

EFFECT OF SEASON ON PLASMA CONCENTRATION OF INSULIN-LIKE GROWTH FACTOR-I AND 1.25-DIHYDROXYCHOLECALCIFEROL IN HORSES

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Abstract. During the summer season, plasma concentrations of insulin-like growth factor I (IGF-I) in Arabian, Anglo-Arabian and Hucul horses as well as colts and fillies were higher compared to the winter season. Significant differences in IGF-I concentration between the seasons were found in Hucul horses ($P \leq 0.01$) and in colts ($P \leq 0.05$). Season had no significant effect on $1.25(\text{OH})_2\text{-D}_3$ concentration in the horse breeds studied. However, plasma concentrations of this hormone were always higher in the summer than in the winter season regardless of the breed (except Anglo-Arabi-ans) and sex. Knowledge and monitoring of the physiological concentrations of IGF-I and vitamin D_3 metabolites in different seasons of the year, combined with analysis of growth rate during these periods may contribute to the improvement of the horse management and feeding system. Because of the association between these hormones and the frequency of developmental orthopaedic diseases, this information could be potentially used in veterinary practice.

Keywords: breed, horses, IGF-I, season, sex, vitamin D_3

INTRODUCTION

Among the many compounds that contribute to the maintenance of body homeostasis, a significant role is played by hormones, which regulate, coordinate and control the activity of most body cells. Hormones exert extensive effects on vital life processes such as metabolism, reproduction, physical and mental development. Growth hormone (GH) is the key regulator of postnatal somatic growth in animals. However, GH measurements are difficult to interpret because of the pulsatile secretion of this hormone. The somatotropic effects of growth hormone can be determined based on insulin-like growth factor (IGF-I), the plasma concentration of which is constant and not subject to diurnal variation, with a long biological half-life [Noble et al. 2007]. IGF-I has been found to influence the proliferation and differentiation of chondrocytes, thus inducing longitudinal bone growth and formation of articular cartilage [Lupu et al. 2001, Yakar et al. 2002]. In horses, IGF-I concentration is

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low at birth, rapidly increases during intensive growth, and stabilizes again at a relatively low level in adulthood [Davicco et al. 1994, Malinowski et al. 1996, Berg et al. 2007].

Vitamin D₃, also known as cholecalciferol, is mainly synthesized in the skin from 7-dehydroxycholesterol, but can be also derived from dietary sources. In the liver, all vitamin D₃ undergoes hydroxylation at the 25-carbon position to produce 25OH-D₃. Before showing biological activity in the small intestine, kidneys and bones, this compound must undergo another hydroxylation at the 1 α -position to produce 1.25-dihydroxycholecalciferol (1.25(OH)₂-D₃), also known as calcitriol [Kawashima and Kurokawa 1986, Maxwell 1994]. The known actions of vitamin D₃ concern 1.25(OH)₂-D₃ while 25OH-D₃ and vitamin D₃ itself exhibits no significant physiological activity. Because 1.25(OH)₂-D₃ is formed in the kidney and acts in the intestine and bones, it can be considered a hormone. It stimulates the intestinal absorption of calcium and phosphorus, bone tissue resorption and bone mineralization, thus protecting against rickets and osteomalacia. Together with parathormone, 1.25-dihydroxycholecalciferol is responsible for maintaining normal plasma concentrations of calcium and phosphorus, and for the normal activities of the nervous and muscular system [Schmidt-Nilsen 1992].

The body's growth and development depends on many factors, both environmental and genetic, as well as on the interaction of all physiological systems, especially the nervous, immune and hormonal systems. In horses, plasma concentrations of IGF-I and vitamin D can be influenced by breed, sex, age, diet and health status [Mäenpää et al. 1988b, Sherman et al. 1990, Cymbaluk and Laarveld 1996, Malinowski et al. 1996, Breidenbach et al. 1998, Sloet van Oldruitenborgh-Oosterbaan et al. 1999, Champion et al. 2002, Lejeune et al. 2007, Noble et al. 2007, Verwilghen et al. 2009].

Of the environmental factors, a significant role in the context of the information presented above can be played by season of the year, the associated photoperiod, and ambient temperature. If seasonal changes in the concentration of these hormones were indeed significant, this information could be used in breeding and veterinary practice and in monitoring somatotrophic processes in the horse. Therefore, the aim of the study was to analyse the effect of season on plasma concentration of IGF-I and 1.25-dihydroxycholecalciferol in horses with regard to breed and sex.

MATERIALS AND METHODS

All procedures used in this study were approved by the First Local Ethics Committee for Animal Experimentation. Subjects were 81 horses of four breeds, born between February and April 2007: Thoroughbred (10 colts and 9 fillies from the Golejewko Horse Stud); Arabian (10 colts and 10 fillies from the Janów Podlaski Horse Stud); Anglo-Arabian (10 colts and 11 fillies from the Janów Podlaski Horse Stud); and Hucul (10 colts and 11 fillies from the Gładyszów Hucul Horse Stud). Only horses that were born healthy and had no major health problems until the end of the experiment were investigated. The areas in which the studs were located had continental, transitional temperate climate and were characterized by similar length of the growth period (about 220 days) and a similar annual precipitation total of 550 mm except the Gładyszów Stud (800 mm).

All horses were weaned at about 6 months of age. After weaning, colts and fillies were kept in separate free-running stables except the Thoroughbreds which were kept in individual boxes. In the summer season during the day, horses remained on pasture with a similar composition of sward, and in the winter they used yards next to the stables. They were fed a conventional diet consisting of meadow hay, oat grain, red carrot and pasture forage, the amounts and proportions of which were adjusted to age, breed, and season. In accordance with the typical breeding system for this breed, Thoroughbreds were sent at about 18 months of age to the Służewiec Racetrack in Warsaw. In two racing stables, they were trained for about 6 months and then ran their first races. Blood samples were collected twice from horses aged 19–21 and 25–27 months, first in December when days were the shortest of the year (winter season), and then in June when days were the longest (summer season). Blood was sampled between 10:00 am and 12:00 pm from the external jugular vein into polystyrene tubes containing an anticoagulant (heparin). Blood was centrifuged at 3000 rpm for 10 min at room temperature. Blood plasma was separated into tubes and frozen 90 min after collection. Samples with blood plasma were thawed immediately before the determinations.

Plasma concentration of IGF-I was determined using a commercial human-specific RIA kit (Immunotech IRMA IGF-I A15729, Beckman Coulter Company, France) according to the manufacturer's instructions.

IGF-I concentration was determined using two monoclonal antibodies against two different epitopes of the hormone molecule, which were non-competitive with each other. The calibrators and samples were incubated with shaking (60 min, 20°C, 350 rpm), in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labelled with ^{125}I . Following incubation, the contents of the tubes were aspirated and then washed to eliminate unbound radioactivity.

The plasma concentration of 1.25-dihydroxycholecalciferol was determined using a commercial human-specific RIA kit 1.25(OH) $_2$ -Vit D $_3$ (Demeditec Diagnostics GmbH, Germany) following the manufacturer's instructions. In this method, extracted samples and controls were placed in containers to separate 1.25(OH) $_2$ -D $_3$ from other vitamin D metabolites. After the samples and controls were eluted, calibrators, samples and controls were incubated in coated tubes. A fixed amount of 1.25(OH) $_2$ -D $_3$ labelled with ^{125}I competed with 1.25(OH) $_2$ -D $_3$ present in the analysed sample or in the calibrator for a fixed number of sites on antibodies immobilized on the walls of polystyrene tube. A day-long incubation at room temperature was followed by aspiration, which interrupted the competitive reaction. The tubes were then washed and aspirated.

The bound radioactivity of both hormones was measured using a Wizard gamma counter. The concentrations of IGF-I and 1.25-dihydroxycholecalciferol, which were directly proportional to their radioactivity, were read from standard curves. The intra- and interassay coefficients of variation were 6.3% and 6.8% for IGF-I and 12.4% and 12.1% for 1.25(OH) $_2$ -D $_3$, respectively.

Because the data did not meet the normal distribution conditions, the results were analysed statistically based on nonparametric analysis of variance. Kruskal-Wallis test was used to determine significance of differences within the analysed factors. Results are presented as means \pm SEM.

RESULTS AND DISCUSSION

During the summer season, when days were the shortest, the mean plasma concentration of IGF-I in Hucul, Arabian and Anglo-Arabian horses was higher compared to the winter season (Fig. 1). In Hucul horses, the difference between the seasons in the concentration of this hormone ($121.52 \text{ ng} \cdot \text{ml}^{-1}$) was highly significant ($P \leq 0.01$). Plasma concentration of IGF-I in Thoroughbred horses was $12.99 \text{ ng} \cdot \text{ml}^{-1}$ lower in the summer season compared to the winter season, but the difference was not significant.

In both colts and fillies, plasma concentration of IGF-I during the longest days of the year was higher compared to the period of the shortest days, but a significant ($P \leq 0.05$) difference was only found for the colts (Fig. 1).

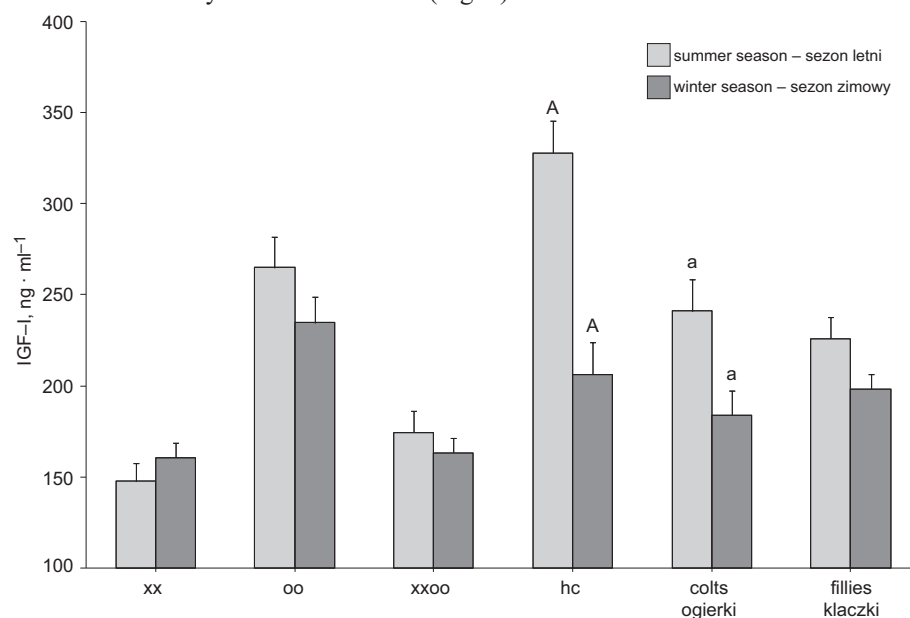


Fig. 1. IGF-I level in blond plasma of horses in the summer season and winter season
Rys. 1. Poziom IGF-I w osoczu krwi koni w sezonie letnim i zimowym

xx – Thoroughbred – konie pełnej krwi angielskiej, oo – Arabian – konie czystej krwi arabskiej, xxoo – Anglo-Arabian – konie angloarabskie, hc – Hucul – konie huculskie.

Numbers denoted with the same letters differ statistically; small letters $P \leq 0.05$; capital letters $P \leq 0.01$. Średnie oznaczone tymi samymi literami różnią się istotnie; litery małe przy $P \leq 0,05$; litery duże przy $P \leq 0,01$.

Season had no significant effect on the concentration of 1.25-dihydroxycholecalciferol in the horse breeds studied. However, in Hucul, Arabian and Thoroughbred horses the mean plasma concentration of $1.25(\text{OH})_2\text{-D}_3$ was higher in the summer than in the winter (Fig. 2). The largest difference in the concentration of this hormone ($3.40 \text{ pg} \cdot \text{ml}^{-1}$) was

found in Thoroughbred horses. Conversely, in Anglo-Arabian horses $1.25(\text{OH})_2\text{-D}_3$ concentration was higher in December when days were the shortest ($10.32 \text{ pg} \cdot \text{ml}^{-1}$) compared to the period of the longest days in June ($8.68 \text{ pg} \cdot \text{ml}^{-1}$). There were no significant correlations between season and plasma concentration of $1.25(\text{OH})_2\text{-D}_3$ in fillies and colts, although in both cases the concentration of the analysed hormone was higher during the summer compared to the winter season (Fig. 2).

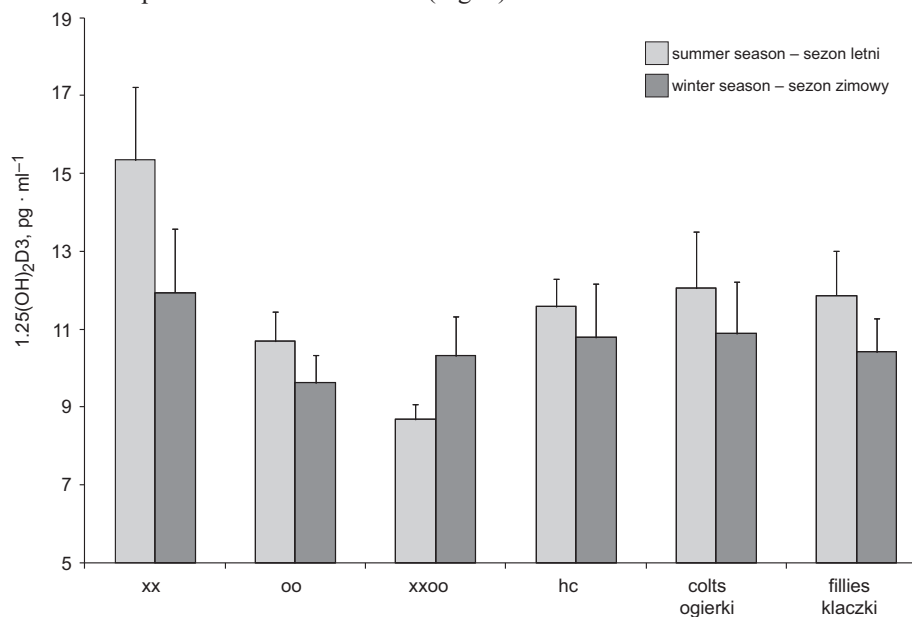


Fig. 2. $1.25(\text{OH})_2\text{-D}_3$ level in blond plasma of horses in the summer season and winter season
Rys. 2. Poziom $1.25(\text{OH})_2\text{-D}_3$ w osoczu krwi koni w sezonie letnim i zimowym

xx – Thoroughbred – konie pełnej krwi angielskiej, oo – Arabian – konie czystej krwi arabskiej, xxoo – Anglo-Arabian – konie angloarabskie, hc – Hucul – konie huculskie.

The present study showed that in most horse breeds studied, the plasma concentration of IGF-I and $1.25(\text{OH})_2\text{-D}_3$ was higher in the summer season when days were the longest, compared to the winter season. A reverse tendency was only observed for IGF-I in Thoroughbred horses and for $1.25(\text{OH})_2\text{-D}_3$ in Anglo-Arabians. Significant between-season differences in IGF-I concentration were found in Hucul horses and in colts.

Hucul horses are the only primitive horses among the analysed breeds and it appears that because of harsh environmental conditions they developed specific characteristics enabling them to respond quickly to changes in the external environment. Likewise, Lejeune et al. [2007], who investigated the dynamics of change in IGF-I in growing Ardennes horses, found that the concentration of this hormone was high during the spring period (March–April), with significantly lower concentrations during the autumn (August–October). According to Noble et al. [2007], although horses have no clear circadian rhythm in pe-

ripheral concentration of IGF-I, such rhythm may be associated with the effect of seasons. When analysing the effect of day length, mean temperatures and nutritive properties of a pasture, it is possible to determine the relationships between the environment and plasma concentrations of IGF-I in animals. Sarko et al. [1994] and Dahl et al. [1997] in cows and Lincoln et al. [2001] in sheep, showed that an increase in mean day temperature and day length is positively correlated to the plasma concentration of IGF-I. In growing Thoroughbred horses, Staniar et al. [2007] observed the mean values of IGF-I to be high in May and June, and low in March. They also found that IGF-I concentration is in direct proportion to temperature and day length. It turned out that in the northern hemisphere, the highest plasma concentrations of IGF-I in animals tend to occur in the spring months. Based on analysis of mean daily weight gains in young Thoroughbreds, Staniar et al. [2004] believe that this is a period of compensatory growth in these horses. It is therefore thought that the higher IGF-I concentrations in the spring-summer period, also reported in the present study, may be associated with a higher growth rate of these horses during that period. This thesis is supported by the studies of Davicco et al. [1994] and Cymbaluk and Laarveld [1996], which proved that the high concentrations of IGF-I are indicative of intensive skeletal growth in young horses. The significantly higher mean concentrations of IGF-I, found in the present study in colts during the summer compared to the winter season may also result from their seasonal reproductive activity. Hess and Roser [2001] showed that IGF-I concentration in both blood plasma and testicular tissue of young colts was highly significantly higher during than outside the breeding season, possibly indicating an increased seasonal production of this hormone. However, according to the authors this concept needs further study because the pattern only concerned colts below 2 years of age. In the present study, of all the breeds only Thoroughbred horses were characterized by a higher (although non-significantly) plasma concentration of IGF-I during the winter compared to the summer season. It seems that the dietary factor could have played a significant role in this case. Because Thoroughbred horses began to be trained on the racetrack in the autumn, their feeding system changed considerably, especially in terms of the protein to energy ratio, compared to the other breeds of horses that were not trained. Treiber et al. [2005] and Staniar et al. [2007] showed plasma IGF-I concentration to be higher in horses fed high-carbohydrate diets compared to the horses receiving carbohydrate-deficient feeds. Likewise, Champion et al. [2002] suggest that the differences in IGF-I concentration in horses from different geographical regions may be due to dietary factors or differences in the management system. The training itself and its different intensities have no significant effect on plasma IGF-I concentration [Noble et al. 2007].

In the present study we observed a tendency for the plasma concentration of $1.25(\text{OH})_2\text{D}_3$ to be higher in the summer compared to the winter season in the horses of all breeds except the Anglo-Arabians. Few publications were found that analysed the effect of season and the associated photoperiod length or temperature on the concentration of vitamin D_3 in horses. However, many studies performed with both humans and different species of animals [Stryd et al. 1979, Smith and Wright 1984, Griffiths and Fairney 1988, Guillemant and Guillemant 1996, Zittermann et al. 1998] show that the seasonal changes in the concentration of vitamin D_3 and its metabolites are related to sunlight ex-

posure, which would explain the higher plasma concentration of the analysed hormone when the days are the longest of the year. Similar findings were reported by Mäenpää et al. [1988a], who observed slightly lower concentrations of 1.25-dihydroxycholecalciferol in pregnant mares in the winter compared to nursing mares in the summer. Meanwhile, the concentration of 25-hydroxyvitamin D was significantly higher (by 47.6%) in the summer months compared to the winter months [Mäenpää et al. 1987]. Also Enbergs et al. [1996] found plasma concentrations of calcidiol to be significantly higher when horses were on pasture compared to the winter period.

In many respects, horses differ considerably from other domestic animals in the metabolism and function of vitamin D₃ and its active form, 1.25-dihydroxycholecalciferol. The concentrations of calcidiol and calcitriol are significantly lower in horses compared to the other species [Mäenpää et al. 1988a, Breidenbach et al. 1998, Harmeyer and Schlumbohm 2004]. The low concentrations of 1.25(OH)₂-D₃, found in the present study could be regarded as a rachitogenic factor in other species of animals. Nevertheless, spontaneous rickets or bone demineralization are very rare to occur in horses. El Shorafa et al. [1979] showed experimentally that it is extremely difficult to induce rickets in horses by limiting vitamin D₃, sunlight, or both. For this reason, the role and significance of this vitamin in calcium homeostasis in the horse should continue to be discussed.

CONCLUSIONS

This study demonstrated that other than IGF-I concentration in Thoroughbred horses and 1.25(OH)₂-D₃ concentration in Anglo-Arabians, the plasma concentration of the analysed hormones was higher in the summer compared to the winter season in the other breeds as well as in colts and fillies. Significant differences in IGF-I concentration between seasons were found in Hucul horses ($P \leq 0.01$) and in colts ($P \leq 0.05$). Knowledge and monitoring of the physiological concentrations of IGF-1 and vitamin D₃ metabolites in different seasons of the year, combined with analysis of growth rate during these periods may contribute to the improvement of the horse management and feeding system. This is particularly important in the winter season, in which deficiency of these hormones may reduce the growth rate of growing horses. Because of the association between IGF-I and vitamin D₃ concentration and the frequency of different developmental orthopaedic diseases [Jeffcott 1996, Sloet van Oldruitenborgh-Oosterbaan et al. 1999], this information could be potentially used in veterinary practice, e.g. when analysing pathogenesis of osteochondrosis.

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WPLYW SEZONU NA KONCENTRACJĘ INSULINOPODOBNEGO CZYNNIKA WZROSTU I ORAZ 1,25 DWUHYDROKSYCHOLEKALCYFEROLU W OSOCZU KRWI KONI

Streszczenie. W sezonie letnim koncentracja IGF-I w osoczu krwi koni czystej krwi arabskiej, angloarabskich i huculskich a także ogierków i klaczek była wyższa w porównaniu z sezonem zimowym. Istotne różnice w stężeniu IGF-I pomiędzy sezonami wykazano u koni huculskich ($P \leq 0,01$) i ogierków ($P \leq 0,05$). Nie stwierdzono istotnego wpływu sezonu na koncentrację $1,25(\text{OH})_2\text{-D}_3$ u badanych ras koni. Obserwowano jednak, że stężenie tego hormonu w osoczu krwi koni bez względu na rasę (oprócz koni angloarabskich) i płeć było zawsze wyższe w sezonie letnim niż zimowym. Znajomość i monitorowanie fizjologicznych stężeń IGF-I i metabolitów witaminy D3 w różnych porach roku w powiązaniu z analizą tempa wzrostu w tych okresach może przyczynić się do usprawnienia systemu utrzymania i żywienia koni. Ze względu na istniejący związek pomiędzy tymi hormonami a częstotliwością występowania ortopedycznych chorób rozwojowych informacje te mogą też być potencjalnie wykorzystane w praktyce weterynaryjnej.

Słowa kluczowe: IGF-I, konie, płeć, rasa, sezon, witamina D3

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