Statin effects beyond lipid lowering—are they clinically relevant?

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Introduction

Currently, five different statins (simvastatin, pravastatin, lovastatin, fluvastatin, and atorvastatin) are approved for treatment of hypercholesterolemia in humans and two new compounds (rosuvastatin and NK-104) are under investigation.1,2 Despite differences in their pharmacokinetic profiles, all statins have at least one characteristic in common: they block the conversion of HMG-CoA to mevalonic acid with consequent attenuation of the biosynthesis of cholesterol (Fig. 1), which is associated with a reduction in serum total and low-density lipoprotein (LDL) cholesterol of as much as 20–31 and 28–42% during chronic treatment.3 Because of these properties, statins have become the most widely prescribed lipid-lowering drugs in patients with elevated serum cholesterol levels. Several large trials demonstrated that statins are not only safe and well tolerated but also significantly decrease cardiovascular morbidity and mortality in hypercholesterolemic patients in both primary and secondary prevention.4–8 However, the striking benefit achieved with statin treatment in patients with a wide range of cholesterol levels, which cannot be attributed to their cholesterol lowering effect alone, has raised the question about the possible presence of additional effects of statins beyond their impact on serum cholesterol levels. Indeed, in recent years a substantial quantity of data has accumulated showing that statins exert various effects on multiple targets, which are independent of their plasma cholesterol lowering properties.

Many of these so-called pleiotropic effects have been shown to be secondary to the inhibition of the
synthesis of isoprenoid intermediates of the mevalonate pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP)\(^9\) and, thus, are completely independent of the intracellular cholesterol biosynthesis (Fig. 1).

Isoprenoids are important attachments for the post-translational modification of a multitude of proteins involved in intracellular signal transduction pathways, including small GTP-binding proteins, which play crucial roles in the regulation of cell growth and differentiation, gene expression, cytoskeletal assembly and cell motility, protein and lipid trafficking, nuclear transport, and host defense.\(^{10,11}\) Whereas geranylgeranylation is

Fig. 1 The effect of statins on the mevalonate pathway. Statins inhibit conversion of HMG-CoA to mevalonate by competitive inhibition of the rate limiting enzyme HMG-CoA reductase. Herewith, statins not only inhibit the cellular production of cholesterol but also the biosynthesis of several intermediates of the mevalonate pathway (e.g. farnesylpyrophosphate and geranylgeranylpyrophosphate). These so-called isoprenoids are essential for the posttranslational modification of several proteins involved in important intracellular signaling pathways (e.g. the small GTP-binding proteins Ras and Rho).
required for activation of most of these small GTP-binding proteins (e.g. Rho, Rac, Rab, Rap), only few are farnesylated (e.g. Ras).10

Another pathway affected by statins seems to be the regulation of the activity of the enzyme cholesteryl ester transfer protein (CETP), which transfers cholesteryl ester to very-low-density lipoprotein (VLDL) and LDL.12 With only few exceptions13 simvastatin and pravastatin decrease plasma CETP activity in normolipidemic individuals and patients with various forms of hyperlipoproteinemia.14–16 The mechanism responsible for this effect is unknown, but could mediate some of the effects of statins beyond CETP effects on lipid metabolism. Indeed, a significant relation between variation at the CETP gene locus and the progression of coronary atherosclerosis has been demonstrated.17 Moreover, the presence of a common DNA variant of the CETP gene appears to predict benefit from treatment with statins in men with coronary heart disease (CHD).17

A novel lipid-independent mechanism of action for statins, which is unrelated to their inhibitory potential on HMG-CoA reductase, has been defined very recently by showing that statins may exert an antiinflammatory effect by binding to a specific site of the lymphocyte function associated antigen-1 (LFA-1) on leukocytes.18 In this study, several statin compounds prevented LFA-1-mediated adhesion and co-stimulation of lymphocytes after binding selectively to a novel site of LFA-1.

Obviously, the considerable quantity of desirable lipid-independent statin properties found in vitro and/or in animal models could easily explain the substantial cardiovascular benefits observed with these drugs in both normo- and hypercholesterolemic individuals, if also present in humans. However, little is known about the possible existence and the clinical relevance of such properties in humans (Table 1). Moreover, the recent withdrawal of cerivastatin from the market because of several reports about fatal cases of rhabdomyolysis in connection with this compound19 has raised major concerns about the possibility that certain pleiotropic statin effects could also be harmful. Thus, this review article, by addressing both the present experimental knowledge about lipid-independent statin effects and the current evidence for their existence in humans as well as several safety issues, aims at examining the question, if it is the proper time (already) to widen the indications for this class of drugs beyond their primary indication as effective cholesterol-lowering agents in patients with hyperlipidemia.

Lipid-independent statin effects in vitro and in animal models

Effect on endothelial function

One of the best documented non-lipid effects of statins is improvement in parameters associated with endothelial function. The complexity of the endothelium as an ‘organ’ and not a simple ‘barrier’ between the bloodstream and the intima has been reviewed elsewhere.20,21 We recently demonstrated that simvastatin preserves coronary endothelial function in experimental porcine hypercholesterolemia in the absence of any lipid lowering effect,22 which translates into preservation of myocardial perfusion response and coronary microvascular integrity during episodes of increased cardiac demand.23 In accordance with these results, pravastatin was shown to improve coronary endothelial function in cynomolgus monkeys, which were pretreated with an atherogenic diet for 2 years, independent of serum lipoprotein concentrations.24 Endothelial dysfunction is characterized by an imbalance between vaso-dilating and vasoconstricting substances, with an impairment of vasodilators such as nitric oxide (NO) and prostacyclin (PGI2) and a predominance of vasoconstrictors such as endothelin-1 (ET-1) and angiotensin II (Ang II). Indeed, the statin-induced improvement in endothelial function is likely achieved by both enhancement of vasodilator and attenuation of vasoconstrictor activity in the vascular wall (Fig. 2).

Effect on nitric oxide bioavailability

One of the major factors responsible for endothelial dysfunction appears to be a reduced stability of the endothelial enzyme generating NO, endothelial nitric oxide synthase (eNOS).25 In 1997 it was demonstrated that statins prevent hypoxia-induced downregulation of eNOS in human endothelial cells by stabilizing eNOS mRNA, leading to an increase in NO production by these cells.26 Subsequently, it was shown that statins exert their salutary effects on eNOS expression predominantly by posttranscriptional mechanisms which are mediated by blocking geranylgeranylation of the small GTP-binding protein Rho due to inhibition of the biosynthesis of GGPP.27,28 A recent report shed light on additional mechanisms involved in the statin-induced upregulation of eNOS activity by demonstrating that simvastatin rapidly activates the serine/threonine kinase Akt (also named protein kinase B) in endothelial cells, which in turn

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Table 1  Lipid-independent effects of the various statins in experimental (in vitro studies and in vivo animal studies) and clinical studies

<table>
<thead>
<tr>
<th>Experimental studies</th>
<th>Clinical studies</th>
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<tbody>
<tr>
<td>Improvement of endothelial function</td>
<td>A;183 C;184 (S, P, L, F158)</td>
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<tr>
<td>eNOS expression and activity ↑</td>
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<tr>
<td>NO synthesis ↑</td>
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<td>ET-1 synthesis ↓</td>
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<td>Antioxidant effects</td>
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<td>Antiinflammatory effects</td>
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<tr>
<td>LECA ↓</td>
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<tr>
<td>EC adhesion molecules ↓</td>
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<tr>
<td>LK adhesion molecules ↓</td>
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<tr>
<td>Proinflammatory cytokines ↓</td>
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<tr>
<td>MHC-II expression ↓</td>
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<td>Carrageenan-induced foot pad edema ↓</td>
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<td>Plaque modifying effects</td>
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<tr>
<td>VSMC proliferation and migration ↓</td>
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<td>VSMC apoptosis ↑</td>
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<td>Neointimal thickening ↓</td>
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<td>MØ proliferation and migration ↓</td>
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<td>MØ MMP expression and secretion ↓</td>
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<td>Antithrombotic effects</td>
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<td>Platelet activation ↓</td>
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<td>TF expression by macrophages ↓</td>
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<tr>
<td>SMC and EC PAI-1 expression ↓</td>
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<td>EC tPA expression ↑</td>
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<td>Antiangiogenic effects</td>
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<tr>
<td>EC migration ↓</td>
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<td>Vascular VEGF expression ↓</td>
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<td>Vascular MMP expression ↓</td>
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<tr>
<td>Proangiogenic (-vasculogenic) effects</td>
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<tr>
<td>EPC ↑</td>
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<tr>
<td>Cardioprotective, antihypertrophic effects</td>
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<tr>
<td>Cardiomyocyte hypertrophy ↓</td>
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<tr>
<td>Infarct size ↓ and cardioprotection ↑</td>
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Clinical studies suggesting only indirect evidence for the existence of a lipid-independent statin effect are in parenthesis, whereas those representing direct evidence are listed without parenthesis. S, simvastatin; P, pravastatin; L, lovastatin; F, fluvastatin; A, atorvastatin; C, cerivastatin; M, mevastatin; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ET-1, endothelin-1; LECA, leukocyte-endothelial cell adhesion; EC, endothelial cell; LK, leukocyte; MHC-II, major histocompatibility complex class II; VSMC, vascular smooth muscle cell; MØ, macrophage; MMP, matrix metalloproteinase; TF, tissue factor; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; EPC, endothelial progenitor cell.
leads to phosphorylation of eNOS resulting in an increase in its activity and enhanced NO production.30 Another recently discovered mechanism by which statins may increase endothelial NO production is their downregulating effect on cellular expression of caveolin-1, which acts as an inhibitor of eNOS activation by forming a heterocomplex with this enzyme.30 Interestingly, the inhibitory effect of atorvastatin on caveolin-1 abundance was observed at considerably lower drug concentrations (0.01 μM) than a promoting effect on eNOS expression (10 μM). However, atorvastatin reduced caveolin-1 expression, which was paralleled by restoration and/or potentiation of basal and stimulated NO production, irrespective of the extracellular cholesterol concentration, this effect was dependent on the inhibition of intracellular cholesterol synthesis and thus, cannot be considered lipid-independent.30 Moreover, the capacity of atorvastatin to reduce caveolin-1 expression may vary from one endothelial cell type to another.31 On the other hand, atorvastatin was also shown to promote the interactions between heat shock protein 90 (Hsp90) and both the protein kinase Akt and eNOS, which are essential for Akt-mediated eNOS activation.30,31 Although the exact mechanism responsible for this effect is unknown, it appears to involve atorvastatin’s ability to inhibit intracellular mevalonate synthesis as well as changes in intracellular calcium concentration.31 The potential clinical importance of all these observations was underscored by the finding that prophylactic treatment of normocholesterolemic mice with statins increased cerebral blood flow, reduced cerebral infarct size and improved neurological function via a NO-mediated mechanism.32 In...
addition, statins were shown to be cardioprotective in animal models subjected to global myocardial ischemia and reperfusion, an effect, again, mediated by an enhanced endothelial release of NO.\textsuperscript{33,34} Other experimental studies in animals\textsuperscript{35,36} and human cells\textsuperscript{37} have confirmed the statins’ lipid-independent ability to upregulate eNOS expression. Notably, the increase of eNOS protein in endothelial cells in response to statin therapy was established to be associated with enhanced release of NO.\textsuperscript{33,38,39}

Taken together, there is now clear evidence from experimental studies that increasing endothelial NO release is a common property of statins which is independent of their impact on cholesterol synthesis.

**Effect on endothelin-1 synthesis**

Although impaired endothelial NO activity is a key variable in the development of endothelial dysfunction and atherosclerosis,\textsuperscript{25,40} vascular reactivity is very complex and involves many substances besides NO. Indeed, normal vascular tone and changes of vascular diameter in order to adapt perfusion to metabolic needs are dependent on a fine balance between vasodilating and vasoconstricting substances. One of the important vasoconstrictory regulators of the vasculature is the peptide ET-1 which is synthesized in endothelial cells. Increased tissue ET-1-like immunoreactivity was found in active coronary atherosclerotic plaques indicating that this peptide might contribute to the exaggerated vasoconstriction associated with acute coronary syndromes.\textsuperscript{41} On the other hand, circulating ET-1 levels were found to be elevated in patients with both advanced atherosclerosis and coronary endothelial dysfunction.\textsuperscript{42,43} The importance of ET-1 in the very early stages of coronary atherosclerosis is highlighted by recent studies demonstrating that endothelin receptor antagonists prevent the development of endothelial dysfunction in experimental hypercholesterolemia and hypertensive humans.\textsuperscript{44–46} Moreover, endothelin receptor antagonism has been shown to reduce atherogenesis in apolipoprotein E-deficient\textsuperscript{47} and LDL receptor-deficient\textsuperscript{48} mice. Vascular ET-1 production is inhibited by endothelium-derived NO.\textsuperscript{49} Thus, statins may attenuate ET-1 synthesis indirectly by increasing NO bioavailability, as described above. On the other hand, it was demonstrated that both atorvastatin and simvastatin reduce the expression of pre-pro-endothelin-1 mRNA and the synthesis of ET-1 in a concentration- and time-dependent manner in bovine aortic endothelial cells in vitro.\textsuperscript{37} This effect was reversed by mevalonate but not by cholesterol, indicating that it is exerted by the inhibitory action of statins on products of the mevalonate metabolism other than cholesterol. Subsequently, it was shown that geranylgeranylation and the presence of Rho proteins are essential for pre-pro-endothelin-1 gene expression,\textsuperscript{50} supporting the hypothesis that the inhibition of isoprenoid synthesis is the main mechanism of action involved in the regulation of ET-1 production by statins. Moreover, the fact that atorvastatin and simvastatin did not affect basal eNOS expression or activity in endothelial cells in the former study, although other investigators reported an increase in basal NO release by cultured endothelial cells in response to atorvastatin,\textsuperscript{30} and that the addition of the competitive NOS antagonist N\textsuperscript{G}-nitro-l-arginine methyl ester (L-NAME) did not revert the inhibitory effect of simvastatin on pre-pro-endothelin-1 gene expression in the latter study, prove that the observed effect of statins on ET-1 synthesis is independent of their impact on NO bioavailability. Given the involvement of ET-1 in the development of endothelial dysfunction and the growing evidence for a contribution of ET-1 to various cardiovascular disease states\textsuperscript{51} these findings suggest a possible role for statins in the therapy of disease states associated with elevated ET-1 levels.

**Antioxidant effect**

Oxidative stress is considered to play a major role in the development of endothelial dysfunction and atherosclerosis. Reactive oxygen species (ROS) and other radicals are involved in the degradation of NO, thereby contributing to a decrease in its bioavailability and to endothelial dysfunction.\textsuperscript{52} We recently demonstrated that simvastatin significantly attenuates the increase in plasma levels of F2-isoprostanes (8-epi-PGF\textsubscript{2α}) and malondialdehyde measured by the thiobarbituric acid-reactive substances (TBARS) assay, both markers of increased oxidative stress in vivo, associated with experimental hypercholesterolemia in the absence of any lipid lowering effect.\textsuperscript{22} For fluvastatin, lipid-independent scavenging of ROS and a reduction of superoxide anion (O\textsubscript{2–})-formation has been shown in vitro and in vivo.\textsuperscript{53–55} In addition, atorvastatin, pravastatin, and cerivastatin may inhibit the NADPH oxidase-dependent O\textsubscript{2–} forming capacity of endothelial cells by preventing isoprenylation of the small GTP-binding protein Rac, which is essential for NADPH activation in vitro and in vivo.\textsuperscript{56–58} Moreover, it was demonstrated that atorvastatin upregulates catalase expression both in rat vascular...
smooth muscle cells (VSMCs) in vitro and in normo-
cholesterolemic, spontaneously hypertensive rats in vivo.58 In the in vitro setting this effect was
inhibited by the addition of L-mevalonate but not 25-hydroxycholesterol, emphasizing that this anti-
oxidant effect exerted by atorvastatin is partly
mediated by a reduction of isoprenoid metabolites
of the mevalonate pathway. Furthermore, a dose-
dependent inhibitory effect on LDL and HDL oxida-
tion was demonstrated for simvastatin.59 Similarly,
chronic administration of fluvastatin and lovastatin
to hypercholesterolemic patients was shown to
reduce the ex vivo susceptibility of LDL to oxida-
tion, which was thought to be partly mediated by
direct binding of the drugs to the phospholipid
fraction of LDL.60 Interestingly, in a recent study
using several oxidation systems, certain ator-
vastatin metabolites but not the parent compound
were found to exert an inhibitory effect on lipo-
protein oxidation in vitro.61 In the same study,
drug-induced inhibition of HDL oxidation was
associated with preservation of HDL-related
paraoxonase, which per se possesses antioxidan-
t properties.61 Moreover, simvastatin was found to
inhibit the ability of activated macrophages to
oxidize LDL in vitro in a dose-dependent manner, an
effect that was reversed by mevalonate.62 Although
not proven in this study, but given the involvement
of isoprenylated small GTP-binding proteins in the
intracellular signal transduction during macrophage
activation, it can be speculated that this particular
antioxidant effect of simvastatin is mediated by
inhibition of the synthesis of isoprenoid inter-
mediates of the mevalonate pathway. Finally, it is
noteworthy that because NO and O2 interact chemi-
cally to neutralize each other, an increase in the
local concentration of O2 is associated with a de-
crease in the concentration of biologically active
NO.56 Thus, the antioxidant properties of statins
may potentiate their effect on NO bioavailability
and vice versa.

Antiinflammatory effect

The interaction between blood leukocytes and the
vascular endothelium represents a crucial inflam-
matory step in the atherogenic process.63 Increased
leukocyte-endothelial cell adhesion (LECA) is
present in hypercholesterolemia64 and can be
induced experimentally by systemic administration
of oxidized LDL.65 Enhanced LECA results from
increased expression of adhesion molecules, such as
P-selectin and intercellular adhesion molecule-1
(ICAM-1), on the surface of endothelial cells.66 It is
known that a decrease in the basal release of
NO from endothelial cells is associated with an
increase in endothelial cell adhesion molecule expres-
sion.64,66-68 A few years ago, it was demon-
strated that fluvastatin significantly attenuates the
LECA responses to platelet-activating factor (PAF)
and leukotriene B4 (LTB4), both leukocyte activat-
ing pro-inflammatory stimuli, in a hypercholes-
terolemic rat model independent of any lipid
lowering effect.69 Furthermore, simvastatin was
shown to attenuate LECA in apolipoprotein
E-deficient, hypercholesterolemic mice in a lipid-
dependent manner.70 Recently, these findings
were extended by demonstrating a significant
reduction of leukocyte-endothelial cell interactions
with simvastatin in a normocholesterolemic rat
model in vivo, which was at least partly mediated
by attenuated up-regulation of P-selectin on
endothelial cells.71 Moreover, it has been shown
that the small GTP-binding protein Rho is essential
for integrin-dependent adhesion of leukocytes to
the endothelium.72 Because geranylgeranylation is
required for Rho activation it can be speculated
that statins modulate LECA at least in part by
inhibition of geranylgeranylation of this protein.73

Beside these antiinflammatory effects on endo-
theelial cell adhesion molecules, statins seem to
exert similar effects on leukocyte adhesion mol-
ecules. Fluvastatin was shown to inhibit adhesive
interaction between monocytes and human umbilici-
ocal vein endothelial cells (HUVECs) by lowering the
expression of LFA-1 and ICAM-1 on monocytes,74
and addition of lovastatin to isolated human mono-
cytes led to a significant reduction of surface
expression of CD11b, which in turn was associated
with decreased CD11b-dependent adhesion of
monocytes to HUVECs.75 Co-incubation with
mevalonate, but not with LDL, reversed the
lovastatin effect, suggesting a crucial role for early
cholesterol precursors of the mevalonate pathway
for the inhibitory effect of statin on integrin
expression and LECA.75 Furthermore, it has been
demonstrated that treatment with simvastatin is
associated with attenuation of CD18 up-regulation
in polymorphonuclear leukocytes (PMNs) in response
to stimulation with LTB4 in normocholesterolemic
rats.33 Recently, cerivastatin was shown to reduce
monocyte adhesion to vascular endothelium under
physiological flow conditions via downregulation of
integrin adhesion molecules (CD11a, CD18, and
VLA-4) and inhibition of actin polymerization via
prevention of Rho translocation to the mem-
brane.76 Thus, statins may affect LECA by various
mechanisms, which depend on their ability to
inhibit HMG-CoA reductase but are independent
of cellular cholesterol biosynthesis. However, very
recently, a novel mechanism of action, which is
unrelated to statin-mediated inhibition of HMG-CoA reductase was found to contribute to the antiinflammatory potential of statins. As pointed out earlier, it was noted that statins, such as lovastatin, block LFA-1 mediated adhesion and co-stimulation of lymphocytes via direct binding to a specific site within LFA-1.18

Another antiinflammatory action demonstrated for several statins is the reduction of the production of pro-inflammatory cytokines. In one study77 fluvastatin and pravastatin were found to significantly inhibit Ang II-induced secretion of interleukin-6 (IL-6) in cultured human VSMCs, whereas in another study78 fluvastatin and simvastatin but not pravastatin reduced production of IL-6 and interleukin-1b (IL-1β) in HUVECs. Moreover, it was found that the reduction of the expression of several pro-inflammatory mediators, such as IL-6 and monocyte chemotactrant protein-1 (MCP-1), exerted by lovastatin in an in vivo model of local acute inflammation is dependent on the impairment of the biosynthesis of non-sterol derivatives arising from the mevalonate pathway.79

In addition, pravastatin and cerivastatin have been shown to inhibit the increased expression of MCP-1 and transforming growth factor-β1 (TGF-β1) associated with chronic eNOS inhibition in rat hearts.80 Importantly, this effect was associated with an upregulation of vascular eNOS protein levels and a consecutive increase in NO bioavailability, indicating again an antiinflammatory role for NO. The observation that similar doses of atorvastatin and pravastatin produced a similar reduction of MCP-1 expression in different arterial vascular beds despite significant differences in their plasma lipid-lowering potential in hypercholesterolemic pigs further supports the existence of such lipid-independent antiinflammatory statin effects in vivo.81

Important support for the existence of lipid-independent antiinflammatory statin properties in vivo, stems from a recent study demonstrating a dose-dependent reduction of carrageenan-induced foot pad edema in mice, which were given single-dose oral simvastatin 1 hour before carrageenan injection.82 Notably, the magnitude of this acute antiinflammatory effect of simvastatin was comparable to that of indomethacin. Moreover, the potent inhibitory effect of simvastatin on foot pad swelling was observed within 4 hours, well before any plasma lipid changes could occur, indicating that simvastatin has specific antiinflammatory properties beyond its lipid-lowering potential.

Furthermore, atorvastatin, lovastatin, and pravastatin were shown to repress interferon γ (IFNγ)-induced expression of major histocompatibility complex class II (MHC-II) molecules on various cell types.83 This effect, suggesting an immunomodulatory role for statins, was limited to antigen-presenting cells requiring co-stimulation by IFNγ, whereas antigen-presenting cells constitutively expressing MHC-II, such as B-cells and dendritic cells, were not affected. Further evidence for an immunomodulatory role for statins stems from a study demonstrating that pravastatin may exert a synergistic effect with cyclosporine regarding the inhibition of cytotoxic T lymphocyte activity in vitro.84

The finding that increased levels of C-reactive protein (CRP), the classic acute-phase reactant and a sensitive marker for inflammation, may predict the risk of developing myocardial infarction,85 and measurement of CRP may provide a useful method of assessing the cardiovascular risk in apparently healthy persons,86 has fueled the interest in this peptide regarding its role in atherogenesis. Importantly, it was recently reported that CRP per se has biological activity by demonstrating that CRP led to a significant increase in the expression of several adhesion molecules and cytokines in endothelial cells.87,88 Notably, these investigators also showed that simvastatin but not aspirin may inhibit this pro-inflammatory effect of CRP.88

In summary, there is now ample experimental evidence indicating that statins may exert a variety of inhibitory effects on inflammatory processes, and that their antiinflammatory potential seems to be mediated to a considerable part independent of cholesterol lowering. In addition, their inhibitory effect on the expression of MHC-II on various cells and the synergistic effect of pravastatin with cyclosporine on cytotoxic T lymphocyte activity suggests an additional immunomodulatory function for this class of drugs. Thus, granted that similar effects also exist in humans and considering the inflammatory nature of atherogenesis89 it can be speculated that the anti-inflammatory and immunological properties of statins might represent one of the important actions of these drugs.

**Effect on macrophage activation and proliferation**

Plaque composition and stability, rather than its volume or the severity of stenosis, are the most important determinants of atherosclerotic complications.90 Unstable plaques, which are prone to plaque fissuring or disruption with superimposed thrombosis, are characterized by a thin fibrous cap,
a high lipid content, few smooth muscle cells, and excess macrophages in the cap.\textsuperscript{91} Macrophages play a key role in destabilizing the plaque by their ability to degrade the extracellular matrix within the plaque by phagocytosis or by production and secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs).

Statins may influence plaque composition by inhibition of cholesterol accumulation in monocyte-derived macrophages either by reducing the availability of free cholesterol, or by reduction of the synthesis of mevalonate and its derivates which are required for cholesterol esterification.\textsuperscript{92} In addition, their antioxidant and antiinflammatory qualities may also contribute to plaque stability. Furthermore, additional not lipid-associated properties of statins primarily affect macrophage activity and plaque stabilization. Interestingly, simvastatin was demonstrated to dose-dependently inhibit migration and MMP-9 secretion of the human monocytic cell line THP-1, an effect that was reversed by the simultaneous addition of mevalonate and its derivates, FPP and GGPP.\textsuperscript{93} Also, fluvastatin and simvastatin decrease secretion of MMP-9 by human and mouse macrophages in culture by their inhibitory action on the mevalonate pathway.\textsuperscript{94}

In conclusion, these macrophage-inhibitory properties, which are due to the inhibitory effect of statins upon protein isoprenylation, suggest a plaque-stabilizing potential for statins that is independent of their lipid-lowering qualities.

**Effect on smooth muscle cell proliferation and apoptosis**

Proliferation and migration of VSMCs are pivotal events in atherogenesis, the pathogenesis of postangioplasty restenosis, venous graft occlusion, and transplant vasculopathy.\textsuperscript{89,95} The isoprenoids generated in the mevalonate pathway are required for both VSMC proliferation and growth.\textsuperscript{96,97} In vitro studies demonstrated that most statins may decrease VSMC proliferation\textsuperscript{96,98,99} and migration\textsuperscript{99,100} in a dose-dependent manner. The non-lipid nature of this effect was first confirmed by the observation that addition of mevalonate and/or certain isoprenoids but not that of squalene, ubiquinone, or LDL prevented the antiproliferative effect of the statins tested.\textsuperscript{96,99,101} Finally, it was shown that statins inhibit platelet derived growth factor (PDGF)-induced VSMC proliferation mainly by reducing isoprenylation of the small GTP-binding protein Rho which is essential for VSMC replication,\textsuperscript{102} although other yet unknown mechanisms seem also to be involved.\textsuperscript{103} Intriguingly, the kinetics of statin-induced inhibition of SMC cholesterol synthesis and inhibition of SMC proliferation seem to differ significantly, as was shown in a study investigating the effect of adding whole-blood sera from fluvastatin-treated patients to cultured human SMCs.\textsuperscript{104} In this study, sera collected 1 hour after the last drug dose led to maximal reduction of cellular cholesterol synthesis, whereas the inhibitory effect on cell growth was most pronounced when sera collected 6 hours after the last drug dose (when fluvastatin concentrations were below the detection limit) were added to the culture media.

Interestingly, compared to the significant antiproliferative potency of cerivastatin and to lesser extent simvastatin, fluvastatin, lovastatin, and atorvastatin, a minimal inhibitory effect on VSMC proliferation has been shown for pravastatin in vitro.\textsuperscript{98,101} Interestingly, similar findings were achieved regarding the impact of statins on VSMC apoptosis. Atorvastatin, simvastatin, and lovastatin, but not the hydrophilic pravastatin may induce VSMC apoptosis in a dose-dependent manner in vitro, an effect that is primarily mediated by the inhibition of protein prenylation.\textsuperscript{97,105} In accordance with these results are in vivo findings demonstrating that lovastatin, simvastatin, and fluvastatin, but not pravastatin, decreased neointimal thickening in a carotid artery model of neointimal proliferation in normcholesterolemic rabbits.\textsuperscript{106} At present, there is no clear explanation for the different antiproliferative and pro-apoptotic behaviour among the various compounds. However, given the hydrophilic nature of pravastatin compared to the other more lipophilic statins, the different effects might be attributed to differences in their ability to penetrate the cell.

**Effect on platelets and the coagulation/fibrinolytic system**

Platelets of hypercholesterolemic patients are more sensitive to aggregating agents than those of normcholesterolemic subjects.\textsuperscript{107} One of the reasons for this observation is an increase in the adenosine diphosphate-induced fibrinogen binding to platelets mediated by LDL in a dose-dependent manner.\textsuperscript{108} Thus, statins may inhibit platelet aggregation by lowering LDL levels in vivo. On the other hand, statins may affect platelet function by changing the cholesterol content of platelet membranes, which alters membrane fluidity.\textsuperscript{109} Among its various beneficial effects NO also inhibits platelet aggregation in vivo and in vitro.\textsuperscript{110,111}
Considering their effect on endothelial NO production, statins may inhibit platelet aggregation indirectly by an increase in NO bioavailability independent of cholesterol levels. In addition, atorvastatin has been shown to upregulate eNOS in thrombocytes and to decrease platelet activation in mice in vivo without affecting cholesterol levels.\(^{112}\) Moreover, statins decrease the levels of isoprostanes, such as 8-epi-PGF\(_{2\alpha}\),\(^{28}\) which are markers of oxidative stress and potent platelet activators, and may thereby inhibit platelet aggregation via their antioxidant effects.\(^{113}\)

In addition to their antiplatelet actions, statins may also exert antithrombotic activities mediated by changes of the coagulation system. For example, simvastatin,\(^{114,115}\) fluvastatin,\(^{115}\) and cerivastatin\(^{116}\) were shown to reduce expression of tissue factor (TF) by cultured human monocyte/macrophages in a dose-dependent manner, an effect that was reversed by co-incubation with mevalonate or all-trans-geranylgeraniol but not cholesterol, indicating its dependence on statin-induced reduction of intracellular GGPP biosynthesis.\(^{115}\) Moreover, it was demonstrated that fluvastatin-induced inhibition of bacterial lipopolysaccharide-stimulated TF gene expression in human monocytes/macrophages is likely mediated via inhibition of the activation of nuclear factor-κB, a pivotal transcription factor involved in the regulation of various inflammatory processes, including atherogenesis.\(^{115,117}\)

Furthermore, statins may shift the fibrinolytic balance within the vessel wall towards increased fibrinolytic activity. Simvastatin was recently shown to inhibit the expression of plasminogen activator inhibitor-1 (PAI-1) from human VSMCs and endothelial cells, while increasing the expression of tissue-type plasminogen activator (tPA) from endothelial cells.\(^{118}\) More light was shed on the mechanism involved in the upregulation of the fibrinolytic potential of endothelial cells in a study demonstrating that lovastatin increases tPA activity and decreases PAI-1 activity in a rat endothelial cell line in a time- and concentration-dependent manner.\(^{119}\) In this study, the lovastatin-induced modification of the endothelial fibrinolytic activity was found to be due to inhibition of Rho geranylgeranylation and disruption of cellular actin filaments.\(^{119}\)

Taken together, the combination of antiplatelet, anticoagulatory, and pro-fibrinolytic effects observed in vitro suggests a particular role for statins in the therapy of disease states associated with an increased thrombogenicity, such as acute coronary syndromes.

Effect on neovascularization

Angiogenesis, the formation of new blood vessels by sprouting of preexisting vessels, is involved in both physiological and pathological processes. On the one hand, angiogenesis is a primary response to local tissue hypoxia,\(^{120}\) and is likely involved in the restitution of blood flow in ischemic diseases such as CHD and occlusive peripheral artery disease. On the other hand, angiogenesis is the hallmark of some diseases like cancer, and excessive vascularization is now considered to be associated also with atherosclerotic plaque formation.\(^{121}\) The process of angiogenesis is very complex and depends on the interaction of several pro- and anti-angiogenic molecules to form functional vessels.\(^{122}\) Vascular endothelial growth factor (VEGF), although representing only one component of the complex angiogenic response, is considered one of the key growth factors involved in angiogenesis.\(^{120}\) Plasma VEGF levels are elevated in patients with hyperlipidemia with or without established atherosclerosis, and lipid lowering with either fluvastatin or fenofibrate results in a significant reduction of VEGF levels.\(^{123}\) Interestingly, VEGF administration is associated with enhanced atherosclerotic plaque progression and increased plaque macrophage and endothelial cell content in cholesterol-fed, apolipoproteinE/apolipoproteinB100-deficient mice,\(^{124}\) suggesting a possible role for VEGF reduction in the prevention of plaque progression and plaque stabilization. In this context, we have recently demonstrated that simvastatin prevents the increase in coronary tissue VEGF expression associated with experimental hypercholesterolemia in a lipid-independent fashion.\(^{125}\) Another important component of the angiogenic machinery are MMPs that are responsible for extracellular proteolysis.\(^{126}\) Fluvastatin, simvastatin, and cerivastatin also inhibit secretion of MMPs by macrophages in vitro and in vivo.\(^{94,116}\) In addition, we showed that simvastatin prevents accentuation of MMPs in the coronary artery wall in a porcine model of hypercholesterolemia in the absence of any change of plasma cholesterol levels.\(^{120}\) Furthermore, statins inhibit endothelial cell migration, possibly by a reduction of Rho geranylgeranylation.\(^{127}\) Finally, our finding that simvastatin prevents vasa vasorum neovascularization in experimental hypercholesterolemia independent of plasma lipid-lowering demonstrates that the inhibitory effects of statins on single components of the angiogenic machinery may translate into an anti-angiogenic effect in vivo.\(^{125}\)

A new mechanism of action for statins was suggested by demonstrating that atorvastatin therapy
led to an early increase in the number and the functional activity of circulating endothelial progenitor cells (EPCs) in patients with stable CHD.\textsuperscript{128} EPCs are bone marrow derived cells that home to sites of neovascularization and differentiate into endothelial cells in situ. Very recently, it was shown that statins induce EPC mobilization from the bone marrow and EPC differentiation via the serine/threonine kinase Akt signaling pathway.\textsuperscript{129,130} Thus, the in situ formation of new blood vessels involving EPCs, or ‘vasculogenesis’, is not limited to the developing embryo but may also occur in the adult organism. Interestingly, one of the important pathophysiological mediators of this process could be VEGF, which promotes EPC mobilization and differentiation in vivo.\textsuperscript{131,132} Obviously, several questions still remain to be answered, such as identification of possible mediators responsible for these angiogenic responses or the role of NO in statin-dependent angiogenesis and vasculogenesis.\textsuperscript{133} As mentioned earlier, simvastatin may enhance eNOS activity and NO production by activating the protein kinase Akt in endothelial cells.\textsuperscript{29} This effect was associated with the induction of angiogenesis in ischemic limbs of normocholesterolemic rabbits\textsuperscript{29} and endothelial NO has been previously implicated in postnatal neovascularization.\textsuperscript{134,135} Thus, statins might also promote angiogenesis indirectly via upregulation of NO production. On the other hand, inhibition of NO-synthesis does not prevent statin-induced increase in EPC numbers in vitro,\textsuperscript{129} arguing against a significant contribution of NO to vasculogenesis.

In summary, statins have the potential to both inhibit and promote neovascularization. Indeed, a recent study\textsuperscript{136} shed more light on the angiogenic aspect of this dual statin role by demonstrating that certain statins exert a biphasic, dose-dependent effect on angiogenesis both in vitro and in vivo. In cultured human endothelial cells, low concentrations of cerivastatin and atorvastatin enhanced endothelial cell proliferation, migration, and differentiation, whereas these parameters as well as endothelial VEGF release were significantly inhibited and endothelial cell apoptosis was increased by higher drug concentrations. These effects of high statin doses on endothelial cells were reversed by the addition of GGPP indicating its lipid-independent nature. In the same study, inflammation-induced angiogenesis in a murine model was enhanced by low-doses of cerivastatin and atorvastatin but inhibited by high-dose statin therapy. On the other hand, the possibility that different vascular beds may show a differential angiogenic response to statins must also be considered.\textsuperscript{137}

### Effect on myocardial hypertrophy, fibrosis, and cardiomyocyte protection

Myocardial hypertrophy and fibrosis represent the non-specific result of an adaptive process in response to various kinds of myocardial injury, and are major determinants of morbidity and mortality in acquired cardiovascular disease.\textsuperscript{138} Neurohormonal activation including an increase in Ang II plays a key role in development of cardiac hypertrophy and fibrosis. Recently certain statins have been shown to inhibit Ang II-mediated cardiac hypertrophy and fibrosis,\textsuperscript{139–141} as well as to block various intracellular signaling pathways involved in cardiac hypertrophy including downregulation of the activity of small GTP-binding proteins of the Rho family.\textsuperscript{50,142–144} A recent study demonstrated that simvastatin induces regression of cardiac hypertrophy and fibrosis in a non-hypercholesterolemic transgenic rabbit model of human hypertrophic cardiomyopathy, which was associated with an improvement in cardiac systolic and diastolic function.\textsuperscript{145} Today, the primary abnormality in this disease is thought to be impaired myocyte function due to mutant contractile proteins, which in turn leads to activation of intracellular signaling molecules and development of cardiac hypertrophy and fibrosis, a process similar to the one observed in other forms of heart failure.\textsuperscript{146} Hence, these results may suggest a role for statins in the treatment and prevention of various forms of cardiovascular disease associated with cardiac hypertrophy and fibrosis. This notion is supported by the observation that cerivastatin was recently demonstrated to improve left ventricular remodeling and function, which was associated with attenuated myocardial expression of collagen I and fetal myosin heavy chain isoenzymes, in a rat model of myocardial infarction-induced chronic heart failure.\textsuperscript{147}

Several years ago, simvastatin has been shown to dose-dependently delay anoxia-induced necrosis of cultured neonatal cardiomyocytes, an effect which has been attributed to inhibition of the sarcolemmal Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, as simvastatin attenuated the increase in intracellular calcium associated with anoxia.\textsuperscript{148} In addition, pravastatin was shown to preserve the infarct size-limiting effect of ischemic preconditioning, that is blunted by hypercholesterolemia, in a hypercholesterolemic rabbit model without normalizing serum cholesterol.\textsuperscript{149} Moreover, as mentioned earlier, several studies confirmed that statins may exert a cardioprotective...
effect in animal models subjected to global myocardial ischemia and reperfusion, which is mostly mediated via enhancement of endothelial NO release. In accordance with these findings are the results of a recently published study, reporting a significant reduction of myocardial lesions, which were induced by acute inhibition of NO biosynthesis, with pravastatin pretreatment in normocholesterolemic rats. Considering that CHD represents the most common cause of chronic heart failure and given the observed effects of statins on myocardial hypertrophy and fibrosis as well as their beneficial properties on ischemic myocardium, it can be speculated that these statin properties, if present in humans, could translate into a major benefit regarding the development of heart failure.

Lipid-independent statin effects in humans

Problems of translating results of experimental studies to clinical practice

Many of the non-lipid effects of statins found in vitro affect processes that are involved in determination of plaque stability. Improvement in endothelial function, reduction of inflammation, and reduction of coagulation and thrombogenicity potentially all contribute to a decreased risk of plaque rupture and its thrombotic complications, which appears to underlie the majority of clinical coronary events. Given the event reduction observed in the large statin trials, it is tempting to assume that the beneficial statin effects found in the experimental setting also exist in clinical practice. However, though some non-lipid statin effects shown in vitro were achieved using nanomolar drug concentrations, a number of in vitro experiments have used concentrations of statins considerably higher than clinically achievable doses in patients. Moreover, different to the clinical setting, most in vivo animal studies have reported these phenomena either at levels of statin exposure too low to modify serum lipids or in models where lipid levels are non-responsive to HMG-CoA reductase inhibition. Hence, the results of in vitro or animal studies are difficult to translate directly to clinical practice, and clinical trials are needed to estimate the clinical significance of the encouraging experimental results. However, the specific statin properties complicate the clinical assessment of non-lipid effects. Modified LDL represents a key mediator of the atherogenic process. Indeed, lowering of serum cholesterol levels per se results in an improvement in many functional abnormalities in hypercholesterolemic individuals. For example, a single LDL apheresis may acutely improve endothelial function in hypercholesterolemic humans, an effect that is mainly mediated by a decrease in oxidative stress or oxidative byproducts. Thus, the improvement in coronary endothelial function observed in hypercholesterolemic patients with or without coronary atherosclerosis in response to lipid-lowering therapy with either lovastatin or pravastatin could be entirely attributed to the lipid-lowering potency of the statins used. This example points out the difficulty in identifying non-lipid statin effects in clinical studies. Because of their excellent lipid-lowering potential, statins always modify serum cholesterol levels to some extent and, thus, it is impossible to differentiate possible lipid-independent effects from those associated with lipid reduction, especially if these effects are complementary. Further, analysis of the basic mechanisms behind an observed statin effect (e.g. addition of isoprenoid intermediates to further define the pathways involved in the mechanism responsible for a specific statin effect) may not be possible in the in vivo setting. The only way to directly assess effects unrelated to modification of plasma cholesterol levels is to study the very short-term statin effects which appear within the first days or even hours after initiation of therapy before changes of plasma cholesterol levels occur. However, it should be kept in mind that there is no guarantee that such short-term effects are extended in chronic long-term therapies. On the other hand, we can only assume the existence of non-lipid effects in humans by indirect evidence, by comparing groups which show similar cholesterol levels despite presence or absence of statin treatment, or by treating normocholesterolemic patients with these drugs.

Indirect evidence for the existence of lipid-independent statin effects in humans

In a post hoc analysis of the WOSCOPS trial, investigators found, by comparing event rates in two subgroups of individuals whose on-treatment LDL cholesterol levels were in the same range (140 to 180 mg dl⁻¹ or 3.62 to 4.65 mmol . l⁻¹) independent of receiving either pravastatin or placebo, that event rates for the two subgroups differed markedly. Subjects treated with pravastatin had a 36% (CI, 9% to 56%) lower risk (P=0.014) than those receiving placebo. Moreover, application of the Framingham risk equation produced a coincidence between predicted and observed coronary event
rate in the placebo group but underestimated the benefit of pravastatin therapy by 31%. Taken together, these data indicate that the benefit observed with pravastatin in moderately hypercholesterolemic men without previous history of CHD cannot be explained by a decrease in LDL alone.

Indirect evidence for the existence of a lipid-independent effect of statins on endothelial function in humans is suggested by a study comparing peripheral endothelial function, as measured as flow-mediated (endothelium-dependent) brachial arterial dilation (FMD), in a group of 23 men with CHD without lipid-lowering medication with that in 22 age- and blood pressure-matched CHD patients with similar lipid levels despite ongoing statin therapy.\(^{158}\) In this study, FMD of the brachial artery was significantly higher in patients receiving statins than in those without any treatment. Moreover, multivariate regression analysis revealed statin use as the only significant predictor of FMD.

In contrast to in vitro studies, two clinical trials failed to demonstrate an effect of pravastatin\(^{159}\) or fluvastatin,\(^{160}\) respectively, on plasma ET-1 levels. The reason for this discrepancy between the results of the in vitro and in vivo studies is not clear but might be due to the different experimental situations or due to intraclass differences between the various statins.\(^{161}\) On the other hand, ET-1 is predominantly secreted abluminally by endothelial cells and plasma ET-1 levels may therefore not entirely reflect its tissue levels.\(^{162}\) Nevertheless, this example points out the problems associated with translating the results of experimental studies to clinical practice.

The existence of lipid-independent antiinflammatory effects of statins in humans is suggested by the finding that several statins were shown to reduce plasma CRP levels in vivo independently of their effect on serum cholesterol.\(^{86,163,164}\) Moreover, a role for an antiinflammatory potential of statins or due to intraclass differences between the various statins.\(^{161}\) On the other hand, ET-1 is predominantly secreted abluminally by endothelial cells and plasma ET-1 levels may therefore not entirely reflect its tissue levels.\(^{162}\) Nevertheless, this example points out the problems associated with translating the results of experimental studies to clinical practice.

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in the acute graft rejection rate in fluvastatin-treated kidney transplant recipients.希望, results of the much larger ‘Assessment of Lescol in Renal Transplantation’ trial would provide a more conclusive answer regarding the possible immunomodulatory properties of statins in transplant recipients.

The role of statins as coagulation modifying agents in vivo is also not clear. Recently and similar to in vitro findings, it was demonstrated that simvastatin reduces the rate of thrombin generation and the expression of TF by monocytes in vivo, an effect that was only in part related to the reduction of serum cholesterol levels. In vivo studies focusing on the effect of statins upon fibrinogen levels have demonstrated inconsistent results. While some studies reported no change or a decrease in fibrinogen levels during treatment with various statins, others found significant increases. The exact reason for these variable results is not clear, although they have been attributed to different actions on fibrinogen-regulating cytokines among the various statin compounds, genetic variation of study populations and measurement variability. To overcome such confounding factors, Rosenson and co-workers recently reported the results of a randomized crossover study which tested the effect of various statins (atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin) on fibrinogen levels in hypercholesterolemic patients with CHD. In this study, using a standardized blood sampling and analysing technique, no effect on plasma fibrinogen levels was found for either of the statins tested, indicating that statins have neither a lipid-dependent nor a lipid-independent effect on fibrinogen levels. The effect of statins on platelet aggregation in humans is also controversial. Even studies using the same compound demonstrated conflicting results. For example, although lipid lowering therapy with simvastatin has been shown to reduce thromboxane biosynthesis in vivo by 50% and to normalize platelet aggregation ex vivo in patients with hypercholesterolemia in one study, a randomized, double-blind, placebo-controlled crossover study of hypercholesterolemic men who were treated with simvastatin for 10 to 12 weeks did not show an effect on platelet function, despite a significant lipid-lowering effect. Interestingly, in a small, randomized, placebo-controlled crossover study, which included 19 hyperlipidemic patients, 1-month atorvastatin treatment (10 mg · day⁻¹) led to a significant increase in platelet eNOS levels. However, different to a study in mice showing a similar effect in the absence of any change in plasma lipid levels, atorvastatin administration to humans was associated with a significant decrease in plasma total cholesterol and LDL cholesterol levels, wherefore the exact mechanism for this potentially beneficial effect of atorvastatin on human platelet eNOS levels remains speculative. Thus, the existence of a clinically relevant lipid-independent effect of statins on platelet function in vivo is questionable. However, the possibility of a beneficial inhibitory effect of statins on activated platelets, as present in acute coronary syndromes, awaits further clarification.

Because of its well-defined mechanism and the fact that a similar effect has not been observed with simple lipid lowering, the increase in the number and functional activity of EPCs found with atorvastatin therapy in patients with CHD represents additional indirect evidence for the existence of non-lipid statin effects in humans.

**Direct evidence for the existence of lipid-independent statin effects in humans**

The most compelling evidence for the existence of clinically relevant lipid-independent effects in humans stems from two small trials investigating the acute effects of atorvastatin and cerivastatin respectively on endothelial function. In the first study an improvement in peripheral arterial endothelial function within 24 hours after initiation of high-dose (80 mg · day⁻¹) atorvastatin treatment was demonstrated in eight healthy, normocholesterolemic men. Withdrawal of atorvastatin was associated with an acute impairment of vascular function within 24 hours. Importantly, the effect of atorvastatin on endothelial function preceded changes in serum cholesterol levels by 24 hours indicating the presence of a cholesterol-independent statin effect in humans. In line with these results are findings of another study reporting a significant improvement in endothelial function of the brachial artery in 27 elderly normo- or mild hypercholesterolemic, diabetic patients after a 3-day therapy with cerivastatin (0.15 mg · day⁻¹) in the absence of any change of lipid profiles. This effect was associated with a significant increase in plasma levels of nitrite/nitrate and cGMP, the second messenger of NO, indicating that it was due to an increase in NO bioavailability. In addition, cerivastatin administration led to a reduction of oxidative stress, as measured by a significant decrease in plasma 8-isoprostane (8-epi-PGF₂α) levels, whereas there was a trend towards lower plasma levels of the inflammation parameters CRP and soluble vascular
These observations, representing direct evidence for the existence of beneficial lipid-independent effects in humans, are of great clinical importance considering that endothelial dysfunction represents the very early stage of atherosclerosis and its presence is associated with an adverse prognosis. Moreover, these immediate effects indicate that statins might be of particular benefit in acute coronary syndromes. Indeed, in the MIRACL trial, the first large-scale trial investigating the effects of early high-dose atorvastatin treatment in acute coronary syndromes, the relative risk for a primary composite endpoint, which included death, non-fatal acute myocardial infarction, cardiac arrest, and recurrent symptomatic myocardial ischemia, was reduced by 16% when atorvastatin therapy (80 mg day$^{-1}$) was initiated within 24 to 96 hours after an acute coronary syndrome. However, this benefit was due mainly to a reduction in recurrent myocardial ischemia, whereas the incidence of the more serious events was not affected. Thus, it is still too early to advocate statins as first-line treatment in acute coronary syndromes, but trials, such as the 'Aggrastat-to-Zocor' study, the 'Pravastatin or Atorvastatin Evaluation and Infection Therapy' study, and the 'Pravastatin in Acute Coronary Syndromes Trial', are under way to further define the role of statins in this setting.

**Potentially harmful lipid-independent statin effects and drug safety**

Given that certain non-lipid statin properties found in experimental studies also exist in humans the possibility that some of these effects might also be harmful in the clinical setting should not be disregarded (Fig. 3).

As pointed out earlier, inhibition of VSMC proliferation in vitro has been well documented for all clinically approved statins except pravastatin. On the one hand this effect might contribute to an attenuation of atherosclerotic plaque development and progression. On the other it might favor destabilization of...
established plaques because VSMCs represent the major source of the collagenous matrix which is essential for the strength of the fibrous cap of the plaque. Similar concerns have to be taken into account regarding the pro-apoptotic effect on VSMCs induced by several statins in vitro. Again, an increase in VSMC apoptosis might be beneficial in the control of neointimal thickening. However, an increased rate of VSMC apoptosis is also found in atherosclerotic lesions and represents a potential contributor to plaque rupture and a major determinant of thrombogenicity and may, therefore, promote the development of acute coronary events. However, again, it has to be noted that the in vitro effects of statins on VSMC proliferation and apoptosis were observed using statin concentrations far higher than those achieved with therapeutic doses in the clinical situation. Thus, the clinical importance of these findings, beneficial or harmful, is seriously questionable.

Another example of a double sided role of lipid-independent statin effects is their impact on neovascularization. As mentioned earlier, statins may potentially either promote or inhibit neovascularization. With respect to the reduction of organ ischemia an increase in new vessel formation may be beneficial. However, regarding the progression of atherosclerosis and the induction of plaque instability an increase in neovascularization may be potentially harmful because the neoformation of microvessels within the vessel wall or the plaque is now thought to contribute to plaque progression and destabilization. In this context it is obvious that inhibition of vessel wall neovascularization may limit plaque progression and contribute to plaque stabilization. In favor of the presence of a mainly inhibitory, and thus beneficial, effect of statins on vessel wall neovascularization in vivo is our recent finding that simvastatin preserves the structure of adventitial vasa vasorum in experimental hypercholesterolemia independent of cholesterol lowering. Moreover, as mentioned earlier, a biphasic effect on angiogenesis was shown for cerivastatin and atorvastatin. These drugs may promote new vessel formation at low concentrations, whereas higher doses inhibit angiogenesis in a lipid-independent manner. However, the effect of statins on blood vessel formation in humans is unclear which adds to the ongoing uncertainty about the long-term effect of statins on the occurrence of cancer, because neovascularization represents a conditio sine qua non for the development of malignant tumors. The concerns about a carcinogenic potential of statins were originally raised by reports that lipid-lowering drugs including statins increase the frequency of several cancers in rodents and refueled by an increased incidence of breast cancer among women taking pravastatin in the CARE trial. However, a recently published case-control study could not confirm an association between statin use and cancer. Furthermore, a meta-analysis of five large statin trials failed to detect an increased risk of fatal or non-fatal cancers in statin-treated individuals. These studies provided some degree of reassurance about the safety of this class of drugs. However, as recognized by the authors, follow-up periods of most trials (5 years) are considered too short for the cancer endpoint. In addition, meta-analyses may have some limitations and their results must be interpreted cautiously. Notably, several recent reports suggest that statins may eventually play a role in cancer prevention or even treatment. In particular, simvastatin, lovastatin, and pravastatin have been shown to selectively inhibit leukemic cell growth. Moreover, lovastatin may promote apoptosis in various tumor cells and high-dose cerivastatin therapy was shown to decrease tumor growth and vascularization in a murine lung cancer model. Given the paucity of long-term data on cancer development and the persisting uncertainty regarding possible carcinogenic effects of statins, ongoing close postmarketing surveillance and studies with longer follow-up are warranted. On the other hand, further studies will have to determine whether statins might represent a new approach to cancer treatment.

Finally, the observation that statin myotoxicity in cultured neonatal rat skeletal muscle cells is associated with selective inhibition of geranylgeranylation of three specific low-molecular-weight proteins raises the question about a possible contribution of lipid-independent statin effects to rhabdomyolysis, the rare but most serious side effect of statin therapy which has recently led to the withdrawal of cerivastatin from the market. However, despite this report, the pathophysiological background of statin-induced muscle damage remains obscure. For example, although statins were shown to decrease oxidative stress in a lipid-independent fashion, a recent study showed that the majority of patients treated with statins and suffering from muscular side-effects had elevated 8-isoprostane (8-epi-PGF₂α) levels in plasma and urine. Discontinuation of statin therapy or successfully changing to another statin compound resulted in normalization of 8-isoprostane levels in all these patients. These findings suggest a significant involvement of oxidative injury in the muscular side-effects
of statins. However, the mechanism and the functional significance of the 8-isoprostane increase in these patients remains to be determined.

In conclusion, although the majority of the currently known lipid-independent effects seems to be desirable, the possibility that some non-lipid effects of statins might be potentially harmful should always be considered.

**Variation of pleiotropy between statins or class effect?**

Different tissue selectivity was demonstrated between lipophilic statins (e.g. simvastatin, lovastatin) and the hydrophilic pravastatin.\(^{202,203}\) Whereas lipophilic compounds can penetrate cell membranes and enter cells in any organ, cellular uptake of pravastatin is dependent on the presence of a specific carrier-mediated mechanism.\(^{202,204}\) This specific uptake mechanism seems to exist in hepatocytes but not in extrahepatic cells, which explains why all statins are similarly effective in inhibiting mevalonate/cholesterol synthesis in hepatocytes, whereas pravastatin has a far weaker inhibitory effect on cellular mevalonate/cholesterol synthesis in extrahepatic cells than the lipophilic compounds.\(^{202,203}\) Thus, reported differences regarding the antiproliferative and proapoptotic effects on VSMCs and the effect on cytokine expression by HUVECs between different statins found in cell culture studies\(^{78,98,101,105}\) are mainly attributed to the relative lipophilicity of the compounds used. Moreover, these differential in vitro properties have raised the question whether the various statins also exert different pleiotropic effects in vivo. Indeed, it has been shown that treatment of Watanabe heritable hyperlipidemic rabbits with fluvastatin resulted in a significantly lower number of intimal VSMCs and expression of type I procollagen mRNA than administration of pravastatin.\(^{205}\) Because administration of both fluvastatin and pravastatin was associated with a significant and similar reduction of serum lipid levels in these animals it can be speculated that the differences in intimal VSMC count and collagen gene expression represent the consequence of the lipid-independent effects observed with these two statins in vitro.\(^{96,98,101}\) Furthermore, treatment with lovastatin, simvastatin, or fluvastatin was shown to reduce carotid neointimal formation in a normocholesterolemic rabbit model, whereas pravastatin had a less pronounced effect.\(^{106}\) Because statin therapy with either compound did not alter plasma cholesterol levels in these animals, the effect on carotid intimal thickening could not be ascribed to the statins lipid-lowering effect. Another example for differential in vivo effects of statins is the finding that administration of either atorvastatin, fluvastatin, or cerivastatin, but not pravastatin, was associated with enhanced myocardial stunning compared with placebo in a canine myocardial ischemia/reperfusion model, although similar reductions of plasma cholesterol levels were achieved with all statins tested.\(^{206}\) These results indicate that differences regarding certain lipid-independent statin effects may also be present in vivo. Thus, it is obvious that not all lipid-independent statin effects represent a class effect. However, the fact that pravastatin and the more lipophilic compounds have demonstrated similar benefit in large clinical trials\(^{4-8}\) suggests that such intra-class differences regarding single lipid-independent statin properties found in vitro or in animal studies do either not exist in humans or are clinically irrelevant. Given their comparable lipid-lowering potential, and granted that lipid-independent properties contribute to the favorable effect of statins on cardiovascular prognosis, the similar benefit observed with lipophilic and hydrophilic compounds in clinical studies raises the question, why pravastatin seems to be as effective as the lipophilic statins regarding its non-lipid properties despite its comparatively low circulating drug levels and its hepatoselectivity.\(^{202,207}\) One interesting hypothesis is that the potent inhibition of mevalonate synthesis in the liver exerted by pravastatin could lead to decreased circulating levels of mevalonate and mevalonate-derived isoprenoids, resulting in mevalonate and/or isoprenoid depletion of extrahepatic cells. However, circulating mevalonate levels show a great physiologic circadian variation which parallels cholesterol biosynthesis, and although administration of simvastatin was shown to be associated with a reduction of circulating mevalonate levels,\(^{208}\) little is currently known about the effect of pravastatin on plasma concentrations of mevalonate or the downstream intermediates of the mevalonate pathway.

In summary, we still know very little about the existence and significance of lipid-independent statin effects in humans. Furthermore, the lack of a clear explanation for the similar clinical benefit observed with various statin compounds, despite obvious differences regarding their lipid-independent properties found in vitro or in animal studies, further fuels the controversy about the existence of clinically relevant non-lipid effects in humans.
Conclusion

Today, there is growing evidence that statins are more than simple lipid-lowering drugs. In recent years a large number of experimental studies confirmed that these compounds exert several potentially beneficial effects by mechanisms unrelated to changes of cholesterol metabolism. In addition, recent evidence suggests that at least some of these effects might also play a role in humans particularly in specific situations, such as acute coronary syndromes. However, we still know very little about the clinical relevance of these lipid-independent statin properties, which is mainly due to the difficulty of differentiating them from those related to modification of plasma cholesterol levels in vivo. In addition, it should be kept in mind that some of the lipid-independent effects found in experimental studies could also be harmful in humans. Thus, it is too early to advocate the use of statins in patients with normal cholesterol levels based on their pleiotropic lipid-independent effects. However, the huge amount of experimental data in favor of such effects cannot be denied and should stimulate the initiation of further studies to clarify their clinical significance.

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