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REVIEW ARTICLE

Statins and bone metabolism

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3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are potent inhibitors of cholesterol biosynthesis. Cholesterol-lowering therapy using statins significantly reduces the risk of coronary heart disease. However, extensive use of statins leads to increases of other undesirable as well as beneficial effects, so-called pleiotropic effects. With respect to these effects, statins augment the expression of bone morphogenetic protein-2, a potent simulator of osteoblast differentiation and its activity, and promote mineralization by cultured osteoblasts, indicating that statins have an anabolic effect on bone. Chronic administration of statins in ovariectomized (OVX) rats modestly increases bone mineral density (BMD) of cancellous bone but not of compact bone. In clinical studies, there are conflicting results regarding the clinical benefits of this therapy for the treatment of osteoporosis. Observational studies suggest an association between statin use and reduction in fracture risk. Clinical trials reported no effect of statin treatment on BMD in hip and spine, and on bone turnover. Statins also may influence oral osseous tissues. Administration of statins in combination with osteoporosis therapy appears to improve alveolar bone architecture in the mandibles of OVX rats with maxillary molar extraction. Statins continue to be considered as potential therapeutic agents for patients with osteoporosis and possibly with periodontal disease. Development of new statins that are more specific and potent for bone metabolism will greatly increase the usefulness of these drugs for the treatment of bone diseases.
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Introduction

Competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins) act at a rate-limiting step in cholesterol synthesis by strongly blocking conversion of HMG-CoA to mevalonate (Goldstein and Brown, 1990; Istvan and Deisenhofer, 2001). Statins, such as simvastatin and atorvastatin, are highly effective cholesterol-lowering drugs widely considered to reduce morbidity from coronary artery disease (Hamelin and Turgeon, 1998; Maron *et al*, 2000). Over 11 million Americans are treated with a statin, and recommendations from a US Government Health Advisory Board suggest that 36 million people could benefit from them. In addition to lowering the serum lipid concentration, statins can decrease platelet aggregation and thrombus deposition (Lacoste *et al*, 1995), promote angiogenesis (Kureishi *et al*, 2000), decrease β-amyloid peptide (Aβ) production which is related to Alzheimer's disease (AD) (Fassbender *et al*, 2001; Kojro *et al*, 2001), and suppress T-lymphocyte activation (Kwak *et al*, 2000). Therefore, the results of many studies have demonstrated the therapeutic impact of the pleiotropic effects of statins and have raised the possibility that the mechanisms of action beyond those of the lipid-lowering activity might be responsible for their beneficial effects in atherosclerotic patients (Bonetti *et al*, 2003; Marz and Koenig, 2003). By modulating the initial part of the cholesterol synthesis pathway, statins decrease the availability of several important intermediate compounds including isoprenoids such as geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP). Isoprenoids are lipids attached by post-translational modification to some proteins such as the small G proteins including Ras and Ras-like proteins (Rho, Rap, Rab, and Ral) (Casey and Seabra, 1996).

The integrity of the skeleton requires a dynamic balance between bone formation and bone resorption, which are controlled by calcitropic hormones and cytokines. When bone resorption exceeds bone formation, diseases of bone metabolism such as postmenopausal osteoporosis can result (Riggs and Melton, 1992). Communication between bone-forming osteoblasts and bone-resorbing osteoclasts is essential, and coupling of bone resorption to bone formation is necessary for the maintenance of healthy bone. Mundy *et al* (1999) first reported that statins stimulate *in vivo* bone formation in rodents and increase new bone

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volume in cultures from mouse calvaria. Recently, we showed that statins stimulate the expression of bone anabolic factors such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) (Maeda *et al*, 2003), and promote osteoblast differentiation and mineralization in MC3T3-E1 cells (Maeda *et al*, 2001, 2004). This review focuses on the action of statins in the regulation of bone metabolism. Furthermore, we describe the multiple actions of statins, including the primary cholesterol-lowering effect, and the pleiotropic effects that benefit the prevention and treatment of lifestyle- and aging-related diseases. Finally, we discuss the effects of statins on the mandible of ovariectomized (OVX) rats.

Cholesterol-lowering effect

While identifying antifungal substances that irreversibly inhibit HMG-CoA reductase, Endo *et al* (1977) isolated new compounds, including mevastatin from *Penicillium citrinum* and lovastatin from *Asperigillus terreus* in 1977. Since that time, natural and synthetic statins have been developed as lipid-lowering drugs. The introduction of a competitive inhibitor of HMG-CoA reductase resulted in two physiologic responses. First, statins bind to HMG-CoA reductase at nanomolar concentrations, leading to competitive displacement of the natural substrate, HMG-CoA, which binds at micromolar concentrations (Istvan and Deisenhofer, 2001). Second, the inhibition of cholesterol biosynthesis is accompanied by an increase in hepatic lipoprotein lipase receptor expression, which promotes uptake and clearance of cholesterol from the blood stream (Veillard and Mach, 2002). Thus, statins have been described as the principal and the most effective class of drug to reduce serum cholesterol concentrations (Figure 1). While all statins inhibit hepatic HMG-CoA reductase to various degrees, important structural differences among the statins effect their lipophilicity, half-life and potency (Illingworth and Tobert, 2001). Two subtypes of statins are commercially available: the natural (fermentation-derived) and the synthetic statins (Figure 2). Molecules derived by fermentation, including simvastatin and pravastatin, have very similar chemical structures. Simvastatin is twice as potent as pravastatin and lovastatin. In contrast, the structures of the synthetic statins, including atorvastatin and fluvastatin, are very different. By altering the basic chemical composition of the mevastatin molecule, drug potency can be increased. Statins differ in their lipophilicity/hydrophilicity (Figure 3), which reflects their potential to cross cellular membranes non-selectively by passive diffusion (Corsini *et al*, 1999). Lipophilic statins such as simvastatin and atorvastatin easily cross the cellular membrane to enter cells, but hydrophilic statins such as pravastatin and rosuvastatin (a newly developed statin) can rely on specific carrier mechanisms in hepatic cells for entry into these cells.

The liver, which is a site of high first-pass hepatic metabolism, is the primary site of action of statins (Lennernas and Fager, 1997). Although their plasma half-life is typically short, their reduction in the

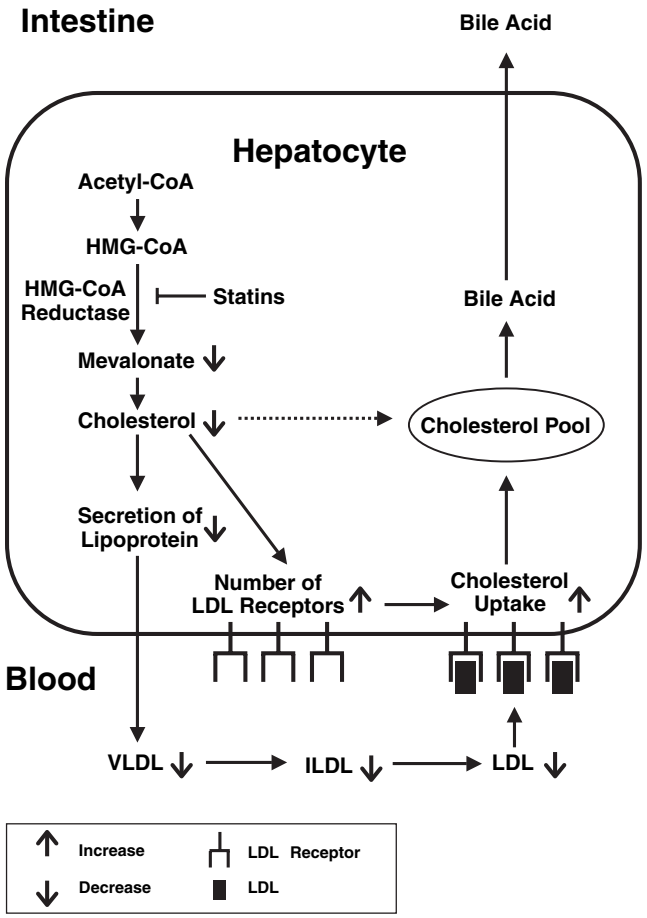

Figure 1 Effect of statins on cholesterol metabolism. Statins inhibit HMG-CoA reductase and decrease the concentration of mevalonate in cells. After statins inhibit cholesterol synthesis in hepatocytes, they increase LDL receptor number and decrease the secretion of lipoprotein. Hepatocytes also increase LDL cholesterol uptake from the blood to maintain the cholesterol pool. Consequently, blood cholesterol concentrations decrease. IDL, intermediate-density lipoprotein

concentration of low-density lipoprotein (LDL) cholesterol is gradual and more sustained, with maximal effects seen after several weeks of the therapy. This reflects their net effect on hepatic LDL receptor expression and clearance of plasma LDL, decreasing very low-density lipoprotein (VLDL) production and metabolism of cholesterol in hepatocytes by 7α-hydroxylases, as well as other factors (Figure 1). Their effects on plasma triglyceride and high-density lipoprotein (HDL) concentrations are also gradual, reflecting changes in the synthesis and secretion of triglyceride-rich lipoproteins, and probable stimulation of HDL apolipoprotein synthesis. Large human clinical studies have demonstrated that statins reduce total serum cholesterol by 15–40%, LDL cholesterol by 20–60%, triglycerides by 10–30%, and increase HDL cholesterol by 5–15% (LaRosa *et al*, 1999). Plasma LDL concentrations are reduced more effectively by the recently developed atorvastatin compared with the older statins such as simvastatin, lovastatin and pravastatin (Furberg, 1999). Treatment with simvastatin at doubled dose causes a 6–7% greater

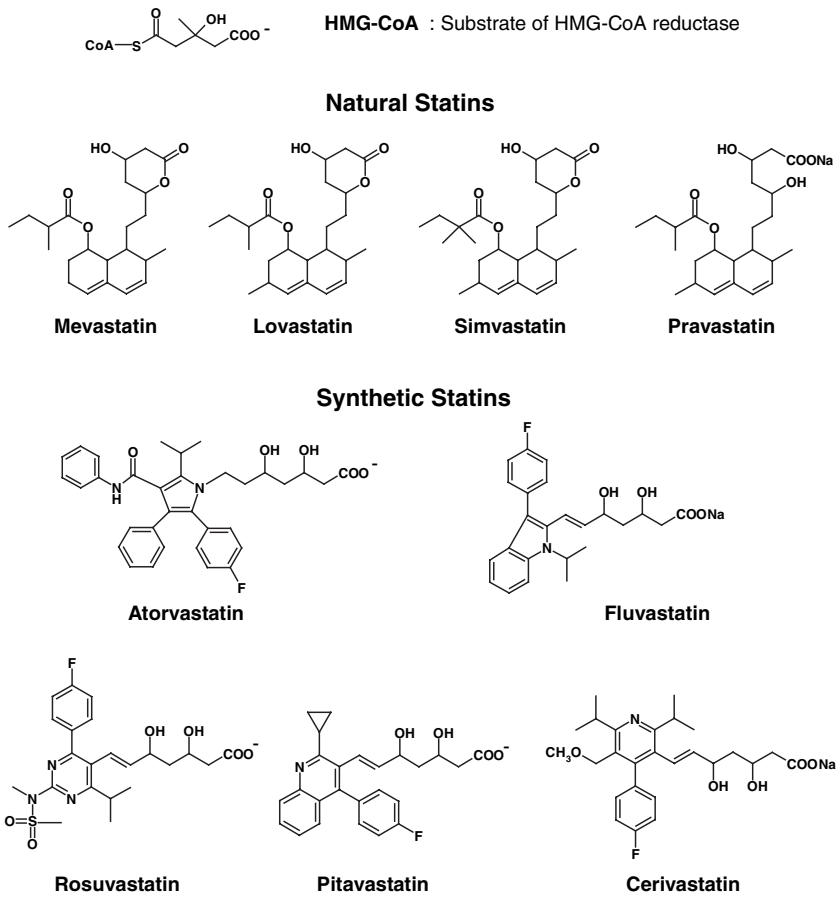

Figure 2 Chemical structures of statins and HMG-CoA, the enzyme substrate. Mevastatin, lovastatin, simvastatin, and pravastatin are natural statins derived by fermentation. Atorvastatin, fluvastatin, rosuvastatin, pitavastatin, and cerivastatin are synthetic statins. Cerivastatin was removed from the market in 2001. Natural statins, except for pravastatin, have a lacton form at the active site, and are converted to the active (open acid) form by cellular esterase

reduction in LDL concentration (Stein *et al*, 2000). The great clinical benefits of statins based on the reduction in cardiovascular morbidity and mortality may be due to not only the lipid-lowering effects of the drugs, but also their pleiotropic effects.

Pleiotropic effects

The pleiotropic effects of statins, summarized in Table 1, include vasodilative, antithrombotic, antioxidant, anti-inflammatory, and immunosuppressive actions (Mundy, 2001). Furthermore, the diverse properties of statins may affect a number of aspects of aging, such as osteoporosis and dementia (including AD) (Waldman and Kritharides, 2003). Although the pleiotropic effects are most often assigned to the effects of statins at extrahepatic sites, their actions on the liver may generate systemic effects with extrahepatic consequences. With respect to the anti-inflammatory effect, statins reduce C-reactive protein (CRP) concentrations in plasma (Ridker *et al*, 1999); this protein is released by the liver in response to interleukin (IL)-6 stimulation. Given their extensive hepatic clearance, some of the pleiotropic effects of statins may be partially attributable to hepatic effects. Statins also affect the synthesis of isoprenoid intermediates of the cholesterol pathway by inhibiting mevalonate production (Figure 4). Isoprenoids such as GGPP and FPP act as covalent modifiers of proteins and play important roles in multiple cellular functions. Prenylation of proteins is a prerequisite for the cell membrane association of both plasma and internal membranes (Zhang and Casey, 1996). Statins mediate their pleiotropic effects by concomitant regulation of other mevalonate metabolites.

The vascular endothelium represents the key regulatory component of the vascular wall and a number of studies shows that statins confer their beneficial effects by modulating endothelium-derived nitric oxide (NO) bioactivity, thereby attenuating endothelial dysfunction and atherosclerotic disease progression (Schachinger *et al*, 2000; Halcox *et al*, 2002; Wolfrum *et al*, 2003). NO synthesis is a crucial mediator of vascular homeostasis and blood flow. Decreases in NO synthesis by vascular endothelial cells promote vasoconstriction, platelet aggregation, and leukocyte recruitment and adhesion (Furchgott and Zawadzki, 1980; Palmer *et al*, 1987; Radomski *et al*, 1990; Huang *et al*, 1995; Mach *et al*, 1999). Knockout mice lacking endothelial NO synthase (eNOS) revealed increased arterial blood pressure and cerebral artery occlusion (Huang *et al*, 1995, 1996). Cerebral blood flow is reduced and postfocal ischemic tissue damage is induced when eNOS activity is inhibited (Huang *et al*, 1994). Enhanced NO production by the administration of L-arginine, the eNOS substrate, protects against stroke after the induction of cerebral ischemia. Statins can directly augment eNOS expression

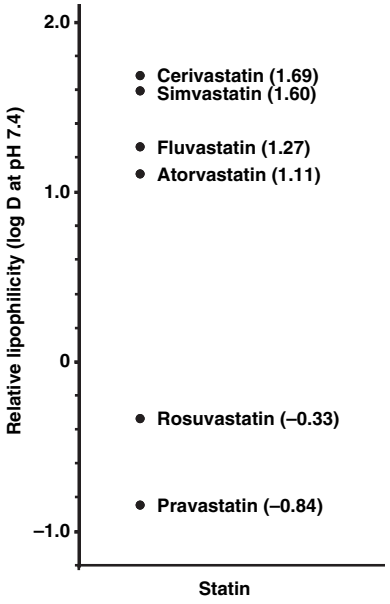

Figure 3 Comparison of the lipophilicity of statins. Cerivastatin is the most lipophilic statin, whereas pravastatin is the most hydrophilic statin

Table 1 Pleiotropic effects of statins

Decrease in coronary artery disease
Decrease in atherosclerosis
Decrease in platelet aggregation and thrombus deposition
Increase in angiogenesis
Decrease in β-amyloid peptide accumulation – related to Alzheimer disease
Decrease in inflammation – anti-inflammatory property
Decrease in cell proliferation – antiproliferative property
Decrease in T-lymphocyte activation – immunosuppressive property
Increase in bone formation – bone anabolic property

in vitro under high cholesterol concentration conditions (Laufs *et al*, 1998). The beneficial effects of statins are absent in eNOS-deficient mice. Statins reduce *in vivo* cerebral infarct size as well as oxidative stress, and improve neurologic function in normocholesterolemic mice (Endres *et al*, 1998). Moreover, statins have additional effects on endothelial cells. Expression of the procoagulant tissue factor induced by thrombin is prevented by simvastatin treatment through inhibition of Rho/Rho kinase and activation of Akt (Eto *et al*, 2002). Statins increase the expression of tissue-type plasminogen activator (Essig *et al*, 1998), and suppress the expression of endothelin-1, a potent vasoconstrictor and mitogenic molecule that regulates vascular tone and remodeling (Hernandez-Perera *et al*, 1998). Collectively, statins clearly improve endothelial function and result in anti-atherothrombotic effects that might be very important for the prevention of acute coronary syndromes (Heeschen *et al*, 2002).

Inflammatory processes play a crucial role in the initiation and progression of atherosclerosis and coronary heart disease such as myocardial infarction. Endothelial dysfunction with vascular injury in response to cardiovascular risk factors is initiated by the migration of leukocytes, including monocyte/macrophages and T lymphocytes. Adhesion molecules, proinflammatory cytokines, and chemokines mediate the extravasation of inflammatory cells. Within atherosclerotic sites, endothelial cells and leukocytes both have increased their expression of numerous adhesion molecules and their receptors, including intracellular adhesion molecule-1, vascular cell adhesion molecule-1, β_1-integrin, β_2-integrin and P-selectin (Nakashima *et al*, 1998; Romano *et al*, 2000; Stalker *et al*, 2001). *In vivo* studies (Nie *et al*, 1997; Shih *et al*, 1999) have shown that blocking these adhesion molecule interactions by the administration of antibodies or gene targeting attenuates the formation of atherosclerotic lesions, indicating a potential therapeutic role for inhibition of leukocyte adhesion and extravasation. Studies *in vitro* demonstrate the beneficial effects of statins by decreasing adhesion molecules, such as monocyte CD11b and the leukocyte function antigen-1 (Weber *et al*, 1997; Weitz-Schmidt *et al*, 2001).

Furthermore, statins suppress the secretion of proinflammatory cytokines including IL-1β and IL-6, but not tumor necrosis factor α (TNF-α). These results support human studies suggesting that statins decrease the number of inflammatory cells in atherosclerotic plaque (Vaughan *et al*, 2000; Crisby *et al*, 2001). NO plays a crucial role in mediating this anti-inflammatory action. The new statin rosuvastatin has significant anti-inflammatory effects via inhibition of P-selectin synthesis by endothelial cells. The protective action of the statin is mediated by vascular endothelial NO (Stalker *et al*, 2001). Rosuvastatin has no effect on leukocyte–endothelium interactions in eNOS-deficient mice, emphasizing the important role of NO in anti-inflammation. Thus, increased NO production by statins could explain the modulation of these leukocyte–endothelium interactions. The suppression of inflammation by statin treatment reduces the production of high-sensitivity CRP, a clinical marker of inflammation produced by the liver in response to proinflammatory cytokines such as IL-6 (Ridker *et al*, 2001). CRP expression is elevated in patients with coronary heart disease. Patients who clinically benefit from statin therapy also have abnormally elevated CRP concentrations (Ridker *et al*, 1998). As statins decrease CRP concentrations in serum (Ridker *et al*, 1999, 2001), statin use would contribute to the prevention and remission of inflammatory diseases.

Proliferation of smooth muscle cells (SMCs) is a major event in the pathogenesis of vascular lesions such as atherosclerosis. Statins, including simvastatin and fluvastatin, dose-dependently reduce SMC migration and proliferation *in vitro* independent of their lipid-lowering properties (Hidaka *et al*, 1992; Munro *et al*, 1994). This inhibitory effect is prevented *in vitro* by the addition of mevalonate, all-*trans* farnesol (F-OH) and all-*trans* geranylgeraniol (GG-OH), but not 2-*cis* GG-OH, squalene or ubiquinone (Raiteri *et al*, 1997). Thus, statins may affect cell growth via interference with

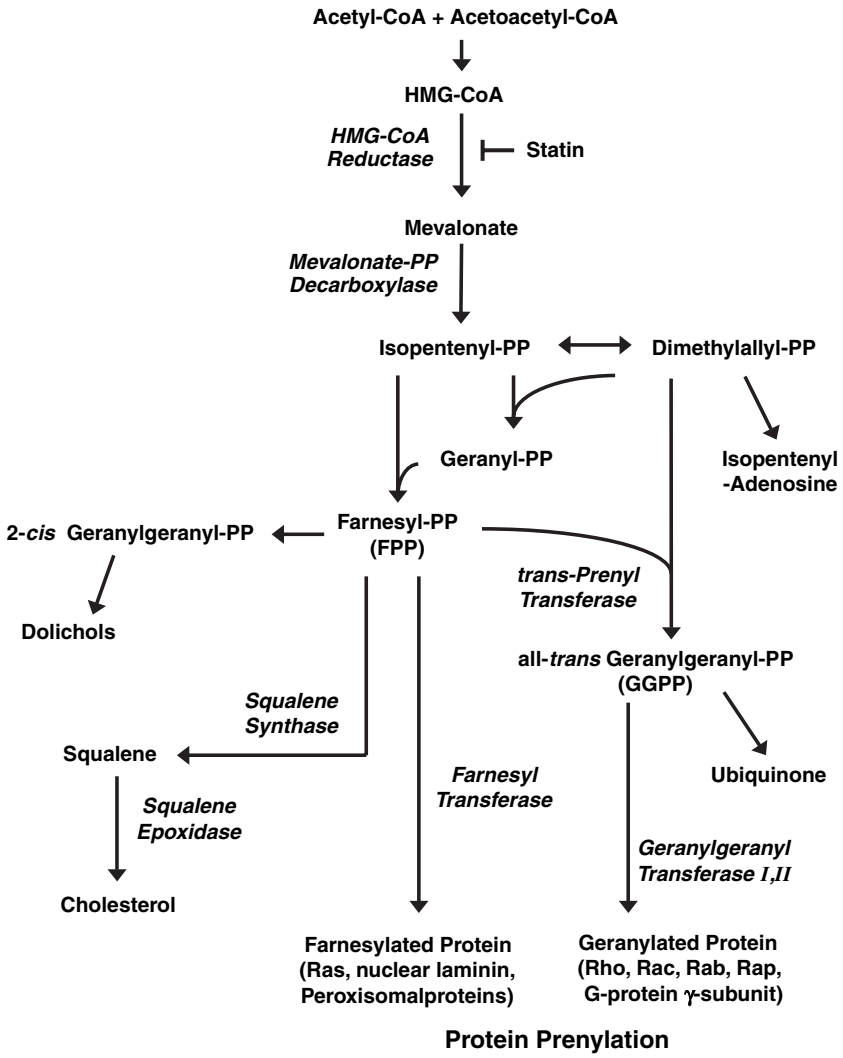

Figure 4 Metabolic pathway of mevalonate and cholesterol. Statins not only block the rate-limiting step in cholesterol biosynthesis but also suppress the synthesis of isoprenoids, such as FPP and GGPP which are required for protein prenylation. Italics in the figure represent enzymes

signaling pathways that require prenylated proteins (Figure 4). Statins delay cell cycling in the G1 and G2/M phases and lead to apoptosis of SMCs. Recent studies have focused on the possible implication of apoptosis in SMC proliferation within atherosclerotic lesions (Rembold, 1996; Guijarro *et al*, 1998). Statins induce apoptosis of endothelial cells and SMCs, an effect that can be reversed by mevalonate, FPP and GGPP (Guijarro *et al*, 1998; Li *et al*, 2002). Because programmed cell death (apoptosis) of vascular SMCs has been identified in physiologic remodeling of the vasculature, SMC apoptosis plays an important role in maintaining the integrity of the vasculature. Increased apoptosis prevents the neointimal thickening seen in early atherosclerosis. Thus, statins may provide strategies for the prevention of neointimal thickening of arteries and the treatment of atherosclerosis.

Atherosclerotic vascular lesions are characterized by the accumulation of lipids, fibrous elements, and immune cell infiltrates. Weakening of the fibrous cap covering an atherosclerotic plaque is characterized by exposure of a highly thrombogenic lipid core covered by a thin fibrous cap composed of SMCs and extracellular matrix (Ross, 1993). Collagen secreted by SMCs is the main component of the fibrous cap, and is responsible for the tensile strength. The degradation of collagen plays a crucial role in the development and subsequent instability of the plaque. Secretion of proteolytic enzymes such as matrix metalloproteinases (MMPs) by SMCs and macrophages influences the fibrous content and instability of atherosclerotic lesions. The shoulder region of the plaque, the margin between the lesion and the unaffected portion of the artery, is inhabited predominantly by foam cell macrophages.

Statins contribute to plaque stability mainly by modifying the cellular composition and physiologic properties of atherosclerotic plaque. Statins suppress monocyte/macrophage infiltration as well as the proliferation and migration of SMCs into the neointima. Statins also decrease the secretion of MMPs, including MMP-1, MMP-3 and MMP-9, by SMCs and macrophages (Aikawa *et al*, 1998; Bellosta *et al*, 1998). Consequently, statins inhibit the progression of important inflammatory processes implicated in atherosclerosis and plaque rupture that might, in part, explain their great clinical benefits in cardiovascular disease.

Statins also act as immunomodulators. Kwak *et al* (2000) analyzed the effect of statins on various features of the control of major histocompatibility complex class II (MHC-II) expression and of subsequent hepatocyte activation. Stains could inhibit inducible MHC-II expression in human endothelial cells and human monocyte/macrophages via inhibition of the promoter IV of the non-DNA binding MHC-II transactivator (CIITA). Thus, statins have beneficial effects as immunosuppressors after cardiac transplantation (Wenke *et al*, 1997). Multiple sclerosis is believed to develop when the body's immune cells, such as T-helper cells, attack myelin, the insulating, fatty sheath around nerve cells. This damages the myelin and the underlying neurons in both the brain and spinal cord, leading to impaired transmission of nerve impulses and progressive physical disability. Youssef *et al* (2002) found that atorvastatin inhibits the expression by brain cells of a pivotal regulatory protein CIITA, which regulates the expression of MHC-II molecule. Statins can act through CIITA and MHC-II molecules to decrease the presentation of 'self' antigen, thereby shifting the pattern of T-helper cell activity. These results raise hopes of a new, oral treatment of multiple sclerosis and related diseases.

Dementia is a clinical syndrome characterized by persistent and usually progressive impairment in multiple cognitive functions. Dementia is grouped into AD and non-AD dementia, the latter including diverse causes such as vascular dementia (VD). AD prevalence increases exponentially after 70 years of age (Evans *et al*, 1989). VD includes dementia caused by disease of large vessels and small vessels, and the two pathologies are not mutually exclusive. The protective roles proposed for statins are in the prevention and treatment of dementia including AD and VD. Statins have been shown to reduce major cerebrovascular events, including stroke and transient ischemic attack (Milani, 2004). AD is characterized by intra- and extravascular deposition of Aβ, and by the presence of neurofibrillary tangles (Kril and Halliday, 2001). The degree of cortical atrophy correlates with the severity of dementia in AD, and this distinguishes it from normal aging. Aβ is a hydrophobic peptide, 39–43 residues in length, which is derived from the proteolytic processing of its precursor, the transmembrane amyloid precursor protein (APP). Aβ tends to form insoluble aggregates, and its apparent toxicity follows fibril formation. Epidemiologic studies indicate that the prevalence of AD is reduced in patients taking a class of cholesterol-lowering drugs such as statins (Wolozin *et al*, 2000). Fassbender *et al* (2001) used both cell culture and *in vivo* studies to demonstrate that inhibiting cholesterol production by statins reduces Aβ production. Kojro *et al* (2001) provided corroborative evidence by showing that the attenuation of cholesterol production induced by statins increases trafficking of APP through the non-amyloidogenic α-secretase pathway. These reports strongly suggest that inhibiting cholesterol production in the brain prevents Aβ production and reduces the accumulation of Aβ that causes AD. As most of the entry of cholesterol into the central nervous system comes from *in situ* synthesis (Dietschy and Turley, 2001), local inhibition of cholesterol synthesis by statins may be particularly important. The use of statins is associated with a significantly lower prevalence of AD in non-randomized study (Wolozin *et al*, 2000). By contrast, the pravastatin in elderly individuals at risk of vascular disease [PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)] study randomized 5804 men and women, 70–82 years of age with a history of vascular disease, to pravastatin 40 mg daily or placebo. The patients were followed for an average of 3.2 years. PROSPER specifically and prospectively measured cognitive function including monitoring for dementia, and found no effect of statin treatment on this population (Shepherd *et al*, 2002). Thus, statin therapy does not appear to have an effect on cognitive function assessed over a 3- to 5-year period, and statins have no documented benefit in preventing dementia. A longer follow-up period may be needed to demonstrate the positive effects of statins on reducing dementia.

Basic studies of the skeletal effects of statins

Osteoporosis is epidemic throughout the world, and is associated with an increase in the incidence of low-trauma fractures in the vertebral spine, femoral neck, and distal radius. Specifically, postmenopausal osteoporosis, which results in pathologic bone fractures, is a major health problem in elderly women. It is typically associated with low bone mass and poor architecture of trabecular bone (Riggs and Melton, 1992; Lips, 1997). Thus, osteoporosis is the most common bone disease, affecting millions of people worldwide and leading to substantial morbidity. Patients with established osteoporosis lose considerable bone mass at critical sites in the skeleton. They also have altered trabecular bone architecture, and require anabolic therapy. Most drugs currently available to treat osteoporosis are antiresorptive agents, including the bisphosphonates, estrogen, selective estrogen receptor modulators, calcitonin, and vitamin D analogues, and have beneficial effects for the patients on preventing further bone loss. All of these drugs are inhibitors of bone resorption that act mainly to stabilize bone mass. Their effects on increasing the bone mass is modest (Christiansen and Lindsay, 1990; Riggs and Hartmann, 2003). Thus, anabolic agents that enhance bone mass and improve the architecture of trabecular bone are crucial in treating established osteoporosis (Margolis *et al*, 1996). The anabolic agents currently under investigation are parathyroid hormone (PTH), fluoride, strontium, and growth hormone. Both PTH and fluorides substantially increase bone formation. However, PTH agents such as human (h) PTH (1–34) must be administered by injection because the agent is a peptide. Fluoride therapy has disadvantages because of the lack of an effect on improving fracture rates. Intermittent administration of hPTH (1–34) to osteoporotic human subjects has been shown to increase bone mass markedly (Neer *et al*, 2001). Currently, several PTH analogs are being investigated, and finally hPTH (1–34) has been approved for use in osteoporosis

treatment in the United States. To date, a bone anabolic drug available for oral administration is not available for the treatment of established osteoporosis.

Osteoporosis and atherosclerosis share the tendency to accelerate after the menopause; both diseases are promoted by inflammatory processes, and many aspects of arterial calcification and bone formation are similar (Tintut and Demer, 2001; Burnett and Vasikaran, 2002). The relationship between osteoporosis and atherosclerosis is supported by the observation that the progression of aortic calcification is most severe in women with most severe metacarpal bone loss (Hak *et al*, 2000). Factors that may promote both processes include estrogen deficiency and increased concentrations of proinflammatory cytokines, such as IL-1, IL-6, and TNF-α (Sakou, 1998).

The biologic effects of statins on bone metabolism were first reported in 1999, when Mundy *et al* (1999) found that statins were potent stimulators of bone formation *in vitro*. Over 30 000 compounds were screened for their ability to stimulate BMP-2 promoter activation of luciferase reporter gene expression in human osteoblast-like osteosarcoma (MG63) and murine osteoblastic (2T3) cells. They thought that osteoblast differentiation should be enhanced by members of the BMP family, including BMP-2, whereas other bone growth factors such as transforming growth factor-β and fibroblast growth factors would stimulate osteoblast proliferation but inhibit osteoblast differentiation (Sakou, 1998). In a landmark study, they showed that statins enhance BMP-2 mRNA expression in cultured mouse and human bone cells, and promote bone formation in murine calvaria maintained in organ culture, strongly suggesting that these drugs have a beneficial effect on bone health. They further reported that simvastatin and lovastatin augment bone formation when injected subcutaneously over the murine calvaria, and that statins increase trabecular bone volume when administered orally to osteopenic OVX rats (Mundy *et al*, 1999). Subsequently, Sugiyama *et al* (2000) reported that lipophilic statins, such as simvastatin and mevastatin, enhance the expression of BMP-2 mRNA and its protein in human osteosarcoma cells, and that simvastatin also activates the BMP-2 promoter linked to a luciferase reporter gene. The statin-mediated activation of the BMP-2 promoter can be abolished by the addition of mevalonate, the downstream metabolite of HMG-CoA reductase, strongly suggesting that it is a result of HMG-CoA reductase inhibition. They also found that hydrophilic statins such as pravastatin do not have the ability to induce BMP-2 expression in osteoblasts. These observations support the notion that the lipophilicity of statins is important in eliciting pleiotropic effects such as bone formation.

We investigated whether simvastatin regulates the differentiation and function of osteoblasts using non-transformed osteoblasts (MC3T3-E1) and rat bone marrow cells. The results indicated that simvastatin enhances alkaline phosphatase activity and mineralization in a dose- and time-dependent fashion (Maeda *et al*, 2001). We also showed that statins such as simvastatin and cerivastatin regulate osteoblast function by increasing the expression of bone sialoprotein (BSP), osteocalcin (OCN), and type I collagen and by suppressing gene expression of collagenases such as MMP-1 and MMP-13 (Maeda *et al*, 2004). Statins also promote osteoblast differentiation by marrow stromal cells. Treatment of pluripotent mouse marrow stromal cells with inhibitors of the cholesterol biosynthetic pathway, such as mevastatin, inhibited the maturation of these cells into functional osteoblast cells (Parhami *et al*, 2002), confirming the crucial role in statins for osteoblast differentiation. Furthermore, we investigated whether bone anabolic factors other than BMP-2 are induced by treatment of osteoblasts with statins. We found that lipophilic statins, simvastatin, atorvastatin and cerivastatin – but not the hydrophilic statin, pravastatin – markedly enhance the expression of VEGF, a bone anabolic factor, in osteoblasts (Maeda *et al*, 2003). This stimulatory effect was abolished with treatment of mevalonate and GGPP, while the addition of manumycin A, a protein prenylation inhibitor, mimicked the statin effect on VEGF expression. Phosphatidylinositol-3 kinase (PI3K) inhibitors, such as LY294002 and wortmannin, inhibit the statin-induced expression. Thus, statins stimulate VEGF expression in osteoblasts via reduced protein prenylation and PI3K pathway activation (Figure 5).

We also demonstrated that the inhibition of the VEGF signaling pathway by a VEGF receptor 2 (Flk-1) kinase inhibitor, SU1498, significantly suppresses mineralization by cultured osteoblasts treated with statins (Maeda *et al*, 2003). Lipophilic statins, such as mevastatin and fluvastatin, augment the mineralization process independent of BMP-2 and Runx2/Cbfa1, a crucial transcription factor for osteoblast differentiation (Izumo *et al*, 2001). A recent study demonstrated that simvastatin enhances VEGF release mainly through the ERK 1/2 MAP kinase pathway in vascular SMCs (Takenaka *et al*, 2003). These studies, including ours, suggest that lipophilic statins promote osteoblast differentiation and bone nodule formation, at least in part, by stimulating VEGF expression in bone tissue. Other studies have shown that VEGF acts through an autocrine/paracrine factor in bone, promoting angiogenesis, ossification, and bone turnover (Gerber *et al*, 1999; Deckers *et al*, 2000). Thus, VEGF also plays a role in statin-induced bone formation.

Pluripotent embryonic stem cells can differentiate *in vitro* into various cell types including osteoblasts and adipocytes. Phillips *et al* (2001) showed that the statin, compactin (mevastatin), stimulates BMP-2 expression at a late stage of differentiation, and consequently promotes osteoblastic differentiation and bone nodule formation. In a study of the mechanism of enhanced expression of BMP-2 by pitavastatin, a newly developed statin, enhanced BMP-2 mRNA expression in primary cultured human osteoblasts. This stimulatory effect was abrogated by the addition of GGPP, an essential molecule for prenylation of small guanosine triphosphate (GTP)-binding proteins such as Rho GTPase, but not by inhibitors of NOS or various protein kinases.

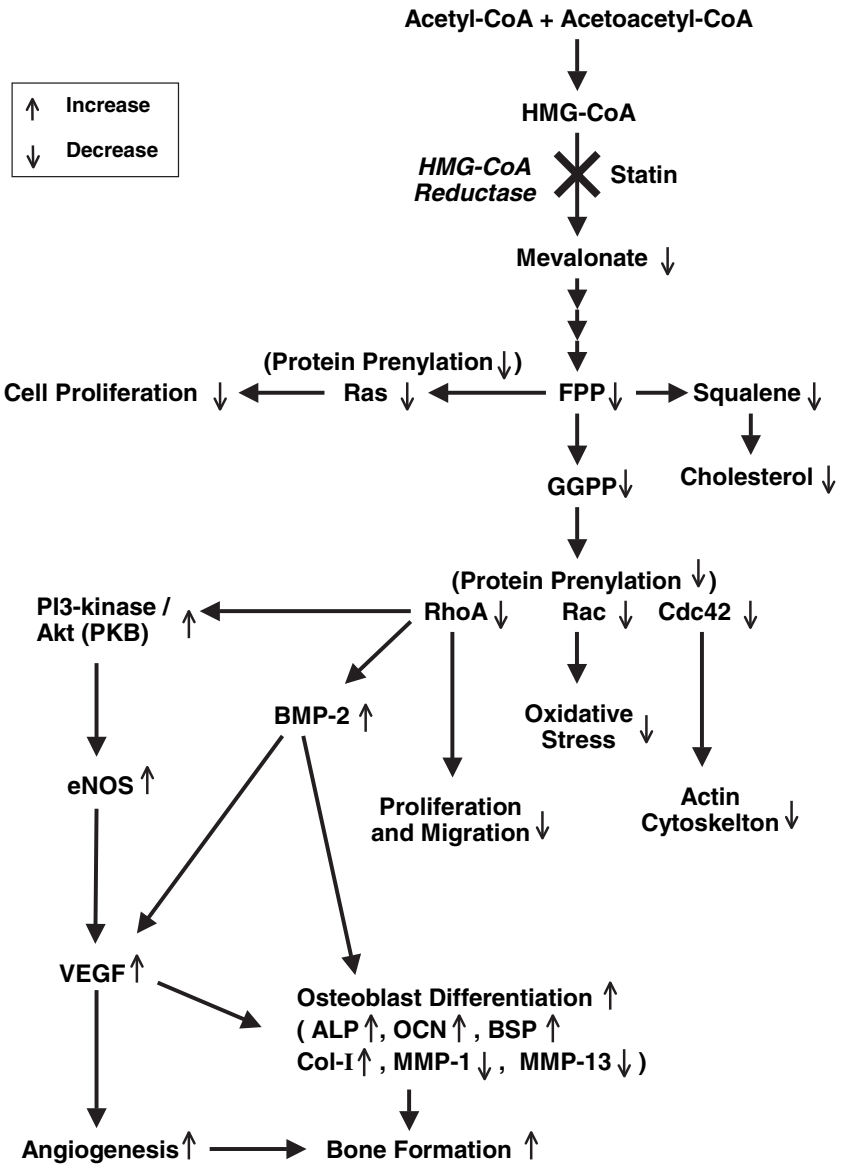

Figure 5 Biologic actions of statins through reduced production of isoprenoids. Decreases in the isoprenylation of signaling molecules, such as Ras, Rho, and Rac leads to modulation of various signaling pathways. Proposed mechanisms of statins on bone formation are also shown. ALP, alkaline phosphatase; OCN, osteocalcin; BSP, bone sialoprotein; Col-I, type I collagen; MMP-1, matrix metalloproteinases-1 (collagenase-1); MMP-13, matrix metalloproteinases-13 (collagenase-3)

A specific inhibitor of Rho-kinase, hydroxyfasudil, upregulates BMP-2 mRNA expression. The authors concluded that Rho-kinase suppresses osteoblast differentiation and statins act mainly as inhibitors of Rho and the Rho-kinase pathway (Ohnaka *et al*, 2001). Osteoblast migration, which plays crucial roles in fracture healing and modeling and remodeling of bone, is accelerated by many growth factors including platelet-derived growth factor (PDGF). Osteoblast migration elicited by PDGF is inhibited by statins, and this previous study suggests that the inhibitory action is mediated by the prevention of Rac prenylation causing a reduction in the phosphorylation of Akt, a downstream target of PI3K (Fukuyama *et al*, 2004). The proposed mechanisms of action of statins on bone formation are depicted in Figure 5.

Although the *in vivo* study by Mundy *et al* (1999) first demonstrated that simvastatin treatment at relatively high doses (5 or 10 mg kg^{-1}) increases trabecular bone volume when orally administered to OVX rats, disparate results with respect to statin-induced effects on bone metabolism have been obtained in subsequent *in vivo* experiments. Maritz *et al* (2001) reported that statins inhibit bone formation and produce a net reduction in bone density in rats. They also noted that simvastatin does not prevent bone loss caused by ovariectomy. Extremely high doses of simvastatin (20 mg kg^{-1} day^{-1}) increase bone formation and resorption, as assessed by quantitative bone histomorphometry. This study concluded that statins do not have *in vivo* anabolic effects on bone in rodents (Maritz *et al*, 2001). By contrast, Oxlund *et al* (2001) found that simvastatin, at an oral dose of 10 mg kg^{-1}, increases cancellous bone by 23%, and the compressive strength of the cancellous bone by 24% in vertebral bone of aged female unovariectomized rats. The *in vivo* effect of simvastatin on the promotion of osteogenesis around titanium implants was

demonstrated in a histologic study in aged rats (Ayukawa *et al*, 2004). Intraperitoneal administration of simvastatin (10 mg kg^{-1}) for 30 days increases both the bone contact ratio to the titanium implant and bone density around the implant, suggesting that statins have the potential to improve osseointegration. An *in vivo* study demonstrated that simvastatin promotes healing of bone fractures in mice (Skoglund *et al*, 2002). As the entire region of fracture repair is involved in bone formation, this observation supports the concept that statins enhance new bone formation *in vivo*.

Orally administered statins are recycled in the enterohepatic circulation, and mostly metabolized in the liver during first-pass circulation. A study of the deposition and metabolism of atorvastatin in rats showed that 73% of the oral dose of the statin was excreted in bile, and administration of multiple doses does not alter bilary metabolic profiles (Black *et al*, 1999). Another *in vivo* study suggests that transdermal administration of statins elicits a greater effect on bone metabolism than oral administration (Gutierrez *et al*, 2001). Therefore, we chose subcutaneous administration of atorvastatin for a study to assess the efficacy of the bone anabolic effects of statins, because atorvastatin has a long half-life compared with other statins. The dose of atorvastatin used (2 mg kg^{-1}) was based on clinically accepted biologically equivalent doses in humans (Illingworth and Tobert, 1994). The bioactivity of the statin at the same dose was higher in our study (Kawane *et al*, 2004) than in other studies with oral administration (Maritz *et al*, 2001; Oxlund *et al*, 2001). Nevertheless, atorvastatin did not improve bone mineral density (BMD) or trabecular bone volume in OVX rats. We failed to demonstrate an anabolic effect of atorvastatin on bone with subcutaneous administration of relatively high doses (10 mg kg^{-1}) for 10 weeks, consistent with the results of Maritz *et al* (2001). By contrast, we found that concomitant injections of atorvastatin (2 mg kg^{-1}) with 17β-estradiol, an antiresorptive agent, or with low-dose (1 μg kg^{-1}) hPTH (1–34), a bone anabolic drug, clearly increases BMD of trabecular bone-rich tissues, such as lumbar vertebrae and femoral metaphysics of OVX rats. These findings demonstrate that statins appear to modestly increase BMD of cancellous bone of OVX rats with submaximal doses of 17β-estradiol and hPTH (1–34) (Kawane *et al*, 2004). Based on these observations, the development of new statins that are more specific for bone will greatly improve the usefulness of these drugs for the treatment of osteoporosis, because the effect of the currently available statins on bone formation *in vivo* is not marked.

The pleiotropic effects of statins, such as increased bone formation, appear to depend on the inhibition of the syntheses of isoprenoid intermediates of the mevalonate pathway that are critical for the post-translational modification of several proteins, including the small GTP-binding Ras or Rho (Zhang and Casey, 1996). Direct inhibition of Rho mediates the increase in eNOS expression by statins (Laufs and Liao, 1998). The activation of the protein kinase Akt may also contribute to NO-mediated effects in endothelial cells (Kureishi *et al*, 2000). Statin-treated animals accumulate marrow-derived endothelial cells in a corneal neovascularization assay. Activation of Akt is a critical signaling of mevalonate (Dimmeler *et al*, 1999, 2001; Llevadot *et al*, 2001). Our previous study, performed in osteoblasts, documented that statins stimulate VEGF expression via activation of the PI3K/Akt pathway to promote osteoblast differentiation (Figure 5) (Maeda *et al*, 2003). Recently, Ongini *et al* (2004) showed that modified pravastatin and fluvastatin with an NO-releasing moiety contribute to the reduction in both SMC proliferation and inflammatory events. As the incorporation of the NO moiety into the statin structure markedly potentiates the non-lipid-lowering action of statins, these NO-releasing statins may be exploited in the treatment of established osteoporosis as bone-anabolic drugs. By contrast, NO signaling may not be involved in the stimulation of BMP-2 expression induced by statins (Ohnaka *et al*, 2001). We hypothesize that statins stimulate bone formation by at least two pathways that are mediated via BMP-2 expression, independent of eNOS synthesis, and via VEGF production which is dependent upon eNOS activity (Figure 5).

Aminobisphosphonates, which are currently used for the treatment of osteoporosis, primarily inhibit osteoclastic bone resorption (Rodan, 1998; Treloar, 2002). They interfered with prenylation of small GTP-binding proteins, such as rho p21, via inhibition of geranylgeranylation, resulting in cytoskeletal disruption and inducing osteoclast apoptosis. The addition of geranylgeranyl abolishes the inhibition of osteoclast activity elicited by bisphosphonates (van Beek *et al*, 1999). Statins affect mevalonate synthesis through HMG-CoA reductase inhibition, and share the ability with aminobisphosphonates to inhibit GGPP formation. Therefore, it is likely that both statins and aminobisphosphonates suppress osteoclast function and reduce osteoclast number. Aminobisphosphonates mainly inhibit FPP synthesis in the mevalonate pathway, and differ in their precise site of action compared with that of statins. Staal *et al* (2003) showed that statins inhibit bone resorption by suppressing osteoclast activity, which correlates directly with the potency of various statins in their inhibition of HMG-CoA reductase activity. However, despite inhibition of the mevalonate pathway in osteoclasts *in vivo*, no inhibitory effect in osteoclast function has been demonstrated on PTH-induced changes in the total serum calcium concentration in thyroparathyroidectomized rats. The reason for the difference between the inhibition of bone resorption in the fetal rat long bone assay *in vitro* and the inability of statins to inhibit bone resorption in these rats is unknown. A possible explanation for the apparent discrepancy may be a consequence of osteoclast overcoming the inhibition of the mevalonate pathway by statins. There are clear differences in the precise sites of action of statins and aminobisphosphonates. Statins do not cause marked inhibition of bone resorption *in vivo*.

Pluripotent mesenchymal stem cells have the capacity to undergo commitment to several cell lineages, including osteoblasts, adipocytes, chondrocytes, and myocytes (Yu *et al*, 1997; Bianco *et al*, 2001). This differentiation process is controlled by several essential transcription factors. Runt-related transcription factor 2/core-binding factor-a 1 (Runx2/Cbfa1) is a critical transcription factor for osteoblast differentiation or osteogenesis (Ducy *et al*, 1997; Frendo *et al*, 1998; Harada *et al*, 1999), whereas peroxisome proliferator-activated receptor (PPAR)-γ2 plays an important role in adipocyte differentiation and adipogenesis (Lazar, 2002). Osteoblasts and marrow adipocytes originate from a common mesenchymal progenitor. Lineage commitment depends on specific transcription factors that simultaneously suppress factors that are required for the expression of the alternate phenotype. Thus, adipogenic agents such as rosiglitazone, a PPAR-γ2 ligand, suppress osteoblast differentiation (Lecka-Czernik *et al*, 2002), suggesting that reciprocal changes in adipogenesis and osteoblastogenesis are typical phenomena in the differentiation of the pluripotent mesenchymal cells. Based on the results of *in vitro* recent studies (Li *et al*, 2003; Song *et al*, 2003), simvastatin and lovastatin inhibit adipogenic differentiation and inversely direct pluripotent cells into the osteoblast lineage. Statins suppress PPAR-γ2 and augment Runx2/Cbfa1 in these cells. Moreover, statins strongly induce BMP-2 expression in murine embryonic stem cells (Phillips *et al*, 2001) and mouse bone marrow stromal cells (Song *et al*, 2003), supporting the concept that BMP-2/Runx2 signaling is crucial for promoting osteoblast differentiation elicited by statins. Concomitantly, lovastatin inhibits adipocyte differentiation by suppressing the expression of fat cell-specific genes, such as PPAR-γ2 and adipocyte-specific protein aP2, and subsequent maturation. Fat represents 50% of the bone marrow space, and the differentiation of other marrow cell phenotypes allows marrow adipocytes to fill the space (Gimble *et al*, 1996). A clinical study showed an inverse relationship between the amount of trabecular bone and adipose tissue in bone marrow (Burkhardt *et al*, 1987). Increased lipid accumulation in the bone marrow has been reported in association with age-related bone loss (Burkhardt *et al*, 1987; Gimble *et al*, 1996; Nuttall and Gimble, 2000). Thus, the inhibitory effects of statins on adipocyte differentiation may confer a benefit for the treatment of osteoporosis.

Clinical studies of the skeletal effects of statins

The most clinically significant measure of the benefit of a drug in the prevention or treatment of osteoporosis is a reduction in the incidence of fractures. A number of observational studies on statin use and fractures have been performed (Bauer, 2003; Yaturu, 2003). Moreover, BMD is the best available quantitative predictor of future osteoporosis fracture. Its relationship to the incidence of fractures is complicated by the interaction with other risk factors for fracture, such as falls, neuromuscular competence, and cognitive impairment (Lips, 1997). Other predictors of osteoporosis fracture risk include high bone turnover rates and increased bone resorption rates. Thus, markers of bone turnover appear to improve the prediction of bone loss and fracture risk (Garnero *et al*, 2000). To establish statins as pharmaceutical agents for the treatment of osteoporosis, randomized, controlled studies are mandatory.

A case–control study of 1222 patients and 4888 control subjects found a relationship between statin use and hip fracture among these 6110 New Jersey residents who were 65 years of age or older (Wang *et al*, 2000). The study found that hip fractures were half as likely to occur among the stain users. After adjustment was made for the extent of statin use during the prior 3 years, current statin use was associated with a 71% reduction in the risk of hip fracture. In this analysis, the authors did not adjust for body weight, although other medication use and health status were included. Furthermore, their population-based case–control study at six health maintenance organizations in the United States reported similar findings (Meier *et al*, 2000). This study used the pharmacy records and other records of 928 women who were 60 years or older, and documented fractures of the hip, humerus, distal tibia, wrist, or vertebrae. These data were compared with records from 2747 control subjects without fracture. The study found that women who use statins for 1 year had fewer fractures than those not reporting statin use. After adjustments were made for age, hospital admissions, chronic disease score, and use of non-statin lipid-lowering medications, they concluded that statins have bone-anabolic effects in humans and thereby decrease the risk of fractures in spite of a modest increase in bone mass by statin use (Chan *et al*, 2000).

Using a general practice-based patient population of 3940 case patients with fractures and 23 379 control patients, Meier *et al* (2000) found a significantly reduced fracture risk in those currently taking or recently receiving statins. The greatest reduction in risk was seen in the risk of hip fractures, although other types of fractures were also reduced. By contrast, van Staa *et al* (2001) reported no reduction in the risk of fractures in a population of 81 880 control patients and 81 880 patients with fractures. Both of the studies (Meier *et al*, 2000; van Staa *et al*, 2001) used a similar database. Although case and control subjects in these studies were selected and analyzed somewhat differently, the explanation for these discordant results remains uncertain.

The Geelong Osteoporosis Study reported a 60% reduction in fracture risk in patients who were treated with statins (Pasco *et al*, 2002). In this cross-sectional study of statin use, BMD and fracture risk in 1375 women, including 573 women with and 802 women without incident fractures, were identified radiologically. Patients receiving statin therapy had fewer fractures than those not reporting statin use. Statin use was associated with a 3% greater adjusted BMD for the femoral neck, and BMD also tended to be greater for the spine and whole body, but did not achieve statistical significance (Pasco *et al*, 2002).

Several studies have examined the relationship between statin use and biochemical markers of bone turnover. One study of stored serum samples was performed to compare simvastatin and atorvastatin for their lipid-lowering effects in 846 patients with hypercholesterolemia (Stein *et al*, 2001). The authors reported that bone-specific alkaline phosphatase (BAP) activity, a marker of bone formation, fell 4.1% after 12 weeks of 40 mg day^{-1} of simvastatin therapy and fell 6.3% among those treated with 80 mg day^{-1} of simvastatin, but did not change with treatment with atorvastatin (20 and 40 mg day^{-1}). A prospective study from Hong Kong reported that serum OCN, another marker of bone formation, rose 85% among 17 men and women treated with 20 mg day^{-1} of simvastatin, while urinary N-telopeptide for type I collagen (NTX), a marker of bone resorption, decreased 5.3% (Chan *et al*, 2001). A clinical trial found that OCN and BAP decreased 24% after 26 weeks of treatment with fluvastatin (20 mg day^{-1}), although there was no placebo group in that study (Watanabe *et al*, 2001). There was a longitudinal study of the effects of simvastatin treatment on BMD and bone turnover in hypercholesterolemic postmenopausal women (Montagnani *et al*, 2003). Simvastatin treatment at a dose of 40 mg day^{-1} for 1 year significantly increased BMD in the lumbar spine and femoral neck in hypercholesterolemic women compared with 30 normocholesterolemic, statin-untreated women. The statin-treated patients had a significant increase in serum concentrations of BAP during 3–12 months of treatment compared with control subjects, while the carboxy-terminal fragment of type I collagen (CTX, a marker of bone resorption) concentration in the serum did not change with statin treatment over a 1-year period. They concluded that statins moderately induce bone formation with a positive effect on BMD, despite the small study population and the short follow-up period in that study (Montagnani *et al*, 2003). Taken together, the effects of statin therapy on biochemical markers of bone turnover are conflicting. Both increased and decreased concentrations of formation and resorption markers have been reported. Statins may have their greatest effects on bone turnover after 4–12 weeks of therapy and may modestly reduce markers of bone resorption.

Two previous trials with cardiovascular endpoints included *post-hoc* analyses to examine fracture risk in the statin and placebo groups. In the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) study, 9014 individuals (17% women) were randomized to receive either pravastatin (40 mg day^{-1}) or placebo, and were followed for up to 6 years. Fractures were reported in 183 subjects in the placebo group and in 175 subjects in the pravastatin group (Reid *et al*, 2001). In the Scandinavian Simvastatin Survival Study, 4444 subjects with coronary artery disease (19% women) received either simvastatin (20–40 mg day^{-1}) or placebo. During an average follow-up of 5.5 years, 155 fractures were reported as an adverse event, but the risk of both non-spine fracture and hip fracture were similar among the simvastatin and placebo groups (Pedersen and Kjekshus, 2000). There are explanations for the negative findings of these two trials, including enrollment of an insufficient number of postmenopausal women, inadequate power to determine a difference in the rate of hip fracture, lack of objective adjudication of fracture, and, in the LIPID trial, use of pravastatin that appears to have little effect on bone metabolism *in vitro* (Sugiyama *et al*, 2000; Maeda *et al*, 2003). Recently, Rejnmark *et al* (2004) assessed the effect of simvastatin on BMD and bone turnover in a randomized, controlled trial in postmenopausal women with low BMD. The study found no effect of 1 year of simvastatin treatment (40 mg day^{-1}) in BMD at the lumbar spine, entire hip, femoral neck, and whole body. Moreover, simvastatin did not affect bone turnover, as assessed by serum concentrations of biochemical markers of bone formation and resorption such as OCN and CTX. By contrast, simvastatin treatment significantly increased BMD in the forearm. It is likely that the BMD at different skeletal sites may respond differently to various pathologic conditions. They also suggested that orally administered simvastatin does not possess a general beneficial effect on bone in an *in vivo* study of humans. Nevertheless, statins continue to be considered potentially useful agents for patients with osteoporosis. A larger sample size, transdermal administration, and a longer duration of treatment may detect the effect of statins on the stimulation of bone formation in humans.

Effects of statins in the oral cavity

A clinical study measured mandibular BMD in osteoporotic women and non-osteoporotic women (von Wowern *et al*, 1994). A large cohort study of older women showed that women on estrogen replacement therapy had significantly lower rates of tooth loss, edentia, and use of dentures than those not on replacement therapy (Paganini-Hill, 1995). Therefore, estrogen replacement therapy in postmenopausal women could be associated with a beneficial effect on mandibular bone mass (Jacobs *et al*, 1996). OVX rats are generally used to determine the roles of hormones and mechanical strain on mandibular bone loss. Recently, Kuroda *et al* (2003) reported that estrogen depletion by ovariectomy results in significant decreases in BMD in the molar region of the mandible without affecting mechanical strain, although the changes in BMD were small. Miller *et al* (1997) showed that intermittent hPTH (1–34) administration at pharmacologic doses profoundly stimulates bone formation in the periosteal and cancellous bone surface of the mandibles of aged OVX rats. Their subsequent study demonstrated again that a high dose of hPTH (1–34) increases bone formation rates on the periosteal and endosteal surfaces of the mandibles of aged rats with an advanced stage of estrogen deficiency when given in combination with the antiresorptive agents such as 17β-estradiol and bisphosphonates (Hunziker *et al*, 2000). These results suggest that mandibular bone mass is less susceptible to antiresorptive agents and bone-anabolic drugs than that of vertebrae and long bones.

It is known that the primary function of bone is locomotion, a function requiring a broader scope of tissue-level inspection to evaluate bones as structures (Frost, 1997). Furthermore, mechanical stimulation augments bone mass *in vivo* through an increase in the number of osteoblasts (Oxlund *et al*, 1998). In recent *in vitro* studies using cultured osteoblasts, mechanical strain led to the activation of the ERK/MAP kinase pathway, stimulating cell proliferation (Boutahar *et al*, 2004), and increased expression of collagenase-3, which plays a role in bone remodeling (Yang *et al*, 2004). An intriguing experiment was performed to try to elucidate the relationship between mechanical loading and estrogen deficiency in rats (Jarvinen *et al*, 2003). Mechanical loading through exercise significantly increased BMD and fracture load in OVX rats, whereas sham-operated rats had no change in these bone parameters with exercise. Thus, estrogen-depleted rats had high degree of responsiveness to mechanical loading. The relationship between estrogen and mechanical stress on mandibular bone volume and structure was studied extensively in an OVX rat model. Extraction of teeth from the upper jaw did not influence BMD or structures of the mandible in sham-operated rats, while maxillary molar extraction led to a decrease in mandibular BMD in the molar region in OVX rats (Elovic *et al*, 1995; Kawane *et al*, 2002). Therefore, functional occlusion is more important in maintaining bone mass and structure in the molar region under estrogen-deficient conditions than that under estrogen-replete conditions. A histomorphologic study by Miller *et al* (1997) showed that intermittent administration of hPTH (1–34), a potent bone-anabolic agent, resulted in detectable increases in mandibular bone formation in OVX rats with functional occlusion despite small increase in the formation rate. They also demonstrated that when antiresorptive agents, such as 17β-estradiol, bisphosphonates and calcitonin, were combined with intermittent administration of hPTH (1–34) in non-molar-extracted OVX rats, most indices of bone formation for the mandibles are markedly increased (Hunziker *et al*, 2000). We found a greater increase in the molar region of the mandibles with subcutaneous injections of hPTH (1–34) in OVX rats with maxillary molar extraction than in OVX rats with functional occlusion (Kawane *et al*, 2002). Therefore, maxillary molar extracted OVX rats represent a suitable animal model to assess the efficacy of drugs with moderate activity on the increment of mandibular bone mass. Using these animals we found a significant increase in the BMD in the mandibular molar region in OVX rats treated concomitantly with atorvastatin and hPTH (1–34) at a very low dose or 17β-estradiol compared with a single administration of these agents. As microcomputed tomography (μCT) allowed us to visualize the microarchitecture of bone in three dimensions, we used μCT to analyze bone structure in the molar region of the mandibles. As shown in Figure 6, atorvastatin treatment in combination with

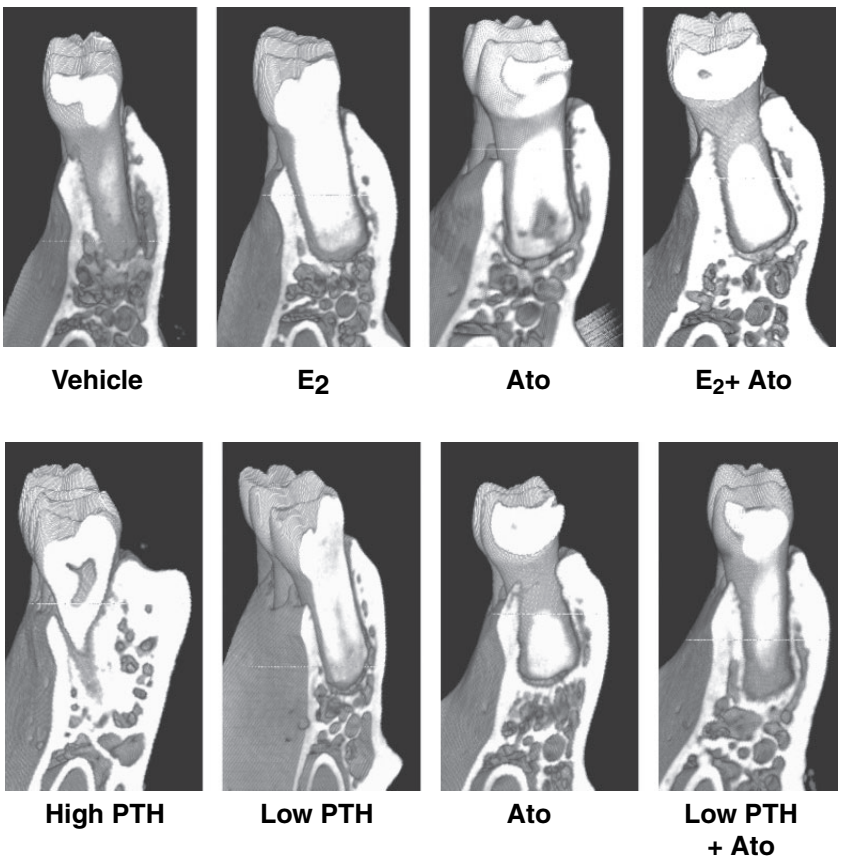

Figure 6 Microcomputed tomographic images of the molar region of mandibles in OVX rats with maxillary molar extraction. The OVX rats were injected subcutaneously with vehicle, atorvastatin (Ato; 2 mg kg^{-1}), 17β-estradiol (E_2; 10 μg kg^{-1}), hPTH (1–34) (low PTH; 1 μg kg^{-1}), or a combination of Ato and the hormone for 8–12 weeks. The high PTH group was injected with hPTH (1–34) at 17.5 μg kg^{-1}

17β-estradiol or hPTH (1–34) at a subeffective dose tended to increase trabecular bone area in OVX rats without functional occlusion (Horiuchi and Terashima, unpublished data). Mandibular BMD was less in osteoporotic women compared with non-osteoporotic women (von Wowern *et al*, 1994), and postmenopausal women with high skeletal BMD had a greater retention of teeth with deep periodontal pockets (Klemetti *et al*, 1993). Osteoporosis or systemic bone loss was associated with loss of periodontal attachment, loss of alveolar bone height, and tooth loss (Tezal *et al*, 2000). Systemic treatment of osteoporosis also acts on oral osseous tissues and influences the progression of periodontal disease. It is generally accepted that effective treatments of established osteoporosis include the combination of antiresorptive and anabolic (stimulating bone formation) agents. It is likely that statin treatment combined with antiresorptive drugs improves oral health. Statins may have beneficial effects for the treatment of dental disease.

Conclusion

Statins have great salutary effects, reducing cardiovascular morbidity and mortality. The clinical benefits of statins may not only be due to lipid-lowering effects but also due to their pleiotropic effects. Recently, statins were found to be potent stimulators of bone formation *in vitro*. Studies *in vitro* clearly demonstrate that statins promote osteoblast differentiation as evidenced by stimulating expression of BMP-2, VEGF, OCN, and BSP, and finally enhanced mineralization. The bone anabolic effect is mediated by inhibition of the mevalonate pathway. By contrast, systemic administration of statins in OVX rats manifests moderate potencies on the stimulation of bone formation, as measured by BMD and mechanical strength. Most animal studies have used oral administration of statins. Parenteral injections allow statins to reach osseous tissues at high concentrations, because orally administered statins enter the enterohepatic circulation and are metabolized in the liver. Therefore, transdermal injections of statins may result in greater effects on bone formation. Most observational studies suggest that statin use is associated with a reduced risk of hip and possibly other fractures. In clinical studies, statin use has only a modest affection on BMD and inconsistent effects on bone turnover. Clinical trials have not shown a significant salutary effect on bone. Nevertheless, statins continue to be considered potential therapeutic agents for patients with osteoporosis. In the future, development of new statins that are more specific for bone will be necessary for the prevention or treatment of osteoporosis. In the oral cavity, the addition of statins in combination with traditional drugs used to treat osteoporosis such as 17β-estradiol and hPTH (1–34), elevates cancellous bone mass in the mandibles, especially in the alveolar bone. It is suggested that statin use may suppress the progression of periodontal disease of osteoporotic patients.

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