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REVIEW

The response of bone, articular cartilage and tendon to exercise in the horse

Elwyn C. Firth

Institute of Veterinary, Animal and Biological Sciences, Massey University, Palmerston North, New Zealand

Abstract

Horses can gallop within hours of birth, and may begin training for athletic competition while still growing. This review cites studies on the effects of exercise on bone, tendon and articular cartilage, as detected by clinical and research imaging techniques, tissue biochemical analysis and microscopy of various kinds. For bone, alterations in bone mineral content, mineral density and the morphology of the mineralized tissue are the most common end-points. Apparent bone density increases slightly after athletic training in the cortex, but substantially in the major load paths of the epiphyses and cuboidal bones, despite the lower material density of the new bone, which is deposited subperiosteally and on internal surfaces without prior osteoclastic resorption. With training of greater intensity, adaptive change is supervened by patho-anatomical change in the form of microdamage and frank lesions. In tendon, collagen fibril diameter distribution changes significantly during growth, but not after early training. The exact amount and type of protracted training that does cause reduction in mass average diameter (an early sign of progressive microdamage) have not been defined. Training is associated with an increase in the cross-sectional area of some tendons, possibly owing to slightly greater water content of non-collagenous or newly synthesized matrix. Early training may be associated with greater thickness of hyaline but not calcified articular cartilage, at least in some sites. The age at which adaptation of cartilage to biomechanical influences can occur may thus extend beyond very early life. However, cartilage appears to be the most susceptible of the three tissues to pathological alteration. The effect of training exercise on the anatomical or patho-anatomical features of connective tissue structures is affected by the timing, type and amount of natural or imposed exercise during growth and development which precedes the training.

Key words articular cartilage; bone; exercise; growth; horse; tendon.

Introduction

The horse is a cursorial animal, with musculo-skeletal structure adapted to maximize locomotor efficiency at an early age to avoid predation. This has been achieved by lessening limb weight by reducing distal muscle mass and the number of bones. The fibular and ulnar shafts are almost vestigial, and distal to the carpus and tarsus, load is borne mainly through the third metacarpal (Mc3) or third metatarsal bone (Mt3). The second and fourth metacarpal/metatarsal bones are much reduced in size, and transfer load across the carpo-metacarpal and tarso-metatarsal joints and thence into the Mc3/Mt3 through interosseous ligaments, which later ossify. There is only one digit (the third), consisting of proximal, middle and distal phalangeal bones, and associated distal sesamoid (navicular) bone and proximal sesamoid bones (PSB) articulating with the palmar medial and lateral condylar surfaces of Mc3 or Mt3.

The digital flexor and extensor muscles tendons are 400–500 mm long. The superficial digital flexor tendon (SDFT) has a muscular origin at the medial humeral epicondyle, and a fibrous origin on the caudomedial aspect of the radius some 10 cm proximal to the antebrachiocarpal joint. The fibrous band (the accessory ligament) joins the tendon immediately proximal to the

Correspondence
Dr Elwyn C. Firth, Institute of Veterinary, Animal and Biological Sciences, Massey University, Palmerston North, New Zealand.
E: E.C.Firth@massey.ac.nz

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antebrachiocarpal joint, and prevents overstretching of the SDF muscle at the instant of maximum load at push-off. The tendon has a cross-sectional area of approximately 1 cm^2. The deep digital flexor tendon (DDFT) is joined by its accessory ligament in the mid-metacarpal region, and passes through the SDFT just proximal to the insertion of the latter. The tendons become fibro-cartilaginous where they pass over the PSB and navicular bones.

The suspensory ligament (SL) is the modified third interosseous muscle and contains striated muscle fibres, which comprise 10–14% of its cross-sectional area (CSA) (Wilson et al. 1991). It passes distally between the second and fourth metacarpal/metatarsal bones, and thence divides into two branches which insert on the abaxial surface of the respective PSB; a thinner branch passes obliquely disto-dorsally over the lateral and medial aspects of the proximal phalanx to join the common digital extensor tendon (CDET).

Growth and development are rapid: in the thoroughbred, birth weight doubles by approximately 6 weeks, 90% of mature height and 66% of mature weight have been reached by 12 months, and 95 and 80% by 18 months, and growth is completed by the fourth year of life (Frape, 1986). Equine joint cartilage has similar appearance and properties to human articular cartilage, and attains its adult thickness (0.6–1.6 mm in the carpus and Mc3) by 5–6 months of age (Firth & Hartman, 1983; Oikawa et al. 1989); of the domestic animals studied, the equine cartilage thickness is closest to that of human cartilage (Stockwell & Meachim, 1979; Frisbie et al. 2005).

Foals can gallop within hours of birth, through advanced neuro-muscular development after normal gestation, and advanced ossification of epiphyses and cuboidal bones combined with as yet unossified epiphyseal cartilage up to 27 mm thick beneath the articular cartilage (Firth & Greydanus, 1987). The subchondral bone 'plate' begins to appear within a few months, although this varies widely between epiphyses and between sites in the same epiphysis. Metaphyseal growth plates (physes) close before birth (for example, middle phalangeal bone, proximal physis of the third metacarpal bone), after some weeks (proximal physis of the proximal phalangeal bone), and up to 4.5 years (proximal femur) (Rooney, 1975). While bone growth rates are maximal in the first months of life, for instance mean daily growth rates between 52 and 83 days of age up to approximately 200 $\mu m\ day^{-1}$ for the physes of the bones of the upper limb (Goyal et al. 1981; Fretz et al. 1984), the thick deformable cartilage protects the newly formed and forming epiphyseal bone at the 'spherical' growth plate of the epiphysis from functional injury associated with normal activity. But these forces do not damage the cartilage either, because reduction in cartilage thickness is exquisitely controlled such that the greater susceptibility to deformation of thick cartilage is avoided. Patho-anatomical changes that may occur if forces are excessive include osteochondrosis (Hurtig & Pool, 1992) and trabecular microfracture and retained cartilage (Firth & Poulos, 1984), although the relationship between these remains unclear, as does the possibility of trabecular fragility rather than excessive force contributing to their presence.

Growth of muscle requires stretch of the sarcomere (Goldspink et al. 1995), presumably imposed on one muscle group by contraction of its antagonists during movement *in utero,* or later during activities such as rising from the lying position, standing, locomotion and playing activities. Bone adaptation has been suggested to be modulated by high strain (of around 3000 $\mu\varepsilon$) associated with vigorous activities (Rubin & Lanyon, 1982). The same inductive mechanism may also be true of cartilage, as evidenced by the association of differing biomechanical influences at various sites of the joint and biochemical heterogeneity between those sites (Brama et al. 2000). *In vivo* measurement of the biomechanical response in cartilage or tendon tissue is much more difficult than in bone, and thus the characteristics of the forces required to cause change in the former are not well known.

In addition, quantifying the amount of exercise is difficult in animals which are not confined. For instance, recording all the incidents of high strain (rate) activities that occur during a 24-h day at various phases of an animal's life is challenging, more so when considering that high-frequency signals associated with habitual activities such as standing, posture maintenance or walking might also influence tissue architecture (Rubin et al. 2002). Optimal orthopaedic health must be largely influenced by environmental effects during the period of growth and development, and yet all animal research and production environments involve confinement, and sedentarism in young people is apparently increasing. In neither young animals nor humans is the amount of 'natural' exercise that is ideal for musculo-skeletal function in later life known, and is one reason why forced exercise during growth is contentious. Horses often begin training in their second year of life, and this,

together with the morbidity and mortality associated with athletic pursuits in horses, has driven considerable study of the effects of exercise in the horse. This review cites literature describing the responses to exercise of bone, tendon and articular cartilage, the three musculoskeletal tissues which contribute to a major extent to equine morbidity and mortality.

Bone

The horse is genetically programmed for rapid early skeletal development. During late pregnancy, CSA of the Mc3 shaft increases and changes from circular to conical, rendering it capable of weight-bearing immediately after birth (Oikawa et al. 1991). Histological and microradiographic examination of the Mc3 of random source Thoroughbred horses showed that after birth, rapid periosteal apposition of circumferential trabeculae connected by radial trabeculae to the parent cortex results in faster cortical expansion and larger total surface area for bone deposition than circumferential lamellar, simple primary osteonal and plexiform mechanisms of periosteal bone formation (Stover et al. 1992). Resorption cavities and incompletely filled secondary osteons were in the Mc3 of 1- and 2-year-old horses, and several generations of secondary osteons, fewer resorption spaces and incompletely filled osteons were in bone from horses aged three or more years. The proportion of circumferentially orientated collagen fibres increased with age (Stover et al. 1992), as did the orientation of collagen in the caudal aspect of the radius (Riggs et al. 1993). The amount of exercise associated with such changes was unknown as the subjects were from random sources.

Bone development during early growth was investigated in a study involving three groups of Warmblood foals (van Weeren & Barneveld, 1999). One group of 14 foals was confined to a box stall from birth to age 5 months, a trained group was kept in similar box stalls but additionally exercised daily through an increasing number of gallop sprints from the age of 1 week, and a third group was at pasture continuously. At 5 months of age, eight foals from each group were randomly selected and euthanized, the remaining 19 foals were allowed free access to pasture for an additional 6 months, and were euthanized at 11 months of age. At the age of 5 months, CSA of Mc3 was determined using peripheral quantitative computed tomography and was significantly larger in the pastured group than in the boxed group, and volumetric bone mineral density (BMD_v) in a region of interest in the dorsal cortex was significantly higher in the boxed group. These between-group differences had disappeared in the foals aged 11 months. The mean density of Mc3 cortical bone was less at 11 months old than at 5 months. In the apical level of the PSB, trabecular BMD_v was higher in the trained group at 5 months, but was less at 11 months in the foals that had been in the trained group than in the foals that had been confined (Cornelissen et al. 1999). It was concluded that confinement up to 5 months of age resulted in a retardation of normal development, which was compensated for when the restriction on exercise was lifted. There was some indication that the sprinting led to an overstimulation of bone, resulting in less active mineral deposition in the longer term.

The lower BMD_v at 11 than at 5 months of Mc3 diaphysis (Cornelissen et al. 1999) is not in agreement with changes in density in the Mc3 BMD_v of pastured foals (Grace et al. 2003), and may have to do with the after-effects of the stall rest and sprint exercise regimens, different choice of area of interest analysed, or the higher cortical density threshold used in the analysis, which would exclude voxels of lower density (the threshold is the value chosen which defines the value above which voxels are included in the cortical fraction and those below the threshold are assigned as trabecular within the CT program). A lower threshold density would include newly formed low-density cortical bone (Grace et al. 2003), which contributes significantly to bone strength because of the peripheral site at which it is predominantly deposited. Together the increase in diaphyseal size and density contributes to increasing the resistance to bending and torsion, through effects on cross-sectional moment of inertia, and on modulus, which is proportional to cortical bone mineral density within the physiological range, in horses (Les et al. 1994) and in rats (Ferretti et al. 1996).

In the medial aspect of the third carpal bone of the same foals, 5 months of sprint and pasture exercise was associated with significantly elevated BMD_v (measured using dual X-ray absorptiometry), but differences between groups were not present at age 11 months (Firth et al. 1999a). Five-month-old weanlings confined in stalls and subjected to 82 m day^{-1} sprinting for 5 days per week for 8 weeks appeared to have greater Mc3 cortical size and mineral content (assessed by radiographic bone aluminum equivalence) than did foals confined in stalls or in a 992-m^2 yard (Hiney et al. 2004).

Controlled training exercise of thoroughbred horses was undertaken at Bristol in the 1990s. The first study, into the effect of exercise on tendon, lasted 18 months and compared 2-year-old trained horses (galloped on a treadmill three times weekly, trotted on a mechanical horse walker three times weekly, and walked for 40 min 6 days per week) with control horses exercised at the walk only for 40 min daily for 6 days per week. The third carpal bone of trained horses had a thicker cortical shell, and thickening of trabeculae, resulting in an increased bone mass and mineral density as assessed by dual X-ray absorptiometry (Firth et al. 1999b), associated with local forces passing through the dorsal load path of the carpus. In a second study, using a similar exercise regimen for only 18 weeks, the third carpal bone responded in a similar way, and the difference between trained and controls was similar to that of the 18-month study (Firth et al. 1999c). Mechanical testing to failure of metacarpal mid-diaphysis specimens had a slightly higher toughness, and higher impact strength, which was correlated positively with the amount of microcracking produced during testing. Increased loading was associated with an enhanced ability to microcrack and increase toughness (Reilly et al. 1997).

Study of the alterations in the epiphysis of Mc3 of the same horses showed the main trabeculae to be robust sagittal plates running in the sagittal direction with less significant mediolateral connections, which imparts maximum strength in the sagittal plane, but minimal resistance to fracture propagation along it (Boyde et al. 1999). In the distal Mc3 epiphysis, the adaptive anatomical change may lead to excess stiffening within the condyles owing to extensive new bone formation, and this may lead to a patho-anatomical change, namely concentration of strain at the condylar grooves, which is a common site of fracture (Riggs & Boyde, 1999).

There was greater thickening of trabeculae and lower porosity on histomorphometric examination in PSB of horses trained for 5 months and a cumulative distance of 152 km on less compliant tracks (dirt) than on wood-chip tracks (Young et al. 1991a). Thoroughbred horses aged 2 or 3 years that had raced or been in race training had greater area fraction and stiffness (determined biomechanically by indentation) in the dorsal aspect of the radial facet of the third carpal bone than did untrained horses. Area fraction and stiffness were closely related, and were significantly greater in horses with patho-anatomical change than without (Young et al. 1991b).

Horses exercised on a treadmill 5 days per week for 6 months had greater volumes of higher bone density on computed tomography and were significantly more lame than horses given only slow exercise at hand (Kawcak et al. 2000). This was possibly due to the greater distance cantered when compared with the 18-month study referred to above. Radionuclide uptake in the metacarpal condyles, but not in the carpal joints, was greater in exercised horses than in control horses. Exercised horses also had a higher subchondral bone density in the metacarpal condyles than control horses, but such differences were not detected in the carpal bones.

To determine the response to very early exercise, before clinical signs associated with patho-anatomical change had occurred, a group of 2-year-old horses was trained on sand and grass racetracks for 13 weeks, known as the Massey University Grass Exercise Study (Firth et al. 2000, 2004a; Boyde, 2003), and produced findings similar to those in the treadmill studies. In Mc3 (Firth et al. 2005) and third carpal bones (Firth & Rogers, 2005) the regional increase in bone mass by 13 weeks produced peak BMD_v approaching that of diaphyseal cortex on quantitative computed tomography examination. The bone immediately beneath the calcified cartilage was less dense (probably due to higher remodelling in the immediate subarticular area) than bone some millimetres deeper. Radiographs of thin transverse slices showed that the regional bone mass and density was increased at least partly by progressive thickening of the walls of the trabecular plates, and formation of columns orientated normal to the articular surface at nodes where parts of the trabecular cylinders join. As more bone was accrued on more of the trabecular surfaces between nodes, stiffness would increase further until the cavities become filled and densification is complete (Firth & Rogers, 2005). However, exactly where and under what exercise or age conditions this occurs has yet to be determined. In both the third carpal and the Mc3 bones of trained horses, backscattered electron microscopic examination showed that new bone was applied directly to trabecular surfaces without prior resorption (Boyde et al. 2001). The bone volume fraction was higher but the material mineral density was less than in control horses because of the lower mineral density of the new lamellar bone that had been formed (Boyde & Firth, 2005).

In the Mc3 diaphyseal shaft of these animals (Firth et al. 2005), bone density, as assessed by peripheral quantitative computed tomography, was greater in horses that had been trained at high speed, but density

did not contribute to bone strength as much as increase in the CSA. Confocal fluorescence laser microscopy showed that active osteons, defined as Haversian systems containing calcein label, were fewer, of smaller diameter at the time of calcein injection and had a greater bone apposition rate in trained than in untrained horses. Horses that had cantered but not galloped had similar bone density to those that had galloped, presumably due to the alteration in remodelling associated with cantering. But horses that had cantered but not galloped had bone CSA, mineral content, periosteal circumference and bone strength similar to control (untrained) horses, indicating that the Mc3 had not grown in response to the cantering exercise. Caution is required because the number of horses examined was small, but the observation was consistent with previous studies in which metacarpal enlargement occurred in treadmill-trained horses (McCarthy & Jeffcott, 1992), increased duration of training was associated with greater dorso-palmar bone diameter and higher cross-sectional moment of inertia (Sherman et al. 1995), and gait velocity of > 12 m s^{-1} was required for significant stimulation of Mc3 dorsal cortex (Davies et al. 1999). Most training involves lower velocities, which may inadequately adapt the bone for forces sustained in competitive racing (Verheyen & Wood, 2004), and patho-anatomical change in the form of metacarpal periostitis may occur less in horses that do more galloping during training (Nunamaker, 2002). All of these underline the concept that bone tissue will adapt if the forces acting upon it cause the deformation (strain) sustained by the bone to exceed certain values, but if that strain value is not exceeded then the bone will not respond to increase its resistance to the deforming forces (Frost et al. 2002).

The implication of fast exercise in terms of adaptive change in functional anatomy is that doubling the minimum second moment of inertia (induced by mechanical means) resulted in > 100-fold increase in fatigue resistance, thus reducing the likelihood of stress fractures (Warden et al. 2005). Of course, such massive increase in size is not desirable in the horse in evolution or in training, as larger bones are heavier, with an associated functional disadvantage. However, suitable exercise programmes to enhance skeletal structural properties, introduced during early growth or early training, would seem to be the most effective approach to attempting to reduce stress fracture incidence, which is one of the main categories of bone injury affecting equine athletes (Verheyen & Wood, 2004).

Tendon

Electron microscopic studies of collagen showed the fibril diameter distribution in tendon and ligaments of neonatal foals was unimodal and became bimodal (Parry et al. 1978; Davankar et al. 1996). The mass average collagen fibril diameter increased to a maximal value of approximately 170 nm at 12–18 months and 240 nm at almost 2 years of age in the SDFT and DDFT, respectively. Collagen fibril index, which expresses the area covered by collagen fibrils, peaked at 70–79% at the same ages. The distinctive waveform of the collagen bundles of tendon, visualized as light and dark banding under polarized light, is termed 'crimp'. Quantification of crimp structure is determined by measuring the crimp angle and crimp period length (Patterson-Kane et al. 1997a). Crimp angle and crimp length of the digital flexor tendons of horses reduced from birth until approximately 2 years of age, and mature (hydroxylysylpyridinoline) cross-linking increased rapidly to almost adult levels by 12–18 months of age (Patterson-Kane et al. 1997b).

The effect of exercise on tendon development was studied in the three groups of foals referred to above, experiencing stall rest, sprint exercise superimposed on stall rest, and pasture exercise to 5 months of age, and then all having access to paddock exercise from 6 to 11 months of age (Cherdchutham et al. 1999, 2001). By 5 months of age there were more small-diameter fibrils in the peripheral and central regions of the SDFT of foals that had been exercised compared with those confined to a large box stall; pasture exercise induced more change than did the artificial sprinting exercise. Such development of small-diameter fibres did not occur in the confined foals until after they had been permitted pasture exercise from 6 to 11 months of age. Previous exercise regimen influenced collagen restructuring, as development in the two confined groups lagged behind the pastured group at 11 months of age. The previous sprinting exercise superimposed upon stall confinement may have had negative effects on tenocyte metabolism in the longer term.

The structure of the SDFT changes with age (Crevier-Denoix et al. 1998): fibre undulation and cellularity, and interfasicular space decreased from 5 years of age, and activity induced a decrease in the number and size of the interfasicular spaces. Less undulation in fibres in the proximal and middle metacarpal zones was related to changes in the stress–strain curve and relative weakness within the SDFT. Focal chondroid metaplasia and

fibrocartilage on the dorsal aspect of the sesamoidean region are related to the compression forces acting there (Gillard et al. 1979). Reduction of the crimp angle (but not crimp length or fibril mass average diameter) in the central but not the peripheral region of the SDFT occurred normally with age in wild horses, presumably because tendons of older animals would have undergone a higher number of loading cycles (Patterson-Kane et al. 1997a). Particularly in the central regions where focal 'core' lesions of tendonitis usually occur, training accelerated normal age-related change (Patterson-Kane et al. 1997b), by increasing the frequency and/or the magnitude of load cycles experienced by the tendon.

Treadmill training of 2-year-old horses for 18 months, but not for 18 weeks, resulted in a decrease in collagen fibril mass average diameter in the SDFT (Patterson-Kane et al. 1997d) and change in crimp morphology (Patterson-Kane et al. 1998a) compared with age cohorts which were not exercised at high speed. This effect was not found in the CDET of the same horses, underlining the functionally distinct nature of these two tendons resulting in fundamentally different responses to high-speed exercise. It was subsequently shown that the SDFT is a stiffer structure than the CDET, and differences in the matrix molecular composition, including water and total sulfated glycosaminoglycan content, allow it to remain more elastic as a material, permitting its energy-storing function (Batson et al. 2003). Presumably such differences in function and structure resulted in less change in the collagen fibril diameter distribution in the DDFT and SL after 18 months of exercise (Patterson-Kane et al. 1998b).

The CSA, determined ultrasonographically and/or *in vitro* using photographs of cross-sections of four digital tendons of 2-year-old horses trained on treadmills for 18 months, was not significantly different from those of horses exercised at the trot. The same applied to the flexor tendons after the 18-week study, but there was significant hypertrophy of the CDET. Comparison of the DDFT from the 2- and 3-year-old horses at the end of 18 weeks and 18 months of exercise, respectively, revealed an age-related increase in CSA but not in the SDFT. The results suggest a structure-specific hypertrophic response to the imposed training regime (Birch et al. 1999). It is noteworthy that CSA measurement should perhaps be regarded as an index of growth or development and not *per se* as an index of resistance to tensile load, because CSA was inversely proportional to the collagen content, dry weight percentage and tendon fibre percentage (Riemersma & De Bruyn, 1986), and dry matter content and total collagen content were not significantly different between normal and 'degenerated' tendons (Birch et al. 1998).

In Dutch Warmblood horses housed except for 2–4 h of pasture exercise six times per week and exercise at various gaits on a walker one, three or five times per week from the age of 3 months, the CSA of the SDFT was not significantly affected by exercise at 14 months of age whereas all the other tendons and ligaments did show adaptive change (van den Belt, 1995). In early race training of thoroughbreds, an increase in size of the SDFT was ultrasonographically detected, although the fact that some horses developed clinical signs means that more than adaptive structural change was probably occurring (Gillis et al. 1993). Training of 2-year-old thoroughbreds for 13 weeks on sand and grass was associated with an ultrasonographically determined increase in mean CSA of the SDFT (Perkins et al. 2004), which was substantiated by tissue examination of the tendons. The CSA, weight and volume of the SDFT and CDET were greater in the trained than in control group, and the lack of clinical or histological abnormality led to the presumption that this change was adaptive to the training (Firth et al. 2004b).

In a more recent study (Kasashima et al. 2002), a control and an exercise group of thoroughbreds received 4 h of pasture exercise from 2 to 15 months of age and the exercise group had an additional short period of treadmill exercise daily. The exercise programme resulted in a significantly larger tendon CSA in the exercise group at several, but not all, time points, which may be attributed to levels of variance. A significantly greater rate of increase in tendon CSA with time in the exercised compared with the control group suggested that tendon development can possibly be modulated by exercise during growth. However, the type and timing of the exercise regimen is likely to be critical, as the mean CSA of the SDFT of a large group of pastured foals exercised from 10 days to 18 months of age for 1030 m 5 days per week at velocities of 4.2–12 m s^{-1} was not significantly different from that of a matched group receiving only pasture exercise (Moffat, 2004).

The final response of equine tendon to exercise is well documented, at least in the SDFT, in which partial rupture preceded by localized macroscopic degenerative changes, increase in total sulfated glycosaminoglycan content and proportion of type III collagen, and decrease in collagen-linked fluorescence occur in the core but

not the peripheral region. These changes probably contribute to a decrease in mechanical properties and the subsequent clinical manifestation of the patho-anatomical change (Birch et al. 1998), usually in the mid-metacarpal region of the SDFT. The nature of events preceding longitudinal tears in SDFT at the level of the metacarpo-phalangeal joint (Wright & McMahon, 1999) has not yet been elucidated.

Cartilage

Biochemical analysis of proximal articular cartilage of the proximal phalanx at two sites, one of which sustains intermittent high-level peak loads and the other lower but continuous load, was conducted in neonatal and 5- and 12-month-old horses. Significant site differences typical of those in adults (Brama et al. 2000a) for DNA, glycosaminoglycan (GAG), collagen content and hydroxylysine content were present in 5- and 12-month-old horses but not between the sites in the neonates. Water content and hydroxylysylpyridinoline (HP) cross-links were not different in the two sites at any of the three ages. At both sites, water, DNA and GAG decreased during maturation whereas collagen content, hydroxylysine content and HP cross-links increased. Postnatal adaptation, resulting in biochemical and therefore biomechanical heterogeneity, which is important for future tissue strength and resistance to injury, occurs early and rapidly, possibly because collagen turnover is extremely low at older ages (Brama et al. 2000b).

Because there was no biochemical heterogeneity in the cartilage of neonatal foals, as occurs in sheep (Little & Ghosh, 1997), the two sites in the metacarpo-phalangeal joint were investigated in foals subjected to different exercise regimens during the first months of life. The water, GAG, DNA, total collagen, hydroxylysine and HP content were measured in articular cartilage (Brama et al. 1999). No effect of exercise on the water or DNA content was found. GAG content was greater in exercised foals at 5 months, but differences had disappeared after 6 months of access to pasture. Moderate exercise had no apparent effect on any of the collagen parameters but had a beneficial, but reversible, effect on the GAG component.

Differences in heterogeneity between the two sites were present for GAG, DNA, collagen and hydroxylysine in exercised foals aged 5 months but only for GAG and DNA in confined foals. For some collagen-related parameters, the delay in attainment of heterogeneity was not compensated for after the additional 6-month period of moderate exercise (Brama et al. 2002). Short bouts of heavy exercise superimposed on a confinement regimen appear to have adverse effects on long-term viability of the tissues and may lead to an impaired resistance to injury (Van De Lest et al. 2002; Billinghurst et al. 2003). The exercise regimen that is ideal for cartilage adaptive change remains unknown.

Conditioning exercise for 1030 m at a velocity up to 12.5 m s^{-1} for 5 days per week on a grass and sand track up to 18 months of age did not markedly affect articular cartilage structure or function. There was marked site-associated variation, with sites medial and lateral to the sagittal ridge of Mc3 showing signs of early degeneration, with low indentation stiffness and collagen content, and relatively high water content (Nugent et al. 2004), compared with the control group undertaking spontaneous exercise at pasture only. This concurs with more viable chondrocytes in the metacarpo-phalangeal joint of foals in the conditioned than control group, and in the palmar than dorsal sites of the same joint (Dykgraaf, 2003).

In 2-year-old horses undergoing 19 weeks of high-intensity treadmill training or low-intensity exercise, cartilage from the dorsal aspect of the carpal bones was less permeable, thinner and had loss of chondrocyte alignment compared with palmar cartilage. Cartilage of strenuously trained animals was significantly less stiff, had reduced superficial toluidine blue staining, showed more fibrillation and chondrocyte clusters than that from gently exercised animals (Murray et al. 1999a), and had greater fibronectin staining (Murray et al. 2000) and interterritorial cartilage oligomeric matrix protein distribution (Murray et al. 2001).

In the dorsal sites sampled, trained horses had thicker calcified (but not hyaline) cartilage than did control horses, pooled hyaline cartilage thickness in trained and control horses was not significantly different, and hyaline cartilage thickness from dorsal sites was not significantly different from that in palmar sites (Murray et al. 1999b). By contrast, after 13 weeks of training on sand and grass tracks of 2-year-old horses that had been raised at pasture (Firth et al. 2004a), third carpal bone articular cartilage was thicker in dorsal than palmar sites (although many more sites sampled in Murray's study may have reduced the significance of difference). At the sites of thickest cartilage, mean hyaline cartilage thickness in trained horses was significantly greater than in untrained horses (Firth & Rogers, 2005). In this

first evidence that the amount of hyaline cartilage is responsive to exercise in the horse, histological examination of the sites showed that staining of the hyaline matrix was more basophilic, chondrocytes were more numerous adjacent to the tidemark, and chondrocyte palisades and chromatin were more obvious through their increased basophilic staining. No patho-anatomical change was evident, and thus the thickness difference was presumed to be adaptive to the forces associated with exercise. This concurs with recent work showing that in childhood, strenuous activity promoted development of knee cartilage, without pain and significant injury, and children who had had no vigorous activity had 22–25% less cartilage than even mildly active children (Jones et al. 2003). It also concurs with the significant effect of exercise on newly synthesized proteoglycan in the third carpal bone of horses exercised for 6 weeks compared with horses that had been boxed for the same period (Palmer et al. 1995), with the difference in rate of proteoglycan synthesis in metacarpo-phalangeal joint cartilage of 2-year-old horses trained on a treadmill for 19 weeks compared with that of control horses (Bird et al. 2000), and with the increase in cartilage thickness after moderate (Kiviranta et al. 1988) but not strenuous running (Kiviranta et al. 1992) in dogs.

After the 13 weeks of training on sand and grass tracks (Firth et al. 2004a), the degree of patho-anatomical change in the metacarpo-phalangeal joint cartilage, in the form of wear-lines and fibrillation, was more severe than suggested by the lack of clinical signs, and was accompanied by loss of the site-specific differences in collagen parameters present in the control horses. Cartilage from the trained group had an increase in water content and a decrease in HP cross-linking correlated with the presence of wear-lines. It was suggested that the training provoked microdamage, which altered and loosened the collagen network of the articular cartilage (Brama et al. 2000c). Macroscopic lesions were not present in the carpal joints of the same horses, and this predilection for patho-anatomical change in the metacarpo-phalangeal joint was previously observed in horses trained for 6 months on a treadmill (Kawcak et al. 2000), and in wild horses (Cantley et al. 1999) in which the degree of change was related to age, presumably due to greater cumulative cyclic load-bearing activity.

The forces acting on cartilage would be expected to affect the subchondral bone also, and the level of exercise in very young foals obviously altered bone density of the compact bone of the subchondral bone plate in the third tarsal bone (Barneveld & van Weeren, 1999b). This prompted study of collagen parameters of subchondral bone, which revealed that exercise affected both post-translational modifications of the collagen component and site-related topographical differences of subchondral bone. Exercise influenced calcium content and levels of HP and lysylpyridinoline cross-links at the intermittently loaded site but not at the constantly loaded site of the proximal phalangeal bone, levels of lysyl-hydroxylation and lysylpyridinoline cross-linking being lower at the former than the latter site (Brama et al. 2001). The epiphyseal bone alters dramatically with exercise (Firth et al. 1999b; Firth & Rogers, 2005) and is only arbitrarily separable from the subchondral bone. But the latter is clearly the region at which the bone response to exercise first occurs in the epiphyses or cuboidal bones, either as the subchondral plate forms during maturation of the epiphysis or as the bone beneath articular cartilage responds after the subchondral 'plate' is obviously present, by thickening and becoming contiguous with the adaptive density changes in the deeper bone (Firth et al. 2005). The role of subchondral bone in disease has been reviewed (Kawcak et al. 2001).

In early osteoarthritis, study of the patho-anatomical and biochemical changes in articular cartilage or subchondral bone (Radin, 1999) has dominated the investigation of response of articular calcified cartilage (ACC). In areas of high stress on the articular cartilage, the ACC may or may not alter its thickness (Murray et al. 1999a; Norrdin et al. 1999; Firth & Rogers, 2005). In 18-month-old horses at pasture, the mean linear accretion rate was 0.89 μm day^{-1} with much higher rates in some regions (Doube et al. 2005). In addition, the suggested concentration of strain at the condylar grooves may lead to cracking, beginning in the ACC and propagating into the subchondral bone (Riggs & Boyde, 1999; Boyde et al. 2001; Boyde, 2003). In random source horses, race training exercise has been associated with macroscopically visible cracks at the sites of condylar fractures (Radtke et al. 2003), in contrast to horses without such an exercise history, although the involvement of ACC, as opposed to that of subchondral bone, was not indicated (Stepnik et al. 2004). High-stress sites of the joint had thinner, more irregular ACC, indicating that subchondral remodelling involves the ACC layer (Norrdin et al. 1998) and in overload arthrosis, breaks in ACC appeared to lead to collapse and cartilage infolding (Norrdin et al. 1999). ACC may be implicated even earlier in the pathogenesis of osteoarthritis if the higher stiffness, hypermineralization and

abrasive role of ACC (Ferguson et al. 2003) are confirmed in the horse.

Clearly, ACC is highly responsive to exercise, and shows a wide range of morphology and patho-anatomical change. Microcracks in ACC have been detected in many sites in racehorses in training, and can be filled with calcified material in an apparently reparative response to overload exercise regimens (Boyde, 2003). A recent new finding in 2-year-old horses is the presence of canals through ACC, mainly in a site which is relatively less loaded than other sites, on the sagittal ridge of Mc3. The canals appear to result from osteoclastic resorption (cutting cones) penetrating from bone through to the non-mineralized hyaline articular cartilage, possibly connecting the extracellular fluid of bone and cartilage (Boyde & Firth, 2004).

Discussion

It is difficult to determine accurately the amount of exercise undertaken by animals (and people). The approaches of determining the effects of abolishing exercise to a greater or lesser degree by casting (Buckingham & Jeffcott, 1991; Richardson & Clark, 1993; Van Harreveld et al. 2002a,b) or confinement (Barneveld & van Weeren, 1999a; Reichmann et al. 2004), although useful in animal management terms, are artificial and might not directly advance information on the amount and timing of additional exercise to optimize functional anatomy of the musculoskeletal system. However, it is highly likely that the so-called 'natural' conditions of animals raised at pasture are also artificial, in that the areas available for exercise are much less than in the natural environment, and may place behavioural constraints on movement as well. This needs to be considered in all studies cited in this review, with the possible exception of the Bristol studies cited above in which the control horses were exercised daily at slower gaits for known periods.

In the horse, natural exercise is mostly achieved through movement at one of several gaits, with force being applied to the bones through muscle and ground reaction forces, higher forces at faster gaits. The tissues respond to new regimens not previously experienced by the tissues, during growth as play and other activities increase. Athletic training is another way in which a novel exercise regimen is imposed, and in the horse this has usually started by 4 years of age, and in some breeds at 1.5 years. No study on response of tissues to new exercise in old horses has been conducted, and old horses that are still athletically active (Brosnahan & Paradis, 2003) are likely to have been so for many years. This review thus has dealt with the response to exercise in horses that are still growing. Growth is relatively easy to measure, whereas the amount of exercise an individual undertakes is not. Only initial attempts at quantifying exercise in trained horses in a cumulative workload index have been presented (Rogers & Firth, 2004), which did not account for characteristics of the surface on which the exercise is undertaken, for very brief peak force activities, or for habitual low-amplitude activities such as standing and posture maintenance (Rubin et al. 2002).

Although an individual's tissue response to an exercise regimen will presumably depend on differences such as conformation, kinematic 'action', muscle fibre composition, genetic propensity and environmental factors, it will also be dependent on the effects of previous and concurrent exercise. This is because adaptive change is likely to be greater after the first exposures to a given new regimen than after long exposure to it. Adaptation occurs after a suitable number of cycles of a given regimen, tissue strength improves to resist deforming forces, continued morphological response to the regimen declines, there is 'maintenance' of tissue morphology and strength, and the regimen is no longer 'novel'. Conversely, if activity declines continually, then the tissue strength declines, although how profoundly and how rapidly is so far unclear. Regularly repeated events affect the response of the tissue to subsequent activity (Milgrom et al. 2000; Lappe et al. 2001), even through activities performed in childhood or adolescence (Fredericson et al. 2005).

In summary, bone appears to be highly responsive to exercise in the horse, in terms both of rapidity and of magnitude of the response: adaptive change occurred within as little as 8 weeks (Hiney et al. 2004) and apparent density was up to 37% greater in trabecular bone of trained than control horses (Firth et al. 1999b, 2005), for reasons which have strong ultrastructural evidence (Boyde, 2003). The effect of withdrawal of a particular exercise regimen (which is a different effect than that of confinement) has not yet been investigated directly, but has been suggested to be of importance because return to racing after spelling from earlier racing was strongly associated with humeral fracture risk in horses (Carrier et al. 1998).

The capability of tendon to respond adaptively to exercise in terms of size, composition and tensile strength

appears to be limited. In elastic energy-storing tendons, an increase in size may reduce function because stiffness increases as size increases. The obvious response of the CDET to exercise, accompanied by little effect in the SDFT (Birch et al. 1999), may indicate that the effect of exercise on the SDFT is more through the increased capability of the CDET to increase stiffness of the SDFT at the instant when such increased stiffness would add to the energy the latter tendon can store during the stance phase and release at push-off. The lack of response of the flexor tendons with increasing work and age accounts for the greater risk of tendon injuries in older horses (Takahashi et al. 2004). Beginning conditioning exercise in younger, rather than older, equine athletes that may increase resistance to later tendon injury has been suggested (Smith et al. 1999). Determining the exact regimen for such conditioning exercise is not complete, but the regimen used did not apparently affect tendon health (Moffat, 2004) or joint cartilage (Dykgraaf, 2003).

Cartilage appears to be the most susceptible of the three tissues to patho-anatomical abnormality in younger horses (Kawcak et al. 2000; Firth et al. 2004a). It is unclear why such lesions occur in horses without training exercise, and why some horses are more susceptible than others. Perhaps the reduced exercise activity available to domesticated horses, which are raised in an environment far more confined than the natural one, precludes adaptive change in cartilage ideal for even normal, let alone athletic, activity later in life. It is clear that cartilage does respond to exercise, but the amount of 'strenuous' (Jones et al. 2003) exercise that is maximally beneficial but innocuous to equine articular cartilage requires further study.

Lastly, the stage of life when 'additional' exercise applied over the 'normal' exercise will be effective is not clear. If sufficient impact or muscle force is applied to bone, it responds quickly (Pead et al. 1988; Dodds et al. 1993) and can respond up to later ages (Ryan et al. 1994). The response of articular cartilage to additional exercise was suggested to occur only before 5 months or less due to the relative inertness of collagen after maturity (Brama et al. 2002), although hyaline cartilage thickness was greater in horses trained as 2-year-olds (Firth & Rogers, 2005). Equine tendon also appears to respond sluggishly to regimens so far imposed in young horses, although this may be because methods have been insufficiently precise. For the latter two tissues, the opportunity for conditioning intervention appears to be limited, and confined to early life. Acquiring further knowledge on the optimal exercise regimens for each tissue (including number of cycles, peak force, how often delivered, age range when delivered) will assist in deciding what should be regarded as 'normal' activity in a 'control' exercise regimen in research, in determining the duration and features of 'rest' in athletic training programmes, and in defining the characteristics of exercise which animals and people should undertake in order to attain, retain or regain health.

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