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Lovastatin effects on bone mineral density in postmenopausal women with type 2 diabetes mellitus

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Abstract The objective of this study was to examine the effects of lovastatin on bone mineral density (BMD) of postmenopausal women with type 2 diabetes mellitus (DM). The study was an open-label clinical trial conducted from March 2002 to November 2003. Fifty-five postmenopausal women age 54–67 years with type 2 DM were allocated to lovastatin-treated and control (without lovastatin) groups based on low-density lipoprotein cholesterol (LDL-C) >130 or ≤130 mg/dl. The first group received lovastatin (20 mg daily titrated every 3 months to keep LDL-C less than 130 mg/dl) for a total of 18 months. The second group received their own diabetic regimen without statin. The BMD of the lumbar spine (L_1–L_4), femoral neck, Wards triangle, trochanter and total hip was measured by dual-energy X-ray absorptiometry at baseline and after 18 months. In the 28 women treated with lovastatin, the BMD increased in lumbar spine (from 0.946 (0.122) to 0.978 (0.135) g/cm^2, $p<0.01$) and Ward's triangle (from 0.685 (0.123) to 0.780 (0.186) g/cm^2, $p<0.01$). In the 27 women not treated with statin, the changes in BMD at all bone sites were not statistically significant. BMD was higher in femoral neck (1.2% *vs.* -2.7%, $p<0.05$), Ward's triangle (13.9% *vs.* 3.3%, $p<0.05$), trochanter (-0.1% *vs.* -2.9%, $p<0.05$), total hip (1.2% *vs.* -1.4%, $p<0.05$) and lumbar spine (3.4% *vs.* 1.2%, $p>0.05$) at the end of the study. Treatment with lovastatin may prevent bone loss in postmenopausal women with type 2 DM.

Key words Bone mineral density • Lovastatin • Statins • Postmenopause • Diabetes mellitus

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Introduction

Osteoporosis is the most common metabolic bone disease, characterised by low bone mass and structural deterioration of bone tissue, leading to bone fragility and increased susceptibility to fractures [1]. The most important cause of osteoporosis in postmenopausal women is increased bone resorption due to oestrogen deficiency [2–4]. Known osteoporosis preventive and therapeutic agents such as bisphosphonates, oestrogens, selective oestrogen-receptor modulators, calcitonin, vitamin D analogues, calcium supplement and ipriflavone work by decreasing bone resorption [5–7]. Although they reduce the fracture risk and bone turnover, they exhibit a weak, or moderate, effect on bone mineral density (BMD). Therefore, effective clinically acceptable drugs that stimulate new bone formation and improve trabecular microarchitecture with subsequent enhancement in BMD are urgently needed. Animal and *in vitro* studies suggest that cholesterol-lowering 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors (statins) can both increase bone formation and decrease resorption [8, 9].

Reviews of the growing body of information from observational and experimental studies regarding the effect of statins on risk of fracture or BMD in humans show inconsistent results [10–17]. Some of these studies

show no effect [11, 15] or a positive effect [10, 13, 14, 16] or different associations for men and women [12], indicating a complex and often paradoxical relationship. The explanations for these discrepancies could include study design, inadequate control of confounders and differences in study populations.

There have been conflicting reports about BMD in patients with type 2 diabetes mellitus (DM) [2, 18–24]. In some studies BMD was reduced [2, 21]; in others BMD was increased [18–20, 24] or unchanged [22, 23]. In some studies on postmenopausal women with type 2 DM, HMG-CoA reductase inhibitors decreased risk of fracture [12, 13]. Therefore, it is still to be determined whether statins can be used for the management of BMD in postmenopausal diabetic women. We conducted an open-label clinical trial to compare BMD in lovastatin-treated patients with low-density lipoprotein cholesterol (LDL-C)>130 and in controls with LDL-C ≤130 mg/dl.

Patients and methods

Patients

In an open-label clinical trial, 60 consecutive postmenopausal women with type 2 DM referred to the Isfahan Endocrine and Metabolism Research Center outpatient clinic who consented to be enrolled in the research were selected from March 2002 till November 2003. Tenets of the current version of the Declaration of Helsinki were followed, institutional ethical committee approval was granted, and the nature of the trial and possible adverse effects of the drug were explained to the patients. After detailed discussion with the endocrinologist, each patient made a final decision and signed a consent form.

The patients were not randomised, but were grouped according to their LDL-C at baseline. The treatment group (n=30) had LDL-C >130 mg/dl and the control group (n=30) LDL-C ≤130 mg/dl, in order to avoid the ethical question of leaving hypercholesterolaemic patients without drug therapy for a long time.

Patients with no serious concomitant medical problems, such as history of renal, cardiac, liver or neoplastic disease, Cushing's syndrome or thyroid dysfunction and who were available for follow-up for 18 months were eligible for the study. Patients with chronic gastrointestinal disorders such as chronic diarrhoea and malabsorption, or disabling joint diseases or taking levothyroxine, anticonvulsants, corticosteroids, calcium supplements, vitamin D, bisphosphonates, oestrogen or diuretics during the observation period were also excluded.

All patients had been on their own diabetic regimen for 3 months and none took statins. At the beginning of the study, patients' age, height, weight, duration of diabetes, and postmenopausal period were recorded. Body mass index (BMI) was calculated by dividing weight (kg) by square of height (m).

The lovastatin-treated group received lovastatin (manufactured by Pursina Pharmaceutical Co., Tehran, Iran) 20 mg daily as a starting dose. The dose was titrated every 3 months to keep LDL-C <130 mg/dl. The maximum increment was 10 mg at each visit. Mean (standard deviation (SD)) of lovastatin dose was 34.6 (9.7) mg daily. Serum creatin phosphokinase (CPK) and hepatic aminotransferases (AST & ALT) were measured before and after 4 weeks of lovastatin treatment and then at 3-month intervals throughout the study period in the lovastatin-treated group. A liver enzyme increment to more than 3 times the upper limit of normal range or increase of CPK with clinical symptoms and signs of muscle involvement, drug intolerance or noncompliance among patients or increase of LDL-C to >130 mg/dl in the control group were also exclusion criteria. The intervention lasted 18 months. At the same time, patients in the control group were on their own diabetic regimen without statin intake.

Methods

After an overnight fast, venous blood samples were taken for the measurement of cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), glycosylated haemoglobin (HbA1c), creatinine, calcium, phosphorus, alkaline phosphatase, CPK, and AST and ALT using an autoanalyser (Liasys, AMS, Italy). HbA1c was measured by chromatography-spectrophotometry. Total cholesterol, triglyceride and HDL-C were assayed by calorimetry. LDL-C was calculated by Friedwald equation (if triglyceride level was <400 mg/dl) [25]. Calcium and phosphorus were measured by calorimetry (cresolphthalein complex and molybdate). AST and ALT, and CPK were measured by an optimised standard (IFCC) and creatinine by the JAFFE method. Measurement of HbA1c and lipid profile was repeated every 3 months in both groups.

BMD of the lumbar spine (L_1–L_4), femoral neck, Ward's triangle, trochanter and total hip was measured by dual-energy X-ray absorptiometry (DXA) with a Lunar DPX-L 3000 densitometer scanner (Madison, WI, USA) with acquisition software version 1.31 by the same technician using the same machine at baseline and after 18 months in both groups. Precision was 1.0% for spine and hip. BMD was expressed as raw bone density (g/cm^2). Percentage BMD increment after follow-up compared with baseline was calculated as: ((follow up BMD - baseline BMD)÷baseline BMD)x100.

We used World Health Organization (WHO) criteria to categorise the BMD values in terms of low bone mass (osteopenia and osteoporosis). T-score of -1 to -2.5 SD was defined as osteopenia and less than -2.5 SD as osteoporosis [26].

Statistical analysis

On the basis of previous estimates of standard deviation of 2.5 and accounting for pair-wise comparisons, we calculated that 30 patients per treatment group would be required to provide the study with 80% power to detect (with a two-sided alpha of 0.05) a mean difference in BMD from baseline of 1.5 g/cm^2. Comparison between groups that had LDL-C >130 mg/dl and LDL-C ≤130 mg/dl was made using Student's t-test for independent samples; comparisons between baseline and post-treatment values were made by paired-Student's t-test. Results are expressed as mean (standard deviation (SD)) and p<0.05 was considered statistically significant. All statistical tests were two-sided. The analyses were done on a personal computer using SPSS for Windows (SPSS Inc., Chicago, IL, USA).

Results

Patient compliance was good, with only 5 dropouts. In the lovastatin-treated group, adverse events were reported in one patient (diarrhoea), and one patient was noncompliant. Three patients were excluded from the control group because of noncompliance. Fifty-five patients (28 lovastatin-treated and 27 controls) who met the entry criteria were enrolled for the study. The patients had a mean (SD) age of 60 (3.1) years (range 54–67 years). Mean duration of diabetes and menopause was 10.8 (4.9) and 9.3 (3.1) years, respectively. Mean (SD) menopausal age was 49 (2.7) years.

At baseline, the lovastatin-treated group had higher total cholesterol, LDL-C and total hip BMD than the control group. Otherwise, the two groups were generally well matched at baseline with regard to age, BMI, duration of diabetes, menopause and other characteristics (Table 1). There was no statistically significant difference between BMD of proximal hip at all 3 sites and spine. The mean (SD) ages of lovastatin-treated and control groups were 60.2 (3.1) and 59.8 (3.2) years. In the lovastatin-treated group, 14 and 11 patients were treated with either insulin or oral hypoglycaemic agents (OHAs). In the control group, 12 and 16 patients were on insulin or OHAs. Others used diet for glycaemic control. First measurement of BMD showed 71.6% low bone mass in at least one bone site in the spine or proximal hip (51% osteopenia and 20.6% osteoporosis).

Changes in BMD before and after the trial are shown in Table 2. The average BMD in spine increased from baseline by 0.032 (95% CI; 0.052, 0.012) g/cm^2 in the lovastatin-treated, compared with an increase of 0.012 (95% CI; -0.006, 0.029) g/cm^2 in the control group. BMD at Ward's triangle was increased by 0.095 (95% CI; 0.033, 0.157) g/cm^2 in the lovastatin-treated group, compared with an increase of 0.021 (95% CI; -0.021, 0.062) g/cm^2 in controls. In the lovastatin-treated group, BMD increased significantly at the spine and Ward's triangle. After 18 months, the lovastatin-treated group gained 3.4% more BMD at the spine, 1.2% at the femoral neck, 13.9% at Ward's triangle and 1.2% at total hip.

In the control group, BMD at total hip (1.4%), femoral neck (2.7%) and trochanter (2.9%) decreased and at spine (1.2%) and Ward's triangle (3.3%) increased during the study period. None of these changes were statistically significant.

After 18 months, BMD was statistically higher in the lovastatin-treated group than in the control group at all bone sites except spine (Table 3).

Table 1 Characteristics and BMD of postmenopausal women with type 2 diabetes on lovastatin-treated and diabetic regimen (control) groups

Characteristics	Treatment group at baseline		
	Lovastatin and diabetic regimen (*n*=28) Mean (SD)	Diabetic regimen (*n*=27) Mean (SD)	Difference (95% CI)
Age (years)	60.2 (3.1)	59.8 (3.2)	0.4 (-1.4, 2.1)
BMI (kg/m^2)	28.9 (3.9)	28.7 (3.8)	0.2 (-1.9, 2.4)
Duration of DM (years)	11.3 (4.8)	10.4 (5.1)	0.9 (-1.8, 3.6)
Menopausal duration (years)	9.7 (2.9)	9.0 (3.4)	0.7 (-1.1, 2.5)
Menopausal age (years)	49.9 (2.6)	50.2 (2.9)	-0.3 (-1.8, 1.1)
HbA1c (%)	10.0 (1.3)	9.5 (1.0)	0.5 (-0.1, 1.2)
Total cholesterol (mg/dl)	275.6 (19.4)	197.1 (31.3)	78.5 (64.1, 93.0)*
Triglyceride (mg/dl)	256.1 (115.4)	201.3 (89.9)	54.8 (-2.2, 111.7)
LDL-C (mg/dl)	174.5 (21.3)	112.0 (29.1)	62.5 (48.2, 76.6)*
HDL-C (mg/dl)	42.4 (10.9)	40.4 (8.2)	2.0 (-3.3, 7.2)
Serum phosphorus (mg/dl)	4.5 (0.6)	4.2 (0.7)	0.3 (-0.1, 0.7)
Calcium (mg/dl)	9.2 (0.5)	9.1 (0.6)	0.1 (-0.2, 0.4)
Alkaline phosphatase (IU/l)	218.8 (47.6)	213.2 (42.9)	5.6 (-23.7, 36.5)
BMD			
Lumbar (L_1–L_4) (g/cm^2)	0.946 (0.122)	0.971 (0.181)	-0.025 (-0.108, 0.058)
Femoral neck (g/cm^2)	0.826 (0.098)	0.788 (0.151)	0.038 (-0.031, 0.106)
Ward's triangle (g/cm^2)	0.685 (0.123)	0.646 (0.195)	0.039 (-0.050, 0.126)
Trochanter (g/cm^2)	0.750 (0.087)	0.701 (0.149)	0.049 (-0.016, 0.115)
Total hip (g/cm^2)	0.942 (0.104)	0.867 (0.162)	0.075 (0.002, 0.148)*

CI, confidence interval, *p<0.001

Table 2 Changes in BMD between 1st and 2nd DXA scans (18-month intervals) in postmenopausal women with type 2 diabetes on lovastatin and on diabetic regimen (control)

	2nd DXA scan	1st DXA scan	Difference (95% CI)
Lovastatin-treated group			
Lumbar (L_1–L_4) (g/cm^2)	0.978 (0.135)	0.946 (0.122)	0.032 (0.012, 0.052)*
Femoral neck (g/cm^2)	0.836 (0.114)	0.826 (0.098)	0.010 (-0.041, 0.020)
Ward's triangle (g/cm^2)	0.780 (0.186)	0.685 (0.123)	0.095 (0.033, 0.157)*
Trochanter (g/cm^2)	0.749 (0.096)	0.750 (0.087)	-0.001 (-0.021, 0.019)
Total hip (g/cm^2)	0.953 (0.109)	0.942 (0.104)	0.011 (-0.009, 0.029)
Control group			
Lumbar (L_1–L_4) (g/cm^2)	0.983 (0.195)	0.971 (0.181)	0.012 (-0.006, 0.029)
Femoral neck (g/cm^2)	0.767 (0.143)	0.788 (0.151)	-0.021 (-0.044, 0.002)
Ward's triangle (g/cm^2)	0.667 (0.177)	0.646 (0.195)	0.021 (-0.021, 0.062)
Trochanter (g/cm^2)	0.681 (0.149)	0.701 (0.149)	-0.020 (-0.041, 0.002)
Total hip (g/cm^2)	0.855 (0.164)	0.867 (0.162)	-0.012 (-0.029, 0.004)

DXA, dual-energy X-ray absorptiometry; *CI*, confidence interval, *p<0.01

Table 3 Comparison of BMD in postmenopausal women with type 2 diabetes on lovastatin and diabetic regimen (control) at the end of the study

	Lovastatin-treated (n=28)	Control (n=27)	Difference (95% CI)
Lumbar (L_1–L_4) (g/cm^2)	0.978 (0.135)	0.983 (0.195)	-0.005 (-0.095, 0.086)
Femoral neck (g/cm^2)	0.836 (0.114)	0.767 (0.143)	0.069 (0.001, 0.139)*
Ward's triangle (g/cm^2)	0.780 (0.186)	0.667 (0.177)	0.113 (0.014, 0.211)*
Trochanter (g/cm^2)	0.749 (0.096)	0.681 (0.149)	0.068 (0.001, 0.135)*
Total hip (g/cm^2)	0.953 (0.109)	0.855 (0.164)	0.098 (0.023, 0.173)*

CI, confidence interval , *p<0.05

In the lovastatin-treated group, plasma total cholesterol decreased from 275.6 (19.4) to 218.9 (24.3) mg/dl (p<0.001), triglyceride from 256.1 (115.4) to 214.2 (89.6) mg/dl (p<0.01) and LDL-C from 174.5 (21.3) to 130.4 (20.1) mg/dl (p<0.001). HDL-C increased from 42.4 (10.9) to 44.4 (7.9) mg/dl (p=0.26). HbA1c decreased from 10% (1.3) to 8.6% (1.1) (p<0.001). In the control group, no changes were observed in lipid profile, but triglyceride level decreased from 201.3 (89.9) to 174.3 (67.8) mg/dl (p<0.05) and HbA1c from 9.5% (1.0) to 8.5% (1.1) (p<0.001).

Percentage change in total cholesterol (-20.6% *vs.* 0.7%; p<0.001) and LDL-C (-24.9% *vs.* 6.7%; p<0.001) was higher in the lovastatin-treated group than in the controls.

At the end of the study period, BMI was not significantly different between the lovastatin-treated and control groups (29.6 (4.8) and 29.5 (3.9), p=0.6, respectively).

Discussion

An improvement in BMD and profound reduction in cholesterol concentration in a lovastatin-treated compared with a control group suggest that 18 months of therapy with lovastatin can prevent bone loss in postmenopausal patients with type 2 DM.

Despite the findings of anti-resorptive and bone anabolic properties of statins in animal experiments and *in vitro* studies [8, 9], conflicting results have been reported on the effects of statins on human bone in patients with and without type 2 DM [10–17]. The present results are consistent with other studies suggesting increased BMD in those using statins [10, 12, 13, 16, 27, 28], but some studies have not supported the hypothesis that these drugs can reduce bone loss and fractures [14, 15, 17, 29–32]. In a retrospective observational study, Chung et al. [12] reported that treatment with HMG-CoA reductase inhibitor could prevent bone loss in patients with type 2 DM and our findings confirmed this. Other studies [26–28] have shown a relationship between HMG-CoA reductase inhibitor use and a reduction in hip fracture. In a cross-sectional study done on Japanese patients with type 2 DM by Wada et al., patients who were on statins had lower BMD than the control group [33]. Like those in our control group, the patients in Wada et al.'s control group were not hypercholesterolaemic, and the lower BMD of patients on lovastatin may be due to the effect of hypercholesterolaemia on dif-

ferentiation of osteoblasts, which is inhibited by oxidised LDL-C and by hyperlipidaemia itself [13]. Nakashima et al. measured BMD of the distal one-third of the radius at baseline and after 2 years in patients with type 2 DM on HMG-CoA reductase and a control group. The annual rate of change in BMD of the radius had a positive correlation with HMG-CoA reductase therapy [13]. In the Nakashima study, the case group had already been on statins at baseline [13]. We designed our study as a clinical trial so our patients at baseline had not been on statin, bisphosphonate or oestrogen treatment, thus avoiding the previous effect of HMG-CoA reductase inhibitors on BMD. In another study with short duration of follow-up, Braatvedt et al. designed a double-blind, placebo-controlled, cross-over study of 12 weeks of placebo or 40 mg/day of atorvastatin with an 8-week wash-out period [11]. They measured markers of bone turnover in patients with type 2 DM and concluded that atorvastatin had no significant effect on bone turnover in humans, in spite of its effect in mice. It is not clear whether lengthening the Braatvedt et al. study would have shown effects of atorvastatin on bone turnover and positive changes in BMD and reduction of fracture risk. But it is unlikely, as most active agents, even relatively weak ones, have significant effects on bone turnover within 12 weeks [11]. On the other hand, longer duration of study seems not to be ethical. In another clinical trial, Rejnmark et al. designed a double-blind, placebo-controlled study of 52 weeks of placebo or 40 mg/day of simvastatin in 82 post-menopausal non-diabetic women and showed no effect of simvastatin on BMD at the hip or spine [32]. However, a significant increase in BMD was found in response to simvastatin at the forearm.

Although *in vitro* and animal studies as well as several clinical studies [10, 12, 13, 16, 27, 28] suggest that HMG-CoA reductase inhibitors increase the bone mass by enhancing bone morphogenetic protein-2-mediated osteoblast expression, several clinical studies have failed to demonstrate an effect of statins on biochemical markers of bone turnover and BMD [11, 32]. Current statins do differ in protein binding, hydro- or lipophilicity, and extent of liver metabolism, and are not designed to have specific effects on bone metabolism [9, 11, 13, 33]. Thus, these drugs may differ from each other with respect to delivery to bone, which may account for differences in reported effects on markers of bone turnover. If different kinds of HMG-CoA reductase inhibitors could have had different effects on bone metabolism and density, in Japanese and Korean patients who used to take different types of HMG-CoA reductase inhibitors, beneficial effects of statins on BMD should not have been shown [12, 13]. It is probable that other confounding factors not identified in a non-randomised placebo-controlled clinical trial may affect such findings. Moreover, as statins are re-circulated in the enterohepatic circulation, the drug concentration in bone environment may be very low. Therefore, it has been suggested that the increased BMD associated with use of statins more likely is due to factors associated with lipid-lowering treatment in general than a specific pharmacological action of statins on bone cells. Alternatively, a so-called healthy drug user effect has been suggested as a possible explanation, i.e., a tendency for patients who are compliant with preventive medicine to have fewer illness than non-users of preventive medicine, even after controlling for known risk factors. It is suggested that the increased BMD in statins users found in this study as well as other epidemiological studies most likely is due to healthy drug user effect. Therefore, the mechanism of action by which statins may increase BMD in type 2 DM remains to be revealed.

We found no unusual or unexpected safety risks with lovastatin therapy in this population with type 2 DM. Although several findings are relevant to the effect of statins on BMD, the study has some limitations. The major issue is the open label nature and the lack of randomisation. Hypercholesterolaemic patients are usually treated with HMG-CoA reductase inhibitors, so ethically a "true" control group cannot exist. For this reason, we studied type 2 DM with and without significant hyperlipidaemia, which provided a group treated with HMG-CoA reductase inhibitors and a control group. DM may itself affect bone metabolism, and there are some inconsistent results regarding the BMD in patients with type 2 DM [2, 18–21]. However, a high BMI may protect against bone loss in patients with type 2 DM [18]. By contrast, hyperglycaemia and proteinuria can cause an increase of calciuria and decrease new bone formation by osteoblasts [21, 34]. In our study, the two groups were generally well matched at baseline with regard to age, BMI, duration of diabetes, menopause, calcium, phosphorus, alkaline phosphates and other characteristics. At the end of the study, BMI did not change and glycaemic control reflected in HbA1c level was improved to the same extent in both groups. Thus, higher BMD in the lovastatin-treated group could not be due to BMI change or better glycaemic control [18, 21, 35]. Cholesterol concentration decreased in the case group, which may explain higher BMD of lovastatin-treated patients, as hypercholesterolaemia can inhibit osteoclast action [13]. Therefore, the effect of lovastatin on BMD, in our study, may be due to its direct effects on bone or its antihypercholesterolaemic action.

Although the two groups were generally well matched at baseline, our results may still be influenced by potential confounding factors that are not included, e.g., smoking, physical activity, vitamin D insufficiency and calcium/vitamin D supplements. Although residual confounding may be present owing to imperfect measurement of calcium, phosphate, alkaline phosphatase and lipid levels in this study, it is unlikely to account for more than a portion of the remaining excess risk. A "healthy drug users" effect may also have influenced the results, leading to artificially low risk estimates among statin users.

In conclusion, our data further support the contention that statins may be useful in the prevention of osteoporosis and may increase BMD in patients with type 2 DM. The encouraging results obtained in this trial warrant further study, in a larger-scale, probably randomised (in non-hyperlipidaemic type 2 DM), blinded trial.

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References

1. Anon (1993) Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 94:646–650
2. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM (1995) Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med 332:767–773
3. Ettinger B, Pressman A, Sklarin P, Bauer DC, Cauley JA, Cummings SR (1998) Associations between low levels of serum estradiol, bone density, and fractures among elderly women: the study of osteoporotic fractures. J Clin Endocrinol Metab 83:2239–2243
4. Slemenda C, Longcope C, Peacock M, Hui S, Johnston CC (1996) Sex steroids, bone mass, and bone loss. A prospective study of pre-, peri-, and postmenopausal women. J Clin Invest 97:14–21
5. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB; Women's Health Initiative Investigators (2003) Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290:1729–1738
6. Cranney A, Wells G, Willan A, Griffith L, Zytaruk N, Robinson V, Black D, Adachi J, Shea B, Tugwell P, Guyatt G (2002) Meta-analyses of therapies for postmenopausal osteoporosis. II. Meta-analysis of alendronate for the treatment of postmenopausal women. Endocr Rev 23:508–516
7. Riggs BL, Hartmann LC (2003) Selective estrogen-receptor modulators – mechanisms of action and application to clinical practice. N Engl J Med 348:618–629
8. Liao JK (2002) Isoprenoids as mediators of the biological effects of statins. J Clin Invest 110:285–288
9. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G (1999) Stimulation of bone formation in vitro and in rodents by statins. Science 286:1946–1949
10. Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, Ensrud KE, van der Klift M, Pols HA (2004) Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Intern Med 164:146–152
11. Braatvedt GD, Bagg W, Gamble G, Davidson J, Reid IR (2004) The effect of atorvastatin on markers of bone turnover in patients with type 2 diabetes. Bone 35:766–770
12. Chung YS, Lee MD, Lee SK, Kim HM, Fitzpatrick LA (2000) HMG-CoA reductase inhibitors increase BMD in type 2 diabetes mellitus patients. J Clin Endocrinol Metab 85:1137–1142
13. Nakashima A, Nakashima R, Ito T, Masaki T, Yorioka N (2004) HMG-CoA reductase inhibitors prevent bone loss in patients with Type 2 diabetes mellitus. Diabet Med 2:1020–1024
14. Pedersen TR, Kjekshus J (2000) Statin drugs and the risk of fracture. 4S Study Group. JAMA 284:1921–1922
15. Reid IR, Hague W, Emberson J, Baker J, Tonkin A, Hunt D, MacMahon S, Sharpe N (2001) Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 357:509–512
16. Wang PS, Solomon DH, Mogun H, Avorn J (2000) HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 283:3211–3216
17. Whitfield JF (2001) Statins: new drugs for treating osteoporosis? Expert Opin Investig Drugs 10:409–415
18. Wakasugi M, Wakao R, Tawata M, Gan N, Koizumi K, Onaya T (1993) Bone mineral density measured by dual energy X-ray absorptiometry in patients with non-insulin-dependent diabetes mellitus. Bone 14:29–33
19. van Daele PL, Stolk RP, Burger H, Algra D, Grobbee DE, Hofman A, Birkenhager JC, Pols HA (1995) Bone density in non-insulin-dependent diabetes mellitus. The Rotterdam Study. Ann Intern Med 122:409–414
20. Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, Jamal SA, Black DM, Cummings SR (2001) Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab 86:32–38
21. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ; Blue Mountains Eye Study (2001) Diabetes and risk of fracture: The Blue Mountains Eye Study. Diabetes Care 24:1198–1203
22. Gregorio F, Cristallini S, Santeusanio F, Filipponi P, Fumelli P (1994) Osteopenia associated with non-insulin-dependent diabetes mellitus: what are the causes? Diabetes Res Clin Pract 23:43–54
23. Sosa M, Dominguez M, Navarro MC, Segarra MC, Hernandez D, de Pablos P, Betancor P (1996) Bone mineral metabolism is normal in non-insulin-dependent diabetes mellitus. J Diabetes Complications 10:201–205
24. Isaia GC, Ardissone P, Di Stefano M, Ferrari D, Martina V, Porta M, Tagliabue M, Molinatti GM (1999) Bone metabolism in type 2 diabetes mellitus. Acta Diabetol 36:35–38
25. Amayo AA, Kirera S (2004) Comparison of calculated and direct low density lipoprotein cholesterol determinations in a routine laboratory. East Afr Med J 81:154–158
26. Kanis JA, Johnell O, Oden A, Jonsson B, De Laet C, Dawson A (2000) Risk of hip fracture according to the World Health Organization criteria for osteopenia and osteoporosis. Bone 27:585–590
27. Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, Donahue JG, Goodman MJ, Gurwitz JH, LaCroix AZ, Platt R (2000) Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 355:2185–2188
28. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H (2000) HMG-CoA reductase inhibitors and the risk of fractures. JAMA 283:3205–3210

29. Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Nicholson GC (2002) Statin use, bone mineral density, and fracture risk: Geelong Osteoporosis Study. Arch Intern Med 162:537–540
30. Sirola J, Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S (2002) Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int 13:537–541
31. van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C (2001) Use of statins and risk of fractures. JAMA 285:1850–1855
32. Rejnmark L, Buus NH, Vestergaard P, Heickendorff L, Andreasen F, Larsen ML, Mosekilde L (2004) Effects of simvastatin on bone turnover and BMD: a 1-year randomized controlled trial in postmenopausal osteopenic women. J Bone Miner Res 19:737–744
33. Wada Y, Nakamura Y, Koshiyama H (2000) Lack of positive correlation between statin use and bone mineral density in Japanese subjects with type 2 diabetes. Arch Intern Med 160:2865
34. Grasser WA, Baumann AP, Petras SF, Harwood HJ Jr, Devalaraja R, Renkiewicz R, Baragi V, Thompson DD, Paraklar VM (2003) Regulation of osteoclast differentiation by statins. J Musculoskeletal Neuronal Interact 3:53–62
35. Inaba M, Nishizawa Y, Mita K, Kumeda Y, Emoto M, Kawagishi T, Ishimura E, Nakatsuka K, Shioi A, Morii H (1999) Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D3 in patients with type 2 diabetes. Osteoporos Int 9:525–531