Final Report for NZRB Contract

Effect of exercise on canals in articular calcified cartilage of equine third carpal bones

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This report is a brief overview of the work carried out on the above contract. A manuscript is currently being prepared by the equine resident who was carrying out this study which will contain full experimental details and results, including electron microscopy images. A copy of the paper will be forwarded to the NZRB when completed.

Summary

The third carpal bone responds to exercise by adaptive remodelling in subchondral bone and articular calcified cartilage. This is important in preventing pathological change. Canals, or holes, penetrating the articular calcified cartilage and thought to contain vascular tissue, have been reported in numerous species. However, the significance of these holes is as yet unclear, as they have been reported in both diseased and non-diseased joints.

The aim of this study was to determine whether the presence of these canals is a normal adaptive response to exercise, by comparing the same dorsal sites in both exercised and control horses, or whether loading is a greater factor, by comparing sites from the intermittently highly loaded dorsal intercarpal site with a palmar site and sites within the low-motion carpometacarpal joint.

Cadaveric sections were taken from the radial facet of the third carpal bones from six exercised and six control horses. Bone mineral density was measured and compared between the groups. The numbers of holes penetrating the calcified cartilage were compared in four regions subject to different loading regimens. The morphology of the sections was also studied.

Bone mineral density was significantly greater in the exercised horses. There were significantly less holes in the dorsal aspect of the bone in exercised horses than in other sites, with no significant difference between the numbers of holes in the intercarpal compared with the carpometacarpal joint sites. Adaptive remodelling therefore appears to have a dominant influence on the numbers of holes present. Imaging of the bone sections also revealed differences in the appearance of the holes. These morphological variations have not been previously described in the literature. While further work is still needed to elucidate the possible relationship between these holes and diseased joints, these results provide valuable new information, particularly morphologically, that may assist in interpreting the significance of the presence of these holes in equine articular calcified cartilage.

Introduction

Many unanswered questions remain concerning the microscopic changes which occur before macroscopic lesions become present in equine hyaline articular cartilage (HAC), calcified cartilage (ACC) and subchondral bone (SCB). Studies of the third metacarpal bones of the thoroughbred racehorse indicate that the earliest changes of osteoarthritis and osteochondral disease occur in the ACC. Localised alterations in the thickness of this tissue in response to exercise could be physiological or pathological, and warrants further investigation so that its role in growth and development, response to environmental stimuli and behaviour in disease and ageing can be better understood.

ACC forms the interface between HAC and SCB, apparently attenuating the shear forces that are transmitted from the joint surface to the underlying bone. Penetrating holes between the SCB and the ACC, thought to contain vascular tissue, have been reported in numerous species including humans, horses, rabbits and dogs but the significance of these holes is as yet unclear, having been reported in both diseased and non-diseased joints. It has been theorised that these holes could be involved in bone remodelling in early osteoarthritic development and in supplying nutrition of the ACC when reparative growth is needed.

In the horse, the radial facet of the third carpal bone is susceptible to injury. Carpal osteochondral lesions in thoroughbred racehorses have been well described in the literature, and a link between training intensity and lesion incidence has been suggested. The objective of this study was to determine if the number of holes penetrating the ACC layer differs between regions of different loading regimes, namely the proximal and distal aspects of the third carpal bone within the middle carpal and carpometacarpal joints respectively. The former has a high range of motion and reduced cartilage contact area at some phases of the stance phase, whereas the latter has almost no range of motion.

It was hypothesised that the number of holes in the ACC surface of the dorsal radial facet would be significantly different from that in the palmar radial facet of the third carpal bone of exercised horses and that the number of holes in the ACC surface of the intercarpal joint would be significantly different from that in the carpometacarpal joint.

Materials and Methods

Animals & tissue source

Previously untrained 19-month old thoroughbred fillies with no lameness or discernible carpal disease were randomly assigned to an exercise group (Group 1: treadmill exercise, n=6) or a control group (Group 2: walked only, n=6). Group 1 horses were intensively trained to mimic preparation for racing. This included progressive work on an inclined high speed treadmill or trotting for 10 minutes in each direction on the horse walker. Group 2 horses were walked daily for 20 minutes in each direction. All horses were stabled when they were not being exercised. After 19 weeks of the assigned exercise regime, all horses were euthanased and tissue was harvested. The right carpi were removed at euthanasia and frozen at -20°C prior to sectioning before storage in ethanol until use in the present study. A full thickness 6 mm sagittal slab was sectioned from each third carpal bone commencing 5 mm medial to the ridge dividing the radial and intermediate facets.

Peripheral quantitative computed tomography (pQCT)
Each sagittal slab was orientated to include the proximal and distal articular surfaces in the pQCT scanner, ensuring that two 2 mm thick tomographic sections were both included within the bone slab. Bone mineral density (BMD) measurements were made of the entire slab in addition to ten contiguous 2 mm x 2 mm blocks along the proximal intercarpal and distal carpometacarpal joint surfaces.

Sample site selection for electron microscopy
Utilising prior knowledge of pathology, loading site information and BMD values, sites were selected for further examination by electron microscopy. These sites were 4-8 mm (DPr) and 16-20 mm (PaPr) from the most dorsal proximal aspect of the sagittal section and 4-8 mm (DDi) and 16-20 mm (PaDi) from the most distodorsal point along either joint surface (summary table below). Samples at these sites were 6 x 4 x 2 mm in size and were sectioned using a diamond saw.

Site on third carpal bone	**Anatomical location**
Dorsoproximal (DPr)	4-8 mm palmar to the dorsal rim of the proximal surface
Palmaroproximal (PaPr)	16-20 mm palmar to the dorsal rim of the proximal surface
Dorsodistal (DDi)	4-8 mm palmar to the dorsodistal edge
Palmarodistal (PaDi)	16-20 mm palmar to the dorsodistal edge

Scanning electron microscopy (SEM) & image analysis
A pilot trial was conducted to optimise conditions for HAC removal and ACC surface preparation prior to performing scanning electron microscopy (SEM). HAC was removed by immersion in tergazyme and hydrogen peroxide at varying concentrations, temperatures and time periods to determine these optimal conditions. SEM images were examined for the presence of holes penetrating the ACC surface from the SCB and other potentially significant morphological features. The diameter of holes was measured and their morphology recorded. Other variations in the appearance of the ACC surface, including depressions, dimples and openings were noted where they clearly differed from the typical features of chondrocyte lacunae.

Statistical analysis
The number of holes at each site of interest, relationship to BMD and the effect of exercise were examined using a Kruskal Wallis rank test. Bonferroni correction was applied with significance set at $p \leq 0.012$.

Results

pQCT
BMD of the whole sagittal section was significantly greater in the exercised horses compared with the control horses ($p=0.004$). BMD values were significantly greater in the cortical bone of the dorsal regions of the joint when compared with the palmar regions in the exercised horses ($p=0.003$). No statistically significant differences were seen between the four selected sites in the control horses.

SEM & image analysis
Different types of holes were observed based on size and morphology.

Large holes were oval, circular or triangular in appearance and had an irregular edge with a border of matrix which was approximately one third of the diameter of the hole. The matrix contained few or no chondrocytes and often had numerous small narrow cracks running perpendicular to the hole edge, giving this matrix a more uniform appearance than the rest of the extracellular matrix. These holes varied in diameter from 23 to 150 microns. In some cases, bony trabeculae could be viewed through these holes and occasionally osteocytes and osteoclasts were also noted.

Smaller holes were identified which were often found in groups of 3 or 4. They were surrounded by a depressed larger region of smooth, flat matrix, sometimes with a further depression around some or all of the holes. No normal chondrocyte lacunae were present in these flat areas. These holes varied in diameter from 6 to 32 microns.

A further type of small hole was noted. These were not surrounded by the depressed regions which characterised the other small holes. They appeared similar in morphology to chondrocyte lacunae, but could be distinguished from them as the holes were larger than those expected for lacunae.

An interesting morphological feature of the ACC surface in some samples was the presence of dimples. These dimples sometimes contained small holes and sometimes did not. Horses that had one site exhibiting dimples were more likely to have other sites with dimples. However, dimples were not found to be present more often at one site than another.

Statistical analysis
The number of large holes was significantly lower in the dorsal sites (DPr & DDi) compared with the palmar sites (PaPr & PaDi) when all horses were compared ($p=0.004$). This statistical difference remained when the dorsal and palmar regions of the control horses were examined ($p=0.006$). There were no statistically significant differences when only the strenuously exercised group were compared.

The number of small holes was not significantly different between site or between strenuously exercised and control horses.

There was no significant difference between the number of holes, either large or small, between the two proximal sites (intercarpal joint) and the two distal sites (carpometacarpal joint).

Discussion
The adaptive remodelling response of equine carpal bones to intensive exercise has been well described and is thought to be related to the degree of compressive loading in this region. The expectation was that the exercised horses in this study would have greater BMD on the dorsal aspects of the third carpal bones whereas those that had not undergone simulated race training would have no difference in bone density between the dorsal and palmar regions of the bone. The BMD pattern seen in this study was therefore as expected.

The sites selected for microscopy analysis were consistent with the data on pathology of this bone. The common classifications of radial facet fracture would involve the site chosen as the dorsoproximal region of interest, as would frontal plane slab fractures. Sites of subchondral ischaemia and sclerosis, bone lysis, cartilage fibrillation and degeneration would also be at least partly involved in the site chosen for the dorsoproximal region of interest.

It is interesting that this adaptive response of the underlying SCB has an apparently greater effect on the morphology of the ACC than the specific motion regimens of the two different joints. The hypothesis that there would be a difference in the number of holes between the intercarpal and carpometacarpal joints was rejected. A marked difference between the common site of pathology and other sites studied had been expected but this did not prove to be the case.

There does appear to be an association between the presence of holes and the adaptive remodelling response to exercise. There was a trend towards increased holes on the palmar carpometacarpal aspect of the third carpal bone which may be consistent with thinner or less calcified ACC at this point or with the proximity to one of the palmar intercarpal ligaments. There does not appear to be a significant difference between hole formation in the intercarpal and carpometacarpal articular surfaces.

This work is clinically relevant in presenting and describing the occurrence of holes seen in the ACC in exercised and non-exercised horses, with no gross pathology. An important finding is that the compressive forces and/or the change in bone density that occurs in the dorsal part of the third carpal bone appears to equally affect the underlying SCB in the carpometacarpal joint and the intercarpal joint and that this seems to drive the decrease in large holes, rather than the differences in joint motion.

There are few reports and even fewer images of ACC samples prepared and oriented in the same way as was undertaken in this study. Many of the images that do exist have no scale bars, making a comparison of the holes described in this study difficult to compare with other reports. Several of the morphological features observed in these samples have not been previously described or illustrated in the literature and are therefore being reported for the first time. It is possible that some may be consistent with osteoclastic resorption on the ACC surface and may therefore play a significant, although as yet still unknown, role in equine orthopaedic disease.

A larger number of horses would be needed to consolidate these findings. Studies in older racing and non-racehorses, particularly those with carpal pathology, would be relevant to further investigate the effects of exercise and pathology on ACC holes.