Osteoarthritis is a chronic disorder of diarthrodial joints and is characterized by progressive deterioration of the articular cartilage, subchondral sclerosis, and osteophyte formation. This disease results in pain when bearing weight and during joint movement. Osteoarthritis accounts for approximately 60% of all lameness in athletic horses, with an annual cost in the United States as high as $1 billion. The pathogenesis of idiopathic osteoarthritis, in which there is no obvious initiating cause such as intra-articular trauma or infection, is still poorly understood and likely multifactorial. Genetic predisposition, abnormal joint conformation, underuse or repeated overuse of a limb, and subtraumatic injuries to joint tissues are all factors that might contribute to the development of osteoarthritis. Many of these factors are thought to influence the ability of a joint to effectively withstand or adapt to the relatively extreme mechanical loads associated with high-performance activities.

Musculoskeletal tissues respond readily to intensive exercise through adaptive changes that can subsequently increase their physiologic threshold of safe function. However, substantial overloading or overuse can lead to gradual degeneration of the tissue and an increase in the incidence of microinjury, with eventual development of clinical lesions. The intensity, duration, and frequency of loads associated with exercise programs are likely to be important in determining both positive adaptive responses and destructive pathologic changes. Negative consequences to tendon and articular cartilage are associated with early intensive exercise. Conversely, early exercise programs of mod-

Influence of early conditioning exercise on the development of gross cartilage defects and swelling behavior of cartilage extracellular matrix in the equine midcarpal joint

Woong Kim, ME; Chris E. Kawcak, DVM, PhD; C. Wayne McIlwraith, BVSc, PhD; Elwyn C. Firth, BVSc, PhD; Brian H. McArdle, PhD; Neil D. Broom, PhD

Objective—To investigate the influence of early conditioning exercise on the development of gross cartilage defects and swelling behavior of cartilage extracellular matrix (ECM) in the midcarpal joint of horses.

Animals—12 Thoroughbreds.

Procedures—6 horses underwent early conditioning exercise from birth to 18 months of age (CONDEX group), and 6 horses were used as control animals (PASTEX group). The horses were euthanized at 18 months of age, and the midcarpal joints were harvested. Gross defects of the cartilage surface were classified and mapped. Opposing surfaces of the third and radial carpal bones were used to quantify swelling behavior of the cartilage ECM.

Results—A wide range of gross defects was detected in the cartilage on the opposing surfaces of the bones of the midcarpal joint; however, there was no significant difference between the CONDEX and PASTEX groups. Similarly, no significant difference in swelling behavior of the cartilage ECM was evident between the CONDEX and PASTEX groups.

Conclusions and Clinical Relevance—In the study reported here, we did not detect negative influences of early conditioning exercise on the prevalence of gross defects in cartilage of the midcarpal joint or the quality of the cartilage ECM as defined by swelling behavior. These results suggested that early conditioning exercise may be used without negative consequences for the midcarpal joint and the cartilage ECM of the third and radial carpal bones. (Am J Vet Res 2009;70:589–598)
erate to low intensity are associated with little or no effect on articular cartilage and some positive effects on tendon. Improved understanding of thresholds for exercise intensity may aid in raising athletic horses with increased musculoskeletal strength and enhanced resistance to exercise-related injuries and degeneration of joint tissue.

Biologically, articular cartilage is most active in an animal's early growth phase and becomes relatively inactive with maturation. Although functionally advantageous under physiologic conditions, this relative inactivity of articular cartilage in adults limits the ability of cartilage to repair itself when weakened or damaged by disease or trauma. Investigations have been conducted into the use of early exercise before the athletic phase of training to enhance the adaptive potential of joint tissues. It is hypothesized that the quality of the developing cartilage matrix is positively influenced by a program of early exercise before an animal reaches maturity; this would subsequently reduce the risk of joint injury and the development of osteoarthritis. However, it remains unclear as to what might constitute a beneficial versus a potentially harmful program of early exercise for immature equine athletes.

To investigate the effects of early exercise on equine musculoskeletal health, Thoroughbreds were subjected to a conditioning exercise regimen from birth to 18 months of age and compared with control horses as part of the collaborative projects of the GERA. The influence of conditioning exercise on both the development of macroscopic cartilage defects and alterations in swelling behavior of cartilage ECM in the midcarpal joint was evaluated. We hypothesized that there would be increased swelling of the cartilage ECM in horses in an early exercise program, compared with the results for the control group; an increased number of defects on the midcarpal joint surfaces in the early exercise group, compared with results for the control group; more severe swelling of cartilage ECM at sites that overlapped or were adjacent to defects, compared with results for sites not adjacent to defects; and more severe swelling of cartilage ECM in sites in the opposing surfaces of the third and radial carpal bones known to have increased loads and stress, compared with results for sites known to have decreased loads and stress. To test these hypotheses, we used tissues from the same control and exercised horses evaluated in other reports of the GERA collaboration. In the study reported here, our objectives were to map and characterize the distribution of gross defects in cartilage of the midcarpal joint with respect to known sites of high stress and vulnerability to osteochondral disease and to determine whether there was any correlation between these defects and swelling behavior of the cartilage ECM.

Materials and Methods

**Horses**—Twelve Thoroughbred foals were blocked on the basis of sex and sire and were allocated into 2 groups at birth, a control group (PASTEX group [n = 6]) and an early exercised (ie, conditioned) group (CONDEX group [6]). The horses were housed at Massey University, Palmerston North, New Zealand, and the study and its procedures were approved by the Massey University Animal Ethics Committee.

**Exercise program**—From birth to approximately 18 months of age (the typical age at which race training is initiated), both groups were housed in pastures with full freedom of movement. However, the CONDEX group had additional exercise (1,030 m/d on 5 d/wk). The exercise was performed on an oval track, and every day, the running direction (clockwise or counterclockwise) was changed from the direction of the preceding day. The exercise program started at a mean ± SD age of 21 ± 19 days and ended when the horses were approximately 18 months old. The CONDEX horses were exercised such that mean target speeds were regulated by use of 2 all-terrain vehicles. The horses were constrained by use of an 8-m horizontal pole attached to the rear of each vehicle, which formed a barrier across the track.

![Figure 1](image_url) —Schematic illustrations of the procedure used to obtain serial slices of cartilage to evaluate swelling behavior of the ECM from the surfaces of the third and radial carpal bones. A—Sampling sites (1 through 4) of cartilage from the opposing surfaces of the third and radial carpal bones known to have increased loads and stress. To test these hypotheses, we used tissues from the same control and exercised horses evaluated in other reports of the GERA collaboration. In the study reported here, our objectives were to map and characterize the distribution of gross defects in cartilage of the midcarpal joint with respect to known sites of high stress and vulnerability to osteochondral disease and to determine whether there was any correlation between these defects and swelling behavior of the cartilage ECM.
The exercise program had 3 phases. During phase 1 (from birth to weaning), which had a duration of approximately 120 days, the mean target speed was 5.4 m/s. During phase 2 (from weaning to first sprint), which had a duration of approximately 100 days, the mean target speed was 7.5 m/s. Phase 3 was during approximately the last 300 days of the study; the mean target speed was 9.6 m/s with a brief sprint at a rate of 12.5 m/s for 129 m. During the 18-month exercise period, none of the horses had obvious lameness or prolonged periods of carpal joint effusion; joint effusion in both groups was mild and similar between the groups.

Sample collection and processing—Horses were euthanized via gunshot at a commercial slaughterhouse as approved by New Zealand regulations at a mean ± SD age of 535 ± 30 days. The midcarpal joints were incised and examined for evidence of general and local defects of the cartilage surface and then classified as normal (smooth surface without any surface undulation or fibrillation [ie, superficial disruption]), mild defects (some undulation but no fibrillation), or severe defects (fibrillation with or without loss of cartilage). These sites were photographed, with special attention given to the opposing surfaces of the third and radial carpal bones.

Four adjacent sites on each of the midpoints of the opposing surfaces of the third and radial carpal bones were marked with a temporary fuchsin stain to identify a consistent sampling region that would yield contiguous osteochondral blocks free of obvious disruption (Figure 1). The intent was to provide serial slices of cartilage matrix through the entire zonal depth. Sites 1 and 2 were toward the dorsal aspects of the third and radial carpal bones and corresponded to locations where it is known that there are high stresses attributable to high loads and where regional adaptive bone changes and osteochondral fractures have been reported.5,27,28 The sites toward the palmar aspects (3 and 4) were chosen as locations with comparatively lower loads. Sites 1 to 4 on the radial carpal bone matched exactly with sites 1 to 4 on the opposing surface of the corresponding third carpal bone.

Osteochondral blocks with en face dimensions of approximately 4 × 4 mm were sawn from the 4 sites on each of the opposing surfaces of the third and radial carpal bones (Figure 1); all osteochondral blocks were maintained in a fully hydrated condition. The blocks were blotted dry and further processed by use of a custom-built cutting system to make a double-cruciform vertical cut of defined dimensions (2.2 × 2.2 mm) through the full depth of the articular cartilage. Each block was then oriented in the vise of a sledge microtome, and 30-µm-thick serial sections of initial en face dimensions (2.2 × 2.2 mm) were obtained. Each osteochondral block yielded between 5 and 20 transverse serial slices, depending on the cartilage thickness, which varied across the joint surface. All cartilage thickness values were normalized to 1, such that the slice closest to the articular surface had a value approaching 0 (1/n, where n is the number of slices) and the slice closest to the subchondral bone had a value of 1 (n/n).

The slices were immersed in 0.15M saline (NaCl) solution and allowed to swell freely for a minimum of 24 hours at 4°C. All of the slices were then digitally photographed under identical magnification (2.5X) to determine their in-plane swollen dimensions relative...
to their standard as-cut dimensions (Figure 2). Swelling strains were calculated as the percentage increase in area (ie, \(\text{[postswelling area} - \text{preswelling area}] / \text{preswelling area}\} \times 100\)) by use of an image analysis software program.

**Statistical analysis**—The swelling strain data sets were analyzed by use of linear mixed modeling to determine whether there were significant influences of several variables on swelling behavior of the cartilage ECM. Variables analyzed included treatment (PASTEX or CON-DEX group), limb (left or right), bone of origin (third or radial carpal bone), sampling site (osteochondral site 1, 2, 3, or 4), and depth of cartilage slice beneath the articular surface (normalized as 0 to 1).

Because there were 6 horses nested within each treatment, horse was set as a random effect (ie, the horses were considered to be a random sample from a population, and our conclusions were generalized to this population). During the analysis, we encountered excess complexity in the variance structure and adjusted the analysis to anticipate 2 additional effects, horse-specific degenerative changes among the horses (likelihood ratio test; \(\chi^2 = 389.2; 4 \text{ df}; P < 0.001\)) and biological swelling variance with depth of cartilage slice among horses (likelihood ratio test; \(\chi^2 = 74.9; 4 \text{ df}; P < 0.001\)). These additional effects were set as random variables within the model. The fixed effects to be estimated included a full expansion that included all of the interaction terms of treatment, limb, bone of origin, sampling site, and depth of cartilage slice. Depth was included as a linear and a quadratic term.

**Results**

Site-specific cartilage defects—Horses in the CON-DEX and PASTEX groups had a wide range of gross defects in the articular cartilage of the bones of the proximal and distal rows of the midcarpal joint, except for 1 horse in the CON-DEX group that did not have any cartilage lesions detected. The defects ranged from slight discoloration of the cartilage to full-thickness loss. The defects were not randomly distributed across the joint surfaces; rather, they were situated at specific sites toward the dorsal or palmar aspects of the second, third, radial, and intermediate carpal bones. These recurring defect sites were identified and labeled as sites A through F (Figure 3).

Site A corresponded to the approximate midpoint toward the dorsal aspect of the radial facet of the proximal surface of the third carpal bone and the corresponding site on the oppositional surface on the radial carpal bone (Figure 4). It contained a group of nondisruptive cartilage defects consisting of mild to moderate local whitening or deep-
er undulations in the cartilage; the articular surface was continuous despite the defects. The dorsal aspect of site A was located approximately 4 mm from the dorsal edge of the radial facet of the third carpal bone and the corresponding site on the oppositional surface of the radial carpal bone. Size of the defects was approximately 20 × 10 mm on the third carpal bone and approximately 15 × 10 mm on the radial carpal bone. They were found in 1 CONDEX and 2 PASTEX horses.

Site B contained a substantial portion of the proximal surface of the third carpal bone and a portion of the medial facet of the proximal surface of the second carpal bone (Figure 5). The cartilage at this site had a smooth and undisrupted surface but was unusually thin and sufficiently translucent to reveal the color of the underlying bone, and in some instances, it was a distinct reddish color. There was a relatively abrupt increase in thickness of the cartilage at the defect boundary. These defects were of various sizes, ranging from approximately 10 × 10 mm to approximately 12 × 50 mm, and they were found in 2 CONDEX and 2 PASTEX horses.

Site C represented a focal area on the proximal surface of the radial facet of the third carpal bone that was adjacent to the notch on the bone’s palmar aspect (Figure 6). The cartilage defects at this site were relatively small (< 10 × 10 mm) and consisted of severe local disruptions. Gentle probing of these defects revealed abnormal softening of the cartilage with disruption of the articular surface that involved a substantial portion of the cartilage thickness. This defect type was found in only 1 PASTEX horse.

Site D was located toward the palmar aspect of the ridge adjoining the radial and intermediate facets of the proximal surface of the third carpal bone (Figure 7). The defects at this site varied in severity. The mild form of a site D defect consisted of a narrow indentation along the ridge. Moderately severe defects had increased depth and width of the indentation, and the most severe defects had a localized region of partial- or full-thickness cartilage loss with bone exposure. Site D defects were found in 4 PASTEX and 3 CONDEX horses.

Site E was located on a small area of ridge adjoining the radial and intermediate facets of the proximal surface of the third carpal bone, close to the notch on the bone’s palmar aspect (Figure 8). The
defects at this site consisted of near-parallel cracks 2 to 3 mm in length that partially traversed the ridge. These defects were found in only 1 CONDEX horse.

Site F was located on the ridge between the lateral and medial facets of the proximal surface of the second carpal bone (Figure 9). The defects at this site ranged from mild surface undulations with no obvious cartilage disruption to more severe forms that involved superficial loss of cartilage with exposure of macroscopically roughened cartilage matrix. These defects were found in 2 PASTEX and 2 CONDEX horses.

Oppositional and bilateral symmetry of cartilage defects—With the exception of site B defects, defects at all other sites were symmetrically arranged on oppositional sites between proximal and distal rows of the midcarpal joint bones, bilateral sites between the left and right limbs, or both oppositional and bilateral sites (Figure 10). Most (6/8) site A defects had an intrajoint oppositional symmetry such that a change located toward the dorsal aspect of the proximal surface of the third carpal bone was matched by a defect on the corresponding distal surface of the radial carpal bone. Most (24/25) site C, D, E, and F defects had a bilateral symmetry such that their location on the surface of the second or third carpal bone was matched on the same site on the contralateral limb.

Measurements of ECM swelling—Curves were fitted to plots of mean swelling strain of cartilage ECM versus cartilage depth for each combination of sampling site (1, 2, 3, or 4), bone of origin (third or radial carpal bone), and limb (left or right). Analysis of the fitted curves from the CONDEX and PASTEX groups indicated that, overall, there was little difference in swelling strain between the 2 groups. There was only 1 of 16 sites (sampling site 2 in the left radial carpal bone) where the swelling strain of the cartilage ECM from the CONDEX group was higher than that of the PASTEX group (Figure 11).

An overall effect of early exercise on the mean swelling strain of cartilage ECM was not detected ($F_{1,6} = 0.07; P = 0.795$). Mean ± SE was $16.93 ± 3.227\%$ for the CONDEX group and $15.71 ± 3.248\%$ for the PASTEX group. The difference between the means had wide variability (mean, 1.23%; 95% CI, $-9.132\%$ to $11.580\%$), which indicated that the power to detect a difference between the groups was low (6 horses/treatment; total of 12 horses in the study). Thus, the mean swelling strain of cartilage ECM from the CONDEX group could be as much as 12% higher or 9% lower, compared with the mean swelling strain from the PASTEX group.

Although the early conditioning did not have a significant overall effect on swelling behavior of the cartilage ECM, there was an influence of the biological variables on swelling behavior. There was a significant ($F_{3,16} = 6.54; P = 0.002$) difference in the pattern of swelling behavior among sampling sites (Figure 12). A significantly ($P = 0.002$) higher swelling strain of 7.4% was detected in the sites of high stress toward the dorsal aspect of the oppositional surfaces of the third and radial carpal bones, compared with the sites of low stress toward the palmar aspect of these surfaces. Swelling strain of cartilage from the third carpal bone was higher than that from the radial carpal bone in the dorsal sites.

Figure 12—Plots of the mean ± SE values for the swelling strain of cartilage ECM for each sampling site (1 through 4) that represent the overall (combined values of the treatment groups, bones of origin, left limb, and right limb) mean swelling strains at each sampling site (A); the mean swelling strains (combined values of the treatment groups, left limb, and right limb) for the third carpal bone (black diamonds) and the radial carpal bone (white squares) at each sampling site (B); and the mean swelling strains (combined values of the treatment groups and bones of origin) for the left (black diamonds) and right (white squares) limbs at each sampling site (C). See Figures 1 and 3 for remainder of key.
(site X bone interaction; \( F_{3,90} = 2.86; P = 0.041 \)); there was also a limb interaction (site X limb interaction; \( F_{3,90} = 4.43; P = 0.006 \)) on the pattern of swelling behavior detected among the sampling sites. Cartilage defects at or close to a sampling site significantly (\( F_{1,90} = 4.23; P = 0.04 \)) influenced swelling behavior of the cartilage ECM, increasing the mean swelling strain by 4.2%, compared with the swelling strain of cartilage ECM from sampling sites not associated with cartilage defects.

Of the many interaction terms involving treatment, only those involving the shape of the curve that related mean swelling strain to cartilage depth were significant. The linear part of the quadratic expression depended on the treatment, limb, bone of origin, and sampling site within the bone (ie, the depth X treatment X site X bone X limb interaction was significant; \( F_{3,79} = 2.36; P = 0.037 \)). The quadratic term depended on the same factors through the 2 simpler interactions; thus, depth\(^2\) X treatment X site X limb (\( F_{3,79} = 3.43; P = 0.021 \)) and depth\(^2\) X treatment X bone X site (\( F_{3,79} = 3.16; P = 0.029 \)) were also significant.

In relation to the 4 stated hypotheses, there was no significant difference in the swelling strains between the 2 groups; there were more defects, but not a significantly larger number, found in the PASTEX group than the CONDEX group; there was a significant (\( P = 0.04 \)) increase (4.2%) in swelling strain of the cartilage ECM in sampling sites that also contained or were adjacent to a defect, compared with the swelling strain of sites not adjacent to a defect; and there was a significant (\( P = 0.002 \)) increase (7.4%) in swelling strain in the sampling sites that also contained or were adjacent to a defect, compared with the swelling strain of sites not adjacent to a defect, compared with the swelling strain of sites not adjacent to a defect, and there was a significant (\( P = 0.002 \)) increase (7.4%) in swelling strain in the sampling sites that contained or were adjacent to a defect.

In the discussion, the authors pointed out that the defects identified in the present study could be precursors for later secondary osteoarthritic development in the joint.

**Discussion**

These 2 cohorts of horses have been used by others in the GERA collaboration to investigate the effects of early exercise on equine musculoskeletal health. Adverse clinical effects of exercise were not detected. Another report had similar results and also revealed no significant changes in the biochemical and biomechanical properties of the cartilage of the distal articular surface of the metacarpal condyles. A positive effect of exercise on chondrocyte viability was reported. Examination of the proximal articular surface of the proximal phalanx found no significant difference in the cartilage degeneration index between the CONDEX and PASTEX groups, but site-related differences in biochemical variables between groups and within each group were detected. It was concluded that there was a measurable effect of conditioning exercise on the cartilage of the proximal phalanx. By use of cartilage defect mapping and site-specific cartilage matrix swelling measurements, the study reported here investigated whether the quality of the articular cartilage in the equine midcarpal joint might be adversely influenced by conditioning exercise.

A variety of cartilage defects was detected in all of the horses studied, except 1. The defects ranged from subtle changes (eg, cartilage discoloration, thinning, and irregularities in thickness) to moderate to severe disruption (eg, fibrillation, erosion, and superficial to full-thickness cartilage loss). However, none of the defects was associated with osteoarthritic changes or intra-articular injuries such as osteophyte formation, subchondral bone erosion, or chip (slab) osteochondral fractures. The cartilage defects detected were not associated with any systemic inflammatory response or lameness in the horses; they were considered to be nonimporant preclinical lesions. However, it was known that repeated cyclic fatigue or acute traumatic loads induce osteoarthritic degeneration of the cartilage. The defects identified in the present study could be precursors for later secondary osteoarthritic development in the joint.

For example, at high speeds, there can be hyperextension of the carpal joint attributable to muscle fatigue. This results in transmission of the dynamic axial loads through a restricted area located toward the dorsal aspect of the opposing surfaces of the third and radial carpal bones, which have bilateral symmetry but not oppositional symmetry. Interestingly, 24 (596%) defects on sites C, D, E, and F, which were located toward the palmar aspect of the proximal surface of the third carpal bone, had bilateral symmetry but not oppositional symmetry with the radial or intermediate carpal bones (Figure 10). The symmetric distribution of defects at particular sites could be related to joint morphology and the load environment of the midcarpal joint.

By contrast, the defects at sites C, D, E, and F, which are located toward the palmar aspect of the proximal surface of the third carpal bone (Figure 10) and developed only bilaterally, might be caused by secondary forces generated from the high dynamic loads. The carpal joint dissipates the force from axial dynamic loads by transferring the force into the intercarpal ligaments. These ligaments restrain the dorsal movements of the carpal bones, thus preventing carpal injury. During this process, shear forces may be generated between the surfaces of the proximal and distal row of the midcarpal bones. This could cause repeated microtrauma in the ridge sites of the proximal cartilage surfaces of the second and third carpal bones (Figures 7 and 9). However, this proposed mechanism is insufficient to explain the origin of the bilateral defects at sites C and E (Figures 6 and 8). Defect formation relating to the load environment and midcarpal joint congruency is an area of potential research.

A comparison of defects in the CONDEX and PASTEX groups revealed no significant differences between the groups. Studies on other joints from these same 2 cohorts of horses support our finding that there was no clear correlation between the early
exercise treatment and the amount of visible damage detected. We therefore conclude that the early exercise program had no significant influence on the prevalence of defects in the midcarpal joint.

Swelling behavior of the cartilage ECM is thought to be dependent on the integrity of the collagen fibrils, fibrillar network, and GAG content. Hydrated cartilage provides a deformable stress-attenuating layer that covers the bone ends. The ultralow permeability of the cartilage matrix determines cartilage behavior such that the response of the cartilage to load is strongly influenced by the rate of load application. During a slowly applied load, the matrix deforms by a process of consolidation in which the water is gradually and passively removed, and the applied load is progressively transferred to the increasingly compacted solid components (ie, the collagen fibrils and GAGs). Conversely, at high rates of load application, the cartilage responds as a relatively stiff, elastic medium with little removal of fluid. Thus, matrix permeability is crucial to the mechanical properties of cartilage and to its role in load bearing. Changes in this permeability are influenced by the overall state of the matrix and its constituents, including the connectivity of the fibrillar network. Such changes may be adaptive in response to an altered mechanical environment or as a direct or indirect consequence of degeneration.

Although GAGs provide the potential for hydration of the matrix, it is the integrity of the collagen network that determines the final swelling magnitude of the tissue in a given aqueous environment. In osteoarthritis, the interconnectivity within the collagen network is reduced, which induces the network to destruct into more aggregated fibrillar bundles with less effective entrapment of the hydrated GAGs. Therefore, a hallmark of osteoarthritic change in the cartilage matrix is an increased degree of swelling. However, higher amounts of swelling do not automatically imply that the tissue is osteoarthritic; the concentrations of collagen and GAG in cartilage vary depending on joint type, site within the joint, and cartilage depth. Furthermore, in horses, the age and the exercise regimen (type, timing, and intensity) influence the composition of the ECM. Overall, the CONDEX and PASTEX groups had similar depth and site-dependent swelling characteristics. Analysis of the swelling strain versus depth plots (Figure 11) indicated that the cartilage had classic swelling behavior (ie, lowest in the surface layer, increasing to a maximum in the middle zone, and diminishing in the deep zone). This characteristic pattern of swelling reflects the natural zonal differentiation of the cartilage matrix. The topographic variation in swelling behavior of the cartilage ECM among the 4 sampling sites (Figure 12), in which the dorsal sites (1 and 2) had significantly higher swelling strains than the palmar sites (3 and 4), indicated a positive correlation between the amounts of swelling strain and weight bearing that exist in different areas of the joint (ie, increased swelling strain in the high-load environment that exists in the dorsal region).

The dorsal aspect of the radial facet of the proximal surface of the third carpal bone, a common site for cartilage loss, osteochondral fracture, and ischemic scarring, had several site-specific defects. These nondisruptive defects, which would otherwise be classified as nonpathologic, were associated with increased swelling strain. This indicated that the technique used in the study reported here has the potential to detect subtle changes in matrices that do not have overt disruption. The dorsally oriented sampling sites from the third carpal bone had swelling strains that were slightly higher than the opposing site on the radial carpal bone (Figure 12). This could reflect slightly different load patterns for these 2 opposing contoured surfaces. We also detected apparent laterality in the horses as evidenced by the limb effects on the mean swelling strains in both the CONDEX and PASTEX groups. This was surprising given that the CONDEX horses were trained bidirectionally.

We did not detect differences in the swelling behavior of the cartilage ECM between the CONDEX and PASTEX groups. This suggests that early exercise may have failed to induce significant changes in the ECM in the surfaces of the third and radial carpal bones of the midcarpal joint. Potential joint-specific effects of early conditioning were evaluated in 2 other studies, in which investigators analyzed the cartilage from different joints in these same 2 cohorts of horses. In one of those studies, the cartilage at defined sites on the lateral and medial condyles of the metacarpal bone was evaluated, and 2 cartilage sites in the proximal phalanx with known differences in load patterns were evaluated in the other study. Both of those studies revealed significant topographic variation in the ECM components. However, significant differences in collagen-related changes and GAG concentrations between the PASTEX and CONDEX groups were detected in only 1 study. The imposed workload for the CONDEX horses increases the rate of remodeling of GAGs and collagen type II, which induces earlier cartilage maturation. However, negative effects of the imposed workload associated with lower collagen concentrations in the region that has a high peak load were also reported. In that study, investigators suggested that the lower collagen content in the high-load region of CONDEX horses could arise from a failure of normal collagen development or from actual collagen loss. However, these explanations contradict results of other studies that revealed a relationship between high intermittent load with higher concentrations of collagen and lower GAG concentrations in areas that have low load during nonathletic activities. If similar maturation-related changes had developed in the comparatively high-load region of sampling sites 1 and 2 in the present study, it would have been expected that the CONDEX group would have had a lower swelling strain, compared with that of the PASTEX group. However, such differences were not detected, which was a finding also supported by another study.

A major limitation of the present study was the low statistical power attributable to the small number of horses in each treatment group. This limited the amount of tissue that was allocated to the swelling strain analyses and concurrently provided tissues for parallel microstructural investigations. With respect to the tech-
nique that was used to evaluate swelling behavior, the choice of a thickness of 30 µm for the sections could be criticized as insufficient to represent in vivo ECM behavior. However, when compared with the scale of the fibrillar network, a 30-µm-thick section would contain a substantial depth of ECM structure and enable investigators to detect meaningful differences in swelling behavior.

The findings of the study reported here and other studies in the GERA collaboration have revealed that early exercise treatment may have an effect in some joints. However, there was no evidence that early conditioning induced alterations in the midcarpal joint that were more deleterious, compared with alterations that develop naturally from typical exercise in pastured horses.

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