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The influence of age on bone metabolism in mares during late pregnancy and lactation

Natalija Filipović [a,*], Zvonko Stojević [a], Nataša Plevnik [a], Tomislav Mašek [a], Nikica Prvanović [a], Zvonimir Tuček [b]

[a] Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb 10000, Croatia
[b] Centre for Animal Reproduction of Croatia, Bani 83, Zagreb 10020, Croatia

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ABSTRACT

The concentrations of carboxyterminal cross-linked telopeptide of type I collagen (CTx), osteocalcin (OCN), parathyroid hormone (PTH) and insulin-like growth factor (IGF-I) were measured in the blood serum of two age groups of mares during late pregnancy (70–50 days before foaling), during early lactation and at the peak of lactation (10–30 and 55–80 days after foaling, respectively). During late pregnancy, the PTH was higher in older (8–19 years old), compared to younger animals (3.5–4 years old) ($P < 0.05$). The OCN was higher in younger group during late pregnancy ($P < 0.05$). IGF-I was higher in the younger group during early lactation, in comparison to late pregnancy and the peak of lactation ($P < 0.05$). IGF-I did not differ between two age groups of mares. The results indicate on the differences in adaptation of bone metabolism to late pregnancy and lactation in older animals, in comparison to younger animals, reflected by elevated PTH secretion.

1. Introduction

Pregnancy and lactation are periods of significant influence on bone metabolism. The intensive foetal growth, the mineralization of the foetal skeleton and substantial quantities of calcium lost by milk cause an increase in the need for calcium and phosphorus. That need is partially satisfied with the resorption of minerals in bones (Liesegang et al., 2000; Yoon et al., 2000). In women, rodents and ruminants, it has been established that late pregnancy and lactation significantly influence the extent of bone remodelling (Kovacs, 2005; Kovacs and Kronenberg, 1997; Liesegang et al., 2000, 2006, 2007). The rate of bone remodelling could be estimated by measuring the concentrations/activities of the bone remodelling markers in blood or urine (Delmas, 1995). These markers are indicators of the activity of bone cells and they include two groups: the markers of bone synthesis and the markers of bone resorption (Delmas, 1995; Lepage et al., 2001). Carboxyterminal cross-linked telopeptide of type I collagen (CTx) is a product of degradation of the type I collagen. Its concentration in the blood is a reliable and sensitive marker of bone resorption in humans (Christgau et al., 1998; Rosen et al., 2000). Osteocalcin (OCN) is a bone tissue-specific protein that is released in the blood in greater quantity during the elevated rate of bone synthesis (Camarda et al., 1987). Measurement of its blood concentration is used as an indicator of the rate of bone synthesis (Kruse and Kracht, 1986). Calcium and phosphorus metabolism is regulated by several hormones, among them being PTH, calcitonin and 1,25 $(OH)_2$-vitamin D_3 (calcitriol), where the latter plays the most important role (Blair et al., 2002). PTH, the hormone secreted by the parathyroid glands, increases the rate of bone resorption. That effect is a consequence of the increased number in osteoclasts and their activities (Rosol and Capen, 1997). In addition, PTH also has anabolic effects in the bone tissue (Swarthout et al., 2002). PTH increases renal reabsorption of calcium and decreases renal reabsorption of phosphates. It increases the synthesis of calcitriol in kidneys, which increases bone resorption and absorption of calcium and phosphates from the intestines (Rosol and Capen, 1997). Secretion of PTH from the parathyroids is regulated by the concentration of calcium ions in the blood plasma (Chiba et al., 2000). In addition to the PTH, many other hormones influence bone turnover. For instance, a significant influence of growth hormone (GH) on bone remodelling has been observed and is thought to be partially mediated by the insulin-like growth factor (IGF-I; Ohlsson et al., 1998; Zhang et al., 2002). IGF-I is a peptide that has a similar structure as insulin and mediates plenty of metabolic effects of GH (Mauras and Haymond, 2005). It is secreted mostly by the liver, but many other tissues produce IGF-I too (D'Ercole and Calikoglu, 2001). IGF-I has autocrine/paracrine as well as endocrine effects (Mauras and Haymond, 2005). In addition to its important metabolic roles, a correlation between the blood concentration of IGF-I and bone

* Corresponding author. Tel.: +385 21 557 804; fax: +385 1 2441 390.
E-mail address: natalija.filipovic@mefst.hr (N. Filipović).

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remodelling markers in equine has been observed (Davicco et al., 1994; Jackson et al., 2003; Noble et al., 2007; Price et al., 2001).

Data exist about measurements of concentrations of bone remodelling markers in horses, related to the influence of diseases of the locomotor system, training and age (Lepage et al., 2001). In addition, the influence of late pregnancy and early lactation on bone metabolism has been studied in mares (Filipovic et al., 2010). By measuring bone alkaline phosphatase, the lower activity of osteoblasts, along with the lower concentration of total calcium, has been observed during late pregnancy in mares, indicating a low level of ability to maintain normocalcaemia in mares during the period of the highest need of the foetus for this mineral (Filipovic et al., 2010). As opposed to most of the previously investigated species, in the beginning of lactation of mares no considerable increase of bone resorption occurs, indicating that the absorption from the digestive tract in mares is sufficient to satisfy the need for calcium and phosphates during lactation (Filipovic et al., 2010). Age is an important factor that influences bone remodelling (Boyde and Kingsmill, 1998). In younger animals, the modelling of the skeleton takes place, which is connected with higher concentrations of bone remodelling markers in the blood (Nagaraja et al., 2007). In aged animals, the regulation of calcium and phosphorus metabolism successively diminishes due to the lower activity of osteoblasts and osteoclasts (Leeming et al., 2006). Pastoret et al. (2007) observed the significant correlation between age of Ardenner horses and a concentration of osteocalcin in serum. Nevertheless, the same study, opposite to expected, failed to record a significant correlation between the concentration of CTx-I in serum and age of those animals.

In mares, the influence of age on bone metabolic parameters during lactation has not yet been completely investigated. In a novel study, Greiner et al. (2012) explored the concentrations of osteocalcin and CTx-I in the blood serum of 19 Haflinger mares during the second half of the pregnancy. They reported on the significant correlation of age and CTx-I concentration in the blood serum of mares during this period. To investigate the influence of age on bone metabolism during the late pregnancy and early lactation in mares, we measured the concentrations of CTx, osteocalcin and PTH in the blood serum of two separate age groups of mares. Since a correlation between the blood concentration of IGF-I and bone remodelling markers in equine has been observed, we explored whether there are potential differences in bone metabolism of two age groups of mares related to the variations in systemic IGF-I concentrations.

2. Material and methods

2.1. Animals and blood sampling

The study was conducted on mares of the Croatian cold-blood horse breed, divided into two groups based on age: the first group included animals between 3.5 and 4 years of age (n = 6) and the second group included mares between 8 and 19 years of age (n = 7). Samples for blood analyses were taken in three experimental periods: late pregnancy (70–50 days before foaling), early lactation (10–30 days after foaling) and peak of lactation (55–80 days after foaling). The animals were kept grazing during the day, and during the night they were in a stable. They were fed additionally with 10 kg of haysilage, 3 kg of oat and 10 kg of hay per animal and Biosaxon vitamin-mineral licks (Salinen, Austria). All procedures performed on animals were approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb and the Veterinary Medicines Directorate of the Ministry of Agriculture of the Republic of Croatia. Samples were always taken between 10.00 a.m. and 11.00 a.m. before morning grazing. Blood was taken from the *v. jugularis externa* and collected in plain BD Vacutainer® tubes (BD Diagnostics, Plymouth, UK). Samples were centrifuged at 1500 g for 15 minutes at room temperature. Aliquot samples were stored at –70 °C.

2.2. Reagents and analysis procedures

2.2.1. CTx assay

The concentration of CTx was determined by using the commercial kit Serum CrossLaps® ELISA (Nordic Bioscience Diagnostics, Denmark). The Serum CrossLaps® ELISA is based on two highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR, where the aspartic acid residue (D) is β-isomerized. In order to obtain a specific signal in the Serum CrossLaps® ELISA, two chains of EKAHD-β-GGR must be cross-linked. The assay uses a mixture of a biotinylated antibody and a peroxidase-conjugated antibody in a one-step incubation. A complex between CrossLaps® antigens, biotinylated antibody and peroxidase-conjugated antibody is generated, and this complex binds to the streptavidin surface via the biotinylated antibody. Detection limit of the test is 0.02 ng/mL.

2.2.2. Osteocalcin assay

The concentration of osteocalcin was determined by using the commercial kit (Metra Osteocalcin, Quidel Corp., San Diego, CA, USA) that has been validated previously in horses (Hoyt and Siciliano, 1999). The assay is a competitive immunoassay. It uses osteocalcin coated strips, a mouse anti-osteocalcin antibody, an anti-mouse IgG–alkaline phosphatase conjugate and a pNPP substrate to quantify osteocalcin in serum. Detection limit of the assay is 0.45 ng/mL.

2.2.3. PTH assay

The concentration of PTH was determined by using the commercial kit Intact PTH (parathyroid hormone) ELISA (Biomerica, Newport Beach, CA, USA). The test is a two-site ELISA (enzyme-linked immunosorbent assay) for the measurement of the biologically intact 84 amino acid chain of PTH. Antibodies used in the Biomerica PTH ELISA were goat polyclonal antibodies purified by the affinity chromatography to be specific for well-defined regions on the PTH molecule. The peroxidase labelled antibody recognizes only the N-terminal region or the 1–34 amino acid sequence of the PTH molecule, whereas the biotinylated antibody is specific to the 39–84 segment. Accordingly, only the intact PTH, which requires binding by both the enzyme conjugated and biotinylated antibodies, can be detected by this assay. The sensitivity of the assay is 0.9 pg/mL.

2.2.4. IGF-I assay

The concentration of IGF-I was determined by using the commercial kit IGF-I equine ELISA (DRG Instruments GmbH, Marburg, Germany). The test is intended for the determination of the IGF-I concentration in the equine blood plasma or serum. The method requires an acid extraction with ethanol, which cleaves IGF-I from IGFBP-3, since IGFBP-3 interferes with the detection method. The assay uses antibodies with high affinity and specificity for two separate epitopes on the IGF-I molecule. The first monoclonal antibody for IGF-I is bound to polystyrene wells, and the second monoclonal antibody is conjugated to an alkaline phosphatase (AP).

The sensitivity of the assay is 4.9 ng/mL. The cross-reactivity for IGF-II is <0.01%, for insulin < 0.1%, and for GH < 0.1%.

2.3. Statistical analysis

The results were statistically analyzed by using the package software Statistica 9 (StatSoft, USA) for the Kolmogorov–Smirnov test of normality and the analysis of variance (Repeated-Measures ANOVA), Tukey's post hoc HSD test. The differences were considered statistically significant if $P < 0.05$.