Beyond the Laboratory
Clinical Implications for Statin Pleiotropy

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Abstract—Results from large-scale clinical trials of lipid lowering with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have led to a revolution in the management of atherosclerosis. In addition to their potent effect on serum lipid levels, statins influence several other cellular pathways, including those involving inflammatory, oxidative, and thrombotic processes. These effects clearly have the potential to beneficially modify the atherogenic process, and it has been suggested that they contribute to the impressive results seen in the clinical trials. We review the clinical evidence for benefits of statin therapy that are distinct from their effect on lipid biology. In particular, we address three key issues: the role of statins in diseases not traditionally associated with elevated cholesterol levels; whether clinical benefits are seen with statin therapy before an effect on lipid levels; and whether the magnitude of clinical benefit observed with statin therapy is unrelated to the degree of cholesterol reduction. At present, low-density-lipoprotein lowering seems to be the primary mechanism underlying the clinical benefits of statin therapy and should remain the focus of risk-reduction strategies in clinical practice. (Circulation. 2004;109[suppl II]:II-42–II-48.)

Key Words: lipids ■ atherosclerosis ■ inflammation ■ prevention

Cardiovascular (CV) disease (CVD) is the leading cause of morbidity and mortality in the developed world, with atherosclerotic vascular disease responsible for the majority of these deaths. Elevated cholesterol, low-density–lipoprotein cholesterol (LDL-C) in particular, has long been established as a strong, independent risk factor for coronary artery disease (CAD). Over the past decade, the use of the potent cholesterol-lowering 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) has revolutionized the approach to the management of atherosclerosis. Data from many definitive, large, randomized, multicenter clinical trials have now established a clear role for statins in both primary and secondary prevention.

Since the publication of the Scandinavian Simvastatin Survival Study (4S), which demonstrated a 30% relative risk (RR) reduction in mortality in high-risk subjects with symptomatic CAD and elevated cholesterol levels (213 to 310 mg/dL), similarly impressive results in subsequent studies have led to wider statin usage in subjects who are at lower risk of atherosclerosis and its complications.

Statins antagonize the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in hepatic cholesterol synthesis. This leads to a reduction in the synthesis and secretion of lipoproteins by the liver, as well as upregulation of LDL receptors on hepatocytes, increasing clearance of circulating apolipoprotein E- and B-containing lipoproteins. Although statins reduce LDL levels particularly effectively, they also lower the levels of remnant particles, including very low-density lipoprotein remnants and intermediate-density lipoprotein. Consequently, statin therapy effectively lowers plasma concentrations of non–high-density lipoprotein (HDL) cholesterol, including LDL-C levels. From extensive experimental study of the influence of statins on the underlying biological mechanisms of the atherosclerotic process, it has become clear that, in addition to their beneficial lipid-lowering effects, they possess a multitude of other effects that may contribute to their clinical benefits. These include increased expression of endothelial nitric oxide (NO) synthase; reduced production of endothelin-1 and generation of reactive oxygen species; improvement of the thrombogenic profile; reduction in inflammation via reduced expression of inflammatory cytokines, chemokines, and adhesion molecules; and lowering of C-reactive protein (CRP) levels; and inhibition of other aspects of the atherosclerotic process, such as macrophage growth and smooth muscle cell (SMC) migration and proliferation. The pathways involved in these processes are discussed extensively in other articles in this supplement and elsewhere. The term pleiotropy has been applied to encompass these non–LDL-mediated effects of statin therapy. In its strictest definition, pleiotropy is a genetic term describing the ability of a single gene to influence multiple phenotypic traits in an organism. Although there are obvious parallels, use of this term to describe the diverse effects of statin therapy has the potential to lead to confusion, as it is still unclear how many of these different effects may also be attributable to the effects of LDL locally in the vessel...
wall. In this article, we review the clinical evidence for non–lipid-mediated benefits of statin therapy in the context of the well-recognized benefits of LDL lowering. We also discuss the consequent implications for statin therapy in subjects with or at risk of developing atherosclerotic vascular disease, as well as for other conditions that may be favorably influenced by their use.

It is difficult to design clinical studies that differentiate the potential impact of statins on the multiple cellular processes involved in the disturbed vascular biology from therapy-related effects mediated by changes in lipids. It is equally challenging to determine reliably whether mechanisms other than lipid lowering are responsible for the impressive results seen with statin use in complex, large-scale clinical trials. It is possible to attribute many of the diverse effects of statin therapy directly to reduction in levels of highly toxic modified LDL, which can themselves promote various inflammatory processes in the arterial wall. These include chemotraction for monocytes and T lymphocytes, induction of expression of growth factors and adhesion molecules in endothelial cells, and pro-mitogenesis for macrophages and SMCs, as well as binding of complement and CRP,12,13 which, in turn, may increase free radical production, enhance thrombogenic potential, and lead to endothelial dysfunction. Furthermore, plasma cholesterol levels may also inaccurately reflect lipid biology in the arterial wall, where there may be important qualitative and quantitative differences compared with circulating lipoproteins. In clinical practice, the diverse effects of statin therapy may have a differing degree of relevance at different stages during the long evolution of the atherosclerotic process. This slow process, which develops over decades, is very difficult to reproduce in experimental models of atherogenesis, in which the more “acute” vascular phenotypes that have been developed are invariably different from the “real-life” situation in humans. This makes extrapolation of findings from laboratory studies difficult. It is also possible that the different mechanistic effects of statins may vary in their relative importance in stable and unstable disease. Finally, not only is it unclear which mechanisms are responsible for the potential clinical benefits of statins in conditions such as stroke, renal failure, dementia, and osteoporosis, but the role of LDL, if any, in the pathogenesis of these conditions is also not fully understood.

The lines of evidence supporting an etiologic role for cholesterol in atherosclerosis are incontrovertible and well known. National statistics indicate a close relationship between cholesterol levels and the incidence of CVD. In keeping with these observations, data from large-scale, prospective population cohorts, such as the Framingham study, have clearly shown a strong, independent association between serum cholesterol levels and risk of CAD.14 Similarly, pathologic studies have demonstrated an independent relationship between abnormal lipids and atherosclerotic lesion burden, even in children and young adults without clinical evidence of atherosclerosis.15 The strength of this association, as well as the importance of elevated cholesterol levels in early disease, is emphasized by the observation that children who have died from trauma, and aborted fetuses of hypercholesterolemic mothers, already have evidence of early atherosclerotic lesions present in their aortas at postmortem.16 Finally, the consistent reduction in CV morbidity and mortality observed in the large-scale lipid-lowering trials confirms the critical role of cholesterol in the pathogenesis of atherosclerosis.2–8

Although cholesterol lowering likely accounts for many of the benefits seen with statin therapy, three lines of evidence suggest that non–lipid-mediated effects may play a role in the clinical effects of these agents: the impact of statins on diseases not traditionally linked to disordered lipid metabolism; the temporal relationship between LDL lowering and clinical benefit after initiation of statins; and the evidence that the observed clinical benefits from statins may be greater than can be explained solely by their effect on lipid levels.

Outcome Is Improved by Statins in Conditions Thought Not to Involve LDL in Their Pathogenesis

The epidemiological association between elevated serum cholesterol and stroke risk is controversial. Data from a large meta-analysis of 45 prospective cohort studies suggested no clear relationship between cholesterol and stroke (except perhaps in those <45 years of age).17 However, the etiology of a significant proportion of these events was not definitively established. In particular, ischemic and hemorrhagic stroke are associated with different risk-factor profiles. A careful analysis of the 350 000 subjects screened for the Multiple Risk Factor Intervention Trial (MRFIT) demonstrated an inverse relation between the serum cholesterol level and the risk of death from hemorrhagic stroke that was overwhelmed by the positive association of higher serum cholesterol levels with death from nonhemorrhagic stroke and total CVD.18 The pathology of carotid artery occlusion, responsible for a significant proportion of ischemic strokes, has many similarities to that observed in the coronary circulation.19 There is also clear evidence of an impact of cholesterol on carotid disease from an early stage, which is likely to contribute to this relationship.20 Statin therapy can induce favorable structural changes in the carotid wall over a 4-year period,21 and over a similar timescale, several primary and secondary prevention trials with statins have demonstrated a significant reduction in ischemic stroke, without an increase in hemorrhagic stroke (Figure 1).2,3,7,22 Although non–LDL-mediated effects of statins probably favorably modulate atherothrombosis in the aorta and the carotid arteries and reduce the tendency for plaque disruption and arterial thromboembolism, a recent meta-analysis of lipid-lowering trials has shown that the reduction in stroke was quantitatively related to the level of cholesterol reduction.23 Studies using fibrates have also demonstrated significant reduction in the incidence of stroke. The Bezafibrate Infarction Prevention (BIP) registry demonstrated that both HDL and LDL have an impact on stroke rate, and that these relationships were themselves modulated by triglyceride levels.24 The Veterans Affairs HDL Intervention Trial (VA-HIT) demonstrated a 31% reduction in stroke in gemfibrozil in men with CAD and low HDL.25 Thus, management of dyslipidemia with a different class of drugs can also produce significant stroke reduction. These clinical data from large-scale studies emphasize the impact of
dyslipidemia on the pathogenesis of stroke and suggest that the beneficial effects of statins can largely be attributable to their lipid-modifying effects.

There is both biological plausibility and early clinical evidence for an effect of statins in a range of other diseases not normally associated with dyslipidemia. These include renal failure, dementia, osteoporosis, and tumor growth. However, the pathophysiology of some of these conditions may involve changes in the arterial wall. For example, atherothrombotic carotid and cerebrovascular disease probably accounts for a substantial proportion of cases of dementia. Tumor growth and renal disease also involve important vascular changes. The role of LDL in these processes and the impact of lipid lowering by statins remain unclear. Inhibition of HMG CoA by statins reduces generation of downstream metabolites of mevalonic acid such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These are involved in the control of numerous cellular processes implicated in other non–lipid-mediated effects of statins and may play a role in regulation of osteoclast number and activity. In this way, statins have the potential to attenuate bone loss. There are, however, conflicting data from small studies examining the clinical benefit in osteoporosis, with both negative and positive results reported. Secondary analyses of the large CV prevention studies have shown no reduction of fracture risk by statins.

Indeed, the lack of prospective, placebo-controlled, randomized clinical trials primarily testing the effect of these drugs on clinically relevant outcomes and their relationship to LDL lowering and non-LDL effects makes it difficult to draw any firm conclusions regarding the clinical significance of non–lipid-mediated effects of statins in the treatment of any of these conditions.

**Clinical Benefits of Statin Therapy Are Apparent Before Lipid Levels Change Appreciably**

The rapid time course of the vascular and clinical benefits reported for statin therapy has been used as evidence for non–lipid-mediated pleiotropic effects. Tsunekawa et al found that brachial-artery flow-mediated dilation in elderly subjects with diabetes improved after just 3 days of cerivastatin therapy. This preceded a measurable change in serum lipid levels and CRP. Measurements were repeated at 3 months, at which stage there was no further improvement in endothelial function despite a significant fall in cholesterol levels and CRP. Similarly, Wassman and colleagues demonstrated an improvement in coronary vascular endothelial function 24 hours after a single oral dose of 40 mg of pravastatin. In contrast, Treasure et al demonstrated that initiation of statin therapy in subjects with CAD awaiting angioplasty was associated with a fall in total cholesterol and LDL-C levels by 12 days but was not associated with any difference in the coronary vasodilator response to acetylcholine. Other studies have demonstrated a rapid improvement in endothelium-dependent vascular function along with a fall in lipids in subjects with hypercholesterolemia and without clinical atherosclerosis. Although these studies did not show an association between the magnitude of changes in LDL and vascular function, neither set out to determine the temporal relationship between these measures. Similarly, cessation of predominantly statin-based lipid-lowering therapy in hypercholesterolemia leads to a rise in cholesterol levels and deterioration in endothelium-dependent vasodilation after only two weeks, with rapid improvement in both lipid levels and endothelial function after reinitiation of therapy.

Recent studies have focused on the potential for rapid improvement in clinical outcome in patients with unstable atherosclerotic disease. In the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial, 3086 subjects with recent acute coronary syndromes were randomized to high-dose atorvastatin (80 mg/d) or regular care. Within 4 months, there was a significant reduction in the composite end point of death, nonfatal myocardial infarction, cardiac arrest with resuscitation, or significant recurrent myocardial ischemia. Of particular note, the incidence of stroke, a prespecified secondary end point, was reduced by 50% with atorvastatin therapy (Figure 1). Although the fall in CRP levels was greater in patients receiving atorvastatin, it is difficult to attribute these impressive early clinical benefits to non–lipid-mediated effects of atorvastatin therapy, given the significant concurrent fall in cholesterol levels (40%) seen in the atorvastatin group within 6 weeks.

In experimental studies, the adverse effects of LDL on the vascular endothelium occur extremely rapidly. Vergnani showed that exposure of endothelial cells to LDL, but particularly oxidized LDL, resulted in marked attenuation of NO production in culture within 60 minutes. A similarly rapid interaction between vascular function and LDL in the
acute clinical setting is elegantly illustrated by Tamai and colleagues, who demonstrated that endothelium-dependent vasodilation and generation of NO improved significantly immediately after an acute reduction of LDL by a single session of apheresis. The degree of cholesterol lowering in this study (60%) was similar in magnitude to that which is achievable using aggressive statin therapy. In a study of chronic statin therapy designed to evaluate the relationship between changes in LDL and biomarkers of inflammation, the time course of statin-mediated modulation of CRP matched that of the fall in LDL-C.

Thus, the highly dynamic and close relationship observed between LDL levels and vascular function and clinical events makes it difficult to attribute early beneficial effects of statin therapy to alteration of non–lipid-mediated pathways.

Clinical Benefits Observed With Statin Therapy Are Greater Than Can Be Explained by Their Effect on Lipid Levels Alone

The third line of argument supporting the clinical relevance of non–lipid-mediated effects of statins has been the apparent dissociation between the degree of LDL reduction and the magnitude of vascular changes and clinical outcomes. Angiographic studies have suggested that the significant clinical event reduction observed in the large statin trials is out of keeping with the relatively modest improvement in coronary angiographic appearances. A likely explanation for this may be the relative insensitivity of angiography to demonstrate important changes in the vascular wall, as has recently been highlighted by intravascular ultrasound. The Reversal of Atherosclerosis with Lipitor (REVERSAL) trial used this technique to investigate the effect of varying intensity of statin therapy on plaque burden. Results from this study, reported at the recent 2003 American Heart Association Scientific Sessions, demonstrated that more aggressive therapy with 80-mg atorvastatin halted progression of coronary plaque growth in patients with CAD. In contrast, coronary plaque burden continued to progress in patients treated with 40 mg daily of pravastatin, even in those achieving target LDL levels. It is likely that lipid lowering contributes greatly to the observed clinical benefits, as illustrated by an earlier meta-analysis of plaque-regression lipid-lowering trials demonstrating that the reduction in clinical events with lipid lowering is similar to the reduction in angiographic progression.

Two large trials using statins in high-risk cohorts have recently demonstrated clinical benefit that appeared at first sight to be unrelated to baseline LDL levels. In the Heart Protection Study (HPS), more than half the subjects enrolled had LDL levels equal to or lower than current recommendations, and a similar relative risk reduction was seen with simvastatin across the range of entry LDL levels (Figure 2). Similarly, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA), an impressive reduction in events was seen with atorvastatin therapy in high-risk hypertensive subjects with average cholesterol levels. Although these observations may be due to lipid-independent effects of statin therapy, it is more likely that they reflect the fact that even average LDL levels are almost certainly too high in these at-risk populations. Furthermore, this issue may be further confounded by a greater absolute risk reduction in subjects with higher cholesterol levels at higher absolute risk, equating to a lower absolute risk reduction in subjects with lower cholesterol levels who are at lower absolute risk of major vascular events when expressed in terms of relative risk reduction.

Secondary retrospective analyses of studies such as the West of Scotland Coronary Prevention Study (WOSCOPS) that extrapolate the data to suggest that the magnitude of clinical benefit observed with pravastatin was greater than would be predicted on the basis of the observed reduction of LDL should also be interpreted with caution, particularly given the direct relationship observed between the achieved level of LDL-C and event rate in the major primary and secondary prevention statin trials (Figure 3). Additionally, a meta-analysis of clinical studies with other classes of drugs shows a clear reduction in major CV events, of the order of 10% for each 10% fall in cholesterol. A significant improvement in outcome related to improvement in HDL-cholesterol levels was also observed with gemfibrozil in VA-HIT. Prospective studies comparing the effect of aggressive lipid lowering with high-dose atorvastatin versus more modest statin therapy—for example, the Treating to New Targets (TNT) study, in which 10 000 patients with coronary disease will be randomized to high-dose (80 mg/d, target LDL 75 mg/dL) or low-dose (10 mg/d, target LDL 100 mg/dL) atorvastatin—should further our understanding of this relationship.

To explore the mechanisms and potential clinical relevance of non–lipid-mediated effects of statin therapy, Libby and colleagues have studied a cynomolgus monkey model of atherosclerosis fed with an atherogenic diet. They compared the effect of pravastatin treatment with placebo on various aspects of the vascular biology of atherosclerosis, using adjustment of dietary cholesterol content to keep serum lipid levels similar in the two study groups. They found that pravastatin therapy produced cholesterol-independent improvement in endothelial-cell vasodilator function, decreased macrophage content, and increased SMC content in atheroma...
specimens, along with lowering vascular cell adhesion molecule-1 interleukin (IL)-1β and tissue factor expression, reducing the macrophage population in the arterial intima, and increasing interstitial collagen. This provides evidence that statins favorably affect plaque biology to reduce inflammation, matrix destruction, and thrombotic potential.

There has been enormous interest in the use of circulating markers of systemic inflammation for risk prediction in the clinical setting. High-sensitivity (assay) CRP has emerged as an independent biomarker of clinical outcome in retrospective analysis of large cohort studies. Secondary analyses of many of the major trials have also examined levels of inflammatory biomarkers and found that the benefits of statin therapy were most apparent in groups with elevated CRP. In the Cholesterol and Recurrent Events (CARE) study, a raised CRP level was associated with an increased risk of events in subjects receiving placebo, but not in subjects with elevated CRP levels receiving pravastatin. Furthermore, the risk reduction in patients receiving a statin was greater in subjects with higher CRP levels for a similar degree of cholesterol reduction than in those with lower levels. The Vascular Basis Study Group looked at the effect of aggressive versus conventional lipid lowering in patients with hypercholesterolemia (CURVES). They found that all three statins lowered CRP significantly and to a greater extent after 3 months. Taken together, these data might suggest that the non–lipid-mediated effects of statin therapy are clinically relevant. To further explore these issues, a number of small studies have examined the relationship between inflammation and varying intensity of statin therapy. Jialal and colleagues compared the effect of simvastatin, atorvastatin, and pravastatin on inflammation at doses found to produce similar lipid-lowering effects as those seen in the Comparative Dose Efficacy Study of Atorvastatin versus Simvastatin, Pravastatin, Lovastatin, and Fluvastatin in patients with hypercholesterolemia (CURVES). They found that all three statins lowered CRP significantly and to a similar extent after 6 weeks of therapy, but did not influence IL-6 levels. The Vascular Basis Study Group looked at the effect of aggressive versus conventional lipid lowering in subjects with atherosclerosis and myocardial ischemia. They found that, compared with moderate therapy with lovastatin, intensive lipid lowering with atorvastatin was associated with a greater and more rapid decline in both CRP and LDL-C (Figure 4). Of particular note were the similarities seen in the time course and degree of reduction in these parameters. The Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) study compared the effect of aggressive lipid lowering with atorvastatin (80 mg/d) and with pravastatin (40 mg/d) in a randomized trial. Atorvastatin therapy lowered both LDL-C and CRP to a greater extent than pravastatin after 3 months. These effects were sustained at 12 months. The potential clinical impact of these findings was highlighted by the concurrent demonstration of regression of carotid intima-media thickness in the atorvastatin group, whereas no change in this parameter was seen with pravastatin. Taken together, the results from these studies suggest that the effect of statin therapy on systemic inflammation appears to be dose dependent and closely mirrors the influence of these drugs on cholesterol levels.

The controversial issue of the importance of non–lipid-mediated effects of statins can only be resolved by prospective clinical trials. The potential role for statins in the prevention of clinical events in persons with evidence of systemic inflammation will be addressed in the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), which will enroll 15 000 patients with average cholesterol (<130 mg/dL) and elevated levels of CRP (>2.0 mg/dL) to investigate the effect of rosuvastatin in the primary prevention of major CV events. Subgroup analysis of the TNT study should also shed light on the relationship between the degree of lipid lowering and reduction of inflammation and the impact of these changes on clinical outcome.

At present, the combined published clinical data show a linear relationship between LDL level and event rate in both primary and secondary prevention trials (Figure 3), with no primary outcome data for non–lipid-mediated effects of statin therapy that alter the interpretation of this relationship.

Figure 3. Decrease in event rates with successively lower concentrations of LDL cholesterol achieved with statin or placebo in major primary and secondary prevention trials. This supports the argument that clinical benefit is directly related to the degree of LDL cholesterol achieved. Rx indicates statin-treated study arm; and Pl, placebo-treated study arm. Adapted with permission from Ballantyne CM.1

Figure 4. LDL and high-sensitivity (assay) C-reactive protein levels (left and right panels, respectively) over 12 months in the intensive LDL-reduction (circles) and modest LDL-reduction (squares) groups. Reproduced with permission from Kinlay S et al.28
Conclusions

Incontrovertible evidence now exists for a clinical benefit from statin therapy that is due to LDL lowering. A clear biological explanation for this benefit is provided by a wealth of experimental and clinical studies demonstrating that LDL is a potent initiator of endothelial dysfunction, vascular inflammation, and atherogenesis. Non-lipid-mediated effects revealed in experimental models may well also have a positive impact on many of these processes, as well as on important novel pathways. However, it is hard to draw definitive conclusions about these effects from the clinical trials thus far, due to the overwhelmingly clear relationship between LDL levels and clinical outcome. Despite consistent and conclusive evidence of the benefit of statins in populations with high risk-factor profiles, widespread undertreatment of dyslipidemia exists worldwide. Although further research will likely extend the indications for statin therapy, current clinical efforts should remain focused on adequate reduction of LDL-C with statins in at-risk populations.

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