Remodeling of the Navicular Bone in Response to Exercise—A Controlled Study

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Treadmill exercise resulted in increased subchondral bone remodeling in the navicular bone, without evidence of arteriosclerosis. The role of subchondral bone remodeling in the pathogenesis of navicular disease is likely to be further defined by the development of diagnostic techniques, which can be used to better assess bone remodeling. Author's address: Rood and Riddle Equine Hospital Lexington, KY 40580 (Sandler); Equine Orthopaedic Research Laboratory, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; (Kawcak, McIlwraith, Norrdin). © 2000 AAEP.

1. Introduction

Although navicular disease has been recognized as a cause of lameness in the horse for more than 200 years, there still remains widespread disagreement among investigators as to the pathogenesis of the disease. Predisposing factors, such as genetic predisposition, poor conformation, a large bodyweight to hoof size ratio, and improper shoeing have been suggested. Unreliable diagnostic methods, as well as the lack of a reproducible model, have hindered progression towards full understanding of the disease.

Many lesions are associated with navicular disease in the horse, including fibrocartilage erosion on the flexor surface of the bone, rarefaction and osteitis of the cortex, chronic synovitis/bursitis, and tearing of the fibers of the deep digital flexor tendon, associated with cavitation and degeneration of cancellous bone. Wright et al. described full thickness defects in the palmar surface fibrocartilage, palmar cortex erosion, medullary lysis, deep digital flexor tendon (DDFT) surface fibrillation, and DDFT core lesions and adhesions between the DDFT and the navicular bone in horses with navicular disease. These changes were not seen in age matched controls. It has been suggested that lesions associated with navicular disease are the result of repeated concussion of the navicular area leading to degenerative disease involving the navicular bone, navicular bursa, and the deep digital flexor tendon. Arteriosclerosis resulting in ischemia and necrosis, due to decreased blood flow to the navicular region, has also been implicated in the pathogenesis of navicular lesions; however, evidence of arteriosclerosis has been demonstrated in the navicular region of sound horses as well.1

Pathologic bone remodeling and increased intraosseous pressure have also been associated with navicular lesions and heel pain.1,3 Increased concussion, due to poor conformation (upright pasterns, small hooves, etc.), excess use, or improper shoeing, are thought to lead to super-physiologic stresses on
the flexor surface of the navicular bone. These stresses may result in increased modeling and remodeling of the navicular bone, which can result in subchondral bone sclerosis, bone marrow fibrosis, impaired venous drainage, increased intrasosseous pressure, and bone pain associated with vascular distention. Increased bone loading due to strenuous exercise and reduction in the ability of degenerative cartilage matrix to disperse forces transmitted to the subchondral bone appear to result in the subchondral bone sclerosis. However, adaptive subchondral bone thickening can result from exercise alone in clinically normal horses. There may exist a point at which increased subchondral bone remodeling may become pathologic. Therefore, normal changes in the navicular bone in response to exercise must be determined in order to identify pathologic changes in subchondral bone.

The goal of this study was to evaluate early radiographic and histologic effects of exercise on the navicular bones of clinically normal young horses. Histologic investigation of navicular disease has not to this point included evaluation of early exercise-induced subchondral bone response.

2. Materials and Methods

Horses

Twelve clinically normal two-year-old horses were randomly assigned to 2 groups—treadmill exercised (TE) or hand-walked (HW) controls. All horses were clinically evaluated and were accepted into the study only if they were free of forelimb lameness and had negative response to phalangeal flexion. The TE horses originally started 2 min at the trot (10–12 mph), then 3 min at a gallop (20 mph), then 2 min at the trot (total time of 7 minutes). Over time (36 days), TE horses moved up to a gallop speed of 26 mph. They were exercised five days per week throughout the study (6 mo). Horses in the HW group were hand-walked for 7 min, 5 days a week. Following the six-month exercise regimen the horses were re-evaluated for lameness. In order to determine bone formation, each horse was given two double labels of intravital stain. On days 81 and 91 (after exercise began), each horse was given 20 mg/kg of calcein intravenously, and on days 181 and 191, each horse was given 25 mg/kg of oxytetracycline intravenously.

Gross Dissection

The horses were euthanized at the end of the study by administration of an overdose of pentobarbital sodium and the entire hoof capsule of both forelimbs of each horse were removed through the proximal interphalangeal joint. After routine radiographs of the navicular region (lateralomedial, 60° dorsopalmar, and 45° palmaroproximal-palmarodistal) and blindly evaluated, P2 was dissected away at its articulation with the coffin bone (P3), allowing for visual evaluation of the articular surface of the navicular bone. The articular surface of the navicular bone was then photographed in situ and evaluated for gross lesions (Fig. 1). In order to facilitate the removal of the navicular bones each hoof wall was transected on the mid-saggital plane from the toe to heel with a band-saw, carefully following the center of the navicular bone (Fig. 1). The navicular bones were then dissected from the hoof capsule with care to avoid disruption of the articular and fibrocartilaginous surfaces.

Histologic Evaluation

Using established methods, both decalcified and non-decalcified histologic sections of each navicular bone were prepared for evaluation. A 1-cm slice was cut first from the axial edge of the lateral half of each navicular bone and placed in 10% buffered formalin for 48 h, then decalcified in Hematoxylin and eosin (EDTA) (Fig. 2). The blocks were then embedded in paraffin and 7 μm sections were mounted onto slides. The decalcified sections of each navicular bone were stained with H&E and were subjectively evaluated for bone marrow fibrosis, articular cartilage damage, and bone damage.

Non-decalcified sections were prepared using 1-cm slices, which were cut from the axial edge of the medial half of each navicular bone and placed in buffered formalin (Fig. 2). Non-decalcified sections were stained with basic fuchsin using established methods. At the end of the staining protocol, the sections were cut into 150 μm sections and ground by hand to 120 μm. These were cleaned and mounted onto glass slides. Four 120-μm sections were made of each section and were utilized to measure bone area, vascular area, marrow area, and microdamage. Total tissue area (T.Ar—mm²), bone area (B.Ar—mm²), and vascular area (Vs.Ar—mm²) were measured. The bone area was determined using point counting at 100× using a 25-point ocular grid. The percent bone area (B.Ar/T.Ar—%) was calculated to normalize bone area for bone size.
The percent vascular area within bone (Vs.Ar/B.Ar—%) was calculated to have a true impression of vascular density within a known area of bone. In order to determine bone formation, one section from each bone was used to measure bone surface (B.Pm—mm), and surface length labeled with oxytetracycline and calcein (O-sL.Pm—mm and C-sL.Pm—mm, respectively). Oxytetracycline labels were normalized to the length of the bone surface within each section (O-sL.Pm/B.Pm—% and O-dL.Pm/B.Pm—%). Calcein labels could not be normalized to bone surface since it was given three months prior to the end of the study and therefore was not on any bone surface. Calcein was therefore normalized to tissue area (C-sL.Pm/T.Ar—mm/mm² and C-dL.Pm/T.Ar—mm/mm²). Oxytetracycline labels were also normalized to tissue area (O-sL.Pm/T.Ar—mm/mm² and O-dL.Pm/T.Ar—mm/mm²) for comparison to calcein labels.

Microdamage was measured both by detecting diffuse matrix staining and by detecting microcracks. Diffuse staining was defined as stain uptake in the bone matrix that obliterated the ability to distinguish canaliculi. These areas were also quantified by point counting, and the area of diffuse staining normalized to bone area (Ddx.Ar). Microcracks were defined as sharp defects within bone matrix that took up basic fuchsin stain within the crack as well as in the surrounding bone matrix. Microcracks were further defined as matrix cracks (Cr) or delamination cracks (D-Cr) that occurred within the osteonal bone parallel to the bone surface. The number and length of each microcrack were determined and normalized to the total bone area of the slide. The microcracks and delamination cracks were added together as a measurement of total microcracks (Tt-Cr).

Data were compared between TE and HW horses. ANOVA was used to compare effects of treatment and limb on dependent variables. Significance was set at p < 0.05.

3. Results

Clinical Examinations
All treadmill-exercised horses became lame during the study. Most TE horses became lame approximately three months into the exercise program. All TE horses demonstrated significantly more severe lameness after phalangeal flexion.

Gross Evaluation
No obvious lesions or discoloration of the articular surface of the navicular bones were noted in either group.

Radiographs
Radiographic findings in the exercised group included mild increases in the size of the vascular foramina, irregular margins at the attachment sites of the DDFT, and sclerosis of the spongiosa. One control horse (HW) had radiographic evidence of elongated flexor cortices, moderate increases in the size of the vascular foramina, and small enthesiophytes.

Decalcified Histology
Subjectively, the exercised horses had thicker trabeculae and denser subchondral bone plates, as compared to the control horses (Fig. 3). Additionally, the bone in the exercised horses appeared to be less porous than that of the control horses. Areas of active remodeling of subchondral bone were evident in 5 of 6 exercised horses and 3 of 6 control horses. No evidence of partial or full thickness fibrocartilage loss was appreciated in either group.

Non-decalcified Histology
The subchondral area (B.Ar/T.Ar) of the dorsal sections of the navicular bones from the TE group had significantly more bone than HW horses (Fig. 4, p = 0.01). The palmar subchondral bone area (B.Ar/T.Ar) was also significantly greater in the TE group as compared to the HW group (Fig. 4, p = 0.0086). Dorsal and palmar vascular density in the dorsal and palmar subchondral bone plates were also significantly greater in the TE group, (Fig. 4, p = 0.0016 and p = 0.0051 respectively). The amount of delamination microcracks (Fig. 5) in the proximal and distal ends (MdLsc/T.Ar) were significantly greater for the TE group, than the HW group (p = 0.0228). The percent of double calcein label, (DdLSc/T.Ar) and (PdLSc/T.Ar) in the exercised group was significantly greater than for the controls. However, the percent of single label (DsLSc/T.Ar) and (PsLSc/T.Ar) was greater in the HW group than the TM group.

4. Discussion
There was an increase in both bone area and vascular area within the dorsal and palmar subchondral.
bone plates of the navicular bones in the exercised horses as compared to the controls. The exercised horses had a greater percent of bone labeled with calcein. This is indicative of increased bone formation early in the exercise regimen. The increase in bone area observed in the exercised group of horses was in the form of increased thickness of the trabeculi and decreased porosity of the subchondral bone plates. This was similar to the increase in trabecular bone previously seen by Wright et al. in the navicular bones of navicular disease patients. Wright also demonstrated that horses with navicular disease had significantly more palmar navicular subchondral bone area, compared to age matched controls. Therefore it appears that increased remodeling, as adaptation to exercise, is similar to pathologic change. However, Wright et al. detected an increase in subchondral area filled with more porous bone, in contrast to the decrease in porosity demonstrated in the current study. Because the subjects in the current study were euthanized after only six months of exercise, we may have detected an initial increase in bone density that would have later been followed by an increase in osteoclastic activity and a subsequent increase in porosity. The results of the current study are in support of Ostblom et al.’s conclusion that increased activation of bone remodeling; and not ischemic necrosis, is likely the cause of pathologic change in navicular disease. This is supported by our findings of increased vascularity within the subchondral bone. Maintenance of bone formation rates equivalent or

Fig. 3. Examples of a non-decalcified slide stained with basic fuchsin from a TE horse (A) and a HW horse (B). Note the increased thickness of the trabeculi and density of the subchondral bone plates in the exercised horse (A).

Fig. 4. Graph representing percent bone area for the dorsal (DB.Ar/T.Ar) and palmar (PB.Ar/T.Ar) subchondral bone plates and dorsal (Dvs.Ar/B.Ar) and palmar (Pvs.Ar/B.Ar) vascular area. Different letters indicate significant differences between TE and HW groups at p < 0.05.
greater than resorption rates may be important in maintaining navicular bone health. In addition, based on the increase in bone area observed in the current study, some amount of exercise may be beneficial in maintenance of navicular disease cases. However, similar to osteochondral disease, subchondral bone sclerosis may lead to overlying cartilage damage. The overall lack of change in the articular cartilage and fibrocartilage in the exercised group of the current study suggests that subchondral bone responds early and rapidly to exercise. Significance of microcracks in the ends of the navicular bones of the TE horses is unknown. However, microcracks are known to stimulate remodeling, therefore remodeling in the ends may have occurred later had the study period been longer.

Future studies are needed to identify thresholds of disease, and at which point adaptive remodeling may become pathologic. Subchondral bone of the navicular bone appears to dynamically respond rapidly and may be the tissue of choice to accurately define disease states. In the future, methods that measure bone density, such as Dual Energy X-ray Absorptiometry (DEXA) and computed tomographic osteoabsorptiometry could help define pathogenesis of disease in the hopes of identifying early change in the subchondral bone of navicular horses.

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References and Footnote