Effects of early exercise on metacarpophalangeal joints in horses

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Objective—To determine the effects of exercise at an early age on tissues in the metacarpophalangeal joints of horses.

Animals—Twelve 18-month-old horses.

Procedures—All horses were pasture reared, but 6 horses had additional exercise starting at 3 weeks of age until 18 months of age. At that time, computed tomography, articular cartilage metabolism evaluation, and histologic assessments of synovial membrane, articular cartilage, and subchondral bone were performed.

Results—Exercised horses had fewer gross lesions, less articular cartilage matrix staining in the dorsal aspect of the condyle, greater bone fraction in the dorsolateral aspect of the condyle, and higher bone formation rate, compared with nonexercised horses.

Conclusions and Clinical Relevance—Exercise at a young age may be protective to joints, although more research is needed to characterize changes in articular cartilage matrix. Results suggested that exercise can be safely imposed at an early age. (Am J Vet Res 2010;71:405–411)

Musculoskeletal injury in horses is common and usually results from chronic pathological changes in the osteochondral tissues. Synovitis and primary articular cartilage defects may be early events in these changes and are not uncommon in these athletes. Musculoskeletal injury can lead to removal from exercise for treatment, retirement because of severity of disease, and, in some cases, euthanasia following catastrophic injury. Consequently, horse owners endure substantial monetary loss because of these injuries, and the racing industry is constantly scrutinized for humane concerns.

Although diagnosis and treatment of musculoskeletal injuries have improved substantially over the past several decades, there is still a need for improvement in prevention of these diseases. One potential method for improving musculoskeletal strength is introduction of exercise at an early age, which has been performed in horses. It is known that most tissues will respond to the stresses that are placed on them. However, there is an optimum level at which strength can be maximized, and there are lower and upper limits for which joint loading can be damaging. Because stall confinement is known to inhibit or reduce musculoskeletal tissue mass, the present management scheme for rearing racehorses can be questioned because it means they spend most of their time in a stall and do not undergo substantial exercise except when brought to the racetrack.

There is evidence that exercise during childhood may lead to maintenance of bone strength later in life and a subsequent decrease in fracture rate. This observation led to the goal of the present study, which was to determine the effects of exercise at an early age on tissues in the MCP joints of horses. In the midcarpal joints of the same horses reported in the present study, there were no significant differences between exercised and pasture-reared horses; however, the influence of early exercise on the MCP joint needs to be determined because of the high rate of injury in that joint. The hypothesis was that horses that were exposed to exercise early in life would have superior articular cartilage and subchondral bone characteristics with fewer gross lesions, compared with horses that had not undergone imposed exercise.

Materials and Methods

Twelve New Zealand Thoroughbred foals were bred, fed similarly, and raised in paddocks as part of a large study performed by the Global Equine Research Alliance. As part of that larger study, the midcarpal joints of the same horses were evaluated, including evaluation of gross and material properties from joint tissues. All

MCP  Metacarpophalangeal
SOFG  Safranin-O fast green
GAG  Glycosaminoglycan

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The MCP joints from each horse were dissected and evaluated grossly for signs of articular cartilage erosion, wear lines, or fragments. Samples of tissues were obtained for histologic assessment of synovial membrane, articular cartilage, and subchondral bone and for evaluation of articular cartilage GAG content and synthesis at certain locations (Figure 1).

Synovial membrane histologic examination—Synovial membrane and joint capsule were harvested from the dorsal and palmar aspects of the joint (Figure 2) and placed in neutral-buffered 10% formalin for H&E staining. Five-micrometer-thick sections were evaluated and graded by an evaluator unaware of treatment assignments for cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis, and vascularity. Each variable was graded and reported as a numeric value ranging from 0 to 4, with 0 representing the most severe change. A cumulative score was also calculated.

Articular cartilage histologic examination—Articular cartilage pieces (5 mm³) were obtained from each joint (Figure 1) and stored in neutral-buffered 10% formalin for 7 days followed by routine histologic processing. One-half of the 5-µm sections obtained were stained with H&E and the remainder with SOFG. Collection locations were chosen to represent the dorsal and palmar aspects of the third metacarpal articular surface. Sections stained with H&E were evaluated without knowledge of treatment groups for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation (chondrocyte division within a lacuna), and focal loss of cells. Numeric values ranging from 0 to 4 were assigned to each measured variable, as described, with a higher value indicating a more pathological change. A cumulative score was also determined, and location of sample collection was taken into account. Articular cartilage sections stained with SOFG were evaluated without knowledge of treatment groups for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones. Numeric values ranging from 0 (indicating no stain uptake) to 4 (for...
normal stain uptake) were assigned to each measured variable, and a cumulative score was calculated. A higher value indicated better staining, indicative of higher GAG content of the articular cartilage matrix.

**Subchondral bone histologic evaluation**—Osteochondral samples (2 mm thick) were obtained from the palmar and dorsal aspects of the third metacarpal condyles in a frontal plane fashion (Figure 2). Because calcein green was given at 19 and 8 days prior to euthanasia, the dorsal and palmar subchondral bone samples were obtained for morphometric analysis. This included evaluation of bone volume fraction and bone formation rate. Specifically, bone formation rate was evaluated in 4 areas of interest on each condyle (Figure 3). These sites included the subchondral bone area and trabecular bone area in the parasagittal groove (axial) and middle of the condyle (abaxial) on the lateral and medial aspects of each slice. The wedge-shaped sections of bone between the slices were block stained in basic fuchsin and sliced 150 µm thick for evaluation of micromage.22 This included evaluation of crack density and fraction of diffuse microdamage in each area.

**Articular cartilage metabolism**—Articular cartilage was also obtained for evaluation of GAG content and synthesis. The total articular cartilage GAG content was measured by use of a reported 1,9-dimethyl methylene blue technique.23 Articular cartilage pieces were obtained aseptically from the articular surface of the third metacarpal condyle (Figure 1), and each piece was stored at −80°C prior to further processing and analysis. Samples were processed in duplicate, and results were recorded as micrograms of GAG per milliliter of digest. For analysis of GAG synthesis, articular cartilage samples were also aseptically collected from the same area in each joint, and 35SO4 incorporation was measured by use of reported methods.23 Samples were processed in duplicate, and the results are reported as counts per minute per milligram of dry weight.

**Statistical analysis**—Results were analyzed by use of a mixed-model ANOVA unless otherwise indicated. Residual plots were constructed to test for fulfillment of model assumptions; if model assumptions were not met, data transformation was performed and reported. The following fixed effects were considered: exercise treatment, limb, site on joint surface (dorsal vs palmar), and all interactions among main effect variables. The subject (horse) within treatments was considered a random effect. When individual comparisons were made, a least squares mean procedure was used and P < 0.05 was considered significant. All data are reported as mean ± SE.

**Results**

**Computed tomography**—There was no significant influence of exercise on any variable of computed tomographic analysis. A mild density gradient was seen in 2 limbs from the exercise group and 3 limbs from the pasture group (Figure 4).

**Gross assessment**—More grossly apparent lesions were detected in the pasture group, compared with the exercise group, such as mild partial-thickness erosions (which included variables such as visually detectable fibrillation and roughening of the articular cartilage surface), osteochondritis dissecans, and chondroid fibrillation. There were no grossly visible lesions in the MCP joints of the exercise group.

**Synovial membrane histologic findings**—No significant effects of exercise, limb, or location within the joint on histologic variables of synovial membrane were found, including cellular infiltration, intimal hyperplasia, subintimal edema, subintimal fibrosis, and vascularity. Consequently, there was no significant effect of exercise on total score (Table 1).

**Articular cartilage histologic and metabolism findings**—An effect of site on focal cell loss was detected that was significantly (P = 0.035) higher in the palmar aspect of the third metacarpal condyle, compared with that in the...
Table 1—Mean ± SE scores for histologic features of the synovial membrane (SM) and articular cartilage (AC) in pasture and exercise groups of horses. For SM and AC scores, higher numbers indicate higher degrees of pathological change. For Safranin-O staining, higher values indicated better staining, indicative of higher GAG content of the articular cartilage matrix.

<table>
<thead>
<tr>
<th>Histologic variable</th>
<th>Exercise group</th>
<th>Pasture group</th>
</tr>
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<tbody>
<tr>
<td>SM cell infiltration</td>
<td>0.08 ± 0.06</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>SM intimal hyperplasia</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>SM subintimal edema</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>SM subintimal fibrosis</td>
<td>0.96 ± 0.04</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td>SM vascularity</td>
<td>0.83 ± 0.76</td>
<td>1.13 ± 0.51</td>
</tr>
<tr>
<td>Total synovial membrane score</td>
<td>1.88 ± 0.18</td>
<td>2.21 ± 0.17</td>
</tr>
<tr>
<td>AC fibrillation</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>AC necrosis</td>
<td>0.13 ± 0.07</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>AC—chondrone formation</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>AC—focal cell loss</td>
<td>1.88 ± 0.15</td>
<td>1.71 ± 0.15</td>
</tr>
<tr>
<td>Total articular cartilage score</td>
<td>2.00 ± 0.16</td>
<td>1.79 ± 0.18</td>
</tr>
<tr>
<td>Tangential zone</td>
<td>0.99 ± 0.50</td>
<td>0.42 ± 0.10</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>2.27 ± 0.10</td>
<td>2.21 ± 0.12</td>
</tr>
<tr>
<td>Radiate territorial matrix</td>
<td>3.64 ± 0.10</td>
<td>3.59 ± 0.10</td>
</tr>
<tr>
<td>Radiate interterritorial matrix</td>
<td>1.27 ± 0.13</td>
<td>1.29 ± 0.75</td>
</tr>
<tr>
<td>Total safranin O score</td>
<td>7.77 ± 0.97</td>
<td>7.50 ± 0.31</td>
</tr>
</tbody>
</table>

Figure 5—Bar graph of bone volume fraction (mean ± SD) in the exercise group of horses (white bars), compared with the pasture group of horses (black bars). DL = Dorsolateral, PL = Palmarolateral, PM = Palmaromedial. 

Figure 6—Bar graph of bone formation rate (mean ± SD) in the subchondral and trabecular bone of the exercise group of horses (white bars), compared with the pasture group of horses (black bars). SCB abaxial = Subchondral bone area at the abaxial site. SCB axial = Subchondral bone at the axial site. TB abaxial = Trabecular bone area at the abaxial site. TB axial = Trabecular bone area at the axial site. There was a significant (P < 0.05) difference between groups at all sites.

with the medial condyle, when pooled over site and group (lateral condyle, 0.24 ± 0.017%; medial condyle, 0.21 ± 0.016%). In addition, diffuse microdamage was higher in the abaxial areas of subchondral bone areas, compared with the axial areas (abaxial, 0.15 ± 0.017%; axial, 0.089 ± 0.011%). There were significantly more microcracks in the axial region of trabecular bone, compared with the abaxial region (axial, 0.0041 ± 0.0012/ mm²; abaxial, 0.00067 ± 0.00036/mm²).

There was a significant effect of joint location and the interaction between location and treatment on bone volume. Specifically, there was significantly higher bone fraction in the dorsal aspect of the joint, compared with the palmar aspect (dorsal, 0.753 ± 0.009%; palmar, 0.728 ± 0.010%), and significantly higher bone volume in the axial and abaxial aspects of subchondral zone, compared with the trabecular zone (Figure 5). In particular, there was significantly higher bone fraction in the dorsolateral aspect of the condyles of the exercise group, compared with the pasture group. There was significantly higher bone formation rate in the exercise group, compared with pasture group when pooled over location and site (exercise group, 0.00039 ± 0.000022 mm/d; pasture group, 0.00024 ± 0.000011 mm/d).

There was significantly higher bone formation rate in the palmar aspect of the condyles, compared with the dorsal aspect. Significantly higher bone formation rate was detected in all 4 sites in the trabecular and subchondral bone of the exercise group, compared with the pasture group (Figure 6).

Discussion

The imposition of exercise at an early age was safe and did not induce musculoskeletal damage. This agrees with previous evidence that this exercise regimen induced no adverse effects on clinical, imaging, or metabolic aspects of these horses.24,25 Results of the present study indicated that imposed exercise at an early age may have been modestly beneficial, as exemplified by a lower prevalence of gross lesions and higher bone formation rate in the exercise group, compared with the pasture group.
Computed tomographic osteoabsorptiometry was used as a means of assessing bone density pattern in the third metacarpal bone, allowing for assessment of density distribution in any plane. It is not uncommon to see a sharp density gradient across the palmar aspect of the third metacarpal condyle, with the lowest density at the parasagittal groove, compared with higher density below the condylar surface and at the midsagittal ridge below the articular cartilage. There appeared to be no effect of exercise on the presence, or subjective severity, of the density gradient across the palmar aspect of the third metacarpal condyle, suggesting that other factors such as geometric properties of the bones of the MCP joint, limb conformation, or MCP joint load may dictate the pattern. This variation in bone density corresponded to changes in calcified cartilage and articular cartilage properties seen in the same horses.

In particular, the parasagittal groove area not only had lower bone density than did the surrounding area, but also thickening of calcified cartilage of relatively low mineralization and articular cartilage with signs of early degeneration, including low reflectance score, low indentation stiffness, low collagen content, and high water content. This spectrum of change was likely because of a gradient of loading across the condyles, which early exercise appears not to have affected.

The reason that there were more gross lesions in the pasture group, compared with exercise group, was not apparent. Some of these lesions may represent developmental lesions, but the partial-thickness erosions were unexpected. However, in the study by Kim et al., the investigators found an unexpectedly high prevalence of partial-thickness erosions in the midcortical joints of the pasture group and the exercise group. There was no significant difference in the prevalence of lesions between the 2 groups. However, the reason for performing the present study was that joints may react differently; in the present study, there was higher prevalence of lesions in the pasture group, compared with the exercise group. An argument could be made that the articular cartilage from the exercise group developed better material properties, but a study investigating that effect revealed no difference in material properties between the groups. van Weeren et al. also found no significant differences in India ink staining on the proximal articular surface of the first phalanx between the pasture and exercise groups. However, they did find that the articular cartilage collagen of the exercise group had more mature biochemical characteristics, compared with that from the pasture group, leading one to conclude that enforced exercise may be protective of articular cartilage. Another possibility is that the exercise group may have been less likely to freely exercise when in pasture, leading to a lower incidence of exercise-induced cartilage damage.

Another possibility is that the exercise group may have developed synovial effusion of the MCP joint during the study and the exercise group had significantly higher effusion scores at 15 months of age, compared with the pasture group. This effusion was transient and apparently caused no lasting pathological effects on the synovial membrane.

The increase in focal cell loss in the palmar aspect of the joint, compared with the dorsal aspect, was in agreement with the findings of Nugent et al., in which reflective index scoring of India ink–stained samples from these same horses revealed that this index of degeneration was higher in the palmar aspect, compared with that in the dorsal aspect. In addition, decreased material properties and higher water content in the palmar aspect were detected in that study, compared with the dorsal aspect. However, those results differ from those in a study by Dykgraaf et al., in which higher chondrocyte numbers and viability in the palmar aspect, compared with the dorsal aspect, were detected. The reason for this difference is unknown, but could be because of differences in sampling site and size of tissue samples. Tissues for the study reported here were significantly larger and were acquired peripherally (farther from the midpoint of the condylar surface) to the sites used by Dykgraaf et al. and therefore possibly subjected to different loads. The larger area of analysis also allowed for a more global assessment of changes in cell characteristics. The SOFG staining of the tangential layer in the present study was significantly lower (ie, more compromised in the dorsal aspect of the exercise group), compared with the palmar aspect of the exercise group and the dorsal aspect of the pasture group. There appears to be a possible negative effect of early exercise on the tangential zone of articular cartilage matrix that affects the dorsal medial aspect of the joint. However, there was no grossly visible effect on the joint surfaces, and further mechanical testing would be needed to verify this effect on articular cartilage.

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The higher amount of diffuse-staining microdamage in the lateral condyle, compared with the medial condyle, could have been caused by increased loading on the lateral condyle because of conformation or use. There was no significant effect of exercise, so it was unlikely to have been caused by exercising on an oval track. Diffuse microdamage was highest in the abaxial aspect of the trabecular bone. Trabecular bone is most reflective index scoring of India ink–stained samples from these same horses revealed that this index of degeneration was higher in the palmar aspect, compared with that in the dorsal aspect. In addition, decreased material properties and higher water content in the palmar aspect were detected in that study, compared with the dorsal aspect. However, those results differ from those in a study by Dykgraaf et al., in which higher chondrocyte numbers and viability in the palmar aspect, compared with the dorsal aspect, were detected. The reason for this difference is unknown, but it is likely attributable to an unknown force distribution at that site. This is similar to the findings of van Weeren et al. in the first phalanx of the same horses, in which GAG concentrations were lower in articular cartilage of the first phalanx of the metatarsophalangeal joints in the exercise group.

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The increase in microcrack numbers in the axial aspects of the trabecular and subchondral bone areas was likely reflective of the damage that typically occurs there. As stated, this is the site of osteochondral fracture in horses as well as degenerative changes in both calcified cartilage and articular cartilage in this same group of horses. Therefore, the spectrum of...
changes seen in this area is consistent with overload arthrosis.

The volume fraction of bone in the subchondral area was higher dorsally than on the palmar aspect, which seems unusual. Most studies have found that in the third metacarpal bone, the volume fraction is highest in the palmar aspect in athletic horses. However, the samples in the present study were from young horses (18 months of age) that had not entered race training properly, and all other studies were in older horses, most of which had undergone substantial training. If this is the reason, then the question must be asked as to why early exercise did not cause the palmar aspect to have higher bone fraction than the dorsal aspect. One reason may be that the level of induced exercise given in this study was not sufficient to induce significantly greater bone volume by 18 months of age. Because the dorsal bone volume is influenced by articulation between the third metacarpal bone and the first phalanx, the results suggested that this articulation may undergo substantially higher peak loading than at the palmar articulation of the third metacarpal bone and the proximal sesamoid bones early in life. Further studies are needed to determine the stresses across these articulations and the effects of development on changing stresses within the joint. Also, the dorsal aspects of the metacarpal condyles are smaller in cross-sectional areas, compared with the palmar aspects, requiring a higher volume of bone to withstand loading. The higher fraction of bone in the dorsolateral aspect of the exercise group was interesting and may truly represent a reflection of asymmetric loading because of exercise.

Bone formation rates did reflect the present loading scheme of the third metacarpal bone because it was highest in the exercise group, compared with the pasture group, and in the palmar aspect, compared with the dorsal aspect of the bone. This scheme represents a possible adaptive point at which the third metacarpal–proximal sesamoid bone articulation undergoes more loading, compared with the third metacarpal–first phalanx articulation. This change was seen in all 4 areas of interest in the exercise group, compared with the pasture group. If this is true, then bone formation in the palmar aspect of the joint may be delayed, compared with that in the dorsal aspect, leading to bone adaptation later in life during training. This may in fact be a sensitive time at which training could induce an inappropriate adaptive response, possibly leading to future injury. Conversely, the lower bone volume fraction in the palmar aspect, compared with that in the dorsal aspect, in the face of increased bone formation could be a manifestation of increased bone remodeling in the palmar aspect. Further work is needed to characterize the bone formation cascade within the third metacarpal condyles during development.

A coordinated change in the entire osteochondral complex because of exercise, consisting of higher bone formation rate and lower dorsal cartilage matrix staining, was detected in this study and was consistent with previous results. Because these studies used the same group of research animals, we were able to objectively identify the changes that occurred in all layers of the osteochondral complex. This led us to conclude that changes in the tissues of the parasagittal groove were indicative of early degeneration, most likely because of intense loading and intense gradient formation of loading. This was evident in the trabecular bone pattern in this area that revealed a spectrum of change in orientation from vertical to horizontal or disorganization in the parasagittal groove. This pattern was suggested by the nonaxial loading in this area, which might be influenced by biomechanical factors such as bone shape.

Results of the present study suggested that exercise from an early age was not harmful to the MCP joint and induced modest, benign changes in the osteochondral tissues. However, regardless of exercise, an apparent degenerative process within the parasagittal groove was evident in all horses, as exemplified by a disorganized trabecular pattern in the subchondral bone. Nugent et al also found degenerative articular cartilage indices indicative of articular cartilage matrix compromise in the parasagittal groove of the same samples. It is difficult to assume that this was a truly degenerative process in a group of sound, healthy young horses. However, the parasagittal area of the distal third metacarpal joint is a site with a higher prevalence of pathological change in both the bone and articular cartilage, and such early changes detected in this study may be an early phase that may lead to damage. Further investigations are needed to determine whether these changes are traumatic or developmental and to identify factors that make this degenerative change become clinically apparent.

References