Lameness in horses is a common cause of lost training days and early retirement. The most common cause of lameness is joint disease, which has been estimated to be the cause of lameness in 60% of cases.1 There are several methods of treating and managing joint disease, and use of a particular treatment can be dictated by its site of action. Specifically, a treatment’s effect on bone, articular cartilage, or soft tissues can have an effect on how that treatment is used in a clinical setting. Therefore, it is important to determine the tissues that respond favorably or negatively to a specific treatment.

Subchondral bone is a potential site of action for treatment. Subchondral bone is commonly involved in joint disease and progression of sclerosis, and ischemia of SCB can lead to joint disease.2,4 Changes in the SCB can lead to osteochondral fragmentation, osteoarthritis, catastrophic injury, and osteoarthritis.2 Any modification of these processes may have a positive impact on disease progression, allowing for management of lameness.

Extracorporeal shock wave therapy has also been used to treat horses with osteoarthritis.5,6 A report involving a small number of clinical cases indicated that ESWT can be safe to use for management of osteoarthritis in horses. However, it was impossible to determine the types of cases that were effectively treated with ESWT or that did not respond.6 In a larger study, Kawcak et al7 evaluated the effects of extracorporeal shock wave therapy and polysulfated glycosaminoglycan treatment on subchondral bone, serum biomarkers, and synovial fluid biomarkers in horses with induced osteoarthritis.

Effects of extracorporeal shock wave therapy and polysulfated glycosaminoglycan treatment on subchondral bone, serum biomarkers, and synovial fluid biomarkers in horses with induced osteoarthritis

Chris E. Kawcak, DVM, PhD; David D. Frisbie, DVM, PhD; C. Wayne McIlwraith, BVSc, PhD, DSc

Objective—To evaluate effects of extracorporeal shock wave therapy (ESWT) and polysulfated glycosaminoglycan treatment (PSGAGT) on subchondral bone (SCB), serum biomarkers, and synovial fluid biomarkers in horses with induced osteoarthritis.

Animals—24 healthy 2- to 3-year-old horses.

Procedures—An osteochondral fragment was created on the distal aspect of the radial carpal bone in 1 middle carpal joint of each horse. Horses were randomly allocated to receive local application of ESWT (days 14 and 28; n = 8), PSGAGT (IM, q 4 d for 28 days; 8), or a sham ESWT probe (placebo; days 14 and 28; 8). Serum biomarkers were measured every 7 days, and synovial fluid biomarkers were measured every 14 days. Bone density was measured by use of computed tomography on days 0 and 70, and microdamage and bone formation variables were compared among groups at the end of the study (day 70).

Results—There was no significant effect of ESWT or PSGAGT on any bone variable. Serum osteocalcin concentration was significantly greater in horses that received ESWT, compared with placebo-treated horses, and serum concentration of the C-terminal telopeptide of type I collagen was significantly higher in horses that received ESWT, compared with placebo- and PSGAG-treated horses. Concentrations of the synovial fluid epitope CS846 were significantly higher in joints with osteoarthritis treated with ESWT.

Conclusions and Clinical Relevance—Treatment of osteoarthritis with ESWT had no effect on SCB but did induce increases in serum biomarkers indicative of bone remodeling. Treatment of osteoarthritis with PSGAG had no effect on SCB or biomarkers. (Am J Vet Res 2011;72:772–779)
er clinical study, McCarroll and McClure\(^7\) reported that 80% of 74 horses with osteoarthritis of the tarsometatarsal and distal intertarsal joints were improved by at least 1 lameness grade 90 days after treatment. There were no consistent changes on radiographs, although the authors reported that they felt subjectively that horses with osteophytes appeared to improve consistently after treatment.\(^7\) McCarroll and Weinberger\(^7\) also noted that ESWT can be beneficial for treating osteoarthritis of the distal intertarsal and tarsometatarsal joints when used in conjunction with intra-articular administration of medication. These subjective clinical reports warrant objective experimental studies on the effects of ESWT.

The effects of ESWT on bone healing have been studied extensively with mixed results. Results of multiple clinical studies\(^8\)–\(^10\) indicate that ESWT increases the ability to heal nonunion and malunion fractures in cortical bone. However, the exact mechanism that induces this response is unknown, although a recent study\(^11\) revealed upregulation of mesenchymal stem cells, vascular endothelial growth factor, and transforming growth factor-\(\beta\) at the fracture sites. A study\(^12\) in which the acute effects of ESWT were evaluated in rabbits found that cavitation induced substantial hemorrhage and trabecular displacement and subperiosteal hemorrhage, leading to substantial appositional cortical bone formation but poor trabecular remodeling. However, in mature cortical bone, researchers found little appositional cortical bone growth and delayed healing at a tibial defect in sheep.\(^13\) Therefore, the effect appears to be site specific within the bone. Frisbie et al\(^14\) have also detected clinical sign–modifying effects of ESWT in an osteochondral fragment model of osteoarthritis in horses.

Polysulfated GAG treatment has been used IM in horses for years and, in a survey of practitioners, was thought to be moderately effective for treating joint disease.\(^15\) However, there is no protective effect of PSGAGT for joints treated by injection with methylprednisolone acetate.\(^16\) Todhunter et al\(^17\) detected variable effects of intra-articular PSGAGT and exercise on scintigraphically observed bone remodeling in joints with articular cartilage defects. They noted that there was an effect of treatment on the noninjected joint and concluded that systemic PSGAGT could have an effect on bone remodeling. Therefore, further work is needed to determine the effects of systemic PSGAGT on bone.

Considering the widespread use of ESWT and PSGAGT for equine joint disease, there is a need to objectively determine in an experimental setting the effects of each in horses. A previous report\(^18\) of data from the experimental study reported here noted substantial clinical sign–modifying effects from ESWT. The purpose of the study reported here was to further elucidate the mode of action of ESWT by determining its effects in horses with surgically induced osteoarthritis, specifically assessing the response of SCB and biochemical markers of bone and articular cartilage metabolism.

**Materials and Methods**

**Osteochondral fragment model**—Following approval of the Colorado State University Animal Care and Use Committee, 24 healthy 2- to 3-year-old mixed-breed male and female horses were used in this study. Prior to being admitted into the study, assessment of body condition scores, carpal radiography, lameness examination, assessment of response to carpal flexion, and assessment of synovial effusion were performed to ensure all measured variables were within reference ranges. The Colorado State University osteochondral fragment model was used to evaluate the efficacy of PSGAGT\(^a\) and ESWT.\(^18\)–\(^21\) The horses were randomly assigned to PSGAGT, ESWT, or placebo (control) groups. On day 0, following routine preparation for surgery, all horses underwent bilateral arthroscopic surgery of the middle carpal joints to ensure there were no preexisting abnormalities. During this procedure, an osteochondral fragment was created in 1 randomly selected middle carpal joint of each horse (osteoarthritic joint). This was accomplished by use of an 8-mm curved osteotome directed perpendicular to the articular cartilage surface of the distal aspect of the radial carpal bone at the level of the medial synovial plica. The edges of the osteotome were visually verified to be in line with the dorsal edge of the bone prior to creation of the fragment. The fragment was allowed to remain adherent to the joint capsule proximally. A motorized arthroburr was used to debride the exposed SCB of the parent bone. A 15-mm-wide defect bed for the 8-mm-wide fragment, measured visually during each surgery with a 7-mm-wide arthroscopy probe, was created, and the debris was not actively flushed from the joint, thus inducing osteoarthritis. The opposite joint (control joint) received a sham operation. The arthroscopic portals were closed by use of 2-0 nylon suture in a simple interrupted pattern. The limbs were bandaged, and the horses were allowed to recover from general anesthesia. Bandages were changed every 3 to 5 days and maintained for 2 weeks. Sutures were removed 10 days after surgery.

Each horse was housed in a 3.65 × 3.65-m stall unless otherwise noted. Beginning on day 14, all horses were exercised on a high-speed treadmill 5 d/wk until the end of the study at day 70. Each day, the horses were subjected to a 2-minute trot (16 to 19 km/h) and 2-minute gallop (32 km/h), followed by a 2-minute trot to simulate the strenuous exercise of race training. Calcitonin (20 mg/kg, IV) was administered on days 59 and 70 to calculate mineral apposition rate and to determine the effects each treatment may have had on SCB remodeling. Oxytetracycline (25 mg/kg, IV) was also administered on days 28 and 35 to assess the same variables.

**Treatment**—Horses in the placebo group had a shield of bubble wrap placed over the scan head to block the shock waves. The same numbers of shock waves were administered, but the air in the bubble wrap effectively prevented transmission of the shock waves to the skin of the horse. Horses in the placebo and ESWT groups received 2,000 shock waves at an energy of 0.14 ml/mm\(^2\) on study day 14. Eight areas (4 areas on the proximal and 4 areas on the distal aspect of the capsule of the middle carpal joint) were exposed to 200 pulses each. The remaining 400 pulses were delivered over the area of the osteoarthritis fragment. On day 28, 1,500 shock waves at an energy of 0.15 ml/mm\(^2\) were delivered. Two hundred pulses were delivered to 6
areas (3 areas on the proximal aspect and 3 areas on the
distal aspect of the capsule of the middle carpal joint),
and 300 pulses were delivered over the area of the
osteoarthritis fragment. The probe head had a focal point
of 1.2 mm. Starting on day 0, PSGAG (300 mg, IM, q 4 d
for 28 days) was administered to horses in the PSGAGT
group. Confinement and exercise were not altered with

treatment.

Computed tomography—Computed tomographic
osteabsorptiometry was performed on both carpal
joints prior to surgery and again after euthanasia to
quantify density changes attributable to osteoarthritis
and treatment. Each forelimb was scanned in a com-
puted tomographic scanner at 1-mm-thick contiguous
slices. Each limb was scanned with a density phantom.
Scans were converted into milligram per milliliter den-
sity and rendered in 3-D for calculation of joint surface
density. Specifically, mean bone densities for the radio-
carpal bone and opposing radial facet of the third carpal
bone were calculated.

Histologic examination—Horses were humanely
euthanized according to Colorado State University In-
stitutional Animal Care and Use Committee protocols
on day 70. Bone remodeling and microdamage were
evaluated on nondecalcified sections obtained from
both joints of each horse. At necropsy, a 10-mm sagi-
tally directed osteochondral section was obtained from
the radial carpal bone and the apposing radial facet of
the third carpal bone, which corresponded to the
region of the third carpal bone, which corresponded to
the center of the section from each joint was evaluated
on day 0. PSGAG (300 mg, IM, q 4 d for 28 days) was administered to horses in the PSGAGT group. Confinement and exercise were not altered with
treatment.

Microdamage was evaluated on the distal dor-
sal aspect of the radial carpal bone in the area of the
osteocondral fragment, if present, to assess the ef-
fects of osteoarthritis and treatment on microdamage
in osteoarthritic and control joints. To clarify, ESWT ef-
fects would be present only on the osteoarthritic side,
whereas the effects of PSGAGT could occur on both the
osteoarthritic and control side because of the systemic
nature of PSGAG administration. This area was specifi-
cally identified as the distal dorsal quadrant of the entire
sagittal plane of the radial carpal bone. Four 120-µm
sections were each evaluated, and the data were
combined and reported for each bone. Bone area in
both the fragmented area (damaged zone, if present,
caused by the osteochondral fragment in osteoarthritic
joints) and nonfragmented area (nondamaged zone in
osteoarthritic and control joints) was calculated (per-
centage of bone by region), and the number of micro-
cracks (cracks/mm²) and their lengths (mm/mm²) were
normalized to the bone area.

Biochemical markers—Beginning on day 0 until
the end of the study (day 70), a blood sample was ob-
tained once per week from a jugular vein in each horse.
This was done in the morning prior to sedation, ex-
ercise, and anesthesia. Hepatic and renal values were
evaluated on day 0. Serum from the blood sample was
stored at –80°C for further analysis. A modified 1,9-
dimethylmethylene blue dye–binding assay was used
on papain-digested samples to determine GAG concen-
tration as a marker of cartilage matrix degradation.

Concentrations of the epitope Col CEQ were mea-
sured by use of an ELISA as a marker of type II collagen
degradation. A competitive ELISA was used to estimate
concentrations of the C1,2C, which provides a mea-
sure of type I and II collagen degradation. By subtracting the concentration of Col CEQ from the concentration of type I collagen, degradation could
indirectly be determined. Concentrations of the epitope
Description of the content: The document appears to be a scientific article discussing the results of a study involving horses. The article includes statistical analysis, results, and a table of mean values for bone density and bone turnover markers.

**Results**

**Computed tomography**—Radial carpal bone density pooled over all treatment groups increased significantly (P < 0.001) over the duration of the study; however, there was no significant effect of osteoarthritis or osteoarthritis, treatment, or time on third carpal bone density.

**Histologic examination**—There was poor uptake of oxytetracycline in the bone; however, the reasons for this were unclear. Therefore, only the calcine labels, indicative of bone activity between days 39 and 70, were measured. There was no significant effect of osteoarthritis or treatment on percentage of bone, trabecular thickness, DLMS, mineral apposition rate, or area of diffuse labeling (Table 2). The effect of osteoarthritis on SLMS of calcine pooled over all treatment groups nearly reached significance (P = 0.051).

There was no significant effect of osteoarthritis on the percentage of radial carpal bone composed of the damaged zone, microdamage in the damaged zone and nondamaged zone, or length of microdamage in the damaged zone (Table 3). Increased length of microdamage nearly reached significance (P = 0.099) in the nondamaged zone of osteoarthritis joints, compared with control joints.

**Biochemical markers**—All data were log transformed to meet assumptions of normality. Concentrations of serum CS846 differed significantly over time but not by treatment or treatment-by-time interaction. Specifically, concentrations of CS846 decreased significantly (P < 0.001 on days 42 and 49). Concentrations of serum CPII decreased significantly over time but not by treatment or treatment-by-time interaction. Specifically, concentrations of CPII were increased significantly (P < 0.001) on days 28 and 70. Concentrations of serum GAG were increased significantly on days 14 and 35 (P = 0.007 and P = 0.006, respectively). A significant (P = 0.037) treatment-by-time interaction was detected for serum GAG (Figure 1). In both ESWT and PSGAGT groups, concentrations of serum GAG were decreased significantly (P = 0.02 and P = 0.025, respectively) on day 21, compared with day 14, and both were increased to baseline concentration on day 35. For se-

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**Table 1**—Mean ± SD bone density values (mg/mL) at various times in control horses (n = 8), horses that received ESWT (8), and horses that received PSGAGT (8), after surgical induction of osteoarthritis.

<table>
<thead>
<tr>
<th>Bone</th>
<th>Control</th>
<th>Control osteoarthritis</th>
<th>PSGAGT</th>
<th>PSGAGT osteoarthritis</th>
<th>ESWT</th>
<th>ESWT osteoarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial carpal bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.708 ± 0.066</td>
<td>0.702 ± 0.094</td>
<td>0.642 ± 0.080</td>
<td>0.657 ± 0.094</td>
<td>0.675 ± 0.050</td>
<td>0.685 ± 0.053</td>
</tr>
<tr>
<td>Day 30</td>
<td>0.740 ± 0.065</td>
<td>0.722 ± 0.045</td>
<td>0.684 ± 0.088</td>
<td>0.705 ± 0.083</td>
<td>0.713 ± 0.053</td>
<td>0.731 ± 0.034</td>
</tr>
<tr>
<td>Third carpal bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.731 ± 0.051</td>
<td>0.729 ± 0.037</td>
<td>0.672 ± 0.079</td>
<td>0.689 ± 0.106</td>
<td>0.691 ± 0.070</td>
<td>0.711 ± 0.080</td>
</tr>
<tr>
<td>Day 70</td>
<td>0.722 ± 0.061</td>
<td>0.712 ± 0.039</td>
<td>0.713 ± 0.104</td>
<td>0.663 ± 0.120</td>
<td>0.699 ± 0.071</td>
<td>0.688 ± 0.070</td>
</tr>
</tbody>
</table>

**Table 2**—Mean ± SD values for third carpal bone (proximal surface) variables in the same horses as in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Control osteoarthritis</th>
<th>PSGAGT</th>
<th>PSGAGT osteoarthritis</th>
<th>ESWT</th>
<th>ESWT osteoarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of bone</td>
<td>68.26 ± 11.10</td>
<td>65.31 ± 11.23</td>
<td>71.11 ± 15.83</td>
<td>63.31 ± 14.17</td>
<td>69.87 ± 14.19</td>
<td>62.60 ± 14.84</td>
</tr>
<tr>
<td>Trabecular thickness (mm)</td>
<td>0.404 ± 0.142</td>
<td>0.434 ± 0.109</td>
<td>0.396 ± 0.124</td>
<td>0.475 ± 0.286</td>
<td>0.470 ± 0.282</td>
<td>0.431 ± 0.240</td>
</tr>
<tr>
<td>SLMS (mm/mm)</td>
<td>0.253 ± 0.156</td>
<td>0.302 ± 0.179</td>
<td>0.353 ± 0.221</td>
<td>0.450 ± 0.270</td>
<td>0.409 ± 0.270</td>
<td>0.365 ± 0.305</td>
</tr>
<tr>
<td>DLMS (mm/mm)</td>
<td>0.043 ± 0.033</td>
<td>0.063 ± 0.035</td>
<td>0.160 ± 0.222</td>
<td>0.182 ± 0.252</td>
<td>0.171 ± 0.070</td>
<td>0.134 ± 0.109</td>
</tr>
<tr>
<td>Mineral apposition rate (mm/μd)</td>
<td>0.002 ± 0.003</td>
<td>0.003 ± 0.006</td>
<td>0.003 ± 0.003</td>
<td>0.002 ± 0.001</td>
<td>0.001 ± 0.001</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>Diffuse area (mm²/mm³)</td>
<td>0.010 ± 0.006</td>
<td>0.011 ± 0.005</td>
<td>0.017 ± 0.010</td>
<td>0.020 ± 0.010</td>
<td>0.011 ± 0.008</td>
<td>0.018 ± 0.008</td>
</tr>
</tbody>
</table>
rum Col CEQ, there was no significant effect of treatment or treatment-by-time interaction, but there was a significant ($P < 0.001$) effect of time, with Col CEQ concentrations highest at day 42. Concentrations of serum C1,2C also changed significantly over time but not by treatment or treatment-by-time interaction. Specifically, concentrations of serum C1,2C were increased significantly ($P < 0.001$) on day 21. Concentrations of serum osteocalcin were significantly different by treatment and time but not by treatment-by-time interaction. Specifically, concentrations of osteocalcin were significantly ($P = 0.033$) higher in horses treated with ESWT, compared with controls, and were increased significantly ($P = 0.002$) from day 0 until the remainder of the study (Figure 2). Concentrations of serum CTX I differed significantly by treatment and time but not by treatment-by-time interaction. Specifically, concentrations of CTX I were significantly ($P = 0.038$) higher in horses treated with ESWT, compared with controls and those treated with PSGAG. Concentrations of CTX I were increased significantly ($P < 0.001$) in all horses from day 0 and were highest at day 42 (Figure 3).

Concentrations of synovial fluid CS846 differed significantly by osteoarthritis and treatment-by-osteoarthritis interaction. Concentrations of CS846 were significantly higher in osteoarthritic joints, specifically in osteoarthritic joints from ESWT-treated horses, compared with controls (Figure 4). Concentrations of synovial fluid CPII changed significantly ($P < 0.001$) over time. Specifically, concentrations of synovial fluid CPII were decreased from baseline on days 14 and 42 and then were increased significantly thereafter. Synovial fluid GAG was significantly influenced by osteoarthritis, time, and osteoarthritis-by-time interaction but not by treatment. Concentrations of synovial fluid GAG were significantly higher in osteoarthritic joints, compared with control joints ($P < 0.001$); higher on day 28, compared with day 42 ($P < 0.001$); and higher in osteoarthritic joints on days 42, 56, and 70, compared with control joints ($P = 0.002$, $P = 0.017$, and $P < 0.001$, respectively). Concentrations of synovial fluid Col CEQ were found to change significantly ($P = 0.002$) over time. Specifically,

### Table 3—Mean ± SD values for radial carpal bone (distal surface) variables in the same horses as in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control osteoarthritis</th>
<th>PSGAGT osteoarthritis</th>
<th>ESWT osteoarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of damaged zone</td>
<td>0.00 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Cracks in damaged zone (cracks/mm²)</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>Crack length in damaged zone (mm/mm²)</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td>Crack length in nondamaged zone (mm/mm²)</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.01</td>
</tr>
</tbody>
</table>

Figure 1—Log mean ± SEM serum GAG concentrations at various times in control horses ($n = 8$), horses that received ESWT (8), and horses that received PSGAGT (8), after surgical induction of osteoarthritis. *Significant ($P < 0.05$) difference from baseline value. *Significant ($P < 0.05$) decrease on day 21, compared with day 14, in horses that received PSGAGT or ESWT.

Figure 2—Log mean ± SEM serum osteocalcin concentrations in the same horses as in Figure 1.

Figure 3—Log mean ± SEM serum CTX I concentrations in the same horses as in Figure 1.
concentrations of synovial fluid C1,2C were increased significantly ($P < 0.001$) on days 14 and 28. Activities of synovial fluid bone alkaline phosphatase were significantly affected by time, osteoarthritis, and treatment-by-time interaction. Activities of synovial fluid bone alkaline phosphatase increased significantly ($P < 0.001$) from day 0 to peak on days 28 and 42, then decreased significantly ($P < 0.001$) thereafter. Activities of synovial fluid bone alkaline phosphatase were significantly increased ($P = 0.005$) higher in osteoarthritic joints when pooled over treatment.

### Discussion

Application of ESWT is safe and provides clinical sign–modifying effects to horses with osteoarthritis induced by experimental osteochondral fragmentation.$^{14}$ No adverse effects were detected with ESWT or PSGAGT in the present study. As has been reported,$^{14}$ evaluation of the data indicated that ESWT and PSGAGT do not cause an increase in signs of pain or induce any damage to the middle carpal joint. In that study,$^{14}$ PSGAGT provided no clinical sign–modifying effects. The results presented here are a follow-up to that report in an attempt to better understand the effects of ESWT and PSGAGT, with an emphasis on bone metabolism. There were no effects of ESWT or PSGAGT on physical measurements of bone density, morphology, and microdamage, but the treatments did appear to have physiologic effects on bone, as indicated by changes in serum biomarkers indicative of bone turnover.

Computed tomographic analysis was used as a clinical measure of bone density in this study to assess the effects of osteoarthritis and treatment.$^{25}$ Analysis of both the distal aspect of the radial carpal bone and proximal aspect of the third carpal bone revealed a differential response to exercise. The radial carpal bone increased in density over the duration of the study, most likely in response to exercise, regardless of the presence of osteoarthritis, whereas the third carpal bone was unchanged throughout the study. This differential response is not uncommon, and it has been seen in other species.$^{3,27}$ However, in a previous study$^{2}$ of the effects of exercise on clinically normal joints conducted over a 6-month period, there was no differential response between radial carpal and third carpal bones, although bone remodeling in the metacarpophalangeal joint was significantly greater, indicating possible effects of exercise intensity and time on bone remodeling in specific joints. Given that the present study was performed over 56 days of exercise, the radial carpal bone may increase in density earlier than the third carpal bone, which may increase to the density of the radial carpal bone over 6 months. A previous study$^{28}$ by our group that used the same model of osteoarthritis found no difference between osteoarthritic and exercise-controlled bones, but a repeated-measure analysis was not performed because computed tomography was performed only at the end of that study. In addition, those data indicate that ESWT had no effect on SCB density, whether it was in control or osteoarthritic joints. In fact, ESWT did not affect the increased bone formation in the proximal aspect of the third carpal bone that was seen in a previous version of this model.$^{29}$ Evaluation of the data from the present study revealed that mineralization variables increased in all osteoarthritic joints (albeit nonsignificantly for most variables), compared with controls (except for DLMS in PSGAG-treated joints), but to a lesser degree than previously reported.$^{29}$ Unlike in the previous study, in which a direct effect or an effect on signs of pain or limb use could induce changes in bone variables, debris was allowed to remain in the joint in the present study.

The increase in microdamage length in the radial carpal bones of joints with osteoarthritis nearly reached significance; however, no other indicators of microdamage were changed because of osteoarthritis or treatment. There is concern that ESWT can induce microdamage and increase bone remodeling.$^{30}$ However, in the present study, an increase in neither microdamage nor bone formation in response to microcracking was seen in joints treated with ESWT. Therefore, at the clinically relevant to high dose used in the present study, which did impart clinical sign–modifying effects, ESWT was safe to bone and other tissues assessed. Because, to the authors’ knowledge, there are no published reports of variability in ESWT doses used clinically and the effects that each imparts, the authors can only give subjective impressions of the clinical relevance of doses used in the study. The treatment protocol used in the present study was chosen by the authors to provide what was thought to be a minimal dose (200 pulses/location) over the proximal and distal joint capsule attachment. Although the total dose of 1,500 to 2,000 pulses was high for a discrete lesion, this dose was diluted over a relatively large area and the total dose should be interpreted accordingly. The authors used an energy setting of 0.14 mJ/mm$^2$ as the upper limit in acute lesions and 0.13 mJ/mm$^2$ in chronic lesions.

Although ESWT and PSGAGT induced no significant effects on SCB, there were changes in serum biomarkers of bone turnover attributable to ESWT that were of interest. Concentrations of serum osteocalcin were significantly higher in horses that received ESWT, compared with those in untreated horses. Concentrations of serum osteocalcin are reported$^{24}$ to increase in
exercised horses both with and without osteochondral fragmentation by use of the same model that was used in this study. In the present study, the increase was seen in the pooled data, regardless of time and the time-by-treatment interaction. Serum osteocalcin has a variable response to exercise and disease. Jackson et al31-33 reported that concentrations of serum osteocalcin increased in horses with dorsal metacarpal disease, but decreased in horses that were exercised on a treadmill in a separate study. Therefore, serum osteocalcin concentration appears to be affected by the types of exercise and treatment.31-33 An increase in serum osteocalcin concentration has been seen in another ESWT study34; specifically, serum osteocalcin concentrations were increased with ESWT in human patients that attained healing of nonunion fractures, compared with concentrations in those patients that underwent ESWT but did not attain healing of nonunion fractures. It was concluded in that study34 that the ESWT dose was sufficient to stimulate a bone-remodeling response in the patients that attained healing as opposed to a failure of that dose to stimulate remodeling in the patients that did not attain healing. Histologically, this response occurs in newly formed bone35; however, that did not occur in the present study because SCB from joints that received ESWT did not have a significant increase in new bone, compared with other treatments. Extracorporeal shock wave therapy induces release of osteocalcin from mature osteoblasts on trabecular bone at the site of treatment without apposition of new bone,35 which may have occurred in the present study. Therefore, although measurement of biochemical markers may have been sensitive enough to detect changes caused by ESWT, their clinical importance may be questionable because of a lack of bone change.

There is concern about a potentially important decrease in osteocalcin that might occur with general anesthesia; however, general anesthesia was used in all horses and at the same time in this study. In addition, because of circadian rhythm changes with osteocalcin, all serum samples were obtained in the morning during the same time period.

Concentrations of serum CTX I were also significantly increased in horses treated with ESWT, compared with concentrations in horses that received PSGAGT and control horses. Serum CTX I concentrations are a measure of bone resorption, and in this study, significant increases occurred within 2 weeks after application of treatment. However, this did not lead to any detectable physiologic or structural changes in the SCB at the end of the study. It is likely that increased serum osteocalcin and CTX I concentrations caused by ESWT were insufficient for detection via the bone analysis techniques (computed tomography and histology) used in this study. In addition, these changes likely occurred well before the bone samples were obtained after euthanasia. Other serum markers of joint disease followed patterns similar to a recently published report35 in horses. Concentrations of serum GAG were increased significantly on days 14 and 35 in all horses, then were decreased significantly on all test days thereafter in that study.34 However, in the present study, concentrations of serum GAG were decreased in ESWT- and PSGAGT-treated horses, compared with concentrations in controls, on day 21. This was likely a treatment response because serum GAG concentration typically increases in horses with induced osteoarthritis.14,24 Overall, serum GAG, osteocalcin, C1,2C, CS846, and CPII concentrations did not consistently increase over the duration of the study as in the previous study.24 The same was true for some of the synovial fluid biomarkers. The reason for this was unclear, although the horses in the previous report were exercised for 21 days prior to induction of osteoarthritis (which was intended to decrease the variability introduced by differing exercise patterns prior to onset of the study), which may have influenced those findings, making direct comparison difficult.

Synovial fluid biomarkers of osteoarthritis changed in affected horses (increased CS846 and GAG concentrations), which has been reported in a similar study.24 Regardless, synovial fluid CS846 concentrations increased in osteoarthritic joints with ESWT without a corresponding change in synovial fluid GAG concentrations. Therefore, ESWT may have stimulated aggrecan synthesis in articular cartilage without the stimulus of degeneration. This allowed for effective differentiation of the effects of ESWT from the typical biomarker response to osteoarthritis. However, this change only occurred at the biochemical level because no change in gross damage was seen.14 Activities of the only bone biomarker to be tested in synovial fluid, bone alkaline phosphatase, were increased in osteoarthritic joints, compared with concentrations in controls, especially on days 14 and 42, but did not change with treatment. Increased serum bone alkaline phosphatase activity has been detected in horses with naturally occurring carpal joint disease.36

Overall, on the basis of findings of the present study, there were no clinically relevant effects of ESWT on SCB in horses with induced osteoarthritis. There were indications of increased bone remodeling on the basis of the significant increase in serum osteocalcin, which may be a justification for further research into the appropriate dose of ESWT for various diseases. The doses used here were effective for providing clinical sign–modifying effects in horses with osteoarthritis but may not be appropriate for inducing trabecular and SCB remodeling and thus healing, as has been reported in other studies. Although others have reported both an intra-articular and apparent systemic effect of PSGAGT on bone remodeling by use of nuclear scintigraphy,14 we failed to find any influence of PSGAGT on structural, physiologic, or biochemical indications of bone metabolism. It was interesting to note that there was a significant decrease in serum GAG concentration in PSGAGT-treated horses on day 21, compared with the concentration on day 14, which may indicate a modest effect on articular cartilage at that point, the effects of which are unknown.

a. Adequan IA, Luitpold Pharmaceuticals Inc, Shirley, NY.
c. Sigma-Aldrich, St Louis, Mo.
d. Picker PQ CT, Phillips Medical, Barlow, Wash.
f. Image Pro Imaging System, Media Cybernetics, Silver Spring, Md.
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