesion progresses, macrophages die and eventually give rise to areas of necrosis filled with extracellular debris, cholesterol crystals, proteases, and procoagulant/thrombotic material. These advanced plaques can lead to fibrous cap thinning, plaque rupture or erosion, and acute thrombotic vascular events such as myocardial infarction and stoke. The timeline shows that the earliest stages occur in the teen years in members of industrialized societies. Green arrows indicate progression; orange arrows, the potential for regression. The earliest stages are the most easily reversible by lowering plasma apoB LPs (large orange arrows), and clinical studies have shown tremendous benefit from risk factor reduction at this stage of life. Moreover, future, complementary therapies directed at interfering with LP retention are likely to be most feasible in the earliest stages, because the later stages involve more complex mechanisms of LP retention. The complexity of advanced lesions, including accelerated LP retention, renders them less readily reversible (small orange arrows). Nonetheless, clinical trials have clearly demonstrated that risk factor reduction at this stage, particularly lowering of plasma apoB LPs, can have substantial benefit in terms of primary and secondary prevention of vascular disease. This benefit undoubtedly arises from the eventual removal of atherogenic components of retained LPs and from regression of lesional elements, including inflammatory cells.

These observations, which extend previous findings on the earliest human lesions,[13] likely represent snapshots of lipoprotein retention before and just after the initiation of local biological responses and thus provide strong support for the Response-to-Retention model in the pathogenesis of human lesions. Most important, the tremendous success of low-density lipoprotein (LDL)–lowering therapy in the prevention of cardiovascular disease in humans[14–16] represents a direct prediction of the Response-to-Retention model. Finally, the model provides an important framework for integrating the various processes that initiate and then promote atherothrombotic vascular disease, including the aforementioned chronic and maladaptive inflammatory response that has received increasing attention over the last decade.[17,18]

Many reviews on atherogenesis have appeared over the years, each emphasizing a particular aspect of the process.

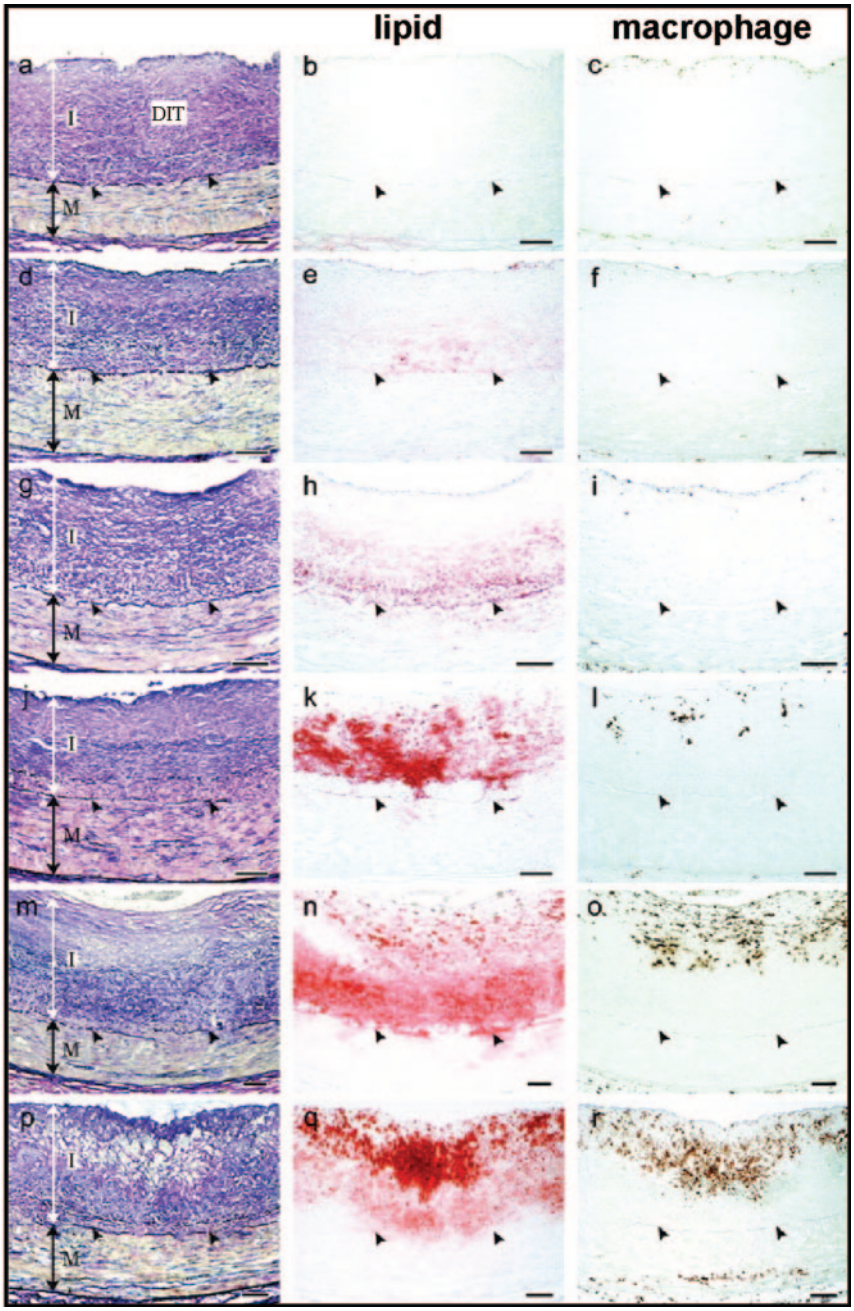


**Figure 2.** Progression of early atherosclerosis in humans. The images, from Nakashima et al,[11] show the earliest stages of atherogenesis in human right coronary artery autopsy specimens. The images portray a series of events that precisely reflect the Response-to-Retention model of atherogenesis: early diffuse intimal thickening (DIT) without lipids or macrophages (preretention in proteoglycan-rich susceptible areas), lipid accumulation preceding macrophage infiltration (retention), and increasing lipid accumulation associated with macrophage accumulation and then foam cell formation (early responses to retention). Lipids were stained with Sudan IV, and macrophages were identified by anti-CD68 immunohistochemistry. The images in the left column were stained with elastica van Gieson (EVG). I indicates intima; M, media; arrowheads, internal elastic lamina; bar, 100 $\mu$m. Reproduced with permission from the authors and publisher. Copyright © 2007, the American Heart Association.

Among these, models placing inflammation, endothelial alterations, and oxidation as the initiating and/or central process have received the widest coverage. What makes the emphasis on retained lipoproteins as the key initiating step in atherogenesis so crucial? The answer lies in the concept that understanding the root cause of a disease provides the foundation for the most effective therapy. By way of analogy, tuberculosis is a disease that has a strong inflammatory component that, like the maladaptive inflammatory response in atherosclerosis, is associated with influx and then persistence of macrophages and T cells, high levels of inflammatory cytokines, elevated plasma levels of C-reactive protein, and endothelial cell changes.[19] The treatment for this "inflammatory" disease is, of course, the elimination of the root cause—*Mycobacterium tuberculosis*—through the use of antibiotics. Likewise, the most successful therapy for atherothrombotic vascular disease in humans—lowering plasma LDL concentrations—attacks the root cause of atherogenesis, which is subendothelial apoB lipoprotein retention. Although it is theoretically possible that future therapies directed at the inflammatory, endothelial, or oxidative components of lesion progression may prove successful as adjunct strategies, such therapies have not been shown to be useful thus far and will likely never be used in the absence of drugs or other manipulations that lower plasma levels of atherogenic lipoproteins.[20]

The role of inflammation in atherosclerosis has been the most widely emphasized feature of atherogenesis over the last decade,[17,18] so a few key points in this area bear emphasis. First, a snapshot of the critical juncture between

lipoprotein retention and the earliest responses to retention supports the notion that inflammation is a consequence of apoB lipoprotein retention, not a de novo initiating factor.[18] For example, Hajra and colleagues[21] showed that although nuclear factor-κB (NF-κB) may be "primed" in susceptible regions of the arterial tree of *Ldlr*$^{-/-}$ mice, NF-κB activation occurred only in the setting of hypercholesterolemia. Similar results were found in a study examining NF-κB–induced endothelial inflammatory markers in normolipidemic versus hyperlipidemic mice.[22] Second, claims have been made attributing the success of statins to their putative role as antiinflammatory drugs.[23] However, long-term risk reduction is very similar among statin and nonstatin approaches to lowering plasma LDL concentration. Therefore, the LDL-lowering action of statins is clearly the most important mechanism by which they decrease the long-term risk of cardiovascular disease.[24,25] That antiinflammatory or other effects of statins can partially explain their ability to decrease short-term risk in the setting of acute coronary syndromes is a plausible hypothesis,[26] but it represents an entirely different concept from the prevention or reversal of atherosclerosis per se. Third, the most important aspect to understand about the local inflammatory response to retained lipoproteins is that it is deranged and maladaptive. If the reaction functioned helpfully in this circumstance, the macrophages that entered the arterial wall and consumed the retained and modified apoB lipoproteins would then simply leave.[27] Instead, they persist, secreting a variety of molecules that accelerate lipoprotein retention, plaque instability, and clotting on rupture. Strategies to convert this maladaptive response into a healthy cleanup function may be possible under certain circumstances.[27]

In the simplest construction, the Response-to-Retention model points to 3 areas of therapeutic focus: prevent the entry and subsequent subendothelial retention of apoB-containing lipoproteins, particularly at an early age; prevent or reverse the maladaptive biological responses to retained lipoproteins; and promote the removal from the arterial wall of the unusually dangerous components derived from retained and modified lipoproteins. In this review, we focus on the first area, with an emphasis on molecular mechanisms, recent advances, and therapeutic implications. In particular, we address 3 factors that influence lipoprotein retention: (1) the likelihood that plasma apoB lipoproteins will enter and then become retained within the subendothelium and trigger atherogenic biological responses; (2) the physical interaction between lipoproteins and subendothelial matrix molecules; and (3) the role of accessory proretentive molecules, notably lipoprotein lipase (LpL), secretory sphingomyelinase (S-SMase), and secretory phospholipase $A_2$ ($sPLA_2$). For the other 2 areas—prevention of the biological responses to retained lipoproteins and removal/regression of lesional material—the reader is referred to recent reviews of these topics.[2,18,27–30] In addition, the full rationale and detailed description of the Response-to-Retention theory itself can be found in the original article and subsequent reviews and in recent reviews by other groups.[1,2,4,31–33]

## Likelihood of ApoB Lipoprotein Entry and Then Retention in the Subendothelium

### Concentration, Age of Onset, and Duration of Elevation

Lipoprotein entry and retention within the subendothelium and hence atherogenesis depend on sustained plasma levels of apoB lipoproteins. Lipoprotein size, charge, and composition and endothelial permeability may influence lipoprotein entry, but less certainty exists in these areas. Features of the arterial wall such as susceptible versus resistant areas, lesions versus healthy segments, and diabetic versus nondiabetic vasculature also may affect lipoprotein retention, as discussed in a following section. Here, we wish to emphasize several key points on the relationship of plasma apoB lipoprotein levels to retention and atherogenesis. First, describing plasma lipoprotein concentrations as "high" or "low," relative terms that are based on the unnatural distribution of lipoproteins levels in industrialized populations, can be misleading. For example, when confronted with coronary artery disease in patients with so-called "low" LDL (eg, in the 100-mg/dL range), a tendency exists for investigators to deemphasize the key role of apoB lipoproteins in atherogenesis in favor of inflammation or endothelial alterations.[17] However, these patients teach us that a subendothelium that is particularly susceptible to retention or maladaptive responses to retained lipoproteins (eg, because of genetic or environmental factors) requires lower levels of circulating apoB lipoproteins to initiate the atherogenic process. An important example of this principle is the increased susceptibility of diabetic patients to coronary artery disease compared with nondiabetic patients with the same plasma levels of LDL, possibly related to their altered arterial matrix.[34] As such, aggressive LDL lowering is particularly successful in lowering coronary artery disease risk in diabetic subjects.[35–37]

The second point is often introduced as "How low should we go?"—ie, is there a plasma LDL level below which atherogenesis will not occur even in the setting of extreme arterial wall susceptibility? Although the answer to this question in humans is not definitively known, several lines of evidence point to plasma LDL levels <40 mg/dL as being nonpermissive for progression of atherosclerotic heart disease in subjects with preexisting disease and perhaps <70 mg/dL if maintained throughout the entire lifetime. For example, members of hunter-gatherer societies or subjects with familial hypobetalipoproteinemia, who typically have LDL levels ≤40 mg/dL as a result of a rare mutation in LDL biosynthesis, do not develop heart disease even when they reach middle age or older.[38,39] Moreover, epidemiological studies and LDL-lowering clinical trials show a curvilinear relationship between low LDL and decreased risk for heart disease that, when extrapolated to "zero risk," intercepts the *x* axis at plasma LDL levels of ≈40 mg/dL.[40] The degree to which apoB lipoproteins can be safely lowered to these nonpermissive levels by drugs and lifestyle remains to be seen.[41] However, a post hoc analysis of the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) trial, in which patients with acute coronary events were treated with 80 mg/d atorvastatin, showed that the subpopulation of

subjects who responded with LDL levels in the 20- to 40-mg/dL range had no increase in drug-related side effects and, as predicted, had the lowest incidence of subsequent cardiovascular events.[42] A recent study showed that treatment of subjects with very low LDL values (average ≈50 mg/dL) with statins to further lower their LDL was associated with a marked improvement in survival and prevention of acute coronary syndromes over a 2-year period.[43] The improvement was observed whether or not the subjects had a history of ischemic heart disease or diabetes mellitus. Statin use in this study was not associated with an increase in malignancy, liver dysfunction, or rhabdomyolysis.[43]

In summary, the Response-to-Retention model directly supports the concept of "lower is better." However, lowering circulating apoB lipoproteins LDL may have other beneficial effects in addition to decreasing the probability of arterial wall lipoprotein retention such as improving endothelial function and promoting the exit of macrophages from lesions.[44,45] In this regard, more mechanistic data are needed to assess the relative importance of direct effects of lowering circulating lipoproteins versus effects mediated through decreasing subendothelial lipoprotein retention.

The third point—often stated as "How early should we go?"—is related to the concept that age of onset and subsequent duration of lipoprotein elevation are important determinants of lipoprotein entry and then retention in the arterial wall and atherogenesis. We know that atherosclerosis begins at a young age in industrialized societies. This concept was first demonstrated in autopsy studies showing that young victims of the Korean and Vietnam wars had extensive coronary atherosclerosis that was directly proportional to their conventional cardiovascular risk factors, notably hypercholesterolemia.[46] Similar results were found in the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) and Bogalusa Heart studies.[46] Indeed, the Bogalusa study showed that high plasma LDL levels in children predict carotid intimal-medial thickness when the individuals reach adulthood.[46]

An argument for early intervention in individuals at risk comes from an important concept of the Response-to-Retention model, namely that the pathophysiological links among plasma apoB lipoprotein levels and consequent lipoprotein retention and atherosclerosis accelerate as vascular disease becomes established and progresses (Figure 1). In particular, the decades-long interval between onset of retention and clinical coronary artery disease and the fact that retention is amplified once lesions become established (below) predict that an elevated level of plasma apoB lipoproteins is much more dangerous when established early in life than later in life, even when equalized for total duration. This concept, which is elegantly supported by a number of recent clinical studies (below), represents a subcategory in the broader arena of primary prevention of cardiovascular disease. In this regard, one should not be dissuaded by meta-analyses showing that primary prevention in adults through LDL lowering is less effective than secondary prevention.[47] In addition to inherent problems in interpreting meta-analyses, the burden of morbidity and mortality of first cardiac events in the very large population of low- to moderate-risk individuals is tremendous.[48]

Lloyd-Jones and colleagues[49] showed that individuals who reach 50 years of age with presumably lifelong optimal values for just 4 conventional cardiovascular risk factors, including plasma cholesterol, have a coronary artery disease risk that is much lower than would be predicted from a similar risk profile that resulted from therapeutic interventions in middle age. For example, compared with 50-year-old individuals with 2 conventional risk factors, these individuals eliminated >90% of their lifetime risk,[49] indicating how well a robust regimen of early management could prevent this lethal and disabling disease. Similarly, Loria et al[50] found that coronary artery calcium in middle-aged adults was predicted more accurately by the risk factor profile 15 years earlier than by current risk factors, even when risk factors changed during the interval. In a third study, Cohen and colleagues[25] described common nonsense mutations and sequence variations in a protein called PCSK9 that results in ≈30% lowering of plasma LDL in the affected population. PCSK9 normally promotes the degradation of hepatic LDL receptors, and the aforementioned polymorphisms result in a dysfunctional PCSK9 protein. As a result, the subjects have higher expression of LDL receptors in the liver, which leads to decreased plasma LDL by the same overall mechanism of statins, namely increased plasma LDL clearance.[25] Thus, this substantial population of humans represents a model for statin-like reductions in LDL, with the critical distinction that the reductions are present throughout life rather than being initiated later in life, as is the usual case with statins. These individuals, despite the presence of other risk factors, had an incidence of atherosclerotic heart disease that was reduced by as much as 88%. Most important, the ratio of percent risk reduction to percent LDL lowering in subjects with PCSK9 polymorphisms was ≈3:1, whereas that in noncarriers whose LDL is lowered after vascular disease has already developed is ≈2:1.[14,15,25] Note that PCSK9 plays no known role in inflammation; thus, its beneficial effects are almost certainly related to lower LDL, not "pleiotropic" effects. Finally, several recent studies have shown that statin therapy can reverse signs of vascular dysfunction and atherosclerosis in children with severe hypercholesterolemia, with the greatest benefit observed in those children to whom statins were administered at an early age.[51,52]

On the basis of all of these data, a recent consensus statement from the American Heart Association addressed guidelines for therapy in boys >10 years of age and girls after menarche.[46] The guidelines favor the use of statins when diet and exercise fail to achieve the goal, and aggressive LDL lowering is recommended for those young people, particularly boys, with multiple risk factors such as family history, low high-density lipoprotein, metabolic syndrome, hypertension, and cigarette exposure.[46] A number of ongoing trials are evaluating these new strategies, which will require decades of follow-up. On the basis of the Response-to-Retention model of atherogenesis, we expect that these trials will show lifelong benefits from early intervention, and we support continuing investigation into more widespread use of early-onset lifestyle and medicinal therapy in at-risk young people.

## Lipoprotein(a) and Remnant Lipoproteins

Most clinical and epidemiological data to date support a major role for LDL, with the understanding that subendothelial modifications of LDL such as aggregation, lipolysis, and oxidation (below) contribute to triggering maladaptive, local responses to retained material.[1,29] However, strong mechanistic and correlative data support potent atherogenic roles for other apoB lipoproteins as well, particularly lipoprotein(a) [Lp(a)] and remnant lipoproteins.[40,53]

Lp(a) is a form of LDL that is modified in the liver by covalent attachment of apoB to apo(a), a member of the plasminogen gene family.[54] Lp(a) has been associated with increased risk of atherosclerotic vascular disease in humans.[54] Although the mechanisms of its atherogenicity are not fully known, the increased retentive properties of the unique apo(a) moiety likely contribute to this effect.[55,56] Whether Lp(a), once retained, is more easily modifiable into more atherogenic forms and/or otherwise is particularly potent at eliciting maladaptive responses represent areas of ongoing investigation. For example, a series of experimental and clinical studies by Tsimikas and colleagues[57,58] have shown that Lp(a) is rich in potentially atherogenic oxidized phospholipids, a property shown to be predictive of atherosclerotic vascular disease in humans. In terms of therapy, nicotinic acid can lower Lp(a) levels, but only to a modest degree. The Response-to-Retention model would predict that if not much can be done to lower Lp(a) levels, decreasing plasma LDL and subsequent LDL retention would be the best strategy to decrease the atherogenic response burden in Lp(a)-laden subendothelium. Indeed, the most successful overall treatment strategy for individuals with high levels of Lp(a) is aggressive lowering of plasma LDL.[59]

Remnant lipoproteins originate from intestinally and hepatically derived triglyceride-rich apoB lipoproteins after they undergo partial triglyceride lipolysis.[53] In a substantial subpopulation of humans, especially those with metabolic syndrome or type 2 diabetes mellitus, hepatic clearance of these remnant lipoproteins in the postprandial state is delayed.[53] This resulting increase in remnant lipoprotein circulation time increases the likelihood that the lipoproteins will enter and become trapped within susceptible regions of the arterial wall.[53,60,61] Indeed, direct evidence exists that remnant lipoproteins are retained in lesion-prone areas of the arterial wall and that patients with high plasma levels of remnant lipoproteins are at markedly increased risk for atherosclerotic heart disease.[53,62,63] Thus, another therapeutic directive of the Response-to-Retention model is to lower remnant lipoproteins. This directive is particularly important in view of the ensuing epidemic of insulin resistance–induced heart disease, which is likely driven to a significant extent by remnant lipoproteins. Examples of current drugs that have been shown to decrease remnant lipoproteins or are being explored for this purpose include nicotinic acid, fibric acid derivatives, statins, intestinal cholesterol-absorption inhibitors, and insulin-sensitizing drugs.[64–68] In addition, recent findings related to the mechanisms of remnant lipoprotein lipolysis and their hepatic uptake may suggest novel therapeutic strategies in the future such as FXR activation and angiotensin-II blockade within the liver.[69–73]

## Lipoprotein Properties and Endothelial Permeability

Other possible determinants of lipoprotein retention within the arterial wall include lipoprotein size, other lipoprotein properties (eg, electrical charge and cholesterol enrichment), and endothelial permeability.[74–76] 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.[77] Although the entry of ≈100-nm chylomicron 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.[78] Whether variations in the size of LDL itself can affect permeation is not known. Although so-called small, dense LDL (≈20 nm) may be more atherogenic than larger LDL (≈30 nm),[74] 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 proteoglycans in vitro, and conversion of apoB lipoproteins into a small, dense form by treatment with phospholipase A2 in vitro increases their affinity to proteoglycans.[33,79] It is possible that other properties of LDL might affect the ability of lipoproteins to permeate the endothelium or to interact with subendothelial matrix molecules. For example, Flood et al[80] recently showed that cholesterol enrichment of LDL increases its affinity for arterial wall proteoglycans. This effect is mediated through a conformational change in one of the proteoglycan-binding sites of apoB-100.[80]

Assessing the role of endothelial permeability in lipoprotein retention is hampered by our relatively poor understanding of how atherogenic lipoproteins gain access to the subendothelial space (eg, via transcytosis versus intercellular transport). Moreover, the work of Schwenke and Carew[81] in rabbits suggests 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. 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.[82,83] The uncertainty in this area allows us to only speculate about therapeutic opportunities. Previous work has suggested that blood pressure lowering, an important clinical intervention, reduced endothelial permeability to LDL.[84] A recent study by Orr and colleagues[76] showed that activation of the matrix-specific p21-activated kinase enhanced vascular endothelial permeability to Evan's blue dye in *Apoe*$^{-/-}$ mice. Whether p21-activated kinase increases permeability to apoB lipoproteins or accelerates atherogenesis remains to be seen. If it does, drugs that locally inhibit p21-activated kinase may be worth investigating. Another determinant of lipoprotein permeation may be endothelial cell turnover and apoptosis,[85,86] although no evidence whatsoever exists for frank endothelial "injury" or denudation in common forms of atherogenesis.[1] Nonetheless, it will

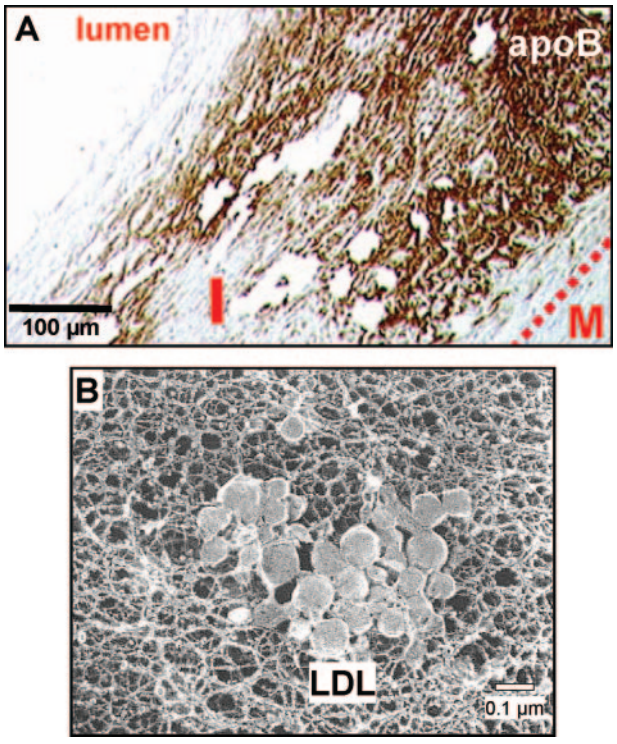


**Figure 3.** ApoB lipoproteins retained in the intima. A, ApoB-100 immunohistochemistry of human atherosclerotic lesions. From Wyler von Ballmoos et al.[130] Reproduced with permission from the authors and publisher. Copyright © 2006, the American Heart Association. B, Freeze-etch electron photomicrograph of an aortic arch intima from a rabbit 2 hours after it was injected with a bolus of human LDL. The image shows fused and aggregated particles enmeshed in the filaments of the extracellular matrix of the intima (I). M indicates media; dotted line, internal elastic lamina. The experimental design was guided by the experimental design of Bragdon et al.[131] From Nievelstein et al.[132] Reproduced with permission from the authors and publisher. Copyright © 1991, the American Heart Association.

be informative to monitor lipoprotein permeation and retention in future studies that attempt to enhance endothelial regeneration.[87]

## The Retention Process Per Se: The Physical Interaction Between Lipoproteins and Matrix Molecules

Subendothelial lipoprotein retention (Figure 3) is mediated by the physical interaction between subendothelial lipoproteins and subendothelial matrix molecules, principally proteoglycans. The reader is referred to recent reviews that describe the species of proteoglycans that are present in susceptible areas of the arterial tree, in diffuse intimal thickening, and in established lesions and how they interact with subendothelial lipoproteins.[3–5,11] Here, we briefly review general principles, in vivo data, regulation, and therapeutic implications.

### Subendothelial Matrix Molecules and Their Roles in Lipoprotein Retention and Early Atherogenesis

Subendothelial matrix molecules are found in the extracellular space and on the surface of cells in the intima and consist of proteoglycans, collagen, elastin, fibronectin, vitronectin, fibulin, and a variety of bone-related matrix molecules.[4,5] Each of these molecules, particularly proteoglycans, have multiple species. Importantly, the types of matrix molecules and molecular species differ in prelesional susceptible, ie, diffuse intimal thickening (above), versus lesion-resistant areas.[1] These differences presumably arise in large part from arterial flow characteristics and almost certainly contribute to the focal nature of atherosclerosis.[1] Moreover, the types and species of matrix molecules become altered as lesions progress, which unfortunately amplifies the process of lipoprotein retention (see penultimate paragraph in this section, below).

Determining which of these molecules participate in apoB lipoprotein retention has been approached through an elegant combination in vitro binding studies, colocalization studies in animal and human atherosclerotic lesions, and genetic manipulations in mouse models of atherosclerosis. Despite their different limitations and strengths, all of these methods point toward extracellular proteoglycans as the most important lipoprotein-retaining molecules in the subendothelium.[3–5] Proteoglycans consist of a core protein to which sulfated sugar polymers are attached.[4,5] Different types of proteoglycans 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 proteoglycan sugars.[3] However, participation of lipoprotein lipids and proteoglycan core proteins also has been reported.[3–5,9]

Proteoglycans that contain side chains of CS appear to play a particularly key role in lipoprotein retention, especially in early atherogenesis.[3,88,89] 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.[3–5,11] 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.[90]

The key advance in establishing causality between lipoprotein-CS interaction and early atherogenesis came from a series of studies in mice expressing apoB-100 with site-directed mutations in its CS-binding region.[9,91] The study was designed to ensure that any differences in atherosclerosis were due to weak binding of the mutated apoB-containing LDL to proteoglycans, not to some other attribute of the mutated LDL such as the inability to bind to LDL receptors. The results showed convincingly that mice expressing the proteoglycan binding–defective LDL had greatly reduced atherogenesis and that this effect was indeed due to decreased interaction of the mutated LDL with arterial wall proteoglycans.[9]

This and subsequent studies raised 2 additional areas of interest. First, the proteoglycan-binding domain on apoB-100 does not exist on the truncated apoB (called apoB-48) that exists on atherogenic remnant lipoproteins (above). If the apoB-48 of remnant lipoproteins lack the apoB-100 proteoglycan-binding site, how do they become retained and initiate atherogenesis? The answer lies in the finding that an otherwise cryptic domain for proteoglycan binding is un-

masked in the truncated apoB-48 of these remnant lipoproteins.[92,93] Second, although the mutant mice described above have less early atherogenesis, later atherogenesis eventually becomes similar between the 2 groups of mice.[93a] This "catch-up" phenomenon strongly suggests that the molecular mechanism of lipoprotein retention changes as lesions progress (Figure 1). Indeed, the work of Schwenke and Carew[94] showed quite clearly that the established lesions are more highly retentive for atherogenic lipoproteins than any prelesional area. Possible mechanisms include alterations in proteoglycan synthesis, including that mediated by lesional macrophages[95,96]; lesion-specific synthesis of other molecules that participate in lipoprotein retention[97]; proretentive lipoprotein modifications in established lesions[98]; decrease in pH[99]; and increased participation of accessory molecules, which are secreted by lesional macrophages.[93a] These mechanisms and possibly others likely contribute to the acceleration of atherosclerosis progression.

Of note, hyperlipidemic mice deficient in the CS-proteoglycan decorin developed larger arterial lesions, whereas those overexpressing decorin exhibited smaller lesions.[3,100] These data, which point to an overall antiatherogenic role for decorin, illustrate that different species of arterial wall proteoglycans 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-$\beta$, a cytokine in the arterial wall that stimulates the synthesis of versican and biglycan CS-proteoglycans with increased LDL affinity.[101] 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.[10] The authors of that study preliminarily concluded that the effect resulted from less arterial lipoprotein retention in the perlecan-deficient mice, consistent with earlier work showing colocalization of apolipoproteins and proteoglycans, including perlecan, in these model lesions.[102]

## Therapeutic Implications of Subendothelial Matrix-Lipoprotein Interactions

In the context of the above discussion, the overall goal would be to develop therapeutic compounds that inhibit subendothelial matrix-lipoprotein interactions. This goal could be approached with a candidate-based approach or through high-volume screening of chemical libraries. Using the former approach, Saxena and colleagues[103] demonstrated that free or high-density lipoprotein–associated apoE, polyarginine, and polylysine could block the interaction of LDL with a complex of extracellular matrix and LpL in vitro. Similarly, Zeng and colleagues[104] showed that a proteolytically released fragment of collagen XVIII called endostatin binds both LDL and biglycan, interferes with LDL-biglycan and -matrix interaction in vitro, and blocks LDL retention and atherogenesis in vivo. This effect of endostatin involves a specific $\alpha$ coil within the molecule.[104] The potential therapeutic potential of endostatin-based compounds is supported by the finding that endostatin expression is decreased in advanced atheromata, which are highly retentive for lipoproteins. A third example of a candidate approach is related to a specific site in apoB-100 that affects LDL retention. In addition to the principal CS-binding site in apoB-100 (site B) that was mutated in the murine atherosclerosis studies described above, apoB-100 contains another CS-binding site (site A) that becomes functional in small, dense LDL and $sPLA_2$-modified LDL and acts cooperatively with site B in increasing proteoglycan-binding activity.[80] A future drug-screening strategy therefore may involve the specific targeting of site A in apoB-100, which would block subendothelial lipoprotein retention without blocking the beneficial process of hepatic LDL clearance.[91] Of interest in this regard, immunization of mice with an apoB-100 peptide containing site A results in reduction of atherosclerosis by $\approx$60% compared with controls given carrier and adjuvant alone.[105]

Another potential approach involves manipulating the synthesis of key retentive proteoglycans or their sugar moieties.[106] Proteoglycan biosynthesis involves core protein synthesis and glycosyltransferase and sulfotransferase reactions.[3,107] Subendothelial proteoglycans are made by smooth muscle cells, endothelial cells, and when atherosclerotic lesions are present, intimal macrophages.[4,95,96] Biosynthesis can be regulated by a number of factors, including transforming growth factor-$\beta$, platelet-derived growth factor, oxidized LDL, and fatty acids.[4,108,109] Therefore, drugs could alter proteoglycan synthesis either directly or by affecting a regulatory factor. If such manipulation resulted in decreased synthesis of the most highly retentive proteoglycans, lipoprotein retention and ensuing atherogenesis could be suppressed.[110] However, the evidence that retentive mechanisms differ between early atherogenesis versus established lesion progression makes these strategies extremely challenging. Moreover, interference with endothelial lipolysis or hepatic catabolism of triglyceride-rich lipoproteins, which also involve lipoprotein-proteoglycan interactions,[71,111] needs to be avoided. Fortunately, the nature of these physiological interactions, which involve triglyceride-rich lipoproteins and heparan sulfate proteoglycans,[69,71] differ from those involved in atherogenesis, which involve the interaction of LDL, remnant lipoproteins, and Lp(a) with mostly CS-containing proteoglycans. Moreover, as mentioned previously, recent developments in our understanding of chylomicron catabolism will likely reveal additional points of distinction.[69–71] Thus, a therapeutic window of opportunity to selectively block proretentive subendothelial matrix-lipoprotein interactions may exist. All in all, a successful approach to this overall goal requires more precise characterization of those lipoprotein-matrix interactions that are most important in human arteries at different stages of atherosclerosis.

## Accessory Molecules That Promote Lipoprotein Retention

In vitro and in vivo studies have provided strong evidence that certain nonmatrix molecules play important roles in lipoprotein retention. The most widely studied of these molecules are LpL, S-SMase, and $sPLA_2$. The roles of these molecules in lipoprotein retention add to our understanding of the pathophysiology and might open up new opportunities for therapeutic manipulation.

### Lipoprotein Lipase

LpL has binding sites for both atherogenic lipoproteins and proteoglycans, and in vitro studies have shown that LpL, which is made by lesional macrophages, can greatly increase the interaction of lipoproteins and proteoglycans through a nonenzymatic bridging mechanism.[1,4,112] To the extent that subendothelial LDL oxidation contributes to atherogenesis, LpL may be particularly important in mediating the subendothelial retention of oxidized LDL, which by itself appears to have decreased affinity for arterial wall proteoglycans at neutral pH.[99,113] In addition, in vitro studies have demonstrated that LpL can enhance the retentive potency of S-SMase (see following section).[114] In vivo studies investigating the effect of LpL deficiency or excess on atherosclerosis must be interpreted with the knowledge of the dual role of this molecule: proatherogenic within the arterial wall by bridging between apoB lipoproteins and matrix but antiatherogenic elsewhere through LpL-mediated lipolysis and hepatic clearance of atherogenic lipoproteins from plasma. Thus, Clee et al[115] showed that global heterozygous deficiency of LpL reduced lesion size in *Apoe*$^{-/-}$ mice despite the presence of dyslipidemia, whereas mice overexpressing LpL in the plasma but not in macrophages exhibited decreased plasma triglyceride and cholesterol and decreased lesion size. In *Ldlr*$^{-/-}$ mice, global heterozygous deficiency of LpL did not lead to increased atherosclerosis despite the presence of dyslipidemia,[116] again consistent with the dual functions of LpL in atherogenesis. Moreover, Babaev and colleagues[117] used bone marrow transplantation to show a proatherogenic role for macrophage-derived LpL in *Ldlr*$^{-/-}$ mice. Most importantly, Wu et al[118] showed that overexpression of catalytically inactive LpL in cholesterol-fed rabbits markedly increased atherosclerotic lesion size in balloon-injured carotid arteries. Similar data were found in mice deficient in LpL or apoE.[119] These data support a model in which vessel wall LpL is proatherogenic, consistent with its nonenzymatic function in lipoprotein-matrix bridging/retention, whereas LpL exposed to plasma is antiatherogenic by promoting the catabolism of atherogenic lipoproteins.

### Secretory Sphingomyelinase

The acid SMase gene gives rise to both lysosomal SMase and S-SMase.[6] S-SMase, which is secreted by endothelial cells and macrophages, can cleave sphingomyelin on the surface of atherogenic lipoproteins, leading to fusion and aggregation of the lipoprotein particles. Aggregation and subsequent fusion of lipoproteins after they enter the subendothelium (Figure 3B) can increase the size of the particles to the point where exit from the arterial wall is prohibited.[6] Moreover, in vitro data have shown that LDL-SM hydrolysis directly increases LDL affinity for arterial wall proteoglycans.[120] Aggregated forms of LDL, including those induced by S-SMase, are avidly ingested by macrophages and are potent inducers of macrophage foam cell formation.[6] Of interest, LpL (see above) acts synergistically with S-SMase to promote lipoprotein retention and foam cell formation in vitro.[114] In vivo evidence supporting a proatherogenic role of S-SMase includes the presence of S-SMase in atheromata and the finding that aggregated lipoproteins extracted from animal and human atherogenic lesions have increased ceramide, the cleavage product of SMase.[6] In terms of establishing causation in vivo, we have recently found that *Apoe*$^{-/-}$ mice lacking S-SMase have decreased development of early atherosclerotic lesions and, most important, decreased retention of atherogenic lipoproteins compared with *Apoe*$^{-/-}$ mice matched for similar plasma lipoprotein levels (Devlin et al, manuscript in preparation). Finally, in the context that a high sphingomyelin content of lipoproteins enhances their susceptibility to S-SMase–mediated hydrolysis,[121] studies have shown an association between high SM content in circulating lipoproteins and an increased risk for aortic atherosclerosis in mice and coronary artery disease in humans.[122]

### Secretory Phospholipase $A_2$

Group IIA and V $sPLA_2$ are enzymes that can cleave lipoprotein phosphatidylcholine to lysophosphatidylcholine and free fatty acids.[8] Like S-SMase, $sPLA_2$ is expressed in animal and human atheromata, and lipoproteins extracted from atherosclerotic lesions show evidence of $PLA_2$-mediated hydrolysis. Lipoproteins hydrolyzed by $sPLA_2$ in vitro are more susceptible to fusion, show a higher affinity for arterial wall–derived proteoglycans, and can promote macrophage foam cell formation.[8,123] The interpretation of in vivo causation studies with group IIA $sPLA_2$ is complicated owing to multiple biological effects of the enzyme, eg, on lipoprotein metabolism and inflammation. Nonetheless, atherosclerosis-susceptible mice lacking this enzyme in all tissues or specifically in bone marrow–derived cells exhibit decreased atherosclerosis.[8] In a similar manner, Bostrom et al[124] reported that *Ldlr*$^{-/-}$ mice overexpressing group V $sPLA_2$ had an increase in lesion size, whereas those lacking this enzyme in bone marrow–derived cells had decreased lesion size. Arterial wall lipoprotein retention studies in mice with genetically altered $sPLA_2$ expression have not yet been reported.

### Therapeutic Implications of Proretentive Accessory Molecules

The mechanistic and in vivo data supporting proretentive and proatherogenic roles of LpL, S-SMase, and $sPLA_2$ are not yet complete, but they clearly raise therapeutic possibilities. In the case of S-SMase and $sPLA_2$, inhibitors of enzymatic activity would be expected to block their putative proretentive actions. However, $sPLA_2$ has important roles in normal physiology,[6,8] so enzyme inhibitors might have to be delivered specifically to the site of lesion development. In contrast, no known physiological role has been established for S-SMase. In particular, all known consequences of mutations in the acid sphingomyelinase gene arise from the lack of its other product, lysosomal SMase. Indeed, a genetically engineered mouse model in which S-SMase was eliminated but lysosomal SMase was preserved showed no signs of the type of central nervous system dysfunction and systemic disease that occurs in complete acid sphingomyelinase deficiency.[125] Thus, S-SMase might be a more amenable target for inhibition. In the case of LpL, potential problems associated with enzymatic inhibition could be avoided by specifically blocking its physical interaction with proteoglycans and/or lipoproteins (ie, its nonenzymatic bridging function). However, it

would be important to avoid blocking the interaction of LpL with chylomicrons and cell-surface scaffolding molecules, a process that is critical for chylomicron hydrolysis and may contribute to hepatic uptake of remnants.[111,112] In this context, recent data suggest that a key endothelial LpL-chylomicron scaffolding molecule on endothelium is not a proteoglycan.[70] Thus, the goal would be to screen for drugs that inhibit the interaction of LpL specifically with atherogenic lipoproteins and subendothelial matrix molecules.

## 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. The unequivocal success of LDL-lowering therapy is a testimony to this overall concept, as is the emerging discussion on how early such therapy should be instituted in at-risk young individuals. In this sense, a critically important goal remains the continued development of drugs that complement the LDL-lowing actions of statins, like cholesterol absorption inhibitors, which are in current clinical use, and inhibitors of PCSK9, apoB transcription, and apoB lipoprotein secretion, which are being developed.[126–129] However, unless future improvements in the potency and safety properties of LDL-lowering drugs and drug combinations enable widespread and early-onset reduction of LDL levels to the 20- to 40-mg/dL range in high-risk individuals, complementary approaches will be needed. We believe that 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. Although not addressed in this review, complementary approaches that work through the removal of atherogenic lipoprotein components from the arterial wall and by promoting regression of the atherogenic responses to retained lipoproteins also offer important therapeutic opportunities and represent a major area of current drug development.[2,27] Finally, whether opportunities lie in preventing biological responses to retained lipoproteins, in particular the maladaptive inflammatory response, remains to be determined.[20] At present, no examples exist of antiinflammatory drugs per se having a beneficial effect on cardiovascular disease specifically through their ability to decrease the inflammatory component of atherogenesis.[20] Nonetheless, ongoing and future research in this area and in other biological responses to retained lipoproteins may someday suggest novel ways to suppress atherogenesis and/or atherosclerotic plaque progression. While these new areas are being explored, efforts to develop new LDL-lowering drug combinations, to improve physician and patient education and patient compliance in the use of LDL-reducing drugs and lifestyle changes, and to explore the use of LDL-lowering therapy in at-risk young subjects represent the best strategies to combat subendothelial lipoprotein retention and the ensuing cardiovascular disease. Examples of ≈90% risk reduction in certain human populations with lifelong low risk factor levels give hope that our extensive understanding of the pathogenesis of this leading killer could lead to its eradication.

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Dr Tabas has received honoraria from Merck and Schering-Plough and is a consultant for Merck. Dr Williams has received honoraria as a member of the ARA Research Awards Committee, Pfizer, and is the inventor of a number of patents on the use of phospholipid liposomes to promote reverse lipid transport in vivo. Drs Tabas and Williams are coinventors of patents on therapeutic manipulations of S-SMase. Dr Borén has received honoraria from Sanofi-Aventis and has a patent on methods and tools for identifying compounds that modulate atherosclerosis by affecting LDL-proteoglycan binding.

## References

1. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol*. 1995;15:551–561.
2. Williams KJ, Tabas I. Lipoprotein retention–and clues for atheroma regression. *Arterioscler Thromb Vasc Biol*. 2005;25:1536–1540.
3. Williams KJ. Arterial wall chondroitin sulfate proteoglycans: diverse molecules with distinct roles in lipoprotein retention and atherogenesis. *Curr Opin Lipidol*. 2001;12:477–487.
4. Khalil MF, Wagner WD, Goldberg IJ. Molecular interactions leading to lipoprotein retention and the initiation of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004;24:2211–2218.
5. Chait A, Wight TN. Interaction of native and modified low-density lipoproteins with extracellular matrix. *Curr Opin Lipidol*. 2000;11:457–463.
6. Tabas I. Secretory sphingomyelinase. *Chem Phys Lipids*. 1999;102:123–130.
7. Pentikainen MO, Oksjoki R, Oorni K, Kovanen PT. Lipoprotein lipase in the arterial wall: linking LDL to the arterial extracellular matrix and much more. *Arterioscler Thromb Vasc Biol*. 2002;22:211–217.
8. Rosengren B, Jonsson-Rylander AC, Peilot H, Camejo G, Hurt-Camejo E. Distinctiveness of secretory phospholipase A2 group IIA and V suggesting unique roles in atherosclerosis. *Biochim Biophys Acta*. 2006;1761:1301–1308.
9. Skålén K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, Borén J. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature*. 2002;417:750–754.
10. Vikramadithyan RK, Kako Y, Chen G, Hu Y, Rikawa-Hirasawa E, Yamada Y, Goldberg IJ. Atherosclerosis in perlecan heterozygous mice. *J Lipid Res*. 2004;45:1806–1812.
11. Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arterioscler Thromb Vasc Biol*. 2007;27:986–989.
12. Nakashima Y, Chen YX, Kinukawa N, Sueishi K. Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age. *Virchows Arch*. 2002;441:279–288.
13. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia: intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*. 1997;100:2680–2690.
14. Gotto AM Jr, LaRosa JC. The benefits of statin therapy: what questions remain? *Clin Cardiol*. 2005;28:499–503.
15. Hackam DG. Intensive reduction of low-density lipoprotein-cholesterol: implications of recent trials. *Am J Cardiovasc Drugs*. 2006;6:367–371.
16. Ford ES, Ajani UA, Croft JB, Critchley JA, Labarthe DR, Kottke TE, Giles WH, Capewell S. Explaining the decrease in U.S. deaths from coronary disease, 1980–2000. *N Engl J Med*. 2007;356:2388–2398.
17. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.

18. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685–1695.
19. Perez RL, Rivera-Marrero CA, Roman J. Pulmonary granulomatous inflammation: from sarcoidosis to tuberculosis. *Semin Respir Infect*. 2003;18:23–32.
20. Blankenberg S, Yusuf S. The inflammatory hypothesis: any progress in risk stratification and therapeutic targets? *Circulation*. 2006;114: 1557–1560.
21. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. *Proc Natl Acad Sci U S A*. 2000;97:9052–9057.
22. Orr AW, Sanders JM, Bevard M, Coleman E, Sarembock IJ, Schwartz MA. The subendothelial extracellular matrix modulates NF-kappaB activation by flow: a potential role in atherosclerosis. *J Cell Biol*. 2005; 169:191–202.
23. Schonbeck U, Libby P Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? *Circulation.* 2004;109(suppl II):II-18–II-26.
24. Sirtori CR, Calabresi L, Marchioli R, Rubins HB. Cardiovascular risk changes after lipid lowering medications: are they predictable? *Atherosclerosis*. 2000;152:1–8.
25. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272.
26. Ray KK, Cannon CP, Ganz P. Beyond lipid lowering: what have we learned about the benefits of statins from the acute coronary syndromes trials? *Am J Cardiol*. 2006;98:18P–25P.
27. Williams KJ, Feig JE, Fisher EA. Rapid regression of atherosclerosis: insights from the clinical and experimental literature. *Nat Clin Pract Cardiovasc Med*. In press.
28. Glass CK, Witztum JL. Atherosclerosis. the road ahead. *Cell*. 2001;104: 503–516.
29. Lusis AJ. *Atherosclerosis Nature*. 2000;407:233–241.
30. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest*. 2006;116:3090–3100.
31. Williams KJ, Tabas I. The response-to-retention hypothesis of atherogenesis, reinforced. *Curr Opin Lipidol*. 1998;9:471–474.
32. Gustafsson M, Flood C, Jirholt P, Borén J. Retention of atherogenic lipoproteins in atherogenesis. *Cell Mol Life Sci*. 2004;61:4–9.
33. Camejo G, Hurt-Camejo E, Wiklund O, Bondjers G. Association of apo B lipoproteins with arterial proteoglycans: pathological significance and molecular basis. *Atherosclerosis*. 1998;139:205–222.
34. Kanter JE, Johansson F, LeBoeuf RC, Bornfeldt KE. Do glucose and lipids exert independent effects on atherosclerotic lesion initiation or progression to advanced plaques? *Circ Res*. 2007;100:769–781.
35. Camejo G, Olsson U, Hurt-Camejo E, Baharamian N, Bondjers G. The extracellular matrix on atherogenesis and diabetes-associated vascular disease. *Atheroscler Suppl*. 2002;3:3–9.
36. Tannock LR, Chait A. Lipoprotein-matrix interactions in macrovascular disease in diabetes. *Front Biosci*. 2004;9:1728–1742.
37. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:7–22.
38. Roberts WC. Preventing and arresting coronary atherosclerosis. *Am Heart J*. 1995;130:580–600.
39. Linton MF, Farese RV Jr, Young SG. Familial hypobetalipoproteinemia. *J Lipid Res*. 1993;34:521–541.
40. Genser B, Marz W. Low density lipoprotein cholesterol, statins and cardiovascular events: a meta-analysis. *Clin Res Cardiol*. 2006;95: 393–404.
41. LaRosa JC. Means and ends of statins and low-density lipoprotein cholesterol lowering. *J Am Coll Cardiol*. 2007;50:419–420.
42. Wiviott SD, Cannon CP, Morrow DA, Ray KK, Pfeffer MA, Braunwald E. Can low-density lipoprotein be too low? The safety and efficacy of achieving very low low-density lipoprotein with intensive statin therapy: a PROVE IT-TIMI 22 substudy. *J Am Coll Cardiol*. 2005;46: 1411–1416.
43. Leeper NJ, Ardehali R, Degoma EM, Heidenreich PA. Statin use in patients with extremely low low-density lipoprotein levels is associated with improved survival. *Circulation*. 2007;116:613–618.
44. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Karas RH. Comparison of flow-mediated dilatation of the brachial artery in coronary patients with low-density lipoprotein cholesterol levels <80 mg/dl versus patients with levels 80 to 100 mg/dl. *Am J Cardiol*. 2005;95:93–95.
45. Angeli V, Llodra J, Rong JX, Satoh K, Ishii S, Shimizu T, Fisher EA, Randolph GJ. Dyslipidemia associated with atherosclerotic disease systemically alters dendritic cell mobilization. *Immunity*. 2004;21:561–574.
46. McCrindle BW, Urbina EM, Dennison BA, Jacobson MS, Steinberger J, Rocchini AP, Hayman LL, Daniels SR. Drug therapy of high-risk lipid abnormalities in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee, Council of Cardiovascular Disease in the Young, with the Council on Cardiovascular Nursing. *Circulation*. 2007;115: 1948–1967.
47. Thavendiranathan P, Bagai A, Brookhart MA, Choudhry NK. Primary prevention of cardiovascular diseases with statin therapy: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2006;166: 2307–2313.
48. Lauer MS. Primary prevention of atherosclerotic cardiovascular disease: the high public burden of low individual risk. *JAMA*. 2007;297: 1376–1378.
49. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PW, Wolf PA, Levy D. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation*. 2006;113:791–798.
50. Loria CM, Liu K, Lewis CE, Hulley SB, Sidney S, Schreiner PJ, Williams OD, Bild DE, Detrano R. Early adult risk factor levels and subsequent coronary artery calcification. *J Am Coll Cardiol*. 2007;49: 2013–2020.
51. Avis HJ, Vissers MN, Stein EA, Wijburg FA, Trip MD, Kastelein JJ, Hutten BA. A systematic review and meta-analysis of statin therapy in children with familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2007;27:1803–1810.
52. Rodenburg J, Vissers MN, Wiegman A, van Trotsenburg AS, van der GA, de GE, Wijburg FA, Kastelein JJ, Hutten BA. Statin treatment in children with familial hypercholesterolemia: the younger, the better. *Circulation*. 2007;116:664–668.
53. Twickler T, Dallinga-Thie GM, Chapman MJ, Cohn JS. Remnant lipoproteins and atherosclerosis. *Curr Atheroscler Rep*. 2005;7: 140–147.
54. Anuurad E, Boffa MB, Koschinsky ML, Berglund L. Lipoprotein(a): a unique risk factor for cardiovascular disease. *Clin Lab Med*. 2006;26: 751–772.
55. Lundstam U, Hurt-Camejo E, Olsson G, Sartipy P, Camejo G, Wiklund O. Proteoglycans contribution to association of Lp (a) and LDL with smooth muscle cell extracellular matrix. *Arterioscler Thromb Vasc Biol*. 1999;19:1162–1167.
56. Edelstein C, Yousef M, Scanu AM. Elements in the C terminus of apolipoprotein [a] responsible for the binding to the tenth type III module of human fibronectin. *J Lipid Res*. 2005;46:2673–2680.
57. Tsimikas S, Brilakis ES, Miller ER, McConnell JP, Lennon RJ, Kornman KS, Witztum JL, Berger PB. Oxidized phospholipids, Lp (a) lipoprotein, and coronary artery disease. *N Engl J Med*. 2005;353: 46–57.
58. Tsimikas S, Kiechl S, Willeit J, Mayr M, Miller ER, Kronenberg F, Xu Q, Bergmark C, Weger S, Oberhollenzer F, Witztum JL. Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. *J Am Coll Cardiol*. 2006;47:2219–2228.
59. Angelin B. Therapy for lowering lipoprotein (a) levels. *Curr Opin Lipidol*. 1997;8:337–341.
60. Moreton JR. Atherosclerosis and alimentary hyperlipidemia. *Science*. 1947;106:190–191.
61. Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation*. 1979;60:473–485.
62. Rapp JH, Lespine A, Hamilton RL, Colyvas N, Chaumeton AH, Tweedie-Hardman J, Kotite L, Kunitake ST, Havel RJ, Kane JP. Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler Thromb*. 1994;14:1767–1774.
63. Chung BH, Tallis G, Yalamoori V, Anantharamaiah GM, Segrest JP. Liposome-like particles isolated from human atherosclerotic plaques are structurally and compositionally similar to surface remnants of triglyceride-rich lipoproteins. *Arterioscler Thromb*. 1994;14:622–635.
64. Xi H, Akishita M, Nagai K, Yu W, Hasegawa H, Eto M, Kozaki K, Toba K. Potent free radical scavenger, edaravone, suppresses oxidative stress-induced endothelial damage and early atherosclerosis. *Atherosclerosis*. 2007;191:281–289.

65. Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. *Arterioscler Thromb Vasc Biol*. 2006;26:1144–1149.
66. Kolovou GD, Anagnostopoulou KK, Salpea KD, Daskalopoulou SS, Mikhailidis DP. The effect of statins on postprandial lipemia. *Curr Drug Targets*. 2007;8:551–560.
67. Bays HE, Ose L, Fraser N, Tribble DL, Quinto K, Reyes R, Johnson-Levonas AO, Sapre A, Donahue SR. A multicenter, randomized, double-blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia. *Clin Ther*. 2004;26:1758–1773.
68. Abbasi F, Chu JW, McLaughlin T, Lamendola C, Leary ET, Reaven GM. Effect of metformin treatment on multiple cardiovascular disease risk factors in patients with type 2 diabetes mellitus. *Metabolism*. 2004; 53:159–164.
69. MacArthur JM, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, Esko JD. Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J Clin Invest*. 2007;117:153–164.
70. Beigneux AP, Davies BS, Gin P, Weinstein MM, Farber E, Qiao X, Peale F, Bunting S, Walzem RL, Wong JS, Blaner WS, Ding ZM, Melford K, Wongsiriroj N, Shu X, de Sauvage F, Ryan RO, Fong LG, Bensadoun A, Young SG. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab*. 2007;5:279–291.
71. Williams KJ, Fuki IV. Cell-surface heparan sulfate proteoglycans: dynamic molecules mediating ligand catabolism. *Curr Opin Lipidol*. 1997;8:253–262.
72. Anisfeld AM, Kast-Woelbern HR, Meyer ME, Jones SA, Zhang Y, Williams KJ, Willson T, Edwards PA. Syndecan-1 expression is regulated in an isoform-specific manner by the farnesoid-X receptor. *J Biol Chem*. 2003;278:20420–20428.
73. Williams KJ, Liu ML, Zhu Y, Xu X, Davidson WR, McCue P, Sharma K. Loss of heparan N-sulfotransferase in diabetic liver: role of angiotensin II. *Diabetes*. 2005;54:1116–1122.
74. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res*. 2002;43:1363–1379.
75. Kuznetsov AS, Missyul BV. The charge and atherogenicity of low density lipoproteins. *Ukr Biokhim Zh*. 1992;64:3–19.
76. Orr AW, Stockton R, Simmers MB, Sanders JM, Sarembock IJ, Blackman BR, Schwartz MA. Matrix-specific p21-activated kinase activation regulates vascular permeability in atherogenesis. *J Cell Biol*. 2007;176:719–727.
77. Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin Chem*. 1995; 41:153–158.
78. Proctor SD, Vine DF, Mamo JC. Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. *Curr Opin Lipidol*. 2002;13:461–470.
79. Hurt-Camejo E, Camejo G, Sartipy P. Phospholipase A2 and small, dense low-density lipoprotein. *Curr Opin Lipidol*. 2000;11:465–471.
80. Flood C, Gustafsson M, Pitas RE, Arnaboldi L, Walzem RL, Borén J. Molecular mechanism for changes in proteoglycan binding on compositional changes of the core and the surface of low-density lipoprotein-containing human apolipoprotein B100. *Arterioscler Thromb Vasc Biol*. 2004;24:564–570.
81. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits, II: selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis*. 1989;9:908–918.
82. Nielsen LB, Nordestgaard BG, Stender S, Kjeldsen K. Aortic permeability to LDL as a predictor of aortic cholesterol accumulation in cholesterol-fed rabbits. *Arterioscler Thromb*. 1992;12:1402–1409.
83. Weinberg PD. Rate-limiting steps in the development of atherosclerosis: the response-to-influx theory. *J Vasc Res*. 2004;41:1–17.
84. Bretherton KN, Day AJ, Skinner SL. Effect of hypertension on the entry of 125 I-labelled low density lipoprotein into the aortic intima in normal-fed rabbits. *Atherosclerosis*. 1976;24:99–106.
85. Simionescu M. Implications of early structural-functional changes in the endothelium for vascular disease. *Arterioscler Thromb Vasc Biol*. 2007; 27:266–274.
86. Chen YL, Jan KM, Lin HS, Chien S. Relationship between endothelial cell turnover and permeability to horseradish peroxidase. *Atherosclerosis*. 1997; 133:7–14.
87. Goldschmidt-Clermont PJ, Creager MA, Losordo DW, Lam GK, Wassef M, Dzau VJ. Atherosclerosis 2005: recent discoveries and novel hypotheses. *Circulation*. 2005;112:3348–3353.
88. Galis ZS, Alavi MZ, Moore S. Co-localization of aortic apolipoprotein B and chondroitin sulfate in an injury model of atherosclerosis. *Am J Pathol*. 1993;142:1432–1438.
89. Cardoso LE, Mourao PA. Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL. *Arterioscler Thromb*. 1994;14: 115–124.
90. Talusan P, Bedri S, Yang S, Kattapuram T, Silva N, Roughley PJ, Stone JR. Analysis of intimal proteoglycans in atherosclerosis-prone and atherosclerosis-resistant human arteries by mass spectrometry. *Mol Cell Proteomics*. 2005;4:1350–1357.
91. Borén J, Olin K, Lee I, Chait A, Wight TN, Innerarity TL. Identification of the principal proteoglycan-binding site in LDL: a single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. *J Clin Invest*. 1998;101:2658–2664.
92. Flood C, Gustafsson M, Richardson PE, Harvey SC, Segrest JP, Borén J. Identification of the proteoglycan binding site in apolipoprotein B48. *J Biol Chem*. 2002;277:32228–32233.
93. Goldberg IJ, Wagner WD, Pang L, Paka L, Curtiss LK, DeLozier JA, Shelness GS, Young CS, Pillarisetti S. The NH2-terminal region of apolipoprotein B is sufficient for lipoprotein association with glycosaminoglycans. *J Biol Chem*. 1998;273:35355–35361.
93a. Gustafsson M, Levin M, Skålén K, Perman J, Fridén V, Jirholt P, Olofsson SO, Fazio S, Linton MF, Semenkovich CF, Olivecrona G, Borén J. Retention of low-density lipoprotein in atherosclerotic lesions of the mouse: evidence for a role of lipoprotein lipase. *Circ Res*. August 30, 2007. DOI: 10.1161/CIRCRESAHA.107.149666. Available at http://circres.ahajournals.org. Accessed September 18, 2007.
94. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits, I: focal increases in arterial LDL concentrations precede development of fatty streak lesions. *Arteriosclerosis*. 1989;9: 895–907.
95. Chang MY, Olin KL, Tsoi C, Wight TN, Chait A. Human monocyte-derived macrophages secrete two forms of proteoglycan-macrophage colony-stimulating factor that differ in their ability to bind low density lipoproteins. *J Biol Chem*. 1998;273:15985–15992.
96. Maor I, Hayek T, Hirsh M, Iancu TC, Aviram M. Macrophage-released proteoglycans enhance LDL aggregation: studies in aorta from apolipoprotein E-deficient mice. *Atherosclerosis*. 2000;150:91–101.
97. Lees AM, Deconinck AE, Campbell BD, Lees RS. Atherin: a newly identified, lesion-specific, LDL-binding protein in human atherosclerosis. *Atherosclerosis*. 2005;182:219–230.
98. Auerbach BJ, Bisgaier CL, Wolle J, Saxena U. Oxidation of low density lipoproteins greatly enhances their association with lipoprotein lipase anchored to endothelial cell matrix. *J Biol Chem*. 1996;271:1329–1335.
99. Sneck M, Kovanen PT, Oorni K. Decrease in pH strongly enhances binding of native, proteolyzed, lipolyzed, and oxidized low density lipoprotein particles to human aortic proteoglycans. *J Biol Chem*. 2005; 280:37449–37454.
100. Al Haj ZA, Caligiuri G, Sainz J, Lemitre M, Demerens C, Lafont A. Decorin overexpression reduces atherosclerosis development in apolipoprotein E-deficient mice. *Atherosclerosis*. 2006;187:31–39.
101. Little PJ, Tannock L, Olin KL, Chait A, Wight TN. Proteoglycans synthesized by arterial smooth muscle cells in the presence of transforming growth factor-beta1 exhibit increased binding to LDLs. *Arterioscler Thromb Vasc Biol*. 2002;22:55–60.
102. Kunjathoor VV, Chiu DS, O'Brien KD, LeBoeuf RC. Accumulation of biglycan and perlecan, but not versican, in lesions of murine models of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2002;22:462–468.
103. Saxena U, Auerbach BJ, Ferguson E, Wolle J, Marcel YL, Weisgraber KH, Hegele RA, Bisgaier CL. Apolipoprotein B and E basic amino acid clusters influence low-density lipoprotein association with lipoprotein lipase anchored to the subendothelial matrix. *Arterioscler Thromb Vasc Biol*. 1995;15:1240–1247.
104. Zeng X, Chen J, Miller YI, Javaherian K, Moulton KS. Endostatin binds biglycan and LDL and interferes with LDL retention to the subendothelial matrix during atherosclerosis. *J Lipid Res*. 2005;46:1849–1859.
105. Fredrikson GN, Soderberg I, Lindholm M, Dimayuga P, Chyu KY, Shah PK, Nilsson J. Inhibition of atherosclerosis in apoE-null mice by immunization with apoB-100 peptide sequences. *Arterioscler Thromb Vasc Biol*. 2003;23:879–884.

106. Little PJ, Ballinger ML, Osman N. Vascular wall proteoglycan synthesis and structure as a target for the prevention of atherosclerosis. *Vasc Health Risk Manag*. 2007;3:117–124.
107. Clarke MC, Figg N, Maguire JJ, Davenport AP, Goddard M, Littlewood TD, Bennett MR. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nat Med*. 2006;12:1075–1080.
108. Olsson U, Bondjers G, Camejo G. Fatty acids modulate the composition of extracellular matrix in cultured human arterial smooth muscle cells by altering the expression of genes for proteoglycan core proteins. *Diabetes*. 1999;48:616–622.
109. Rodriguez-Lee M, Ostergren-Lunden G, Wallin B, Moses J, Bondjers G, Camejo G. Fatty acids cause alterations of human arterial smooth muscle cell proteoglycans that increase the affinity for low-density lipoprotein. *Arterioscler Thromb Vasc Biol*. 2006;26:130–135.
110. Merrilees M, Beaumont B, Scott L, Hermanutz V, Fennessy P. Effect of TGF-beta(1) antisense S-oligonucleotide on synthesis and accumulation of matrix proteoglycans in balloon catheter-injured neointima of rabbit carotid arteries. *J Vasc Res*. 2000;37:50–60.
111. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res*. 1996;37:693–707.
112. Williams KJ, Fless GM, Petrie KA, Snyder ML, Brocia RW, Swenson TL. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins: roles for low density lipoprotein receptors and heparan sulfate proteoglycans. *J Biol Chem*. 1992;267:13284–13292.
113. Oorni K, Pentikainen MO, Annila A, Kovanen PT. Oxidation of low density lipoprotein particles decreases their ability to bind to human aortic proteoglycans: dependence on oxidative modification of the lysine residues. *J Biol Chem*. 1997;272:21303–21311.
114. Tabas I, Li Y, Brocia RW, Wu SW, Swenson TL, Williams KJ. Lipoprotein lipase and sphingomyelinase synergistically enhance the association of atherogenic lipoproteins with smooth muscle cells and extracellular matrix: a possible mechanism for low density lipoprotein and lipoprotein(a) retention and macrophage foam cell formation. *J Biol Chem*. 1993;268:20419–20432.
115. Clee SM, Bissada N, Miao F, Miao L, Marais AD, Henderson HE, Steures P, McManus J, McManus B, LeBoeuf RC, Kastelein JJ, Hayden MR. Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis. *J Lipid Res*. 2000;41:521–531.
116. Semenkovich CF, Coleman T, Daugherty A. Effects of heterozygous lipoprotein lipase deficiency on diet-induced atherosclerosis in mice. *J Lipid Res*. 1998;39:1141–1151.
117. Babaev VR, Patel MB, Semenkovich CF, Fazio S, Linton MF. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in low density lipoprotein receptor-deficient mice. *J Biol Chem*. 2000;275:26293–26299.
118. Wu X, Wang J, Fan J, Chen M, Chen L, Huang W, Liu G. Localized vessel expression of lipoprotein lipase in rabbits leads to rapid lipid deposition in the balloon-injured arterial wall. *Atherosclerosis*. 2006;187:65–73.
119. Wang J, Xian X, Huang W, Chen L, Wu L, Zhu Y, Fan J, Ross C, Hayden MR, Liu G. Expression of LPL in endothelial-intact artery results in lipid deposition and vascular cell adhesion molecule-1 upregulation in both LPL and ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2007;27:197–203.
120. Oorni K, Pentikainen MO, Ala-Korpela M, Kovanen PT. Aggregation, fusion, and vesicle formation of modified low density lipoprotein particles: molecular mechanisms and effects on matrix interactions. *J Lipid Res*. 2000;41:1703–1714.
121. Schissel SL, Jiang XC, Tweedie-Hardman J, Jeong TS, Camejo EH, Najib J, Rapp JH, Williams KJ, Tabas I. Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH: implications for atherosclerotic lesion development. *J Biol Chem*. 1998;273:2738–2746.
122. Jiang XC, Paultre F, Pearson TA, Reed RG, Francis CK, Lin M, Berglund L, Tall AR. Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2000;20:2614–2618.
123. Boyanovsky BB, van der Westhuyzen DR, Webb NR. Group V secretory phospholipase A2-modified low density lipoprotein promotes foam cell formation by a SR-A- and CD36-independent process that involves cellular proteoglycans. *J Biol Chem*. 2005;280:32746–32752.
124. Bostrom MA, Boyanovsky BB, Jordan CT, Wadsworth MP, Taatjes DJ, de Beer FC, Webb NR. Group V secretory phospholipase A2 promotes atherosclerosis: evidence from genetically altered mice. *Arterioscler Thromb Vasc Biol*. 2007;27:600–606.
125. Marathe S, Miranda SR, Devlin C, Johns A, Kuriakose G, Williams KJ, Schuchman EH, Tabas I. Creation of a mouse model for non-neurological (type B) Niemann-Pick disease by stable, low level expression of lysosomal sphingomyelinase in the absence of secretory sphingomyelinase: relationship between brain intra-lysosomal enzyme activity and central nervous system function. *Hum Mol Genet*. 2000;9:1967–1976.
126. Issandou M. Pharmacological regulation of low density lipoprotein receptor expression: current status and future developments. *Pharmacol Ther*. 2006;111:424–433.
127. Williams KJ, Fisher EA. Atherosclerosis: cell biology and lipoproteins: three distinct processes that control apolipoprotein-B secretion. *Curr Opin Lipidol*. 2001;12:235–237.
128. Zimmermann TS, Lee AC, Akinc A, Bramlage B, Bumcrot D, Fedoruk MN, Harborth J, Heyes JA, Jeffs LB, John M, Judge AD, Lam K, McClintock K, Nechev LV, Palmer LR, Racie T, Rohl I, Seiffert S, Shanmugam S, Sood V, Soutschek J, Toudjarska I, Wheat AJ, Yaworski E, Zedalis W, Koteliansky V, Manoharan M, Vornlocher HP, MacLachlan I. RNAi-mediated gene silencing in non-human primates. *Nature*. 2006;441:111–114.
129. Data demonstrate in vivo activity with systemic RNAi therapeutics targeting PCSK9 for hypercholesterolemia: advancements with antagomirs, potential RNAi therapeutics that target microRNAs. Alnylam Pharmaceuticals. Available at: http://phx.corporate-ir.net/phoenix.zhtml?c=148005&p=irol-newsArticle&ID=919540&highlight=. Accessed June 2007.
130. Wyler von Ballmoos M, Dubler D, Mirlacher M, Cathomas G, Muser J, Biedermann BC. Increased apolipoprotein deposits in early atherosclerotic lesions distinguish symptomatic from asymptomatic patients. *Arterioscler Thromb Vasc Biol*. 2006;26:359–364.
131. Bragdon JH, Boyle E, Havel RJ. Human serum lipoproteins, II: some effects of their intravenous injection in rats. *J Lab Clin Med*. 1956;48:43–50.
132. Nievelstein PFEM, Fogelman AM, Mottino G, Frank JS. Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. *Arterioscler Thromb*. 1991;11:1795–1805.