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Effect of Statin Therapy Duration on Bone Turnover Markers in Dyslipidemic Patients

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Abstract

Background and Purpose: *Statins are cholesterol-lowering drugs decreasing bone resorption by inhibition of the farnesyl diphosphate synthase step in the mevalonic acid pathway and therefore are believed to have beneficial effects on bone status. The objective was to examine the relationship between statin therapy duration and bone turnover markers in dyslipidemic patients.*

Patients and Methods: *Two hundred and eighty subjects were divided into five groups depending on duration of statin therapy: (controls 0 yrs); (0.1-1.5 yrs); (2-5 yrs); (6-10 yrs); (11-30 yrs). ELISA method was applied on fasting serums using bone formation markers: Osteoprotegerin (pmol/l) and Osteocalcin (ng/ml) and bone resorption markers: sRANKL (pmol/l) and CrossLaps (ng/ml). In statistical analysis, multiple regressions were used.*

Results: *A common influence of studied predictor variables was statistically significant for sRANKL, Serum CrossLaps and osteocalcin (P<0.001), while statistical significance was not found for osteoprotegerin. The largest shares of contributions were recorded in Model 2 for the statin group (40%) and BMI (36%) and in Model 1 for statin group (35%) and total cholesterol (28%).*

Conclusions: *Statins showed favorable influence on osteocalcin and sRANKL, indicating improved bone metabolism in patients with longer duration of statin therapy.*

INTRODUCTION

Dyslipidemia and osteoporosis are two clinically important medical issues with high prevalence in modern society. Nowadays, cholesterol is associated with atherosclerosis and to all its consequences that every year affect a large number of victims around the world. Osteoporosis is also a very common disease of modern times whose prevalence increases due to lifestyle and aging of population. In the course of cholesterol synthesis, statins inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) of the mevalonate pathway, which significantly reduces the cardiovascular risk. It was found, however, that statins have other positive - pleiotropic effects. Thus, statins lower platelet aggregation, promote angiogenesis, reduce the production of beta amyloid peptide which is associated with Alzheimer's disease, suppress the activation of T cells *(1, 2, 3)*. A positive effect on bone was also reported. Increased formation of bone tissue seems to depend on inhibition of the synthesis

of isoprenoid mediators of the mevalonate pathway which are important for posttranslational modification of certain proteins, such as Ras and Rho proteins *(4)*. In 1999, Mundy reported in *vivo* impact of statins that increased bone mass of mice's skulls *(5)*. It was recently discovered that statins stimulate the expression of anabolic factors such as bone morphogenetic protein 2 (BMP-2) and vascular endothelial growth factor (VEGF) *(6)*. It is believed that statins activate the expression of BMP-2 in osteoblasts, as confirmed by deactivating expression of BMP-2 after administration of mevalonate metabolites of HMG-CoA reductase. The latter effect of the BMP-2 was found only with lipophilic statins *(5)*. Some statins have also been described to regulate the function of osteoblasts by expressing bone sialoprotein (BSP), osteocalcin, and type I collagen *(7)*. Statins enhance the differentiation of osteoblasts from stromal cells *(8)*. The migration of osteoblasts plays an important role in the healing of fractures as well as during bone modeling and remodeling. It is stimulated by many growth factors, including platelet derived growth factor (PDGF).

By reducing isoprenilation of the signaling molecules in mevalonate pathway (Rac-Akt), statins stimulate PDGF and thereby the healing of the bone *(9)*. Clinical studies confirmed the positive effect of statins on bone mass density and bone remodeling *(8, 9, 10)*. In a clinical study conducted by Montagnani et al. bone mineral density, as the gold standard for diagnosis of osteoporosis, and the concentration of bone remodeling markers were assessed in patients using statins. Statins exhibit the best effect on bone remodeling after 4 to 12 weeks of treatment when a slight reduction in bone resorption markers was described *(10)*. However, some other studies, such as the LIPID (Long Term Intervention in Ischemic Disease) and Simvastatin Survival Study, did not show such changes in bone remodeling *(11)*. Some statins inhibit differentiation of the bone marrow mesenchymal cells in fatty cells and thus direct pluripotent cells to differentiate into osteoblasts *(12, 13)*. Statins also suppress the expression of adipocyte-specific genes PPR-gamma-2 and adipocyte specific protein P2 and their maturation *(13)*. The latter fact is important since the loss of bone mass was related to their increased accumulation in the bone, and also to an increased percentage of fat in the body *(14, 15, 16)*.

Positive effect of statins has not been confirmed in the prevention or treatment of osteoporosis. Researchers suspect that the cause can be found in the following: the application of too low doses of statins, differences in the chemical structure of statins, and or their metabolism in the liver. Thus, some researchers have tried to replace oral preparations with transdermal or topical preparations *(17)*. Local application of gelatin sponges rich in statin helped the healing of mandibular defect in rats *(18)*. As documented in the recent scientific literature, the key factors in the regulation of bone turnover, i.e. the receptor activator of nuclear factor kappa B ligand (RANKL) and its receptor RANK, are essential for the development, activation and apoptosis of osteoclasts *(19)*. Osteoprotegerin (OPG) or RANKL decoy receptor is a natural antagonist of RANKL and it is produced by osteoblasts and endothelial cells. Recent studies suggest that statins could inhibit osteoclast activity indirectly by activating osteoblasts that produce osteoprotegerin, *(20, 21)*. So far no study was conducted to examine the relationship between statins and RANK/RANKL/OPG system. The aim of this study was to correlate the duration of statin treatment with bone turnover markers in patients with dyslipidemia.

PATIENTS AND METHODS

Patients

This study included women with dyslipidemia who were treated with statins (N = 200). The control group consists of women (N = 80) who first presented for specialist examination because of suspected dyslipidemia. Considering the period of treatment with statins, respondents were divided into four subgroups: Subgroup 1 consists of control; subgroup 2 consists of patients who were treated with statins between 0.1 and 1.5 years; subgroup 3 consists of patients who were treated with statins between 2 and 5 years; subgroup 4 consists of patients who were treated with statins between 6 and 10 years, and subgroup 5 consists of patients who were treated with statins between 11 and 30 years. Along with antilipemic, some of the the subjects received antiresorptive therapy for osteoporosis. Therefore, respondents were labeled with 0 - not receiving treatment for osteoporosis/dyslipidemia and 1 - receiving therapy for osteoporosis/dyslipidemia.

Methods

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed according to manufacturer's protocol. Concentrations of the following bone turnover markers: C-terminal telopeptide of type I collagen (Serum CrossLaps); osteocalcin; soluble receptor activator of NF-kappaB (sRANKL); osteoprotegerin (OPG) as well as parameters of lipid status (total cholesterol, high density lipoproteins-HDL, low-density lipoproteins-LDL and triglycerides) were determined in the sera collected from fasting subjects. Blood samples (8 ml) were centrifuged to obtain the sera (4 ml). Following manufacturer's instructions, the values of the bone turnover markers were expressed in appropriate units of measure: RANKL (pmol/l), OPG (pmol/l), osteocalcin (ng/ml) and serum CrossLaps (ng/mL), vitamin D (nmol/l) (Biomedica Gruppe, Vienna, Austria).

Anthropometry

Body weight was determined using portable electronic scales (Seca, Hamburg, Germany), with an accuracy of ± 0.1 kg. Body height was measured using the moving sta-

TABLE 1
Descriptive characteristics of respondents (N=280).

Anthropometry and age	Mean± SD	Minimum	Maksimum
Age (years)	65.82±6,4	50	81
Body weight (kg)	74.78±14,17	50	118
Body height (cm)	162.92±8,14	147	186
Body mass index, BMI (kg/m2)	27.59±4.18	18,5	40
Waist circumference (cm)	96.01±10.80	68	118
Hip circumference (cm)	103.43±9.36	81	130
Waist to hip ratio, WHR	0.9	0.7	1.1
Hemodynamic and lipid parameters			
Systolic blood pressure (mmHg)	132.75±19.23	90	185
Diastolic blood pressure (mmHg)	83.61±11.32	50	105
Pulse rate (/min)	73,86±11,86	52	122
Total triglycerides (mmol/l)	1.37±0.5	0,5	2.7
Cholesterol (mmol/l)	5.60±1.32	2.6	9.2
LDL (mmol/l)	3.53±1.1	1.5	5.8
HDL (mmol/l)	1.52	0.8	3
Bone remodeling parameters			
OPG (pmol/l)	3.23±1.33	0.43	7.26
sRANKL (pmol/l)	3.64±3.75	-0.35	19.12
Serum CrossLaps (ng/ml)	22.22±14.54	3.07	60.32
Osteokalcin (ng/ml)	14.26±10.77	4	45

LDL-low density lipoproteins; HD- high density lipoproteins; OPG-osteoprotegerin; sRANKL-soluble receptor activator of NFkappaB ligand; Serum CrossLaps- serum C-terminal telopeptide of type I collagen

diometer (Seca, Hamburg, Germany) with an accuracy of ± 0.5 cm. Body mass index, BMI (kg/m2) was calculated from the known values of the body height and body weight. Waist to hip ratio (WHR, cm) was measured from the known values of the waist and hip circumference.

Statistical analysis

Statistical analysis was performed using the software Statistica 8.1 (StatSoft). Descriptive statistics was used to display all the examined variables. Kolmogorov-Smirnov test was used to determine normality of distribution. Results were normally distributed and multiple regression analysis was performed. The results were considered statistically significant at $P < 0.05$.

Medical ethics

Medical data were collected in accordance with ethical and bioethical principles. Privacy (medical secret) and confidentiality were ensured for respondents of the survey. The study had been preceded by an informative meeting with the principal investigator and other co-workers who participated in the study. The subjects were personally acquainted with the importance, objectives and plan of research and voluntarily signed the informed consent to participate in the study.

RESULTS

Kolmogorov-Smirnov test showed normal distribution of respondents. Their age ranged from 50 to 81 years. The average age of respondents was 66 years. Most respondents ranged between 60 and 70 years.

Tables 2 – 4 show the results of multiple regression analysis with shares of contribution of independent predictors to criterion variables, i.e. bone turnover markers (Osteocalcin, Serum CrossLaps, sRANKL and Osteoprotegerin). Prediction variables in Model 1 are age (years),

TABLE 2

The share of contribution of predictor variables for sRANKL.

Model	R	R^2	F	P
1 (a)	0.70	0.50	3.17	0.000
2 (b)	0.71	0.50	3.74	0.000

Model		ß	SE?	r	P	Share of contribution (%)
1 (a)	Age (years)	0.33	0.11	0.37	0.005	12
	Statin group (1-5)	-0.78	0.22	-0.45	0.001	35
	WHR	0.54	0.22	0.31	0.021	17
	Total cholesterol (mmol/l)	-0.93	0.41	-0.30	0.030	28
	LDL (mmol/ml)	0.82	0.37	0.29	0.032	24
2 (b)	Age (years)	0.34	0.11	0.38	0.004	13
	Statin group (1-5)	-0.81	0.22	-0.55	0.000	45
	WHR	0.55	0.22	0.32	0.016	18
	Total cholesterol (mmol/l)	-0.94	0.41	-0.30	0.023	28
	LDL (mmol/l)	0.86	0.37	0.16	0.022	14

sRANKL (soluble receptor activator of NFkappaB ligand); β - regression coefficient; SEβ- standard error of β coefficient; r-correlation coefficient; p <0.05

TABLE 3

The share of contribution of predictor variables for Serum CrossLaps.

Model	R	R^2	F	P
1 (a)	0.55	0.31	3.17	0.03
2 (b)	0.60	0.36	3.74	0.04

Model		ß	SE?	r	P	Share of contribution (%)
1 (a)	Body mass index (kg/m^2)	-1,1	0,50	-0,30	0,031	33
2 (b)	Body mass index (kg/m^2)	-1,2	0,50	-0,32	0,016	38
	Therapy for osteoporosis (yes/no)	-0,3	0,14	-0,55	0,043	17

Serum CrossLaps (C-terminal telopeptide of type I collagen); β - regression coefficient; SEβ- standard error of β coefficient; r-correlation coefficient; p <0.05

statin group *(1-5),* statin therapy (yes/no), body height (cm), body weight (kg), body mass index (kg/m^2), waist circumference (cm), hip circumference (cm), waist to hip ratio (WHR), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol (mmol/l), high density lipoproteins (HDL, mmol/L), low-density lipoproteins (LDL, mmol/l), triglycerides (mmol/ l), pulse rate (/min). In Model 2, prediction variables are the same as in Model 1, with inclusion of the variable therapy for osteoporosis (yes/no). If β is negative, relationship is inversely proportional, while positive β indicates proportional relationship. Osteoprotegerin was not significantly predicted by Model 1 or 2 and therefore is not shown.

DISCUSSION

In this investigation, it was determined that average waist circumference in subjects was 96 cm and the average

TABLE 4
The share of contribution of predictor variables for Osteocalcin.

Model	R	R^2	F	P
1 (a)	0.96	0.92	41.03	0.000
2 (b)	0.96	0.93	40.39	0.000

Model		ß	SE?	r	P	Share of contribution (%)
1 (a)	Statin groups (1-5)	1.24	0.08	0.89	0.000	10
	Statin therapy (yes/no)	-0.32	0.09	-0.45	0.001	14
	Systolic blood pressure (mmHg)	0.15	0.07	0.30	0.025	5
	Diastolic blood pressure (mmHg)	-0.19	0.06	-0.30	0.005	6
2 (b)	Statin groups (1-5)	1.23	0.08	0.90	0.000	10
	Statin therapy (yes/no)	-0.32	1.85	-0.57	0.000	18
	Systolic blood pressure (mmHg)	0.15	0.06	0.31	0.021	5
	Diastolic blood pressure (mmHg)	-0.16	0.07	-0.32	0.018	5

β - regression coefficient; SEβ- standard error of β coefficient; r-correlation coefficient; p <0.05

of the waist to hip ratio was 0.9, which placed our respondents into the risk group for the development of metabolic syndrome. An average BMI in the subjects was around 28 kg/m^2, which is bordering on severe obesity (BMI > 30 kg/m^2). The association between body weight or BMI and bone mineral density at the spine or hip has already been established *(22)*. When it is not possible to make bone densitometry, BMI is an important diagnostic criterion in the diagnosis of osteoporosis. Protective effect of obesity on development of osteoporosis in women is explained by the specific role of adipose tissue as a source of estrogen *(22)*.

BMI is a good indicator of bone metabolism, as confirmed by our result of inversely proportional relationship between Serum CrossLaps and BMI (Table 3). Thus, higher values of BMI are associated with lower serum Serum CrossLaps, suggesting less resorption of bone.

Multiple regression analyses revealed the largest shares of contributions recorded in Model 2 for the statin group (40%) and BMI (36%) and in Model 1 for statin group (35%) and total cholesterol (28%).

Dyslipidemia and bone remodeling markers

Hyperlipidemia represents an increase in cholesterol and/or triglycerides in the blood, with a low value of high density lipoprotein (HDL), which leads to atherosclerosis. This disorder of metabolism of lipids is called dyslipidemia and is predicted to grow. Dyslipidemia is one of the most important risk factors for cardiovascular diseases which are the most common cause of death *(23, 24,25)*. When the scientific literature notifies about the relationship between dyslipidemia and bone metabolism, two links are emphasized: dyslipidemia is the basis for vascular calcifications as a result of advanced atherosclerosis of the blood vessels; oxidized low-density lipoproteins (LDL) inhibit differentiation of pre-osteoblasts into osteoblasts *(23, 24)*. The literature has described a positive correlation between the bone mineral density and the concentration of triglycerides, and a negative correlation between the bone mineral density and the concentration of high density lipoproteins (HDL) in postmenopausal women *(23, 24, 25, 26)*.

The results of this study showed that a common influence of studied predictor variables was statistically significant for sRANKL, Serum CrossLaps and osteocalcin (P<0.001), while statistical significance was not found for osteoprotegerin. The analysis of the influence of individual variables revealed a statistically significant and inversely proportional relationship between total cholesterol and sRANKL (Table 2). Furthermore, it was found that total cholesterol has equal share of contributions (28%) for sRANKL in statin-treated patients, as well as in patients who, along with statins, took drugs for osteoporosis. Not only do our results indirectly confirm the above mentioned research of positive correlation between blood lipids and bone status but generally it can be said that a rise in cholesterol concentration coincides with reduced values of sRANKL, suggesting a role of choles-

terol in bone remodeling. This could have an inhibitory effect on the function of osteoclasts, resulting in reduced bone degradation. Actually, sRANKL is a ligand that binds to the RANK receptor on osteoclasts, which activates osteoclast-mediated resorption of bone tissue *(20, 21).*

By increasing the concentration of triglycerides, the level of high density lipoproteins (HDL) lowers, and small dense particles called low-density lipoproteins (LDL) appear in circulation. LDL particles are atherogenic and considered to be the main risk factor for cardiovascular diseases and the leading cause of mortality in patients with diabetes mellitus *(29).* Mean LDL levels in our participants were greater than 3.4 mmol/l and as such are considered critical values with the risk associated to increased values (> 4.1 mmol/l).

We found statistically significant and exactly proportional relationship between LDL cholesterol and sRANKL, with a 24% share of contribution in patients taking lipid lowering drugs, and with 14% decrease in patients who took antiresorptive therapy along with antilipemic therapy. LDL particles are elevated in obese people, but their links with sRANKL and other inflammatory cytokines are increasingly discussed in medical literature, with special reference to the metabolic syndrome *(27, 28).*

Inflammatory cytokines are physiologically secreted by fatty tissue. This refers to cytokines that have endocrine, autocrine and paracrine effect, such as leptin, adiponectin, TNF-?, resistin, IL-6 and others. Adiponectin is the principal hormone of adipose tissue with positive effects on metabolism. Its secretion stimulates weight loss. The main effect of adiponectin is directed through the activation or inhibition of the nuclear transcription factor kappaB (NF-kappaB) *(28, 29)*. Along with adiponectin, fat cells in obesity release proinflammatory cytokines, such as TNF-? which activates NF-kappaB. Our result of a positive correlation between LDL and sRANKL is in line with these facts and further indicates the possibility of LDL to stimulate secretion of proinflammatory cytokines from fat cells, which could affect bone metabolism in terms of increased bone resorption. The finding of a lower share of contribution of LDL in patients with antiresorptive therapy indicates lower serum sRANKL in their blood, which may be associated with reduced bone loss. This fact indirectly indicates the good choice of therapy for osteoporosis.

Statins and osteoporosis

Statins are applied in primary prevention in patients with hypercholesterolemia that persists despite adequate dietary regime conducted, and in secondary prevention in patients with myocardial infarction or ischemic stroke. Administration of statins is recommended in diabetic patients with total cholesterol above 5 mmol/l. Statins lower serum LDL cholesterol by 30 to 50% by inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase in the liver and thus indirectly cause an increase in the number of LDL receptors in the liver cells, which leads to an increased removal of LDL cholesterol from the blood *(23-26)*. Besides anti-atherosclerotic effects, statins have additional effects on the bone, i.e. formation of osteoclasts is suppressed and activity of osteoblasts is increased. According to recent knowledge, statins may be useful in acute inflammatory syndrome leading to reduction of morbidity and mortality in sepsis syndrome *(23-26).*

The precise mechanisms by which statins act on the increased bone formation are explained by scientists who have studied the mechanisms of the action of bisphosphonates on bone metabolism. A focal point in the events provoked by these mechanisms is the synthesis of cholesterol. In the first step of this synthesis, statins inhibit the enzyme HMG-CoA reductase which converts HMG-CoA to mevalonate. In the second step, mevalonate is converted to geranyl pyrophosphate which is converted to farnesyl pyrophosphate by action of farnesyl pyrophosphate synthase. Bisphosphonates inhibit farnesyl pyrophosphate synthase *(23-26, 30, 31).* Osteoclasts use certain lipid products formed in the synthesis of cholesterol, and formation of these lipid products is inhibited by bisphosphonates and statins. Therefore, both of these medications lead to apoptosis of osteoclasts, which is reflected in the dynamics of bone turnover. Patients receiving both anti-lipidemic and anti-resorptive therapy have positive balance between bone resorption and formation, with reduced incidence of osteoporotic fractures *(23-26, 30, 31)*. The strongest effect on inhibiting cholesterol pathway was observed for alendronate and risedronate and lipophilic statins such as simvastatin and lovastatin *(10, 26).*

So far studies have determined positive correlation of statins with parameters of bone densitometry, but there are few investigations involving bone turnover markers. Results of some studies suggest that atorvastatin enhances the effect of bisphosphonates in osteoporosis, while other studies have identified a reduced level of serum alkaline phosphatase in patients taking risedronate and atorvastatin *(26).* Our results suggest that osteocalcin is positive predictor in patients treated with statins. Statin groups and osteocalcin are exactly proportional, with the share of contribution of 10% (Table 4).

Based on our results and consideration of favorable influence of statins on sRANKL, it can be concluded that bone metabolism is improved in patients with prolonged duration of statin therapy.

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