LEADING ARTICLE

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# The Pleiotropic Effects of HMG-CoA Reductase Inhibitors

## Their Role in Osteoporosis and Dementia

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## Abstract

HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis and its inhibition exerts profound effects on cellular metabolism. Inhibitors of this enzyme are used in clinical practice to lower plasma cholesterol levels and are commonly collectively referred to as 'statins'. A number of *in vitro*, *in vivo* animal, and clinical studies suggest that properties of statins other than cholesterol lowering may be of biological importance. These diverse properties are often referred to as 'pleiotropic' and suggest that statins may affect a number of diseases of ageing. In this article we review the biological plausibility and clinical evidence of a role for statins in modulating two diseases of ageing: osteoporosis and dementia (including Alzheimer's disease). In both diseases, there is a sound cellular and laboratory basis for a plausible therapeutic effect of statins. In the case of osteoporosis, there are conflicting data regarding clinical benefit, with both negative and positive results reported. In particular, secondary analyses of randomised, controlled studies have shown no reduction of fracture risk by statins. In the case of dementia there are fewer clinical studies but there is clear anticipated benefit in macrovascular dementias attributable to statin-mediated reduction of the risk of stroke. Overall, there are a lack of prospective, placebo-controlled, randomised data testing statins and modulation of the risk of osteoporosis-related fracture or of clinical dementia, where these are primary outcomes. Until such data are available, the use of statins appears promising but cannot be recommended as a primary therapeutic modality for either condition.

HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis and its inhibition exerts profound effects on cellular metabolism. Inhibitors of this enzyme such as pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin, cerivastatin (voluntarily withdrawn) and rosuvastatin (currently investigational in the US) are used in clinical practice to lower plasma cholesterol levels, and are commonly collectively referred to as 'statins'. In this article we specifically address the effects of statins in two diseases of ageing which are somewhat distinct from macrovascular atherosclerosis: osteoporosis and dementia.

The primary clinical pharmacological function of statins is to inhibit HMG-CoA reductase (in the liver), which results in reduced hepatic cholesterol synthesis and consequent upregulation of hepatic low-density lipoprotein (LDL) receptor expression and LDL clearance.[1] By upregulating LDL receptor expression and decreasing LDL production,

statins lower LDL cholesterol and significantly reduce cardiovascular morbidity and mortality in populations at high risk of coronary events (for example, see references[2,3]). Statins also exert important effects on other lipoprotein classes: elevating high-density lipoprotein (HDL), lowering triglycerides and altering levels of plasma apolipoproteins.

The reduction of plasma cholesterol is of undoubted importance in mediating the clinical benefit of statins.[4] However, a number of *in vitro*, *in vivo* animal, and clinical studies suggest that properties other than cholesterol lowering may be of biological importance.[5-9] These diverse properties are often referred to as 'pleiotropic' (Gk. 'pleio'- meaning many, and 'tropos'- meaning manner) indicating diverse modes of action.

Most inclusively, all *in vitro* and *in vivo* effects of statins independent of those attributable to reduction of plasma LDL cholesterol levels can be considered 'pleiotropic'. However, the distinction is problematic as lowering plasma cholesterol itself generates multiple biological consequences. For example, it is known that greater plaque lipid content, thinning of the collagen cap, increased content of macrophages and increased local expression of collagen-degrading matrix metalloproteinases all increase the risk of atherosclerotic plaque rupture. Dietary reduction of LDL cholesterol in rabbits favourably modifies all these processes,[10] indicating that the reduction of LDL cholesterol in itself has complex consequences. Similarly, the acute lowering of LDL cholesterol following apheresis (removal of plasma LDL by filtration[11]) produces immediate improvement in endothelial function,[12] indicating that reduction of LDL cholesterol can directly affect arterial function.

## 1. Cellular Actions and Pharmacology of Statins

### 1.1 Cellular Cholesterol Biosynthesis

Statins block cholesterol synthesis through interference with mevalonate pathway (figure 1 [13]). Mevalonate is present in virtually all living cells and the activity of HMG-CoA reductase has been positively correlated with mammalian cell growth. Downstream metabolites of mevalonic acid such as geranylgeranylpyrophosphate and farnesylpyrophosphate are important for isoprenylation of cellular proteins involved in regulation of cellular growth, cell-to-cell signalling and apoptosis.[7,14] Isoprenoids bind to a range of nuclear envelope proteins and growth-regulating proteins (including G proteins ras, rho, rac), stabilising their attach-

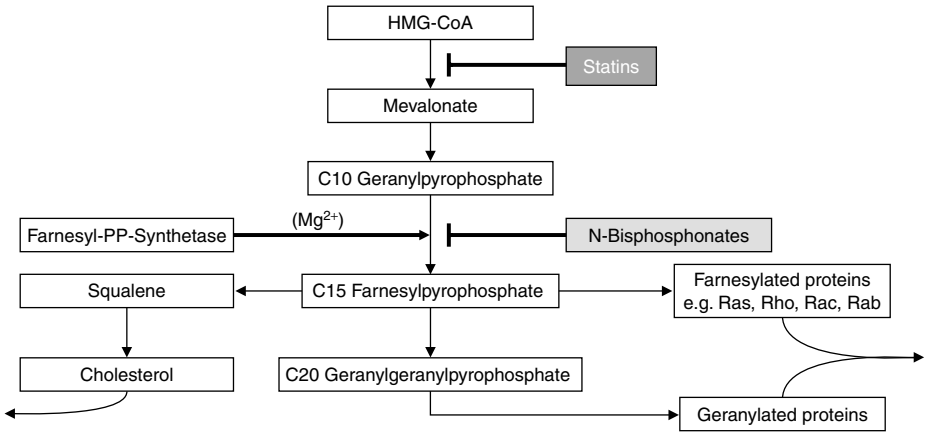


**Fig. 1.** Simplified mevalonate pathway and the site of action of HMG-CoA reductase inhibitors (statins) and bisphosphonates.

ment to cell membranes. Interference with isoprenylation by statins affects cell growth, cell proliferation and apoptosis, and has been proposed to have antineoplastic potential. The ubiquitous presence of the mevalonate pathway and the diversity of end-products affected by isoprenoid synthesis implies that modulation of this pathway can have far reaching consequences in a range of body tissues and in a range of clinical conditions.

### 1.2 Pharmacology of Statins Relevant to Pleiotropic Properties

Statins demonstrate different degrees of absorption, water solubility, pharmacokinetics and pharmacodynamics (table I). The liver is the primary site of action of statins and these agents have a high rate of first pass hepatic metabolism.[15,16] Although their plasma half-life is typically short, their reduction of LDL cholesterol is gradual and much more sustained, with maximal effects seen after several weeks of therapy. This reflects their net effect on hepatic LDL receptor expression and clearance of plasma LDL, decreased very low-density lipoprotein (VLDL) production, and metabolism of hepatocyte cholesterol by 7a-hydroxylase, as well as other factors. Their effects on plasma triglyceride and HDL are also gradual, reflecting changes in the synthesis and secretion of triglyceride rich lipoproteins, and probable stimulation of HDL apolipoprotein synthesis.[17]

Although pleiotropic effects are most often assigned to local effects of statins in extrahepatic sites, their actions on the liver may generate systemic anti-inflammatory effects with extrahepatic consequences. For example, statins reduce plasma C-reactive protein (CRP) levels[25] (which is released by the liver in response to interleukin [IL]-6). Moreover, as the liver has roles in the detoxification of xenobiotics and potential neurotoxins,[26] changes in hepatocyte physiology due to altered cholesterol synthesis and plasma membrane composition after HMG-CoA reductase inhibition may also exert systemic effects. Given their extensive hepatic clearance, that the pleiotropic effects of statins may be attributable to hepatic effects may require further evaluation.

### 1.3 Extrahepatic Concentrations of Statins

Statins have primarily been designed to inhibit hepatic HMG-CoA reductase with intentional high hepatic selectivity, as significant extra-hepatic concentrations theoretically raise the potential of unwanted side effects. Ironically, in the context of apparent pleiotropic effects the accumulation of statins in extra-hepatic tissues may be of therapeutic importance. The concentration of unbound drug in plasma or extracellular fluid, the effective membrane permeability of drug, and the concentrations and potency of active metabolites are all potential determinants of peripheral effects (reviewed in Lennernas and Fager[15]).

Membrane transport via both active carrier-mediated or passive processes is thought relevant to statins. Passive transport is principally determined by the ability of drugs to partition into lipophilic membranes, and this is approximated by lipophilicity or octanol/water partition co-efficient (table I). Statins differ widely in this property, pravastatin and rosuvastatin, for example, having low lipophilicity and simvastatin and lovastatin having high lipophilicity. Penetration of the blood brain barrier is expected to parallel lipophilicity,[15] and this may be relevant to the development of adverse effects such as insomnia, and to local actions of statins on CNS cholesterol synthesis. However, the extent to which blood brain barrier penetration by statins occurs in humans, and whether there is any predictable relationship between lipophilicity and concentrations in human bony tissues, is unknown.

The clinical significance of apparent lipophilicity is not straightforward. Lipophilicity varies according to whether one considers the pro-drug, the active drug or its polar metabolite. For example, lovastatin and simvastatin are administered as inactive lactone pro-drugs which are much more lipophilic than the active hydroxy acid forms, and these in turn are much more lipophilic than pravastatin.[16] Although the hydrophilicity of pravastatin will inhibit its passive penetration of cell

**Table I.** Selected pharmacokinetic properties of statins[a]

| Parameter | Atorvastatin | Cerivastatin | Fluvastatin[b] | Lovastatin | Pravastatin | Rosuvastatin | Simvastatin |
|---|---|---|---|---|---|---|---|
| **Absorption (%)** | 30 | 98 | 98 | 30 | 34 | NA | 60-85 |
| $t_{max}$ (h)[c] | 2-3 | 2.5 | 0.5-1 | 2-4 | 0.9-1.6 | 3.0 | 1.3-2.4 |
| $C_{max}$ (μg/L)[c] | 27-66 | 2 | 448 | 10-20 | 45-55 | 37.0 | 10-34 |
| **Bioavailability (%)** | 12 | 60 | 19-29 | 5 | 18 | 20 | 5 |
| **Distribution** | | | | | | | |
| Protein binding (%) | 80-90 | >99 | >99 | >95 | 43-55 | 88 | 94-98 |
| Lipophilicity: | | | | | | | |
| octanol : water[d] | 1482 : 1 | 29.5 : 1 | 1738 : 1 | 18 620 : 1 | 0.6 : 1 | NA | 47 860 : 1 |
| log D[e] | 1.11 | 1.69 | 1.27 | NA | -0.84 | -0.33 | 1.60 |
| **Metabolism** | | | | | | | |
| Hepatic metabolism (%) | >70 | NA | >68 | >70 | 46-66 | Minimal | 78-87 |
| Active metabolites | + | + | – | + | – | NA | + |
| CYP (predominant) | CYP3A4 | CYP3A4 | CYP2C9 | CYP3A4 | Negligible | CYP2C9 CYP2C19[f] | CYP3A4 |
| **Excretion** | | | | | | | |
| $t_{1/2}$ (h) | 15-30 | 2.1-3.1 | 0.5-2.3 | 2.9 | 1.3-2.8 | 18-20 | 2-3 |
| Urinary (%) | Negligible | 30 | 6 | 10 | 20 | 10 | 13 |
| Faecal (%) | Major | 70 | 90 | 83 | 71 | 90 | 58 |

a Table is reproduced with permission from Corsini et al.[18] and includes additional data from references.[19-22]

b For fluvastatin extended release preparation, a single dose of 80mg generates $t_{max}$ of 4h, $C_{max}$ 61μg/L and $t_{1/2}$ of 4.5h.[23]

c $t_{max}$ and $C_{max}$ values derived from single 40mg dose.

d Lipophilicity expressed as ratio of concentration of drugs in octanol/water from Corsini et al.[18]

e Lipophilicity determined as log D (log to base 10 of the concentration of distribution of active hydroxy forms of statins between 0.01 mol/L phosphate buffer pH7.4 and octanol [1 : 100v/v]) was determined by gradient HPLC (Buckett et al.[22]). The most lipophilic statin is simvastatin and the most hydrophilic is pravastatin. Discrepancies in relative lipophilicities between references[18] and [22] may relate to the respective use of lactone or active hydroxy preparations.

f Rosuvastatin appears to be metabolised only to limited degree in humans,[21] but pharmacological inhibition of CYP2C9 and CYP2C19 does inhibit metabolism of rosuvastatin in hepatocyte cultures.[24]

**$C_{max}$** = peak serum concentration; **CYP** = cytochrome P450; **HPLC** = high performance liquid chromatography; **NA** = not available; **$t_{1/2}$** = half-life; **$t_{max}$** = time to $C_{max}$; + indicates yes; –indicates no.

membranes (and the blood brain barrier), because it undergoes lesser hepatic extraction and demonstrates less protein binding than other statins, the effective free plasma concentration and active extrahepatic distribution of pravastatin may be much higher than that of lipophilic agents.[15] However, hepatoselectivity of pravastatin is promoted by its uptake via carrier-mediated processes.[16] Rosuvastatin[20,21,27] appears to be relatively hydrophilic with only limited hepatic metabolism (table I), and may behave similarly to pravastatin in terms of tissue distribution.

### 1.4 Summary of Pharmacokinetic Considerations and Possible Peripheral Actions of Statins

As there are multiple competing pharmacokinetic properties, it is extremely difficult to anticipate peripheral actions and tissue uptake of statins from current data. Our ability to extrapolate the *in vitro* effects of drugs to clinical conditions is particularly limited if drugs do not achieve meaningful concentrations at their site of proposed action. Clinical data are essential for the evaluation of apparent non-lipoprotein-related or pleiotropic actions.

## 2. Statins and Osteoporosis

### 2.1 Background

Osteoporosis is endemic throughout the world and is associated with an increase in the incidence of low-trauma fractures (mostly of the vertebral spine, femur and distal radius).[28] Elderly women are particularly at risk, as approximately 40% of women will experience one or more fractures after age 50 years.[28] The financial burden of osteoporosis is enormous, with a predicted cost of hip fractures in the US in the year 2050 of $US131.5 billion.[29] Risk factors for osteoporosis include prevalent fracture, postmenopausal state in women, positive family history of hip fracture, history of smoking, inadequate calcium and vitamin D intake, low body mass, sedentary life style, and being Caucasian or Asian.

Bone mineral density (BMD) is the best available quantifiable predictor of future osteoporosis fracture. However, its relationship to incidence of fractures is complicated by interaction with other risk factors for fracture such as falls, neuromuscular competence and cognitive impairment.[28] Other predictors of osteoporosis fracture risk include high bone turnover rates and increased bone resorption rates. Markers of turnover appear to improve the prediction of bone loss and of fracture risk.[30-32]

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 held in common.[33,34] The relationship is supported by the observation that the progression of aortic calcification is most severe in women with most severe metacarpal bone loss.[35] Factors which may promote both processes include oestrogen deficiency, parathyroid hormone excess, (oxidised) lipoprotein lipids,[36,37] proinflammatory chemokines and cytokines such as IL-1, IL-6, and tumour necrosis factor (TNF)-α, and obesity-related factors such as elevated plasma leptin levels.[38-41]

### 2.2 *In Vitro* Pharmacological Modulation of Osteoporosis

At a cellular level, osteoporosis can most simply be considered to result from an imbalance between bone formation and resorption. Bone is deposited by osteoblasts, which are derived from bone marrow stromal cells, and resorbed via the action of osteoclasts, which are of macrophage lineage. Factors that decrease osteoclastic bone resorption or increase osteoblastic bone formation, or modulate the cycle frequency of bone remodelling, can be expected to reduce the progression of osteoporosis. The regulation of the differentiation and function of osteoblasts and osteoclasts is complex, and our understanding of these has been enhanced by the molecular delineation of certain rare human genetic diseases.[42] Recently identified determinants of bone remodelling include the LDL

receptor-related protein 5, core binding factor α1 and osteoprotegerin.[42]

With ageing, there is relative reduction of osteoblast activity and life span, and in the perimenopausal state hormone deprivation is associated with relatively increased osteoclast activity.[43] Most currently available proven therapies (including bisphosphonates and selective oestrogen receptor modulators) inhibit bone resorption,[44,45] but a number of agents (such as statins) have the potential to promote bone deposition [46,47] and may be found in the future to interact synergistically with anti-resorptive therapies.

Bone morphogenetic protein (BMP)-2 plays an important role in osteoblast differentiation and bone formation. In a landmark study, Mundy et al. found that statins enhance expression of BMP-2 m-RNA in cultured mouse and human bone cells.[48] They confirmed the significance of their results *in vivo* by demonstrating that lovastatin and simvastatin increased bone formation when injected subcutaneously over the calvaria of mice and increased cancellous bone volume when orally administered to rats. Others have found that BMP-2 is induced in cell cultures by simvastatin but not pravastatin, via inhibition of the HMG-CoA reductase pathway.[49]

Aminobiphosphonates, which are currently used for the treatment of osteoporosis, primarily inhibit osteoclastic bone resorption.[44,50] They interfere with prenylation of small guanosine triphosphate (GTP)-binding proteins such as rho p21 via inhibition of geranylgeranylation, and the resultant cytoskeletal disruption and apoptosis inhibits osteoclast activity. Adding geranylgeranol reverses the above-mentioned effect.[50] Statins can also affect the resorption of bone.[50] Statins affect mevalonate synthesis through HMG-CoA reductase inhibition, and thus share with aminobisphophonates the ability to inhibit formation of geranylgeranyl phosphate (see figure 1) and to inhibit osteoclast function and reduce osteoclast number.[51] Although statins and bisphosphonates share effects via the mevalonate pathway, they differ in their precise site of action (figure 1). It is not clear at this stage which of the two effects, enhanced bone deposition or reduced bone resorption, is more important for the roles of statins *in vivo*.

### 2.3 *In Vivo* Human Studies

The most clinically significant measure of benefit in the prevention or treatment of osteoporosis is a reduction in the incidence of fractures. However, at this time most clinical studies regarding osteoporosis, fracture-risk and statins have been observational. Some data have been derived from post-hoc analyses from randomised studies designed to investigate the effect of statins on vascular events, and from smaller studies using surrogate endpoints, such as improved or sustained BMD or changes to bone turnover. To date, no randomised, controlled trials with primary endpoints of either modulation of BMD or incidence of fractures have yet been published.

#### *2.3.1 Biochemical Studies*

Biochemical studies in humans are inconsistent in their evidence that statins promote bone deposition. Chan et al. found that serum osteocalcin levels increased 4 weeks after therapy, whereas other bone markers including serum bone-specific alkaline phosphatase, urine deoxypyridinoline and urine cross-linked N-telopeptides of type I collagen did not show any significant changes.[52] In a retrospective study of patients with type 2 diabetes mellitus, Chung et al. found that use of statins increased BMD in men and attenuated bone loss in women without significant difference between different statins.[53] A recent randomised, controlled study found no effect of simvastatin on markers of bone formation (bone-specific alkaline phosphatase) or bone resorption (N-telopeptides and C-terminal propeptide of type 1 collagen) in osteopenic women.[54]

#### *2.3.2 Bone Mineral Density Studies*

Edwards et al.[55] investigated the effect of statins (most commonly simvastatin and pravastatin) on BMD in a population-based cohort of 41 women taking statins (median duration of statin use of 48

months) and 100 age-matched controls. BMD of the spine and hip was greater in patients taking statins after adjustment for age, height, weight and hormone replacement therapy. Similarly, in a retrospective study of 69 individuals with type 2 diabetes, BMD decreased in the 36 not receiving a statin, but increased after 15 months in the 33 receiving pravastatin, lovastatin or simvastatin, even after adjusting for fasting glucose level, glycosylated haemoglobin ($HbA_{1C}$), insulin levels and C-peptide.[53] The fact that all three agents were similarly effective indicates that lipophilicity is unlikely to be important in determining effects on bone density, and questions the relevance of *in vitro* studies suggesting variation between statins in their effect on bone metabolism.

In contrast to the above, in a small prospective study of 31 postmenopausal women with elevated cholesterol levels, there was a trend towards greater preservation of BMD of the lumbar spine in those randomised to fluvastatin 20mg than in those randomised to treatment with pravastatin 10mg, with no consistent difference in their effects on markers of bone metabolism.[56] In the absence of a placebo group, the significance of this result is unclear.

#### 2.3.3 Fracture Risk

In a 2-year case-control study (928 cases and 2747 controls), women with 13 or more completed statin prescriptions had a reduced risk of low trauma fracture (odds ratio 0.48, 95% CI 0.27–0.83) relative to women with no record of statin usage.[57] There was no benefit in women receiving fewer than 13 prescriptions of statins. There was no positive association between fracture risk and use of non-statin, lipid-lowering drugs (clofibrate, cholestyramine, colestipol, gemfibrozil and probucol), suggesting a class-specific effect. The results were not controlled for patient height and weight, and confounding factors cannot be excluded. Moreover, the biochemical effects of these non-statin, lipid-lowering drugs are so diverse that meaningful conclusions regarding mode of action are not possible.

Using a general-practice-based patient population of 3940 case patients with fractures and 23 379 controls, Meier et al.[58] found significantly reduced fracture risk in those currently taking or recently receiving statins. The greatest reduction in risk was seen for the risk of hip fractures, although other types of fracture were also reduced. Fibric acid derivatives (fibrates) were associated with a trend to reduction in risk but this was not significant. Despite using a similar database to Meier, Van Staa et al. found no reduction in the risk of fracture[59] in a population of 81 880 controls and 81 880 patients with fractures. Importantly, duration of statin use and dose of statin had no bearing on the risk of fracture.

In a careful case-control study of 6110 patients aged over 65 years, 1222 cases were identified on the basis of hip fracture, and compared with 4888 age and sex-matched controls for the incidence of fracture in the preceding 180 days and 3 years. The adjusted relative risk of hip fracture was reduced by approximately 50% by statin use in the previous 180 days or 3 years.[60] The effect was dependent on the duration of therapy, and was specific to statins and not other lipid-lowering drugs. The effect of obesity could not be accounted for, although a recent study presented in abstract form suggests a protective effect of statins may exist even when allowing for body mass index.[61]

An important secondary analysis of the large, double-blind, placebo-controlled Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study has recently been published. This study randomised 9014 patients (17% women) with a recent history of acute myocardial infarction and/or unstable angina pectoris, and cholesterol between 4.0–7.0 mmol/L, to pravastatin or placebo, and found that pravastatin did not protect against the development of osteoporotic fracture.[62] However, the study had several limitations with regard to this end-point: only a small number of female patients were enrolled; the mean age was only 62 years; and, somewhat predictably in view of the age and sex distribution, only a small number of fractures were observed in the study popu-

lation (101 with placebo and 107 with pravastatin). Similar results were obtained for various subtypes of fractures, for women only and for patients over 65 years. Whether the negative effect is specific for pravastatin or related to the study population will be clarified in ongoing studies.

Pederson and Kjekshus have recently reported on a retrospective analysis of the randomised, controlled Scandinavian Simvastatin Survival Study (4S).[63] In this population of 4444 patients (aged 35–70 years, 19% women), 155 patients experienced fractures during a mean follow up of 5.5 years. There was no reduction in the risk of fracture following treatment with simvastatin. Limitations of this analysis include the inability to distinguish fractures resulting from severe trauma from those more directly related to osteoporosis and the fact that the study population was not at high risk of developing osteoporotic fracture.

The Heart Protection Study (HPS) has recently been published.[64] 20 536 adults (40–80 years of age) with a history of coronary heart disease, occlusive vascular disease, diabetes or treated hypertension were randomised to simvastatin 40mg or placebo, and followed up for an average of 5 years. Pre-specified tertiary outcomes included the rate of hospitalisation for fractures. Hospitalisation for all fractures, excluding road-traffic-related accidents, were identical in the statin and placebo groups, as was the incidence of fractures related to osteoporosis (hip, wrist, or spine). These negative data are consistent with retrospective analyses from the LIPID and 4S cohorts.

### 2.4 Conclusions

Data accumulated from *in vitro* and animal studies support the feasibility of statin-mediated effects on bone. Differences in study design, study population, duration of treatment and statistical analyses complicate synthesis of conclusions derived from clinical studies published to date. Data suggesting improved BMD and reduced bone turnover in humans is inconsistent.

Statistically significant inverse associations between statin use and hip fractures were reported in three non-randomised studies.[57,58,60] In four other studies, three of which were randomised and controlled (LIPID, 4S and HPS), the results were negative.[59,62-64] As these represent the largest retrospective analysis and the largest prospective studies to date, the negative results must be taken seriously.

Although it is possible that patients taking statins for other indications may be favourably reducing their risk of fracture, there is insufficient evidence to justify prescription of statins for the prevention or treatment of osteoporosis instead of established therapy. This must remain the case as long as the extent of benefit relative to established therapy is unknown. Large randomised, placebo-controlled trials currently in progress should resolve these issues.

## 3. Statins and Dementia

One of the most widely discussed, ostensibly non-macrovascular, roles proposed for statins has been in the prevention and or treatment of dementia, including Alzheimer's disease (AD). Relative to osteoporosis, there is little clinical data describing the use of statins and AD, and much of our current knowledge requires extrapolation from our understanding of pathological processes. The pathological processes causing dementia are complex and often overlap, making attribution of disease-specific benefit difficult. We will focus on the most common types of dementia, AD and vascular dementia (VD), and begin by introducing important pathological components of the two conditions and their potential overlap. Pathological overlap is a particularly important issue if statin-mediated effects are to be attributed to pleiotropic rather than lipid-lowering properties.

### 3.1 Pathology of Dementia

Dementia is a clinical syndrome characterised by persistent and usually progressive impairment in multiple cognitive functions. Traditionally, dementias are grouped into AD and non-AD dementias, the latter including diverse causes such as VD, dementias with extrapyramidal features (such as

dementia with Lewy bodies) and fronto-temporal dementias.[65] AD prevalence increases exponentially after 70 years of age.[66] VD includes dementia due to disease of large vessels and of small vessels, and the two pathologies are not mutually exclusive.

#### 3.1.1 Pathology of Alzheimer's Disease (AD)

AD is characterised by intra- and extra-vascular deposition of β-amyloid protein, and the presence of neurofibrillary tangles.[67] The degree of cortical atrophy correlates with the severity of dementia in AD and this distinguishes AD from normal ageing. There is consistent substantial neuronal loss, which parallels the pattern of neurofibrillary pathology, and there is disease of cholinergic, serotoninergic and noradrenergic loci. Small vessel disease can cause a clinical syndrome indistinguishable from AD, and dementia with Lewy bodies can have clinical and pathological overlap with AD.

β-Amyloid (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. Aβ peptides are released from cells after endosomal/lysosomal hydrolysis of re-internalised APP, and deposition in the brain and microvasculature precedes the onset of dementia by years. Neurofibrillary tangles (NFT) are paired helical filaments of the microtubule-associated protein tau, which stabilises microtubules allowing fast axonal transport. NFT are not unique to AD, but do co-localise with sites of neuronal loss and correlate with measures of functional decline. Inflammatory processes are also active in AD as acute phase proteins and proteins of the complement pathway are found in Aβ plaques, and these may derive from microglia.[67]

#### 3.1.2 Clinical and Pathological Overlap of AD and Vascular Dementia (VD)

In macrovascular VD the greater the volume of infarcted tissue the greater the risk of dementia and multiple sites of infarction interact to increase this risk. Although some vascular pathology exists in up to 38% of patients with dementia at autopsy, only <10% have pure vascular pathology sufficient to account for dementia.[65] In VD due to microvascular disease, leukoencephalopathy and microinfarction may be the most frequent pathology.[68]

Clinically, AD is marked by insidious memory loss followed by gradual progression to dementia without focal neurological signs. In contrast, vascular or multi-infarct dementia is characterised by cognitive impairment in several domains, and radiological or clinical evidence of neurological deficits consistent with stroke.[65,69] However, patients with small vessel disease may present in a manner identical to that of AD and microvascular disease may be the most frequent pathology underlying vascular dementia.[68]

There is both overlap and synergy between AD and VD pathology in promoting dementia. Patients with AD have more dementia if they also have coexistent cerebrovascular disease, and pre-existing degenerative processes appear to contribute to post-stroke dementia.[70] Cerebrovascular disease may in part contribute to AD pathology by promoting non NFT-mediated neuronal loss and exacerbating Aβ plaque formation.

These considerations indicate that clinical distinction of types of dementia is difficult and that attribution of disease-specific effects of statins in dementias may be unreliable. In any individual, beneficial effects of statins may therefore arise from their effects on VD or AD or their combination.

### 3.2 Modification of Risk Factors for Vascular Disease and Dementia

#### 3.2.1 Statins Reduce the Risk of Stroke

It is well recognised that statins reduce the incidence of stroke, as evidenced from large, randomised, controlled trials. The 4S, the Cholesterol and Recurrent Events Study (CARE), and the LIPID study reported significant reduction in stroke rates by between 19–31%.[2,71,72] Consequently, statins can be expected to protect against development of dementia related to large vessel disease, at least to the extent attributable to reduc-

ing cerebrovascular events. Reduced risk of stroke may be attributable to many properties of statins including cholesterol reduction, restoration of vascular endothelial function and antithrombotic effects.[8]

The HPS, which randomised patients to simvastatin 40mg or placebo, included as a pre-specified tertiary outcome measures of cognitive impairment.[64] Despite a highly significant 25% reduction in the risk of stroke in participants taking simvastatin, there was no difference in the incidence of cognitive impairment as detected by telephone interview nor was there a difference in the incidence of reported dementia. Relative deficiencies in these data include the limited cognitive evaluation performed, the presumably low (but unspecified) baseline incidence of dementia in this population and the lack of comparative baseline data in the two groups. Nevertheless, these data do not support a clinically important effect of statins on the risk of dementia in patients receiving these medications for established indications.

#### 3.2.2 Vascular Risk Factors Affect the Risk of AD as Well as Increasing the Risk of Stroke

Cholesterol accumulates in senile AD plaques, and promotes both amyloid fibril formation and the interaction of Aβ with cell membranes.[73-75] There is a strong association between plasma cholesterol level and the degree of Aβ accumulation in the brains of rabbits and mice.[76,77] Epidemiological studies support the experimental association, demonstrating increased risk of AD in humans with elevated plasma cholesterol levels. Other risk factors for vascular disease, such as the apo E4 phenotype and mid-life hypertension, also increase the risk of later AD.[78-81] In conjunction with supportive *in vitro* data, it is reasonable to conclude that reduced progression or incidence of AD could follow reduced systemic or cerebral cholesterol concentrations, both of which may be affected by statins.

Plasma lipoproteins do not cross the blood brain barrier, CNS cholesterol input being either entirely or predominantly synthesised *de novo* in the CNS.[82] Cholesterol output from the CNS is facilitated by conversion of cholesterol to 24-OH cholesterol, which crosses the blood brain barrier to enter the plasma,[82] and plasma and cerebrospinal fluid levels of 24-OH cholesterol are increased in patients with AD.[83] Statins reduce the plasma 24-OH cholesterol level, indirectly suggesting they affect CNS cholesterol synthesis.[84] Animal models indicate that high-dose statins can reduce intracellular and extracellular concentrations of AD Aβ peptides *in vivo*,[85] supporting that inhibition of brain cholesterol synthesis may inhibit the development of AD.

#### 3.2.3 Statins Affect Endothelial Function and the Inflammatory Response

Statins may also affect the development of dementia by the modulation of two further processes: endothelial function and inflammation. A recent study has identified that pre-treatment with a statin up-regulates endothelial nitric oxide synthase (eNOS) augmenting cerebral blood flow and reducing cerebral infarct size in mice.[86] The beneficial effects were absent in eNOS-deficient mice and occurred via the mevalonate pathway, being reversed by mevalonate and geranylgeranylphospate but not farnesylphosphate. Rho negatively regulates eNOS expression and statins, by blocking Rho geranylgeranylation, stabilise eNOS mRNA.[87]

AD pathology has been associated with an inflammatory glial response to neuronal injury,[88] raising the possibility that anti-inflammatory drugs may have a place in its therapy.[89] However, this is far from resolved, as other studies have suggested anti-inflammatory drugs improve neuropsychological test scores but do not affect extent of inflammatory glia, plaques or tangles.[90] As statins reduce plasma CRP levels,[25] and can modulate the behaviour of antigen presenting cells,[91,92] direct anti-inflammatory effects of these agents on the glial response are a speculative but interesting possibility for disease modification.

### 3.3 Statins and AD

#### 3.3.1 In Vitro Studies

Recently lovastatin and simvastatin were found to reduce levels of Aβ peptides in cultures of cor-

tical neuronal and hippocampal cells *in vitro* and after *in vivo* administration.[85] This effect may be related to the reduction in plasma cholesterol and brain cholesterol by statins, as local cholesterol depletion by statins or cyclodextrins also reduces the deposition of Aβ in neuronal cultures.[93] Cyclodextrins are cyclical oligomers of glucose with a hydrophobic interior, which solubilise and promote the cellular elimination of hydrophobic molecules such as cholesterol and other sterols (for example, see references[94,95] ), but do not exert direct effects on HMG-CoA reductase. Given that most input of cholesterol into the CNS comes from *in situ* synthesis,[82] local inhibition of cholesterol synthesis by statins may be particularly important.

#### 3.3.2 Clinical Studies

The use of statins has been associated with significantly lower prevalence of AD in non-randomised studies. In a retrospective cross-sectional study of patients from three different hospitals, Wolozin et al. found that pravastatin and lovastatin, but not simvastatin, significantly reduced the risk of developing AD.[96] None of the statins reduced the rate of transient ischaemic attacks and the prevalence of co-existent cerebrovascular disease was not known. Important limitations were the lack of case-control design (thus drug groups were unmatched for other risk factors), and the inability to control for the duration of medication usage or plasma cholesterol level. The lack of effect of simvastatin relative to pravastatin and lovastatin is not easily explained by efficacy of cholesterol lowering or lipophilicity, as simvastatin more closely resembles lovastatin in these respects than does pravastatin. This also does not agree with blood brain barrier penetrance, as lovastatin and simvastatin have high penetrance, and pravastatin has negligible penetrance.[97-99] It may be explained by shorter duration of therapy in the simvastatin group in this study.[100]

In a nested case-control study from the UK-based General Practice Research Database undertaken between 1992 and 1998, 284 cases with dementia were compared with 1080 controls.[101] After adjustment for age, sex, hypertension, history of coronary artery disease, history of cerebral ischaemia, body mass index and smoking, use of statins reduced the risk of developing dementia by 70%, compared with those without statin treatment or other lipid-lowering drugs (relative risk 0.29; 95% CI 0.15–0.63, p = 0.002). Important for comparison with Wolozin et al.,[96] simvastatin was the most commonly prescribed agent and appeared effective. Numbers were insufficient to delineate drug-specific or duration-specific effects.

In a small, recently published study, lovastatin induced a dose-dependent reduction in the plasma concentration of Aβ peptide.[102] Although indirect, this is interesting data supporting modulation of a biological process relevant to AD.

In a randomised, placebo-controlled, double-blind study of 209 adults (mean age 46 years), 6 months treatment with lovastatin exerted no significant effect on cognitive functions. Indeed, placebo exerted slightly greatly improvements in attention and psychomotor speed than did lovastatin.[103] These results do not indicate cognitive improvement in healthy young adults, but are of uncertain relevance to elderly populations or those with pre-existing cognitive impairment.

### 3.4 Conclusions

At this time, there are clear data indicating that statins reduce the incidence of stroke and are therefore likely to reduce macrovascular dementia. There is biological plausibility that lowering plasma cholesterol or CNS cholesterol will also lessen the severity of AD pathology; however, the delineation of VD and AD on clinical grounds is extremely difficult. Whether the cholesterol-dependent effects follow penetration of the blood brain barrier and arise from local effects on cells of the CNS, or follow plasma LDL reduction requires clarification. The former would raise the possibility that mevalonate-dependent endothelial changes may be relevant.

At this time there is insufficient controlled clinical data to recommend initiation of treatment with statins in patients with early or advanced AD. For patients with hypercholesterolaemia and risk fac-

tors for ischaemic heart disease and stroke, the evidence supporting the use of statins is quite clear. Forthcoming studies such as the prospective study of Pravastatin in the Elderly at Risk (PROSPER)[104] will help assess and quantify the cognitive benefits attributable to statins in patients at risk of vascular disease.

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