Bone 44 (2009) 316–319

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Correlation of quantitative computed tomographic subchondral bone density and ash density in horses

M.G. Drum [a,*], C.M. Les [b], R.D. Park [c], R.W. Norrdin [d], C.W. McIlwraith [a], C.E. Kawcak [a]

[a] Equine Orthopaedic Research Center, Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80523, USA
[b] Bone and Joint Center, Henry Ford Hospital, Detroit, MI 48202, USA
[c] Department of Environmental and Radiologic Health Sciences, Colorado State University, Fort Collins, CO 80523, USA
[d] Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, USA

ARTICLE INFO

Article history:
Received 11 January 2008
Revised 25 August 2008
Accepted 27 August 2008
Available online 19 September 2008

Edited by: H. Genant

Keywords:
Ash density
Quantitative computed tomography
Subchondral bone
Equine
Metacarpophalangeal

ABSTRACT

The purpose of this study was to compare subchondral bone density obtained using quantitative computed tomography with ash density values from intact equine joints, and to determine if there are measurable anatomic variations in mean subchondral bone density. Five adult equine metacarpophalangeal joints were scanned with computed tomography (CT), disarticulated, and four 1-cm^3 regions of interest (ROI) cut from the distal third metacarpal bone. Bone cubes were ashed, and percent mineralization and ash density were recorded. Three-dimensional models were created of the distal third metacarpal bone from CT images. Four ROIs were measured on the distal aspect of the third metacarpal bone at axial and abaxial sites of the medial and lateral condyles for correlation with ash samples. Overall correlations of mean quantitative CT (QCT) density with ash density (r=0.82) and percent mineralization (r=0.93) were strong. There were significant differences between abaxial and axial ROIs for mean QCT density, percent bone mineralization and ash density (p<0.05). QCT appears to be a good measure of bone density in equine subchondral bone. Additionally, differences existed between axial and abaxial subchondral bone density in the equine distal third metacarpal bone.

Published by Elsevier Inc.

Introduction

Quantitative computed tomography (QCT), is a powerful tool to noninvasively expand our knowledge of normal and abnormal joint anatomy. Several studies [1–6] have been performed in humans to verify that bone mineral density measurements obtained using QCT, which measures volumetric bone density, are accurate representations of true bone mineral density. The majority of these studies focused on samples from trabecular bone. Cortical and subchondral bone are significantly more dense, and thus the higher density of subchondral bone may influence QCT density measurements due to beam hardening effects. Beam hardening occurs in areas of X-ray attenuation, such as subchondral bone, where the mean energy of the X-ray beam passing through the bone is increased and results in adjacent structures having falsely low measured densities [7]. This phenomenon can also occur in the center of highly dense materials. However, bone reconstruction algorithms used by the CT computer aim to reduce these effects, and are thought to be adequate to clinical and research requirements [8].

One study that investigated the relationship between cortical QCT density and ash density found only a moderate correlation between the two indices [9]. In horses, research is very limited, but one study found that cylinders of equine trabecular and cortical bone correlated strongly with QCT density [10]. Another study found cross-sectional area, thought to be related to ash content, is strongly correlated to QCT cortical bone density [11]. Other studies in dogs have found similar strong correlations of calcium content to QCT density [12]. No correlations have been reported for subchondral bone density measured using QCT and ash density in humans or animals. Regional variations in subchondral density have been documented using QCT in dogs and humans [12,13]. QCT subchondral density variations have been subjectively evaluated in horses [14], but specific anatomic variations in subchondral density have not been measured in horses. Knowledge of density variations in specific anatomic areas would be useful in identifying sites for subchondral measurement in the detection of early joint disease in horses.

With newer more powerful computer applications, three-dimensional volumes of subchondral bone surfaces can be created. QCT density values obtained from three-dimensional models of equine subchondral bone have not been previously published. Therefore, the accuracy of QCT equine subchondral bone density measurements obtained using three-dimensional modeling programs is also

* Corresponding author. Fax: +1 865 974 5554.
E-mail address: mdrum@utk.edu (M.G. Drum).

8756-3282/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bone.2008.08.129

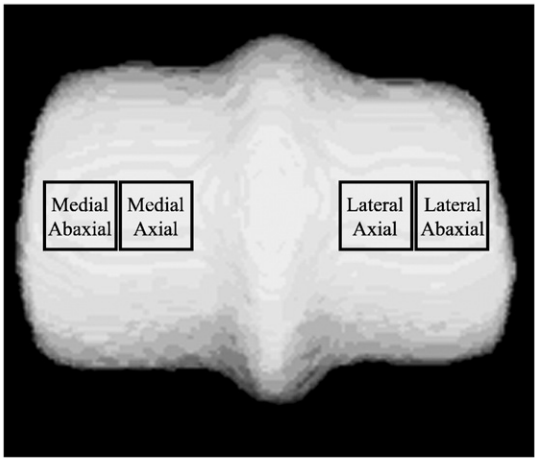

Fig. 1. Three-dimensional model of the equine distal third metacarpal bone with the four 1-cm^2 regions of interest.

unknown. The accuracy of subchondral bone density values obtained using QCT from three-dimensional models needs to be determined before applying this technology to clinical and experimental applications in the horse. The goals of this study were to evaluate QCT as a measure of subchondral bone density in distal third metacarpal bone of horses, and to determine if there are regional density variations in distal third metacarpal bone. We hypothesized that equine bone density measured using apparent ash density would correlate well with mean bone density measured using QCT, and that there is a measurable anatomic difference of subchondral bone density in the equine distal third metacarpal bone.

Materials and methods

Five left metacarpophalangeal (MCP) joints from two racing and three non-racing horses were used. Racing horses were both two-year-old female Thoroughbreds euthanized for non-musculoskeletal diseases or injuries, while non-racing horses were a 21-year-old Arabian gelding, a 10-year-old Quarter Horse female, and a four-year-old mixed breed female euthanized for non-musculoskeletal diseases or injuries.

The distal portion of each third metacarpal bone was scanned via CT after removing the metacarpophalangeal joint at the level of the mid-metacarpus to simulate *in vivo* scanning of equine MCP joints. No other alterations were made to the MCP joint, and the hoof and surrounding soft tissues left intact. A Picker PQ CT scanner (Philips Medical, Barthow, WA USA) was used to perform the CT scans on the intact MCP joints with settings at 140 kVp, 512×512 matrix, 18-cm field of view, and a 1.5-mm slice thicknesses. A tri-calcium density equivalent CT phantom (Computerized Image Reference Systems Inc., Fairfax, VA, USA) was scanned at the end of each CT scan for bone density calibration. The densities used for calibration were cylinders of tri-calcium phosphate approximately 1-cm wide embedded in a solid plastic cylinder of approximately 3-cm in diameter.

After intact joint scanning, the MCP joint was disarticulated and the distal third metacarpal condyle was removed using a band saw approximately 3 cm proximal to the medial condyle. Finally, four 1-cm^2 regions of interest (ROI, Fig. 1) were cut and removed to a depth of 1-cm from the surface of the medial condyle using a precision cutting system (Exakt Trennschleifsystem, Exakt-Apparatebau, Norderstedt, Germany) to create approximately 1-cm^3 bone samples for ashing.

Three-dimensional modeling of the distal third metacarpal condyle CT scans was performed using a specially designed PC-based application (OsteoApp, Research Systems Inc., Boulder, CO USA and Colorado State University, Fort Collins, CO USA). A pixel threshold of 500 Hounsfield Units [16] was used to isolate the distal third metacarpal bone for rendering into a three-dimensional model. The four regions of interest were placed precisely on the distal articular surface for each CT scan (Fig. 1) by determining the coordinates, in millimeters, of each corner of the square ROI in relation to the medial, dorsal, and palmar joint edges of the actual distal third metacarpal bone specimen. Coordinates, in millimeters, were then reproduced in OsteoApp on the distal third metacarpal bone articular surface of the three-dimensional model. Mean density values were calibrated using a tri-calcium phosphate density phantom and a simple linear regression for each scan.

Cartilage was removed from the surface of the bone cubes using a #10 scalpel blade and cubes were sonicated for 10 min to remove residual soft tissue. Prior to ashing, samples were transferred to fresh physiologic saline and frozen at −20 °C for storage and transport. Samples were ashed using a previously published protocol [15], where an apparent dry density (ρ_d) was obtained after placing samples in a 100 °C vacuum oven for 24 h and weighed using a precision scale. Apparent ash density (ρ_a) was measured by heating samples in a muffle furnace at 800 °C for 24 h and weighed using a precision scale. Percent mineralization was calculated by dividing apparent ash density by apparent dry density (ρ_a/ρ_d). Ash density and percent mineralization were correlated to QCT mean bone density using a Pearson's correlation coefficient. A mixed-model analysis (Proc Mixed, Statistical Analysis Software, Cary, NC USA) for differences in mean QCT density, mean ash density and mean percent mineralization were performed by side (medial/lateral), anatomic sector (axial/abaxial), and specific ROI site (lateral abaxial, lateral axial, medial abaxial,

Table 1
Pearson's correlation coefficients (r) and p-values by region and specific ROI, for correlations between mean QCT density and physical measurements, for ash density and percent mineralization

Region	Ash density (r)	Percent mineralization (r)
All regions	0.82 (p<0.0001)	0.93 (p<0.0001)
Lateral	0.87 (p=0.0011)	0.93 (p=0.0001)
Medial	0.84 (p=0.0025)	0.84 (p=0.0024)
Axial	0.85 (p=0.0016)	0.63 (p=0.048)
Abaxial	0.93 (p=0.0001)	0.79 (p=0.0065)
Lateral axial	0.75 (p=0.14)	0.92 (p=0.027)
Lateral abaxial	0.97 (p=0.0073)	0.92 (p=0.026)
Medial axial	0.63 (p=0.26)	0.91 (p=0.033)
Medial abaxial	0.96 (p=0.0096)	0.73 (p=0.16)

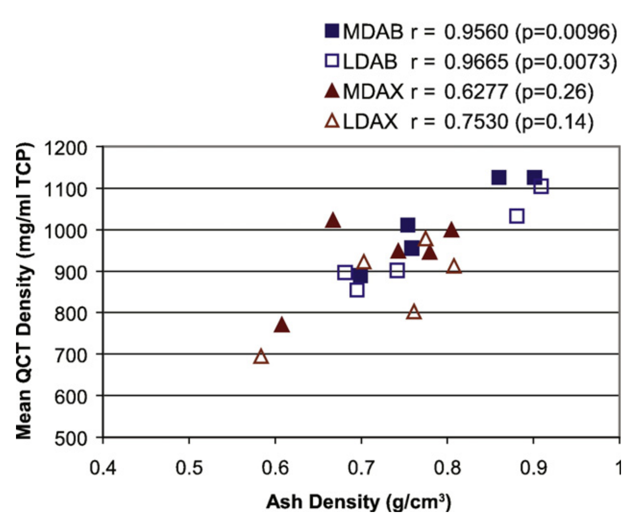

Fig. 2. Mean QCT density vs. ash density. MDAB = Medial abaxial, LDAB = Lateral abaxial, MDAX = Medial axial, LDAX = Lateral axial. Overall correlation was r = 0.8176. Individual correlations and p-values are listed by ROI site.

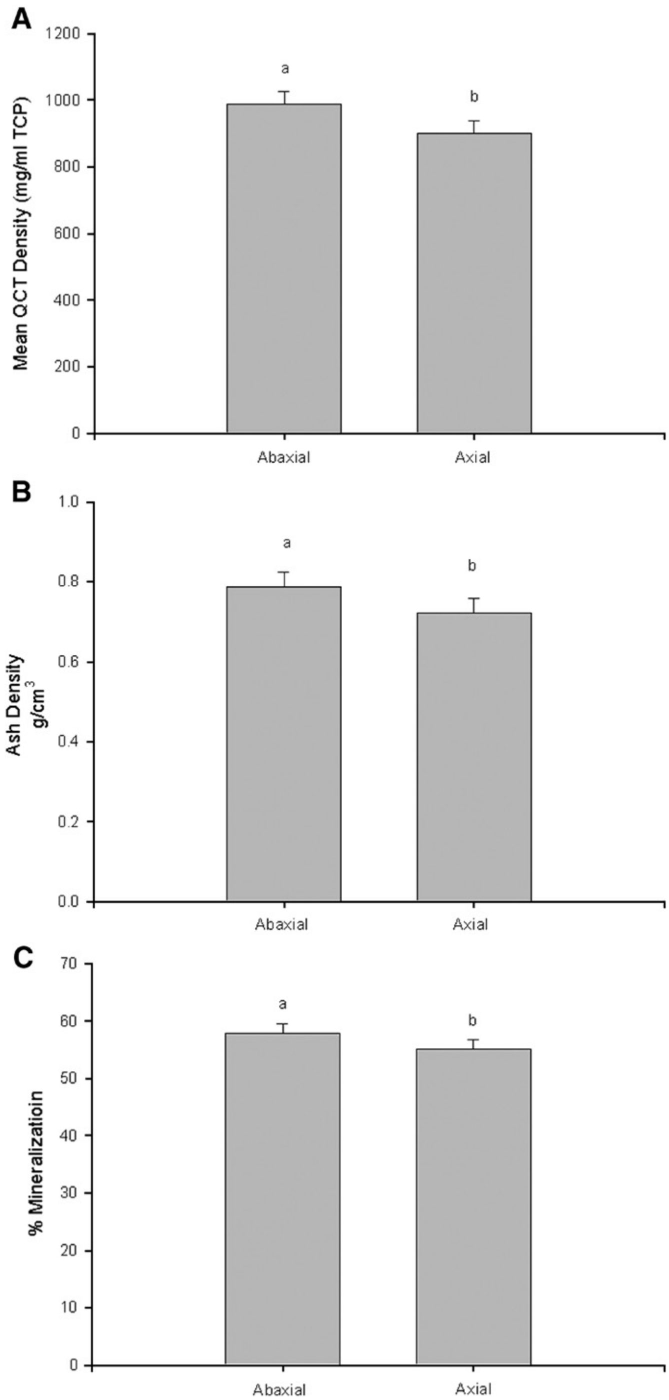

Fig. 3. (A) Mean QCT density by ROI sector (Abaxial 989.55±37.43, Axial=900.02±37.43) (B) Mean ash density by ROI sector (Abaxial=0.7884±0.036, Axial=0.7231±0.036) (C) Mean percent mineralization by ROI sector (Abaxial 57.90±1.67, Axial=55.13±1.67). Different letters indicate significant differences at $p<0.05$, and error bars represent standard error of the mean.

medial axial) with horse as a random factor. Results were considered significant with $p<0.05$.

Results

The correlation between QCT density and physical measurements, pooled for all ROIs, was $r=0.82$ ($p<0.0001$) for ash density and $r=0.93$ ($p<0.0001$) for percent mineralization. Individual correlations for specific condylar side, anatomic sector, and ROI sites are listed in Table 1, and plotted for mean QCT density vs. ash density (Fig. 2). All pooled correlations for condylar side and anatomic sector were significant ($p<0.05$) except lateral and medial axial ash density, and medial abaxial percent mineralization.

Mean QCT density, ash density and percent mineralization were not significantly different between lateral and medial ROIs ($p>0.10$). For axial and abaxial sites, mean QCT density, mean ash density, and mean percent mineralization of abaxial sites were significantly higher than axial sites ($p=0.02$, $p=0.01$, and $p=0.007$ respectively, Figs. 3A–C). No significant differences were found at each individual ROI site for mean QCT density, mean ash density, and percent mineralization.

Discussion

Determining the ability of quantitative computed tomography (QCT) to measure subchondral bone density in equine whole bone cadaver specimens is crucial in determining if QCT is a valid diagnostic modality for evaluating equine subchondral bone density in clinical diseases, such as osteoarthritis and subchondral bone disease. The current study evaluated the relationship of QCT subchondral bone mineral density to apparent ash density and percent mineralization.

Although there has been a report of good correlations with pre-cut QCT bone density values to apparent ash density [1], it was unknown if this relationship existed with equine subchondral bone. The overall correlation coefficient of ash density to QCT subchondral bone density in this study was high, and fell within the range of previously reported correlations between ash density and cortical or trabecular bone [2]. Subchondral bone density measured using three-dimensional QCT models appears to correlate strongly with ash density and percent mineralization even in areas with significantly different densities.

Abaxial regions of interest (ROIs) consistently had higher values of QCT subchondral bone density, ash density and percent mineralization compared to axial ROIs. This trend is in accordance with natural density variance found in an incongruously loaded joint [13]. The variability in QCT measurements between axial and abaxial sites could be affected by beam hardening. However, this is unlikely due to the same decreased ash density observed in the axial ROIs. More likely it is due to naturally occurring density variations of the subchondral bone in horses, which may be further influenced by individual horse factors such as age or exercise level. Site specific alterations in equine subchondral bone density have been demonstrated in the proximal phalanx [17], but not in the distal third metacarpal bone. This is most likely due to the difficult nature of imaging the curved surface of the distal third metacarpal bone, hence the need to validate a new method of measuring subchondral bone density with QCT.

Significant correlations were not found in individual ROI axial sites. This may be due to small sample size and/or high variability in axial QCT density measurements. One limitation of this study in general was the relatively small number of samples. However, the impact of small sample size is greater for comparing individual regions of density. By pooling the samples for an overall correlation, the power was increased and the main objective of this study was fulfilled. Further study of the variability in axial and abaxial subchondral density would be useful to determine if this is a trend indicative of early joint disease in horses.

Overall, QCT density is accurate in measuring subchondral bone density in the equine third metacarpal condyle from three-dimensionally created models when compared with ash density, which is considered the gold standard for measuring bone mineral content.

Acknowledgments

Supported by the Colorado State University College Research Council. The authors are grateful to Billie Arceneaux for performing the CT scans, Carola Pechey for performing the ash analysis, and Dr. Phillip Chapman for assistance with the statistical analysis.

References

[1] Ciarelli MJ, Goldstein SA, Kuhn JL, Cody DD, Brown MB. Evaluation of orthogonal mechanical properties and density of human trabecular bone from the major metaphyseal regions with materials testing and computed tomography. J Orthop Res 1991;9:674–82.

[2] Hayes WC, Piazza SJ, Zysset PK. Biomechanics of fracture risk prediction of the hip and spine by quantitative computed tomography. Radiol Clin North Am 1991;29: 1–18.

[3] Takada M, Engelke K, Hagiwara S, Grampp S, Genant HK. Accuracy and precision study in vitro for peripheral quantitative computed tomography. Osteoporos Int 1997;6:207–12.

[4] Kaneko TS, Bell JS, Pejcic MR, Tehranzadeh J, Keyak JH. Mechanical properties, density and quantitative CT scan data of trabecular bone with and without metastases. J Biomech 2004;37:523–30.

[5] Schneider S, Breit SM, Grampp S, Künzel WW, Liesegang A, Mayrhofer E, Zentek J. Comparative assessment of bone mineral measurements obtained by use of dual-energy X-ray absorptiometry, peripheral quantitative computed tomography, and chemical–physical analyses in femurs of juvenile and adult dogs. Am J Vet Res 2004;65:891–900.

[6] Glüer CC, Reiser UJ, Davis CA, Rutt BK, Genant HK. Vertebral mineral determination by quantitative computed tomography (QCT): accuracy of single and dual energy measurements. J Comput Assist Tomogr 1988;12:242–58.

[7] Curry TS, Dowdey JE, Murry RC. Christensen's physics of diagnostic radiology. 4th ed. Philadelphia: Lea & Febiger; 1990. p. 289–322.

[8] Langton CM, Njeh CF. The physical measurement of bone. Bristol and Philadelphia: Institute of Physics Publishing; 2004.

[9] Snyder MS, Schneider E. Estimation of mechanical properties of cortical bone by computed tomography. J Orthop Res 1991;9:422–31.

[10] Les CM, Keyak JH, Stover SM, Taylor KT, Kaneps AJ. Estimation of material properties in the equine metacarpus with use of quantitative computed tomography. J Orthop Res 1994;12:822–33.

[11] Waite K, Nielsen BD, Rosenstein DS. Computed tomography as a method of estimating bone mineral content in horses. J Equine Vet Sci 2000;20:49–52.

[12] Markel MD, Morin RL, Wikenheiser MA, Robb RA, Chao EY. Multiplanar quantitative computed tomography for bone mineral analysis in dogs. Am J Vet Res 1991;52:1479–83.

[13] Muller-Gerbl M. The subchondral bone plate. Adv Anat Embryol Cell Biol 1998;141: III–XI.

[14] Kawcak CE, McIlwraith CW, Norrdin RW, Park RD, Steyn PS. Clinical effects of exercise on subchondral bone of carpal and metacarpophalangeal joints in horses. Am J Vet Res 2000;61:1252–8.

[15] Les CM, Whalen RT, Beaupré GS, Yan CH, Cleek TM, Wills JS. The X-ray attenuation characteristics and density of human calcaneal marrow do not change significantly during adulthood. J Orthop Res 2002;20:633–41.

[16] Maule M, Gerhards H. Densitometrical study of the distal thoracic limb of the horse by means of quantitative computed tomography. Pferdeheilkunde 2004;20:153–8.

[17] van der Harst MR, DeGroot J, Kiers GH, Brama PA, van de Lest CH, van Weeren PR. Biochemical analysis of the articular cartilage and subchondral and trabecular bone of the metacarpophalangeal joint of horses with early osteoarthritis. Am J Vet Res 2005;66:1238–46.