Scientific Article

The quantitative assessment of photodensity of the third carpal bone in the horse

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Abstract

AIM: To determine whether variation in x-ray-beam angle significantly affected photodensity of the third carpal bone (C3) in the horse using the tangential radiographic view, and indirectly determine whether radioabsorptiometry (RA) could be used to assess differences in bone mineral density (BMD) of C3 between exercised and non-exercised horses.

METHODS: The left distal carpal row was isolated post-mortem from 2-year-old Thoroughbred horses that had been either exercised (n=7) for up to 14 weeks using a standard training regimen for racehorses, or not exercised (n=7). The effect of variation in x-ray-beam angle on photodensity was determined on these isolated carpal bones *in vitro*. Image analysis was used to assess photodensity (compared to a known thickness of aluminium) of four regions of interest (ROI) in C3 and one ROI in the fourth carpal bone (C4) of 14 isolated distal rows of carpal bones of the horse. The isolated carpal bones were placed flat on a x-ray cassette and radiographed at 90° (i.e. with the x-ray beam perpendicular to the cassette). The x-ray-beam angle was varied in the dorsal sagittal plane by 5° increments to a total of 15° from 90° and from a base angle of 60° (the x-ray-beam angle at which the tangential view is taken in clinical cases).

RESULTS: Variation in beam angle of <10° from 90° significantly affected photodensity, and photodensity was significantly affected when the angle was varied <5° from 60°. When taken at an x-ray-beam angle of 60°, the abaxial aspect of the radial facet of C3 had a consistently higher photodensity than the rest of C3 and C4. The photodensity of the third and fourth carpal bones was higher in exercised horses than in non-exercised horses.

CONCLUSION: As variation in x-ray-beam angle significantly affected photodensity, RA using the tangential view is not considered clinically applicable for assessing BMD of C3 and the accuracy of subjective assessment of BMD of C3 using the tangential view in horses is questionable.

KEY WORDS: *Third carpal bone, radioabsorptiometry, photodensity, bone mineral density, horse*

Introduction

Lameness is the most common cause of interference with a performance horse's training schedule (Jeffcott et al 1982; Rossdale et al 1985; Lindner et al 1992). The carpus is a common site of injury, and carpal-bone fractures during training or racing account for 1–8% of all injuries in some populations (Mohammed et al 1991; Wilson et al 1993; Mizuno 1996), with C3 being a frequently injured carpal bone (Schneider et al 1988). In response to exercise, the subchondral bone models by increasing its density and modifying its architecture. Remodelling occurs concurrently; however, when damage to the bone exceeds its ability to repair, pathological lesions within the subchondral bone occur. Because some *in vivo* and *in vitro* studies demonstrated traumatic loading-induced damage to the subchondral bone and calcified cartilage before inducing damage to articular cartilage (Silyn-Roberts and Broom 1990; Verner et al 1992), it has been proposed that early detection of increased BMD may be helpful in preventing degenerative joint disease and fractures (DeHaan et al 1987).

Numerous methods of non-invasive quantitative bone-mineral analysis have been developed to aid the early detection of osteoporosis in humans. One technique, RA, may be clinically applicable for quantitatively assessing the BMD of C3 of horses. RA estimates BMD by measuring radiographic photodensity and comparing it to a material of known photodensity (Yang et al 1994; Yates et al 1995). The technique involves taking a conventional radiographic image that includes a reference standard in a predetermined place, converting the radiograph into a digital image and interpreting the data using an image-analysis program (Yates et al 1995). The technique does not require expensive equipment and the patient is not required to be motionless for a prolonged period of time.

To measure BMD within the dorsal aspect of C3 using RA, the bone must be positioned free from other superimposing structures, which is achieved *in vivo* using the flexed dorsoproximal-dorsodistal oblique (tangential) radiographic view of the distal row of carpal bones. In this view, the x-ray beam travels in a palmaroproximal to dorsodistal direction. Clinical experience has suggested that small changes in x-ray-beam angle appeared to result in variation of the amount of the dorsal aspect of C3 visualised. The procedure for taking the tangential view of C3 in a live horse is variable. It is difficult to accurately reproduce x-ray-beam-C3 angle, the position of C3, and the angle at which

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ANOVA	Analysis of variance
BMD	Bone mineral density
C3	Third carpal bone
C4	Fourth carpal bone
RA	Radioabsorptiometry
ROI	Region(s) of interest

the x-ray beam penetrates the x-ray cassette. In order to determine whether RA could be used to accurately assess the BMD of C3, it must be established whether variation in the angle at which the x-ray beam penetrates C3 (C3-beam angle) significantly affects the photodensity measured.

RA is an accurate method for assessing BMD when the object-beam angle is 90° (Cosman et al 1991). The objectives of the *in vitro* study presented here were to determine whether variation in x-ray-beam angle, in 5° increments, from the base angles of 60° (the C3–beam angle recommended in the literature for the tangential view of the distal row of carpal bones in the horse) and 90° significantly affected photodensity of specific ROI in the third and fourth carpal bones and; to establish the effect of exercise on the BMD of these bones isolated at necropsy from horses in two groups maintained under different exercise regimens.

Materials and methods

Fourteen 2-year-old female Thoroughbred horses were accustomed to being ridden for the first time ('broken-in') over a 6-week period in 1997. The control group (n=7) was confined to pens (25 m x 8 m) on pasture and the exercised group (n=7) were boxed at night, turned out into small pens during the day, and followed a standard commercial training regimen for Thoroughbred racehorses under the direction of a licensed professional horse trainer for up to 14 weeks, according to a protocol approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand. The horses were euthanised at 754±28 days of age for other reasons, and the left distal carpal row was isolated and immersed in 98% ethyl alcohol for a minimum of 9 months.

A Picker Explorer (Picker International Ltd, GEC Company, Wembley Middlesex, UK) mobile x-ray machine was used to expose medical grade HR G-30 film (Fuji Photo Film Company Limited, Tokyo, Japan) at a focal distance of 1,000 mm, in cleaned cassettes containing medium intensifying screens. A template was designed to ensure the metal cube, wedge, circle (used for calibration – see below) and distal row of carpal bones would be in a similar position for every radiograph (Figure 1).

The isolated carpal bones were placed on a cassette and radiographed at an x-ray-beam angle of 90° (control). X-ray-beam angle was defined as the angle between the x-ray tube head and the horizontal axis, horizontal being 0° and vertical being 90°. The vertical x-ray-beam angle was the angle the tube head deviated in the vertical plane. The horizontal x-ray-beam angle was the angle the tube head deviated in the horizontal plane. X-ray cassette-beam angle was defined as the angle at which the x-ray beam struck the surface of the cassette containing radiographic film, horizontal being 0° and vertical being 90°. The vertical x-ray cassette-beam angle was the angle at which the x-ray beam struck the x-ray cassette in the vertical plane. The horizontal x-ray cassette-beam angle was the angle the x-ray beam struck the x-ray cassette in the horizontal plane.

A pilot study using isolated forelegs revealed the x-ray-beam angle could be varied by up to 15° before the dorsal aspect of C3 became completely concealed by either the distal radius or the proximal third metacarpal bone. Radiographs were taken at 5° increments up to 15° from vertical; thus, with the x-ray beam travelling in a

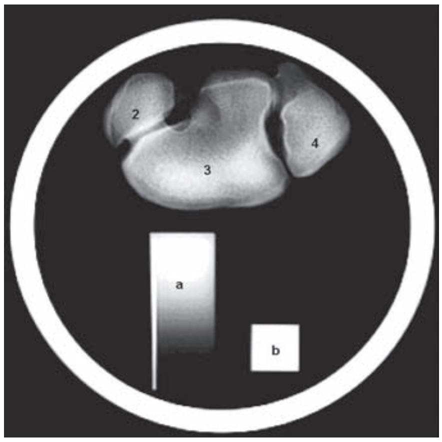

Figure 1. Radiographic image of the isolated distal row of carpal bones from a Thoroughbred filly, taken using an x-ray-beam angle of 90°. The calibration circle surrounds the bones (2=second, 3=third, 4=fourth carpal bone) and encloses the aluminium wedge (a) and stainless-steel cube (b) used for calibration.

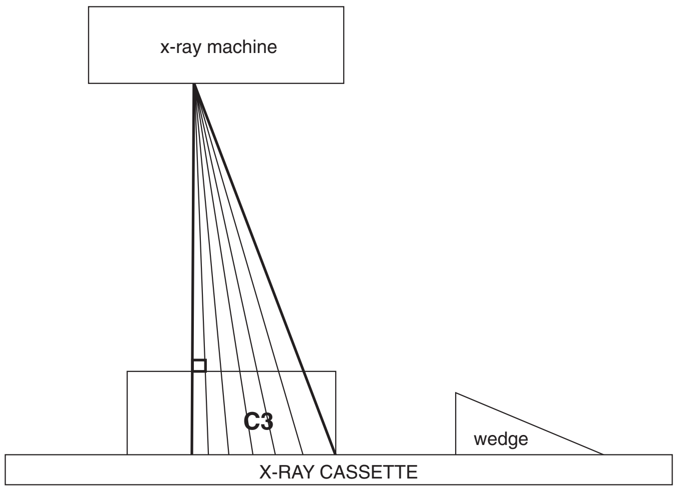

Figure 2. Radiographs were taken initially at an x-ray-beam angle of 90° (which is illustrated as the bold line originating from the x-ray machine to the left of the diagram) and 60° (which is illustrated as the bold line originating from the x-ray machine to the right of the diagram) with the x-ray beam travelling in a palmaroproximal to dorsodistal direction in relation to the isolated carpal bones. The finer lines between the two bold lines represent x-ray-beam angles at 5° increments from either 90° or 60°.

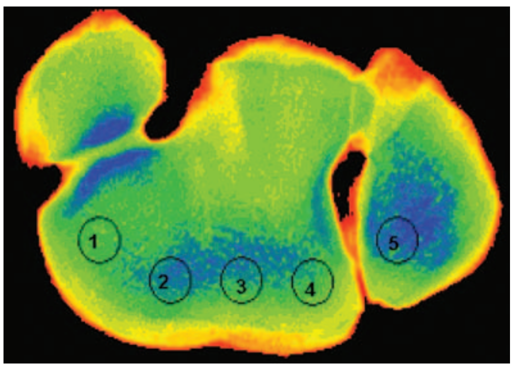

Figure 3. Calibrated image of an isolated distal row of carpal bones from a Thoroughbred filly, taken at 90° with the regions of interest (ROI) labelled 1 to 5. ROI 1 was within the abaxial aspect of the radial facet, ROI 2 was within the axial aspect of the radial facet, ROI 3 was within the axial aspect of the intermediate facet, and ROI 4 was within the abaxial aspect of the intermediate facet of the third carpal bone. ROI 5 was within the fourth carpal bone.

palmaroproximal to dorsodistal direction (referring to the carpal bones' normal anatomical positioning *in vivo*), the x-ray-beam angles were 85°, 80° and 75°. Radiographs were also taken at 60°, 65° and 70° (Figure 2). Once the x-ray head was set to the correct angle, all 14 C3's were radiographed and the developed radiographs immediately scanned and digitised to prevent accumulation of particles on the film resulting in possible artefacts. The radiographs were scanned using an Agfa Duoscan (Agfa-Gevaert NV, Mortsel, Belgium) desktop, flatbed scanner fitted with a transparency scanning bed. All image analyses were performed using the Vision Image Processing system (Bailey and Hodgson 1988), using macros (a program consisting of a sequence of image processing commands) custom written for this project.

The first macro calibrated the image, determined x-ray cassette-beam angle and allowed photodensity to be measured in units of 0.1 mm of aluminium. Two stainless-steel circles, the first with a 130-mm and the second with a 160-mm external diameter were made. The outer edge was bevelled to provide a sharp edge on the radiograph and digitised image, regardless of x-ray beam angle. The aim of the circle was to calibrate the size (in mm) and aspect ratio (ratio between the height and width) of each pixel. The circle was as large as possible, given the film size used, to maximise the accuracy of calibration by reducing the relative error inherent in the measuring process (Bailey 1995). A 15-mm stainless-steel cube was used to determine the angle at which the radiograph was taken, which was achieved by first determining the dimensions of the cube on an image for which the x-ray cassette-beam angle was exactly 90° (perpendicular to the x-ray cassette). The cube's image on radiographs taken when the x-ray cassette-beam angle varied from 90° allowed determination of the deviation from 90° with a calculated error of ±2°. Both the vertical and horizontal x-ray cassette-beam angles were determined in this way. As the C3 bone was lying on the horizontal x-ray cassette, the x-ray-beam-to-C3 angle was quantified once the x-ray cassette-beam angle had been determined. A smooth surfaced (i.e. non-graduated) aluminium wedge provided a scale of densities for comparison with the photodensity of each ROI within the third and fourth carpal bones.

By knowing the x-ray cassette-beam angle and the distance from one end of the wedge (detectable by the presence of a piece of stainless steel along the length of the wedge) to the density of interest, the average photodensity of each ROI could be calibrated in units equivalent to 0.1 mm increments in thickness of aluminium. The process used allowed the image of the wedge to be corrected for changes in x-ray cassette-beam angle, thereby acting as a standard regardless of changes of angle. The photodensity of the image of the wedge increased linearly until a plateau was reached; the photodensity of all ROI were within this linear range.

An ROI programme was used to determine the site of five ROI in a consistent and repeatable manner within each bone by following a set of predefined rules. From the entire image of C3, 9 mm was removed which resulted in sharp points at which to delineate the widest part of C3 and served to create a line parallel to the front edge of the bone that could be used to site the ROI. As the most dorsal aspect of C3 is at the dorsocentral aspect of the bone not the dorsoproximal aspect, for the 90° image the ROI were placed 6 mm from the most dorsal edge of the bone. The ROI moved dorsally as the angle reduced, and the degree of displacement was dependent on the vertical x-ray cassette-beam angle. ROI 1 and 4 were determined from these points and a line drawn between them. ROI 2 and 3 were evenly spaced between ROI 1 and 4. ROI 5 was determined by extending the line drawn between ROI 1 and 4 and was dependent on the width of C4. ROI 1 was within the abaxial aspect of the radial facet of C3, ROI 2 was within the axial aspect of the radial facet, ROI 3 was within the axial aspect of the intermediate facet, and ROI 4 was within the abaxial aspect of the intermediate facet of C3. ROI 5 was within C4. (Figure 3). The ROI were circles (so orientation was not important), each with a radius of 3 mm and thus an area of 28 mm^2.

Statistical analysis

Statistical analysis was performed on the data (photodensity measured in 0.1 mm of aluminium) generated from the placement of ROI within the distal row of the excised carpal bones. The effect of variation of angle and exercise on photodensity were analysed using analysis of variance (ANOVA) and Type-III sums of squares. All analyses were performed using SPSS 9.0 for Windows (SPSS Inc, Chicago IL, USA). The data generated from the placement of ROI at 60°, 65°, 70°, 75°, 80° and 90° were analysed for effect of variables including horse, ROI (1, 2, 3, 4, 5), angle, and group (exercised *vs* control). Horse was treated as a random effect and nested within group for the purpose of analysis. All other vari-

Table 1. Analysis of variance results for photodensity measured *in vitro* at five dorsally located regions of interest (ROI) on the third carpal bone of horses subjected to exercise or no exercise (Group), using radioabsorptiometry and x-ray-beam angles of 60°, 65°, 70°, 75°, 80°, 85° and 90° from horizontal.

Factor	Type III SS[a]	df[b]	Mean square	F-value	P-value
Between horse:					
Group	26718	1	26718	33.17	<0.001
Error	9666	12	805		
Within horse:					
Angle	23784	6	3964	70.53	<0.001
ROI	11877	4	2969	52.83	<0.001
Angle*Group[c]	746	6	124	2.21	0.041
Angle*ROI[c]	3995	24	166	2.96	<0.001
Group*ROI[c]	2229	4	557	9.92	<0.001
Angle*Group*ROI[c]	749	24	31	0.55	0.958
Error	22930	408	56		

[a] Type III sums of squares
[b] Degrees of freedom
[c] Interaction terms between main effects

Table 2. The mean difference for pairwise comparisons of photodensities measured *in vitro* at five dorsally located regions of interest on the third carpal bone of horses, using radioabsorptiometry and x-ray-beam angles of 60°, 65°, 70°, 75°, 80°, 85° and 90° from horizontal.

Angle I	Angle J	Mean difference[a]	P-value[b]
60°	65°	–5.064	0.002
	70°	–10.586	<0.001
	75°	–11.120	<0.001
	80°	–11.915	<0.001
	85°	–19.638	<0.001
	90°	–21.439	<0.001
65°	70°	–5.523	<0.001
	75°	–6.056	<0.001
	80°	–6.851	<0.001
	85°	–14.574	<0.001
	90°	–16.376	<0.001
70°	75°	–5.340	1.000
	80°	–1.329	1.000
	85°	–9.052	<0.001
	90°	–10.853	<0.001
75°	80°	–0.795	1.000
	85°	–8.518	<0.001
	90°	–10.320	<0.001
80°	85°	–7.723	<0.001
	90°	–9.525	<0.001
85°	90°	–1.801	1.000

[a] Photodensity at Angle (I) – photodensity at Angle (J); unit = photodensity equivalent to 0.1 mm of aluminium.

[b] Bonferroni adjusted p-values using a family-wise alpha of 0.05; standard error for comparisons = 1.267 and degrees of freedom = 24.

Table 3. The mean difference for pairwise comparisons of photodensities measured *in vitro* at five dorsally located regions of interest (ROI) on the third carpal bone of horses using radioabsorptiometry. Comparisons are for ROI (I) – ROI (J), measured using each of two x-ray-beam angles (60° and 90° from horizontal).

Angle	ROI (I)	ROI (J)	Mean difference[a]	P-value[b]
90°	1	2	–1.882	1.000
		3	4.073	1.000
		4	6.709	0.184
		5	1.595	1.000
	2	3	5.955	0.362
		4	8.591	0.026
		5	3.477	1.000
	3	4	2.636	1.000
		5	–2.478	1.000
	4	5	–5.114	0.718
60°	1	2	18.796	<0.001
		3	22.559	<0.001
		4	23.258	<0.001
		5	14.364	<0.001
	2	3	3.763	1.000
		4	4.562	1.000
		5	–4.432	1.000
	3	4	0.799	1.000
		5	–8.195	0.040
	4	5	–8.994	0.016

[a] Photodensity at ROI (I) – photodensity at ROI (J); unit = photodensity equivalent to 0.1 mm of aluminium.

[b] Bonferroni adjusted p-values using a family wise alpha of 0.05; standard error for comparisons = 2.834 and degrees of freedom = 408.

ables were treated as fixed effects. Bonferoni adjusted *t*-tests were used to compare means between levels within significant effects from the ANOVA, and the p-value was set at 0.05.

Results

The main effects of angle, exercise and group, and the interaction between group and angle, ROI and angle, as well as group and ROI were significantly associated with ROI photodensity (Table 1). The three-way interaction between group, ROI and angle was not significant. Estimates of observed power conducted in conjunction with the ANOVA were 0.48 for the 3-way interaction term (group*angle*ROI), 0.78 for the group*angle interaction, and 1.0 for all other terms in the model. These estimates confirmed that sample sizes and measurement precision were sufficient to confidently detect significant effects.

Effect of angle on photodensity

When ROI were in a dorsal position there was significant variation (p<0.05) in photodensity when the angle varied by as little as 5° from 60°, and 10° from 90° (Table 2).

Effect of ROI on photodensity

At an object beam angle of 90°, the photodensity of ROI 2 significantly differed from ROI 4 (p=0.026), however all other ROI were not significantly different from each other (Table 3). At an object beam angle of 60°, the photodensity of ROI 1 was significantly higher than all other ROI (p<0.001), and ROI 5 was significantly more photodense than ROI 3 (p=0.04) and 4 (p=0.016) (Table 3).

Effect of exercise on photodensity

Exercise significantly increased photodensity in each ROI; differences in photodensity (measured in units equivalent to 0.1 mm increments in the thickness of aluminium) between exercised and non-exercised horses averaged –11.78, –19.81, –18.00, –16.13 and –8.11 for ROI 1, 2, 3, 4, and 5, respectively (SE=1.773, df=117, p<0.001 for all comparisons). In the non-exercised group of horses, the photodensity of ROI 5 was significantly greater than those in ROI 2, 3 and 4 (p<0.001), but was significantly less than that seen in ROI 1 (p=0.002) (Table 4). In the exercised group, ROI 1 and 2 were significantly more photodense than all other ROI (p<0.021) (Table 5).

Evaluation of the group*angle interaction (data not shown) demonstrated that the measured increase in photodensity in exercised *vs* non-exercised horses was greater at 60° than at other angles though the effect of exercise remained significant at all angles.

Discussion

The indirect aim of this study was to determine if RA could be used to objectively assess variation in BMD within the dorsal aspect of C3 of the horse. Because the atomic number, specific gravity and attenuation coefficient of bone mineral and aluminium are similar, photodensity of bone can be accurately measured by comparison against an aluminium standard. However, conversion from photodensity to bone mineral concentration is more complex (Colbert and Bachtell 1981). Additional factors that must be taken into consideration include attenuation of photons by the bone and aluminium, the role of scatter, and photon/film interactions. The advent of computer-assisted RA has enabled these factors to be accounted for by using a series of complex equations (Colbert and Bachtell 1981); however, within this study these factors could not be accounted for and the results are discussed in terms of photodensity, using a scale of equivalent absorption through aluminium of known thickness. As a measure of BMD is not being used, comparison with studies using other methods of non-invasive bone mineral analysis was based on the assumption that photodensity, in terms of mm of aluminium, was directly proportional to BMD.

Photodensity changed significantly when the x-ray-beam angle varied >5° from 90° and <5° from 60°. This most likely occurred because when the angle reduces to 60° from 90°, the path length increases nonlinearly. While small deviations from a nominal angle of 90° only increase the path length slightly, any angular deviation from a nominal angle of 60° has a significant effect on the path length. For example, if the actual angle is 80° rather than 90°, the path length is increased by only 1.5%. However, a 10° error at 60° will increase the path length by 13% (50°) or decrease the path length by 7.8% (70°). As a result of this, any measurements made at 60° are significantly more sensitive to errors in angle than similar measurements made at 90°.

Table 4. The mean difference for pairwise comparisons of photodensities measured using radioabsorptiometry *in vitro* at five dorsally located regions of interest (ROI) on the third carpal bones of a group of non-exercised horses.

ROI (I)	ROI (J)	Mean difference[a]	P-value[b]
1	2	12.092	<0.001
	3	14.973	<0.001
	4	16.740	<0.001
	5	5.687	0.002
2	3	2.880	0.579
	4	4.648	0.023
	5	–6.405	<0.001
3	4	1.768	1.000
	5	–9.286	<0.001
4	5	–11.053	<0.001

[a] Photodensity at ROI (I) – photodensity at ROI (J); unit = photodensity equivalent to 0.1 mm of aluminium.

[b] Bonferroni adjusted p-values using a family-wise alpha of 0.05; standard error for comparisons = 1.515 and degrees of freedom = 408.

Table 5. The mean difference for pairwise comparisons of photodensities measured using radioabsorptiometry *in vitro* at five dorsally located regions of interest (ROI) on the third carpal bones of a group of exercised horses.

ROI (I)	ROI (J)	Mean difference[a]	P-value[b]
1	2	4.055	0.077
	3	8.750	<0.001
	4	12.386	<0.001
	5	9.375	<0.001
2	3	4.694	0.021
	4	8.340	<0.001
	5	5.307	0.005
3	4	3.636	0.168
	5	0.614	1.000
4	5	–3.023	0.466

[a] Photodensity at ROI (I) – photodensity at ROI (J); unit = photodensity equivalent to 0.1 mm of aluminium.

[b] Bonferroni adjusted p-values using a family-wise alpha of 0.05; standard error for comparisons = 1.515 and degrees of freedom = 408.

To our knowledge, the effect of variation in angle on photodensity when applied to RA has not been documented. The findings of this study indicate that due care must be taken in application of object-beam angle (in this case C3-beam angle) when radiographing areas for analysis using RA and when comparing tangential views of C3 (taken at a C3-beam angle of 65°) with radiographs of isolated C3 taken at 90°. Our results indicate that variation in x-ray-beam angle of 25° significantly affected photodensity, and there is unlikely to be a good objective correlation between the two views. These results are in direct contrast to the study by Uhlhorn et al (1998), in which there was a significant relationship between the photodensity taken at a C3-beam angle of 65° and those taken at 90°. The likely reason for the difference between these studies is that those authors compared the radiographs subjectively rather than quantitatively.

The discrepancy of photodensity of ROI 1 and all other ROI between 60° (C3-beam angle of the tangential view) and 90° was thought to be due to the prominent transverse ridge that is present on the medial dorsocentral aspect of C3. When the C3-beam angle is at 60°, the amount of bone traversed is significantly higher at the abaxial aspect of the radial facet compared with the rest of C3, resulting in higher photodensity. In contrast, when the C3-beam angle is 90°, the x-ray beam does not travel through the bone on the dorsocentral aspect of the radial facet and the photodensity of the entire radial facet and the axial aspect of the intermediate facet are not significantly different.

In all microradiographic and BMD studies we have reviewed (Young et al 1989; Young et al 1991; Ulhorn et al 1998; Firth et al 1999a), C3 has been assessed in a proximodorsal or lateromedial direction, and comparison with the present study can be made only for data collected at a C3-beam angle of 90°. A study assessing subchondral bone stiffness and bone density of C3 of horses found no change in stiffness between the intermediate and radial facets (Young et al 1991). This has also been found when objectively assessing the BMD of C3 using dual x-ray absorptiometry (Firth et al 1999a). In both studies, there was only one area of interest in the radial facet and one in the intermediate facet of C3. In the study by Young et al (1991), the area of interest in the radial facet was between ROI 1 and 2, and in the intermediate facet between ROI 3 and 4. In the study by Firth et al (1999a), the area of interest in the radial facet was in a similar position to ROI 2 in the study presented here, and in the intermediate facet it was in a similar position to ROI 3. Thus, the results from the present study support the conclusions of Young et al (1991) and Firth et al (1999a).

ROI 5 was within C4 and the mean photodensity, as well as the photodensities of the individual horses, were always within the range of photodensities evident for the ROIs located within C3. It has been suggested that C4 be used as a control when subjectively assessing the photodensity of C3 from the tangential radiographic view (O'Brien et al 1986; DeHaan et al 1987). Thus, C3 would be classified as not radiographically sclerotic if the subjective photodensity and trabeculation were the same as those of C4. In this study, the objective assessment of photodensity at a C3-beam angle of 60° indicated that the photodensity of C4 was similar to that of the axial aspect of the radial facet, significantly less than the abaxial aspect of the radial facet, and significantly more than the axial and abaxial aspect of the intermediate facet. When the C3-beam angle was 90°, the photodensity of C4 was not significantly different from any ROI in C3. These findings suggest that radiographing C3 using the tangential view results in the abaxial radial facet always being more photodense than C4, which could result in false positive diagnoses of radiographic sclerosis in the radial facet.

The bones used in this experiment came from horses used in a short-term exercise study. The results indicated that short-term exercise significantly affected the photodensity of all ROI in the dorsal aspect of C3, and are consistent with those of Firth et al (1999ab) who found that exercise resulted in increased BMD of C3. Exercise also resulted in significantly higher photodensity of C4 and, as a result, the photodensity of C4 in exercised horses was not significantly different from that of the intermediate facet. As the horses did not appear to have pathological changes in their C3, no comment can be made as to whether C4 continues to model (that is, the bone modifies its architecture and increases BMD in response to stress) or if it reaches a particular BMD and only remodels (that is, damaged tissue is replaced with an equal amount of new bone tissue and there is no nett increase in BMD). This study indicated that C4 responds to the same stresses as C3 and models accordingly, which may place doubt on whether C4 should be used as a radiographic control in the subjective assessment of carpal radiographs.

The clinical usefulness of RA appears limited when applied to C3 because the C3-beam angle must be reproduced within 5° each time the tangential view is taken to avoid effects of beam angle on radiodensity. This may not be feasible with the current practical constraints involved in taking radiographs of live horses. An indirect conclusion of this study is that the current method of subjective evaluation of the photodensity of C3 using the tangential view may be relatively inaccurate. A retrospective study evaluating sclerosis of C3 found no significant relationship between C3 sclerosis, lameness or prognosis (Uhlhorn and Carlsten 1999), which appears to support the indirect conclusion of this study.

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References

Bailey DG. Pixel calibration techniques. *Proceedings of the New Zealand Image and Vision Computing Workshop*. Pp 37–42, 1995

Bailey DG, Hodgson RM. VIPS – a digital image processing algorithm development environment. *Image and Vision Computing* 6, 176–84, 1988

Colbert C, Bachtell RS. Radiographic absorptiometry (photodensitometry). In: Cohn SH (ed). *Non-invasive Measurements of Bone Mass and Their Clinical Application.* Pp 51–85. CRC Press Inc, Boca Raton, USA, 1981

Cosman F, Herrington B, Himmelstein S, Lindsay R. Radiographic absorptiometry: a simple method for determination of bone mass. *Osteoporosis International* 2, 34–8, 1991

DeHaan CE, O'Brien TR, Koblik PD. A radiographic investigation of third carpal bone injury in 42 racing Thoroughbreds. *Veterinary Radiology* 28, 88–92, 1987

Firth EC, Delahunt J, Wichtel JW, Birch HL, Goodship AE. Galloping exercise induces regional changes in bone density within the third and radial carpal bones of Thoroughbred horses. *Equine Veterinary Journal* 31, 111–5, 1999a

Firth EC, Van Weeren PR, Pfeiffer DU, Delahunt J, Barneveld A. Effect of age, exercise and growth rate on bone mineral density (BMD) in third carpal bone and distal radius of Dutch Warmblood foals with osteochondrosis. *Equine Veterinary Journal* 31 (Supplement), 74–8, 1999b

Jeffcott L, Rossdale P, Freestone J, Frank CJ, Towers-Clark PF. An assessment of wastage in Thoroughbred racing from conception to 4 years of age. *Equine Veterinary Journal* 14, 185–98, 1982

Lindner A, von Wittke P, Dingerkus A, Temme M, Sommer H. Causes and rates of training failure in Thoroughbreds. *Equine Athlete* 5, 3–7, 1992

Mizuno Y. Fractures of the carpus in racing Thoroughbreds of the Japan racing association: prevalence, location and current modes of surgical therapy. *Journal of Equine Veterinary Science* 16, 25–31, 1996

Mohammed HO, Hill T, Lowe J. Risk factors associated with injuries in Thoroughbred horses. *Equine Veterinary Journal* 23, 445–8, 1991

O'Brien TR, DeHaan CE, Arthur R. Third carpal bone lesions of the racing Thoroughbred. *Proceedings of the American Association of Equine Practitioners* 31, 515–24, 1986

Rossdale P, Hopes R, Winfield Digby N, Offord K. Epidemiological study of wastage among racehorses 1982 and 1983. *Veterinary Record* 116, 66–9, 1985

Schneider RK, Bramlage LR, Gabel AA, Barone LM, Kantroitz BM. Incidence, location and classification of 371 third carpal bone fractures in 313 horses. *Equine Veterinary Journal* 6 (Supplement), 33–42, 1988

Silyn-Roberts H, Broom ND. Fracture behaviour of cartilage-on-bone in response to repeated impact loading. *Connective Tissue Research* 24, 143–56, 1990

Uhlhorn H, Carlsten J. Retrospective study of subchondral sclerosis and lucency in the third carpal bone of Standardbred trotters. *Equine Veterinary Journal* 31, 500–5, 1999

Uhlhorn H, Ekman S, Haglund A, Carlsten J. The accuracy of the dorsoproximal-dorsodistal projection in assessing third carpal bone sclerosis in Standardbred trotters. *Veterinary Radiology and Ultrasound* 39, 412–7, 1998

Verner MJ, Thompson RC, Lewis JL, Oegema TR. Subchondral damage after acute transarticular loading: An *in vitro* model of joint injury. *Journal of Orthopedic Research* 10, 759–65, 1992

Wilson JH, Howe SB, Jensen RC, Robinson RA. Injuries sustained during racing at racetracks in the U.S. in 1992. *Proceedings of the American Association of Equine Practitioners* 39, 267–8, 1993

Yang S, Hagiwara S, Engelke K, Dhillon MS, Guglielmi G, Bendavid EJ, Soejima O, Nelson DL, Genant HK. Radiographic absorptiometry for bone mineral measurement of the phalanges: precision and accuracy study. *Radiology* 192, 857–9, 1994

Yates AJ, Ross PD, Epstein RS. Radiographic absorptiometry in the diagnosis of osteoporosis. *American Journal of Medicine* 98, 41–7, 1995

Young A, O'BrienTR, Pool RR. Exercise-related sclerosis in the third carpal bone of the racing Thoroughbred. *Proceedings of the American Association of Equine Practitioners* 34, 339–46, 1989

Young DR, Richardson DW, Markel MD, Nunamaker DM. Mechanical and morphometric analysis of the third carpal bone in Thoroughbreds. *American Journal of Veterinary Research* 52, 402–9, 1991

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