Evaluation of the influences of exercise, birth date, and osteochondrosis on plasma bone marker concentrations in Hanoverian Warmblood foals

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Objective—To determine whether plasma concentrations of bone turnover markers in growing Hanoverian foals are influenced by age, housing conditions, or osteochondrosis.

Animals—165 healthy foals and 119 foals with osteochondrosis.

Procedures—Foals were allocated according to birth date and housing management into groups of early-born (born before March 31, 2001; n = 154 foals, 88 of which were healthy and 66 of which had osteochondrosis) and late-born (born after March 31, 2001; 130 foals, 77 of which were healthy and 53 of which had osteochondrosis) foals. Plasma osteocalcin and carboxyterminal propeptide of type I collagen concentrations were analyzed as markers of bone formation, and carboxyterminal telopeptide of type I collagen concentration was analyzed as a marker of bone resorption. Foals underwent radiographic evaluation to screen for osteochondrosis.

Results—Plasma concentrations of osteocalcin, carboxyterminal propeptide of type I collagen, and carboxyterminal telopeptide of type I collagen decreased with age, but these changes were more distinct in late-born foals than in early-born foals. Neither sex nor predisposition to develop osteochondrosis affected the pattern of bone marker changes in either group.

Conclusions and Clinical Relevance—An age-related decrease in concentrations of bone markers was seen during the first 200 days of life. Changes in bone marker concentrations were similar for foals with osteochondrosis and healthy foals. The correlation between the decrease in bone marker concentration and date of birth indicates that there are differences in skeletal development between early- and late-born foals. (*Am J Vet Res* 2007;68:1319–1323)

Osteochondrosis has been described as a disturbance in endochondral ossification in fast-growing animal species and humans.[1,2] Suspected etiologic factors for osteochondrosis in horses include genetic predisposition, endocrinologic dysfunction, biomechanical stress, nutritional imbalances, and rapid growth.[2] Osteochondrosis appears to affect bone metabolism in addition to the damage it causes to cartilage. Lower bone mineral density was reported in foals with the most severe osteochondrosis scores in 1 study.[3] Bone metabolic activity in animals with osteochondrosis is also reflected by serum osteocalcin concentration, a marker of bone turnover and mineralization, and in an earlier study,[4] osteocalcin concentrations were significantly correlated with severity of osteochondrosis in foals during the first months after birth. In the same study,[4] sprint exercise or pasturing in 5-month-old Dutch Warmblood foals was associated with higher bone mineral density than in foals kept predominantly in stalls.[3] Additionally, calcium concentration and pyridinoline cross-links increased significantly less in subchondral bone in foals confined to stalls than in those raised on pasture or trained.[5] Correspondingly, stall confinement in Arabian weanlings and yearlings has been associated with lower bone mineral density than in those maintained on pasture.[6,7] A transient decrease in serum osteocalcin concentration has also been observed in foals transferred from pasture to stables.[8] In general, exercising

Abbreviations

PICP	Carboxyterminal propeptide of type I collagen
ICTP	Carboxyterminal telopeptide of type I collagen

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young racehorses is known to increase bone mineral density on the dorsal aspect of the third carpal bone after several months of training.[9] These bone remodeling processes are necessary for the skeleton to adapt to mechanical stresses. Changes in bone metabolism are reflected by bone markers in blood and urine, and bone markers are useful for monitoring skeletal growth and development to assess risks to skeletal modeling.[10] In horses, several bone markers may be used to monitor both bone matrix formation and degradation: for the former, PICP and bone-specific alkaline phosphatase are measured, whereas for the latter, total deoxypyridinoline, ICTP, and the telopeptide of type I collagen are measured.[11,12] Furthermore, osteocalcin is the primary noncollagenous protein of bone that is synthesizd by osteoblasts, and osteocalcin is assumed to be associated with mineralization. Osteocalcin is believed to be a marker of osteoblastic activity, not a marker that primarily reflects metabolism of bone matrix.[13] The objective of the study reported here was to determine whether bone markers in growing foals follow characteristic patterns influenced by age, housing conditions, and osteochondrosis.

Materials and Methods

This study was part of a larger study investigating associations between body weight, feeding practices, housing conditions, and genetics and development of osteochondrotic lesions in Warmblood foals. The overall study included 629 Hanoverian Warmblood foals (308 colts and 321 fillies) born from December 26, 2000, to July 1, 2001, on 83 farms in Germany. Most mares and foals were housed in stalls from November 2000 until March 2001 and were turned out to pasture all day and night from the second half of April 2001 until October 2001. During the monitoring period, blood samples were collected every second month from all foals. At the first sampling point, foals were 1 to 49 days old. Because of farm breeding routines, some foals were omitted from examination and blood collection 1 or more times. Information on osteochondrosis lesions was collected by obtaining radiographs from foals at 6 to 9 months of age[a,b] (**Table 1**). Radiographic examination included 90° lateromedial views of both femoropatellar joints, 135° plantarolateral-dorsomedial views of the tibiotarsal joints, and 90° lateromedial views of the dorsal aspect of the distal portions of the metacarpus and metatarsus. A subpopulation of 284 foals (165 healthy foals and 119 foals with osteochondrotic lesions) was included in the present study for monitoring bone marker concentrations in blood. The criteria for inclusion in that subpopulation were ≥ 5 foals on a farm and similarities in feeding and housing conditions.

Foals were allocated into 2 groups according to birth date and type of housing. Early-born foals were those born before March 31, 2001 (n = 154 foals, 88 of which were healthy and 66 of which had osteochondrosis), and late-born foals were those born after March 31, 2001 (130 foals, 77 of which were healthy and 53 of which had osteochondrosis). Both groups of foals were further subdivided into age groups of 0 to 50 days, 51 to 100 days, 101 to 150 days, and 151 to 200 days. Because blood was sampled on 3 days every second month during the observation period but age classification included 4 categories, the 151 to 200 days group included only 108 foals.

Sample collection—Jugular venous blood samples were collected, and blood was placed in tubes containing lithium heparin (20 U of heparin/mL of blood[1]) and EDTA (15% tripotassium EDTAC[a]). Blood samples were centrifuged (1,559 × *g* for 10 minutes) within 10 minutes after collection. Plasma samples were frozen in liquid nitrogen at –196°C for 1 week and then at –20°C until analysis.

Statistical analysis—Plasma osteocalcin concentration was analyzed by use of a competitive ELISA[d] that has been validated for use in horses.[10] The assay measures newly synthesized, intact osteocalcin, with monoclonal mouse antibody and human osteocalcin used as standards. All samples were initially diluted 1:11 with assay wash buffer. Analyses were performed according to the manufacturer's instructions. The assay detection limit was 0.45 µg/L, and coefficient of variation was 4.6%. Plasma PICP concentration was analyzed with a commercially available radioimmunoassay[e] validated for use in horses.[10] Plasma samples were diluted 1:5 with the zero standard. Concentrations of PICP were measured in 100-µL plasma samples according to the manufacturer's instructions. The limit of sensitivity was 1.2 µg/L, and the coefficient of variation was 4.5%. Plasma concentrations of ICTP were measured in 100-µL samples by use of radioimmunoassay[f] according to a described method.[10] Assays were performed according to the manufacturer's instructions. The assay detection limit was 0.5 µg/L, and coefficient of variation was 5.1%.

Data for bone markers were summarized and expressed as mean ± SD. Normal distribution of the data was tested by use of the Shapiro-Wilk W test. Because data were not normally distributed, nonparametric tests were used. The effects of time were tested by use of the

Table 1—Number (%) of foals with radiographically determined osteochondrosis lesions by site in 629 Hanoverian Warmblood foals[a,b] and in a subpopulation of 284 of those foals that included 165 healthy foals and 119 foals with osteochondrosis.

	Site of lesion			
Affected foals	Metacarpophalangeal and metatarsophalangeal joints	Tibiotarsal joint	Femorotibial joint	Total
In overall population	134 (52)	86 (33)	39 (15)	259*
In the subpopulation	66 (50)	46 (34)	22 (16)	134†

*33 foals developed osteochondrosis lesions in several joints. †15 foals developed osteochondrosis lesions in several joints.

Kruskal-Wallis 1-way ANOVA, and differences among the effects of sex, birth date, and predisposition to osteochondrosis were tested by use of the Mann-Whitney *U* test. For all comparisons, values of $P < 0.05$ were considered significant.

Results

Plasma concentrations of osteocalcin, PICP, and ICTP decreased significantly during the first 200 days of life, with the decrease in plasma osteocalcin concentration being most distinct (change from 0 to 50 days to 51 to 100 days, $P < 0.05$; change from 51 to 100 days to 101 to 150 days and from 101 to 150 days to 151 to 200 days, $P < 0.05$) and the changes in PICP and ICTP concentrations being more moderate (change from 0 to 50 days to 51 to 100 days, $P < 0.05$; change from 51 to 100 days to 101 to 150 days and from 101 to 150 days to 151 to 200 days was not significant).

Decreases in plasma osteocalcin, PICP, and ICTP concentrations were more pronounced in late-born foals than in early-born foals (**Figures 1–3**), whereas sex and predisposition to develop osteochondrosis had no influence on the course of bone marker changes in either group. The ratio of PICP to ICTP concentrations increased during the first 200 days after birth, with the highest increase detected in late-born foals (**Tables 2 and 3**). Concomitantly, the ratio of osteocalcin to PICP or ICTP concentration decreased with age, with a more pronounced decrease detected in late-born foals.

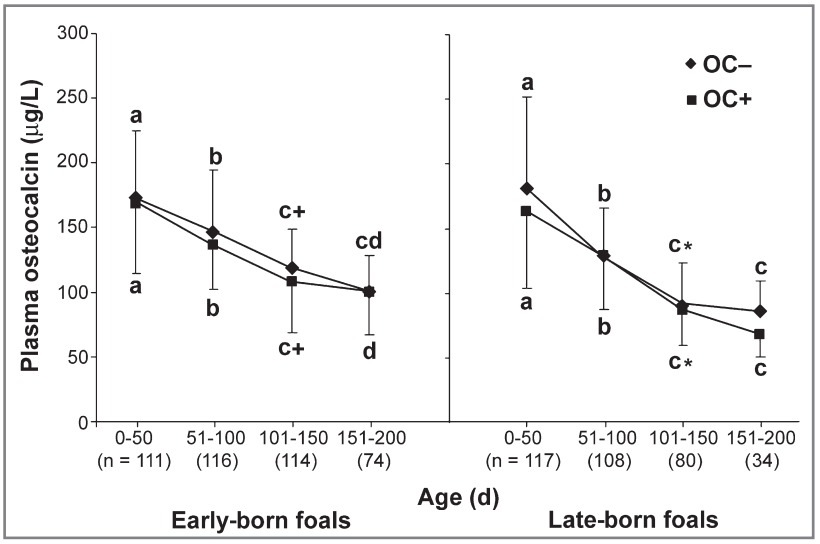

Figure 1—Graph depicting mean ± SD plasma osteocalcin concentrations by birth date (early- vs late-born) and whether affected by osteochondrosis in 284 Hanoverian Warmblood foals. OC– = Not affected with osteochondrosis. OC+ = Affected with osteochondrosis.[a-d] Mean values with different letters are significantly ($P < 0.05$) different between early- and late-born foals. +,*Mean values for OC– with different symbols are significantly ($P < 0.05$) different between early- and late-born foals. +,*Mean values for OC+ with different symbols are significantly ($P < 0.05$) different between early- and late-born foals.

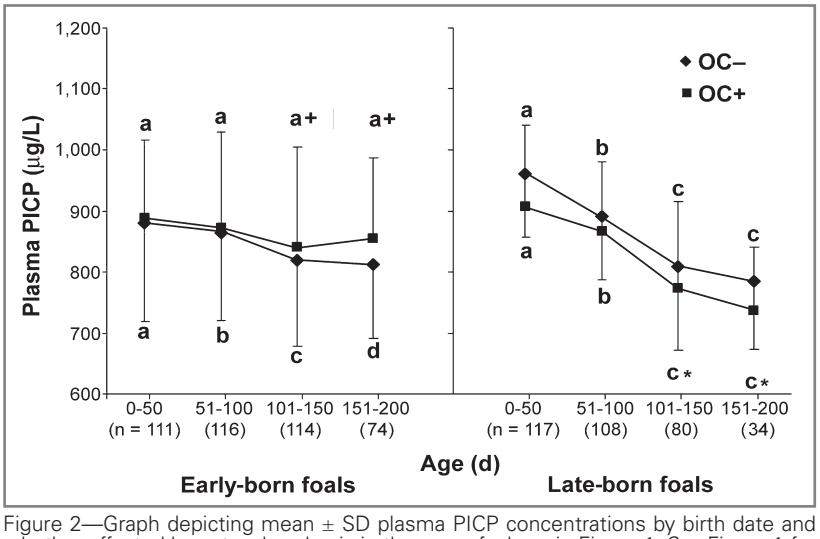

Figure 2—Graph depicting mean ± SD plasma PICP concentrations by birth date and whether affected by osteochondrosis in the same foals as in Figure 1. *See* Figure 1 for key.

Discussion

Active growth begins immediately after birth in horses and continues at the maximum rate until they are 9 months of age, at which time the rate of growth decreases until adult size is attained.[14] As has been reported in other studies,[11,12] concentrations of bone markers measured in the present study were negatively correlated with age. On the other hand, another marker of bone resorption, telopeptide of type I collagen, increases in concentration in Dutch Warmblood foals during the first months after birth.[15] However, concentrations of osteocalcin and ICTP were higher and those of PICP were lower in the present study than values reported in an earlier study.[10] These discrepancies may be attributable to genetic differences between Warmblood foals and Thoroughbred foals because differences in osteocalcin concentrations between different horse types have been reported.[16]

In the present study, bone marker concentrations were not different between healthy foals and those with osteochondrosis. These findings are partly in contradiction to those of other investigators,[4] who found a positive association between osteocalcin concentration and severity of osteochondrosis in Dutch Warmblood foals. Because we recorded only the total osteochondrosis score, no conclusions could be drawn about differences in osteocalcin concentration and severity of osteochondrosis.

The most striking results from the present study were the differences in bone turnover between early- and late-born foals. Although there were no differences between healthy foals and those that developed osteochondrosis lesions, the decrease in osteocalcin, PICP, and ICTP concentrations was more distinct in late-born foals than in early-born foals. The ratio between osteocalcin and ICTP concentrations was higher in early-born

foals between days 101 and 150, indicating enhanced bone turnover rate in those foals. Furthermore, the ratio between PICP and ICTP concentrations was higher in late-born foals between days 150 and 200, especially in foals that did not develop osteochondrosis lesions, suggesting increased type I collagen formation in this group.

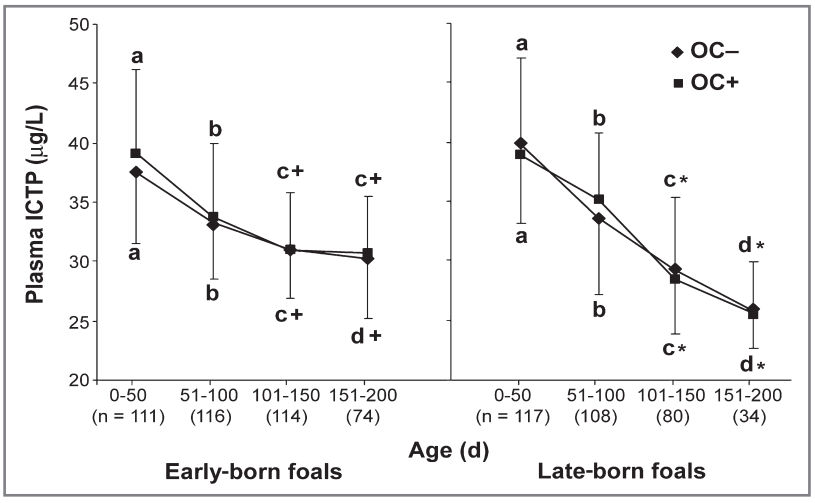

Figure 3—Graph depicting mean ± SD plasma ICTP concentrations by birth date and whether affected by osteochondrosis in the same foals as in the preceding figures. *See* Figure 1 for key.

Table 2—Summary of age-related changes in plasma osteocalcin, PICP, and ICTP concentrations in the same subpopulation of foals as in Table 1. Values represent concentrations in units of µg/L and are expressed as mean ± SD.

Age (d)	Osteocalcin	PICP	ICTP
1–50	172 ± 61.2[a]	913 ± 139[a]	38.8 ± 6.92[a]
51–100	135 ± 41.2[b]	876 ± 127[b]	34.0 ± 5.85[b]
101–150	104 ± 34.6[c]	821 ± 145[c]	30.1 ± 5.00[c]
151–200	91.6 ± 28.6[d]	812 ± 127[c]	29.0 ± 5.00[c]

[a-d]Different superscripts indicate significantly ($P < 0.05$) different values within a column.

Among other factors, such as nutrition, the different patterns in bone turnover could be attributed to differences in exercise, which the early-born foals lacked because they were kept indoors, whereas late-born foals were turned out to pasture immediately after birth. Stall confinement in the first month after birth apparently delayed the process of bone maturation. The incidence of osteochondrosis was 10% higher in early-born foals than in late-born foals, which underlines the importance of pasture exercise in prevention of osteochondrosis.[g]

Our findings corroborated those from an earlier study,[5] in which significant differences in subchondral bone development in 5-month-old Warmblood foals confined in stalls and those maintained on pasture were reported. The lack of exercise during stall confinement affected collagen maturation at the site that bears weight during pasture exercise. Calcium and pyridinoline contents were higher at this site in pastured foals than those in confined foals. However, implementation of a standardized training program led to changes in subchondral bone maturation in growing foals, similar to those with pasture exercise.[5] Our findings also supported those from a 2003 study,[15] in which the bone turnover rate reflected by bone marker concentrations was lower in pastured foals than in foals kept in stalls or trained. Furthermore, bone mineral density in the dorsomedial area of the third carpal bone was lower in

Table 3—Mean ± SD values for ratios of osteocalcin-to-PICP concentration, osteocalcin-to-ICTP concentration, and PICP-to-ICTP concentration by birth category (early- vs late-born), age subgroup, and osteochondrosis status (affected or nonaffected) in foals in the same subpopulation of foals as in the preceding tables.

	Age (days)			
	Early-born foals (born before 31 March)		Late-born foals (born after 31 March)	
Variable	OC–	OC+	OC–	OC+
Osteocalcin-PICP				
1–50	0.21 ± 0.08[a]	0.20 ± 0.07[a]	0.19 ± 0.07[a]	0.17 ± 0.07[a]
51–100	0.17 ± 0.06[b]	0.16 ± 0.05[b]	0.14 ± 0.05[b]	0.15 ± 0.05[b]
101–150	0.17 ± 0.04*[,b]	0.13 ± 0.04†[,c]	0.11 ± 0.03†[,c]	0.11 ± 0.04†[,c]
151–200	0.13 ± 0.05[c]	0.11 ± 0.04[c]	0.11 ± 0.03[c]	0.10 ± 0.02[c]
Osteocalcin-ICTP				
1–50	4.8 ±1.3[a]	4.3 ± 1.1[a]	4.7 ± 1.9[a]	4.2 ± 1.5[a]
51–100	4.4 ± 1.3[a,b]	4.1 ± 1.1[a]	3.8 ± 1.3[b]	3.8 ± 1.0[a]
101–150	3.8 ± 0.9*[,b]	3.6 ± 1.1*[,b]	3.0 ± 0.9†[,c]	3.2 ± 0.8†[,b]
151–200	3.3 ± 0.9*[,b,c]	3.5 ± 1.4*[,b]	3.3 ± 1.1*[,b,c]	2.7 ± 0.8†[,b]
PICP-ICTP				
1–50	23.8 ± 5.1[a]	23.3 ± 5.8[a]	25.7 ± 4.1[a]	24.8 ± 7.4[a]
51–100	26.6 ± 5.2[b]	26.4 ± 6.2[b]	26.9 ± 5.1[a,b]	25.6 ± 4.9[a]
101–150	26.6 ± 5.8[b]	27.8 ± 6.7[b]	28.9 ± 7.1[b]	28.4 ± 8.4[b]
151–200	27.6 ± 4.8*[,b]	27.9 ± 6.2*[,b]	31.1 ± 4.2†[,b,c]	29.3 ± 3.8*[,†,b]

*,† Different symbols indicate significantly ($P < 0.05$) different values within a row. OC– = Not affected with osteochondrosis. OC+ = Affected with osteochondrosis. *See* Table 2 for remainder of key.

foals housed in stalls.[3] These findings suggest that lower rates of skeletal remodeling during growth resulted in increased bone density. Similarly, children with 10% greater bone mass at the end of puberty had concentrations of bone turnover markers 50% less than those of children with lower bone mass.[17] However, it is interesting that our findings were most obvious 100 days after foaling despite the fact that the early-born foals were housed in stalls, especially during the first 100 days after birth. We concluded that bone markers reflected long-term effects on skeletal development and a delay in bone maturation.

In addition to bone metabolism, cartilage metabolism is also positively affected by pasture exercise in growing foals. Earlier authors have reported[18] that cartilage metabolism in pasture-exercised foals was more active and proteoglycan synthesizing capacity was higher than those in foals confined to stalls. Those authors concluded that pasture exercise was best for development of a healthy musculoskeletal system, whereas stall confinement resulted in retardation of cartilage development.

Results of the present study indicated that lack of exercise in early-born foals leads to retardation of bone maturation, reflected by a more prolonged decrease in bone marker concentrations. Delayed bone metabolism could increase the risk for musculoskeletal injury. Routine analysis of bone markers that reflect bone turnover appears to be useful for monitoring bone metabolism in growing foals in the first months after birth. However, despite the fact that bone metabolism is affected by osteochondrosis, our results indicated that bone marker concentrations are not useful for detecting differences between healthy foals and foals that develop osteochondrosis.

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