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Review

Statins, bone formation and osteoporosis: hope or hype?

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ABSTRACT

Osteoporosis is a major health problem affecting both men and women. Statins, besides their action as lipid-lowering agents, seem to have additional pleiotropic properties, among them a beneficial effect on bone mineral density. The entirety of experimental and the majority of clinical studies as well as the only relevant meta-analysis suggest that statins have an anabolic effect on bone metabolism. Statins, osteoporosis and adipogenesis share the same pathway, RANKL/OPG. It would appear that an imbalance in this pathway could be responsible for the manifestation of some metabolic disorders such as diabetes mellitus, atherogenesis, multiple myeloma, osteoporosis. Possibly in the future, drugs which can intervene in this biochemical and pathophysiological cascade, like statins, in a variety of doses, could be used for the management of ectopic ossification syndromes and other bone disorders, even as an additive treatment. Until then, further large longitudinal randomized controlled studies for each statin separately are required to confirm this hypothesis.

Key words: Adipogenesis, Bone formation, Bone mineral density, OPG, Osteoporosis, RANKL, Statins

INTRODUCTION

Osteoporosis is a serious health problem not only because it affects the quality of life but also and more importantly because it is associated with morbidity and mortality as well as economic burden. According to the WHO, osteoporosis is a "systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue which leads to bone fragility and susceptibility to fracture". Bone mineral density (BMD) in osteoporotic patients is less than -2.5 SD compared with the BMD of young

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people (T-score<-2.5 SD). BMD of people without osteoporosis is usually above -1SD.[1]

Osteoporosis is a more frequent disease in women than in men,[2] although mortality due to osteoporotic fractures is higher in men than in women.[3] In addition to this, post-menopausal women suffer from osteoporosis and osteoporotic fractures at a higher frequency than pre-menopausal women.[4]

Through adult life there is a dynamic progress which is called "bone remodeling". Bone remodeling is well established throughout the literature and involves both systemic and non-systemic factors.[5] It is well known that in this procedure an important role is played by the system of receptor activator of nuclear factor kappa b ligand (RANKL)-osteoprotegerin (OPG),[6] some cytokines and bone morphogenetic proteins (BMPs) (Figure 1).[7]

On the other hand, it has been well known since the 70s that bone loss in osteoporotic patients is associated with increase of adipose tissue in bone marrow.[8] Mesenchymal stem cells are pluripotent cells with a high mitotic index and are involved in the differentiation of adipocytes under the regulation of genes and transcription factors. Adipose tissue is considered as a separate endocrine gland, responsible for the secretion of adipokines (leptin, adiponectin) and hormones (vitamin D3, estrogen, etc.) and is involved in the pathophysiology of some entities. Leptin controls the RANKL/OPG axis by inhibiting the expression of RANKL and inducing OPG to create pre-osteoblasts and mononuclear cells in circulation. The diversion of an adipocyte into osteoblast is considered to be a multifactorial process regulated by all these factors (Figure 2). In addition to this, it is well known that statins, osteoporosis and adipogenesis share the same pathway, RANKL/OPG.[9,10] It appears that an imbalance in this pathway could be responsible for the manifestation of some metabolic disorders such as diabetes mellitus,

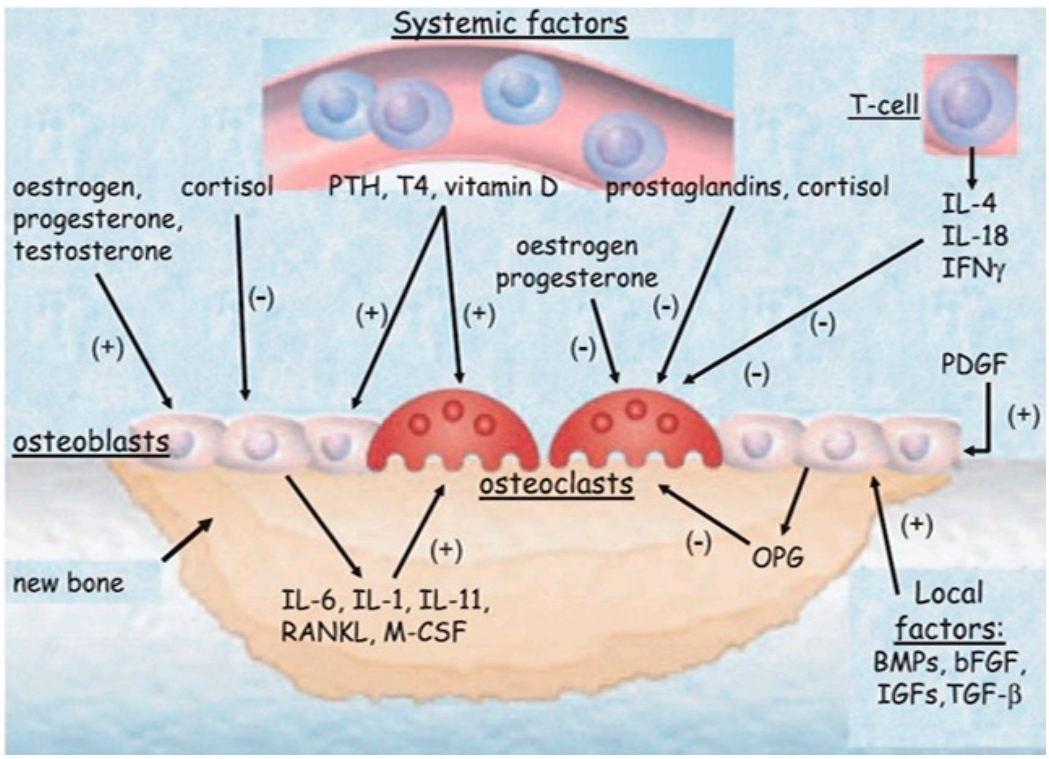

Figure 1. Bone metabolism enhanced by growth factors like bone morphogenetic proteins (BMPs), transforming growth factors beta (TGF-β), insulin growth factors (IGFs), fibroblast growth factors (FGFs). Systemic factors can also enhance osteoblast differentiation and proliferation. Systemic factors and locally produced growth factors can also induce activation of osteoclasts. Interleukins, prostaglandins and M-CSF produced from osteoblasts also induce the formation of osteoclasts. RANKL binds its receptor RANKL and induces the formation of osteoclasts. OPG inhibits RANKL binding to RANK.

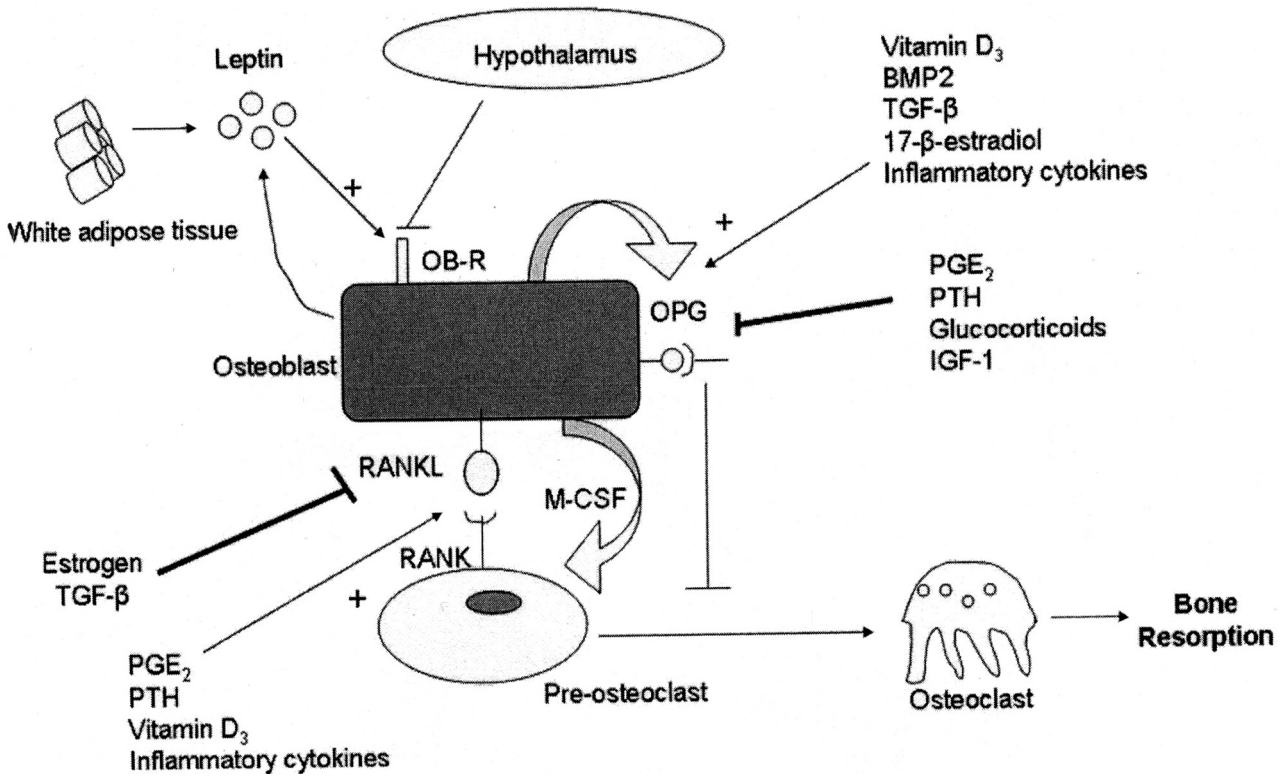

Figure 2. The interaction of adipose tissue and other factors in the differentiation of osteoblasts (Savopoulos 2011).

atherogenesis, multiple myeloma, osteoporosis. We have also seen that fat and bone tissue interaction altered by activation or silencing of genes, signaling molecules and transcription factors.[11]

The purpose of our review is to investigate whether, according to the available clinical data, there is a relation between statins and osteoporosis and thus to pose queries regarding new pathways which may enhance our knowledge about the prevention and management of osteoporosis. Clinical studies, systematic reviews and meta-analyses were searched for in PUBMED and EMBASE/EXCERPTA MEDICA databases. Computerized search of the databases was accomplished by using the combination of keywords and Medical Subject Heading terms such as: statins, aminobiphosphonates, osteoporosis, RANKL, OPG, BMP, HMG-CoA reductase inhibitors, BMD, adipose tissue. We limited our search to articles published between June 2007 and October 2011 that were at least accompanied by an English abstract. We have found that there is a meta-analysis including all the clinical studies until June 2007.

CURRENT MANAGEMENT OF OSTEOPOROSIS

It is now generally accepted that first-line agents for the management of osteoporosis are the aminobiphosphonates. These drugs act to decrease bone resorption by inhibition of the farnesyl diphosphate synthase, which is a step in the mevalonic acid pathway.[12] 3-Hydroxy-3-Methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) inhibit the same pathway at an earlier point and may also antagonize osteoclasts by increasing expression of osteoprotegerin (Figure 3).[13] In order to improve BMD and prevent osteoporosis, we have used many different drugs based on two treatment strategies: i) to inhibit the osteoclast activity and ii) to stimulate the osteoblast activity. First-line agents to avoid bone resorption are the biphosphonates like alendronate, risedronate, ibandronate and zoledronic acid.[14,15] Other drugs used are teriparatide, a recombinant form of parathyroid hormone,[16] selective estrogen receptor modulators (SERMs), hormone replacement therapy, calcitonin, calcitriol and vitamin D analogues.[17-19] We also have agents with a different

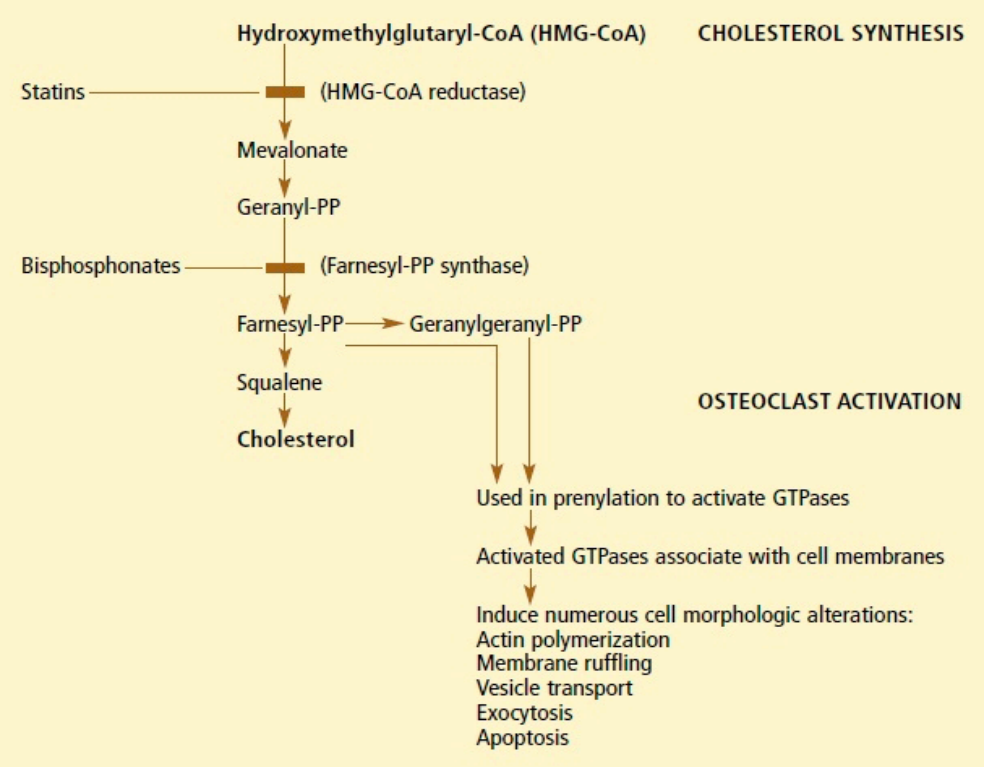

Figure 3. The interaction between HMG-CoA reductase inhibitors and bIphosphonates in the mevalonate pathway.

mechanism of action: bone forming through osteoblast modulation and antiresorptive through osteoclastic inhibition like strontium ranelate.[20]

STATINS AND BONES: INTRIGUING INTERACTIONS

Lipid-lowering therapy and pleiotropic effects

Statins compose a drug class broadly characterized as lipid-lowering agents.[21] These agents can be subgrouped according to their hydrophobic or hydrophilic nature. Hydrophobic statins (simvastatin, lovastatin) enter the liver by the hepatic portal vein, while the hydrophilic statins (rosuvastatin, pravastatin, fluvastatin) require active transport into the cell.[22] Statins inhibit mevalonic acid synthesis and, as a consequence, there is a decrease in the amount of total cholesterol and decreased levels of low density lipoproteins (LDL).[23] All statins have favorable effects on cardiovascular diseases, the nervous system, the immune system, the skeletal system, tumor growth.[24,25] There is emerging interest in the pleiotropic effects of this class of drugs, e.g. Srivastava and colleagues[26] who examined the possible action of atorvastatin in acute phase reaction in children after intravenous biphosphonate infusion, but who, however, failed to demonstrate a positive result.

Statins and bones: metabolism and clinical implications

Molecular biology and genetics reveal that both vascular and osteoblast biology have a common pathway: RANK/RANKL/OPG.[27-29] With regard to this issue, there is growing interest concerning the possible mechanism and the impact of statins on bones on either the experimental or the clinical level. Mundy and colleagues in 1999 were the first to report an anabolic effect of statins in cultured mouse and human bone cells. Both simvastatin and lovastatin enhanced the expression of bone morphogenetic protein-2 (BMP-2) mRNA.[30]

Experimental studies

Several experimental studies illustrate the effect of statins in bone metabolism either in vitro or in vivo. Table 1 displays the main characteristics of various

Table 1. Main characteristics of animal models showing the effect of statins in bone metabolism

Author	Method	Statin	Outcome	Comments
Saraf et al 2007[31]	21 rabbits osteotomized in left femur, 2 groups (statin fed, non-statin fed)	Simvastatin 120mg/kg/day	Simvastatin group 4 weeks no fracture gap and in 4 weeks remodeling and repairing better observed, 8 weeks no abnormal mobility, 4 and 8 weeks more stiff and strength	Simvastatin had significant radiographic, mechanical, biochemical and histological differences in fracture healing, SIM abolic effect in bones
Wang JW et al 2007[32]	2-month old female ovariectomized rats (no data available about the number)	Simvastatin 10mg/kg/day	Callus cross-section area significantly enlarged 1 and 2 weeks; maximal load was▲ at 2 and 4 weeks, significant ▲ of MLW, MLV and MAR	Local application of simvastatin could promote fracture healing in OVX rats
Nyan et al 2007[33]	45 rats in three groups: i) no treatment, ii) calcium sulfate, iii) statin & calcium sulfate	Simvastatin for 8 weeks	i) Control group BMD 0.88-0.97, ii) calcium sulfate group BMD 51.7-69.6, iii) third group BMD 52.3-69.3	1 mg Simvastatin and calcium sulfate combination promote bone formation
Skoglund et al 2007[34]	70 rats in 6 groups: i) 20 mice sb inj SIM, ii) 10 sub inj vehicle, iii) 10 sub inj vehicle and SIM, iv) 10 sub inj vehicle and SIM, v) 10 by tube SIM and vi) by tube vehicle	i) Simvastatin 20mg, ii) continuous vehicle substance, iii) simvastatin5mg/kg/day, iv) simvastatin 10mg/kg/day, v) simvastatin 0.1mg/kg/day	i) Daily injections no effect, ii) continuous systemic delivery 160% larger force at failure, iii) continuous local delivery 170% larger force at failure and twofold larger energy uptake	Simvastatin (+) effect on fracture area if applied directly
Zhibin Du et al 2008[35]	54 female rats in 3 groups: i) OVX and Sham-operated, ii) OVX+ Simvastatin, iii) OVX	Simvastatin 5mg/kg/day	28 days i) SHAM BD 25.1±9.19 ii) OVX BD 9.81±4.18 iii) OVX+SIM BD 19.63±7.01 84 days i) SHAM BD 31.74±10.29 ii) OVX BD 15.72±5.05 iii) OVX+SIM BD 24.67±4.32	Simvastatin influences bone healing around titanium implant. Anabolic effect of SIM on bone metabolism
Funk et al 2008[36]	Females rats with induced arthritis (no data available about the number)	Simvastatin 20mg/kg/day subcutaneously	i) Prevented early and late joint inflammation, ii) suppressed the periarticular bone destruction occurring late in the course of disease, iii) osteocalcin levels unaltered	i) Antiinflammatory and antiresorptive joint-protective agent in RA, ii) inhibition of HMG-CoA reductase may be therapeutically useful in preserving periarticular bone in RA joints
Pengde et al 2008[37]	54 rabbits in two groups (statin, non-statin) and 16 rabbits as controls	Lovastatin 5mg/kg/day for 14 weeks	Osteonecrosis > in placebo treatment, < serious adipogenesis and bone death in statin group; size and area of fat cells in bone marrow < in statin group	i) Lovastatin can also preserve hematopoietic cells and ▼ the bone fat volume, ii) combination with steroids preserve the bone mass by inhibiting adipogenesis

Table 1. (continued)

Author	Method	Statin	Outcome	Comments
Gutierrez et al 2008[38]	In 2 female rats they created fractures and they put Kirschner wires and given Lovastatin either transdermally or orally	Lovastatin transdermally 0.1-5mg/kg/day Lovastatin orally 5-25mg/kg/day for 5 days	Transdermal LV enhanced repair in week 2 and 6. BMD of callus area▲ and stiffness▲ 90%. LV p.os in high doses(10-25mg/kg) showed ▲ in stiffness, no change in other biochemical properties	Transdermal administration of LV in low doses accelerates fracture healing, 10-fold the lipid lowering dose required to produce any effect when administered orally
Kaji H et al 2008[39]	Mouse osteoblastic MC3T3-E1 and rat osteoblastic UMR-106 cells	Pitavastatin, mevastatin, and simvastatin	1) Pitavastatin induced the expression of TGF-β, and cycloheximide, antagonized the ▲ levels of pitavastatin on Smad 3.2) pitavastatin antagonized dexamethasone- or etoposide-induced apoptosis in a dose-dependent manner	Statin suppressed cell apoptosis partly through TGF-beta-Smad3 pathway in osteoblastic cells
Alam S et al 2009[40]	12 adult male Japanese white rabbits, into 3 experimental groups and 1 control group	1) 10 mg of a statin dissolved in 0.2 mL water with an ACS 2) 5 microg rhBMP-2 with an ACS 3) only the ACS	1) No significant differences between the statin/ACS group and rhBMP-**2**/ACS group at **1**, **2**, and 4 weeks after surgery	Statin suppressed cell apoptosis partly through TGF-beta-Smad3 pathway in osteoblastic cells
Uyar Y et al 2009[41]	63 rats divided into 7 groups i) non-OVX ii) OVX iii) OVX+RSN iv) OVX+AV v) OVX+E2 vi) OVX+RL vii) OVX+CC	Atorvastatin	OVX+AV vs OVX femur & femur midschaft BMD and three-point bending test ▲ OVX+RSN vs OVX femur midschaft BMD ▲ OVX+ E2, RL and CC vs OVX no changes in femur midschaft BMD	RSN prevented the ▼ in BMD, AV maintained mechanical characteristics of bone and also prevented the ▼ in BMD as RSN
Hanayama R 2009[42]	Fructose-fed insulin resistant model rats	Fluvastatin, pravastatin	RANKL, M-CSF, TRAP ▲ by fluvastatin	Fluvastatin significantly attenuated osteoclast differentiation and activation through a blockade of the classical mevalonate pathway and an antioxidant action, leading to prevention of osteoporosis
Ayukawa et al 2009[43]	60 male rats in 2 groups (statin, control)	Simvastatin 100μl of 1mg/ml	5 day SIM group larger new bone area, TRAP-multinucleated cells < in SIM group, in SIM group BALP and BMP-2 mRNA▲ and cathepsin K▼, RANKL depressed. In 10 day no differences	SIM▲ bone area, this effect did not continue after the end of administration, osteoclast suppression may be the consequence of RANKL depression

MLW: mineralization width; MLV: mineralization volume; MAR: mineral opposition rate; BMD: bone mineral density; SIM: simvastatin; OVX: ovariectomized; SHAM: sham-operated group; ACS: atelocollagen sponge; LV: lovastatin; RA: rheumatoid arthritis; RSN: rosuvastatin; AV: atorvastatin; E2: 17β-estradiol; RL: raloxifene; CC: clomiphene citrate; RANKL: receptor activator of nuclear factor kappa-b ligand; M-CSF: macrophage clony stimulating factor; TRAP: tartrate resistant acid phospatase; BALP: bone specific alkaline phosphatase; TBV: trabecular bone volume; OC: osteocalcin; VEGF: vascular endothelial growth factor; TGF: transforming growth factor; LFB: left femoral bone; LS: lumbar spine; TCP: tricalcium phosphates; DBBM: demineralized bovine bone matrix; <: less; >: higher; (+): positive; (-): negative; ▲: increased; ▼: decreased.

Table 1. (continued)

Author	Method	Statin	Outcome	Comments
Ho et al 2009[44]	54 OVX and Sham operated female rats	Simvastatin 10-20mg/kg/day for 6 weeks	Sham less TBV than OVX, SIM ▲ TBV and osteoblast number, osteoblastic cells with immune-stained BMP2, collagen type 1, OC ▲ by SIM 20mg	SIM might promote bone formation via ▲ osteoblast numbers and matrix protein levels
Pauly et al 2009[45]	Fractures produced into 200 female rats and stabilized intramedullary. Divided into 4 groups depending on the wires and statin. 9 animals of each group tested	In second group simvastatin low dose 3μg/implant and in the third group 50 μg/implant	Progressed callus consolidation in BMP2 and statin group, high dose SIM▲ stiffness and elevated maximum load	Dose-dependent effect and improved fracture healing under local application of SIM
Chang Liu et al 2009[46]	48 rats inplanted carriers with or without SIM and divided into 2 groups	Simvastatin	TGF-β1, BMP-2, VEGF mRNA ▲ in both groups after one week. TGF-β1, BMP-2 mRNA ▲in 1 after 1,2,4 weeks and VEGF mRNA▲ after 1,2 weeks	Local administration of SIM can influence alveolar bone remodeling by regulating the expression of growth factors
Shung-Hsiung Chen et al 2009[47]	27 female rats underwent bilateral OVX divided into 3 groups, i) control, ii) Aromasin group, iii) SIM + Aromasin group	Simvastatin 6.5mg/kg 5 times per week for 12 weeks	After 1 month: i) LFB BMD 0.4926±0.0332 and LS BMD 0.2858±0.011, ii) LFB BMD 0.46084±0.058 LS BMD 0.3318±0.0056, iii) LFB BMD 0.4524±0.024 LS BMD 0.3034±0.019. After 3 months LS BMD: i) 0.3883±0.0259, ii) 0.3174±0.0071, iii) 0.3702±0.0095	Aromasin catabolic effect on skeletal system and SIM may have a therapeutic application in the treatment of osteoporosis to counterbalance the adverse effects of Aromasin
Nyan M et al 2009[48]	Bilateral 5-mm-diameter calvarial defects were created in adult Wistar rats	Simvastatin 0, 0.01, 0.1, 0.25 and 0.5 mg combined with alpha-TCP particles or left empty	1) 0.25 and 0.5 mg caused inflammation of the soft tissue at the graft site, control and other doses did not, 2) alpha-TCP with 0.1 mg simvastatin (TCP-0.1) group yielded significantly > bone volumes than untreated control group at all three time points, 3) the percentage of defect closure, bone mineral content and bone mineral density were also > in the TCP-0.1 group	When combined with alpha-TCP particles, 0.1 mg simvastatin is the optimal dose for stimulation of the maximum bone regeneration in rat calvarial defects without inducing inflammation and it could be applied as an effective bone graft material.
Wang et al 2010[49]	30 mice in two groups	Lovastatin 10mg/kg once at the time of fracture	1) Lack of Nf1 in osteoblasts delays bone healing, 2) lack of Nf1 in osteoblasts▼ callus biomechanical properties, 3) extensive osteoid surfaces and impaired osteoclast function may prevent proper callus remodeling in $Nf^{-/-}$ mice, 4) Lovastatin microparticle treatment improves bone healing and mechanical properties in $Nf^{-/-}$ mice	i) Dysfunctions caused by loss of Nf1 in osteoblasts impair callus maturation and weaken callus mechanical properties, ii) local low dose of lovastatin may improve fracture healing

Table 1. (continued)

Author	Method	Statin	Outcome	Comments
Goes P et al 2010[50]	Periodontitis was induced by ligature placement around the upper second left molar in a total of 24 male Wistar rats (± 200 g)	Groups of 6 animals received via oral gavage either saline or AV (1, 3 and 9 mg/kg) during 11 days.	1) ATV (9 mg/kg) caused a significant ▲ on gray tone variation of over 48% when compared to saline, indicating greater radiographic density, 2) AV (9 mg/kg) ▼ alveolar bone loss by over 47% ($p<0.05$), when compared to the group of untreated animals	ATV was able to prevent alveolar bone loss seen on a Sligature-induced periodontitis model.
Lima et al 2011[51]	64 rats in 4 groups, i) no treatment, ii) DBBM, iii) SIM & DBBM, iv) SIM& DBBM	Simvastatin 2.2mg/50µl in third group and simvastatin 0.5mg/50µl in fourth group	Third group lowest BMD in 30 days, in 60 days simvastatin groups < BMD, on 30 day second and third group (-) impact on bone formation, on 60 day none of the combinations impaired bone formation	High local doses of simvastatin caused an intense inflammatory reaction, SIM & DBBM have negative impact on bone repair

MLW: mineralization width; MLV: mineralization volume; MAR: mineral opposition rate; BMD: bone mineral density; SIM: simvastatin; OVX: ovariectomized; SHAM: sham-operated group; ACS: atelocollagen sponge; LV: lovastatin; RA: rheumatoid arthritis; RSN: rosuvastatin; AV: atorvastatin; E2: 17β-estradiol; RL: raloxifene; CC: clomiphene citrate; RANKL: receptor activator of nuclear factor kappa-b ligand; M-CSF: macrophage clony stimulating factor; TRAP: tartrate resistant acid phospatase; BALP: bone specific alkaline phosphatase; TBV: trabecular bone volume; OC: osteocalcin; VEGF: vascular endothelial growth factor; TGF: transforming growth factor; LFB: left femoral bone; LS: lumbar spine; TCP: tricalcium phosphates; DBBM: demineralized bovine bone matrix; <: less; >: higher; (+): positive; (-): negative; ▲: increased; ▼: decreased.

studies performed mostly in animal models. Although Lima et al (2011)[51] demonstrated controversial, and even negative, effects of statins on bone repair, the vast majority of the studies in Table 1 support the beneficial role of this group of drugs. The administration of statins presents anabolic effects by promoting osteoblast activity and suppressing osteoclasts. As a result, statins act effectively on bone formation, inhibition of BMD decrease and, in general, on fracture healing and osteoporosis prevention. However, we should take into account that these studies refer to different animal models, they used different doses and the result was a local phenomenon, beyond the established cholesterol-lowering effect of statins. Nevertheless, certain studies underline the remarkable increase of relevant growth factors (TGFβ-1, VGF), revealing a possible explanation for the statins and bones interaction.

Further studies performed in vitro, in cell cultures (Table 2), support the previous findings, thus clarifying the potential mechanism of the beneficial effect of statins on bone metabolism. The expression of genes as BMP-2, *COLLIA1*, *osteocalcin (OC)* (which demonstrate an anabolic effect) and depression of others like RANKL (leading to suppression of osteoclast activity), all stimulated by statins, may regulate the role of this class of drugs in bone formation. Hughes A et al in 2007[52] found that hydrophobic and hydrophylic statins can inhibit osteoclast function in vitro, thereby showing a possible class effect, although stronger evidence supports the role of lipophilic agents as simvastatin (Pagkalos et al[60]).

Observational studies

An interesting meta-analysis of clinical studies since 2007 by Uzzan et al[61] showed that statins have a positive effect on BMD in various sites. In particular, the better effect on BMD was found by lipophylic statins (simvastatin, lovastatin). The authors proposed that statins could be used for the management of osteoporosis, but the minimum concentration required for the beneficial effects on bone remains to be determined.

Several clinical studies since then have demonstrated the positive effect of statins on bones. Table 3 displays the main characteristics of these studies and their effect on BMD and bone biochemical markers.

Table 2. Main characteristics of studies in cell cultures investigating the role of statins in bone metabolism

Author	Method	Statin	Outcome	Comments
Hughes A et al 2007[52]	Mouse macrophage like cells, osteoclast like cells from rabbit bone marrow	Rosuvastatin, pravastatin, simvastatin, cerivastatin	i) Order of potency for inhibition of bone resorption CER>SIM>RSV>PRA ii) PRA inhibited resorption at concentrations >50μM iii) Single injections of RSV and CER sufficient to prenylate bone marrow cells	i) Hydrophobic and hydrophylic statins can inhibit osteoclast function in vitro ii) > doses of statins can inhibit protein prenylation in osteoclasts in vivo iii) > doses of CER or RSV mildly prevent the bone loss
Ruis-Gaspa et al 2007[53]	Cultures of bone specimens of 3 post menopausal women and MG3 osteosarcoma like cells.Cells incubated with the presence of statin	Simvastatin or atorvastatin fro 10^{-6}M -10^{-9}M	COLLIA1, OC, BMP2 gene expression significant ▲, similar effects to MG3 cells	SIM and atorvastatin stimulatory effects in COLLIA1, OC, BMP2 genes, (+) effect in osteoporosis
Ahn KS et al 2008[54]	Mouse macrophage cells, human breast adenocarcinoma and multiple myeloma cells exposed to statins and RANKL	Simvastatin	i) Osteoclast ▼ with▲ concentrations of simvastatin, ii) the inhibitory effect▼ in time-dependent manner, iii)inhibited osteoclastogenesis induced by tumor cells	Simvastatin inhibits the RANKL-induced NF-kappaB activation, suppresses osteoclastogenesis, therapeutic potential in osteoporosis and in cancer-related bone loss.
Yamashita et al 2008[55]	Cultures of mouse myoblast cell line C2C12 treated with BMP-2, TNF-a, SIM	Simvastatin	SIM no effects on Runx2 and ALP activity, SIM reversed TNF-a inhibition of BMP-induced Smad 1, 5, 8 phosphorylation, SIM ▲ expression of Smad in C2C12 cells exposed to TNF-a, SIM suppressed TNF-a phosphorylation of ERK1/2 and SARK/JNK, FPP and GGPP reversed the SIM effects on TNF-a induced activation of Ras/Rho/MARK pathway	SIM supports BMP-induced osteoblasts differentiation through antagonizing TNF-a-to-Ras/Rho/MARK pathway and augmenting BMP-Smad signaling suggesting potential usage of SIM to inflammatory bone damage
Monjo et al 2010[56]	Cultures of mouse osteoblastic cell line MC3T3-E1	Different concentrations of rosuvastatin (0.001-10μ M)	< concentrations of RSV were protective against cell death and > showed cytotoxicity	RSV promotes osteoblast differentiation and regulates the expression of Slco1a1 which may constitute the transport system for RSV across the cell membrane in mature osteoblasts
Yamashita et al 2010[57]	Mouse osteoclast line cell MLC-6 from mouse bone marrow co-cultured with mouse chondrocytes	Simvastatin	SIM suppressed osteoclastic activity and▲ RANK, TRAP and cathepsin K expression, SIM activated ERK, SARK/JNK, AKT pathways and inactivated Ras, Src phosphorylation suppressed by SIM	SIM inhibits osteoclastic differentiation through inhibiting Src and enhancing MARK/AKT pathways
Chen et al 2010[58]	Cell cultures of mice osteoblast like cells after 3 days examined the mitochondria osteoblastic activity with various concentrations of SIM	Simvastatin	With 10^{-6}M SIM ALP enhanced and BMP-2, ALP, sialoprotein, type I collagen up-regulated, RasGRF1 and phosphoRasGRF1 ▼	i) SIM can promote osteoblast viability and differentiation via Smad/Erk/BMP-2 pathway, ii) statins stimulate osteoblast differentiation in vitro

Table 2. (continued)

Author	Method	Statin	Outcome	Comments
Zhou et al 2010[59]	An ITB composed of hADSCs and hPRP was preliminarily constructed, but its osteogenic capability needs improving	Simvastatin	0.01 microm, 0.1 microm, and 1 microm SIM induce hADSCs' osteoblastic differentiation in vitro accompanied with non-inhibition on cell proliferation, > ALP activity, more mineralization deposition and more expression of osteoblast-related genes such as OC, Cbfa1 , BMP-2, VEGF, and basic FGF	1) Simvastatin at 1 mlcrom seemed the most optimal concentration due to its high osteocalcin secretion in media, 2) simvastatin at optimal concentrations can be used to promote this ITB's osteogenesis
Pagkalos et al 2010[60]	ESCs, derived from the inner cell mass of the blastocyst	Simvastatin	1) Simvastatin induces murine ESC differentiation toward the osteogenic lineage in the absence of osteoinductive supplements, 2) simvastatin concentration in the micromolar range and > was toxic to the cells and that an effective concentration for osteoinduction is 0.1 nM	Lipophilic simvastatin may provide a novel pharmacologic agent for bone tissue engineering applications

CER: cerivastatin; SIM: simvastatin; PRA: pravastatin; RSV: rosuvastatin, COLLIA1: collagen type I a 1; BMP-2: bone morphogenetic protein-2; OC: osteocalcin; RANKL: receptor activator of nuclear factor kappa-b ligand; TNF-a: tumor necrosis factor alpha, FPP: farnesyl pyrophosphate, GGPP: geranylgeranyl pyrophosphate, TRAP: tartrate resistant acid phosphatase, ALP: alkaline phosphatase, ITB: injectable tissue engineered bone, hADSCs: human adipose-derived cells; h PRP: human platelet rich plasma; Cbfa1: core binding factor alpha 1; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; ESCs: embryonic stem cells; >: higher; <: lower, (+): positive; ▲: increased; ▼: decreased.

All these studies found a significant change either in BMD or in bone markers, except for three studies which found no statistically significant correlation.

Among these studies, there are four controlled studies, one cross-sectional, three open-label, one open randomized and one cohort study. Although not all of them are characterized by a strictly controlled study design, the vast majority reveal a positive effect on BMD and bone biochemical markers. Furthermore, as shown in Table 1 the controlled studies, even though not establishing the effects on BMD, showed significant changes in bone markers, which corroborates the hypothesis of the correlation between statins and bone formation.

DISCUSSION

The pleiotropic effect of statins has led clinicians to investigate their potential use among other entities, such as bone metabolism. Uzzan et al,[61] in their aforementioned meta-analysis, found that statins have a positive effect on BMD in various sites. Although the authors concluded that there was a modest but statistically significant favorable effect of statins on BMD, thus confirming the results of previous studies, we are as yet far from an evidence-based recommendation of statins as a useful therapeutic modality in osteoporotic patients, even as a complementary one. In addition, more data are needed to support the use of statins for prevention of bone fracture.

This perception has mainly been developed by experimental studies, there being a lack of observational studies to clarify the field. The majority of the literature showed an increase in BMD or in bone markers. Several reasons might be advocated to explain the discrepancies. In fact, the doses used in experimental models which provided a favorable effect were much higher than the doses used in clinical practice. In addition, implementation of treatment was in a short-term perspective. Although obesity and physical activity were associated with prevention of fracture risk, they were neither controlled nor quantified. Thus, the control groups in most of the studies were small, thereby not reflecting an equal, comparative population and thus leading to bias.

On the other hand, statins could be prescribed

Table 3. Main characteristics of the studies showing effect either in BMD or in bone markers with the use of statins

Author	Study design	Statin	Period of use of statins	Patients	Age	Effect on BMD	Bone markers
Uysal et al 2007[62]	Cross-sectional	Simvastatin;		37 women with type 2 diabetes	No available data	Borderline, no significant increase	No data available
Majima et al 2007[63]	Open label	Atorvastatin 10mg/day	3 months	22 hypercholesterolemic Japanese males	62.36±10.1	No data available	Ca 9.58-9.44 BALP 22.42-21.76 NTX 15.84-12.20
Majima et al 2007[64]	Open control	Pitavastatin 1mg/day	3 months	101 hypecholesterolemic Japanese (57 men, 44 women, 63 users, 35 non-users)	58.6±12	No data available	Ca 9.56-9.44 P 3.38-3.38 BALP 23.39-23.09 NTX 14.19-12.52
Safaei H et al 2007[65]	Open label clinical trial	Lovastatin 20mg/day	18 months	55 diabetic post-menopausal women	54-67	LS 0.946-0.978 Ward's triangle 0.685-0.780	No available data
Bone GH et al 2007[66]	Prospective randomized double-blind, placebo-controlled, dose ranging comparative	Placebo, atorvastatin 10, 20, 40, 80mg/day	52 weeks	626 dyslipidemic postpmenopausal women with T-score 0 to -2.5	40-75	No significant change	No significant change
Pérez-Castrillón JL et al 2008[67]	OR	Atorvastatin 10-20mg/day and 40-80mg/day	1 year	62 patients (35 males, 27 females)	60	GG genotype LS 1.107-1.129 (p=0.0001)	No data available
Patil S et al 2009[68]	Prospective double-blind RCT	Simvastatin 20mg/day	12 weeks	62 patients (31 users, 31 non-users)	25-81	No significant change	BALP 23.5-28.2 OC 19.5-18.9 P1NP 61.8-65.9 CTX 0.20-0.21
Yavuz B et al 2009[69]	Prospective cohort	Rosuvastatin 10-20mg/day	8 weeks	91 hyperlipidemic patients	59±12.5	No available data	25(OH)D 14-36.3 1.25(OH)2D 22.9-26.6 OC 3.5-3.6 BALP 17.7-9.5 Ca 9.4-9.3 P 3.1-3.1
Kanazawa et al 2009[70]	Open label randomized	Rosuvastatin 2.5mg/day		36 Japanese	60.1±7.4 (users), 64.7±2.7 (non-users)	No data available	BALP 29.7-33.1 NTX 46.4-51.6 DPD 5.8-6.4
Cheungsamarn et al 2010[71]	Prospective RCT	Simvastatin 40-80mg/day	18 months	212 patients (106 users, 106 non-users, 63 male, 149 female)	>40	BMD increased (p<0.01)	Bone formation increased (p <-.0001) bone resorption reduced (p=0.017)

BMD measured in gr/cm3; LS¦: lumbar spine; FN: femoral neck; TH: total hip; Troch: trochanter; OR: opens randomized; RCT: randomized control trial.

in people with lower risk for fractures. Moreover, most of the studies attribute the interaction of statins with bone metabolism to a class-effect mechanism rather than to an individual drug effect. We have to stress here that we do not have extensive data on the pharmacological effects of statins in non-hypercholesterolemic patients.

All the available data from the literature, including evidence from experimental studies as well as from the vast majority of observational studies and the results of a single meta-analysis, suggested that there is a positive effect of statins on BMD, although another meta-analysis by Bauer et al[72] showed evidence that the beneficial effects on BMD and on fracture risk are observational, while many limitations and the placebo-controlled trials did not demonstrate any clear-cut benefit. However, the in vitro and some clinical studies (Chuengsamarn et al[71]) suggest that statins inhibit bone resorption and stimulate bone formation, having a dual action on bone metabolism. Therefore, in the future statins might gain a position among drugs used for the prevention and management of osteoporosis, taking into account that clinicians already have a good deal of experience in prescribing statins, for other indications, and feel familiar with this drug family. Their anabolic and anti-resorptive effects on bone make them an ideal candidate for osteoporosis treatment.

In conclusion, statins, osteoporosis and adipogenesis share a major pathway, that of RANKL/RANK/OPG. Moreover, fat and bone tissue interaction is altered by activation or silencing of genes signaling molecules and transcription factors. Possibly in the future drugs which intervene in this biochemical and pathophysiological cascade, like statins, in a variety of doses, could be used for the management of ectopic ossification syndromes and other bone disorders like osteoporosis and multiple myeloma, even as an adjuvant therapy. Until then, further large longitudinal randomized controlled studies for each statin separately are required to confirm this hypothesis.

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