OSTEOCYTE LOSS IS ASSOCIATED WITH PATHOLOGICAL REMODELING OF THE NAVICULAR BONE IN HORSES

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Introduction

Failure of protective adaptive mechanisms is an important factor contributing to development of skeletal weakening, osteoporotic fracture, and stress fracture in human beings. In the horse, distal limb bones often experience large cyclic loads, which lead to fatigue injury of bone. Consequently, the horse is a useful model in which failure of functional adaptation is common. Pathological remodeling of the palmar sesamoid (navicular) bone of the distal interphalangeal joint is a common cause of foot pain and thoracic limb lameness in domesticated horses. Lameness is often bilateral in horses affected with navicular syndrome (NS) [1]. Extensive functional adaptation of the navicular bone occurs over time. Therefore, the loads experienced by the navicular bone are likely influenced by athletic activity and the use of metal shoes, which attenuate the viscoelastic composite of the tissues of the foot [2]. While bones may be well adapted to habitual cyclic loading, accumulation of microcracking may develop if cyclic loading is atypical or rapidly applied. Management of domesticated horses makes it likely that bone loading is often atypical. The osteocyte network is an important mechanotransduction pathway; a healthy population of osteocytes appears important for the maintenance of bone mass. We hypothesized that development of pathological remodeling of the equine navicular bone and local loss of bone mass would be associated with specific regional adaptive changes including development of microdamage and loss of osteocytes.

Methods

Horses. Left and right navicular bones were collected from: (1) Thoroughbred racehorses without NS (n=10, age 2-7 years); (2) Unshod ponies without NS (n=10, age 1-7 years); and (3) Horses with clinically diagnosed NS (n=7, age 10-20 years). Ponies are rarely affected with NS, whereas Thoroughbreds are commonly affected. All horses were euthanized for reasons unrelated to this study.

Specimen Preparation and Bone Density Microscopy. High-detail dorso-palmar radiographs were made. Volumetric bone mineral density (vBMD, g/cm3) was determined in sagittal and parasagittal slices using pQCT (Fig. 1). One navicular bone from each pair was then randomly selected, and sagittal and parasagittal 125μm calcified sections were prepared after bulk-staining in 1% basic fuchsin.

Histomorphometry. Sagittal and parasagittal bone sections were further divided into two regions: (1) The articular cortex underlying the synovial articulation with the middle and distal phalanges, and (2) the flexor cortex underlying the flexor surface. Sections were examined using bright-field, circularly polarized light, and epifluorescent UV light microscopy. For each region-of-interest, cortical thickness (Ct.Th, mm), bone volume fraction (B.Ar/T.Ar, %), microrack surface density (Cr.S.Dn, μm²/mm²), osteocyte density (Oc.N/B.Ar, #/mm²), and resorption space density (Rs.N/T.Ar, #/mm²) were determined. Collagen orientation was determined by birefringence in polarized light. Representative Z-stack images were also obtained using confocal laser microscopy using a krypton/argon laser with a 568nm excitation and a 585nm emission filter (Bio-Rad MRC-1024, Hercules, CA). Confocal images were scored for diffuse matrix injury and disruption to the lacunocanalicular network in a blinded fashion by three observers using a visual analogue scale.

Statistics. Repeated-measures ANOVA and a Bonferroni post-hoc t-test were used for normally distributed data. For data that were not normally distributed, the Friedman and Kruskal Wallis ANOVA tests were used and the Wilcoxon matched-pairs test were used. Results were considered significant at P < 0.05.

Results

Navicular adaptation. Development of cystic lesions associated with the bone vasculature was confirmed on radiographs of horses with NS (Fig. 1). In NS horses, vBMD was decreased in sagittal sections, when compared with parasagittal sections (P < 0.05). Ct.Th was increased in the flexor cortex, when compared with the articular cortex in all groups (P < 0.001). Networks of large intracortical vessels were often associated with resorption spaces. Multiple tendumas were also seen, particularly in horses with NS. Secondary osteons within both cortices were orientated in a horizontal lateral-to-medial direction; osteonal bone collagen was similarly orientated. B.Ar/T.Ar was decreased in horses with NS, when compared with Thoroughbreds and ponies (P < 0.001).

Osteocyte network and remodeling. Low osteocyte densities were evident in all bones. Oc.N/B.Ar density ranged from 34 to 248 cells per square millimeter. Loss of osteocytes was most prominent immediately adjacent to the calcified cartilage layer. Ot.N/B.Ar was decreased in horses with NS, when compared with Thoroughbreds and ponies (Fig. 2). Ot.N/B.Ar was decreased in sagittal versus parasagittal sections (P < 0.005) and decreased in the articular versus flexor cortex (P < 0.005). Micropetrosis was also a prominent feature of bones from NS horses. Rs.N/T.Ar was increased in ponies, when compared with Thoroughbreds (P < 0.005). Rs.N/T.Ar was also higher in ponies, when compared with horses with NS (P = 0.07).

When viewed with confocal microscopy, diffuse matrix staining, indicating diffuse microdamage, and extensive disruption to the network of osteocytes was evident in all bones. The connectivity of osteocytes was also reduced in all bones, particularly bones from horses with navicular disease. Disruption of canaliculi was increased in the articular cortex, when compared with the flexor cortex (P<0.01).

Microcracking. Cr.S.Dn was increased in NS horses, when compared with Thoroughbreds and ponies (P < 0.01).

Fig. 1. Dorso-palmar radiographs of navicular bones from a Thoroughbred (A) and a 19-year old horse with NS (B). Enlargement of foramina and associated cortical resorption is apparent (arrows). PS – sites of the parasagittal section, S – site of sagittal section.

Fig. 2. Photomicrograph of a sagittal calcified section of navicular bone from a 17-year-old horse with NS. Micropetrosis and loss of osteocytes was evident. Micropetric bone matrix typically exhibited poor update of basic fuchsin after bulk-staining. Diffuse matrix staining, indicating diffuse microdamage, was also often seen (arrows). Scale bar = 50μm.

Discussion

The presence of horizontal secondary osteons suggests the navicular bone is predominantly loaded in cyclic dorso-palmar bending. With such loading, it would be expected that cyclic strains are greatest in the sagittal part of the bone; the region with the greatest loss of osteocytes and vBMD in NS horses. The significant accumulation of navicular bone microcracking in NS horses suggests mechanical overload may promote osteocyte loss and associated micropetrosis over time. Endochondral ossification of articular cartilage was also a prominent feature in pathological bones and likely promotes joint degeneration over time [3]. Taken together, these data suggest that loss of osteocytes, but not increased resorption space formation, is associated with the development of uncoupled pathological adaptive remodeling and local loss of bone mass in response to chronic cyclic mechanical overload of bone in this model. Use of metal shoes may be a key factor promoting atypical loading and eventual adaptive failure.

REFERENCES


53rd Annual Meeting of the Orthopaedic Research Society
Paper No: 0012