Institute of Clinical Biochemistry and Diagnostics, Medical Faculty and University Hospital; Radioisotope Laboratories and Vivarium, Faculty of Medicine, Charles University; Department of Radiobiology, Faculty of Health Sciences, University of Defence, Hradec Kralove, Czech Republic

The role of atorvastatin in bone metabolism in male albino Wistar rats

I. Gradosova , H. Zivna , K. Svejkovska , V. Palicka, A. Tichy , P. Zivny

Received February 17, 2011, accepted March 18, 2011

Iveta Gradosova, Institute of Clinical Biochemistry and Diagnostics, Medical Faculty and University Hospital, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
gradosovai@lfhk.cuni.cz

Pharmazie 66: 606–610 (2011) *doi: 10.1691/ph.2011.1525*

Objective: Statins have been widely used for the treatment of hypercholesterolemia, and recent studies have shown that these drugs also affect bone metabolism. The aim of this experiment was to follow the effect of atorvastatin on bone metabolism in male albino Wistar rats. *Methods*: Our study was carried out on 16 rats (240 ± 10 g) which were randomly divided into 2 groups of 8 animals. The control group (CO) was given *aqua pro injectione* (0.2 mL/100 g BW; gavage) and the experimental group atorvastatin suspension (AT; 0.3 mg in 0.2 mL *aqua pro inj.*/100 g BW; gavage) daily for 8 weeks. We examined serum markers of bone turnover using ELISA – C-terminal crosslinking telopeptide of type I collagen (CTX-I), total osteocalcin (total OC), procollagen type I N propeptide (PINP) and bone alkaline phosphatase (bone ALP). We investigated bone morphogenetic protein-2 (BMP-2) in the proximal tibia using Western blot analysis. Additionally, we measured bone mineral density (BMD). The femurs were used for a three-point bending test and compression test of the femoral neck. *Results:* After 8 weeks of atorvastatin administration, a significant decrease was found in serum level of bone ALP to 30% vs. CO ($p = 0.005$). PINP, CTX-I and OC did not change significantly. The expression of BMP-2 was increased. There were no significant differences in BMD measurements, three-point bending test or compression test of the femoral neck. *Conclusions:* Our results suggest that atorvastatin has a positive effect on bone metabolism in rats by maintenance of BMD and the biomechanical characteristics of bone. Atorvastatin influenced bone metabolism by decreasing bone ALP, and probably in consequence increasing expression of BMP-2 in rats.

1. Introduction

Despite significant improvements in the treatment of osteoporosis (OP) and cardiovascular diseases, these diseases are the most frequent cause of morbidity and mortality in our aging population (Parhami et al. 2000). Patients diagnosed with osteoporosis suffer from bone tissue losses of more than 50% in critical areas of the skeleton, which are characterized by disruption of trabecular bone microarchitecture (Garrett and Mundy 2002). Drugs prescribed e.g. for high blood pressure or cholesterol to patients with OP should by chosen so that they do not negatively affect bone metabolism and exacerbate the loss of bone tissue.

Atorvastatin belongs to the group of statins that are considered to be some of most effective hypocholesterolemics. Besides the ability to reduce cholesterolemia, statins proved to have so called pleiotropic effects such as the improvement of endothelial dysfunction, reduction of inflammatory response, stabilization of atherosclerotic plaque and reduction of thrombogenic response (Liao 2002). In addition, many studies have been published that show the effect of statins on bone metabolism (Jadhav and Jain 2006).

Atorvastatin inhibits the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase during cholesterol synthesis. Inhibition of the enzyme results in reduced conversion of HMG-CoA to mevalonate and subsequent isoprenoid precursors. Inhibition of the enzyme reduces cholesterol mainly in liver cells (Stern et al. 2000). Reduced cholesterol availability leads to increased liver low-density lipoprotein (LDL) receptor synthesis and increased removal of LDL from the bloodstream. Hence, there is a decrease in the total amount of cholesterols, LDL and triglycerides, and an increase in high density lipoprotein (HDL) level (Malhotra and Goa 2001).

The above-mentioned isoprenoid precursors, which include especially farnesyl pyrophosphate and geranylgeranyl pyrophosphate are very important for posttranslational modification (prenylation) of GTP binding proteins (also called small GTPases) – Rho, Rac and Rab. These proteins are activated after the prenylation and participate in various signal transduction events such as cytoskeleton modeling. The prenylation adds a lipid chain that anchors the small GTPases into the membrane of the osteoclasts. This step is necessary for correct osteoclast ruffled border formation that serves for attachment to a bone segment, prior to release of vesicles containing proteolytic enzymes and acids into the space sealed by the osteoclasts. These acids and proteolytic enzymes dissolve underlying bone. Lack of intermediates of cholesterol synthesis due to the effect of the statin leads to apoptosis of osteoclasts and subsequent reduction of bone resorption (Coxon and Rogers 2003).

Another factor affecting bone tissue is bone morphogenetic protein-2 (BMP-2). Bone morphogenetic proteins, members of the transforming growth factor (TFG)-β superfamily, promote

differentiation of mesenchymal cells into chondrocytes and osteoblasts, as well as the differentiation of osteoprogenitor cells into osteoblasts (Lieberman et al. 2002). Mundy et al. (1999) as the first researchers presented the fact that expression of BMP-2 gene in bone cells is enhanced by statins.

Furthermore, it was found that atorvastatin enhances the *in vitro* expression of other osteoblastic differentiation markers, notably bone alkaline phosphatase (bone ALP), osteocalcin (OC), type-I collagen, and osteoprotegerin (OPG) (Viereck et al. 2005; Ruiz-Gaspa et al. 2007). By reducing protein prenylation, atorvastatin also stimulates the expression, mediated through the phosphatidylinositol-3-kinase (PI3K) pathway, of vascular endothelial growth factor (VEGF), which promotes differentiation of osteoblasts (Maeda et al. 2003).

Bone metabolism is routinely monitored by assessing the serum level of markers of bone turnover. Specific markers of bone formation are bone ALP, OC and procollagen type I N and C propeptide (PINP, PICP). Bone ALP is located in the membranes of osteoblasts, from which it is released into the serum on their activation. It correlates with the intensity of bone formation, and is involved in the formation and mineralization of osteoid (Brown et al. 2009). Osteocalcin is a small non-collagen protein produced by osteoblasts, dependent on vitamins D and K, and conferring a greater affinity to calcium and hydroxyapatite. OC fragments released into the bloodstream are considered one of the most sensitive markers of bone formation (Lee et al. 2000). PINP and PICP are propeptides of procollagen I before its integration into bone as type-I collagen. Their serum concentration reflects bone formation (Garnero et al. 2008). Conversely, during degradation of type-I collagen, N- and C-terminal crosslinking telopeptide of type I collagen (NTX-I, CTX-I) are released into the circulation, enabling estimation of the resorption of bone (Herrmann and Seidel 2008).

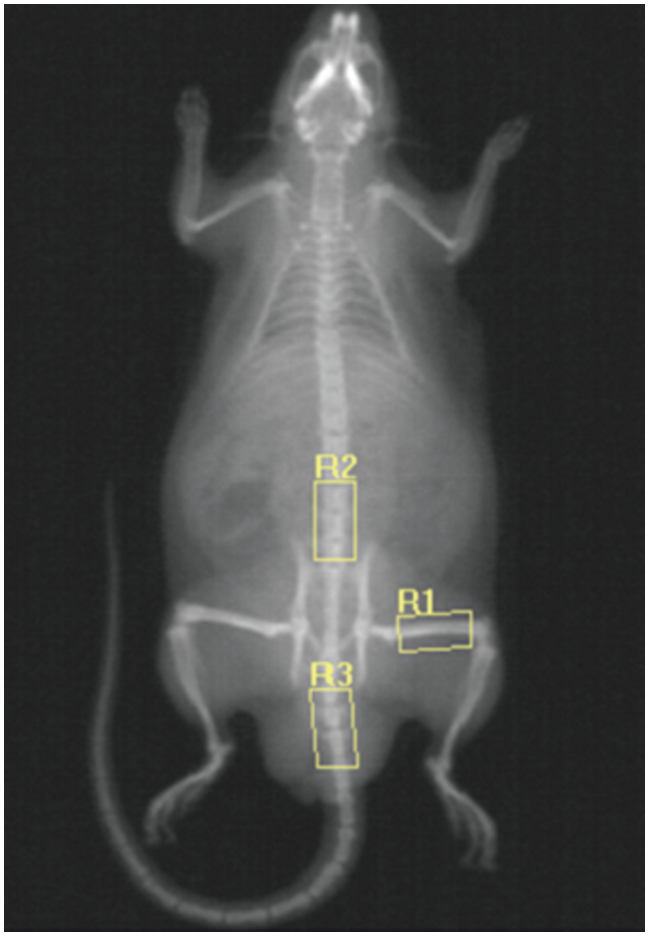

Fig. 1: Dual-energy X-ray absorptiometry (DXA) scan of the whole body of the rat with signed measured bone areas: R1 femur, R2 lumbar vertebrae and R3 caudal vertebrae

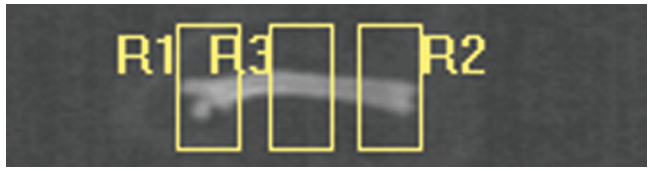

Fig. 2: Scanned image of the left femur. BMD of the entire femur, the proximal femur (R1), the femoral shaft (R3) and the distal femur were determined using computer software (DXA; QDR-4500A Elite; Hologic, Waltham, MA, USA)

2. Investigations and results

We examined the effect of atorvastatin (at a dose of 0.3 mg/100 g BW) on bone metabolism in male albino Wistar rats. Serum markers of bone turnover – C-terminal crosslinking telopeptide of type I; total osteocalcin; procollagen type I N propeptide; and bone alkaline phosphatase – were measured with enzyme immunoassay kits. BMP-2 expression in proximal tibia extract was evaluated using Western blot analysis. Bone mineral density (BMD) was analyzed using dual energy X-ray absorptiometry (DXA). Scans were recorded for three skeletal areas (lumbar and caudal vertebrae, and femur), and for the left femur (the whole femur, the proximal femur, the femoral shaft and the distal femur). The mechanical properties of the whole femur were measured by three-point bending test, and the proximal femurs were used for a compression test of the femoral neck using a special electromechanical custom-made testing machine.

After 8 weeks the group of rats given atorvastatin showed statistically significant decrease of serum level of bone ALP to 30% ($p = 0.005$), compared to the control group (Fig. 1a). There were no differences between the atorvastatin and control groups in the serum concentration of PINP, OC or CTX-I ($p = 0.19$, 0.80 and 0.37) (Fig. 1b, c, d).

In the lumbar vertebrae, caudal vertebrae and femoral areas, no difference was revealed in BMD values between the atorvastatin group and control group. There was no significant difference in BMD values between the groups for the left entire femur, proximal femur, femoral shaft and distal femur. Results related to BMD are given in the Table. There were no significant differences between the two groups in maximal load values for the femoral shaft or femoral neck.

Results from Western blot analysis showed that the BMP-2 protein level was enhanced in the proximal tibia for the atorvastatin group compared with the control group (Fig. 2).

3. Discussion

Osteoporosis and atherosclerosis are frequent diseases in the elderly. Patients with cardiovascular disease should be prescribed drugs to control high blood pressure and cholesterol, but side effects of this treatment may affect bone metabolism. In better cases the effect is positive, but for clinical practice it is necessary to know whether this therapy does or does not cause bone loss.

In our experiment we chose atorvastatin, one of the most frequently prescribed statins. It is indicated mostly for elderly people at high risk of cardiovascular disease to reduce hypercholesterolemia, mainly LDL-CHOL. It has been found that lipid oxidation products not only promote atherogenesis but also inhibit differentiation of osteoblasts, thus negatively affecting bone tissue (Parhami et al. 2000). In our experiment we followed the effect of the drug on bone metabolism in healthy adult male

Table: Effect of atorvastatin on bonemineral density (BMD) in three skeletal areas and left femur, andbiomechanical properties of the femur three-point bending test andcompression test of femoral neck in male albino Wistar rats

Measurement (unit)	Aqua pro inj.	Atorvastatin	p
Lumbar vertebrae (g/cm^2)	0.223 ± 0.014	0.217 ± 0.012	0.33
Caudal vertebrae (g/cm^2)	0.222 ± 0.012	0.234 ± 0.010	0.07
Femur areas (g/cm^2)	0.189 ± 0.021	0.175 ± 0.013	0.13
Left femurs			
Entire femur (g/cm^2)	0.109 ± 0.042	0.118 ± 0.038	0.67
Proximal femur (g/cm^2)	0.121 ± 0.039	0.139 ± 0.042	0.39
Femoral shaft (g/cm^2)	0.102 ± 0.055	0.110 ± 0.049	0.77
Distal femur (g/cm^2)	0.092 ± 0.050	0.080 ± 0.050	0.68
Maximal load of the femoral shaft (N)	173.50 (168.25–191.00)	173.00 (160.00–187)	0.44
Maximal load of femoral neck (N)	144.50 (118.75–61.25)	144.00 (122.25–177.00)	0.56
Right femurs			
Maximal load of the femoral shaft (N)	192.00 (173.25–205)	175.50 (162.50–202.50)	0.27
Maximal load of femoral neck (N)	144.50 (96–153.75)	139.00 (131.00–142.00)	0.70

albino Wistar rats. The bone metabolism was not affected by any other pathological process (obesity, dyslipidemia or arterial hypertension). No significant BMD changes were observed in our study in the measured areas after 8 weeks of atorvastatin administration. The unchanged BMD may be attributed to male sex hormones (Binkley 2006).

There are few studies concerned with change in the mechanical properties of bone with atorvastatin administration. Uyar et al. (2009) concluded that atorvastatin at a dose of 50 mg/kg BW in rats after ovariectomy both prevented a decrease in BMD and created a protective effect on mechanical properties of bone. Our data showed that atorvastatin administration (0.3 mg/100 g BW) had no significant influence on the biomechanical properties of femur.

In our experiment, the serum level of bone ALP significantly decreased to 30% of its initial value due to the effect of atorvastatin, which indicates suppression of osteoblastic activity. The serum concentration of PINP, CTX-I and OC showed only a small decrease, not statistically significant. Some human studies have shown a decrease in serum level of bone ALP after treatment with statins (Rosenson et al. 2005; Yavuz et al. 2009; Rejnmark el al. 2002, Hatzigeorgiou and Jackson 2005). In a randomized controlled trial of simvastatin at the high dose of 80 mg/day, treatment lowered serum bone ALP and osteocalcin (Rosenson et al. 2005). Yavuz et al. (2009) reported that bone ALP levels were decreased during rosuvastatin treatment without change in osteocalcin levels. In a cross-sectional study of 140 postmenopausal women who had been treated for more than 2 years with a statin (primarily simvastatin, atorvastatin), plasma levels of bone turnover markers (bone ALP, OC and CTX-I) were lower than in controls (Rejnmark el al. 2002). A meta-analysis found improved hip bone mineral density and a decrease in bone ALP levels with statin treatment (Hatzigeorgiou and Jackson 2005). Our findings, together with previous reports (Rosenson et al. 2005; Yavuz et al. 2009; Rejnmark el al. 2002, Hatzigeorgiou and Jackson 2005), suggest a potent effect of statins on reducing bone turnover. However, further investigations are required in order to clarify the mechanism of how the statins affect bone ALP.

The vast majority of published results suggest that statins have beneficial effects on bone *in vitro*. Studies on cell cultures of osteoblasts exposed to statins show stimulation of bone formation, mediated by increased expression of bone morphogenetic protein-2. The effect of statins appears to be different *in vitro* than *in vivo*, the antiresorptive effect being more predominant in the former (Jadhav and Jain 2006). Some studies however do not identify with this statement (Maritz et al. 2001). It is hence desirable to explore further the influence of statins on bone metabolism. We were able to demonstrate that atorvastatin increases BMP-2 synthesis in proximal tibia. Increased expression of BMP-2 may be a subsequent reaction to the inhibition of osteoblasts (decreased bone ALP) with atorvastatin.

Several studies have been presented in which it was suggested that there was a link between hypercholesterolemia and osteoporosis. Tintut et al. presented that hypercholesterolemia

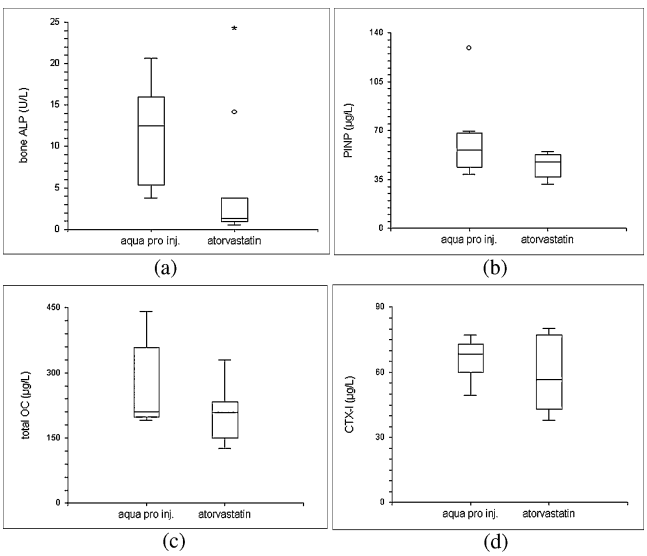

Fig. 3: Effect of atorvastatin (AT; 0.3 mg/100 g BW) on bone formation in male albino Wistar rats after an 8-week administration. The figure represents the change of concentration of a) bone alkaline phosphatase (bone ALP); b) procollagen yype I N propeptide (PINP); c) total osteocalcin (OC); and d) C-terminal crosslinking telopeptide of type I collagen (CTX-I) compared to the control group. Data are expressed as box plots. the box extends from 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). Whiskers extend down to the smallest value and up to the largest. The points outside the ends of the whiskers are outliers. *p<0.05

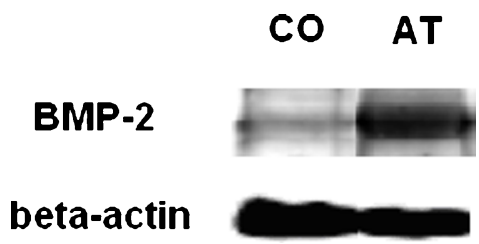

Fig. 4: Effect of atorvastatin on BMP-2 protein synthesis in proximal tibia (CO–control, AT–atorvastatin)

enhances differentiation of osteoclasts and resorptive activity both *in vivo* (Tintut et al. 2004) and *in vitro* (Tintut et al. 2002). Majima et al. (2008) found in their work that women and men with hypercholesterolemia have a significantly higher serum NTX-I level compared with control groups. In another study, Majima et al. (2007) investigated the influence of atorvastatin on bone turnover in male patients with hypercholesterolemia. After 3-months treatment the NTX-I significantly decreased. On the other hand, *in vitro* studies show that oxidation products of lipids and lipoproteins inhibit the differentiation and function of osteoblasts (Tintut et al. 2004; Parhami et al. 1999). Atorvastatin might have a beneficial effect on bone metabolism both indirectly by the compensation of hypercholesterolemia as well as by the direct effect of slowing down bone turnover.

Given the proven evidence of the effect of atorvastatin on bone metabolism it would be appropriate to examine these findings also in humans with hypercholesterolemia treated with atorvastatin.

There are limits of reproducibility of the study: The experiment was carried out on an animal model of intact rat. In the future it will be appropriate to verify these results on rats with induced hypercholesterolemia.

In summary, atorvastatin affects not only the level of serum cholesterol but has other non-lipid effects as well, including effects on bone tissue. Our findings in male albino Wistar rats indicate that an 8-week administration of atorvastatin (0.3 mg/100 g BW) results in a reduction of bone turnover by suppression of osteoblastic activity. Increased expression of BMP-2 was probably a reaction to the inhibition of osteoblasts with atorvastatin. We presume that the effect of atorvastatin on bone should be mainly positive – mediated not only by the treatment of hypercholesterolemia, but also by any significant changes in BMD and biomechanical characteristics. Further studies are required to confirm our results.

4. Experimental

4.1. Animals

The study was carried out according to experimental protocol approved by the Animal Welfare Committee of the Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic (File Nr. 2709/2009–30). Adult male albino Wistar rats (Biotest Ltd., Konarovice, Czech Republic) were used with body weight 240 ± 10 g at the beginning of the experiment. The rats were located in the vivarium of the Medical Faculty of Charles University, Hradec Kralove, Czech Republic, 4 in each plastic cage, and bred in standard conditions (12 h light and 12 h dark, temperature 22 ± 2 °C, air humidity 30–70%). They were fed a standard lab diet (ST-1, VELAS Ltd., Lysa nad Labem, Czech Republic), with drinking water *ad libitum*. The rats were weighed every day to adjust the drug dose to their body weight.

4.2. Drugs

We applied the hypolipidemic drug atorvastatin (Atorvastatin-ratiopharm; Ratiopharm GmbH, Ulm, Germany) for this experiment. *Aqua pro injectione* (sterile water for injection„ FRESENIUS"; Fresenius Kabi Italia Ltd, Verona, Italy) was used as solvent and placebo. Midazolam (MIDAZOLAM TORREX 5MG/ML; Torrex Chiesi Pharma GMBH, Vienna, Austria) and ketamine (NARKAMON SPOFA 1%; SPOFA a.s. Prague, Czech Republic) were used for analgosedation.

4.3. Experimental design

The rats were randomly divided into 2 groups of 8 animals each. The control group (CO) was given *aqua pro injectione* at a dose 0.2 mL/100 g body weight of rat (BW). The experimental group received atorvastatin (AT; 0.3 mg in 0.2 mL *aqua pro inj.*/100 g BW). The drug and placebo were each applied orally by gavage daily in the morning for 8 weeks.

4.4. Bone mineral density measurements

After 8 weeks the bone mineral density (BMD; g/cm^2) was measured in rats during analgosedation (5 mg midazolam/kg BW and 100 mg ketamine/kg BW; i.p.) in the Osteocentre of the University Hospital in Hradec Kralove, Czech Republic. BMD was measured by means of dual-energy X-ray absorptiometry (DXA) on a Hologic Delphi A device. Before measurements, a tissue calibration scan was performed with the Hologic phantom for the small animal. BMD was evaluated in lumbar and caudal vertebrae and in femoral areas (Fig. 3) by an appropriate PC program (DXA; QDR-4500A Elite; Hologic, Waltham, MA, USA).

After sacrifice of the animals, both femurs were collected immediately, carefully cleaned of adhering soft tissue and the left femurs were scanned by DXA on the Hologic Delphi A. For scanning, the femurs were placed on saline-moistened gauze and oriented such that the scanner beam passed from anterior to posterior. The BMD of the femur was measured on the scan field by use of computer software (DXA; QDR-4500A Elite; Hologic, Waltham, MA, USA). The areal BMD was determined from four regions of interest: the entire femur, proximal femur (R1), femoral shaft (R3) and distal femur (R2) (Fig. 4).

4.5. Serum analysis

The animals were sacrificed by blood collection from the bifurcation of the abdominal aorta in general ether anesthesia. Blood samples (about 10 mL) were allowed to clot, and centrifuged at 3500 rpm for 10 min at 4 °C. Serum was removed and stored at − 80 °C until analysis. Serum levels of C-terminal crosslinking telopeptide of type I collagen (CTX-I) were measured using a commercially available enzyme immunoassay (EIA) kit (RatLaps^{TM} EIA, Immunodiagnostic Systems Ltd., UK) according to the manufacturerĭs instructions. The detection limit of this assay was 2.0 ng/mL. Total serum osteocalcin (OC) levels were measured with a commercially available EIA kit (Rat-MID^{TM} Osteocalcin EIA, Immunodiagnostic Systems Ltd., UK). The detection limit of this assay was 50.0 ng/mL. Serum concentration of procollagen type I N propeptide (PINP) was determined by EIA kit (Rat/Mouse PINP EIA, Immunodiagnostic Systems Ltd., UK). The sensitivity of the method was 0.7 ng/mL. Bone ALP was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Rat bone alkaline phosphatase, Wuhan Uscn Sciences Co., Ltd, China). The sensitivity of the method was 1.6 U/L.

4.6. Biomechanical testing procedure

The femurs were wrapped in saline-moistened gauze and stored at −80 °C. The mechanical properties of the femurs were performed and measured using a special electromechanical custom-made testing machine (Martin Kosek & Pavel Trnecka, Hradec Kralove, Czech Republic). A few hours before biomechanical testing, the femurs were thawed at room temperature and continuously moistened with isotonic saline solution. All specimens were tested at room temperature.

The femurs were subjected to the three-point bending test. Before performing the test, the diameter of the midshaft of the femurs and the length from the top of the femur head to the distal point of the medial condyle were measured with a sliding micrometer (OXFORD 0–25MM 30DEG POINTED MICROMETER, Victoria Works, Leicester, England). Their midpoints were marked with a waterproof marking pen. The femur was placed on a holding device with the two support points located at a distance 18 mm apart. A small stabilizing preload to 10 N was applied at the medial surface of the diaphysis. A constant deformation rate of 6 mm/min (Turner and Burr 1993) until maximal load failure and bending stiffness were recorded. The three-point bending test was carried out at midfemur in the anteroposterior direction. Breaking strength (maximum load, N) was recorded.

The proximal femurs were used for a compression test of the femoral neck. The distal end of the broken femur was potted using self-curing adhesive methacrylate resin (Spofacryl-SpofaDental Ltd.; Jicin, Czech Republic) into custom-made casting containers, and was so fixated. A vertical load was applied by a cylinder to the top of the femoral head. A small stabilizing preload to 10 N was applied and advanced at a constant speed of 6 mm/min until fracture of the femoral neck. Breaking strength (maximum load, N) was determined. All bones were analyzed by the same operator.

4.7. Protein extraction, SDS-PAGE, and Western blotting

After animal sacrifice, both tibiae were excised to remove skin, muscles and tendons, and frozen at –80 °C until used. Bone segments of the proximal region were obtained and minced in a solution consisting of RIPA buffer (50 mM Tris-HCl, pH 8.0, with sodium chloride, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS), and protease inhibitors (aprotinin 2 μg/mL, leupeptin 0.5 μg/mL, and benzamidine 50 μg/mL). The resulting slurry was disrupted and homogenized with a MagNA Lyser instrument (Roche Applied Science, Germany) during three cycles at 6500 rpm for 20 s with intermediate cooling of 5 min in the MagNA Lyser Cooling Block. The tissue homogenate was centrifuged at 10,000 G at 4 °C for 10 min. The supernatant was withdrawn and stored at −80 °C. The samples were pre-

pared as a pool. A pool was created by mixing 25 μg proteins of sample from the 8 rats.

The samples containing 200 μg of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide gel. After electrophoresis (200 V; 70 min), proteins were transferred (100 V; 120 min) to polyvinylidene difluoride (PVDF) membrane (BioRad, Hercules, CA, USA). The membrane was blocked for 1 h with 5% non-fat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBST). The membrane was incubated with primary antibody anti-BMP-2 at 1:250 (Abcam, Cambridge, UK) overnight at 4 °C, followed by incubation with a secondary peroxidase-conjugated antibody at 1:1000 (Dako, High Wycombe, UK) for 1 hr at room temperature. After washing, the immunoreactive bands were visualized using a chemiluminescence (ECL) detection kit (Roche, Boehringer Mannheim, Deutschland) by exposure onto X-ray film (Foma, Hradec Kralove, Czech Republic). Equal loading of proteins onto the gel was confirmed by immunodetection of beta-actin.

4.8. Statistical analysis

The data obtained from the experimental group were compared to that from the control group. The collected data were subjected to statistical analysis using the NCSS 2007 software package (Number Cruncher Statistical System, Kaysville, Utah, USA). Differences in concentrations of bone markers between groups were evaluated by Kolmogorov-Smirnov test. Comparisons of BMD measurements in different groups were performed using unpaired t-test. Data of biomechanical testing were analyzed by unpaired t-test, Mann-Whitney test and Kolmogorov-Smirnov test. For BMD results are expressed as mean ± standard deviation (SD), and in PINP, CTX-I, total OC, bone ALP, three-point bending test, and compression test of the femoral neck as median and 25th and 75th percentiles. The value $p < 0.05$ was considered as statistically significant.

Acknowledgements: This study was supported principally by Research Project MZO 00179906, by a grant of the Medical Faculty of Charles University, Hradec Kralove, Czech Republic and also by the grant SVV-2010–62051. We gratefully acknowledge the skilful technical assistance of Dagmar Jezkova, Katerina Sildbergerova and Lenka Mervartova. The authors are grateful to Ian McColl MD, PhD for assistance with the manuscript.

References

Binkley N (2006) Osteoporosis in men. Arg Bras Endocrinol Metabol 50: 764–774.

Brown JP, Albert C, Nassar BA, Adachi JD, Cole D, Davison KS, Dooley KC, Don-Wauchope A, Douville P, Hanley DA, Jamal SA, Josse R, Kaiser S, Krahn J, Krause R, Kremer R, Lepage R, Letendre E, Morin S, Ooi DS, Papaioaonnou A, Ste-Marie LG (2009) Bone turnover markers in the management of postmenopausal osteoporosis. Clin Biochem 42: 929–942.

Coxon FP, Rogers MJ (2003) The role of prenylated small GTP-binding proteins in the regulation of osteoclast function. Calcif Tissue Int 72: 80–84.

Garnero P, Vergnaud P, Hoyle N (2008) Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. Clin Chem 54: 188–196.

Garrett IR, Mundy GR (2002) The role of statins as potential targets for bone formation. Arthritis Res 4: 237–240.

Hatzigeorgiou C, Jackson JL (2005) Hydroxymethylglutaryl-coenzyme A reductase inhibitors and osteoporosis: a meta-analysis. Osteoporos Int 16: 990–998.

Herrmann M, Seibel MJ (2008) The amino- and carboxyterminal cross-linked telopeptides of collagen type I, NTX-I and CTX-I: a comparative review. Clin Chim Acta 393: 57–75.

Jadhav SB, Jain GK (2006) Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol 58: 3–18.

Lee AJ, Hodges S, Eastell R (2000) Measurement of osteocalcin. Ann Clin Biochem 37: 432–446.

Liao JK (2002) Beyond lipid lowering: the role of statins in vascular protection. Int J Cardiol 86: 5–18.

Lieberman JR, Daluiski A, Einhorn TA (2002) The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am 84-A: 1032–1044.

Maeda T, Kawane T, Horiuchi N (2003) Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. Endocrinology 144: 681–692.

Majima T, Komatsu Y, Fukao A, Ninomiya K, Matsumura T, Nakao K (2007) Short-term effects of atorvastatin on bone turnover in male patients with hypercholesterolemia. Endocr J 54: 145–151.

Majima T, Shimatsu A, Komatsu Y, Satoh N, Fukao A, Ninomiya K, Matsumura T, Nakao K (2008) Increased bone turnover in patients with hypercholesterolemia. Endocr J 55: 143–151.

Malhotra HS, Goa KL (2001) Atorvastatin: and updated review of its pharmacological properties and use in dyslipidaemia. Drugs 61: 1835–1881.

Maritz FJ, Conradie MM, Hulley PA, Gopal R, Hough S (2001) Effect of statins on bone mineral density and bone histomorphometry in rodents. Arterioscler Thromb Vasc Biol 21: 1636–1641.

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.

Parhami F, Garfinkel A, Demer LL (2000) Role of lipids in osteoporosis. Arterioscler Tromb Vasc Biol 20: 2346–2348.

Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Teriito M, Demer LL (1999) Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. J Bone Miner Res 14: 2067–2078.

Rejnmark L, Buus NH, Vestergaard P, Andreasen F, Larsen ML, Mosekilde L (2002) Statins decrease bone turnover in postmenopausal women: a cross-sectional study. Eur J Clin Invest 32: 581–589.

Rosenson RS, Tangney CC, Langman CB, Parker TS, Levine DM, Gordon BR (2005) Short-term reduction in bone markers with high-dose simvastatin. Osteoporos Int 16: 1272–1276.

Ruiz-Gaspa S, Nogues X, Enjuanes A, Monllau JC, Blanch J, Carreras R, Mellibovsky L, Grinberg D, Balcells S, Díez-Perez A, Pedro-Botet J (2007) Simvastatin and atorvastatin enhance gene expression of collagen type 1 and osteocalcin in primary human osteoblasts and MG-63 cultures. J Cell Biochem 101: 1430–1438.

Stern RH, Yang BB, Hounslow NJ, MacMahon M, Abel RB, Olson SC (2000) Pharmacodynamics and pharmacokinetic-pharmacodynamic relationships of atorvastatin, an HMG-CoA reductase inhibitor. J Clin Pharmacol 40: 616–623.

Tintut Y, Morony S, Demer LL (2004) Hyperlipidemia promotes osteoclastic potential of bone marrow cells ex vivo. Arterioscler Tromb Vasc Biol 24: 6–10.

Tintut Y, Parhami F, Tsingotjidou A, Tetradis S, Territo M, Demer LL (2002) 8-Isoprostaglandin E2 enhances receptor-activated NFkappa B ligand (RANKL)-dependent osteoclastic potential of marrow hematopoietic precursors via the cAMP pathway. J Biol Chem 277: 14221–14226.

Turner CH, Burr DB (1993) Basic biomechanical measurements of bone: a tutorial. Bone 14: 595–608.

Uyar Y, Baytur Y, Inceboz U, Demir BC, Gumuser G, Ozbilgin K (2009) Comparative effects of risedronate, atorvastatin, estrogen and SERMs on bone mass and strength in ovariectomized rats. Maturitas 63: 261 – 267.

Viereck V, Gründker C, Blaschke S, Frosch KH, Schoppet M, Emons G, Hofbauer LC (2005) Atorvastatin stimulates the production of osteoprotegerin by human osteoblasts. J Cell Biochem 96: 1244–1253.

Yavuz B, Ertugrul DT, Cil H, Ata N, Akin KO, Yalcin AA, Kucukazman M, Dal K, Hokkaomeroglu MS, Yavuz BB, Tutal E (2009) Increased levels of 25 hydroxyvitamin D and 1,25-dihydroxyvitamin D after rosuvastatin treatment: a novel pleiotropic effect of statins? Cardiovasc Drugs Ther 23: 295–299.