# The influence of prolactin on bone mineral density (BMD) and some biochemical markers of ovariectomized rats

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**ABSTRACT**: A model of postmenopausal osteoporosis was used in the study. 107 days after ovariectomy the animals were subcutaneously applied 1.0 IU PRL/kg BM or 1.0 IU calcitonin/kg BM for 7 days in single doses. The application of the preparations started again 80 days after the first application. After 194 days the animals were narcotized, blood was collected, and lumbar (L2–L4) and femoral bones were prepared. The material obtained was subjected to densitometric analysis of bone mineral density (BMD). In ovariectomized rats the loss of osseous tissue was observed only in the lumbar bone. The application of prolactin to ovariectomized rats caused an increase in the mineral density of the lumbar bone up to a BMD value equal to that of the control group which had not been subjected to ovariectomy (control healthy group – SHAM). Similar results were obtained in the case of calcitonin. An increase in osteocalcin concentration and activity of isoenzyme alkaline phosphatase (BAL), with a decrease in the activity of isoenzyme acid phosphatase (TRAP) was observed in experimental groups compared to control ones.

**Keywords**: prolactin (PRL); ovariectomy; experimental osteoporosis; bone mineral density (BMD); lumbar bone (L2–L4); femoral bone; female rats

Osteoporosis is a disease characterised by changes in the structure of osseous tissue and a decrease in bone mass. Currently it poses an important social problem. There are numerous research projects concerning not only different mechanisms of that disease formation but also possibilities for its early detection, prevention and successful treatment (Delaney, 2006; Kamel, 2007; Pinkerton and Dalkin, 2007; Briot et al., 2009; Compston et al., 2009).

For that purpose, various models, like the model of experimental postmenopausal osteoporosis in rats, are used. The study demonstrated that in ovariectomized rats, like in humans, there are changes in the structure of osseous tissue. These changes apply to the architecture of a bone, its mechanical strength, and also chemical composition (Peng et al., 1997; MengYoung et al., 2008). Thus, this is a model that allows for the analysis of numerous mechanisms connected with that disease. The influence of hormones like calcitonin or oestradiol on osseous tissue metabolism is currently known. However, as may be expected, other hormones also probably influence this metabolism directly or indirectly. Prolactin seems to be one of such hormones. It is a hormone of the pituitary gland. It exhibits species differences, however is not species specific (Dróżdż et al., 1998). Over 300 effects of its biological activity have been demonstrated in

various animal species (Dróżdż et al., 1998; Nicoll, 1980; Ryszka et al., 2002; Wang et al., 2007; Scotti et al., 2008). The study also demonstrated that it influences calcium management (Krishnamara et al., 1993, 1997; Krishnamara and Taweerathitan, 1995; Tanrattana et al., 2004).

The aim of the present study was to determine the influence of prolactin applied in two 7-day cycles to rats with experimentally induced osteoporosis on the mineral density of lumbar (L2–L4) and femoral bones, and some biochemical indices. Such a model of periodical application was chosen in order to assess if the time-limited prolactin application may influence a decrease in the osseous tissue loss and some biochemical markers. Calcitonin was also given to animals for comparative purposes.

## MATERIAL AND METHODS

**Animals**: Forty female rats of the Wistar strain with the initial body mass at a level of 200–210 g were used in the research. The animals came from a breeding plant of the Central Experimental Animal House at the Medical University of Silesia in Katowice. They were treated in a humane manner and kept in conventional conditions with free access to water and feed (all-mash mixture Murigran). The experiment was conducted with the agreement of the Bioethics Commission of the Medical University of Silesia in Katowice.

**Analysed preparations**: The following hormonal preparations were used in the study:

(1) Biolactin – the preparation containing 100 IU of pig prolactin in lyophilizated form soluble ex temporae; produced by: F.Z.N.P. Biochefa, Polska;
(2) Miacalcic – the preparation containing 100 IU of calcitonin in 1 ml of solution; produced by Novartis, USA.

**Experimental osteoporosis model (ovariectomy)**: 30 female rats were subjected to a treatment of bilateral ovariectomy in halothane narcosis. Ovaries were removed after the previous ligature of uterine horns with vessels (Kalu, 1991).

**Course of the experiment**: Hormonal preparations were applied starting from 107 days after the ovariectomy, after an identification of changes in the density of lumbar bone in OVX females (on the basis of the analysis conducted earlier). Animals were divided into 4 groups, 10 individuals in each:

Control groups:

(1) Females with intact ovaries (SHAM);
(2) Females with removed ovaries (OVX).

Females from both control groups were given 0.9% solution of NaCI.

Experimental groups:

(3) Females with removed ovaries, given prolactin in a dose of 1.0 IU/kg BM (OVX + PRL);
(4) Females with removed ovaries, given calcitonin in a dose of 1.0 IU/kg BM (OVX + calcitonin).

Hormonal preparations and 0.9% solution of NaCI were applied subcutaneously, once a day for 7 days. The volume of a single injection was 0.5 $cm^3$. Body mass of animals was systematically controlled during the study in order to update the doses. After 80 days from the first injection, females were again given the preparations in the same doses according to the same schedule.

After the application of preparations ended, animals were subjected to euthanasia by inhalatory overdosing of halothane and then examined. Blood, femoral and lumbar bones were collected from animals during the examination. Blood collected for a clot was centrifugated (15 minutes per 3 000 rotations) after 3 hours. The serum obtained was stored at a temperature of –20°C until analysis. Bones were cleaned of surrounding soft tissues and connective ones.

**Densitometric analysis**: the assessment of mineral density (BMD) of lumbar (L2–L4) and femoral bones was conducted using a DPX Lunar densitometer, applying a programme for small animals (Small Animals) with appendicular scan option for bones prepared from a body.

Bones for scanning were placed each time in the same position in a plastic box with a smooth, even bottom. A sack filled with rice of the thickness of 3 mm was placed inside the box, (as a simulation and equalization of the amount of soft tissues surrounding bones). Total mineral density of bones (BMD) in g/$cm^2$ was determined in the study.

In order to determine the precision and accuracy of measurements, bones of 2 animals from each group were subjected to densitometric analysis five times, and then the coefficient of variation (*CV*)

was calculated. The mean calculated coefficient of variation (*CV*) was 0.52 ± 0.14% (0.27–0.71) for femoral bones, and 0.65 ± 0.27% (0.3–0.97) for lumbar ones.

All densitometric measurements were done during one day.

### Biochemical study

Spectrophotometric methods, previously validated, were used for quantitative determination of analysed biochemical indices (Int. Conf. on Harmonization).

The following analyses were conducted:

(1) Osteocalcin concentration – using ELIS's method with DAKO Osteokalcin Elis Kit, Denmark. The kit is designed for the determination of osteocalcin concentration in rat serum.
(2) Activity of the bone isoenzyme alkaline phosphatase (BALP) – using a kinetic method with ALP-MPR-3 and ALP-Bone kits of Boehringer Mannheim Company.
(3) Activity of the bone isoenzyme acid phosphatase (TRAP) – using a kinetic method with Enzyline Phosphatase acide optimisé 10 kit of bioMérieux Company.

Mathematical calculations. The results obtained were presented as means, and standard deviations were calculated (SD). Statistical evaluation of the results was conducted on a significance level of $P < 0.05$.

## RESULTS

### Densitometric analysis

BMD values (g/cm$^2$) of lumbar and femoral bones in the particular groups are presented in Table 1.

Differences in bone mineral density between the particular groups were observed only in lumbar bones. After ovariectomy, the mineral density of lumbar bones (group OVX) decreased by 22.5% and was statistically significantly lower ($P < 0.05$) when compared to the control group SHAM. The application of prolactin caused an increase in the lumbar bone density by 28.5% compared to the control group OVX ($P < 0.05$). The density of lumbar bones in the same group of female rats (OVX + PRL) was comparable to BMD value observed in the control group (SHAM). Supplementation of calcitonin results in a smaller effect. The density of lumbar bones in the analysed group (OVX + calcitonin) increased by 16.4% compared to the control group OVX ($P < 0.05$).

Some significant changes in femoral bone density between groups were observed. They may result from the greater motility of this bone.

### Biochemical analysis

Results of the analysis of chosen parameters of osseous tissue synthesis, i.e. osteocalcin concentration, activity of the bone isoenzyme alkaline phosphatase (BALP) and its degradation – activity of the bone isoenzyme acid phosphatase (TRAP) are presented in Table 2.

Ovariectomy resulted in a decrease in the values of parameters characterizing the synthesis of osseous tissue, i.e. osteocalcin concentration by 51%, and activity of the bone isoenzyme alkaline phosphatase (BALP) by 48.9% in the control group of female rats (OXV) compared to the SHAM control group ($P < 0.05$). In turn, in OXV control group the activity of the bone isoenzyme acid phosphatase (TRAP) determining the degradation of osseous tissue increased by 48.1% compared to the SHAM

Table 1. Density of lumbar and femoral bones – BMD

| L.P. | Group | Bone density | | | |
|---|---|---|---|---|---|
| | | lumbar | | femoral | |
| | | (g/cm$^2$) | (%) | (g/cm$^2$) | (%) |
| (1) | SHAM | 0.213 ± 0.010 | 100.0 | 0.239 ± 0.007 | 100.0 |
| (2) | OVX | 0.165 ± 0.014 | 77.5 | 0.236 ± 0.010 | 98.7 |
| (3) | OVX + PRL | 0.212 ± 0.028 | 99.5 | 0.239 ± 0.014 | 100.0 |
| (4) | OVX + calcitonin | 0.192 ± 0.012 | 90.1 | 0.234 ± 0.007 | 97.9 |

Table 2. The influence of ovariectomy and hormonal therapy on biochemical parameters of blood serum in female rats

| L.P. | Group | Osteocalcin concentration | | Activity | | | |
|---|---|---|---|---|---|---|---|
| | | | | BALP | | TRAP | |
| | | (μg/g) | (%) | (IU/l) | (%) | (IU/l) | (%) |
| (1) | SHAM | 3.98 ± 0.97 | | 122.25 ± 19.95 | | 11.82 ± 1.66 | |
| (2) | OVX | 1.95 ± 0.25 | 100.0 | 62.53 ± 27.60 | 100.0 | 17.51 ± 3.80 | 100.0 |
| (3) | OVX + PRL | 4.52 ± 1.35 | 231.8 | 101.03 ± 23.61 | 161.6 | 8.75 ± 3.35 | 50.0 |
| (4) | OVX + calcitonin | 4.93 ± 1.46 | 252.8 | 113.58 ± 17.83 | 181.6 | 8.16 ± 2.19 | 46.6 |

group ($P < 0.05$). The supplementation of prolactin and calcitonin to female rats caused a significant increase in the osteocalcin concentration and activity of the isoenzyme (BALP) in the blood serum of analysed animals compared to the control group OVX ($P < 0.05$). However, a decrease in TRAP activity by about 50% was observed in both analysed groups (OVX + PRL and OVX + calcitonin) as compared to OVX control group and it might suggest an inhibition of the degradation process of osseous tissue ($P < 0.05$).

## DISCUSSION

Calcitonin and oestradiol belong to the most efficient remedies in osteoporosis treatment. However, research is still conducted for new medicines that efficiently prevent an excessive loss of bone mass. The studies of mechanisms causing the occurrence of this disease also continue. These studies are conducted multidirectionally. It seems that one of the factors that may be connected with an excessive loss of osseous tissue is prolactin (PRL). It was demonstrated that in young women with hyperprolactinaemia the mineral density of bones (BMD) was at a level observed in postmenopausal women (Adler et al., 1998). Also, in the study concerning the results of chronic hyperprolactinaemia conducted on rats, it was proved that the long-term high concentration of prolactin causes losses of the osseous tissue. However, as was demonstrated by the authors, the main factor of bone mass loss is rather the deficiency of oestrogens induced by an excess of prolactin that increases the amount of this hormone (Roux et al., 1996). In another study at the same time, a positive influence of prolactin on calcium absorption from intestines was demonstrated. In a study conducted on mature female rats that were given PRL at a dose of 0.02 mg/100 g BM 1 hour before the experiment, an increased concentration of $^{45}Ca$ in serum was noted (Krishnamara et al., 1997). Similar results were obtained in a study on the isolated small intestine collected from female rats of different age. The study demonstrated that prolactin influences the active transport of calcium from duodenum only in sexually mature females. The stimulating activity of prolactin is probably connected with the activation of dihydroxycholecalciferol production in kidneys, however, the influence of other factors is not excluded (Mortensen et al., 1993; Krishnamara and Taweerathian, 1995). A study conducted on the bone turnover of calcium in rats demonstrated that prolactin probably takes part in the bone metabolism. Within 1 hour from the prolactin application in a dose of 0.02 mg/100 g BM the authors noted a significantly higher content of $^{45}Ca$ in analysed bones (especially in femoral, peroneal bone) compared to the group that was not given this hormone. Such an effect was observed when PRL was given 30 minutes before the application of labelled calcium. With simultaneous application of prolactin and calcium, a decrease in $^{45}Ca$ content in analysed bones was observed compared to the control group. It was also noted that an increase in the content of labelled calcium in bones depends on the dose of the applied hormone (Krishnamara et al., 1997). The model worked out by Kalu (1991) was used in the study. After about 100 days since the treatment, a decrease in the density of lumbar bones was observed in animals that were subjected to ovariectomy. Similar changes were noted in femoral bones. It is probable that the absence of changes in the these bones is connected with the young age of animals used in the study, and their considerably higher mobility. Roux

et al. (1996) studying the density of the tailbone of female rats of SPD strain with a body mass of about 200 g at the beginning of the experiment (OVX model) reported no differences in the density of the analysed segment between the group with intact ovaries (SHAM) and the group with removed ovaries (OVX). At the same time the authors conducting another experiment observed considerable changes in the same segment, while the study was conducted on older females (OVX model).

Compared to the control group OVX an increase in the density of lumbar bones of animals that were given the two hormones was observed in the present study. It may prove the positive influence of PRL on the bone metabolism. The results of biochemical markers of the bone turnover may be a confirmation of this finding. We observed a distinct increase in osteocalcin concentration and BALP activity, i.e. markers of the osseous tissue synthesis, and a decrease in TRAP – a marker of the osseous tissue degradation, activity in the serum of animals. It may be supposed that the periodical application of exogenous prolactin in small doses, in short series, may considerably improve the bone turnover of calcium. Comparing the results obtained from densitometric and biochemical analyses, there are distinct differences with the application of PRL and calcitonin to ovariectomized female rats. We observed a greater influence of PRL on the density of lumbar bones than on biochemical indices of the blood serum. In the case of calcitonin a reverse relationship was observed. It might be connected with different mechanism of the activity both hormones. Analysing the distribution of labelled $^{125}$JPRL and $^{125}$J calcitonin we observed clearly marked capture of PRL by the osseous tissue (Ryszka et al., 1995, 2002). In the case of calcitonin we did not observe that capture. We noted, however, an increase in its concentration in blood. The biological availability of calcitonin increased almost 10 times in female rats with osteoporosis compared to the control group (Ryszka et al., 1995). It seems that the applied model of hormonal therapy allows us to avoid problems connected with possible hyperprolactinaemia (with long-term application of medicines), with all the negative results caused by such a state. It is probable that in future, after the mechanisms of the influence of prolactin on the bone turnover were explained precisely, this hormone may also find an application in osteoporosis treatment equally with oestradiol.

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