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Original Article

Assessment of bone response to conditioning exercise in the radius and tibia of young Thoroughbred horses using pQCT

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Abstract

Objectives: To assess the effect of conditioning exercise on bone parameters at multiple sites in the radius and tibia of young Thoroughbred horses. **Methods:** The left and right radius and tibia were obtained from twelve horses, six of which had received conditioning exercise and six which formed the control group. Each bone was scanned at 5% intervals along its entire length using pQCT. **Results:** Bone strength, bone area and periosteal circumference were significantly greater for the group of conditioned horses in both the radius and tibia. Volumetric bone mineral density was lower while bone mineral content, endocortical circumference and polar moment of inertia were higher in the conditioned group of horses but the significance of these differences varied between the two bones. Cortical thickness was not significantly different between the groups in either bone. **Conclusions:** Conditioning exercise stimulated a significant increase in the strength of both bones that could be attributed mainly to an increase in bone size, rather than differences in bone mineral content or density. The radius and tibia exhibited differences in the significance of changes in several bone parameters suggesting that not all bones respond in an identical fashion to imposed exercise.

Keywords: Conditioning Exercise, Thoroughbred Horse, pQCT, Radius, Tibia

Introduction

Successful training of the equine athlete requires a carefully designed exercise programme that optimises physiological adaptation to the demands of an increasing workload. The level of exercise that results in beneficial adaptation of the musculoskeletal system is particularly difficult to determine, since the various tissues do not all respond in the same way[1]. Musculoskeletal injury is a common reason for loss of performance in Thoroughbred racehorses and is significantly influenced by management and training methods[2].

The response of the musculoskeletal system to conventional training programmes used by commercial racing stables has been studied in a group of two-year-old New Zealand Thoroughbred fillies[3]. The effect of exercise was evaluated for various tissues including bone[4-6], cartilage[5] and tendon[7,8]. A major finding from this study was that cancellous bone showed the greatest response to training while changes in cortical bone, articular cartilage and tendon were much less obvious[9], which agrees with findings in other mammals.

The effect of exercise on the musculoskeletal tissues of younger animals prior to training is of further interest. Restriction of exercise by confining a group of foals to stalls during the first months of life was found to result in a delay in the functional adaptation of the articular cartilage collagen network[10] and had lasting negative effects on indicators of articular cartilage metabolism. When these foals were given moderate exercise by allowing them free access to pasture after confinement in box stalls for five months, the articular cartilage failed to show adaptation to the new level of exercise. Similar effects have also been shown for other musculoskeletal tissues such as bone and tendon which respond to variable extents[11].

The spontaneous locomotion activity of one-month-old foals at pasture has been observed to be about double that of older foals and, significantly, consists of more cantering and play[12]. Faster gaits and play activities promote dynamic load-

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ing of bones by "novel" forces which, when combined with relatively high forces of unusual distribution and short duration, is believed to optimise the response of bone to mechanical stimuli[13]. A better understanding of how these natural processes of tissue adaptation occur in very young animals could therefore allow development of programmes that might minimise the risk of musculoskeletal injury when training and athletic competition are undertaken later in life.

A large study undertaken by the Global Equine Research Alliance (GERA) was set up to investigate the effect of early conditioning exercise on young Thoroughbred horses[14]. The results to date have demonstrated that the foals easily tolerated an increase in workload, defined as the product of distance and velocity[15], of approximately 30% over and above natural spontaneous pasture exercise without significant adverse clinical effects on the musculoskeletal system[16,17]. There was apparently little effect of this early conditioning exercise on the superficial digital flexor tendon[18] or tenocyte density of the common digital extensor tendon[19], although no harmful effects were observed in either case. Investigation of the biochemical composition of articular cartilage extracellular matrix showed advancement of the normal maturation process of the matrix components in the conditioned foals but no evidence that this was detrimental[20]. Chondrocyte viability and the quality of articular cartilage in the distal third metacarpal and metatarsal bones were found to be improved by the early exercise[21]. The response of bones in the upper limb is the subject of this study.

Many methods for assessing bone properties in horses have serious disadvantages associated with accessibility, cost, accuracy or precision[22]. Peripheral quantitative computed tomography (pQCT) is a widely applied technique to investigate bone and has been highlighted as a tool that is likely to become increasingly important in future equine orthopaedic studies[23]. pQCT can be used to determine mineral content and cross-sectional area of bone, periosteal circumference, endocortical circumference and volumetric bone mineral density. All of these parameters contribute to bone strength, which is estimated by the strength strain index (SSI)[24]. The ability to quantify bone size, shape and volumetric density and to distinguish between cortical and trabecular bone are significant advantages over traditional planar imaging techniques such as radiography and dual energy X-ray absorptiometry.

The aim of this study was to assess bone response to conditioning exercise in the radius and tibia of young Thoroughbred horses using pQCT. The shapes of these bones are of interest since it has been proposed that their curvature, particularly pronounced in the radius, may ensure a highly predictable loading distribution[25]. A further aim was to investigate variations in bone parameters including size, mineral content, density and strength along the entire length of these bones rather than at a few selected sites. The overall hypothesis was that the conditioning exercise would have been of sufficient intensity to stimulate a response in both the radius and tibia, resulting in statistically significant differences in bone strength between the conditioned and non-conditioned group of horses.

Materials and Methods

Bones

The left and right radius and tibia of twelve Thoroughbred horses, part of the GERA study described previously[17], were defleshed, wrapped in plastic and stored in a freezer at -20°C prior to this study. All twelve horses had been grazed together in the same paddock; six had received conditioning exercise from an average age of three weeks to seventeen months in addition to spontaneous exercise at pasture (CONDEX group), whereas the other six exercised only at pasture (PASTEX group). A detailed description of the exercise protocol has been previously reported[17] and consisted of clockwise and anticlockwise exercise on alternate days around an oval track over a distance of 1030 m per day, five days per week, at increasing speeds as the foals became older. The workload of the CONDEX animals was calculated to be 30% higher than that of the PASTEX group.

Peripheral quantitative computed tomography (pQCT)

The length of each bone was measured from the most proximal to the most distal aspect on the lateral aspect using a steel ruler. Transverse pQCT scans (XCT 2000, Stratec Medical, Pforzheim, Germany) were performed at 5% intervals along the entire length of both the left and right radius and tibia of the twelve horses. The 5% and 95% slices represented the most distal and most proximal scans respectively. All of the bones were scanned in the same orientation, each slice had a thickness of 2 mm and voxel size was 0.4 mm. The outer threshold was set at 280 g/cm^3 and the inner threshold for cortical bone was 710 g/cm^3.

As part of the distal epiphysis of the radius had been removed for analysis in a previous study, the 5% slice for this bone was not obtained. In order to calculate the original length of the radius, nine radii were obtained from other horses to establish the relationship between the length of the bone from the proximal joint surface to the lateral physeal prominence and the total length of the bone. From these measurements, it was estimated that the radii in the study were, on average, 91.5% of their original length, from which the true length and hence the actual distance between the 5% scan sites could be calculated.

Bone mineral content (BMC), bone area, periosteal circumference, endocortical circumference, cortical thickness, volumetric bone mineral density (vBMD) and strength strain index (SSI) were obtained using the manufacturer's software (XCT 6.00B). The SSI data, derived from the polar cross-sectional moment of inertia (pCSMI) and cortical vBMD of the bone scan slice, provided a measure of the ability of the bone to resist torsion.

Statistical analysis

The data obtained from the pQCT software were analysed using a repeated measures general linear model (SPSS version 17.0, SPSS Inc., Chicago) to test for differences between the CONDEX and PASTEX groups and between bones of the left

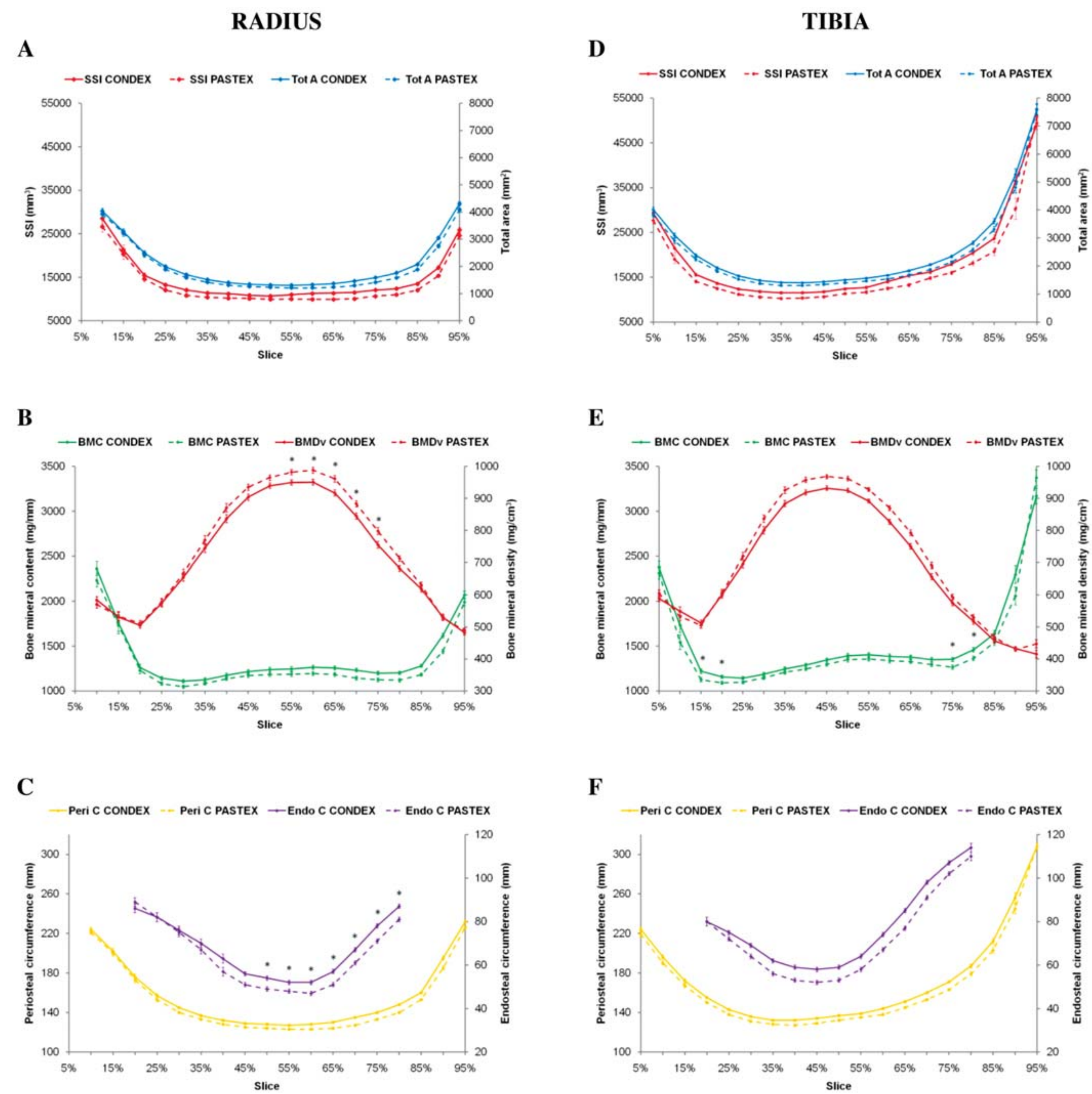

Figure 1. Variation in bone parameters in the radius (**A-C**) and tibia (**D-F**) for conditioned (CONDEX) and control (PASTEX) groups and shown as estimated marginal means for strength strain index [SSI], total area (Tot A), bone mineral content [BMC], volumetric bone mineral density [vBMD], periosteal circumference (Peri C), and endocortical circumference (Endo C). For each parameter the difference between groups was significant by repeated measures analysis except for radius vBMD (graph B), radius Endo C (graph C) and tibia BMC (graph E), where individual slices were analysed by ANOVA and significant differences are marked with an asterix. Error bars represent the standard error of the mean.

and right limbs within each group. The level of significance was set at $p<0.05$. Given the extremely wide variation in bodyweights amongst the 12 horses, which ranged from 398 to 525 kg, and the fact that the heavier horses did not necessarily belong to the exercised group (the lightest and heaviest horse both belonged to the CONDEX group), weight was included as a covariate. As a further check on the effect of weight, an independent samples t-test was performed. After establishing that the variances of the groups were equal, this test showed that there was no significant difference in weights between the two groups [t(10) = 0.100, P = 0.922]. For all parameters, no significant differences between the left and right bones were observed and these data were subsequently pooled. Wilks' lambda was used to investigate within-group effects by determining the significance of the variation in each bone parameter as a function of slice and the interaction of slice and group for each bone.

Results

Figures 1A and 1D illustrate the variation in SSI along the radius and tibia respectively of the CONDEX and PASTEX groups. Bone strength was significantly greater in the CONDEX group for both bones, as determined by repeated measures analysis (radius: P=0.023, tibia: P=0.01). In the radius, SSI decreased markedly between the 10% and 20% slices, then much more gradually to a minimum in the mid-region of the diaphysis before increasing again very gradually to the 85% slice, followed by a marked increase to the 90% and 95% slices. A similar trend in SSI was observed in the diaphysis of the tibia except that there was a greater increase in strength observed between the 50% and 85% slices before the marked increase to the 90% and 95% slices. The change in SSI along the length of the bone was not significant for either the radius (P=0.066) or tibia (P=0.284). There was no significant interaction between slice and group for either bone (radius: P=0.138, tibia: P=0.487).

The variation in bone area of the radius (Figure 1A) and tibia (Figure 1D) was very similar in the CONDEX and PASTEX groups, with the total bone area being significantly greater in the CONDEX group (radius: P=0.025, tibia: P=0.043). Neither the variation in area with slice (radius: P = 0.069, tibia P=0.447), nor the interaction between slice and group (radius: P=0.346, tibia P=0.195), was significant for either bone.

Differences in volumetric bone mineral density (vBMD) in response to exercise were very similar for the radius (Figure 1B) and tibia (Figure 1E). While vBMD was higher in the PASTEX group in both bones, the significance of this finding varied. The higher vBMD of the PASTEX group was significant in the tibia (P=0.040). The variation in vBMD along the length of the tibia was significant (P=0.024), as was the interaction between slice and group (P=0.033). In the radius, the difference in vBMD between the two groups was not significant but the change in density along the length of this bone was significant (P<0.001) as was the interaction between slice and group (P=0.011). Analysis of individual slices of the radius using ANOVA indicated that differences in density between the two groups were significant in the region between the 55% and 75% slices (significant slices are shown in Figure 1B with an asterix, P=0.01).

Differences in bone mineral content (BMC) in response to exercise were similar for the radius (Figure 1B) and tibia (Figure 1E). While BMC was greater in the CONDEX group in both bones, the significance of this finding varied. The greater BMC of the CONDEX group just reached significance in the radius (P=0.050) but was not significant in the tibia. However, analysis of individual slices in the tibia showed significant differences in mineral content for the 15% (P=0.01), 20% (P=0.015), 75% (P=0.019) and 80% (P=0.020) slices (shown by an asterix in Figure 1E). The variation in BMC along the length of the bone (radius: P=0.192, tibia P=0.073), and the interaction between slice and group (radius: P=0.070, tibia P=0.460), were not significant for either bone.

There were identical trends in the differences in periosteal circumference between the CONDEX and PASTEX groups for the radius (Figure 1C) and tibia (Figure 1F), with both being significantly greater in the CONDEX group on repeated measures analysis (radius: P=0.026, tibia: P=0.030). There were significant variations in periosteal circumference along the length of the radius (P=0.027) and tibia (P<0.001) but no significant interaction between slice and group for either bone (radius: P=0.357, tibia P=0.248).

The variation in endocortical circumference in the diaphysis of each bone is shown for both groups of horses for the radius (Figure 1C) and the tibia (Figure 1F). Endocortical circumference was significantly greater in the CONDEX group in the tibia (P=0.013). The variation in endocortical circumference along the diaphysis of the tibia was significant (P=0.032), as was the interaction between slice and group (P=0.004). In the radius there was no significant difference between the two groups except where analysis of individual slices by ANOVA showed a significantly greater endocortical circumference in the CONDEX group for slices 55-80% (significant slices shown by an asterix in Figure 1C). The variation in endocortical circumference along the diaphysis of the radius was significant (P=0.029), as was the interaction between slice and group (P=0.044).

Polar cross-sectional moment of inertia of the total bone area is shown for the radius in Figure 2A and the tibia in Figure 2D. The higher values for the CONDEX group were significant in the radius (P=0.033) but not the tibia. The variation along the bone was significant for the radius (P<0.001) but not for the tibia (P=0.272). There was no significant interaction between slice and group for either bone (radius: P=0.275, tibia: P=0.060).

Average cortical thickness was not significantly different between the two groups in the diaphysis of either bone. Regional differences in thickness were apparent by pQCT, as illustrated by the selected slices in Figures 2B and 2E for the radius and tibia respectively. The appearance of the whole bone is shown in Figure 2C for the radius and Figure 2F for the tibia.

Discussion

The strength of a bone is related to both its structural and material properties[26]. Structural properties, sometimes referred to as bone architectural properties, refer to bone size and shape, which in this study were assessed by determining bone area, polar moment of inertia, periosteal circumference, endocortical circumference and cortical thickness. The material properties of bone tissue are determined by the chemical composition of its organic and inorganic components and its stage of miner-

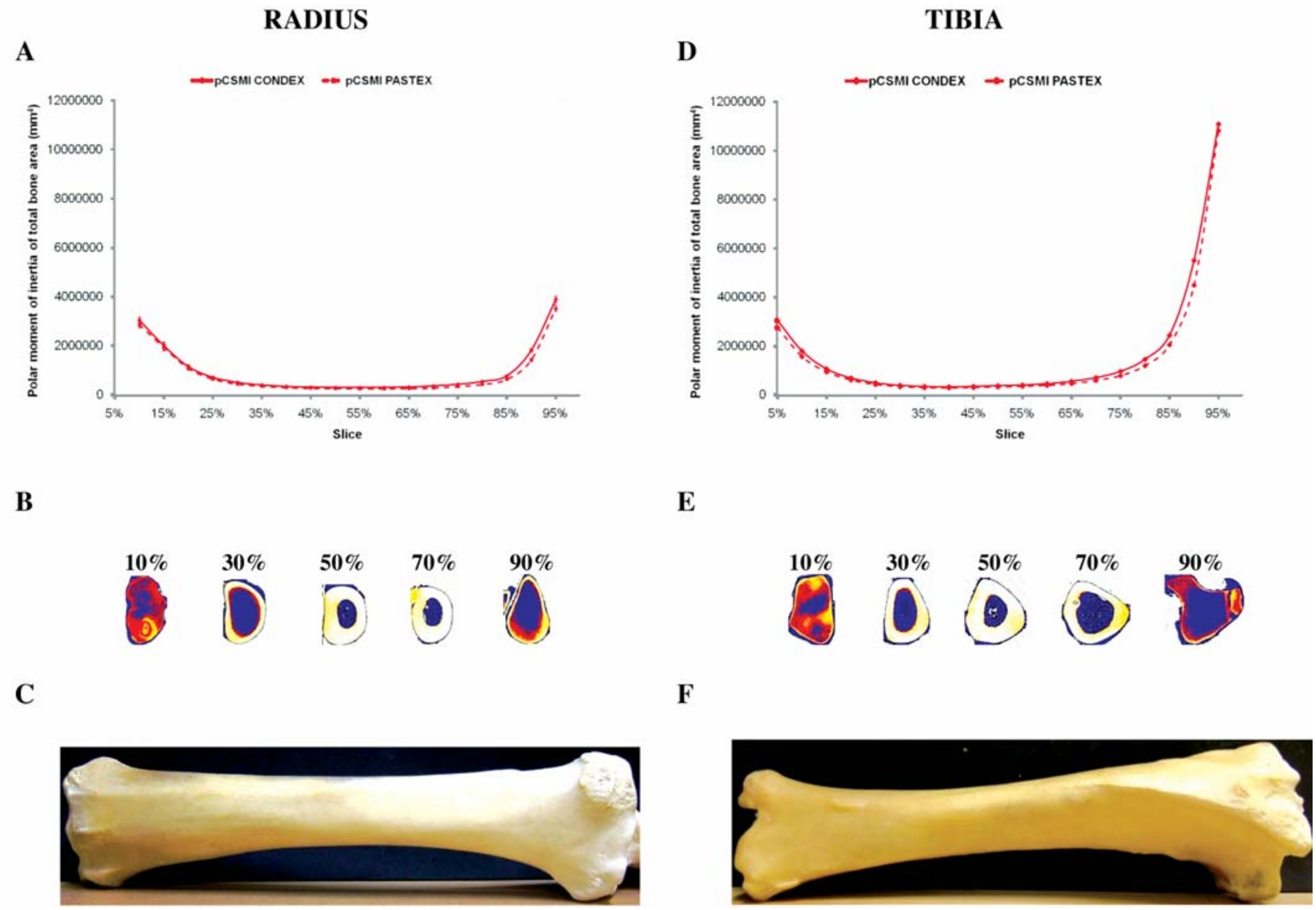

Figure 2. Variation in polar moment of inertia of the total bone area (pCSMI) in the radius (**A**) and tibia (**D**) for conditioned (CONDEX) and control (PASTEX) groups and shown as estimated marginal means. The difference between groups was significant by repeated measures analysis for the radius but not the tibia. Error bars represent the standard error of the mean. Selected pQCT slices are illustrated for the radius (**B**) and tibia (**E**), with the cranial aspect to the right and lateral aspect to the top. The cranial aspect of the whole bone is shown for the radius (**C**) and tibia (**F**), with the distal aspect to the left and proximal aspect to the right.

alisation, with the mineral content known to be highly correlated with the mechanical properties of bone[27]. Microdamage is a further determinant of strength[28]. The amount of microdamage caused by the mild exercise programme imposed on the CONDEX group was considered to be low in relation to what would be expected for horses in race training or competition and was therefore not assessed as part of this study.

The ability to assess structural and material properties of bone simultaneously and integrate these into an index of bone strength, through calculation of SSI, is a key feature of pQCT. SSI data were not used as absolute values of strength in the radius and tibia of the study horses but rather for comparison of diaphyseal bone strength between the CONDEX and PASTEX groups. The data clearly showed that the diaphysis of the radius and tibia responded markedly to the imposed conditioning exercise, resulting in significantly greater bone strength in the CONDEX group. The increase was due mainly to size (geometry) increase of the structure. SSI is less relevant to the epiphysis and metaphysis of a bone since the original development of a bone strength index was intended as a non-destructive equivalent to the mechanical three-point bending test[29] which is not applicable to the ends of a test beam. The extremities of the radius and tibia also have cross-sectional shapes which deviate considerably from the more rounded or oval geometry of the diaphysis, potentially contributing to significant errors in strength estimates.

Bone area was significantly greater throughout the radius and tibia in the CONDEX group of horses. Bone is capable of adapting to changing applied loads, as occurs during exercise, according to the theory of the mechanostat[30]. The mechanostat is a model of bone accretion and loss in response to localised mechanical forces so that if a load is applied to a bone above or below particular threshold levels, the bone will respond to the applied forces by addition or removal of bone mineral. In this way, the mass and architecture of the bone are determined by the required strength of the bone[31].

The significance of findings for parameters related to bone size and shape were different for the radius and tibia. For bones with the same cross-sectional area, the greater the diameter, i.e. the more peripheral the distribution of cortical bone, the stronger the bone will be in bending or torsion due to the increased cross-sectional moment of inertia[32]. It may be expected, therefore, that exercise should induce an increase in CSMI in bones that are subjected to particularly high bending or torsional forces. While pCSMI in both bones was greater for the CONDEX group, this was significant only for the radius. Additionally, although the periosteal circumference of the CONDEX group was significantly greater than that of the PASTEX group for both bones, significant differences in the endocortical circumference of the CONDEX group were found only in the proximal radius. The significance of the slice and group interactions for the endocortical circumference indicated that identical trends were not seen in the two groups for this parameter, reflected in the graphs for the two groups not being parallel in the distal diaphysis, particularly in the radius. The combined effects of increased bone area, polar moment of inertia, periosteal circumference and endocortical circumference in the CONDEX group, although not necessarily significant on their own, appeared to result in an overall significant increase in bone strength in this group of horses for both bones.

The reason for differences in response between the two bones and within regions of the same bone may lie in their shape and the magnitude and direction of applied forces during weight-bearing. In the radius, load distribution is more predictable[33] due to its pronounced curvature in comparison with the tibia. While both bones experience large bending forces, the magnitude and relative contributions of tensile, compressive and torsional strains have been shown to differ markedly within and between the radius and tibia in the horse at walk[34]. Differences in response to exercise may also reflect the different roles of each bone, i.e. the tibia contributes to the propulsive action of the equine hind limb while the radius is involved in the more supportive function of the fore limb.

No significant differences in cortical thickness were observed as a consequence of exercise, even though the CONDEX group had bigger bones and greater endocortical and periosteal circumferences. In the CONDEX group, subjected to the novel forces induced by cantering and galloping, the increase in endocortical and periosteal circumference resulted in an increased bone area which contributed to the overall increase in SSI. Similar histomorphological changes in which both the periosteal and endocortical circumference have increased as the result of exercise occurred in adult rats subjected to increased mechanical usage of the hindlimb[35] and, in juvenile rats, when long-term loading was separated into discrete bouts[36]. These studies, in conjunction with our own findings, illustrate how mechanical loading can optimise the structural integrity of bone. By formation of new bone that is localised to precisely where it is needed, tissue economy is increased by minimising increase in bone mass.

While the pQCT methodology provided only an average cortical thickness, regional variations were apparent as can be seen in Figures 2B and 2E. For instance, the medial and caudal aspects of the radius are thicker than the lateral and cranial aspects. Significant regional differences in cortical thickness of the lower tibial bone shaft in humans have been suggested as being important for modulating bending stress and compensating for areas of reduced vBMD[37]. Quantification of the regional differences in cortical thickness between the two groups was outside the scope of this study and was not performed.

Although BMC was higher in the CONDEX group, this only just reached significance in the radius and was significant in only some individual slices in the tibia. The changes in geometry in response to the exercise apparently led to an increase in bone strength without significant differences in bone mass. This again illustrates tissue economy in action; the exercise induced new bone formation only where it was needed for increased strength and removed bone where it was not required.

Volumetric bone mineral density of cortical bone was the only parameter that was significantly lower in the CONDEX than PASTEX group. There was also a significant interaction between bone slice and group in this parameter, indicating that the trends in vBMD for each group were different, and reflected in the shape of the two graphs which are superimposed in the epiphyseal and metaphyseal regions. The significantly greater bone area in the CONDEX group may have outweighed the relatively minor increase in mineral content, resulting in a lower bone density compared to the PASTEX group. Also, the CONDEX group added new (primary) bone, which is less mineralised than the secondary bone laid down in succeeding months.

The significant increase in diaphyseal bone strength associated with conditioning exercise therefore appears to be largely due to size and shape effects rather than vBMD or BMC. This suggests that the mild conditioning exercise stimulated adaptive geometric changes which, through power laws, are much more important for increased bone strength than any increase in bone mineral density, mineral content or cortical thickness. This importance of bone size and geometry in influencing bone strength in the radius and tibia appear to confirm the findings from studies of other equine bones in which the authors suggested that this effect is more important than bone density[4,38] which increases to only a small extent at slow rather than fast gaits, with faster work appearing to stimulate an increase in bone size.

Although the differences in bone parameters between the CONDEX and PASTEX groups were most apparent in the diaphysis, the metaphyseal regions of bone may warrant more attention in future studies. The metaphysis is not a precisely defined region of bone, as there is no clear transition between it and the diaphysis. By considering its definition as the flared portion of bone at each extremity of the diaphysis[39], in combination with mediolateral density profiles which show the relative amounts of trabecular and cortical bone, the 10%, 15%, 85% and 90% slices were defined as metaphyseal in this study and were associated with changes in the general trends shown by the diaphysis. It may be worthwhile to re-examine these areas using a much narrower scan interval to obtain a more detailed picture of the variation in bone parameters in this region which is associated with a high incidence of fractures during growth[40,41].

Overall, the imposed exercise induced changes in both the radius and tibia which led to a significant increase in the bone strength of the CONDEX group of horses. However, the tibia and radius responded differently in terms of changes to bone density, mineral content, polar cross-sectional moment of inertia and endocortical circumference to effect this strength increase. In the tibia, the CONDEX group exhibited significantly lower total density and greater endocortical circumference but no significant difference in polar cross-sectional moment of inertia or mineral content except in a few individual slices. In contrast, in the radius, the CONDEX group exhibited significantly higher mineral content and polar cross-sectional moment of inertia but no significant difference in total density or endocortical circumference except in the proximal half of the diaphysis. This suggests that each bone responds in an individual fashion to enable it to best resist the forces it is subjected to, presumably as a result of each bone's location, function, shape and architecture. It also shows that the mechanostat is an ideal mechanism for ensuring that bone strength can be modified in a highly localised and efficient manner. While general conclusions may be drawn on the likely effects of exercise on bone, it may not be possible to directly extrapolate results from studies of one bone to predict the likely response of another.

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