significant exercise-related changes in the serum levels of two biomarkers of collagen metabolism in young horses

R. C. Billinghurst D.V.M., Ph.D., Associate Professor†*, P. A. J. Brama D.V.M., Ph.D., Assistant Professor‡, P. R. van Weeren D.V.M., Ph.D., Professor‡, M. S. Knowlton B.S., Research Associate† and C. W. McIlwraith B.V.Sc., Ph.D., Professor†

†Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80523, USA
‡Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Summary

Objective: To identify metabolic biomarkers that can be evaluated in serum for monitoring the effects of exercise on skeletal development in mammals.

Design: Sera of foals from three groups (box-stall rest, pasture and training) were serially collected over the first 5 months of life and assayed for eight biomarkers of cartilage and bone metabolism. Sub-populations from each group were sampled for an additional 6 months of identical exercise.

Results: When expressed as a percentage of baseline values, lower serum levels of the carboxy-terminal propeptides of type II collagen (CPII), and higher serum levels of the cross-linked telopeptide fragments of degraded type I collagen (CTx1) were found in the trained foals compared to the other groups. Significant differences disappeared in those foals sampled during an additional 6 months of identical exercise. The most significant correlations were between serum biomarkers of bone metabolism, being positive between anabolic markers and negative between anabolic and catabolic markers. Serum levels of CTx1 and CPII significantly increased with age in all groups throughout the study.

Conclusions: We have identified two markers of collagen metabolism, CPII and CTx1, as potential serum indicators of the exercise effects on the developing skeletal system in horses. Forced exercise during the first months postpartum appeared to have a negative effect on collagen turnover when compared to levels in pastured foals. Routine monitoring of collagen biomarkers in sera of exercising young mammals may allow for the early detection of abnormalities in skeletal tissue metabolism and for subsequent intervention before permanent damage occurs.

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Introduction

All components of articulating joints participate in load transmission, and failure of the bone, articular cartilage, muscles, ligaments/tendons or nerves of a joint may lead to exercise-induced damage. The ability to detect these changes at an early stage would potentially enhance one’s ability to modulate exercise programs on an individual basis, thereby avoiding irreversible damage to joint structures. One such diagnostic tool, that has been receiving expanding attention for the early diagnosis and monitoring of orthopedic diseases in general, is the evaluation of body fluid levels of byproducts of skeletal tissue metabolism.

In humans, it has generally been shown that the type, intensity and duration of exercise are critical in determining its effect on bone metabolism. For example, serum levels of biomarkers of bone formation, such as bone-specific alkaline phosphatase and osteocalcin, have been shown to either increase¹–⁶ or decrease⁵,⁷–⁹ in human athletes, dependent upon the exercise regimen. Similarly in animals, the effects of exercise on bone metabolism vary with the type and duration of exercise. Significantly greater subchondral bone volume and bone formation have been identified in the metacarpal bones of treadmill-exercised compared to hand-walked 2-year-old horses¹⁰, despite no significant differences in serum osteocalcin levels between the two groups of horses over 6 months¹¹. In other studies, baseline serum osteocalcin levels dropped during a 6-week training program¹², but significantly increased after 6 months of race training¹³ in young Standardbred horses. In a study involving 2-year-old Thoroughbreds, it was shown, by evaluating the serum levels of three biomarkers of bone metabolism serially sampled over 12 months, that there was a general increase in bone turnover with a regimen including treadmill exercise compared to normal daily walking¹⁴. We have shown more recently that the serum levels of a panel of biomarkers of both bone and cartilage metabolism significantly increased with treadmill exercise
in horses and could be used to discriminate exercise-induced changes from pathology due to surgically induced osteoarthritis. As with all other joint tissues, including the subchondral bone, it is generally believed that systemic factors including age, sex and genetics establish basic properties of articular cartilage. However, local biomechanical factors, such as degree of joint loading and deformity, have a significant effect on the final qualities of articular cartilage. It has been hypothesized that regular joint loading during youth is important for the development of a well-organized and strong articular cartilage collagen network, and that this contributes to the prevention of osteoarthritis later in life. The maintenance of normal articular cartilage composition, structure, and function depends on repetitive joint use. Marked decreased use (disuse) or abuse of normal joints through high intensity impact loading may be associated with degenerative changes in the articular surface. Moreover, normal use of abnormal joints, such as malaligned or unstable joints, may increase the risk of degenerative joint disease. To date, few studies have looked at the effect of exercise specifically on articular cartilage and on body fluid levels of biomarkers of articular cartilage metabolism. The two main structural components of the articular cartilage matrix are type II collagen and aggrecan. Changes suggestive of microdamage and weakening of the type II collagen network of articular cartilage have been reported in strenuously exercised young racehorses, whereas moderate exercise has been shown to have a beneficial effect on glycosaminoglycan (GAG) content of articular cartilage in young and adult horses. It has been reported that the serum and synovial fluid levels of keratan sulfate, a GAG found on the individual proteoglycan molecules of aggrecan, are increased in young horses and ponies as a result of exercise.

The young horses used in the current study were part of a larger project designed to investigate the effects of exercise on musculoskeletal development, as well as the occurrence and severity of the developmental orthopedic disease, osteochondrosis. Foals were subjected to stall rest, pasturing or an increasing level of training from 1 week to 5 months of age, after which time a cohort from each group was allowed free pasture access for an additional 6 months. For the training group, an exercise regimen was chosen that was extreme enough to allow for the detection of a treatment effect in the foals during their first 5 months of life. It has already been reported that the exercise regimen used did not appear to have an etiological role in the osteochondrotic joint lesions identified in these foals. However, there were some significant exercise-induced changes noted in the mineral density of certain appendicular bones, in tendon and subchondral bone composition, and in the proteoglycan synthetic capacity of articular cartilage from various joints of the foals.

We hypothesized that the effects of the different levels of exercise on skeletal tissue metabolism in these foals during the first months of life could be detected at the molecular level by evaluating the concentrations of a panel of eight biomarkers of cartilage and bone metabolism in serially sampled blood. From the results of these assays, it may be possible to determine which biomarkers could be used to identify and modulate those exercise regimens that are harmful to proper skeletal development, before there is permanent damage.

Methods

ANIMALS

This study involved 43 Dutch Warmblood foals (23 males, 20 females) that were all born in the same year and housed on the same stud farm in the Netherlands. They were part of a large project investigating the effect of exercise on osteochondrosis and musculoskeletal development in the foal. All sires and 11 of the 43 dams had radiographic evidence of osteochondrosis in the femoropatellar/femorotibial (stifle) or tibiotalar (hock) joints.

EXERCISE REGIMENS

The foals were kept in a paddock with the mares for the first week after birth and then allotted randomly to three groups (blocked for sex and sire) until weaning at 5 months of age. One group (Groupbox) was confined to box stalls of 3×3.5 m (n=14). Another group (Grouptraining) was kept in similar-sized box stalls but also given an increasing number of gallop sprints for 6 days a week (n=14). The number of sprints was 16 from day 8 to 24, 24 from day 25 to 38, and then 32 and 16 sprints on alternate days until 5 months of age. After the sprint training sessions, these foals were given 0.5 h of free exercise in a 48×15 m enclosure. A third group (Grouppasture) was kept at pasture 24 h a day (n=15). At 5 months of age, eight foals were randomly removed from each group for further examinations. The remaining 19 foals were kept together for an additional 6 months under similar conditions of an open loose box with paddock access and no training. The Utrecht University Ethical Committee approved all procedures involving these horses.

SAMPLES

Blood samples were obtained from the jugular veins of the 43 foals in the first week after birth and then monthly until 5 months of age, after which time they were collected at 7, 9 and 11 months in the remaining 19 foals. All blood samples were collected at the same time in the morning (08:00 h) in an attempt to control for the diurnal variation often noted in humans, especially for biomarkers of bone resorption, such as with serum CTX1 measured in this study. The blood was collected into serum tubes and centrifuged at 3000 rpm for 10 min within 30 min of collection. The sera were aliquoted into cryotubes and frozen to –20°C within 90 min. Upon receipt of the frozen samples at the Orthopaedic Research Center of Colorado State University, the aliquots were kept at –80°C until assayed for biomarker levels.

BIOMARKER ANALYSES

Collagen markers

The individual alpha chains of procollagen molecules are synthesized with extensions at both the amino- (N-) and carboxy- (C-) termini called ‘propeptides’. With extracellular fibril formation, there is proteolytic removal of these propeptides from the triple-helical procollagen molecules and these can be detected in tissues and body fluids as indicators of collagen synthesis. The serum levels of the C-propeptide of type I collagen were estimated using a
commercially available radioimmunoassay (PICP, DiaSorin, Stillwater, MN) that has been validated for equine use. In our study, the equine sera were diluted 1:10, and the intra- and inter-assay coefficient of variation (CV) was less than 6 and 7%, respectively. Type II collagen synthesis was estimated using a newly available commercial ELISA (CPII Assay, IBEX Technologies, Montreal, Canada). This assay utilizes the same rabbit polyclonal IgG antiserum employed in a radioimmunoassay for the C-propeptide of type II collagen that has previously been validated for equine use. In our study, the equine sera were diluted 1:2, and the intra- and inter-assay CV for CPII levels was less than 4 and 11%, respectively.

It is believed that the initiating factor in the degradation of the triple-helical collagen molecule is its cleavage into 3/4- and 1/4-length fragments by mammalian collagenases belonging to the matrix metalloproteinase (MMP) family, specifically MMP-1, MMP-8 and MMP-13. The collagenase-generated termini of the individual cleaved alpha chain fragments are ‘neoepitopes’ and antibodies have been produced to detect these in tissues and fluids. We estimated the levels of type II collagen degradation in the foals by using the recently described 234CEQ assay. This immunoassay was developed to react specifically with the collagenase-generated C-termini of the 3/4-length fragment of type II collagen cleavage by collagenases was less than 4 and 14%, respectively. An estimation of type I collagen cleavage by collagenases was made by incorporating the previously described COL2-3/4short ELISA. As this assay detects 3/4-length fragments of both type I and II collagen, the relative amount of collagenase-generated fragments of type I collagen was calculated by subtracting the 234CEQ values for type II collagen cleavage from the COL2-3/4short values for types I and II collagen cleavage. In our study, the equine sera were diluted 1:10, and the intra- and inter-assay CV for COL2-3/4short was less than 11 and 33%, respectively.

We also evaluated overall type I collagen turnover by measuring the serum levels of cross-linked degradation products with a sandwich assay (Serum CrossLaps™ One Step ELISA, Osteometer A/S, Herlev, Denmark) according to the manufacturer’s instructions. This assay recognizes cross-linked and β-isomerized C-terminal telopeptide fragments (CTX1) released into the circulation when type I collagen is degraded by osteoclasts during bone resorption. This ELISA has been validated by the manufacturer for use in the horse. In our study, the equine sera were assayed neat, and the intra- and inter-assay CV for CTX1 was less than 4 and 9%, respectively.

**Noncollagenous protein markers**

Two molecular markers that are believed to be indicative of anabolic processes in skeletal tissues are osteocalcin and the chondroitin sulfate 846 epitope (CS-846). Osteocalcin is a small, noncollagenous protein synthesized mainly by osteoblasts and is believed to be associated with mineralization of newly formed osteoid. A commercial ELISA (NovoCalcin®, Metra Biosystems, Mountain View, CA), that detects equine osteocalcin, was used to assay serum levels of osteocalcin, according to the manufacturer’s instructions. In our study, the equine sera were diluted 1:5, and the intra- and inter-assay CV for osteocalcin was less than 4 and 9%, respectively.

Aggrecan molecules are a main component of the extracellular matrix of articular cartilage and are responsible for the compressive strength of this tissue. Studies have suggested that aggrecan synthesis is reflected by the presence of an epitope called 846, found on some newly synthesized aggrecan molecules. A newly developed commercial ELISA for the 846 epitope (CS-846, IBEX Technologies, Montreal, Canada) was used in our study. It utilizes the same mouse monoclonal IgM antibody employed in a radioimmunoassay for this epitope that has previously been described. In our study, the equine sera were diluted 1:5, and the intra- and inter-assay CV for CS-846 was less than 8 and 22%, respectively. As a general indicator of proteoglycan turnover, the serum levels of sulfated glycosaminoglycans (sGAG) were determined using a previously described dimethylmethylene blue (DMMB) colorimetric assay. In our study, the equine sera were diluted 1:2, and the intra- and inter-assay CV for sGAG levels was less than 10 and 11%, respectively.

**STATISTICAL ANALYSIS**

The measurements obtained for the majority of the biomarkers evaluated in this study (except osteocalcin and PICP) did not follow a Gaussian distribution as determined using a Kolmogorov–Smirnov test. Therefore, all biomarker values were transformed to logarithms (base 10) allowing the use of statistical tests based on a normal distribution. In addition to determining the precision of each assay utilizing equine sera, by calculating the intra- and inter-assay CV% for each assay (as reported above), linearity was confirmed by serially diluting samples and comparing the observed values with the expected values. To compare the effects of both age, exercise group and the interaction of age and exercise group on the serum levels of each of the biomarkers in all foals (n=43) over the first 5 months of age, a repeated measures ANOVA was performed using an autoregressive correlation structure for the repeated measures effect of months. This comparison was also performed for the levels of all biomarkers for those foals (n=19) that were sampled from 0 to 11 months. This was to determine if the exercise regimens used from 1 week to 5 months of age had any lasting effects on serum biomarker levels, after allowing these foals similar exercise from 5 to 11 months of age. Where significance was detected (P<0.05), a Least Squares Means procedure was used involving t tests to detect the significant pair-wise differences in mean serum levels between the exercise groups and between months for that biomarker. Due to significant differences between the groups in the baseline (week 1) serum values of some biomarkers (Table I), the percent change from baseline in the serum levels for each foal at each time point was calculated for each biomarker. This allowed for the detection of exercise effects on the baseline levels, determined before the foals were put into their respective groups. Mean foal weights were not significantly different between the groups (Table I).

Significant correlations between the serum levels of all biomarkers were determined by Pearson correlation analysis, using the logarithmically transformed values. All statistical analyses were performed using SAS software, version 8e (SAS Institute, Cary, NC).
Results

EXERCISE-RELATED CHANGES IN SERUM BIOMARKER LEVELS DURING THE FIRST 5 MONTHS OF LIFE

There was a significant exercise-group effect on the serum levels of the type I collagen degradation biomarker CTx1 ($P=0.032$) and the type II collagen synthesis biomarker CPII ($P=0.005$). The percent change from baseline in the serum levels of CTx1 was significantly less in the Group pasture compared to both the Group training ($P=0.028$) and Group box foals ($P=0.019$). This suggested that free pasture exercise resulted in less type I collagen degradation than either forced strenuous exercise or box-stall rest in these young horses. After an initial drop from baseline values at 1 month (Fig. 1), the mean serum levels of CTx1 increased for all exercise groups, such that by 5 months of age, the groups had reached similar increases from baseline values (Group box $=184.1\pm16.2\%$, Group pasture $=200.2\pm20.1\%$, and Group training $=200.3\pm38.3\%$). Moreover, although there was no significant effect of exercise ($P=0.085$) on the serum levels of the cleavage fragments of type I collagen (calculated as $\text{COL2-3/4C}_{\text{short}}-234\text{CEQ}$), there were significantly higher levels in the Group box foals compared to Group pasture foals ($P=0.042$). This finding further suggests that there was increased type I collagen degradation with the forced exercise in the trained foals during the first 5 months of life.

There were significantly smaller increases from baseline for the serum levels of CPII in the Group training foals compared to both the Group pasture ($P=0.0015$) and Group box ($P=0.035$) foals, suggesting less type II collagen synthesis with forced exercise in these foals during the first 5 months of life. After an initial increase at 1 month of age for all foals (Fig. 2), the CPII levels in the Group box foals stayed essentially the same while the levels in the other two groups decreased at 2 months of age, and more or less increased thereafter. As with the CTx1 levels, all groups reached similar percent increases above baseline values for CPII at 5 months of age (Group box $=131.2\pm11.7\%$, Group pasture $=139.2\pm15.8\%$, and Group training $=144.8\pm11.5\%$).

None of the other biomarkers evaluated in this study revealed significant differences between the groups in terms of their respective percent changes from serum baseline values. Although the differences were not statistically significant, the percent change from baseline in the serum levels of PICP, a marker for type I (bone) collagen synthesis, was greater for the trained and stall-rested foals, compared to the pastured foals, at each sampling point during the 5-month exercise period (Fig. 3). Coupled with the findings of increased type I collagen degradation reported above, this suggests increased bone turnover in both these groups of foals.

THE RESIDUAL EFFECTS OF EXERCISE ON SERUM BIOMARKER LEVELS (0–11 MONTHS)

The serum levels of each biomarker were analyzed for a continuation of significant exercise and age effects over the 6 months following the end of the different exercise regimens in those 19 foals sampled until 11 months of age. Exercise group had no significant effect on the serum levels of any of the biomarkers evaluated in this study when considered over the entire 11 months. In other words, while CTx1 and CPII were significantly related to exercise group up to 5 months of age, these relationships disappeared in
the subsequent 6 months. This suggests that, with subsequent paddock exercise, the foals were able to recover from the previously described effects of the different exercise regimens on collagen metabolism during the first 5 months. This is supported by the fact that significant interaction between exercise group and age was identified for CTx1 ($P_{H11005}=0.0082$) for the entire 11 months, indicating that exercise did not have the same effect on the serum levels of this biomarker at all ages in these foals.

### CORRELATIONS BETWEEN THE SERUM BIOMARKER LEVELS DURING THE FIRST 11 MONTHS OF LIFE

Significant positive correlations were identified between some of the anabolic biomarkers, and between some of the catabolic markers evaluated in this study (Table II). The serum levels of PICP and osteocalcin, two putative markers of bone synthesis, had the highest correlation coefficient for any two biomarkers ($r_{H11005}=0.5821$, $P<0.0001$). The next two highest correlation coefficients were between these same two bone synthesis biomarkers (PICP and osteocalcin) and CS-846, a putative indicator of aggrecan synthesis ($r_{H11005}=0.4289$, $P<0.0001$). The two biomarkers of type I (bone) collagen degradation, CTx1 and COL2-3/4C_short, were also positively and significantly correlated ($r_{H11005}=0.1508$, $P=0.0073$).

The most significant negative correlations were all between a marker of catabolism and a marker of anabolism. The serum levels of the type I (bone) collagen degradation marker, CTx1, were significantly and inversely related to those for bone synthesis markers PICP ($r_{H11005}=-0.4135$, $P<0.0001$) and osteocalcin ($r_{H11005}=-0.4016$, $P<0.0001$), as well as the putative aggrecan synthesis marker CS-846 ($r_{H11005}=-0.4289$, $P<0.0001$). There were also significant negative correlations between the serum levels of the marker of type II (cartilage) degradation, 234CEQ, and the two putative markers of cartilage synthesis, CPII ($r_{H11005}=-0.1975$, $P=0.0004$) and CS-846 ($r_{H11005}=-0.1584$, $P=0.0044$).

### THE EFFECT OF AGE ON SERUM BIOMARKER LEVELS DURING THE FIRST 11 MONTHS OF LIFE

For each of the biomarkers evaluated, except the collagenase-generated neoepitopes of type I and type II

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### Table II: Correlations between the serum concentrations of biomarkers of cartilage and bone metabolism ($n=43$)

<table>
<thead>
<tr>
<th></th>
<th>CS-846</th>
<th>CPII</th>
<th>234CEQ</th>
<th>PICP</th>
<th>CTx1</th>
<th>C2C_short</th>
<th>Osteocalcin</th>
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<tr>
<td>sGAG</td>
<td>0.0310</td>
<td>0.0375</td>
<td>0.0828</td>
<td>−0.0289</td>
<td>−0.0327</td>
<td>0.0658</td>
<td>−0.0162</td>
</tr>
<tr>
<td>CS-846</td>
<td>−0.0333</td>
<td>−0.1584 (0.0044)</td>
<td>0.5204 (&lt;0.0001)</td>
<td>−0.4289 (&lt;0.0001)</td>
<td>0.1706 (0.0024)</td>
<td>0.4426 (&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>CPII</td>
<td>−0.1975 (0.0004)</td>
<td>−0.1362 (0.014)</td>
<td>−0.0934</td>
<td>0.0192</td>
<td>0.0434</td>
<td></td>
<td></td>
</tr>
<tr>
<td>234CEQ</td>
<td>−0.0488</td>
<td>−0.0179</td>
<td></td>
<td>−0.1044</td>
<td>−0.0609</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PICP</td>
<td>−0.4135 (&lt;0.0001)</td>
<td>0.0495</td>
<td>0.5821 (&lt;0.0001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTx1</td>
<td>−0.1508 (0.0073)</td>
<td>−0.4016 (&lt;0.0001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2C_short</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>−0.0157</td>
<td></td>
</tr>
</tbody>
</table>

Values are the Pearson correlation coefficients ($r$) for transformed (log10) marker values. Significant $P$ values (<0.05) are shown in parentheses. See text for definitions (C2C_short=COL2-3/4C_short).
collagens (COL2-3/4C_short and 234CEQ), there was a significant age effect for the serum levels during the first 11 months of life (Fig. 4). For all of the markers significantly affected by age, except CTx1 and CPII, the overall relationship was an inverse one of decreasing serum levels with increasing age (P<0.0001). Conversely, the serum levels of CTx1 (P=0.0001) and, less dramatically, CPII (P=0.027) were positively correlated to age in these foals. As would be expected given the strong correlation between age and weight (r²=0.92), these relationships were maintained for each biomarker and foal weight (not shown). There were no significant differences between any of the biomarkers based on foal gender.

Looking at each of these biomarkers, the mean serum levels of the synthesis biomarkers osteocalcin and CS-846, after increasing during the first month of life, sharply decreased with increasing age to 7 months before leveling off through to 11 months [Fig. 4(A, B)]. The mean serum levels of PICP also decreased with increasing age, but there was no initial increase during the first month of life [Fig. 4(C)]. CPII serum levels gradually increased with age in foals up to 5 months of age, before they leveled off from 7 to 11 months [Fig. 4(D)]. After an initial drop at 1 month of age, the serum CTx1 and GAG levels increased with age from 1 to 5 months in our foals, and this trend continued to 9 months for CTx1 [Fig. 4(E)] but reversed itself for serum GAG levels [Fig. 4(F)]. As described above, there were no significant relationships between the mean serum levels of the collagen cleavage-site neoepitopes and age in these foals [Fig. 4(G, H)], despite a trend for increasing COL2-3/4C_short levels with age.

Discussion

The ability to monitor the molecular activities of skeletal tissues through the evaluation of body fluid levels of metabolic byproducts or ‘biomarkers’ offers the opportunity for identifying disturbances indicative of early structural damage. The goal of this study was to identify which biomarkers, from a panel of well-characterized indicators of anabolic and catabolic processes within bone and cartilage, could monitor exercise-induced changes in these tissues in young, developing horses. Of the eight biomarkers evaluated during the first 5 months of life, only the changes in the serum levels of a specific C-terminal cross-linked telopeptide of type I collagen (CTx1) and the C-terminal propeptide of type II collagen (CPII) were significantly different amongst the exercise groups. Trained foals had significantly higher percent changes from baseline in serum levels for CTx1 (suggesting increased bone collagen degradation), and significantly lower changes from baseline for CPII (reflecting decreased cartilage collagen synthesis), compared to pastured foals. The forced exercise during the early postpartum months may have had a negative effect on collagen metabolism relative to free pasture exercise.

Type I collagen accounts for 90% of the organic matrix of bone and when degraded, small peptide fragments are released into the blood. The Serum CrossLaps™ One Step ELISA used in this study measures the levels of a cross-linked telopeptide fragment of type I collagen (CTx1) containing two chains of the amino-acid sequence EKAHD-β-GGR, where the aspartic acid residue (D) is β-isomerized, as indicated. These fragments have high specificity and sensitivity for monitoring antiresorptive therapy in horses47 and in humans with metabolic bone diseases48. There are few reports of their use to assess the effects of exercise on bone metabolism. Studies involving young human athletes have shown significantly higher serum levels of CTx1 with exercise5,49. Increases in serum levels of ICTP, another cross-linked telopeptide fragment of type I collagen, have been demonstrated with long distance running in regularly exercising humans50 and in young, treadmill-exercised horses12,14.

For the foals in our study, it was previously reported that exercise, and in particular the sprint training, enhanced bone mineral density27. The significantly elevated changes in the serum levels of type I collagen degradation products in our trained foals support the concept of increased bone turnover with increased exercise. This is further suggested by the fact that the changes in the serum levels of the bone formation marker PICP were higher for the trained than for the pastured foals at each sampling point during training. This has also been reported for young horses that were treadmill-exercised for 1 year when compared with walked controls14.

The foals confined to box stalls for 5 months also had significantly higher increases in the serum levels of CTx1 than those at pasture. Higher urinary levels of deoxypyridinoline cross-links have been noted in young confined horses, compared to levels in pastured horses50. These cross-links predominate in bone collagens and, like CTx1 and ICTP, can monitor bone degradation. Although not biomechanically the same as box-stall rest, there were increased serum levels of CTx1 in cosmonauts after only 8 days of space flight51,52. These studies suggest a need for minimal exercise and joint loading for normal bone collagen turnover.

The C-propeptide of type II collagen represents a potential marker of type II collagen synthesis, as its content and release are directly correlated with collagen synthesis in normal and osteoarthritic articular cartilages53. The levels of this propeptide have been measured in body fluids from humans with various arthritides54,55,56 and significantly elevated serum levels have been reported in horses with osteochondral fragmentation56. We recently described a direct correlation between serum CPII levels and the severity of osteochondrosis in the same foals used in the current study57. These findings support those of increased...
CPII levels in synovial fluids from osteochondroitic joints of young horses compared to levels in fluids from normal joints. As with CTx1, there were few published reports on the effects of exercise on body fluid levels of CPII. There was no effect of exercise on the synovial fluid levels of this propeptide in human athletes, even though they had lower levels than nonathletes. Studies in dogs have reported exercise-induced changes in the collagen of articular cartilage, but little in terms of type II collagen content or cross-links. It has previously been noted that there was no exercise effect on the collagen component of articular cartilage specimens from the metacarpophalangeal joints of the 24 foals in the current study sampled at 5 months of age. Our biomarker analyses suggest that forced exercise in horses during early life may result in a significant decrease in type II collagen synthesis compared to either pasture exercise or stall rest.

The significant differences between the exercise groups for the changes from baseline in the serum levels of CTx1 and CPII essentially disappeared at 5 months of age. This is consistent with adaptive responses to the forces being applied by the different exercise regimens within the developing skeletal system of these foals. That the significant exercise-induced differences in the serum levels of CPII and CTx1 disappeared completely with an additional 6 months of free exercise further supports the positive effects of normal exercise on collagen metabolism in foals.

Significant exercise-induced changes in proteoglycan content and synthetic capacity of articular cartilage removed from various joints of these foals have previously been reported. However, we found no significant differences between the groups in terms of the biomarkers that represent aggrecan turnover (sGAG and CS-846). This suggests that the evaluation of these byproducts of proteoglycan turnover in serum may not represent what is happening at the tissue level, perhaps due to rapid processing and dilution of these metabolites upon clearance from the affected joints to the systemic circulation. This is supported by a study involving horses in which there was an increase in the serum levels of the sGAG, keratan sulfate, immediately after training, but a return to pre-training concentrations within 1 h after exercise.

We confirm the previously described age-related decreases in the serum levels of certain biomarkers of skeletal metabolism in horses. We identified however, a significant increase in the serum levels of CPII up to 7 months, and CTx1 up to 9 months of age. This is the first known report of increasing levels of skeletal biomarkers in the body fluids of young horses during the first year of life. The finding for CTx may reflect increasing isomerization and maturity of bone collagen degradation fragments detected by the assay with increasing foal age.

It is important to note that our population of foals was genetically predisposed to develop osteochondrosis and all foals examined at 5 months had osteochondrotic lesions. Thus, there is the potential for this inherent abnormality to confound the effects of exercise on the serum levels of biomarkers of skeletal tissue metabolism. However, it was reported that the exercise regimen used in this study for the first 5 months did not significantly affect the number or severity of osteochondrotic lesions in these foals. Nevertheless, our results need to be confirmed in a larger population of foals, free of any predisposition for, or the presence of orthopedic disease.

We have shown the potential for monitoring the effects of exercise on the skeletal system in the developing animal during the dynamic first postpartum months, by evaluating the serum levels of specific metabolic biomarkers. By measuring the serum levels of biomarkers of cartilage collagen synthesis (CPII) and bone collagen degradation (CTx1), we were able to distinguish the exercise groups and suggest that the pastured foals had greater type II collagen synthesis and less type I collagen degradation than the stall-rested or trained foals. Our findings, based on the serum biomarker analyses, support those from the previously reported tissue biochemical analyses in these foals, in that pasture exercise was the best of the options employed in this study for the development of a healthy musculoskeletal system. Routine sampling and assaying for these biomarkers may allow the identification and monitoring of potentially adverse effects of exercise on skeletal development in animals and humans.

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