

# Evaluation of subchondral bone mineral density associated with articular cartilage structure and integrity in healthy equine joints with different functional demands

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**Objective**—To determine and correlate subchondral bone mineral density and overlying cartilage structure and tensile integrity in mature healthy equine stifle (low magnitude loading) and metacarpophalangeal (high magnitude loading) joints.

**Animals**—8 healthy horses, 2 to 3 years of age.

**Procedure**—Osteochondral samples were acquired from the medial femoral condyle (FC) and medial trochlear ridge (TR) of the stifle joint and from the dorsal (MC3D) and palmar (MC3P) aspects of the distal medial third metacarpal condyles of the metacarpophalangeal joint. Articular cartilage surface fibrillation (evaluated via India ink staining) and tensile biomechanical properties were determined. The volumetric bone mineral density (vBMD) of the underlying subchondral plate was assessed via dual-energy x-ray absorptiometry.

**Results**—Cartilage staining (fibrillation), tensile moduli, tensile strength, and vBMD were greater in the MC3D and MC3P locations, compared with the FC and TR locations, whereas tensile strain at failure was less in MC3D and MC3P locations than FC and TR locations. Cartilage tensile moduli correlated positively with vBMD, whereas cartilage staining and tensile strain at failure correlated negatively with vBMD.

**Conclusions and Clinical Relevance**—In areas of high joint loading, the subchondral bone had high vBMD and the articular cartilage surface layer had high tensile stiffness but signs of structural wear (fibrillation and low failure strain). The site-dependent variations and relationships in this study support the concept that articular cartilage and subchondral bone normally adapt to physiologic loading in a coordinated way. (*Am J Vet Res* 2005;66:1823–1829)

Articular cartilage and underlying subchondral bone play important and coordinated functional roles in joints. Normal articular cartilage transmits joint loads to the underlying subchondral bone. As in other tis-

ues, the functions of articular cartilage and subchondral bone are related to their structure and composition.<sup>1-3</sup> Pathologic changes in structure and function of cartilage and bone can occur in response to abnormal mechanical loading or in disease.<sup>2,5</sup> The pathologic changes of articular cartilage associated with aging, but preceding osteoarthritis, may include articular surface wear as evidenced by India ink staining<sup>5,6</sup> and biomechanical weakening as determined by tensile tests.<sup>2,7,a</sup> Common changes in subchondral bone include alterations in structural-material stiffness<sup>4,8</sup> and **bone mineral density (BMD)**,<sup>9</sup> determined, for example, by dual-energy x-ray absorptiometry.<sup>10</sup> Coordinated changes in cartilage and bone have been hypothesized<sup>4,11-13</sup> to be related, with osteoarthritic cartilage degeneration being associated with increased BMD.<sup>14</sup> The interactive mechanisms by which bone changes may affect cartilage, and vice versa, remain a subject of intensive study. Less studied is the degree to which alterations in cartilage and bone may be coordinated as a normal adaptation to imposed mechanical demands and may serve as a precursor to disease.

Horses have been used as an animal model to study exercise-related effects on the musculoskeletal system, in addition to cartilage degeneration, repair, and regeneration.<sup>15-18</sup> Equine joints offer large articular surfaces for investigation, and articular cartilage thickness and loading magnitudes are similar to those in humans.<sup>19</sup> The equine stifle and **metacarpophalangeal (MCP)** joints have been analyzed in a number of investigations and offer contrasts with respect to normal physiologic loading.

The equine stifle joint has been used to study articular cartilage repair<sup>17,18</sup> because of its biomechanical stability, accessibility, and cartilage thickness, which is similar to that in the human knee.<sup>19</sup> In the stifle joint, the femorotibial articulation has relatively large contact areas subjected to compressive loading, whereas the

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femoropatellar articulation has smaller contact areas, and the trochlea is subjected to intermittent loading.<sup>20</sup> However, the exact load magnitudes applied within the equine stifle joint remain unknown. The medial femoral condyle (FC) of the femorotibial joint and the trochlear ridge (TR) of the femoropatellar joint are often used for studies of cartilage regeneration (Figure 1).<sup>17,18</sup>

In contrast, the MCP joint is frequently affected by osteoarthritic changes, with articular cartilage degeneration accompanied by subchondral bone sclerosis.<sup>21-23</sup> The MCP is a composite hinge joint composed of the distal portion of the third metacarpal (MC3), proximal portion of the first phalanx, and proximal sesamoid bones. During locomotion and stance, the distal surfaces of the MC3 have different applied loading magnitudes (Figure 1). For instance, the sesamoid bones place greater loads on the palmar (MC3P) surfaces of the MC3, compared with those applied on the dorsal (MC3D) surface by the first phalanx.<sup>24-27</sup> During walking, the sesamoid bones place 35% more load on the MC3P surfaces of the MC3, compared with those applied on the MC3D surface by the first phalanx.<sup>25</sup> The differences in physiologic loading between the palmar and dorsal aspects of the MC3 have been proposed to be related to the higher incidence of osteochondral degeneration on the palmar aspect.<sup>21-23</sup>

The MCP and stifle joints have different physiologic demands. Although objective comparisons between regions of the MCP and stifle joints remain unknown, the applied stresses are likely greater within the MCP, as indicated by kinematic,<sup>26,28</sup> ground reaction force,<sup>26,28</sup> and structural differences.<sup>20</sup> Gait kinematic data indicate that the MCP joint acts as the primary shock absorber, whereas the joints of the hind limb (stifle, tarsal, and MCP) work in conjunction to absorb forces.<sup>26</sup> In addition, the forelimb ground reaction forces (2 to 3 times body weight<sup>25,26</sup>) are 33% larger<sup>28</sup> over a smaller cartilage surface area<sup>20</sup> relative to the stifle joint; therefore, the

stresses acting on the MCP (30 to 35 MPa<sup>29</sup>) are estimated to be larger than the stresses acting on the stifle joint. The different physiologic demands of these joints provide an opportunity to determine whether normal cartilage and bone have coordinated variations in composition, structure, and function that may ultimately progress toward osteoarthritic disease.

Previous studies of the relationship between properties of articular cartilage and subchondral bone caused by weight bearing,<sup>30,31</sup> abnormal mechanical loading,<sup>4</sup> and disease<sup>13,32</sup> have used histologic analysis and determination of BMD.<sup>9</sup> Although the exact mechanisms by which cartilage and bone may interact continue to be a mystery, little is known about the structural-mechanical alterations in cartilage and bone that occur in normal adaptation. Associations between cartilage and bone structural-mechanical properties in normal adaptation may provide some insight to precursors to disease. The objectives of the study reported here were to determine the structure and biomechanical properties of the surface region of articular cartilage as well as the subchondral volumetric BMD (vBMD) of healthy mature equine stifle and MCP joints from the same horse and to examine by correlative analysis whether vBMD and cartilage properties are related.

## Materials and Methods

**Tissue acquisition**—Tissue specimens were harvested from 8 horses (2 male and 6 female; 2 to 3 years old; mean  $\pm$  SD weight, 325  $\pm$  43 kg) as part of a study approved by the institutional animal care and use committee. All horses were clinically normal as assessed by routine physical examination including lameness evaluation. Within 4 hours postmortem, bilateral stifle joint and MC3 osteochondral blocks were confirmed to be normal by gross examination of articular surfaces, articular margins, ridges, and synovial membranes, noting the absence of osteophytes. All cartilage surfaces were kept moist during sample preparation with sterile PBS solution with proteinase inhibitors.<sup>33</sup> Osteochondral samples

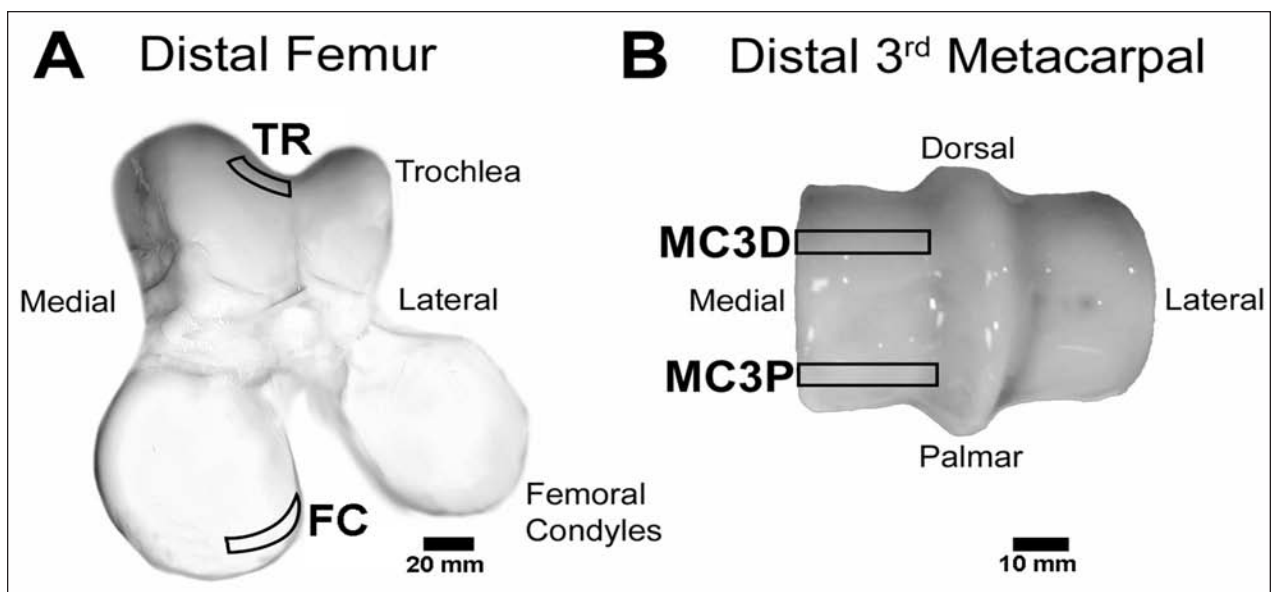


Figure 1—Photographs of the articular surfaces of (A) the distal aspect of the femur and (B) the distal aspect of the third metacarpal bone in a horse. In the femur, specimens were obtained from the medial trochlear ridge (TR) and medial femoral condyle (FC). In the third metacarpal bone, specimens were obtained from the mediiodorsal (MC3D) and mediopalmar (MC3P) aspects of the articular surface.

(10 × 10 × 10 mm<sup>3</sup>), including the articular surface, were prepared by use of a diamond-coated bandsaw<sup>b</sup> with PBS solution as a cooling and hydrating fluid. Samples were obtained from the medial FC and medial TR of the stifle joint (Figure 1) and from the dorsal and palmar aspects of the MC3 condyles, 1 cm dorsal (MC3D) and palmar (MC3P) from the transverse ridge. All samples were stored in gauze soaked in PBS solution with proteinase inhibitors at -20°C until testing.

**Cartilage structure**—India ink staining was used to assess articular surface alterations, and video imaging was used to assess thickness of the articular cartilage, as described.<sup>6</sup> Briefly, India ink staining of the articular surface was quantified as a reflectance score by calibrated digital imaging with gray-scale reference standards, with a value near 1 indicating high reflectance (little ink staining, normal surface) and a value near 0 indicating low reflectance (substantial ink staining, roughened surface). Articular cartilage thickness was measured by microphotography (field of view, 33.6 × 44.8 mm; 28 μm/pixel), with an intrasample variability (coefficient of variation) of approximately 9%.

**Cartilage tensile integrity**—The tensile integrity of the articular cartilage surface layer was determined by biomechanical analysis, essentially as described.<sup>34</sup> Briefly, a thin cartilage section from each specimen, including the intact articular cartilage surface, was sectioned on a microtome. From each section, a tensile specimen (gauge length, 4 mm; gauge width, 0.8 mm) was obtained with the long axis perpendicular to joint motion (Figure 1). By use of a contact-sensing micrometer, tensile specimen thickness was measured (0.33 ± 0.09 mm, 0.40 ± 0.11 mm, 0.39 ± 0.08 mm, and 0.39 ± 0.09 mm for the FC, TR, MC3P, and MC3D locations, respectively). Briefly, a tare load of 0.05 N (0.2 MPa) was applied, followed by a constant displacement (0.25%/s) to a nominal strain of 10% and 20%, while stress relaxation was monitored at each strain level until equilibrium. Then the samples were extended (5 mm/min) until failure. Equilibrium modulus was determined as the slope of the equilibrium stress-strain data by use of linear regression. Dynamic modulus was calculated as the slope of the stress-strain curve from 25% to 75% of failure strain. Strength and strain at failure were the maximum stress and strain at failure, respectively. From a clinical perspective, a decrease in tensile modulus diminishes the ability of articular cartilage to resist indentation type loads at the articular surface,<sup>35</sup> which would be expected during joint loading. In contrast, a decline in tensile strength indicates a reduction in the threshold for cartilage damage associated with joint trauma. All testing was performed with continuous irrigation of PBS solution at 22°C.

**Subchondral BMD**—vBMD (g/cm<sup>3</sup>) of the subchondral bone region was determined as described.<sup>10</sup> The areal BMD (aBMD [g/cm<sup>2</sup>]; scan area perpendicular to the articular surface, 5.2 × 5.2 mm<sup>2</sup>) was assessed through the thickness of each specimen (approx 3 mm) by use of a dual-energy x-ray absorptiometry scanner.<sup>c</sup> The aBMD encompassed all radio dense subchondral tissues, including calcified cartilage, subchondral bone plate, and subchondral trabecular bone. Mean sample thickness was determined along the scan plane with a micrometer.<sup>d</sup> Subchondral vBMD was determined as the aBMD (g/cm<sup>2</sup>) divided by the sample thickness (cm). The intrasample variability (coefficient of variation) was < 1% for aBMD and < 5% for vBMD.

**Statistical analyses**—The effects of sample location (FC, TR, MC3P, and MC3D) and side (right vs left) on cartilage properties and subchondral vBMD were determined via repeated-measures ANOVA with location and side as repeated factors for each horse.<sup>36,e</sup> When significant effects were detected, Tukey post hoc comparisons were performed to determine differences between levels of effect. Side was not found to be a significant factor (*P* > 0.4 for all responses). Tensile equilibrium modulus, tensile dynamic modulus, strength, and strain at failure responses required a square-root transformation, whereas cartilage thickness responses were log transformed to satisfy normal distribution assumptions. Linear regressions were performed to determine whether variations of the cartilage responses were correlated with underlying subchondral vBMD. For all comparisons, *P* < 0.05 was considered significant.

## Results

**Cartilage structure**—Cartilage thickness varied significantly (*P* < 0.001) between sample locations (Table 1). The FC had significantly (*P* < 0.001) thicker cartilage than all other locations. The TR had thicker cartilage than the MC3 locations, which in turn were not significantly different (MC3P vs MC3D; *P* = 0.7).

The extent of India ink staining (reflectance score) varied with location (*P* < 0.05; Table 1). Ink staining was generally greater (lower reflectance scores) in the MC3 locations than in stifle joint locations (less fibrillation; *P* = 0.01). Within joints, there was no significant difference in reflectance scores between FC and TR (*P* = 0.2) or between MC3P and MC3D (*P* = 0.5). Qualitatively, after staining, mild wear lines oriented in the cranio-caudal direction were visible grossly on 25%

Table 1—Summary of normal equine cartilage and subchondral bone properties (mean ± SD) for the medial femoral condyle (FC) and medial trochlear ridge (TR) of the femur and the dorsal (MC3D) and palmar (MC3P) aspects of the distal condyles of the third metacarpal bone in horses.

| Variable   | Femur                       |                             | Third metacarpal          |                             |
|--|-----------------------------|-----------------------------|---------------------------|-----------------------------|
|  | FC                          | TR                          | MC3D                      | MC3P                        |
| <b>Cartilage</b>                                     |                             |                             |                           |                             |
| Cartilage thickness (mm)                             | 2.20 ± 0.33 <sup>*a</sup>   | 1.48 ± 0.13 <sup>†b</sup>   | 0.58 ± 0.11 <sup>*c</sup> | 0.67 ± 0.11 <sup>*c</sup>   |
| Reflectance score                                    | 0.90 ± 0.08 <sup>*a,b</sup> | 0.97 ± 0.06 <sup>†a</sup>   | 0.87 ± 0.11 <sup>*b</sup> | 0.84 ± 0.10 <sup>*b</sup>   |
| Equilibrium modulus (MPa)                            | 4.8 ± 2.9 <sup>†a</sup>     | 7.7 ± 4.2 <sup>†a</sup>     | 17.9 ± 7.9 <sup>†b</sup>  | 13.0 ± 5.6 <sup>†b</sup>    |
| Dynamic modulus (MPa)                                | 20.0 ± 6.8 <sup>†a</sup>    | 31.7 ± 12.4 <sup>†b</sup>   | 44.3 ± 16.4 <sup>†c</sup> | 36.9 ± 8.5 <sup>†c</sup>    |
| Strength (MPa)                                       | 6.7 ± 2.2 <sup>†a</sup>     | 10.7 ± 3.2 <sup>†b</sup>    | 9.9 ± 2.9 <sup>†b</sup>   | 10.6 ± 4.2 <sup>†b</sup>    |
| Strain at failure (μm/μm)                            | 0.68 ± 0.05 <sup>†a-c</sup> | 0.71 ± 0.08 <sup>†b-c</sup> | 0.60 ± 0.09 <sup>†b</sup> | 0.63 ± 0.13 <sup>†b-c</sup> |
| <b>Bone</b>  |                             |                             |                           |                             |
| Volumetric bone mineral density (g/cm <sup>3</sup> ) | 0.78 ± 0.16 <sup>†a</sup>   | 0.62 ± 0.16 <sup>†b</sup>   | 0.94 ± 0.14 <sup>*c</sup> | 1.14 ± 0.10 <sup>*d</sup>   |

\*n = 14. †n = 13. ‡n = 15. §n = 16.  
<sup>a-d</sup> Within a row, values with different superscript letters are significantly (*P* < 0.05) different.

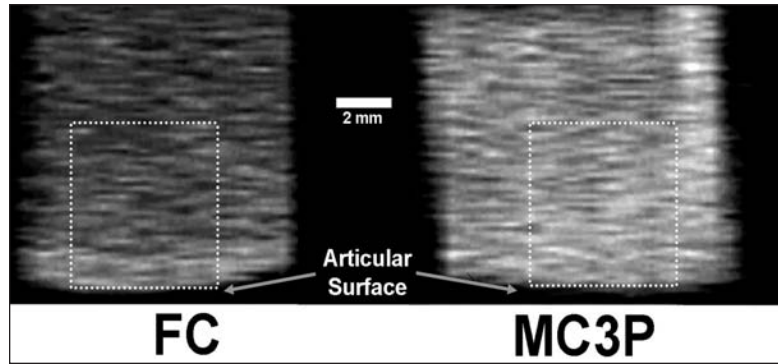


Figure 2—Dual-energy x-ray absorptiometric images of a portion of the medial FC of a femur and a portion of the MC3P of the MC3 from a horse. Dashed lines indicate region of analysis. Bone mineral density is indicated by intensity (whiteness) of the images.

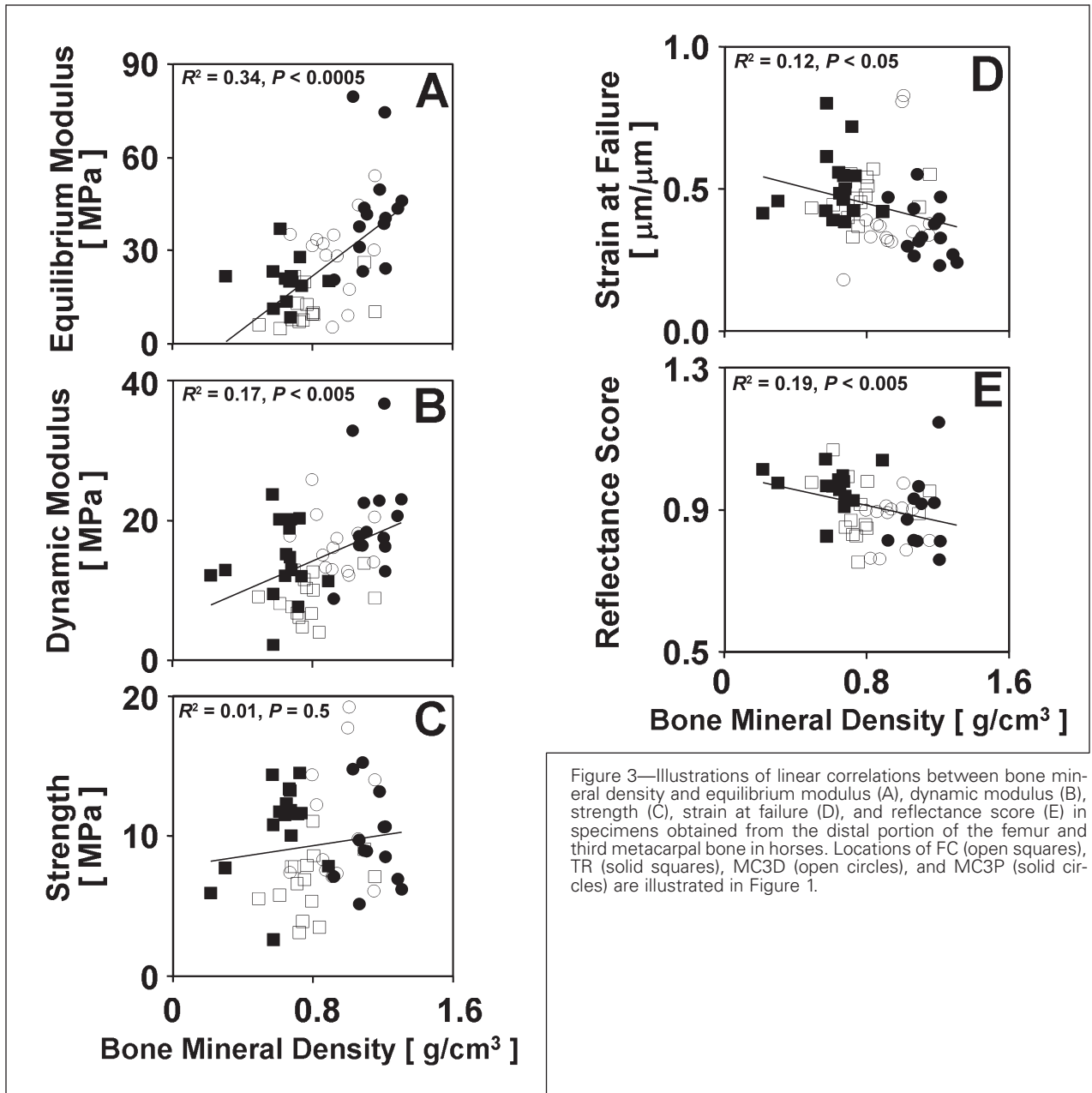


Figure 3—Illustrations of linear correlations between bone mineral density and equilibrium modulus (A), dynamic modulus (B), strength (C), strain at failure (D), and reflectance score (E) in specimens obtained from the distal portion of the femur and third metacarpal bone in horses. Locations of FC (open squares), TR (solid squares), MC3D (open circles), and MC3P (solid circles) are illustrated in Figure 1.



of the MC3D and MC3P articular surfaces but were not evident on the FC and TR surfaces.

**Cartilage tensile properties**—Each of the cartilage tensile properties varied with sample location ( $P = 0.01$ ; Table 1). The tensile equilibrium moduli of the MC3P and MC3D were significantly greater than those of the FC and TR locations, whereas sample locations within the MC3 and stifle joint regions were not significantly different ( $P = 0.2$ ). Tensile dynamic modulus had similar differences (MC3 > stifle joint [ $P < 0.05$ ]). Additionally, FC had a significantly smaller tensile dynamic modulus, compared with all other regions, whereas tensile dynamic moduli were not significantly different between MC3D and MC3P ( $P = 0.12$ ). The tensile strength was smaller in the FC than all other locations ( $P = 0.01$ ) and not significantly different among TR, MC3P, and MC3D ( $P = 0.7$ ). Strain at failure was significantly lower in the MC3P than the TR.

**Subchondral vBMD**—The vBMD varied with location (Table 1;  $P = 0.005$ ). The MC3P location had the largest vBMD ( $P = 0.005$ ) and was 85% denser than the TR location. In addition, the MC3P had 21% greater vBMD than its dorsal counterpart ( $P = 0.005$ ). The FC region had a 26% greater vBMD than the TR location. Qualitatively, the MCP (MC3P and MC3D) had a thicker subchondral bone plate, compared with stifle joint locations (FC and TR; Figure 2).

**Associations of subchondral vBMD with articular cartilage structure and integrity**—The tensile equilibrium modulus and dynamic modulus had a significant positive correlation with vBMD across locations ( $P < 0.001$  and  $P < 0.01$ , respectively; Figure 3). Tensile strength ( $P = 0.5$ ) and cartilage thickness ( $P = 0.1$ ) variations had nonsignificant correlations with underlying vBMD. Failure strain and reflectance score ( $P = 0.005$ ) variations had negative correlations with vBMD across locations ( $P < 0.05$  and  $P = 0.01$ , respectively).

## Discussion

Results of this study provide evidence for coordinated variations in the articular cartilage surface and the subchondral region of 4 joint regions in adult horses that are subject naturally to different types of stresses *in vivo*. Results indicated that in joint regions subject to large stresses and with high subchondral vBMD, the articular cartilage surface was stiff in tension, but also had signs of early degenerative changes with relatively low strain at failure and structural wear (decreased reflectance score with increased India ink staining). The MCP (MC3D and MC3P) had relatively stiff cartilage along with relatively high subchondral vBMD, whereas the stifle joint (FC and TR) had soft cartilage with relatively low subchondral vBMD.

Results may have been affected by the characteristics of the horses studied as well as the details of the experimental methods that were used. Age, sex, weight, exercise, and diet are all potential sources of

variability.<sup>19</sup> The 2 male and 6 female horses were 2 to 3 years old, similar in weight, and had the same exercise regimen and diet for 12 weeks before euthanasia. However, their diet and exercise histories prior to inclusion in the study were unknown. Another possible source of variability was the tensile sample preparation.<sup>37</sup> The methodology assured that all tensile samples were prepared perpendicular to the direction of joint motion, which is likely coincident with split-line direction.<sup>38,39</sup> Lastly, the vBMD analysis region included calcified cartilage, subchondral plate, and subchondral trabecular bone. The subchondral bone plate likely dominated the vBMD values because the subchondral plate is thicker than calcified cartilage and volumetrically denser than subchondral trabecular bone. Therefore, differences in composition, structure, and function of calcified cartilage, subchondral bone plate, and subchondral trabecular bone were not analyzed.<sup>9</sup> Additional mechanical properties of articular cartilage (eg, compression and shear properties) may be useful to characterize because articular cartilage is subjected to a combination of tensile, compressive, and shear loads. Although compressive properties were not measured in the present study, compressive properties (eg, in a confined configuration) of articular cartilage are relatively insensitive to degenerative changes of articular cartilage.<sup>40</sup>

The characteristics of the cartilage surface were consistent with those detected in previous studies and extended the analysis to a variety of joints not studied previously. The cartilage reflectance scores were generally consistent with those found for young normal human FCs<sup>6</sup> and the distal aspect of the MC3 bones.<sup>41</sup> The equilibrium moduli of the TR and FC cartilage (approx 4 to 5 and 20 MPa, respectively) were comparable to those of young adult bovine,<sup>34</sup> canine,<sup>42</sup> and human cartilage<sup>2,43</sup> from the same regions. The pattern of greater tensile strength and equilibrium moduli of FC, compared with TR, has also been detected in bovine and fibrillated human cartilage,<sup>2,34</sup> but the opposite pattern (FC < TR) was detected in normal human adult cartilage.<sup>2</sup> A full understanding of how equine and human cartilages differ remains to be determined. The pattern in adult humans may be related to the age-related wear that affects the weight-bearing FC at an earlier age than the TR.

The MC3 subchondral vBMD variations (MC3D and MC3P) were similar to those previously found for the same locations.<sup>10</sup> The vBMD variations between the distal MC3 locations were similar to those found previously,<sup>10</sup> (0.9 g/cm<sup>3</sup>, after correction of units). The subchondral vBMD values were also similar to the vBMD values found for the human femoral head<sup>9</sup> and FCs.<sup>32</sup>

Like subchondral vBMD, cartilage surface variations are likely related to stress that occurred locally in the joint. The structure and composition of subchondral bone are indicative of its stress history.<sup>44</sup> Similarly, cartilage composition<sup>34,45,46</sup> and function<sup>35</sup> respond to changes in physiologic loading as well. Therefore, it seems likely that the relatively high stress on the MCP caused greater

subchondral vBMD and cartilage tensile stiffness in MC3D and MC3P locations, compared with the FC and TR of the stifle joint. Therefore, it is reasonable to believe the MCP's greater joint stresses caused greater subchondral vBMD and cartilage stiffness in MC3D and MC3P locations, compared with the stifle joint's FC and TR. In addition, the greater subchondral vBMD and stiffer cartilage surface for MC3P coincided with topographic stress differences on the distal aspect of the MC3 (MC3P > MC3D).<sup>25</sup> The relatively high frequency of osteoarthritic changes in the MC3 condyles<sup>21,47,48</sup> may be related to early-stage cartilage surface wear and brittleness, even in apparently normal joints of young adult horses.

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