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HMG-CoA Reductase Inhibitors Increase BMD in Type 2 Diabetes Mellitus Patients*

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ABSTRACT

Recently, it was reported that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors increased bone mineral density (BMD) in mice. We studied the effect of HMG-CoA reductase inhibitors on BMD of type 2 diabetes mellitus by a retrospective review of medical records.

Sixty-nine type 2 diabetic patients were included. The control group (n = 33) did not take HMG-CoA reductase inhibitors. The treatment group (n = 36) was administered either lovastatin, pravastatin, or simvastatin. BMD of the spine, femoral neck, femoral trochanter, and total hip were measured by dual-energy X-ray absorptiometry.

There were no significant differences between control and treatment groups in age, sex, body mass index, glycemic control, and serum insulin levels. In the control group, BMD of the spine significantly decreased (from 1.116 ± 0.165 to 1.081 ± 0.178 g/cm^2) after 14 months. In the treatment group, BMD of the femoral neck significantly increased (from 0.853 ± 0.139 to 0.878 ± 0.147 g/cm^2) after 15 months. In male subjects treated with HMG-CoA reductase inhibitors, there was a significant increase in BMD of the femoral neck and femoral trochanter (from 0.899 ± 0.139 to 0.934 ± 0.139 and from 0.801 ± 0.145 to 0.833 ± 0.167 g/cm^2, respectively), but in female subjects, only BMD of the femoral neck increased (from 0.819 ± 0.132 to 0.834 ± 0.143 g/cm^2). Percentage increments of BMD of the femoral neck, femoral wards triangle, femoral trochanter, and total hip in the treatment group were significantly higher than in the control group (2.32% *vs.* −0.99, 1.77% *vs.* −1.25%, 1.40% *vs.* −1.21%, 0.88% *vs.* −1.03%, respectively). The proportion of subjects who had an increase in BMD of the spine and total hip more than two percentages was significantly larger in the treatment group than in the control group (30.6% *vs.* 15.2% and 30.6% *vs.* 9.1%, respectively). The increased increment in BMD of the treatment group was significantly greater than those in the control group after adjustment for age and body mass index ($P < 0.05$).

These results suggest that HMG-CoA reductase inhibitors may increase BMD of the femur in male patients with type 2 diabetes mellitus. (*J Clin Endocrinol Metab* **85:** 1137–1142, 2000)

IMBALANCE between osteoblastic bone formation and osteoclastic bone resorption is responsible for osteoporosis. The pathophysiology responsible for the discrepancy between bone resorption and formation remains complex. Bisphosphonates inhibit bone resorption and are used to prevent and treat bone loss. *In vitro* and *in vivo* studies indicate that bisphosphonates activate apoptosis of osteoclasts (1) and reduce osteoclast recruitment (2) to reduce bone resorption.

Luckman *et al.* (3, 4) recently reported that aminobisphosphonates inhibit the biosynthetic pathway from mevalonate to cholesterol, which results in a shortage of farnesyl or geranylgeranyl pyruvate. Decreased prenylation of guanosine 5′-triphosphate (GTP)-binding proteins such as Ras induces osteoclast apoptosis. The addition of mevalonate prevented bisphosphonate-induced osteoclast apoptosis. Additional work by Fisher *et al.* (5) suggests that alendronate and other N-containing bisphosphonates inhibit steps in the mevalonate pathway. This is mediated by direct action of alendronate to stimulate 34-kDa kinase in purified osteoclasts (5). This kinase response is blocked by all-trans geranylgeraniol (3–5).

Additional reports indicate an unexpected role for HMG-CoA reductase inhibitors in skeletal tissue. HMG-CoA reductase inhibitors increased new bone formation by osteoblasts in both *in vitro* cell culture systems and *in vivo* mice experiments. The addition of mevalonate prevented bone formation, and this phenomenon was specifically related to bone morphogenetic protein 2 gene expression (6).

Braga *et al.* (7) reported that aminobisphosphonates decreased serum low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein cholesterol levels in postmenopausal women (7). Lovastatin, a HMG-CoA reductase inhibitor, induces osteoclast apoptosis by activating Mst1 kinase, which is also a typical mechanism of aminobisphosphonates (8). Lovastatin inhibits osteoclastogenesis by blocking the cholesterol biosynthetic pathway, resulting in decreased bone resorption (9).

The purpose of this study was to observe the effects of HMG-CoA reductase inhibitors on bone mineral density (BMD) of human subjects with type 2 diabetes mellitus.

Subjects and Methods

Subjects

Sixty-nine type 2 diabetes mellitus Korean patients were identified by a retrospective review of medical records of the Ajou University Hos-

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pital at Suwon, Korea. The treatment group (n = 36) took HMG-CoA reductase inhibitors for hypercholesterolemia. HMG-CoA reductase inhibitors administered included lovastatin (Mevacor; MSD, Whitehouse Station, NJ) [10 mg (n = 1) and 20 mg (n = 8)], pravastatin (Mevalothin, Sankyo Co., Ltd., Tokyo, Japan) [10 mg (n = 14)], and simvastatin (Zocor; MSD) [5 mg (n = 1), 10 mg (n = 11), and 20 mg (n = 1)]. At the same time, the control group (n = 33) did not take HMG-CoA reductase inhibitors and had normal cholesterol levels. The control subjects were randomly selected from medical records, and they were matched for age, sex, body weight, postmenopausal status, and fasting blood glucose levels. All subjects were not taking medication affecting bone mineral metabolism, and none had any history of disease related to bone mineral metabolism.

Methods

Body weight, height, duration of diabetes mellitus, and medications used to control the diabetes and menopausal status and duration were recorded for analysis. Fasting plasma glucose and postprandial 2-h glucose levels were measured by the glucose oxidase method. Serum hemoglobin A_1c levels were measured by ion exchange high-performance liquid chromatography (Bio-Rad Laboratories, Inc. Hercules, CA). Fasting and postprandial 2-h insulin levels were measured by RIA (Dainabot Co., Japan). Fasting and postprandial 2-h C-peptide levels were measured by RIA (Technogenetics Co., Italy). Total cholesterol levels, high-density lipoprotein cholesterol levels, and triglyceride levels in the serum were measured by autoanalyzer (Hitachi 747, Tokyo, Japan). LDL cholesterol levels were calculated by Frederickson equation. Serum levels of calcium, phosphorus, and creatinine were measured by autoanalyzer (Hitachi 747).

BMD of the lumbar spine (L_2-L_4), femoral neck, wards triangle, femoral trochanter, and total hip were measured by dual-energy X-ray absorptiometry (Expert-XL; Lunar Corp.). Precision was 1.0% for the AP spine and total hip. BMDs were measured before and after treatment with HMG-CoA reductase inhibitors. BMD measurements were performed by the same technician using the same machine before and after treatments.

Statistical analysis

Data were analyzed by Student's paired *t* test to compare before and after treatment, by independent Student's *t* test to compare control and treatment subjects, and by multiple regression analysis and analysis of covariance to correlate other variables with HMG-CoA reductase inhibitor usage. Statistics were calculated using SPSS, Inc. for windows program version 7.0 (SPSS, Inc., Chicago, IL). Statistical significance was considered at $P < 0.05$.

Results

Clinical characteristics

There were no significant differences between control and treatment groups in age, sex, body mass index (BMI), menopausal status, duration of diabetes mellitus, and treatment of diabetes mellitus (Table 1). There were also no significant differences between the control and treatment groups in biochemical parameters of fasting and postprandial 2-h glucose levels, hemoglobin A_1c, fasting and postprandial 2-h insulin and C-peptide levels, or serum levels of calcium, phosphorus, and creatinine (Table 2). There were, however, significant differences in serum total cholesterol, LDL-cholesterol, and triglyceride levels between two groups (Table 2).

BMD before and after treatment with HMG-CoA reductase inhibitors

No significant differences were noted in baseline BMD in control and treatment groups. No difference in the prevalence of osteoporosis (WHO criteria) was noted in the control or treatment groups.

TABLE 1. Clinical characteristics of study subjects of effects of HMG-CoA reductase inhibitors on BMDs

	Control (n = 33)	Treatment (n = 36)
Age (yr)	52 ± 12	55 ± 8
Sex (M/F)	15/18	14/22
Pre/postmenopause (n)	5/13	3/19
Height (cm)	159.9 ± 7.0	158.2 ± 8.6
Weight (kg)	66.2 ± 8.1	62.2 ± 8.2
BMI (kg/m^2)	25.7 ± 2.7	24.8 ± 2.2
Duration of DM (month)	38 ± 8	47 ± 9
Treatment of DM		
Diet (%)	7 (21.2)	0 (0.0)
Sulfonylurea (%)	25 (75.8)	32 (88.9)
Insulin (%)	1 (3.0)	4 (11.1)
Interval of follow-up BMD (month)	15 ± 2	14 ± 3
HMG-CoA reductase inhibitor		
Duration (month)		15 ± 7
Type of HMG-CoA reductase inhibitor		
Lovastatin (%)		9 (25.0)
Pravastatin (%)		14 (38.9)
Simvastatin (%)		13 (36.1)

Values are expressed as the mean ± SD.
BMI, body mass index; DM, diabetes mellitus.
No significant differences between control *vs.* treatment groups.

Follow-up interval for measurement of BMD in the control group was 14 ± 2 months and in the treatment group was 15 ± 3 months. In the control group, BMD of the spine was significantly decreased at 14 months (Table 3). Gender differences in BMD were evident during the study period in the control subjects. In male control subjects, there were no significant differences in BMD measurements at follow-up (Table 3). In female control subjects, there was a significant decrease in BMD of the spine (Table 3).

In contrast, BMD of the femoral neck was significantly increased in the treatment group (Table 4). Significant increases in the femoral neck and femoral trochanter BMD were present in the male subjects treated with HMG-CoA reductase inhibitors. In female subjects treated with HMG CoA reductase inhibitors, changes in BMD were less compared with male subjects. Significant increases in BMD of the femoral neck were present in female subjects in the treatment group (Table 4).

BMD changes in two groups

Percentage increment of BMD after follow-up compared with baseline were defined mathematically as (follow-up BMD − baseline BMD) ÷ baseline BMD × 100. Percentage increments of BMD of the all femoral sites in the treatment group were significantly higher than the control group (Fig. 1A). Percentage increments of BMD of the all femoral sites were significantly increased compared to control group in male subjects (Fig. 1B), but not in female subjects (Fig. 1C).

The percentage of subjects who experienced an increase of more than 2% in BMD of the spine or total hip were 15.6% and 9.1% in the control group, compared with 30.6% and 30.6% in the treatment group (Fig. 2A). The percentage of male subjects who experienced a gain in BMD of more than

TABLE 2. Biochemical characteristics of study subjects

	Normal	Control	Treatment
Fasting plasma glucose (mg/dL)	70–110	161.1 ± 45.2	164.7 ± 49.3
Follow-up fasting plasma glucose (mg/dL)	70–110	160.5 ± 51.7	161.1 ± 38.9
HbA1c (%)	3.5–6.5	7.6 ± 1.5	7.9 ± 1.3
Fasting insulin (μU/mL)	3.0–12.0	6.8 ± 4.1	5.5 ± 2.9
Fasting C-peptide (ng/mL)	0.4–4.0	1.84 ± 0.62	2.02 ± 0.88
Serum calcium (mg/dL)	8.4–10.2	9.2 ± 0.3	9.2 ± 0.2
Serum phosphorus (mg/dL)	2.7–4.5	3.5 ± 0.6	3.7 ± 0.4
Serum creatinine (mg/dL)	0.5–1.4	1.0 ± 0.3	0.9 ± 0.2
Total cholesterol (mg/dL)	120–220	192.6 ± 31.3	236.3 ± 39.4[a]
Triglyceride (mg/dL)	35–200	159.5 ± 70.4	209.2 ± 91.2[a]
HDL-cholesterol (mg/dL)	45–100	44.5 ± 7.87	44.6 ± 10.3
LDL-cholesterol (mg/dL)	0–130	117.6 ± 29.4	149.9 ± 37.8

Values are expressed as the mean ± SD.
[a] $P < 0.05$ compared with control group.

TABLE 3. Comparison of BMD (g/cm^2) at baseline and follow-up of the control subjects

	Total				Male				Female			
	Baseline		Follow-up		Baseline		Follow-up		Baseline		Follow-up	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LS	1.116 ± 0.165		1.081 ± 0.178[a]		1.141 ± 0.116		1.116 ± 0.130		1.092 ± 0.198		1.048 ± 0.208[a]	
FN	0.903 ± 0.145		0.894 ± 0.146		0.904 ± 0.096		0.895 ± 0.101		0.902 ± 0.180		0.893 ± 0.178	
FW	0.706 ± 0.178		0.698 ± 0.185		0.700 ± 0.144		0.684 ± 0.144		0.711 ± 0.204		0.709 ± 0.216	
FTR	0.783 ± 0.142		0.775 ± 0.140		0.833 ± 0.115		0.826 ± 0.115		0.737 ± 0.148		0.726 ± 0.143	
TH	0.988 ± 0.149		0.978 ± 0.148		1.015 ± 0.101		1.004 ± 0.103		0.963 ± 0.179		0.956 ± 0.177	

Values are expressed as the mean ± SD.
LS, Lumbar spine; FN, femoral neck; FW, femoral ward; FTR, femoral trochanter; TH, total hip.
[a] $P < 0.05$ baseline *vs.* follow-up.

TABLE 4. Comparison of BMD (g/cm^2) before and after HMG-CoA reductase inhibitor administration of the treatment subjects

	Total				Male				Female			
	Before		After		Before		After		Before		After	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LS	1.035 ± 0.172		1.015 ± 0.186		1.049 ± 0.172		1.051 ± 0.184		1.000 ± 0.172		0.988 ± 0.187	
FN	0.853 ± 0.139		0.878 ± 0.147[a]		0.899 ± 0.139		0.934 ± 0.139[b]		0.819 ± 0.132		0.834 ± 0.143[b]	
FW	0.674 ± 0.162		0.688 ± 0.161		0.721 ± 0.157		0.737 ± 0.155		0.638 ± 0.159		0.651 ± 0.160	
FTR	0.748 ± 0.142		0.763 ± 0.163		0.801 ± 0.145		0.833 ± 0.167[b]		0.708 ± 0.129		0.710 ± 0.144	
TH	0.954 ± 0.149		0.968 ± 0.158		0.987 ± 0.169		1.004 ± 0.174		0.930 ± 0.130		0.933 ± 0.141	

Values are expressed as the mean ± SD.
LS, Lumbar spine; FN, femoral neck; FW, femoral ward; FTR, femoral trochanter; TH, total hip.
[a] $P < 0.001$ comparing before *vs.* after treatment.
[b] $P < 0.05$ comparing before *vs.* after treatment.

2% in the spine and total hip were 20.0% and 6.7% in the control group, compared with 42.9% and 50.0% in the treatment group (Fig. 2B). Female subjects revealed similar trends in BMD of the spine and total hip. The BMD of female subjects increased more than 2% included the spine and total hip (11.8% and 11.1% in the control group and 22.7% and 18.2% in the treatment group, respectively) (Fig. 2C). The effect of the HMG-CoA reductase inhibitor was not different in subjects with osteoporosis or osteopenia at baseline in both groups.

Correlation with other variables

BMD of spine and femur were significantly correlated with age and BMI. BMD changes comparing two groups with respect to usage of HMG-CoA reductase inhibitors were still significant after adjustment for age and BMI (Table 5).

BMDs according to different HMG-CoA reductase inhibitors

We analyzed the change of BMD according to different types of HMG-CoA reductase inhibitors administered. BMD of the spine and total hip changed −3.13 ± 5.06% and 0.05 ± 1.55% in the lovastatin group, 1.66 ± 5.13 and 2.33 ± 3.25% in the pravastatin group, and −1.71 ± 5.49% and −0.09 ± 3.28% in the simvastatin group. There were no significant differences in the change in BMD among the three drugs (lovastatin, pravastatin, and simvastatin).

Discussion

HMG-CoA reductase inhibitors such as lovastatin, pravastatin, and simvastatin block the conversion of HMG-CoA to mevalonate in the cholesterol synthesis pathway. Luckman *et al.* (3, 4) reported that both aminobisphosphonates and HMG-CoA reductase inhibitors increased macrophage

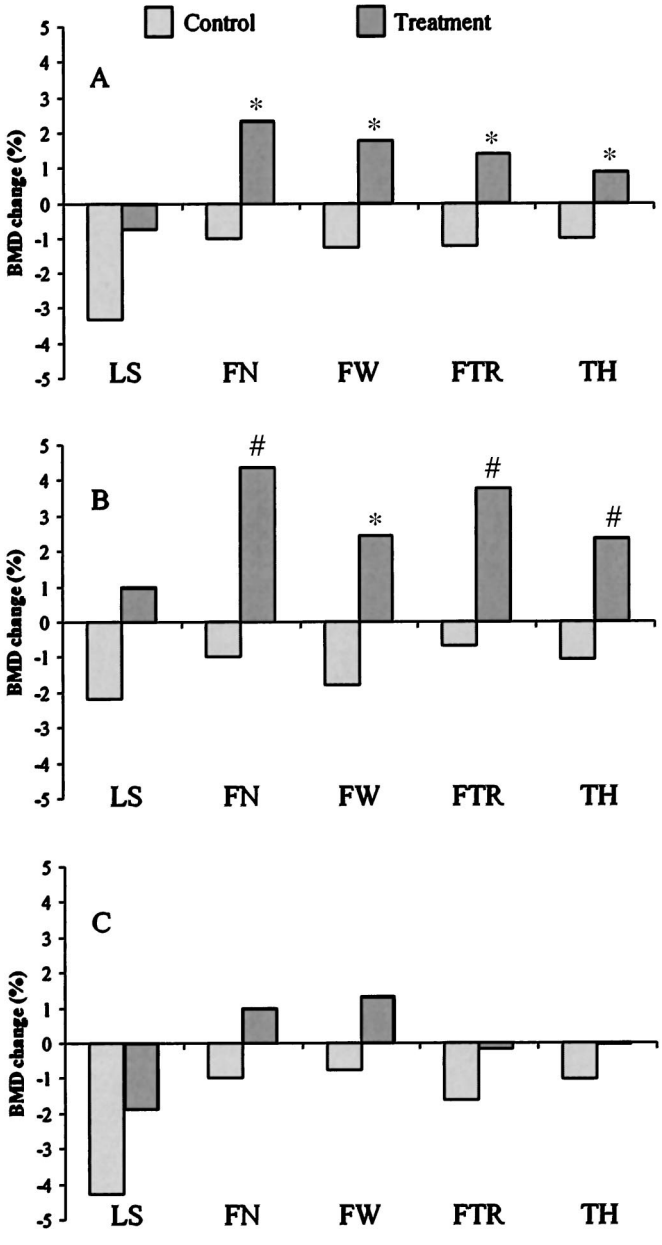

FIG. 1. Comparison of BMD improvement between control and HMG-CoA reductase inhibitor-treated subjects. A, total subjects. B, male subjects. C, female subjects. *, $P < 0.05$; #, $P < 0.001$ compared with control group. LS, Lumbar spine; FN, femoral neck; FW, femoral ward; FTR, femoral trochanter; TH, total hip.

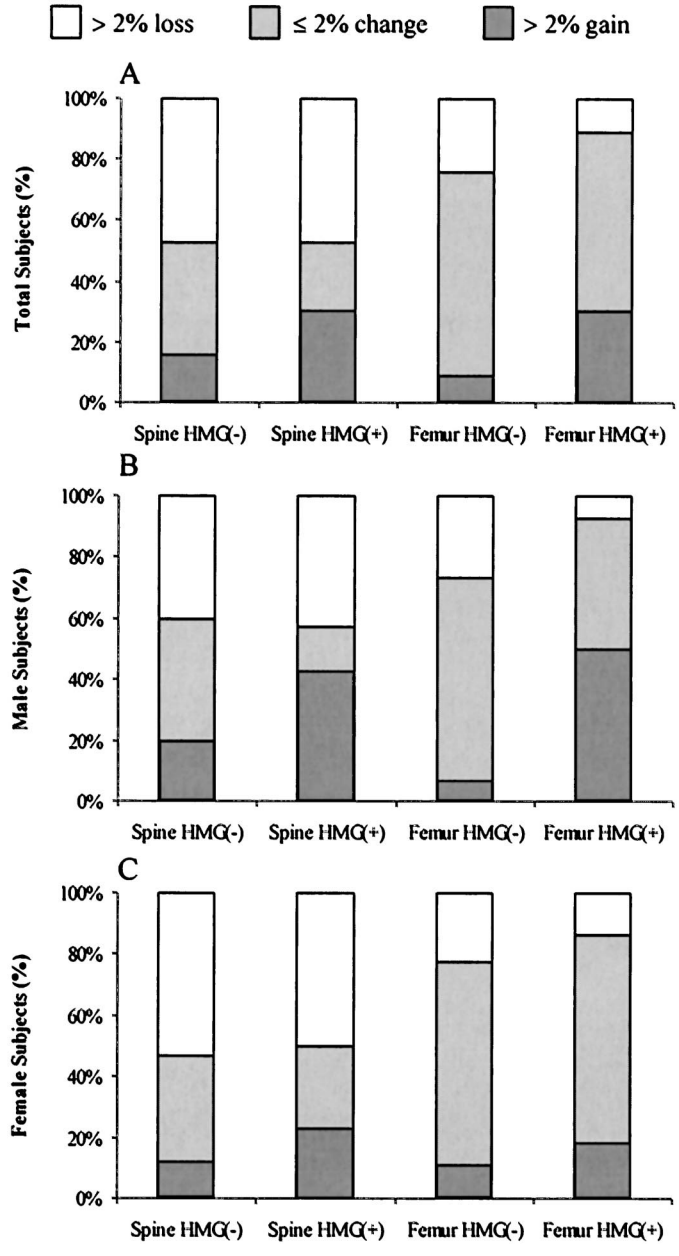

FIG. 2. Proportion of total subjects (A), male subjects (B), and female subjects (C) with a loss of more than 2%, a change of 2% or less, or a gain of more than 2% in BMD of the lumbar spine and total hip. HMG, HMG-CoA reductase inhibitor; LS, lumbar spine; FN, femoral neck; FW, femoral ward; FTR, femoral trochanter; TH, total hip.

(osteoclast-like cells) apoptosis. HMG-CoA reductase inhibitors also increase osteoblastic new bone formation (6).

Recently, Bauer *et al.* (10) reported in abstract form that HMG-CoA reductase inhibitor use may be associated with higher hip BMD and reduced fracture risk.

Initially, we planned to verify the effects of HMG-CoA reductase inhibitors in bone metabolism in patients with primary osteoporosis, but a bias was present because most of these patients had already been treated. Therefore, we selected subjects with type 2 diabetes mellitus, a group known for their lack of hormone replacement therapy usage (11).

Patients with hyperlipidemia, but without diabetes mellitus, usually do not undergo a BMD test. Hypercholesterolemic patients are usually treated with HMG-CoA reductase inhibitors, so ethically a "true" control group cannot exist. For these reasons, we studied type 2 diabetes mellitus with and without hyperlipidemia, which provided a group treated with HMG-CoA reductase inhibitors and a control group.

Diabetes mellitus may itself affect bone metabolism. BMD of type 1 diabetes mellitus patients is decreased compared with normal subjects (12). There are some controversial reports regarding BMD measurements in type 2 diabetes mel-

TABLE 5. The relationship between BMD and HMG-CoA reductase inhibitors

BMD	Adjusted for covariates	Model significance	*P*	R	R^2
Lumbar spine	Age, Sex, BMI, FPG, HbA_{1c}	0.003	0.006	0.52	0.27
Femoral neck	Age, Sex, BMI, FPG, HbA_{1c}	0.005	0.000	0.50	0.25
Femoral ward	Age, Sex, BMI, FPG, HbA_{1c}	0.274	0.019	0.33	0.11
Femoral trochanter	Age, Sex, BMI, FPG, HbA_{1c}	0.034	0.010	0.44	0.19
Total hip	Age, Sex, BMI, FPG, HbA_{1c}	0.009	0.003	0.48	0.23

BMI, Body mass index; FPG, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}.

litus patients (13–15). Hyperinsulinemia and relatively high BMI are protective against bone loss in type 2 diabetes mellitus (16). By comparison, increased calciuria and decreased osteoblastic function due to hyperglycemia may lead to deterioration of bone mass and have been observed in patients with type 2 diabetes mellitus (17, 18). In our study, we matched our subjects for fasting plasma glucose, hemoglobin A_1c levels, and duration of disease. Insulin may act on osteoblasts, enhancing bone formation. For this reason, we adjusted for insulin levels in our study subjects (19, 20). The effects of HMG-CoA reductase inhibitors on BMD were still significant, even after adjustment for insulin and C-peptide levels.

Recent work of Van Beek *et al.* (21) tested the effect of a series of bisphosphonates on bone resorption *in vivo*. All bisphosphonates tested inhibited ^{45}Ca release in murine fetal metatarsals (21). The addition of geranylgeraniol attenuated the inhibition of N-containing bisphosphonates (alendronate, risedronate, or olpadronate) but not of non-N-containing bisphosphonates, clodronate or etidronate (21). In addition, mevastatin, an inhibitor of HMG-CoA reductase, was a potent and direct suppressor of bone resorption. This finding can be reversed by the addition of mevalonate. Geranylgeraniol, but not farnesol, reversed the effect, suggesting protein geranylgeranylation and not farnesylation is essential for osteoclast-mediated bone resorption. Thus, the importance of the mevalonate pathway in osteoclast-mediated bone resorption and its regulation by N-containing bisphosphonates cannot be underestimated.

Differences in the farnesol and geranylgeraniol pyrophosphate synthase activities vary among tissues that have been tested (22). Mevalonate reversed the alendronate-induced inhibition of bone resorption in mouse calvariae (3, 4), in agreement with findings by Fisher *et al.* (5). These studies describe minimal effect of mevalonate on alendronate-induced inhibition of osteoclast formation in cocultures of murine bone marrow with osteoblast-like cells (5). Bone resorption induced by ibandronate, in contrast, was totally overcome by geranylgeraniol, but mevalonate had a small effect (21).

The role of geranylgeranylation in bone resorption remains speculative. Small GTP-binding proteins, such as rho p21, are preferentially expressed in osteoclasts and regulate cytoskeleton organization within the cell. Cytoskeleton organization in osteoclasts is altered by alendronate. It has been proposed that the N-containing bisphosphonates may prevent prenylation of small GTP-binding proteins such as rho p21 by inhibition of geranylgeranylation resulting in cytoskeleton disruption and apoptosis of osteoclasts (4, 5).

Effects of HMG-CoA reductase inhibitors on BMD were more prominent in male subjects compared with females. These differences may be due to physiologic differences of male and female bone loss (23). Osteoporosis with decreased osteoblastic function is the main mechanism of bone loss in men. In contrast, osteoporosis with increased bone resorption associated with estrogen loss during the menopause is the major mechanism of bone loss in women. HMG-CoA reductase inhibitors increased osteoblastic new bone formation both in *in vitro* cell culture systems and *in vivo* animal experiments (6). Our speculation is that HMG-CoA reductase inhibitors will increase BMD favorably in male subjects because of osteoblast-induced new bone formation. Studies of osteoclast apoptosis *in vitro* suggest that this is an additional mechanism by which HMG-CoA reductase inhibitors may decrease bone loss (3, 4). The majority of the female subjects in our study were postmenopausal, and HMG-CoA reductase inhibitors may be helpful in attenuation of increased bone resorption in postmenopausal osteoporosis but not completely compensate for the untreated bone loss associated with estrogen deficiency.

The limitations of this study were a retrospective clinical record review design and a relatively small number of subjects. The study subjects were patients with type 2 diabetes mellitus who might have abnormal bone metabolism. The controls are not hypercholesterolemic subjects, as it would be unethical to deny them lipid-lowering therapy. Despite these limitations, some insights have been gained from this study. The first is that this clinical study supports the published *in vitro* and *in vivo* animal studies. Second, gender differences are noted, reflective of the different pathophysiology regarding bone loss in female and male subjects. The majority of the female subjects were postmenopausal, a time with increased resorption resulting in higher turnover and greater bone loss. HMG-CoA reductase inhibitors may not be able to attenuate the rapid phase of bone loss in the face of total estrogen depletion.

In conclusion, we suggest that HMG-CoA reductase inhibitors prevent bone loss in patients with type 2 diabetes mellitus, even after adjustment for age, sex, BMI, fasting glucose levels, and hemoglobin A_{1C} levels. These findings are consistent with *in vitro* studies, indicating that this class of medications effect osteoclast apoptosis and decrease bone resorption.

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