Pleiotropic Effects of Statins on the Cardiovascular System

Adam Oesterle, Ulrich Laufs, James K. Liao

Abstract: The statins have been used for 30 years to prevent coronary artery disease and stroke. Their primary mechanism of action is the lowering of serum cholesterol through inhibiting hepatic cholesterol biosynthesis thereby upregulating the hepatic low-density lipoprotein (LDL) receptors and increasing the clearance of LDL-cholesterol. Statins may exert cardiovascular protective effects that are independent of LDL-cholesterol lowering called pleiotropic effects. Because statins inhibit the production of isoprenoid intermediates in the cholesterol biosynthetic pathway, the post-translational prenylation of small GTP-binding proteins such as Rho and Rac, and their downstream effectors such as Rho kinase and nicotinamide adenine dinucleotide phosphate oxidases are also inhibited. In cell culture and animal studies, these effects alter the expression of endothelial nitric oxide synthase, the stability of atherosclerotic plaques, the production of proinflammatory cytokines and reactive oxygen species, the reactivity of platelets, and the development of cardiac hypertrophy and fibrosis. The relative contributions of statin pleiotropy to clinical outcomes, however, remain a matter of debate and are hard to quantify because the degree of isoprenoid inhibition by statins correlates to some extent with the amount of LDL-cholesterol reduction. This review examines some of the currently proposed molecular mechanisms for statin pleiotropy and discusses whether they could have any clinical relevance in cardiovascular disease. (Circ Res. 2017;120:229-243. DOI: 10.1161/CIRCRESAHA.116.308537.)

Key Words: cardiovascular diseases cholesterol, LDL hydroxymethylglutaryl-CoA reductase inhibitors rho-associated kinases vascular diseases

Cardiovascular diseases remain the leading cause of death worldwide. The development of coronary atherosclerosis involves a complex interplay between metabolic and inflammatory processes. Mechanistic and genetic evidence shows that ApoB-containing lipoprotein, specifically low-density lipoprotein cholesterol (LDL-C), is causal for atherogenesis. Statins decrease cholesterol biosynthesis and decrease serum LDL-C and triglyceride levels. Landmark clinical trials have demonstrated the efficacy of statins for both the primary and secondary preventions of coronary heart disease. It has been proposed that statins exert both LDL-C-dependent and LDL-C-independent (or pleiotropic) effects. Clinical studies show statin benefits in diseases that are not clearly related to LDL-C (Table 1), but some of the outcomes may be because of direct cholesterol lowering. Decreased gallstone formation could be because of decreased hepatic cholesterol formation; decreased cholesterol reduces platelet aggregation and could lead to less deep vein thrombosis and decreased cholesterol could affect the progression of renal disease by decreasing renal artery atherosclerosis. The clinical significance of the pleiotropic effects of statins in the cardiovascular system remains controversial, given the overwhelming benefits of cholesterol reduction in preventing cardiovascular events.

Pharmacokinetic Properties of Statins

HMG-CoA reductase produces mevalonate and is the rate-limiting enzyme for cholesterol biosynthesis in the liver, and it is competitively and reversibly inhibited by statins through their lactone ring and side chains that help them bind to the enzyme’s active site (Figure 1). Statins were initially identified as metabolites of fungi, have been on the market since 1987, and each vary in their lipophilicity, elimination half lives, and potency (Figure 2). Inhibition of cholesterol synthesis leads to decreased cholesterol production and upregulation of the LDL receptor.

The lipophilic statins cross cell membranes largely by passive diffusion, whereas pravastatin and rosuvastatin require activated carrier-mediated transport with organic anion transporting polypeptide 1B1 and are more selective for hepatic tissues. Similar transporters exist in other tissues, such as organic anion transporting polypeptide 1A4 and organic anion transporting polypeptide 2B1 although their efficacy in transporting hydrophilic statins is unknown. The concentrations of statins and mevalonate in different cell types are incompletely understood. It is unclear whether the pleiotropic effects of statins are because of the hepatic or nonhepatic effects of isoprenoid inhibition.

It is unclear whether statins exert effects independent of mevalonate synthesis inhibition. One article reported that statins...
could bind to an allosteric site within the β2 integrin leukocyte function–associated antigen-1.41 Leukocyte function–associated antigen-1 is involved in leukocyte trafficking and T-cell activation and binds intercellular adhesion molecule-1.42 Intercellular antigen-1 is involved in leukocyte trafficking and T-cell activation and binds intercellular adhesion molecule-1.42 Intercellular adhesion molecule-1 is crucial for the adhesion of monocytes to the endothelium, and it is a biomarker for coronary events that is reduced by atorvastatin.43 However, to date, no consistent mevalonate-independent effects of any statin have been reported.

Evidence of Statin Pleiotropy in Clinical Trials

The concept of anti-inflammatory pleiotropic effects of statins has been tested for perioperative risk reduction. Several studies provide evidence for beneficial effects of statins on atrial fibrillation and outcomes after cardiac surgery.44–47 In contrast, in a study with 1922 patients in sinus rhythm who underwent elective cardiac surgery and received perioperative rosuvastatin 20 mg or placebo, statin therapy did not prevent postoperative atrial fibrillation or myocardial damage.48 Similarly, in a large trial among patients undergoing cardiac surgery, atorvastatin treatment did not reduce the risk of acute kidney injury.49 Cardiac surgery is proinflammatory and rosuvastatin reduced C-reactive protein (CRP) in 1 study, but subgroup analyses are not available from either study by CRP level. Although these clinical studies do not show benefits of statin therapy, they do not exclude whether statin pleiotropy exists, but rather that statins are not beneficial in these diseases.

Additional considerations on the pleiotropic effects of statins come from their effects on CRP. JUPITER (Rosuvastatin to Prevent Vascular Events in Men and Women With Elevated C-Reactive Protein) was the primary prevention trial between rosuvastatin and placebo for 17,802 patients with a LDL-C of <130 mg/dL and a CRP of ≥2.0 mg/L.12 Rosuvastatin reduced LDL-C by 50%, CRP by 37%, and the primary end point by 44%.12 Plotting the expected benefit from JUPITER based on LDL-C lowering on the Cholesterol Treatment Trials’ Collaboration regression line suggests that the realized benefit may be greater than the expected benefit based on LDL-C reduction alone (Figure 3).

In contrast, the recent HOPE-3 study (Cholesterol Lowering in Intermediate-Risk Persons Without Cardiovascular Disease) was a primary prevention trial with rosuvastatin 10 mg that did not have LDL-C or CRP as inclusion criteria and rosuvastatin reduced LDL-C by 26.5% and the coprimary outcomes by 24% and 25%.50 The benefit of rosuvastatin occurred in both high and normal CRP groups, and whereas rosuvastatin did lower CRP, the HOPE-3 study suggests that the benefit of statins may be primarily because of LDL-C lowering.50 In the A-Z trial (Early Intensive vs a Delayed Conservative Simvastatin Strategy in Patients With Acute Coronary Syndromes Phase Z of the A to Z Trial), patients with acute coronary syndrome received either simvastatin 40 mg for 1 month followed by titration to 80 mg versus placebo for 4 months and then simvastatin 20 mg. High-dose simvastatin lowered LDL-C more effectively, but there was no difference in CRP levels at 30 days and the trial did not achieve its prespecified end point.51 From month 4 onward, there was a reduction of CRP in the high-intensity simvastatin group, and the trend to benefit was stronger after 4 months than earlier.51 Both groups had relatively low CRP (2.5 versus 2.4 mg/L) at 1 month, which may explain the lack of effect.51 The MIRACL trial (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) was an acute coronary syndrome trial comparing atorvastatin 80 mg with placebo, and atorvastatin lowered the primary end point in patients with both high and normal LDL-C and lowered CRP by 83%.14,52 Although these data on CRP suggest that statins reduce inflammation, there are no data showing the change in CRP correlates with efficacy and it is unclear whether the benefit seen with CRP reduction on medication is because of statin therapy or a lower baseline CRP, indicating a lower risk cohort.

It is difficult to separate the LDL-C–lowering benefit of statins from their potential pleiotropic effects in clinical trials, given the strong association between elevated cholesterol and coronary heart disease.53 Lowering ApoB-containing lipoproteins, therefore, represents the most important mechanism of statins. Nevertheless, there is cumulative evidence for the existence of pleiotropic effects in humans, but the contribution in addition to LDL-C lowering remains unknown for 2 reasons:44:

1. Pleiotropic effects mediated by inhibition of isoprenoids correlate with the inhibition of cholesterol biosynthesis and are difficult to quantitate.
Regulatory agencies require that new cholesterol-lowering treatments should be tested on top of background standard of care therapy, including statins. This does not allow quantitating potential cholesterol-independent effects of statins because potential pleiotropic statin effects are present in both treatment arms.

Nonstatin LDL-C-lowering therapies could reduce the risk of coronary heart disease, thus confirming the cholesterol hypothesis. A recent meta-analysis suggests that nonstatin therapies that increase the LDL receptor have the same benefits as statins and fall on the Cholesterol Treatment Trialists regression line.\(^{55,56}\) Nonstatin trials often take longer to show a benefit than statin trials. In the LRC-CPPT (Lipid Research Clinic-Coronary Primary Prevention Trial) with cholestyramine, the Program on the Surgical Control of Hyperlipidemias with partial ileal loop bypass surgery, and the IMPROVE-IT trial (Ezetimibe Added to Statin Therapy After Acute Coronary Syndromes) with ezetimibe in addition to simvastatin, benefits occurred after 7.4, 9.7, and 7.0 years, respectively, whereas most of the statin trials showed benefits within 5 years (Table 2).\(^{56-58}\) Interpreting the time to benefit data are difficult because the low event rates early in the trials prevent the separation of survival curves, and both LRC-CPPT and Program on the Surgical Control of Hyperlipidemias were primary prevention trials with lower event rates but many of the statin trials are secondary prevention trials and the IMPROVE-IT trial was conducted until a prespecified total event number was reached. Guidelines have generally recommended lifestyle changes and statins as the first-line pharmacotherapy, with or
without LDL-C targets. At present, nonstatins are indicated only as adjunctive therapy for patients who are unable to reach their lipid goals despite optimal statin therapy.

Studies using clamped cholesterol designs, eg, comparing the effect of statin-mediated LDL-C lowering with equal LDL-C lowering mediated by another intervention (eg, diet) have reported pleiotropic effects of statins in animals including cynomolgus monkeys. Nonstatins such as the inhibitor of Niemann–Pick C1–like protein, ezetimibe, have been used in humans for this purpose. Ezetimibe lowers LDL-C by 15% to 20% and can only be compared with less potent statins, which makes vascular effects more difficult to observe. Ezetimibe reduces cholesterol absorption in the small intestine, and animal data suggest an increase in the LDL-C receptor, but human data are inconsistent. Nevertheless, multiple small studies have attempted to determine whether statins have pleiotropic effects compared with ezetimibe. These studies are characterized by surrogate end points. For example, a randomized study of patients with heart failure with simvastatin 10 mg or ezetimibe 10 mg found a 15% reduction in LDL-C for both groups, but only simvastatin improved radial artery flow-dependent vasodilation, increased functionally active endothelial progenitor cells, and increased superoxide dismutase. Several studies randomized healthy volunteers or patients with coronary heart disease to protocols comparing high-dose statins to a combination of lower dose statins and ezetimibe and reported greater improvement in endothelial function and vascular inflammation with high-dose statins, despite comparable lowering of LDL-C in both groups. Other studies did not find differences between groups, suggesting the absence of statin pleiotropy. Additional evidence for pleiotropy stems from studies that report effects of statins that were observed before serum LDL-C was lowered. All of these studies were relatively small, were performed in heterogeneous patient populations with different outcome measures and duration of therapy, and showed conflicting results. These studies provide interesting data, but do not provide definitive clinical evidence of statin pleiotropy.

The notion of whether statin pleiotropy has clinical relevance in terms of cardiovascular risk reduction may benefit from ongoing trials with the proprotein convertase subtilisin kexin 9 inhibitors (PCSK9i) that lower LDL-C levels by 60% alone and could be compared with a high-dose statin in terms of equivalency in LDL-C reduction. High-dose
statin therapy has demonstrated impressive benefits on plaque reduction that has been postulated to be because of their anti-inflammatory effects in addition to their intensive LDL-C–lowering effects. The GLAGOV (Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound; NCT01813422) results have been recently announced and will be published later this year and demonstrate that the PCSK9i reduce plaque size on top of optimal statin therapy, demonstrating that plaque regression may not be primarily because of pleiotropic effects of statins. Together with CANTOS (NCT01327846), a study testing the inhibition of the pro-inflammatory cytokine IL-1β and CIRT (Cardiovascular Inflammation Reduction Trial; NCT01594333) examining methotrexate, the PCSK9i outcome trials will provide important information on the relevance of the reduction of systemic inflammatory markers for clinical outcomes.

**Diverse Target Points for Statin Actions**

**Statins and Isoprenylated Proteins**

By inhibiting mevalonic acid synthesis, statins prevent the synthesis of isoprenoid intermediates farnesylpyrophosphate (FPP) and geranylgeranylprophosphate (GGPP). FPP and GGPP serve as lipid attachments for the post-translational modification of many proteins, including Ras and the Rho GTPases, which are involved in cell signaling and cell growth. Statins lower LDL-C by a mechanism similar to statins because they increase the LDL receptor–mediated hepatic uptake of ApoB-containing lipoproteins. However, they do not inhibit the mevalonate pathway and would not have similar pleiotropic effects stemming from Rho GTPase inhibition. Despite their potent LDL-C–lowering effects, PCSK9i do not reduce serum markers of inflammation such as CRP, interleukins (IL), or tumor necrosis factor-α. These observations do not exclude anti-inflammatory effects on circulating monocytes or on vascular cells. Together with CANTOS (NCT01327846), a study testing the inhibition of the pro-inflammatory cytokine IL-1β and CIRT (Cardiovascular Inflammation Reduction Trial; NCT01594333) examining methotrexate, the PCSK9i outcome trials will provide important information on the relevance of the reduction of systemic inflammatory markers for clinical outcomes.

<table>
<thead>
<tr>
<th>Nonstatins</th>
<th>Control</th>
<th>Trial</th>
<th>Time to Benefit, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestyramine</td>
<td>Placebo</td>
<td>LRC-CPPT&lt;sup&gt;57&lt;/sup&gt;</td>
<td>7.4</td>
</tr>
<tr>
<td>Partial ileal bypass surgery</td>
<td>No surgery</td>
<td>POSCH&lt;sup&gt;56&lt;/sup&gt;</td>
<td>9.7</td>
</tr>
<tr>
<td>Ezetimibe with simvastatin 40 mg</td>
<td>Placebo with simvastatin 40 mg</td>
<td>IMPROVE-IT&lt;sup&gt;56&lt;/sup&gt;</td>
<td>7.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>Placebo</td>
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</table>
| 4S indicates Scandinavian Simvastatin Survival Study; AFCAPS/Tex-CAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; ASCOT-LLA, Prevention of Coronary and Stroke Events With Atorvastatin in Hypertensive Patients who Have Average or Lower-Than-Average Cholesterol Concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm; CARE, the Effect of Pravastatin on Coronary Events After Myocardial Infarction in Patients With Average Cholesterol Levels, HPS, Heart Protection Study; IMPROVE-IT, Ezetimibe Added to Statin Therapy After Acute Coronary Syndromes; JUPITER, Rosuvastatin to Prevent Vascular Events in Men and Women With Elevated C-Reactive Protein; LIPID, the Long-Term Intervention With Pravastatin in Ischaemic Disease; LIPS, the Lescol Intervention Prevention Study; LRC-CPPT, Lipid Research Clinic-Coronary Primary Prevention Trial; POSCH, Effect of Partial Ileal Bypass Surgery on Mortality and Morbidity From Coronary Heart Disease in Patients With Hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipemias; SPARCL, Stroke Prevention by Aggressive Reduction in Cholesterol Levels; and WOSCOPS, Prevention of Coronary Heart Disease With Pravastatin in Men With Hypercholesterolemia.
modification of heterotrimeric G proteins, including Ras and Rho. Ras and Rho regulate cell proliferation, differentiation, apoptosis, and the cytoskeleton. In endothelial cells (ECs), Ras translocation is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation. Although the inhibition of isoprenoid intermediate synthesis is central to the possible pleiotropic effects of statins, it is unclear whether the primary LDL-C–lowering benefit of statins is because of reduced cholesterol production and reduced mevalonic acid production or upregulation of the LDL receptor. There is a relative paucity of human studies on the levels of FPP and GGPP with chronic statin therapy.

**Statins and Rho/Rho Kinase**

Rho kinases (ROCKs) are protein serine/threonine kinases of 160 kDa that contribute to the downstream effects of Rho GTPases. ROCK shifts to an active open conformation when RhoA binds to ROCK (Figure 4). ROCKs regulate actin cytoskeletal changes through effects on myosin light chain phosphorylation. This affects focal adhesion complex formation, smooth muscle contraction, cell migration, and gene expression.

In human aortic ECs, simvastatin prevented tissue factor induction by thrombin in a Rho/ROCK-dependent manner. In animal models, inhibition of ROCK has cardiovascular effects similar to statins, the ROCK inhibitors (fasudil and Y27632), limit cardiac fibrosis, hypertrophy, and pathological remodeling in response to angiotensin II and N\(^{\circ}\)-nitro-L-arginine methyl ester, transverse aortic constriction, and myocardial infarction. Increased leukocyte ROCK activity is observed in patients with hypertension, pulmonary hypertension, metabolic syndrome, dyslipidemia, coronary artery disease, coronary vasospasm, left ventricular hypertrophy (LVH), and in heart failure with decreased systolic function. Statins reduce ROCK activity (Table 3). Statins have demonstrated the inhibition of leukocyte ROCK activity in humans independent of LDL reduction. ROCK inhibition is a candidate for
mediating statin pleiotropy because of ROCK’s effects on the cardiovascular system, ROCK activity is a biomarker of cardiovascular disease, and ROCK inhibition by statins occurs through cholesterol-independent mechanisms.

**Statins and Rac**

Rac is a 20- to 39-kDa monomeric G protein and a member of the Rho GTPase subfamily. Rac1 modulates phosphorylation of intercellular proteins occludin, vascular endothelial cadherin, and β-catenin, which are critical for tight junction and adherence junction integrity. Rac1 activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and produces reactive oxygen species (ROS) leading to LVH. ROS could also modify LDL to oxidized LDL, which is atherogenic and mediates foam cell formation. Elevated Rac1 and NADPH oxidase activity is seen in rats undergoing transverse aortic constriction, vascular smooth muscle cells (SMC) stimulated by angiotensin II, in saphenous vein grafts and internal mammary artery after coronary artery bypass, in ischemic and nonischemic cardiomyopathy, and atrial fibrillation and is attenuated by statins. The inhibition of Rac1 links statins to reduced ROS and NADPH oxidase activity and may explain some of the pleiotropic actions of statins.

**Statins and the Peroxisome Proliferator–Activated Receptor**

Statins have been shown to activate peroxisome proliferator–activated receptors (PPARs). Statins acutely decrease lipopolysaccharide-related inflammation in wild-type mice but not in PPARα-null mice, independent of cholesterol-lowering mechanisms. Statins increase PPAR-γ activity and inhibit lipopolysaccharide-induced tumor necrosis factor-α and monocytic chemotactic protein-1 activity. The administration of simvastatin in combination with PPAR-γ agonists elicits additive beneficial vascular effects. Atorvastatin reduces advanced glycation end products in rats and attenuates fibroblast proliferation and cardiac fibrosis, which was reversed with the PPAR-γ antagonist GW9662. Statins reduced ROS production by augmenting the mRNA expression of the PPAR-γ coactivator, which is an important regulator of mitochondrial biogenesis. However, statins, especially the high-intensity statins, increase the risk of diabetes mellitus. Thus, the ability of the PPAR-γ agonists, thiazolidinediones, to lower blood sugar is in contrast to the effects of statins on PPAR-γ and demonstrates the complex nature of statin interactions with other pathways, including glucose metabolism.

**Cellular Effects of Statins**

**Statins and the Endothelium**

Endothelial dysfunction is caused by hypercholesterolemia and is characterized by impaired bioavailability of endothelial-derived nitric oxide (NO). Endothelial NO is important for vasodilation, platelet aggregation, vascular smooth muscle proliferation, and endothelial–leukocyte interactions. Statins increase endothelial NO production, in part, by upregulating endothelial NO synthase (eNOS), which may be a pleiotropic effect of statins (Table 4). Although the increase in eNOS is important, it should be noted that much of the animal studies examining eNOS and other cellular targets

| Table 3. Studies Showing Statin Inhibition of Rho Kinase |
|---------------------------------|--------------|-----------------|-----------------|
| **Author** | **Year** | **Sample** | **Effect** |
| In vitro and animal studies | | | |
| Eto et al | 2002 | Human endothelial cells | ↓ Tissue factor induction |
| McNeish et al | 2013 | Rat middle cerebral arteries | ↓ Thromboxane receptor stimulation |
| Massaro et al | 2010 | Human endothelial cells | ↓ COX2 and MMP-9 expression |
| Li et al | 2007 | Bovine pulmonary artery SMCs | ↓ SMC mitogenesis and migration |
| Ma et al | 2012 | Rats with hypertension | ↓ ROCK activity |
| Ohnaka et al | 2001 | Human osteoblasts | ↓ ROCK activity and BMP-2 expression |
| Tramontano et al | 2004 | Human endothelial cells | ↓ Endothelial microparticle levels |
| Gojo et al | 2007 | Rats with diabetes | ↓ Urinary albumin and 8-hydroxydeoxyguanosine excretion |
| Yamanouchi et al | 2005 | Rabbits with normal cholesterol and human endothelial cells | ↓ ROCK activity and carotid intimal hyperplasia |
| Kozai et al | 2005 | Human saphenous vein SMCs | ↓ ROCK activity and carotid intimal hyperplasia |
| Trebicka et al | 2007 | Rats with cirrhosis | ↓ ROCK activity and ↑ eNOS |
| Human studies | | | |
| Rawlings et al | 2009 | Humans with stable CAD | ↓ ROCK activity |
| Liu et al | 2009 | Humans with hyperlipidemia | ↓ ROCK activity |
| Nohria et al | 2009 | Humans with stable CAD | ↓ ROCK activity |

BMP indicates bone morphogenetic protein; CAD, coronary artery disease; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; MMP, matrix metalloproteinase; ROCK, rho kinase; and SMC, smooth muscle cell.
The effects of statins on eNOS expression are not reversed by FPP or LDL-C, indicating that the effect is likely mediated through the geranylgeranylation of RhoA and ROCK signaling.80 Statins also increase eNOS activity by post-translational activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway as eNOS is phosphorylated by Akt.129 Inhibition of the Rho/ROCK pathway activates the PI3k/Akt pathway and cardioprotection.130,131 ROCK is a negative regulator of the Akt pathway, possibly through the activation of phosphatase and tensin homologue.131 Statins also act on caveolin 1, which is an integral membrane protein that binds to eNOS in caveolae and directly inhibits NO production.132 Statins decrease caveolin-1 expression in vitro and in mice, thereby promoting eNOS activity.132 Statins could also exert pleiotropic effects through the transcription factor kruppel-like factor-2. Statins induce kruppel-like factor-2 mRNA in ECs, which may be required for eNOS expression.133 Statins reduce T proliferation through kruppel-like factor-2, which may explain some of their immunomodulatory effects.134 Statins may exert pleiotropic effects by enhancing the mobilization of endothelial progenitor cells. Impaired endothelial progenitor cells are associated with impaired endothelial function and decreased NO levels.135 Atorvastatin increases endothelial progenitor cells in patients with coronary artery disease within 1 week.136 This effect is apparently observed only at low statin concentrations; higher concentrations of statins tend to have angiostatic effects, which may explain why high-intensity statins are able to reduce intraplaque angiogenesis in patients with atherosclerosis.137,138

### Table 4. Pleiotropic Effects of Statins by Cell Type

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Effect</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cells</td>
<td>↑ eNOS expression and activity58,87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Plasminogen activator inhibitor-1 expression, and ↑ Tissue-type plasminogen activator expression58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Endothelin-1 synthesis and expression102</td>
<td></td>
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<tr>
<td></td>
<td>↓ ROS118,119</td>
<td></td>
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<tr>
<td></td>
<td>↑ Peroxisome proliferator–activated receptor-CT and γ expression118–121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Proinflammatory cytokines (IL-1β), IL-6, and cyclooxygenase-2 expression127,128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ CD40 expression160</td>
<td></td>
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<tr>
<td>Vascular smooth muscle cells</td>
<td>↓ Migration and proliferation142,144</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ ROS153,175,178</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ NADPH oxidase activity115,115,116</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ AT receptor expression113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Platelet-derived growth factor141</td>
<td></td>
</tr>
<tr>
<td>Myocardium</td>
<td>↓ NADPH oxidase activity144,151</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ ROS114</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Left ventricular fibrosis and hypertrophy144,149</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Nitric oxide155,156</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Apoptosis152</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>↓ Platelet reactivity153,156</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Thromboxane A2 biosynthesis157</td>
<td></td>
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<tr>
<td>Monocyte/macrophages</td>
<td>↓ Macrophage growth151</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ MMP expression and secretion153,154,155</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Tissue factor expression and activity154,155,156,157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF–α) expression160,170</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Monocyte chemokine–protein–1 secretion160,171,172</td>
<td></td>
</tr>
<tr>
<td>Vascular inflammation</td>
<td>↓ CRP level172,173</td>
<td></td>
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<tr>
<td></td>
<td>↓ Leukocyte-endothelial cell adhesion174,175,176,177</td>
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<tr>
<td></td>
<td>↓ T-cell activation174</td>
<td></td>
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<tr>
<td></td>
<td>↓ Nuclear factor–κB activation143,144</td>
<td></td>
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<tr>
<td>Endothelial progenitor cells</td>
<td>↑ Mobilization of stem cells126</td>
<td></td>
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</tbody>
</table>

AT, indicates angiotensin type 1; CD, cluster of differentiation; CRP, C-reactive protein; eNOS, eNOS; IL, interleukin; MMP, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; and TNF, tumor necrosis factor.

Statins upregulate eNOS through multiple mechanisms. One pathway involves Rho/ROCK signaling. In vitro studies show that Rho inhibition increases eNOS expression.80 Increased ROCK activity downregulates eNOS, and ROCK inhibitors (Y-27632 and fasudil) increase eNOS expression.127,128 The effects of statins on eNOS expression are not used significantly higher doses of statins than are used in clinical practice.

Statins increase eNOS activity by post-translational activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway as eNOS is phosphorylated by Akt.129 Inhibition of the Rho/ROCK pathway activates the PI3k/Akt pathway and cardioprotection.130,131 ROCK is a negative regulator of the Akt pathway, possibly through the activation of phosphatase and tensin homologue.131 Statins also act on caveolin 1, which is an integral membrane protein that binds to eNOS in caveolae and directly inhibits NO production.132 Statins decrease caveolin-1 expression in vitro and in mice, thereby promoting eNOS activity.132

Statins could also exert pleiotropic effects through the transcription factor kruppel-like factor-2. Statins induce kruppel-like factor-2 mRNA in ECs, which may be required for eNOS expression.133 Statins reduce T proliferation through kruppel-like factor-2, which may explain some of their immunomodulatory effects.134

Statins may exert pleiotropic effects by enhancing the mobilization of endothelial progenitor cells. Impaired endothelial progenitor cells are associated with impaired endothelial function and decreased NO levels.135 Atorvastatin increases endothelial progenitor cells in patients with coronary artery disease within 1 week.136 This effect is apparently observed only at low statin concentrations; higher concentrations of statins tend to have angiostatic effects, which may explain why high-intensity statins are able to reduce intraplaque angiogenesis in patients with atherosclerosis.137,138

**Statins and Vascular Smooth Muscle**

The proliferation of vascular SMCs is important in vascular lesion pathogenesis.139 Transplant arteriosclerosis is an immune response directed against donor ECs and vascular SMCs independent of hypercholesteremia that is still attenuated by statins.140 Inhibition of isoprenoid synthesis by statins decreases platelet-derived growth factor–induced DNA synthesis in vascular SMCs by increasing the cyclin-dependent kinase, p27Kip1, which was possibly mediated by Rho GTPase.141 Simvastatin decreases intimal thickening and reduces cellular proliferation, leukocyte accumulation, and platelet-derived growth factor receptor phosphorylation in LDL receptor–deficient mice.142 In vitro, atorvastatin reduces the effects of the proinflammatory cytokine IL-18, which inhibits SMC migration, nuclear factor–κB activation, and matrix metalloproteinase-9 expression.143 In bovine pulmonary artery SMCs, atorvastatin inhibits the migration of pulmonary artery SMC, which was reversed by GGPP and mevalonate, again implicating the potential for the Rho/ROCK pathway in SMC proliferation.144

**Statins and the Myocardium**

The GTP-binding proteins, Ras, Rho, and Rac play a critical role in cardiac hypertrophy.145 Mice without Rac1 showed decreased NADPH oxidase activity and myocardial oxidative stress, confirming that Rac1 is essential for myocardial hypertrophy.146 Rac1 also increases the activity of the mineralocorticoid receptor.147 In vitro, statins increase small GTP-binding
The effect of statins on the myocardium is also mediated through RhoA and ROCK because both lead to increased apoptosis and increased fibrosis, which could lead to the development of LVH and heart failure. Overexpression of RhoA in rat ventricular myocytes increases induced caspase-9 activation, DNA fragmentation, and apoptosis, which are blocked by ROCK inhibitors. Mice with a genetic deletion of ROCK1 had less ischemic reperfusion-related fibrosis compared with wild-type mice. Mice without ROCK2 demonstrated less LVH, fibrosis, and apoptosis when exposed to angiotensin II or transverse aortic constriction compared with wild-type mice. In humans, leukocyte ROCK levels are 4.5x higher in patients with LVH and hypertension than in those with hypertension without LVH and ROCK activity also is increased with LVH in chronic kidney disease. Statins increase NO bioavailability, which increases myocardial blood flow under hypoxic conditions and inhibits IL-6, IL-8, and vascular cell adhesion molecule-1. In vitro studies show that statins reduce mitochondrial dysfunction and cardiomyocyte death.

There is, however, conflicting data about whether statins improve outcomes in nonischemic cardiomyopathy. A cohort study demonstrated decreased mortality and a small randomized controlled trial showed improvement in ejection fraction, symptoms, and lower levels of proinflammatory cytokines. However, both the large randomized GISSI-HF (Effect of Rosuvastatin in Patients With Chronic Heart Failure) and CORONA (Rosuvastatin in Older Patients With Systolic Heart Failure) trials did not show any benefit for either death or the composite end point. Although there are questions about the efficacy of statins for heart failure, it is possible that statin therapy is beneficial if started earlier in the disease course.

Statins and Platelets
Platelets are essential in the pathogenesis of acute coronary syndrome. Hypercholesterolemia is associated with increased platelet reactivity and thrombin generation, which is decreased with pravastatin and is likely both cholesterol associated and LDL-C independent. Mice treated with atorvastatin showed increased eNOS and downregulated platelet factor 4 and β-thromboglobulin in platelets, effects that are absent in eNOS knockout mice. Fluvastatin acts through PPARα and PPAR-γ to reduce platelet aggregation in response to arachidonic acid and decreased platelet aggregation compared with celestimide. Atorvastatin acutely inhibited platelet recruitment, decreased Nox2, Rac1, protein kinase C, platelet phospholipase A2, and thromboxane A2 while increasing NO levels. Finally, the antithrombotic effect was also shown in the JUPITER trial because treatment with rosuvastatin was associated with decreased thromboembolism, an effect that is likely to be unrelated to cholesterol reduction because hypercholesterolemia is not a particularly strong risk factor for venous thromboembolism.

Direct Non-LDL Effects on Statins in Cardiovascular Disease

Statins and Atherosclerosis
Atherosclerosis is a chronic inflammatory process of the vascular wall that is initiated by excessive LDL-C and is mediated by activated macrophages, T lymphocytes, B lymphocytes, and SMCs. Statins are anti-inflammatory and reduce inflammatory cytokines and adhesion molecules and acting on both the innate and adaptive immune responses. Statins reduce Rac1-mediated ROS species production and reduce the oxidation-sensitive inflammatory pathways. Statins decrease inflammatory cytokines such as IL-6, IL-8, and monocyte chemotactic protein-1. In vitro statins inhibited IL-6–induced monocyte chemotaxis and monocyte chemotactic protein-1 expression and inhibited Janus kinase and the signal transducers and activators of transmission pathway, an effect that was reversed by GGPP. Statins reduce matrix metalloproteinase-1, matrix metalloproteinase-3, and matrix metalloproteinase-9 from both SMCs and macrophages in a rabbit model, which was reduced by both GGPP and mevalonate.

In the adaptive immune system, statins have effects on T-cell differentiation. Simvastatin reduced the differentiation of the proinflammatory IL-17 helper T cells and enhanced the production of forkhead box P3+ CD4+ regulatory T cells in a geranylgeranylation-dependent manner. Transforming growth factor-β induces forkhead box P3+ CD4+ regulatory T cells, and simvastatin acts through geranylgeranylation to inhibit the transforming growth factor-β inhibitors Smad6 and Smad7 and therefore increase transforming growth factor-β and forkhead box P3+ CD4+ regulatory T cell expression. C4+ T lymphocytes from patients with acute coronary syndrome induced EC apoptosis through an upregulated tumor necrosis factor–related apoptosis-inducing ligand receptor DR5 on ECs and increased tumor necrosis factor–related apoptosis–inducing ligand expression on T lymphocytes, an effect that was blocked by statins and may provide a pathway for improved plaque stability by statins.

Statins decrease the leukocyte and EC interaction that occurs in atherogenesis. Interacellular adhesion molecule-1 and vascular cell adhesion molecule-1 regulate the migration of leukocytes to ECs and platelet and EC adhesion molecule-1 is involved in leukocytes crossing ECs. Statins inhibit vascular cell adhesion molecule-1 through PPARα and increased NO production. RhoA inhibition inhibits the clustering of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 and decreases monocyte adhesion to ECs. Lovastatin regulates platelet and EC adhesion molecule-1 expression, which was reversed by GGPP and mevalonate, suggesting a role of Rho in regulating leukocyte migration.

Statins and Stroke
Although elevated cholesterol and LDL-C are risk factors for ischemic strokes in many epidemiological studies, it has not been established in every study and the link remains more
controversial than the link for coronary artery disease.181 Nonetheless, statins reduce the risk of stroke by 25% in both the Heart Protection Study and the Treating to New Targets study and 48% in the JUPITER trial.10,182,183 The SPARCL trial (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) demonstrated that atorvastatin is effective for stroke secondary prevention.184 Although there has been debate whether nonstatin cholesterol medications effect stroke incidence, the IMPROVE-IT trial showed the ezetimibe in addition to simvastatin had a 21% reduction in ischemic stroke.56 A potential pleiotropic target for statins in stroke is the effect of statins on eNOS, given that mice without eNOS demonstrate larger infarcts.155 The effects of statins are likely mediated by Rho/ROCK because ROCK inhibitors upregulate eNOS and improve cerebral blood flow in mice.128

Conclusion

Given the cell culture and the animal studies as well as indirect evidence from clinical trials, it remains important to assess whether the non-LDL-C–lowering effects of statins could be replicated by other cholesterol-lowering therapies or by agents that act downstream of isoprenoid synthesis, eg, squalene synthase inhibitors. Unfortunately, all of the current novel hyperlipidemia treatments are tested in patients receiving statins, which will only provide information on how much further to lower serum LDL-C, but does not exclude or include the potential pleiotropic effects of statins. The concept of statin pleiotropy has provided a window of opportunity to test and target other nonlipid-lowering signaling pathways that may affect cardiovascular disease. Agents that target inflammation alone, such as anti-IL-1β therapy (canakinumab) and methotrexate, are currently being tested in secondary prevention trials as adjunctive therapy to lipid lowering.185,186 Furthermore, the ROCK inhibitors, fasudil and ripasudil, which are currently approved in Japan for the treatment of cerebrovascular vasospasm after subarachnoid hemorrhage and to treat glaucoma, respectively, may be of interest as novel therapies for reducing cardiovascular diseases. Finally, the PCSK9i may help provide evidence for statin pleiotropy, especially when low-dose PCSK9i is compared with high-potency statins that are matched for equivalent LDL-C lowering. This design would provide the opportunity to definitively test the clinical relevance of statin pleiotropy on cardiovascular outcomes.

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Disclosures

None.

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