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METHODS OF ASSESSING BONE GROWTH AND DEVELOPMENT IN YOUNG HORSES

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Introduction

When raising and training horses we attempt to maximize production and performance capability, while at the same time minimize the possibility of developmental diseases and athletic injury. Improvements in management of growth, conditioning, and training, both systematically and through trial and error, may have had either positive or negative effects. But these effects have rarely been measured directly, preventing comparison between studies and thus delaying discussion and progress.

One of the objectives of most growing and training regimens is to alter orthopedic tissues so they possess greater capability to withstand the rigors of the intended training and competition. There is no research on the relationship between the outcomes, such as reduced bone and joint disease incidence, and the tissue changes we hoped to induce. This is because of the lack of sensitive, noninvasive, and inexpensive methods of monitoring changes, the difficulty of conducting extensive research investigation in commercial practice, and the cost and technical difficulties of long-term epidemiological studies that depend on accurate diagnosis.

It is quite clear that the costs associated with orthopedic growth problems in horses are huge. These include costs of definitive diagnosis of developmental orthopedic disease (DOD), costs of treatment and prevention, withdrawal from sale, reduced sale price, and the costs of continued research to attempt to determine the causes and prevent the diseases. Despite the recognition of various diseases included under the DOD banner and research attempts to determine pathogenesis and prevention, there is little or no substantial rigorous evidence that prevalence and implications of DOD have been reduced for either individuals or industries as a whole. The comment made more than a decade and a half ago, that DOD is the single biggest problem that horse breeders face, probably remains true today.

Although there is some progress on the research front, the way ahead to cure or prevention is not obvious. This is possibly because we do not have abundant rigorous data on some aspects of growth and development as a whole, and certainly in bone growth and development. It is important that such data be acquired for three reasons. First, some DOD is associated with abnormalities of bone tissue,

but the sensitivity of the methods we have to detect the disease is not high. Second, some DOD is suggested to be due to bone being of subnormal strength, but we have not ever measured this. Third, nutrition management variations may play a part in the cause of some DOD. The changes in nutrition are introduced by breeders and trainers on a subjective basis, to seek an end point in terms of bone development that is not well defined. For instance, how often does one hear of altered regimens to produce “better bone,” “better bone growth,” “harder bones,” or “higher bone density,” when in fact we have little data to know what is normal for these ill-defined parameters at various stages of life or the significance of any change achieved under a particular nutritional management system?

This presentation mentions various ways in which bone can be assessed in the growing horse, and includes a relatively new possibility, peripheral quantitative computed tomography (pQCT), which is the only currently affordable technology that can measure true bone density as well as bone dimension. Some preliminary findings concerning bone development in young pasture-raised horses are also presented.

Noninvasive Methods of Assessing Bone Growth

The reason for any assessment of bone is to estimate its strength so that predictions can be made about its likely capability of function under particular circumstances. There is an absolute requirement for cross-sectional depiction and quantification of bone properties, if understanding of bone physiology in the horse is to increase. This is because, although mineral content has a very large influence on bone strength, it is not only the amount of mineral but the manner in which it is sited in the bone that determines the bone’s resistance to the forces which act upon it.

Only three methodologies offer this, namely quantitative ultrasound (QUS), magnetic resonance imaging (MRI), and computed tomography (CT). Although the list below includes techniques which are unable to provide cross-sectional information, emphasis is placed on those that can. More emphasis is placed on CT because research into the validation of QUS is still in progress, and although portable MRI units of suitable resolution are said to be close to market stage, their cost is likely to restrict their use to research institutes for some time, unlike portable CT units.

DIRECT LINEAR MEASUREMENT

Assessment of bone size is possible in some individual limb bones by direct measurement using bony landmarks such as joint margins and is reasonably accurate in the hands of a single observer (Burbidge and Pfeiffer, 1998). Serial measurements of height measure growth of the whole limb column but contribute only indirectly to the study of a particular bone.

BONE MARKERS

Markers expressed during bone synthesis (type I collagen carboxy-terminal propeptide, the bone-specific isoenzyme of alkaline phosphatase, and osteocalcin), bone remodeling (telopeptide of type I collagen and deoxypyridinoline), and soft tissue turnover (N-terminal propeptide of type III collagen) have been investigated in the horse (Lepage et al., 2001; Price et al., 2001; Carstanjen et al., 2003; Jackson et al., 1996). Markers of bone cell activity and soft tissue turnover follow characteristic patterns of change influenced by age, season, and bodyweight (Price et al., 1995). Markers offer potentially great advantage because their use is relatively noninvasive, and they respond rapidly to changes in the skeleton.

BIOPSY

Postmortem and in vivo ilium biopsy have been used in equine research (Savage et al., 1991a,b) and although invasive appear practical and reliable in investigation of systemic bone responses. Biopsy of distal radius growth plate tissue (Belling and Glade, 1984) does not appear to have been used widely, possibly because of the degree of invasiveness and limited follow-up described. Even when using a much smaller skin incision and biopsy core, and aseptic surgery under general anesthesia (Pearce et al., 1999), biopsy of the distal radial metaphysis, which resulted in little clinically discernible postoperative discomfort, was associated with postoperative slight deviation in the opposite forelimb. Biopsy appears to have little place in growth and development research.

RADIOLOGY

Good-quality radiographs can be examined subjectively, and features of bone assessed to infer features of bone health, such as fracture, periosteal new bone formation, physeal width, cortical width, and patterns of increased or decreased radiodensity. But these are indications of end-stage disease and not development. Radiography is unsuitable for studying abnormal bone development because accuracy is low due to the substantial change required before it is detectable on standard radiographs, and because assessment of presence and significance of the changes is inevitably subjective. Newer technologies of digital radiography and image processing may alter the sensitivity of radiography to detect and quantify more subtle change in bone.

RADIOGRAMMETRY

The measurement of bone dimensions on radiographs allows quantification of the size of parts of the bone. Usually the cortical thickness is measured. The technique

has been used previously in metacarpal and other diaphyses in people (Ruiz-Echarri et al., 1996). The method lacks sensitivity and has a precision error of up to 10%, does not account for the implications of intracortical porosity because the latter cannot be recognized, and cannot detect changes in trabecular bone. However, the technique has been employed in the horse (Jeffcott et al., 1988) and most recently in a radiographic index of dorsal and palmar cortical thickness measurements of the third metacarpal, which was considered sufficiently accurate to measure third metacarpal bone shape (Walter and Davies, 2001).

RADIOGRAPHIC ABSORPTIOMETRY

The technique was the first to quantify mineral content and uses an aluminum wedge in the radiographic field, against which attenuation of the x-rays by the bone in question is compared. Standardization is difficult because of factors affecting the radiographic image, although automated image analysis techniques have led to a resurgence of the technique as a cheap screening tool in bones of the human digit. The optical density is proportional to the bone mineral content. In the human digit the method is precise and accurate (Cosman et al., 1991; Yang et al., 1994; Yates et al., 1995).

In horses, the method was originally described by Meakim and coworkers (1981) using excised bones, in which reproducibility was good and correlation with mineral content in the mid-diaphysis of the third metacarpal was between .88 and .94. The technique is used in equine nutritional research despite several sources of error including variability in the radiographic source, scatter radiation, soft tissue surrounding the bone, and limited linear response of radiographic film (Markel, 1996). As well, its use is limited in areas containing cancellous bone because changes in this fraction may be obscured by the overlying cortical bone. The method is planar, and cannot determine the site of mineral in the bone, and thus cannot estimate bone strength directly. Also, in cancellous bone with anisotropic cancellous architecture (e.g., the third carpal bone), even a very small change in radiographic beam angle can lead to significant change in results (Secombe CJ, MVSc thesis, 2000).

QUANTITATIVE ULTRASOUND

The method was used in the third metacarpi of horses to determine velocity of sound through the bone, velocity being highest where the cortex was thickest (Jeffcott and McCartney, 1985). Combined with single photon absorptiometry, transmission velocity was used to estimate bone strength (McCarthy et al., 1988) and was accurate and precise (Buckingham et al., 1992). Glade et al. related ultrasound transmission parameters to biomechanical testing of ex situ equine bone and concluded that the portability, reproducibility, and simplicity of the

method lent it great promise (1986). The variation in soft tissue between animals and across time can lead to error.

The ultrasound transmission velocity has a reasonable precision (< 1.5%), but the broadband ultrasound attenuation is less precise (Genant et al., 1996). These two parameters are influenced by bone density and weakly by architecture (trabecular number, orientation, and connectivity) (Nicholson et al., 2001). Faster ultrasound speeds through the outer 3-5 mm of third metacarpal bone shaft were associated with thinner third metacarpal dorsal cortex in different ages of training racehorses (Davies, 2002). Similarly, speed of sound measurements obtained by quantitative ultrasonography in axial transmission mode precisely measured superficial cortical bone properties of third metacarpal and other bones (Lepage et al., 2001; Carstanjen et al., 2002; Carstanjen et al., 2003). The technique appears promising and requires validation for use in detection of appropriate parameters for studying bone growth and development.

PHOTON ABSORPTIOMETRY

The attenuation of low-energy photons emitted by a radionuclide source is determined by a detector opposite the source and is related to bone mineral content per unit length of bone. The technique was potentially useful in the calcaneus (Scotti and Jeffcott, 1988) and third metacarpus (Tomioka et al., 1985; Buckingham et al., 1992). The technical limitations of this technique and its successors, single x-ray absorptiometry and dual photon absorptiometry, led to dual x-ray absorptiometry.

DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA)

The radioisotope source is a dual energy x-ray source. The amount of the two different energy x-rays passing through the patient is determined by the detector opposite the source, and the amounts of fat, lean, and bone tissue in the field can be quantified. DXA is precise and accurate, and is used extensively to measure total body mineral mass or mineral mass of a part in people. DXA is also used extensively in animal model research to detect changes in bone mineral.

From the determination of bone mineral content, and the projected area of bone that has been scanned or defined within the scanned area as a region of interest (ROI), an areal or projectional bone mineral density (BMDa) is produced. The use of the word “density” in this context is a confusing misnomer.

The technique does not discriminate between change in real bone density and bone geometry as the bone alters due to growth, disease, or altered morphology. Increase or decrease in cortical thickness, greater or lesser subperiosteal expansion, greater or lesser apposition or resorption at the endosteal surface, and changes in bone mineral content within the cancellous or cortical compartment within the

scanned region cannot usually be discriminated. If the third dimension (parallel to the beam) increases, then BMDa would increase, implying increased strength, although the mechanism for the implication could be either a real (volumetric) density increase, or (third) dimensional increase.

Standard DXA scans provide reliable width and length measurements (Sievanen et al., 1994), and some programs are available for imputing not only geometric parameters but strength indices with some reliability (Carter et al., 1992; Sarin et al., 1999). Such a program is not available for equine limbs.

DXA has been proposed for use in third metacarpal bone mass measurements in racehorses (Grier et al., 1996; Oikawa and Shimazu, 1996). The potential difficulties of the technique have been shown in *ex vivo* study of equine third metacarpal (Hanson and Markel, 1994; McClure et al., 2001; Carstanjen et al., 2003) in which density obtained was not unexpectedly different when the part was scanned in different projections. The mean BMDa was greater in mediolateral projections than in dorsopalmar or craniocaudal projections. As well, soft tissue disposition can alter findings because the algorithms depend on consistent fat and lean tissue ratios for specific regions in humans. Little attention has been paid to this in the horse as most work has centered on the third metacarpus. When studying bone growth *in vivo*, the technique is limited.

MAGNETIC RESONANCE IMAGING (MRI)

MRI depends on proton magnetic dipoles generated by the uneven number of protons in hydrogen, the most ubiquitous atom with an odd number of protons, and in other less abundant atoms. The unpaired protons exert a magnetic dipole of measurable strength and vector. When an external magnetic field is applied, the spin vector of the protons aligns with the external applied magnetic field, producing a low-energy or high-energy state; more of the dipoles align into a low-energy state (align with the external applied magnetic field) than into a high-energy state. As the photons absorb energy from exposure to electromagnetic radiation applied as radiofrequency pulses, the proton enters a higher energy state, altering the vector and plane direction of the proton, after which it reverts (relaxes) to the lower energy state. The relaxation characteristics are different for different tissues. The free induction decay data of the excited protons as they lose phase coherence are detected and analyzed to compose two- or three-dimensional images. The borders of cortical bone can be detected, and MRI analysis of cortical bone seems as promising as QCT or pQCT. So far the modality has not been used to study bone development.

PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY (PQCT)

CT has been used mainly for diagnosis in horses. pQCT machines have been developed for imaging the lower arm and leg in people, the purpose being to

make only a limited number of cross-sectional images of the limb to obtain bone dimensions. The program quantifies the area of, and the bone mineral content of, the cancellous and cortical bone fractions, and determines the volumetric bone density (BMD_v) of each. The density is expressed in volumetric terms since the thickness of the cut is known. This is a tissue density and is the mass of mineral per unit volume of the trabecular or cortical compartment.

Also, a derived strength value can be determined from knowing the center of the bone, the density values, and the distance from the center of all voxels in the slice of bone being examined. Bone that is distributed peripherally in the structural column is more effective in resisting bending and torsion forces.

The stiffness of a structure is a function of the product of some material quality of the bone sampled (such as the elastic modulus), and some parameter expressing the distribution and amount of the tissue (geometric properties of the bone itself). The pQCT does not measure or determine the modulus or intrinsic stiffness, but over a wide range of values the modulus varies linearly with the cortical bone mineral density. The density value of each voxel and its site are determined by CT. The cross-sectional area moment of inertia (CSMI) in either x or y plane expresses the amount of material and its distribution relative to the center of the bone and indicates the efficiency of the cross-sectional architecture of the bone at the level of the slice in resisting bending forces. A similar index expresses resistance to torsion of the longitudinal bone axis and is called polar CSMI. When combined with BMD, such geometric variables are more reliable than either parameter alone in discriminating between strong and weak bones. The product of CSMI and cortical bone density (integrating the pixel area times the square of distance of each pixel from the axis) produces the bone strength index, combining calculation of the geometric properties and the bone material's intrinsic properties at the scan site. This is closely related to the biomechanically determined fracture load of the bone (Ferretti et al., 1996) and is probably the best in vivo estimate available of bone strength.

The radiation source (47 kV and less than 0.3 mA) emits photons which are detected by an array of detectors. The source and detectors move around the limb, which is held in the gantry (140 mm diameter) by supports on either side of it. The limb remains motionless while the detectors and source are moved up or down the limb to take images at other sites as defined in the mask. The mask defines the site of scout scan as well as the voxel size, resolution, number, spacing, and level of the intended scan "slices." The length of the bone is measured from external landmarks, and the level at which the QCT slice is taken is expressed in actual measurement (mm) or in proportional terms (% of bone length) from a reference point. The machine measures the mineral portion of bone by calibration with phantoms of a specific concentration of hydroxyapatite. The attenuation coefficient of each voxel is transformed to a density value in mg/cm³. Radiation dose is very low.

The system produces a cross-sectional image, and data on the following parameters from each 2-mm slice: bone mineral content, bone area, and bone density separated into cortical and cancellous fractions according to a chosen threshold. Further analysis allows determination of parameters such as cortical thickness, circumference, and strength indices. Regions of interest can be used to quantify properties of particular areas within the slice image. Precision in such machines is 0.3-2% (Augat et al., 1998; Sievanen et al., 1998). Because the density obtained is a volumetric density, meaningful interpretation in growing animals is possible.

Our work so far has required the animal to be anesthetized, a limitation which together with the small gantry size has resulted in production of a vertically oriented machine, with a larger gantry, which allows scanning in the standing animal (Desbrosse, unpublished).

Longitudinal pQCT Scanning in Young Horses

Several foals were scanned from a few weeks of age to 410 days of age, and the results of those scans are presented. The intentions of this pilot study were to determine changes in both diaphyseal and epiphyseal bone and to determine if altering calcium intake affected bone development (Grace et al., 2002).

METHODS

The study group consisted of 17 Thoroughbred foals (nine fillies and eight colts) born to mares kept at pasture for the whole pregnancy, fed supplemental hay in winter, and dosed orally every month with 25 mg of selenium selenate. The foals were born and raised at pasture, were wormed at regular intervals, and were examined and weighed every two weeks. The foals were weaned in two groups, one progressively between May 8 to May 14, and the other group abruptly on May 9, 2000. The colts were castrated at between 15 and 17 months of age.

Calcium intake was increased in some of the foals by feeding a supplement containing calcium for 84 days. All the horses were fed pasture and the three treatment groups were rotationally grazed as a single group in 1.5-2 ha paddocks of ryegrass and white clover pasture starting in late May. To increase calcium intakes equivalent to dietary calcium concentrations of 0.35%, 0.63%, or 1.20% DM, 0.5 kg of a pelleted calcium supplement containing barley meal, malt culums, molasses, soybean oil, and 0%, 8%, or 24% calcium carbonate was fed as a single meal at 0830 h for 84 days to each horse individually; all of the supplement was observed to have been consumed.

The foals underwent a series of pQCT scans (XCT2000, Stratec Medizintechnik, Pforzheim, Germany) at approximately six-week intervals. Each foal had a minimum of six scans, with the exception of one brought-in colt which had only three scans due to its later inclusion in the study group. The foals born early in the

breeding season were not scanned until 82 days of age, and foals born later in the breeding season were scanned at younger ages, the youngest being four days old at first scan. Summarized results are presented in Figure 1. The results for phalanx and radius, although not shown here, were similar.

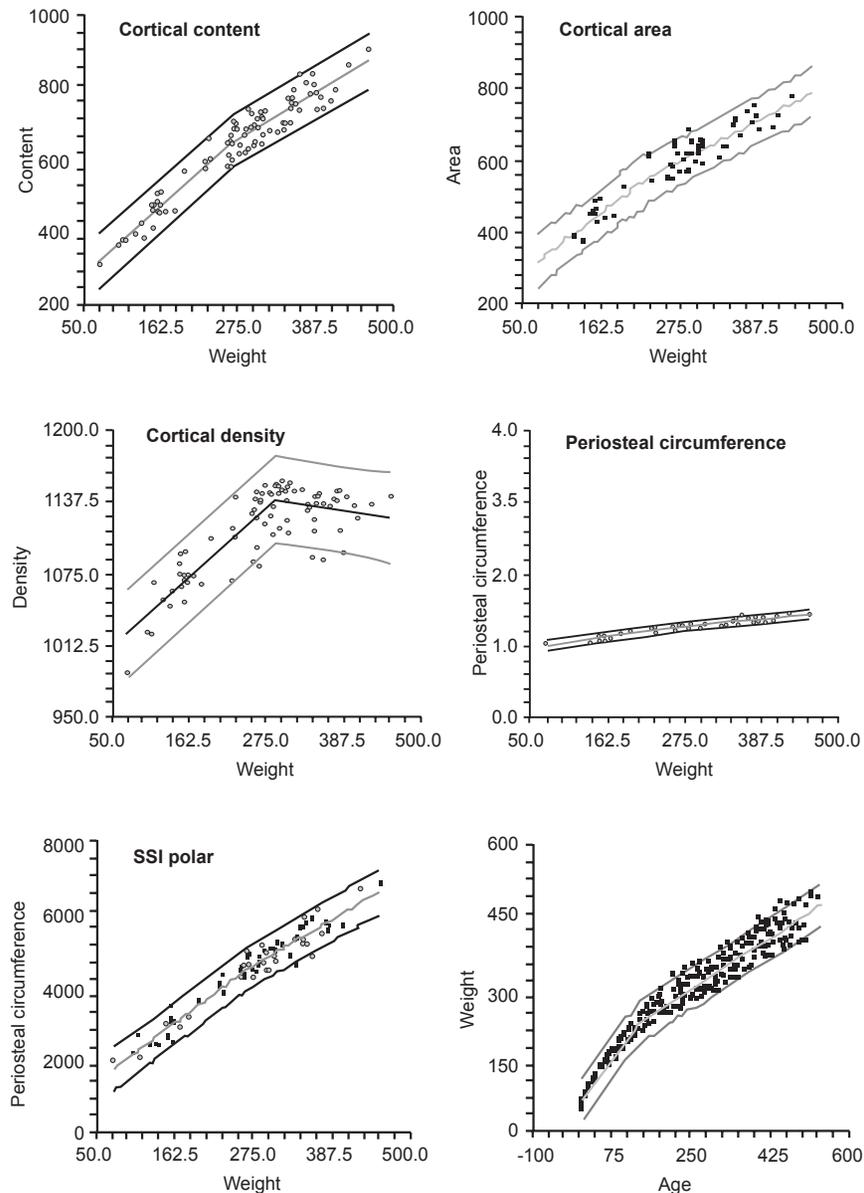


Figure 1. Third metacarpal bone mineral content, area, density, periosteal circumference, strength index, and body weight gain in a group of 17 young horses growing at pasture.

DIAPHYSIS

The bone mineral content and the area it covered in third metacarpal mid-diaphysis increased biphasically, but density plateaued around a mean weight of 275 kg. This indicates that the bone strength (represented as index-expressing resistance to torsion of the bone) increased due to increase in the size of the bone after this weight was achieved, due to continuing increase in periosteal circumference, and not due to density increase. Bone strength was continuing to increase by the end of the study.

There may have been differences between male and female animals, but these are not reported here. The change in bone growth occurred before weaning and long before puberty onset occurs in animals on this breeding farm (Brown-Douglas, PhD thesis 2003). The change in rate of bone growth, as indicated by the inflection points of biphasic growth, was preceded by the most obvious change in body weight growth. This corresponds with current indications that muscle force and bone growth are closely related (Schoenau et al., 2000).

EPIPHYSIS

In early life, the third metacarpal epiphysis cancellous bone is fairly homogeneous, and density was low. By 60 days of age, bone density was already increasing, with dense bone more obvious in areas subjected to highest local articular cartilage contact stress. In the distal third metacarpal epiphysis, by approximately 200 days about 80% of content and area consisted of epiphyseal bone denser than 540 mg/cm³, a fraction not reached until about 400 days for even denser (>710 mg/cm³) bone.

CALCIUM

Increasing calcium intake had no significant effect on daily energy intake, dry matter digestibility, or the apparent absorption of calcium, phosphorus, sodium, and potassium. Regardless of calcium intake, the apparent absorption of calcium was 0.56. The apparent absorption of magnesium decreased from 0.50 to 0.38. Perhaps other forms of calcium would increase bone quality at the most susceptible time (post-weaning, winter) or the time of most rapid growth. The scans before and after the calcium supplementation period revealed bone parameter increases attributable to normal age- and season-related bone development, and not due to calcium supplementation, because the changes were common to all groups, with no significant differences between groups (Grace et al., 2002).

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