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

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