In the past several months, a remarkable transformation occurred in how the field regards lipoproteins, statins, and atherosclerosis. The straw—or tree trunk—that broke this stubborn camel’s back was the prospective, randomized, double-blinded Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT). This key study demonstrated that lowering plasma low-density lipoprotein (LDL) concentrations in humans through the use of a nonstatin—ezetimibe—not only reduced cardiovascular events, but did so to exactly the same extent as LDL lowering by statins. Preliminary data in humans suggest that the same is true of the new PCSK9 inhibitors, which are also nonstatins.\(^3\) Abundant prior data have told the same story for decades (reviewed in a recent American Heart Association Council Statement\(^4\)). For example, lowering plasma LDL concentrations surgically by partial ileal bypass or by random Mendelian inheritance of key polymorphisms that lower plasma levels of LDL, or inheriting polymorphisms that lower levels of cholesterol- and triglyceride-rich apolipoprotein-B (apoB)–containing remnant lipoproteins also reduce human atherosclerotic cardiovascular events.\(^5,6\)

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Though a variety of pleiotropic effects are often attributed to statins, such as acting as antioxidants or anti-inflammatory agents, the bulk of the evidence supports that statins—and ezetimibe—reduce long-term human cardiovascular risk because they do one thing well. They lower plasma LDL levels.

Atherosclerosis has become as pathogenically simple as tuberculosis. What causes tuberculosis? Mycobacterium tuberculosis. What causes atherosclerosis? LDL and other cholesterol-rich, apoB-containing lipoproteins. Diabetes mellitus and smoking increase the risk of tuberculosis—and atherosclerosis—but cannot cause either disease on their own. Tuberculosis and atherosclerosis both involve extensive, and strikingly similar, host immune responses, including a persistent infiltrate of macrophages and T-cells, the development of foam cells, local induction of many of the same antimigration molecules that keep these cells in place,\(^7,8\) and systemic elevations in so-called inflammatory markers, such as plasma C-reactive protein. But no primary immune derangement has ever been shown to cause tuberculosis in the absence of the bacillus—nor to cause atherosclerosis in the absence of abundant apoB-containing lipoproteins.

In this context, the interactions of LDL and other cholesterol-rich, apoB-containing lipoproteins with the vessel wall are now paramount to understanding how a normal artery becomes atherosclerotic and how an existing atherosclerotic plaque worsens, stabilizes, or heals. The new study by Bartels and Christoffersen et al adds fresh insight by comparing the way established murine arterial plaques handle LDL during progression versus regression.\(^9\)

Prior literature indicates that a key process initiates atherogenesis—namely, the subendothelial retention, or trapping, of plasma-derived apoB-containing lipoproteins, particularly LDL and remnants.\(^10,12\) In earliest atherogenesis, the affinity of specific domains of apoB to adhere directly to specific elements of the arterial matrix, particularly at branch points and other areas of nonlaminar flow, drives lipoprotein retention.\(^10,12\) The retained lipoproteins become modified by arterial-wall enzymes and other processes to form a uniquely dangerous accumulation (Figure A). The resulting material provokes a series of strikingly maladaptive responses that include endothelial dysfunction and the recruitment and abnormal persistence of macrophages and T-cells.\(^10,12,14\) Cellular and molecular programs that cause immune cells to remain in place may be adaptive defenses against M. tuberculosis, an acid-fast bacillus that is not killed after phagocytosis and would therefore be spread throughout the body by emigrating macrophages. But retained and modified apoB-containing lipoproteins within the arterial wall inappropriately elicit many of the same evolutionarily conserved antimigration signals.\(^7,12,15\) The result is a crippling of the reticuloendothelial system, which otherwise has a huge capacity that could easily handle a few grams of intramural cholesterol and other debris.

In atherosclerosis, persistent macrophages secrete lipases that perversely accelerate further retention and modification of apoB-lipoproteins within the developing plaque. Many of these cells die, but their carcasses fail to undergo normal disposal by phagocytosis (efferocytosis), leading to necrotic core formation and further harmful immune activation.\(^14\) Persistent, living macrophages in an atheroma also release proteases, which weaken the overlying fibrous cap, and tissue factor, which ensures vigorous clot formation on plaque rupture—and hence the risk of arterial occlusion (Figure A, reviewed in Williams and Tabas\(^12\) and Williams et al\(^15\)).

What about the other direction? How can an existing atherosclerotic plaque stabilize or heal? Over a half-century of studies...
on experimental atherosclerosis in animals, including nonhuman primates, indicates that essentially all features of advanced atherosclerotic plaques can reverse, including seemingly permanent changes, such as necrosis and extracellular lipid accumulations, including crystalline material. The key is drastic improvements in the plasma lipoprotein profile, most commonly, vast reductions in total cholesterol or LDL. Plaque stabilization and shrinkage have been documented in humans as well, and we and others are convinced that these effects will become easier to achieve clinically with new, more potent LDL-lowering agents.

Studies on the cellular infiltrate in experimental atherosclerotic plaques indicate that regression is not merely a rewinding of progression, but instead a resolution response that involves emigration of the maladaptive macrophage infiltrate, followed by the initiation of a stream of healthy, normally functioning phagocytes that clear retained apoB-containing lipoproteins, apoptotic cells, necrotic debris, and other components of advanced plaques (Figure B).

Nevertheless, there has been a conspicuous gap in our knowledge of the interactions of LDL and other cholesterol-rich, apoB-containing lipoproteins with the vessel wall during plaque regression. As far as we know, Bartels and Christoffersen et al are the first to address this crucial issue. Their experimental system was LDL receptor–negative mice fed on a high-cholesterol diet for ≈4 to 5 months to produce sustained elevations in plasma LDL concentrations and to form atherosclerotic plaques in the aorta. Then, weekly doses of an anti-Apob antisense oligonucleotide (ASO) corrected endothelial permeability for LDL entry into the plaques de-creased, and the fractional degradation of LDL that still made it into the plaques fell dramatically (green Xs in Figure B).

Nevertheless, everyone on experimental atherosclerosis and earlier studies of regression in vivo will know that LDL entry into and out of the arterial wall, nor do we understand what makes the local endothelium more permeable to LDL once a plaque develops underneath. Bartels and Christoffersen et al ruled out a nonspecific process by showing that permeability to Evans blue dye does not change during that crucial first week of anti-Apob ASO treatment. Caveolin-1—and by implication unesterified cholesterol in the membrane—might be involved: knockout of this abundant endothelial protein impedes LDL from crossing into the arterial wall and slows atherogenesis in hypercholesterolemic mice.

A crucial question remains: what pool of LDL might be acting on the endothelium to alter permeability to apoB during progression or regression of plaques? The current study, the endothelial barrier might have improved after anti-Apob ASO treatment because there was less LDL in the plasma flowing past these cells so that, say, the endothelial plasma membrane might become less enriched in unesterified cholesterol through aqueous diffusion. Or the key effect of anti-Apob ASO treatment could have been less LDL to pass through the endothelium, a process that itself might induce cellular changes. There would have been less newly retained and aggregated LDL under the endothelium to release fatty acids, modified phospholipids, steroids, ceramides, and other potentially biologically
active molecules. Anti-ApoB ASO treatment decreased the amount of newly degraded LDL, which can be another source of harmful products within the plaque. There could also be a pool of LDL that induces rapidly reversible alterations in other elements of the plaque that were not assessed but communicate with the endothelium.

It should be experimentally feasible to distinguish roles for these different pools of LDL—and the distinct mechanisms by which they could act on the arterial endothelium. For example, would high plasma concentrations of LDL made from mutated apoB₁₀₀ that lacks its domains for binding arterial matrix—a previous technical triumph from the Borén laboratory—still alter endothelial function, or LDL in the presence of antibodies that block its binding to arterial matrix and hence atherosclerosis, or LDL after knockout or overexpression of specific arterial-wall enzymes that are known to accelerate apoB retention, aggregation, and the release of lipolytic products? Would direct injection of LDL into the subendothelial cushion

![Diagram of arterial healing process](http://circres.ahajournals.org/Downloaded_from)
impair endothelial function? To our knowledge, this last approach has not been tried since the 1930s.

One clue comes from prior studies showing that intramural retention and aggregation of apoB-containing lipoproteins occur within mere minutes to hours after the onset of hypercholesterolemia, long before the earliest known signs of endothelial dysfunction. The most straightforward conclusion is that early alterations in endothelial function cannot be a cause, and may be a consequence, of the initial retention and modification of apoB-lipoproteins within the arterial wall. We speculate that newly retained and modified LDL may be the most pathogenic pool during regression as well—and that shrinkage of the fresh pool of retained material, as opposed to the total pool of arterial-wall LDL, allows the overlying endothelium to recover in a few days. As Bartels and Christoffersen et al note, the shapes of the kinetic curves suggest a lag in labeled LDL entry, especially after treatment with the anti-Apob ASO; future studies should confirm and explain this intriguing pattern.

We are on somewhat firmer ground when trying to understand the drop in the fractional degradation of LDL that entered the plaque during early regression. Again, the authors ruled out a nonspecific effect: phagocytic and pinocytic capacities of the cells in the plaques did not measurably decrease during the first week of anti-Apob ASO treatment. At that initial time point, the lack of any significant change in macrophage expression of mRNAs for classical scavenger receptors, mentioned above, is consistent with prior work showing that these molecules are unnecessary for full foam cell formation in murine atherosclerosis.

Instead, the authors’ data suggest less degradation of retained LDL because there was less modification of these particles within the arterial wall, inferred in part from a decrease in overall protein nitrosylation within plaques after 1 week of anti-Apob ASO treatment. One possibility would be lower local levels of enzymes, such as the secretory sphingomyelinate, that promote apoB retention and aggregation. Calmer endothelium secretes less secretory sphingomyelinase basolaterally. Moreover, less cholesterol entry into nearby macrophages would decrease their production of damage-associated molecular patterns that activate endothelium. An additional option would be decreased expression of known receptors on macrophages for uptake of enzymatically aggregated LDL, such as LRPI and the syndecan-4 heparan sulfate proteoglycan. But these are only hypotheses. The mechanistic chain—from rapid correction of atherogenic hypercholesterolemia, to these or other molecular effects, to a sharp reduction in intramural LDL degradation—remains to be discovered.

Most importantly, the rapid decreases in permeability to LDL and in the fractional degradation of LDL that enters the plaque may turn out to be crucial for subsequent plaque stabilization, resolution of maladaptive sterile inflammation, and atherosclerosis regression. The rule of these effects in altering the balance between pro- and antiinflammatory signals for immune cells within the plaque, production and stability of lipid proresolving mediators, such as resolvins and lipoxins, and as noted earlier, the generation of harmful byproducts from modification and degradation of retained LDL will be fruitful areas for study.

Overall, this work provides novel information about endothelial permeability to LDL and then intramural degradation of newly entered LDL during a crucial early time point in a model of atherosclerosis regression. These are completely new findings in an area of considerable scientific and clinical interest, particularly because stronger LDL-lowering medicines—statins and nonstatins—have become approved for clinical use.

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### Disclosures
None.

### References


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