SERUM LEVELS OF COLLAGEN BIOMARKERS REFLECT EXERCISE-INDUCED CHANGES IN CARTILAGE

+++Billinghurst, C; +Knowlton, M; ++Brama, P; **van Weeren, R; *McIlwraith, W
+Colorado State University, Fort Collins, CO

INTRODUCTION
Measuring body fluid levels of specific metabolic byproducts offers the potential for a minimally invasive method of assessing the state of the musculoskeletal system of an individual in both health and disease. These biomarkers may allow for the diagnosis of disease at an earlier stage than is currently possible with imaging and clinical evaluations. There is also evidence that they may allow for the prediction of disease occurrence and progression. However, there are factors, outside of disease, that will affect body fluid levels of many musculoskeletal biomarkers. These include the age and sex of the individual, as well as natural circadian and seasonal fluctuations. It has also been shown that the fluid levels of many markers are affected by recent exercise (1). It was the objective of this study to determine if the effects of different levels of exercise upon the developing musculoskeletal system could be monitored by measuring serum levels of specific molecular markers of cartilage and bone metabolism.

METHODS
This study utilized 43 Dutch Warmblood foals (23 males, 20 females) that were part of a large project investigating the effect of exercise on osteochondrosis and musculoskeletal development of the young horse. After one week of age, the foals were randomly divided into 3 groups, which were subjected to different exercise regimens until weaning at 5 months. Group_box (n=14) were each confined to a 3 x 3.5 m box stall. Group_pasture (n=15) were kept in similar box stalls, but underwent an exercise protocol of an increasing number of 40-m gallop sprints. After sprint training the foals had an additional half-hour of free exercise in a 48 x 15-m enclosure. Group_excel (n=14) were kept with their mothers at pasture for 24 h/day. At age 5 months, 8 foals were randomly selected from each group, euthanized and extensively examined. The remaining 19 foals were kept under exactly the same conditions in 2 similar pens with access to a small paddock for an additional 6 months. There was no further training.

Serum samples were obtained from all foals at 0800 h in the first week after birth, and at age 1, 2, 3, 4 and 5 months. The remaining 19 foals were sampled at age 7, 9 and 11 months at 0800 h. These sera were assayed for levels of COL2-3/4C
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(2) and 234CEQ (3), markers of type I/II and type II collagen degradation, respectively. Collagen synthesis was measured using a commercially available PICP radioimmunoassay for type I (bone) collagen and a CPII ELISA for type II (cartilage) collagen. Bone turnover was further evaluated by using commercially available osteocalcin and CTX-I assays. Proteoglycan metabolism was assessed by measuring serum levels of sulfated glycosaminoglycans using a DMB assay and the chondroitin sulfate (CS) 846 epitope using a commercially available ELISA. The serum concentrations of each marker were compared between the three groups by the non-parametric Mann-Whitney test with significance at P<0.05.

ESSENTIAL RESULTS
The training (forced-exercise) regimen had the most significant negative effects on collagen metabolism during the first 5 months of development, based on the mean serum levels of collagen biomarkers. The degradation of type II collagen of cartilage was significantly increased (P<0.01) and its synthesis significantly decreased (P<0.01) in the trained foals compared to the pastured foals, according to the serum levels of the type II collagen biomarkers 234CEQ (Fig. A) and CPII (Fig. B), respectively. Similarly, a significant increase in type II collagen degradation was noted for the box-stalled (rested) foals compared to the foals at pasture (Fig. A), whereas type II collagen synthesis, although decreased, was not significantly so (Fig. B). There was also a significant increase in type I collagen degradation in the trained compared to the pastured (P<0.01) and box-stalled (P<0.05) foals (Fig. C). This was calculated as the difference in the serum levels of the COL2-3/4C-
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(type I/II collagens) and 234CEQ (type II collagen) neoepitopes for each foal at each time point. No significant differences were identified between the different exercise groups for type I collagen synthesis, based on the mean PICP serum levels (Fig. D).

DISCUSSION
The results of this study suggest that the measurement of the serum levels of certain biomarkers of skeletal tissue metabolism reflect molecular events occurring in the developing animal in response to varying levels of exercise. In particular, it was shown that type II collagen degradation is significantly increased in foals by both increased and decreased levels of exercise compared to normal pasture exercise during the first 5 months of life. Moreover, altered cartilage metabolism is further supported by the finding of decreased levels of a biomarker of type II collagen synthesis in the two non-pastured groups of foals. As previously reported, when the metabolic activity of cartilages from these foals was assessed, it was also concluded that the training protocol appeared to have long-lasting negative effects and that chondrocyte viability was affected long after the cessation of training (4).

These foals were from a population of horses with increased susceptibility to osteochondrosis (OC), a developmental orthopaedic disease that is due to a disturbance in endochondral ossification in the cartilaginous skeletons of these animals. It may be that the significant changes in serum levels of collagen biomarkers described in this study are merely reflective of this predisposed group of horses. However, it should be noted that when the lesions were analyzed in these foals at necropsy, it was concluded that exercise did not significantly affect the number or severity of OC lesions present (5). This suggests that the described changes in collagen biomarker levels are truly reflective of exercised-induced changes in the cartilage of the developing foals. It can be concluded from the serological and pathological changes noted in these foals, that natural pasture exercise is the best for the development of healthy cartilage in young horses. Furthermore, the changes occurring in the cartilages of these animals may be detected by measuring serum levels of molecular markers of collagen metabolism.

REFERENCES
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