



## Bone turnover markers in medicamentous and physiological hyperprolactinemia in female rats

### Markeri koštanog metabolizma u medikamentnoj i fiziološkoj hiperprolaktinemiji kod ženki pacova

Danijela Radojković\*, Milica Pešić\*, Tatjana Ristić†

\*Clinic of Endocrinology, †Center for Medical Biochemistry, Clinic Center Niš, Niš, Serbia

#### Abstract

**Background/Aim.** There is a lack of data on the effects of prolactin on calcium metabolism and bone turnover in hyperprolactinemia of various origins. The aim of this study was to compare the influence of medicamentous and physiological hyperprolactinemia on bone turnover in female rats. **Methods.** Experimental animals (18 weeks old, Wistar female rats) were divided as follows: the group P – 9 rats, 3 weeks pregnant; the group M3 – 10 rats that were intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks, the group M6 – 10 rats that were intramuscularly administrated with sulpirid (10 mg/kg) twice daily for 6 weeks, and age matched nulliparous rats as the control group: 10 rats, 18-week-old (C1) and 7 rats, 24 weeks old (C2). Laboratory investigations included serum ionized calcium and phosphorus, urinary calcium and phosphorous excretion, osteocalcin and serum procollagen type 1 N-terminal propeptide (P1NP). **Results.** Experimental animals in the group P compared to the control group, displayed lower mean serum ionized calcium ( $0.5 \pm 0.2$  vs  $1.12 \pm 0.04$  mmol/L;  $p < 0.001$ ); higher mean serum phosphorus ( $2.42 \pm 0.46$  vs  $2.05 \pm 0.2$  mmol/L;

$p < 0.05$ ); increased urinary calcium ( $3.90 \pm 0.46$  vs  $3.05 \pm 0.58$ ;  $p < 0.01$ ) and significantly increased P1NP ( $489,22 \pm 46,77$  vs  $361,9 \pm 53,01$  pg/mL;  $p < 0.001$ ). Experimental animals in the group M3 had significantly decreased P1NP, compared to the control group. Prolongated medicamentous hyperprolactinemia (the group M6) induced increased serum ionized calcium ( $1.21 \pm 0.03$  vs  $1.15 \pm 0.02$  mmol/L;  $p < 0.001$ ); decreased serum phosphorus ( $1.70 \pm 0.13$  vs  $1.89 \pm 0.32$  mmol/L;  $p < 0.001$ ); decreased osteocalcin and P1NP. **Conclusions.** Physiological hyperprolactinemia does not have such harmful effect on bone metabolism as medicamentous hyperprolactinemia. Chronic medicamentous hyperprolactinemia produces lower serum levels of bone formation markers. Assessment of bone turnover markers in prolonged medicamentous hyperprolactinemia provides an opportunity for earlier diagnosis of bone metabolism disturbances and should be considered as mandatory.

#### Key words:

hyperprolactinemia; pregnancy; sulpiride; rats; osteogenesis; biological markers; calcium; phosphorus; osteocalcin.

#### Apstrakt

**Uvod/Cilj.** Nema dovoljno podataka o efektima prolaktina na metabolizam kalcijuma i koštani promet kod hiperprolaktinemije različitog porekla. Cilj ovog rada bio je da uporedi uticaj medikamentne i fiziološke hiperprolaktinemije na koštani metabolizam kod ženki pacova. **Metode.** Eksperimentalne životinje (ženke pacova soja Wistar, stare 18 nedelja) podeljene su na sledeće grupe: grupa P – devet pacova u trećoj nedelji trudnoće; grupa M3 – 10 pacova, kojima je tokom tri nedelje, dva puta dnevno, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova kojima je tokom šest nedelja, davan sulpirid (10 mg/kg) intramuskularno, dva puta dnevno; starosno odgovarajuće nulipare ženke pacova kao kontrolne grupe: C1 – 10 pacova, starih 18 nedelja i C2 – sedam pacova, starih 24 nedelje. Određivana je koncentracija

jonizovanog kalcijuma i fosfora u serumu, 24-časovno izlučivanje kalcijuma i fosfora urinom, osteokalcin i serumski amino terminalni propeptid prokolagena tipa I (P1NP). **Rezultati.** U poređenju sa kontrolnom grupom eksperimentalne životinje u grupi P imale su snižen jonizovan kalcijum u serumu ( $0,5 \pm 0,2$  vs  $1,12 \pm 0,04$  mmol/L;  $p < 0,001$ ), povišene fosfate u serumu ( $2,42 \pm 0,46$  vs  $2,05 \pm 0,2$  mmol/L;  $p < 0,05$ ), povećano 24-časovno izlučivanje kalcijuma urinom ( $3,90 \pm 0,46$  vs  $3,05 \pm 0,58$  mmol/24 h;  $p < 0,01$ ) i značajno povišen P1NP ( $489,22 \pm 46,77$  vs  $361,9 \pm 53,01$  pg/mL;  $p < 0,001$ ). Značajan pad P1NP zabeležen je u eksperimentalnoj grupi M3 u poređenju sa kontrolnom grupom. Prolongirana medikamentna hiperprolaktinemija u grupi M6 dovela je do porasta jonizovanog kalcijuma u serumu ( $1,21 \pm 0,03$  vs  $1,15 \pm 0,02$  mmol/L;  $p < 0,001$ ), pada fosfata ( $1,70 \pm 0,13$  vs

1,89 ± 0,32 mmol/L;  $p < 0,001$ ) i sniženja koncentracije osteokalcina i P1NP. **Zaključak.** Fiziološka hiperprolaktinemija u manjoj meri utiče na koštani metabolizam nego medikamentna hiperprolaktinemija. Hronična medikamentna hiperprolaktinemija dovodi do pada koncentracije P1NP, što je odraz snižene koštane formacije. Rutinsko određivanje biohemijskih markera koštanog metabolizma u prolongiranoj

ranoj medikamentnoj hiperprolaktinemiji pruža mogućnost ranije dijagnoze poremećaja koštanog metabolizma.

**Ključne reči:**  
**hiperprolaktinemije; trudnoća; sulpirid; pacovi; osteogeneza; biološki pokazatelji; kalcijum; fosfor; osteokalcin.**

## Introduction

Hyperprolactinemia (HP) is a common hypothalamic-pituitary axis disorder. This "abnormal laboratory value" may be caused by any process interfering with dopamine synthesis, its transport to the pituitary gland or its action on lactotroph dopamine receptors<sup>1</sup>. Considering the complexity of various etiologies, HP could be divided into 4 categories: physiological, pathological, medicamentous and functional HP.

The most frequent clinical symptoms of HP, regardless of its origin, are galactorrhea, oligo- or amenorrhea and sterility in women and impotence, libido loss and gynecomastia in men. In last few decades, there is growing evidence of decreased bone mineral density (BMD) and increased activity of bone turnover markers caused by HP. Prolactin (PRL)-secreting pituitary tumors in people<sup>2-6</sup> and rats<sup>7</sup> are associated with osteopenia. Antipsychotic-induced HP can cause osteoporosis and increased risk of hip fracture<sup>8-10</sup>. Pregnancy and prolonged lactation, conditions with physiological HP, can lead to a significant bone loss<sup>11-13</sup>. Although rapid mineralizing neonatal skeleton (during pregnancy) and higher calcium demand for milk production (during lactation) places significant stress on maternal calcium homeostasis, bone loss is usually recovered after weaning<sup>14-16</sup>. Longitudinal studies have shown that there is no detrimental effect of parity and prolonged breast-feeding on long-term bone health<sup>17, 18</sup>.

Although physiological and medicamentous HP have different final effects on skeletal system, there is a lack of data regarding the effects of PRL on calcium metabolism and bone turnover in HP of various origins.

The aim of this experimental study was: 1) to determine if there was a difference in calcium metabolism during pregnancy (physiological HP) and in sulpirid-induced HP (medicamentous HP); 2) to compare the influence of medicamentous and physiological HP on bone turnover markers; 3) and to reveal a possible effect of prolonged medicamentous HP on calcium metabolism and bone turnover markers.

## Methods

### Animals

Pregnant and age matched nulliparous Wistar female rats (18 weeks old) were obtained from the Animal Laboratory Centre Torlak, Institute for Medical Research, Military Medical Academy, Belgrade, Serbia. Experimental study was conducted in Biomedical Research Center, Medical Faculty, University of Niš, Serbia. The weight of experimental animals ranged 290–340 g. They were housed under a

12 : 12 h light-dark cycle (lights on at 06 h) and fed standard chow and water. Room temperature was 23–25°C with average humidity of 50–60%. The study was approved by the Ethical Committee of the Medical Faculty, University of Niš, Serbia.

### Experimental design

Experimental animals were divided into the following groups: 9 rats, 3 week pregnant (P – physiological HP during pregnancy; gestation period in Wistar rats 19–22 days); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks (M3 – medicamentous HP); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 6 weeks (M6 – medicamentous HP). Since bone growth and calcium accretion are normally age dependent, we used age matched nulliparous rats as control groups: 10 rats, 18 weeks old (C1), and 7 rats, 24 weeks old (C2). All rats in each group (pregnant, medicamentous treated and controls) were sacrificed on the same day.

### Laboratory investigations

In order to confirm HP serum PRL levels were measured in all experimental groups and compared with controls. PRL concentration was measured using enzyme-linked immunosorbent assay kit for PRL. The kit is a sandwich enzyme immunoassay for *in vitro* quantitative measurement of PRL in rat serum, plasma and other biological fluids (manufactured by Usck, Life Science Inc.).

All experimental animals were analyzed for serum ionized calcium and urinary calcium, inorganic phosphorus and urinary phosphate. All rats, in each group, were kept in single rat metabolic cages, 24 h before they were sacrificed, in order to collect 24 h urine for calciuresis and phosphorus diuresis. Rats were anesthetized with intramuscular injection of 10% ketamine hydrochloride (0.3 mL *per* animal). Blood samples until exsanguination were taken by puncture of left myocardial ventricle through midline thoracoabdominal incision. Mineral assays were done by the following methods: serum ionized calcium by potentiometric method; urine calcium by photometric colour test (Beckman Coulter, OLYMPUS analyzer); serum and urine phosphate concentration by photometric UV test (Beckman Coulter, OLYMPUS analyzer); The bone turnover markers studied were: osteocalcin (OC) and serum procollagen type 1 N-terminal propeptide (P1NP). The methods used for bone turnover markers were: OC by electrochemiluminescence immunoassay (N-MID Osteocalcin, Cobas, Roche) and P1NP was measured using enzyme-linked immunosorbent assay kit for P1NP (Usck, Life Science Inc.).

*Statistical analysis*

Data were analyzed using SPSS (version 15.0). Continuous (measurable) parameters were presented with mean values ( $\bar{x}$ ) and standard deviation (SD), median (md), maximum (max) and minimum (min) values. The Shapiro-Wilk test was used to determine normality of parameters distribution. Differences were tested by Student's *t*-test for independent samples if the distribution of parameters was normal and Mann-Whitney *U*-test was used if parameters distribution was deviated. We used Student's *t*-test for dependent samples (normal distribution) and Wilcoxon test (deviated distribution) to test statistical significance between continuous parameter values at the beginning and the end of the study.

**Results**

*Changes in prolactin concentration, calcium metabolism and bone turnover markers in physiological hyperprolactinemia*

PRL concentrations were significantly higher during the third week of pregnancy (P), compared with C1 ( $181.80 \pm 29.65$  vs  $105.38 \pm 28.34$  pg/mL;  $p < 0.001$ ) (Table 1).

increased phosphorus compared to C1 ( $2.42 \pm 0.46$  mmol/L vs  $2.05 \pm 0.19$  mmol/L;  $p < 0.05$ ) (Table 2).

Urinary calcium and phosphorus excretion (measured as daily total calcium and daily phosphorus excretion) significantly increased during pregnancy compared to the control group (urinary calcium  $3.90 \pm 0.46$  mmol/24 h vs  $3.05 \pm 0.58$  mmol/24 h;  $p < 0.01$ ; urinary phosphorus  $141.15 \pm 20.65$  mmol/24 h vs  $45.54 \pm 7.99$  mmol/24 h,  $p < 0.001$ ) (Table 2).

*Changes in prolactin concentration, calcium metabolism and bone turnover markers in medicamentous hyperprolactinemia*

Significantly increased PRL levels in sulpirid-treated rats, compared to age matched controls, confirmed the state of medicamentous HP (M3:  $182.03 \pm 57.80$  vs  $105.38 \pm 28.34$  pg/mL;  $p < 0.001$ ; M6:  $148.92 \pm 20.46$  vs  $112.01 \pm 11.92$  pg/mL,  $p < 0.001$ ). Even though lower PRL concentration were verified in M6 in comparison with M3, decrease was not significant ( $148.92 \pm 20.46$  vs  $182.03 \pm 57.80$  pg/mL,  $p > 0.05$ ).

**Table 1**

**Concentration of prolactin, osteocalcin and PINP in experimental groups**

Experimental groups	Prolactin (pg/mL) $\bar{x} \pm SD$	Osteocalcin (ng/mL) $\bar{x} \pm SD$	PINP $\bar{x} \pm SD$
C <sub>1</sub>	$105.38 \pm 28.34$	$17.5 \pm 2.76$	$361.90 \pm 53.01$
P	$181.8 \pm 29.65$	$9.01 \pm 1.09$	$489.22 \pm 46.77$
M <sub>3</sub>	$182.03 \pm 57.8$	$15.28 \pm 2.51$	$309.60 \pm 36.74$
M <sub>6</sub>	$148.92 \pm 20.46$	$13.55 \pm 3.42$	$291.70 \pm 71.03$
C <sub>2</sub>	$112.01 \pm 11.92$	$16.18 \pm 2.0$	$314.86 \pm 50.99$
<i>p</i>	$< 0.001$ (P : C <sub>1</sub> ) $< 0.001$ (M <sub>3</sub> : C <sub>1</sub> ) $< 0.001$ (M <sub>6</sub> : C <sub>2</sub> )	$< 0.001$ (P : C <sub>1</sub> )	$< 0.05$ (M <sub>3</sub> : C <sub>1</sub> ) $< 0.001$ (M <sub>3</sub> : P)

Experimental groups: P – physiological hyperprolactinemia (HP) during pregnancy; M3 – medicamentous HP with a 3-week duration; M6 – medicamentous HP with a 6-week duration; C1 – control group age matched with P and M3; C2 – control group age matched with M6;  $\bar{x} \pm SD$  – mean value; SD – standard deviation.

OC concentrations were significantly lower during pregnancy compared to C1 ( $9.01 \pm 1.09$  ng/mL vs  $17.50 \pm 2.76$  ng/mL,  $p < 0.001$ ) (Table 1).

In medicamentous HP (M3), serum calcium levels were higher, with no significant difference compared to C1 ( $1.15 \pm 0.04$  vs  $1.12 \pm 0.04$  mmol/L) but serum calcium levels

**Table 2**

**Calcium and posphorous serum concentrations and 24-h urine excretion values**

Parameter	C1 (n = 10)	P (n = 9)	M3 (n = 10)	M6 (n = 10)	C2 (n = 7)
Serum ionized calcium (mmol/L), $\bar{x} \pm SD$	$1.12 \pm 0.04$	$0.50 \pm 0.20^*$	$1.15 \pm 0.04$	$1.21 \pm 0.03^*$	$1.15 \pm 0.02$
Serum phosphorous (mmol/L), $\bar{x} \pm SD$	$2.05 \pm 0.19$	$2.42 \pm 0.46^*$	$2.14 \pm 0.48$	$1.70 \pm 0.13$	$1.89 \pm 0.32$
Urine calcium excretion (mmol/24h), $\bar{x} \pm SD$	$3.05 \pm 0.58$	$3.90 \pm 0.46^\dagger$	$4.31 \pm 1.11^\dagger$	$2.88 \pm 0.60$	$3.37 \pm 0.87$
Urine phosphorous (mmol/24h) excretion ( $\bar{x} \pm SD$ )	$45.54 \pm 7.99$	$141.15 \pm 20.65^\ddagger$	$50.58 \pm 9.77$	$53.93 \pm 14.05$	$55.03 \pm 20.37$

\* $p < 0.05$  (P vs C1);  $^\dagger p < 0.01$  (P vs C1; M3 vs C1, M6);  $^\ddagger p < 0.001$  P, M3, M6, C1, C2 (for explanation see under Table 1).

PINP concentrations were significantly higher in physiological HP (P) in comparison to that in the C1 group ( $489.22 \pm 46.77$  pg/mL vs  $361 \pm 53.01$  pg/mL,  $p < 0.001$ ) (Table 1).

Serum ionized calcium concentrations were significantly decreased in pregnancy ( $0.5 \pm 0.2$  mmol/L vs  $1.12 \pm 0.04$  mmol/L,  $p < 0.001$ ) followed by significantly in-

in physiological HP were significantly decreased in comparison with M3 ( $0.5 \pm 0.2$  vs  $1.15 \pm 0.04$  mmol/L,  $p < 0.001$ ). Phosphorus concentrations were not significantly changed during 3 weeks of medicamentous HP compared to C1 ( $2.14 \pm 0.48$  vs  $2.05 \pm 0.19$  mmol/L,  $p > 0.05$ ). Even though lower phosphorus concentration were verified in comparison with pregnant rats, a decrease was not significant ( $2.14 \pm 0.48$  vs  $2.42 \pm 0.46$

mmol/L). With prolongation of medicamentous HP (M6) calcium concentrations continued to rise and serum phosphorus levels to fall. After 6 weeks of sulpirid provoked HP, serum calcium concentrations were significantly increased in comparison with C2 ( $1.21 \pm 0.03$  mmol/L vs  $1.15 \pm 0.02$  mmol/L,  $p < 0.001$ ). There was no significant difference in serum phosphorus concentrations with a prolongation of sulpirid treatment ( $1.70 \pm 0.13$  mmol/L vs  $1.89 \pm 0.32$  mmol/L,  $p > 0.05$ ).

In M3 rats calciuresis was significantly increased compared to the control group ( $4.31 \pm 1.11$  mmol/24 h vs  $3.05 \pm 0.58$  mmol/24 h;  $p < 0.01$ ) while phosphorus diuresis was not significantly changed ( $50.58 \pm 9.77$  mmol/24 h vs  $45.54 \pm 7.99$  mmol/24 h,  $p > 0.05$ ).

With longer duration of medicamentous HP (M6) there were no significant changes in urinary calcium and phosphorus excretion compared to C2 (calciuresis:  $2.88 \pm 0.6$  mmol/24 h vs  $3.37 \pm 0.87$  mmol/24 h; phosphorus diuresis:  $53.93 \pm 14.05$  mmol/24 h vs  $55.03 \pm 20.37$  mmol/24 h).

OC concentrations were decreased in sulpirid-treated rats compared to age matched control groups (M3:  $15.28 \pm 2.51$  ng/mL vs  $17.50 \pm 2.76$  ng/mL; M6:  $13.55 \pm 3.42$  ng/mL vs  $16.18 \pm 2.0$  ng/mL) but without statistical significance.

P1NP concentration in M3 compared to C1 was significantly decreased ( $309.60$  pg/mL  $\pm 36.74$  vs  $361.90 \pm 53.01$  pg/mL,  $p < 0.05$ ) and even more in comparison with physiological HP ( $309.60 \pm 36.74$  pg/mL vs  $489.22 \pm 46.77$  pg/mL;  $p < 0.001$ ). Although the tendency of further P1NP decrease was noticed with longer duration of medicamentous HP, no significant difference was verified ( $291.70 \pm 71.03$  pg/mL, vs  $314.86 \pm 50.99$  pg/mL,  $p > 0.05$ ).

## Discussion

Our study results confirmed the expected PRL increase during pregnancy. The results of mineral analyses and bone turnover markers conducted in this experimental group could be considered as representative for physiological HP.

Calcium homeostasis during pregnancy is changed due to elevated fetus demand for calcium and maternal adaptations. Adaptation mechanisms include increase in intestinal calcium absorption, decrease in urinary calcium excretion or mobilization of maternal bone mineral.

A decrease in total serum calcium concentration during pregnancy has already been reported<sup>19, 20</sup> and usually considered as a consequence of hemodilution and decreased serum albumin<sup>19, 21, 22</sup>. In order to avoid low calcium concentration due to dilutional effect, we measured serum ionized calcium level. Our study results confirmed a significant decrease in ionized calcium, which is in the contrast with previously reported results of unchanged ionized calcium throughout gestation<sup>23, 24</sup> and more consistent with data from several animal models, reporting fall of ionized calcium in late pregnancy<sup>25, 26</sup>. Rapid fetus growing in late pregnancy may exceed the maternal capacity to maintain a normal serum calcium level and result in decreased ionized calcium.

Inorganic phosphorus is very often considered to be a passive companion of calcium fluxes. Studies which evaluate phosphate balance during physiological HP (as pregnancy) are less

common than calcium studies. Serum phosphate levels are usually reported as normal throughout pregnancy in humans and animals<sup>19, 21</sup>. Our study results showed significantly increased serum phosphorus during pregnancy. It is consistent with decreased serum ionized calcium in our experimental study, increased parathyroid hormone during rat pregnancy, reported in previous animal models<sup>21</sup> and a fact that dietary phosphorus is absorbed almost twice as efficiently as dietary calcium<sup>27</sup>.

The increase of urinary calcium excretion during pregnancy is consistent with previous reports<sup>19, 20</sup>. It is considered as a consequence of increased calcium absorption and elevation in glomerular filtration rate (GFR) during pregnancy, which together exceed the reabsorptive capacity of the kidney<sup>19-21</sup>.

Changes in urinary phosphorus excretion, during pregnancy, could be also due to increased dietary intake in late pregnancy, increased absorption and increased GFR during pregnancy.

OC fulfils all three of the following criteria for reliable bone turnover marker: it is osteoblast-produced protein, its increase correlates with increased bone formation, and it has fast response to changes in skeletal homeostasis. A decline in serum OC during pregnancy, in this study is consistent with the findings of previous reports<sup>19, 20, 28, 29</sup>. Decreased OC in pregnancy may be related to hemodilution, fetal contribution<sup>20</sup>, increased renal degradation secondary to increased GFR<sup>29</sup> or lacking of normal values during pregnancy<sup>22</sup>.

P1NP together with carboxy terminal propeptide (P1CP) are a part of the process in which type I procollagen is transformed into type I collagen. Since type I collagen constitutes 90% of bone proteins, it may be considered as very valuable and precise marker of bone formation. Our study results are consistent with limited, previously reported data, showing low P1CP and P1NP concentration in the first trimester<sup>28, 30</sup> with the tendency to rise above normal in the late pregnancy<sup>30, 31</sup>. Higher osteoblastic activity, in physiological HP, could explain faster recovery of bone loss after pregnancy and lactation.

Increased PRL levels in sulpirid-treated rats were confirmed in our study. Therefore, the results of mineral analyses and bone turnover markers, conducted in these experimental groups, could be considered as representative for medicamentous HP. With sulpirid treatment prolongation slightly decreased PRL concentrations were verified. Data from the literature usually cover the issue of different sulpiride effects, according to the low or high dosages<sup>32</sup>. Lower concentration of dopamine antagonist (sulpiride) can block presynaptic dopamine (D) 2 receptors, leading to decreased dopamine synthesis and release. Lactotrophs are released of dopamine inhibition and hyperprolactinemia occurs. Higher sulpiride concentrations are needed to block postsynaptic D2 receptors<sup>33</sup>. There are no literature data showing different effect of sulpiride with longer treatment duration. A possible explanation for decreased PRL concentration with prolongation of sulpiride treatment could be up-regulation of D2 receptors in lactotrophs after longer blocking sulpiride effect or postreceptors downstream of cAMP/calcium signalling which is necessary for PRL release<sup>34</sup>.

Studies conducted to reveal a connection between medicamentous HP and skeletal system are often based on parameters of bone mineral density and biochemical turnover markers. There are less available data about HP influence on calcium and phosphorus levels. Our study results, showing no significant changes in serum ionized calcium and phosphorus, during a 3-week sulpirid-provoked HP, are consistent with limited previously reported data<sup>35-37</sup>. Even though there are growing evidences that prolonged medicamentous HP can lead to decreased bone mineral density<sup>8-10,36</sup>, there are still missing data about calcium and phosphorus changes during those conditions. Our study results revealed a significant calcium increase and phosphorus decrease during longer medicamentous HP. Hypercalcemia in prolonged medicamentous HP could be a result of increased calcium absorption in upper intestine (absorptive hypercalcemia), increased net bone resorption (remodelling hypercalcemia) or increased tubular calcium reabsorption (tubular reabsorptive hypercalcemia). In last decade, many experimental studies confirmed very important and direct PRL role in regulating intestinal calcium absorption<sup>38-41</sup>. All of these studies are based on physiological HP. There are no literature data showing that medicamentous HP also leads to increased intestinal calcium absorption. The findings of PRL receptor mRNA expression in osteosarcoma cell lines<sup>42</sup>, cultured calvaria osteoblasts<sup>43</sup> and in tibia, femur and vertebrae in normal adult rats<sup>44</sup> suggested bones as possible direct targets of PRL. It is still uncertain whether hypercalcemia in prolonged medicamentous HP could be considered as a consequence of direct PRL influence on bones.

Renal tubular dysfunction resulting in excess calcium loss, caused by sulpirid is not so far reported. A significant fall of urinary calcium excretion in prolonged medicamentous HP was not previously reported, to our knowledge, and could be a result of some still unknown mechanisms, switched on to prevent further calcium loss.

Different studies conducted in women with major depressive disorder, with or without borderline personality disorder, before psychotropic medication, or treated with antidepressant, found increased OC<sup>35, 45, 46</sup>. Our study results,

presenting lower OC, but still in normal referent rang, are more consistent with data provided in schizophrenic patients with antipsychotic treatment<sup>36,47</sup>.

There are no previously reported data, to our knowledge, about changes in P1NP in medicamentous HP. Our results, for the first time show statistical significant decrease of this osteoblastic marker in sulpirid-induced HP. Prolonged medicamentous HP leads to further fall of P1NP, reflecting poor osteoblastic activity.

Even though OC and P1NP are bone formation markers their serum levels reflect different aspects of osteoblastic activity. Osteocalcin is mostly produced during the mineralization phase, while procollagen peptides are mostly produced by proliferating osteoblasts<sup>47</sup>.

Limitation of this study is a lack of biochemical markers of bone resorption. Comparison of bone resorption/formation rates could also be very important data about bone remodelling in physiological and medicamentous hyperprolactinemia.

### Conclusion

We demonstrated herein that ionized calcium concentrations were significantly different in physiological and medicamentous hyperprolactinemia (decreased in late pregnancy and increased in sulpirid-induced hyperprolactinemia). Quite opposite influence of physiological and medicamentous hyperprolactinemia on bone formation marker procollagen type 1 N-terminal propeptide, revealed an increased osteoblastic activity in pregnancy and decreased bone formation in sulpirid-provoked hyperprolactinemia. These results provide a possible explanation why pregnancy does not determine such harmful effect on bone metabolism, while medicamentous hyperprolactinemia leads to decreased bone mineral density. The present experimental data of further procollagen type 1 N-terminal propeptide decrease in prolonged medicamentous hyperprolactinemia, provide information on dynamic, time-dependent and origin-dependent osteoregulatory roles of prolactin.

### R E F E R E N C E S

- Mancini T, Casanueva FF, Giustina A. Hyperprolactinemia and prolactinomas. *Endocrinol Metab Clin North Am* 2008; 37(1): 67-99.
- Greenspan SL, Neer RM, Ridgway EC, Klibanski A. Osteoporosis in men with hyperprolactinemic hypogonadism. *Ann Intern Med* 1986; 104(6): 777-82.
- Schlechte J, Khoury G, Kathol M, Walkner L. Forearm and vertebral bone mineral in treated and untreated hyperprolactinemic amenorrhea. *J Clin Endocrinol Metab* 1987; 64(5): 1021-6.
- Biller BM, Baum HB, Rosenthal DI, Saxe VC, Charpie PM, Klibanski A. Progressive trabecular osteopenia in women with hyperprolactinemic amenorrhea. *J Clin Endocrinol Metab* 1992; 75(3): 692-7.
- Vestergaard P, Jorgensen JOL, Hagen C, Hoek HC, Laurberg P, Rejnmark L, et al. Fracture risk is increased in patients with GH deficiency or untreated prolactinomas - a case-control study. *Clin Endocrinol (Oxf)* 2002; 56(2): 159-67.
- Zadrozna-Slwkka B, Bolanowski M, Kaluzny M, Syrycka J. Bone mineral density and bone turnover in hyperprolactinaemia of various origins. *Endokrynol Pol* 2007; 58(2): 116-22.
- Adler RA, Farrell ME, Deiss WP, Krieg RJ, MacLeod RM. Hypercalciuria in a new rat model of hyperprolactinemia. *Metab Clin Exp* 1991; 40(3): 292-6.
- Haddad PM, Wieck A. Antipsychotic-induced hyperprolactinaemia: mechanisms, clinical features and management. *Drugs* 2004; 64(20): 2291-314.
- Howard L, Kirkwood G, Leese M. Risk of hip fracture in patients with a history of schizophrenia. *Br J Psychiatry* 2007; 190: 129-34.
- Muench J, Hamer A. Adverse effects of antipsychotic medications. *Am Fam Physician* 2010; 81(5): 617-22.
- Lotinun S, Limlomwongse L, Krishnamra N. The study of a physiological significance of prolactin in the regulation of calcium metabolism during pregnancy and lactation in rats. *Can J Physiol Pharmacol* 1998; 76(2): 218-28.

12. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. *J Clin Endocrinol Metab* 2001; 86(6): 2344–8.
13. Lotinun S, Limlomwongse L, Sirikulchayanonta V, Krishnamra N. Bone calcium turnover, formation, and resorption in bromocriptine- and prolactin-treated lactating rats. *Endocrine* 2003; 20(1–2): 163–70.
14. Polatti F, Capuzzo E, Viazzo F, Colleoni R, Klery C. Bone mineral changes during and after lactation. *Obstet Gynecol* 1999; 94(1): 52–6.
15. Karlsson C, Obrant KJ, Karlsson M. Pregnancy and lactation confer reversible bone loss in humans. *Osteoporos Int* 2001; 12(10): 828–34.
16. Bezerra FF, Mendonça LM, Lobato EC, Brien KO, Donangelo CM. Bone mass is recovered from lactation to postweaning in adolescent mothers with low calcium intakes. *Am J Clin Nutr* 2004; 80(5): 1322–6.
17. Streeten EA, Ryan KA, McBride DJ, Pollin TI, Shuldiner AR, Mitchell BD. The relationship between parity and bone mineral density in women characterized by a homogeneous lifestyle and high parity. *J Clin Endocrinol Metab* 2005; 90(8): 4536–41.
18. Lenora J, Lekamwasam S, Karlsson MK. Effects of multiparity and prolonged breast-feeding on maternal bone mineral density: a community-based cross-sectional study. *BMC Womens Health* 2009; 9: 19.
19. Cross NA, Hillman LS, Allen SH, Krause GF, Vieira NE. Calcium homeostasis and bone metabolism during pregnancy, lactation, and postweaning: a longitudinal study. *Am J Clin Nutr* 1995; 61(3): 514–23.
20. Ritchie LD, Fung EB, Halloran BP, Turnlund JR, Van LM, Cann CE, et al. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. *Am J Clin Nutr* 1998; 67(4): 693–701.
21. Kovacs CS, Kronenberg HM. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. *Endocr Rev* 1997; 18(6): 832–72.
22. Mahadevan S, Kumaravel V, Bharath R. Calcium and bone disorders in pregnancy. *Indian J Endocrinol Metab* 2012; 16(3): 358–63.
23. Rasmussen N, Frolich A, Hornnes PJ, Hegedus L. Serum ionized calcium and intact parathyroid hormone levels during pregnancy and postpartum. *Br J Obstet Gynecol* 1990; 97(9): 857–62.
24. Dahlman T, Sjöberg HE, Bucht E. Calcium homeostasis in normal pregnancy and puerperium. A longitudinal study. *Acta Obstet Gynecol Scand* 1994; 73(5): 393–8.
25. Garner SC, Peng TC, Toverud SU. Modulation of serum parathyroid hormone and ionized calcium concentrations during reproduction in rats fed a low calcium diet. *J Bone Miner Res* 1988; 3(3): 319–23.
26. Boass A, Garner SC, Schultz VL, Toverud SU. Regulation of Serum Calcitriol by Serum Ionized Calcium in Rats During Pregnancy and Lactation. *J Bone Miner Res* 1997; 12(6): 909–14.
27. Peacock M. Calcium Metabolism in Health and Disease. *Clin J Am Soc Nephrol* 2010; 5(Supplement 1): 23–30.
28. Gallacher SJ, Fraser WD, Owens OJ, Dryburgh FJ, Logue FC, Jenkins A, et al. Changes in calciotropic hormones and biochemical markers of bone turnover in normal human pregnancy. *Eur J Endocrinol* 1994; 131(4): 369–74.
29. Cole DE, Gundberg CM, Stirk LJ, Atkinson SA, Hanley DA, Ayer LM, et al. Changing osteocalcin concentrations during pregnancy and lactation: implications for maternal mineral metabolism. *J Clin Endocrinol Metab* 1987; 65(2): 290–4.
30. Cross NA, Hillman LS, Allen SH, Krause GF. Changes in bone mineral density and markers of bone remodeling during lactation and postweaning in women consuming high amounts of calcium. *J Bone Miner Res* 1995; 10(9): 1312–20.
31. Hellmeyer L, Ziller V, Anderer G, Ossendorf A, Schmidt S, Hadji P. Biochemical markers of bone turnover during pregnancy: a longitudinal study. *Exp Clin Endocrinol Diabetes* 2006; 114(9): 506–10.
32. Rosenzweig P, Canal M, Patat A, Bergougnan L, Zieleniuk I, Bianchetti G. A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. *Hum Psychopharmacol* 2002; 17(1): 1–13.
33. Beaulieu J, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 2011; 63(1): 182–217.
34. Stojilkovic SS, Murano T, Gonzalez-Iglesias AE, Andric SA, Popovic MA, Van GF, et al. Multiple roles of Gi/o protein-coupled receptors in control of action potential secretion coupling in pituitary lactotrophs. *Ann NY Acad Sci* 2009; 1152: 174–86.
35. Aydin H, Mutlu N, Akbas NB. Treatment of a major depression episode suppresses markers of bone turnover in premenopausal women. *J Psychiatr Res* 2011; 45(10): 1316–20.
36. Yang J, Joe S, Lee M, Ko Y, Jung I, Kim S. Effects of long-term combination treatment with valproate and atypical antipsychotics on bone mineral density and bone metabolism in premenopausal patients with bipolar disorder: a preliminary study. *Psychiatry Investig* 2011; 8(3): 256–61.
37. Wyszogrodzka-Kucharska A, Rabe-Jabłońska J. Calcium balance and regulation in schizophrenic patients treated with second generation antipsychotics. *Psychiatr Pol* 2005; 39(6): 1157–71.
38. Tanrattana C, Charoenphandhu N, Limlomwongse L, Krishnamra N. Prolactin directly stimulated the solvent drag-induced calcium transport in the duodenum of female rats. *Biochim Biophys Acta* 2004; 1665(1–2): 81–91.
39. Charoenphandhu N, Limlomwongse L, Krishnamra N. Prolactin directly enhanced Na<sup>+</sup>/K<sup>+</sup>- and Ca<sup>2+</sup>-ATPase activities in the duodenum of female rats. *Can J Physiol Pharmacol* 2006; 84(5): 555–63.
40. Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpantakit J, Krishnamra N, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphoinositide 3-kinase pathway. *Am J Physiol Endocrinol Metab* 2007; 293(1): 372–84.
41. Charoenphandhu N, Krishnamara N. Prolactin is an important regulator of intestinal calcium transport. *Can J Physiol Pharmacol* 2007; 85(6): 569–81.
42. Bataille-Simoneau N, Gerland K, Chappard D, Basle MF, Mercier L. Expression of prolactin receptors in human osteosarcoma cells. *Biochem Biophys Res Commun* 1996; 229(1): 323–8.
43. Clément-Lacroix P, Ormandy C, Lepescheux L, Ammann P, Damotte D, Goffin V, et al. Osteoblasts are a new target for prolactin: analysis of bone formation in prolactin receptor knockout mice. *Endocrinology* 1999; 140(1): 96–105.
44. Charoenphandhu N, Tudpor K, Thongchote K, Saengamart W, Puntheeranurak S, Krishnamra N. High-calcium diet modulates effects of long term prolactin exposure on the cortical content in ovariectomized rats. *Am J Physiol Endocrinol Metab* 2007; 292(2): E443–52.
45. Herrán A, Amado JA, García-Unzueta MT, Vázquez-Barquero JL, Perera L, González-Macías J. Increased bone remodeling in first-episode major depressive disorder. *Psychosom Med* 2000; 62(6): 779–82.
46. Kahl KG, Greggersen W, Rudolf S, Stoekelhuber BM, Bergmann-Koester CU, Dibbelt L, et al. U. Bone mineral density, bone turnover, and osteoprotegerin in depressed women with and without borderline personality disorder. *Psychosom Med* 2006; 60(6): 669–74.
47. Lee T, Chung M, Chung H, Choi J, Kim T, So H. Bone density in chronic schizophrenia with long-term antipsychotic treatment: preliminary study. *Psychiatry Investig* 2010; 7(4): 278–84.

Received on August 26, 2012.

Revised on November 27, 2012.

Accepted on February 18, 2013.