Use of quantitative ultrasonography for noninvasive surveillance of the third metacarpal bone in racing and training Thoroughbreds

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Objective—To use quantitative ultrasonography to evaluate the association between the speed of sound (SOS) at 9 sites in the third metacarpal bone (MCIII) of racing Thoroughbreds with workload accumulation and the effect that MCIII failure has on this association.

Animals—Sixty-two 2- and 3-year-old Thoroughbreds in racing condition.

Procedures—Cumulative work index (CWI) was used to calculate total workload ($\text{CWI}_{\text{total}}$) and also 3 independent CWIs for the various gaits (ie, trot [CWI_{trot}], gallop [$\text{CWI}_{\text{gallop}}$], and race [$\text{CWI}_{\text{race}}$]) used during training and racing. Speed of sound was monitored in horses during the 2007 racing season and compared with the CWIs via regression analysis. Sex, age, limb, and MCIII failure were included as covariates in the model.

Results—SOS was significantly associated with $\text{CWI}_{\text{total}}$ at 8 sites and with independent CWIs of the various gaits at all 9 sites. Progression of SOS in MCIIIs with workload differed significantly in horses with clinical signs of metacarpal bone failure, compared with results for horses with clinically normal MCIIIs, in 1 site by use of $\text{CWI}_{\text{total}}$ and in 5 sites by use of the independent CWIs for the various gaits.

Conclusions and Clinical Relevance—These results indicated that SOS in the MCIII of racing Thoroughbreds followed a constant pattern of progression as workload accumulated. With the development of more precise quantitative ultrasonography devices, SOS corrected for amount of activity may be used to identify horses at risk of bone failure. (*Am J Vet Res* 2009;70:1484–1493)

Musculoskeletal injuries are the most common clinical problem in performance horses and horses used for pleasure riding and are the main cause of lost training days and economic losses in Thorougbreds.[1,2] Thus, they represent an important problem in the equine industry. Bone failure manifested as fractures and dorsal metacarpal disease (more commonly known as bucked shins) is the leading cause of morbidity and fatalities among racing Thoroughbreds.[3] In these horses, stress fractures are a threat to their performance and may lead to catastrophic injuries. There is growing evidence that catastrophic breakdown injuries result from a chronic nonadaptive bone response to exercise characterized by uncoupled bone remodelling, microdamage accumulation, and lack of appropriate repair at predictable sites.[4,5]

The MCIII in horses is particularly susceptible to high-strain cyclic loading that results in bone fatigue.[6] Thoroughbreds race at speeds exceeding 15 m/s. During such activity, surface strains in excess of 5,000 με are applied to the mid-diaphysis of the MCIII.[7] Dorsal metacarpal disease, diaphyseal stress fractures, and condylar fractures are common problems in the racing industry, despite the efforts of many groups who have

ABBREVIATIONS

BMD	Bone mineral density
CWI	Cumulative work index
$\text{CWI}_{\text{gallop}}$	Cumulative work index for gallop
CWI_{race}	Cumulative work index for race
$\text{CWI}_{\text{total}}$	Total cumulative work index
CWI_{trot}	Cumulative work index for trot
MCIII	Third metacarpal bone
QUS	Quantitative ultrasonography
QUSbase	Baseline quantitative ultrasonography value for each horse
SOS	Speed of sound

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conducted studies of the ability of the MCIII of horses to adapt to exercise. Additional research is necessary to improve our understanding of bone failure and the etiopathogeneses of catastrophic fractures.[8]

The effects of exercise on the appendicular skeleton have been characterized. In a simplistic view of Wolff's law, bone is deposited where it is needed and resorbed where it is not needed, which allows bones to adapt to different forces imposed on the skeletal system during daily activity.[9] The result of this bone deposition-resorption cycle in the equine metacarpus is the modification of its mass and geometry at macrostructural and microstructural levels, which results in the modification of mechanical properties on a site-to-site anatomic basis.[10] An inadequate or abnormal deposition-resorption response to exercise can lead to mechanical weakening and sharp gradients in properties of bone in adjacent sites, which can lead to increases in stress and make the bone at risk for failure under loads experienced during training and racing.

Current diagnostic tools available to evaluate in vivo bone mechanical properties and adaptation at macroscopic and microscopic levels have several limitations because complex nonmobile equipment, the need for general anesthesia, long study times, or use of ionizing radiation limits their usefulness in a clinical setting.

In racing Thoroughbreds, current imaging techniques such as radiography and scintigraphy do not predict bone failure; thus, development of diagnostic tools capable of monitoring the adaptive response of bone to exercise would be beneficial and may be useful in identifying those horses at high risk of bone failure. Quantitative ultrasonography is a recognized noninvasive technique that measures elements of bone quality, such us microarchitecture, bone elasticity, and BMD,[11] although the exact algorithm relating all these elements with SOS is still unknown.[12] Quantitative ultrasonography measures the propagation of sound waves through a material. It is based on the fact that an ultrasound wave is a moving mechanical wave, and mechanical properties of the transport medium (eg, bone) alter the speed of the wave.[13]

In human medicine, QUS is routinely used to evaluate and monitor metabolic bone diseases,[14] to evaluate the effects of exercise in children[15,16] and adults,[17,18] and to predict stress fractures.[14,19] It has been validated for use in measuring bone properties of the MCIII, radius, and tibia in horses.[20] It can be used sequentially on horses for the assessment of bone quality, which makes it a potential stall-side monitoring tool for use in detecting horses at risk of having a fracture or in the development of safer training regimens. If horses with incipient bone failure can be identified, then training involving reductions in speed and distance may allow the metacarpus to remodel and repair damage before there is a catastrophic bone failure.

Bone adaptation is a function of the mechanical environment to which the bone is exposed. Therefore, it would appear most appropriate to study the evolution of SOS on the basis of workload, rather than on the basis of time. To our knowledge, only 1 study[21] has been conducted to evaluate the association of axial SOS and workload in racehorses. In that study, workload was quantified as a categorical variable and was not associated with SOS. In another study,[22] investigators described a CWI that quantifies the amount of work performed for each gait, taking into account the distances and speeds for each gait. The objective of the study reported here was to evaluate the association of workload (quantified as CWI) with SOS and the effect that MCIII failure has on this association.

Materials and Methods

Animals—Sixty-five 2- and 3-year-old Thoroughbreds in racing condition were identified for use in the study. Horse trainers were solicited to enter horses in the study through the Ontario Horsemen's Benevolent and Protective Association. Inclusion criteria included a low likelihood that a horse would be claimed or leave training, no evidence of musculoskeletal conditions, and no ongoing health problems that would affect training.

Horses at Woodbine Racetrack in Toronto were included in the study. The study was performed during the 2007 racing season; the study started in June and finished the first week in December. The horses belonged to 10 training stables. Horses were housed at the racetrack or at local training centers, and training practices were not changed for the horses during the study. Speed of sound measurements obtained during the study were not divulged to the trainers.

Horses were trained 5 to 7 d/wk. The typical training routine for this group of horses consisted of a warm-up period, which included trotting for a distance of 400 to 1,600 m, that was followed by galloping for a distance of 1,600 to 2,400 m. Horses rested the day after a workout and rested for 3 days after a race. After a rest period, some trainers used only trotting at distances of 2,400 to 3,200 m to exercise their horses. Training was performed on a track with a dirt surface or an all-weather surface.[a]

Between training sessions, horses were housed in box stalls and fed in accordance with the trainer's management practice. No efforts were made to control or manipulate any aspects of horse management.

Study design—A prospective cohort study was conducted to study the associations between workload (quantified as CWI) and SOS in the MCIII and to investigate the suitability of QUS as a minimally invasive technique to monitor bone-associated changes related to exercise in racing Thoroughbreds. Speed of sound measurements were obtained at monthly intervals from June to November. At every monthly SOS measurement, trainers or trainer's assistants were interviewed to record details regarding the medical and exercise history of the horses. Stress was placed on the orthopedic medical history and procedures affecting the limbs, such as cryotherapy and shock wave therapy. Date of birth of each horse was also recorded to evaluate the influence of age on SOS, which was considered a categorical variable. Horses that left the exercise program for > 4 weeks were excluded from data analysis. Criteria for MCIII failure included clinical, radiographic, or scintigraphic diagnosis of dorsal metacarpal disease, metacarpal disease in the second or fourth bones (ie, splint bones), or any type of metacarpal fracture; all of these conditions would be expected to affect a racehorse's performance and cause a decrease in SOS of the MCIII.

QUS for SOS measurements—A multisite QUS device[b] (which consisted of a portable computer, proprietary software, and ultrasound probe) was used to measure SOS. The manufacturer's largest straight handheld probe that created pulsed acoustic waves at a center frequency of 1.25 MHz (bandwidth, 0.7 to 1.8 MHz) was used.[c] Because of logistic reasons, a second probe was used a few times during the later stages of the study. Speed of sound was measured in an axial direction by placing the long axis of the probe parallel to the long axis of MCIII in standing horses that were bearing full weight on the forelimbs. For acoustic coupling, alcohol was first sprayed on the MCIII, and then a multipurpose ultrasound gel[d] was liberally applied to facilitate transmission of sound waves. Most measurements were obtained after a horse had completed a training session; measurements were obtained at approximately the same time each day. Measurements were obtained at monthly intervals, with approximately 30 days between subsequent measurements. Horses were not sedated for SOS measurements, but physical restraint was used as needed.

Speed of sound was measured in 9 sites of the MCIII of both forelimbs (**Figure 1**). The MCIII was divided into 3 equal parts from medial to lateral and from proximal to distal. The sites were defined as the 3 aspects (medial, dorsal, and lateral) at 3 regions (proximal, middle, and distal) and identified with numbers from 1 to 9.

Speed of sound measurements were performed by 3 investigators. During typical operation of the QUS, 3 scanning sequences of 10,000 measurements were used at each location. The software required that the 3 mean measurements had variances of < 1.2% at the 95th and 25th percentiles to enable it to calculate the definitive SOS value for that site as the mean of the 95th percentiles. Verification of the QUS system was performed daily by use of a temperature-corrected model[e] in which SOS for a certain temperature was known; this ensured the probe and QUS system were operating properly. To pass the verification, SOS needed to pass through the model with a variability of ± 50 m/s (calculated as the difference between the expected and measured SOS in the model), which corresponded to a variability < 3%.

Additional in-house evaluations were performed to assess day-to-day variance originating from the QUS system. An MCIII was obtained from the cadaver of a racing Thoroughbred, cleaned of soft tissues, and frozen at –40°C in multiple layers of gauze sponges soaked in lactated Ringer's solution and 2 layers of plastic food wrap. On each successive day of system verification, the MCIII was thawed before use. Twenty-two locations were drawn in ink on the MCIII to ensure precise probe placement. Duplicate measurements of these 22 sites and additional measurements of the temperature-corrected model were obtained with the 2 QUS probes on 5 days. Measurements were made in laboratory-controlled ambient conditions. Between each set of these 5 measurements, the QUS system was exposed to conditions typical of the field study, including transport and use at the racetrack in less controlled environmental conditions.

Workload—The $\text{CWI}_{\text{total}}$ described in another study[22] was used to quantify workload. This index was obtained by determining the sum of the products of the speed of each gait by the distance exercised at that gait since the beginning of the study (ie, $\text{CWI}_{\text{total}}$ = [distance × speed at a trot] + [distance × speed at a gallop] + [distance × speed while racing]) and is considered adimensional because it does not have a physical meaning. The CWI of each of the 3 gaits (ie, CWI_{trot}, $\text{CWI}_{\text{gallop}}$, and CWI_{race}) was also considered independently. The $\text{CWI}_{\text{total}}$ was calculated starting on the day the initial SOS was measured, and values were added for each of the days on which subsequent SOS measurements were collected. Distances a horse exercised at a trot and at a gallop were recorded on flow sheets provided to the trainers or collected directly from the training logs. Distance and times for workouts and races were acquired from the official database[f] compiled by The Jockey Club. To calculate the mean speed of horses at a trot and at a gallop, 44 horses were timed with a stopwatch for a distance of 200 m. Official times from races and workouts were obtained from the official datatabase[f] and used to calculate mean speed at these gaits. Mean of the speeds at each gait was used as the speed for calculation and standardization of the CWI.

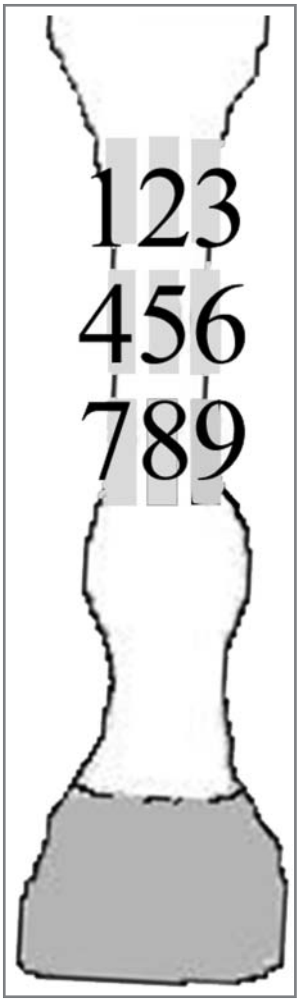

Figure 1—Schematic depicting the location of the 9 sites at which SOS was evaluated by use of QUS in the MCIII of Thoroughbreds. Left corresponds to medial and right to lateral.

Statistical analysis—Data from the QUS probes and day-to-day comparisons on the frozen-thawed MCIII were compared by use of a 3-way ANOVA with probe, day, site, and the interactions as main effects. Results of a Shapiro-Wilk test and examination of the residuals of the in-house data revealed that a logarithmic transformation was appropriate and normalized the data distribution.

Two general mixed models were estimated for each site in the metacarpus. The first model considered the effect of each gait (CWI_{trot}, $\text{CWI}_{\text{gallop}}$, and CWI_{race}), and the second model used the sum of the 3 gaits (ie, $\text{CWI}_{\text{total}}$). Initially, sex, age, limb, MCIII failure, QUS probe, and the work index were included in the models. Interactions of factors as well as linear and quadratic effects were included in the full model while accounting for the random effect of horse and day. Only variables with a value of $P < 0.05$ were considered significant, although variables were still included in the final model when the value was $P \geq 0.05$ but < 0.10. To account for the initial variation of each horse, QUSbase (ie, SOS on day 0 for each horse) was included as a covariate in the models. The 95% tolerance intervals were estimated as 2.9 × SD. A Shapiro-Wilk test and examination of the residuals were used to assess distribution of the data.

Results

Of the 65 horses initially recruited, 62 met the inclusion criteria and were included in the study. Of these horses, 22 did not complete the study (6 SOS measurements) because they were sold, retired from racing because of inadequate performance, or had been moved to other racetracks, but the data collected until they were lost to follow-up monitoring were included in the analysis; 6 were removed because of unspecified soft tissue lesions; 2 were removed because of severe dorsal metacarpal disease; 2 sustained condylar fractures of the MCIII (1 horse) or third metatarsal bone (1 horse); 1 had a pelvic fracture; 1 had an incomplete, nondisplaced (ie, saucer) fracture on the left MCIII; 1 had laminitis with rotation of the third phalanx; 1 had lameness associated with sclerosis of the third carpal bone; 1 had an undiagnosed lameness of the hind limb; and 1 had suspected soreness of the sesamoids of the forelimbs. Thus, only 24 horses completed the entire study (there were 62 horses in the study in June, 56 in July, 51 in August, 41 in September, 34 in October, and 24 in November).

During data collection, quality verification of the QUS revealed differences between the expected and measured SOS on the temperature-corrected model that was at times larger than the manufacturer's recommendations. Analysis of the data obtained for the 2 QUS probes with respect to day-to-day variation revealed that the probes did not differ significantly (intersite and interday mean ± SD SOS was 3,854 ± 562 m/s and 3,887 ± 490 m/s for probes 1 and 2, respectively). However, measurements collected on different days differed significantly ($P < 0.001$). The progression of the differences between the measured and expected SOS measurements on the temperature-corrected model during the period of the study was examined to identify any possible patterns, but a clear pattern was not detected. When the differences of both probes for the expected SOS and measured SOS of the temperature-corrected model phantoms were pooled, the differences ranged from –171 to 148 m/s, which corresponded to a variability of 8% of the magnitude of SOS measurements obtained from the MCIII in the in vivo study.

CWI—The mean speeds for each gait were 5 ± 1 m/s and 10 ± 2 m/s for trotting and galloping, respectively (n = 62 horses). During workouts and races, the mean speeds were equivalent (16 ± 1 m/s). Therefore, they were pooled and referred to as the race gait with a mean speed of 16 ± 1 m/s. These mean velocities were used to calculate the various CWIs.

Progression of $\text{CWI}_{\text{total}}$ and each of the 3 work indices over time was plotted (**Figure 2**). It was evident that $\text{CWI}_{\text{gallop}}$ accounted for most of the $\text{CWI}_{\text{total}}$, followed by CWI_{trot} and then by CWI_{race}, which accounted for a low fraction of the $\text{CWI}_{\text{total}}$.

Association between $\text{CWI}_{\text{total}}$ and SOS—Linear patterns of the data were plotted for the 3 aspects (medial, dorsal, and lateral; **Figure 3**) and 3 regions (proximal, middle, and distal; **Figure 4**). There was an approximation of the overall progression of SOS by aspect and region as CWI accumulated. At 8 of 9 sites, $\text{CWI}_{\text{total}}$ was significantly associated with SOS (P values: site 1 = 0.002, site 2 < 0.001, site 3 = 0.010, site 4 < 0.001, site 5 < 0.001, site 6 < 0.001, site 8 < 0.001, and site 9 = 0.003; **Table 1**).

When considering the CWI for each of the various gaits separately, CWI was associated with SOS at

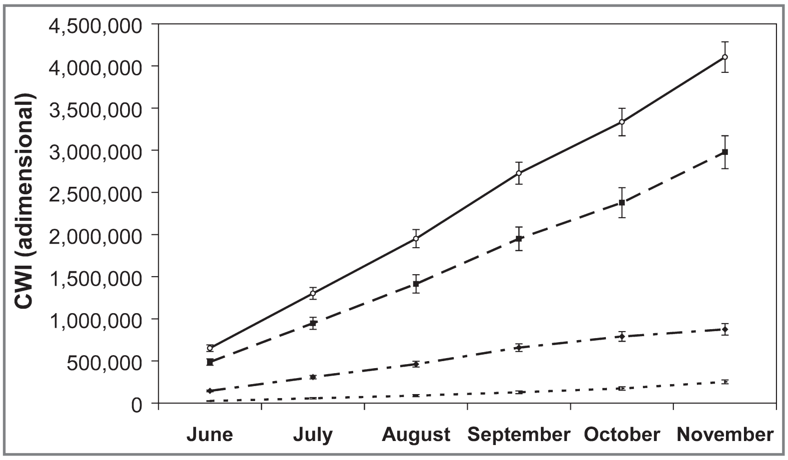

Figure 2—Progression of the mean of the $\text{CWI}_{\text{total}}$ (white circles and solid line), $\text{CWI}_{\text{gallop}}$ (black squares and dashed line), CWI_{trot} (black diamonds and dashed-and-dotted line), and CWI_{race} (dotted line) for SOS measurements obtained at monthly intervals from 62 racing and training Thoroughbreds. The bars represent 95% confidence intervals.

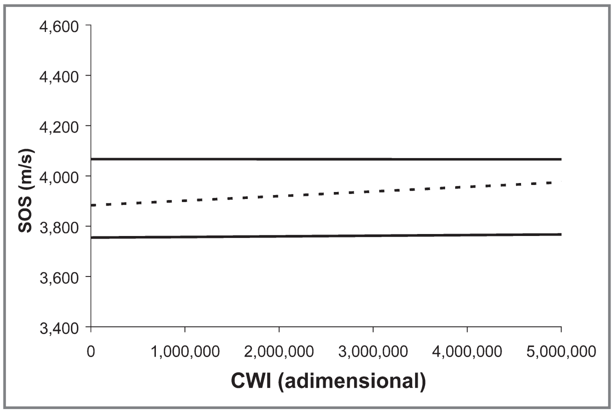

Figure 3—Overall progression of SOS measured for each aspect (lateral, upper solid line; dorsal, lower solid line; and medial, dotted black line) of the MCIII in 62 racing and training Thoroughbreds on the basis of $\text{CWI}_{\text{total}}$. The association between SOS and $\text{CWI}_{\text{total}}$ was correlated for each aspect (lateral, $R^2 = 0.2028$; dorsal, $R^2 = 0.0403$; and medial, $R^2 = 0.0023$).

all sites, with higher R^2 values than when considering $\text{CWI}_{\text{total}}$ (**Table 2**). The CWI_{trot} was associated with SOS at 8 sites (*P* values: site 1 = 0.002, site 2 < 0.001, site 3 < 0.001, site 5 = 0.004, site 6 = 0.030, site 7 = 0.040, site 8 < 0.001, and site 9 < 0.001). The $\text{CWI}_{\text{gallop}}$ was significantly associated with SOS at all 9 sites (*P* values: site 1 < 0.001, site 2 = 0.006, site 3 = 0.004, site 4 < 0.001, site 5 = 0.002, site 6 < 0.001, site 7 = 0.040, site 8 < 0.001, and site 9 = 0.001). The CWI_{race} was significantly associated with SOS at 4 sites (*P* values: site 4 = 0.001, site 6 = 0.002, site 7 = 0.040, and site 9 = 0.002) and was associated (but not significantly) at 3 sites (site 1 = 0.05, site 2 = 0.07, and site 5 = 0.05). Therefore, CWI_{trot} and $\text{CWI}_{\text{gallop}}$ had more influence on SOS than did CWI_{race}.

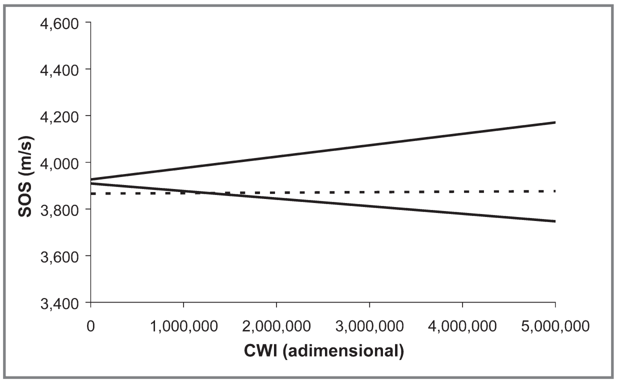

Figure 4—The SOS measured for each region (proximal, upper solid line; middle, dotted line; and distal, lower solid line) of the MCIII in 62 racing and training Thoroughbreds on the basis of $\text{CWI}_{\text{total}}$. The association between SOS and $\text{CWI}_{\text{total}}$ was correlated for each region (proximal, $R^2 = 0.0792$; middle, $R^2 = 0.0414$; and distal, $R^2 = 0.0487$).

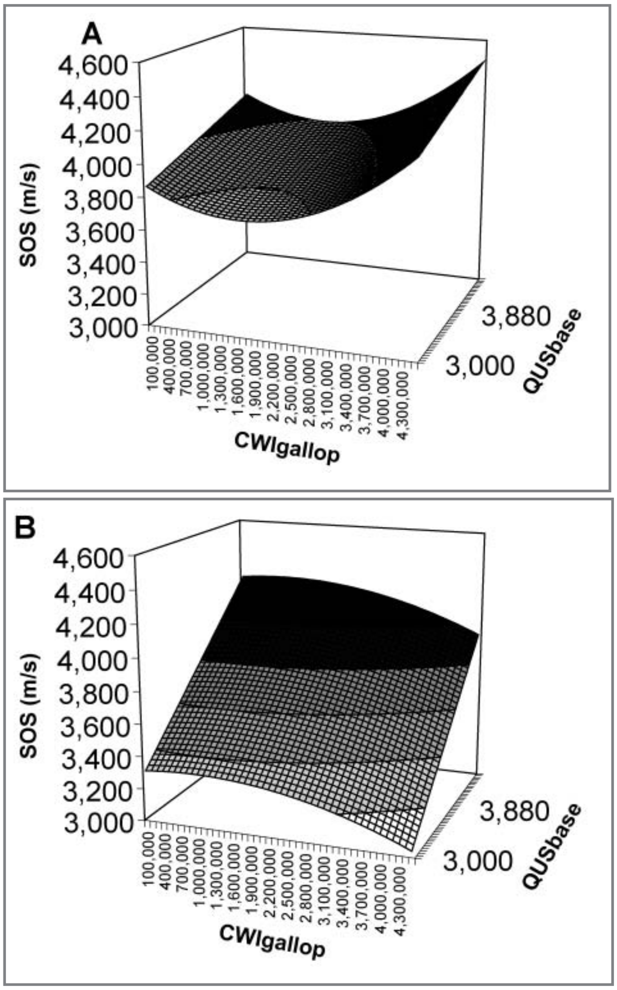

Figure 5—Graphs of the regression model for site 7 in 3-year-old gelding Thoroughbreds without (n = 46 Thoroughbreds; A) and with (16 Thoroughbreds; B) MCIII failure. Notice the differences in SOS progression as $\text{CWI}_{\text{gallop}}$ accumulates for a fixed CWI_{trot} and CWI_{race}. The association was correlated ($R^2 = 0.13$).

Equations estimated by the regression models allowed calculation of the mean of the expected SOS for a particular CWI and QUSbase (**Tables 3 and 4**). However, the 95% tolerance interval, which is the range that included 95% of the measurements in our study, ranged from 401.58 to 751.60 m/s for $\text{CWI}_{\text{total}}$ and from 398.02 to 736.80 m/s for the gait-related CWIs (**Table 5**). This indicated that the data had high variability.

Effect of age—Of the 8 sites at which SOS was significantly associated with $\text{CWI}_{\text{total}}$, the association between SOS and $\text{CWI}_{\text{total}}$ differed significantly between 2- and 3-year-old horses at sites 2 and 4. The most remarkable difference was the coefficient for $\text{CWI}_{\text{total}}^2$, which was positive for 2-year-old horses and negative for 3-year-old horses. This indicated that in 2-year-old horses, the SOS decreased initially and then increased at an increasing rate, whereas in 3-year-old horses, the SOS had a more pronounced initial increase and its rate of increase then decreased progressively.

For the gait-related CWIs, the association with SOS was affected by age at 7 sites (sites 1, 2, 4, 5, 7, 8, and 9). This indicated that the gait-related CWIs were more sensitive to the effects of age than was $\text{CWI}_{\text{total}}$.

Effect of sex—Sex had a significant effect on the association between $\text{CWI}_{\text{total}}$ and SOS at 5 sites (sites 1, 4, 5, 6, and 7). Geldings had the lowest intercept, the highest coefficient for $\text{CWI}_{\text{total}}$, and the lowest coefficient for $\text{CWI}_{\text{total}}^2$; thus, their SOS was the lowest at the beginning of the study but had the greatest increase throughout the study. Fillies had the lowest slope for $\text{CWI}_{\text{total}}$ and the highest coefficient for $\text{CWI}_{\text{total}}^2$ at 3 sites (sites 1, 4, and 6); thus, SOS in fillies had the smallest rate of increment. At the remaining site (site 7), colts had the highest rate of increment, which was slightly higher than the rate of increment for the fillies. These findings indicated that there were patterns of variation for the association between $\text{CWI}_{\text{total}}$ and SOS within sex that persisted in various sites.

The association between the gait-related CWIs and SOS was affected by sex at 5 sites (sites 1, 4, 6, 7, and 9). Thus, they were not more sensitive for use in detecting variations on SOS progression attributable to sex, compared with those attributable to $\text{CWI}_{\text{total}}$.

Effect of limb—Speed of sound was associated differently with $\text{CWI}_{\text{total}}$, depending on the limb evaluated, at 6 sites (sites 1, 2, 4, 5, 6, and 7). At the 2 medial sites for which limb had an effect (proximal and middle, corresponding to sites 1 and 4), the SOS-CWI curve had a higher intercept, slightly lower slope, and higher coefficient for $\text{CWI}_{\text{total}}^2$ on the left limb than on the right limb; thus, the initial SOS was higher in the left limb but did not increase as much as in the right limb. At sites 2 and 5 (dorsal aspect), limb only affected the intercept of the SOS-CWI curve and was lower on the left limb; therefore, SOS followed the same progression in both limbs but with lower values for the left limb. At the only lateral site (site 6) for which limb affected the curve, there was a lower intercept, higher CWI coefficient,

Table 1—The *P* values for variables included in each model of CWI_{total} for each of the 9 sites in the MCIII of 62 racing and training Thoroughbreds.

Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
CWI	0.002	< 0.001	0.010	< 0.001	< 0.001	< 0.001	NI	< 0.001	0.003
QUSbase	< 0.001	< 0.001	0.568	< 0.001	< 0.001	0.004	< 0.001	< 0.001	0.008
MCIII failure	NI	NI	NI	NI	NI	0.019	0.039	NI	NI
Limb	0.036	0.017	NI	0.009	< 0.001	0.018	0.009	NI	NI
Sex	< 0.001	NI	NI	0.006	0.05	0.004	0.019	NI	NI
Age	NI	0.002	NI	0.057	NI	NI	0.037	NI	NI

Variables were considered to have a significant effect on the CWI-SOS association at values of $P < 0.05$.
NI = Not included in final model.

Table 2—The *P* values for variables included in each model for the gait-related CWIs for each of the 9 sites in the MCIII of 62 racing and training Thoroughbreds.

Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
CWI_{trot}	0.002	< 0.001	< 0.001	0.060	0.004	0.030	0.040	< 0.001	< 0.001
CWI_{gallop}	< 0.001	0.006	0.004	< 0.001	0.002	< 0.001	0.040	< 0.001	0.001
CWI_{race}	0.050	0.070	NI	0.001	0.050	0.002	0.040	NI	0.002
QUSbase	< 0.001	< 0.001	NI	< 0.001	< 0.001	0.004	< 0.001	< 0.001	0.004
MCIII failure	0.047	NI	0.009	0.037	0.039	0.026	0.009	NI	NI
Age	0.002	0.006	NI	0.008	0.036	NI	0.038	0.037	0.018
Limb	0.038	NI	NI	0.001	< 0.001	0.01	0.007	NI	NI
Sex	0.002	NI	NI	0.006	NI	0.004	0.038	NI	0.007

See Table 1 for key.

Table 3—Equations for regression models correlating CWI_{total} and SOS for each of 9 sites and differences for sex, limb, age, and MCIII failure in 62 racing and training Thoroughbreds.

Site	R^2	Variable*	Equation of regression model for CWI_{total}
1	0.41	Right limb of 3-year-old geldings	$y = 560.40 + 0.7469z + 0.002683x - (544 \times 10^{-12} \bullet x^2) - (5.54 \times 10^{-7} \bullet z \bullet x) + (1.16 \times 10^{-13} \bullet z \bullet x^2)$
		Right limb of 3-year-old fillies	$y = 185.33 - 0.00025x + (6.47 \times 10^{-11} \bullet x^2)$
		Right limb of 3-year-old colts	$y = 126.89 - 0.00017x + (4.74 \times 10^{-11} \bullet x^2)$
		Left limb of 3-year-old geldings	$y = 70.1514 - 0.00013x + (3.12 \times 10^{-11} \bullet x^2)$
2	0.53	Right limb of 3-year-old geldings	$y = 1698.66 + 0.4909z + 0.000203x - (23 \times 10^{-12} \bullet x^2)$
		Right limb of 2-year-old horses	$Y = 1668.42 - 0.3891z - 0.00015x + (3.74 \times 10^{-11} \bullet x^2)$
		Left limb of 3-year-old horses	$y = -691.14 + 0.1873z$
		Left limb of 2-year-old horses	$y = -56.0116$
3	0.28	Right limb of 3-year-old geldings	$y = 3{,}449.09 + 0.1336z + 0.00003x + (1.6 \times 10^{-11} \bullet x^2)$
4	0.36	Right limb of 3-year-old geldings	$y = 1{,}650.28 + 0.4906z + 0.000318x - (617 \times 10^{-13} \bullet x^2)$
		Right limb of 3-year-old fillies	$y = 116.65 - 0.00017x + (4.2 \times 10^{-11} \bullet x^2)$
		Right limb of 3-year-old colts	$y = 122.65 - 0.00014x + (3.59 \times 10^{-11} \bullet x^2)$
		Left limb of 3-year-old geldings	$y = 89.2201 - 0.00013x + (2.28 \times 10^{-11} \bullet x^2)$
		Right limb of 2-year-old geldings	$y = 26.0222 - 0.00008x + (2.18 \times 10^{-11} \bullet x^2)$
5	0.45	Right limb of 3-year-old geldings	$y = 2{,}121.12 + 0.393z + 0.000045x$
		Right limb of all fillies	$y = 3.3151$
		Right limb of all colts	$y = -56.5154$
		Left limb of all geldings	$y = -100.57$
		Left limb of all colts	$y = 101.69$
		Left limb of all fillies	$y = 50.2171$
6	0.36	Right limb of 3-year-old geldings	$y = 3{,}020.03 + 0.2311z + 0.000043x + (2.01 \times 10^{-12} \bullet x^2)$
		Right limb of all fillies	$y = -836.11 + 0.2139z - 0.00011x + (3.28 \times 10^{-11} \bullet x^2)$
		Right limb of all colts	$y = 1{,}723.92 - 0.4245z - 0.00014x + (3.2 \times 10^{-11} \bullet x^2)$
		Left limb of all geldings	$y = -88.6666 + 0.000148x - (338 \times 10^{-13} \bullet x^2)$
		Right limb of all geldings with MCIII failure	$y = 10.3751$
		Right limb of all fillies with MCIII failure	$y = 7.0874$
		Right limb of all colts with MCIII failure	$y = -146.71$
7	NS	NA	NA
8	0.14	Right limb of 3-year-old geldings	$y = 2{,}111.55 + 0.4303z + 0.000295x + (2.88 \times 10^{-11} \bullet x^2) - (1.04 \times 10^{-7} \bullet z \bullet x)$
9	0.08	Right limb of 3-year-old geldings	$y = 3{,}363.31 + 0.1776z - 0.00011x + (1.74 \times 10^{-11} \bullet x^2)$

*The first equation for each site corresponds with the right limb of 3-year-old geldings; additional variables that had a significant effect on the model for each site are included.
NA = Not applicable. NS = Not significant. x = CWI_{total}. y = SOS. z = QUSbase.

Table 4—Equations for regression models correlating gait-related CWIs and SOS for each of 9 sites and differences for sex, limb, age, and MCIII failure in 62 racing and training Thoroughbreds.

Site	R^2	Variable*	Equations of regression model for gait-related CWIs
1	0.47	Right limb of 3-year-old geldings	$y = 470.42 + 0.7972z - 0.00087t + 0.003951g + 0.002085r + (8.65 \times 10^{-10} \bullet t^2) - (1.04 \times 10^{-9} \bullet g^2) - (3.236 \times 10^{-9} \bullet r^2) + (1.89 \times 10^{-10} \bullet t \bullet g) - (1.69 \times 10^{-9} \bullet t \bullet r) - (8.37 \times 10^{-10} \bullet g \bullet z) + (2.15 \times 10^{-13} \bullet g^2 \bullet z)$
		Right limb of 3-year-old fillies	$y = 152.88 - 0.00024t - 0.0002g + (4.14 \times 10^{-10} \bullet t^2) + (4.51 \times 10^{-11} \bullet g^2)$
		Right limb of 3-year-old colts	$y = 69.2150 + 0.0012t - 0.00046g - (1.03 \times 10^{-9} \bullet t^2 +) + (1.21 \times 10^{-10} \bullet g^2)$
		Right limb of 2-year-old geldings	$y = -9.0669 + 0.001049t - 0.00042g - (806 \times 10^{-12} \bullet t^2) + (1.27 \times 10^{-10} \bullet g^2)$
		Left limb of 3-year-old geldings	$y = 57.7894 - 0.00011g - 0.00049r + (4.63 \times 10^{-11} \bullet g^2)$
		Right limb of 3-year-old geldings with MCIII failure	$y = 44.8626 - 0.002320r + (8.68 \times 10^{-9} \bullet r^2)$
2	0.58	Right limb of 3-year-old geldings	$y = -200.14 + 0.9920z + 0.008036t + 0.000151g + 0.000498r - (7.54 \times 10^{-9} \bullet t^2) - (242 \times 10^{-13} \bullet g^2) - (2.62 \times 10^{-9} \bullet r^2) - (2.04 \times 10^{-6} \bullet t \bullet z) + (1.98 \times 10^{-12} \bullet t^2 \bullet z)$
		Both limbs of all 2-year-old horses	$y = 1{,}706.07 - 0.4181z - 0.00013g + (5.25 \times 10^{-11} \bullet g^2)$
3	0.36	Right limb of 3-year-old geldings	$y = 4{,}286.5 + 0.000253t - 0.00003g - (7.54 \times 10^{-10} \bullet t^2) + (2.91 \times 10^{-11} \bullet g^2)$
		Both limbs of all horses with MCIII failure	$y = 141.14 - 0.000713t + (663 \times 10^{-12} \bullet t^2)$
4	0.41	Right limb of 3-year-old geldings	$y = 1{,}688.42 + 0.4989z - 0.00003t + 0.000292g + 0.000751r + (1.42 \times 10^{-10} \bullet t^2) - (811 \times 10^{-13} \bullet g^2) - (2.25 \times 10^{-9} \bullet r^2)$
		Right limb of 3-year-old fillies	$y = 94.9431 - 0.00017g + (5.35 \times 10^{-11} \bullet g^2)$
		Right limb of 3-year-old colts	$y = 143.24 - 0.00022g + (7.09 \times 10^{-11} \bullet g^2)$
		Right limb of 3-year-old geldings	$y = -8.372 - 0.0001g + (4.28 \times 10^{-11} \bullet g^2)$
		Left limb of 3-year-old geldings	$y = -56.1269 - 0.00132r + (3.67 \times 10^{-9} \bullet r^2)$
		Right limb of 3-year-old geldings with MCIII failure	$y = 10.2641$
5	0.45	Right limb of 3-year-old geldings	$y = 2{,}179.88 + 0.3693z + 0.000119t + 0.000054g - 0.00033r$
		Right limb of all 2-year-old horses	$y = 19.1552$
		Left limb of all 3-year-old horses	$y = -72.7397$
		Right limb of all 3-year-old horses with MCIII failure	$y = 810.02 - 0.2035z$
6	0.36	Right limb of 3-year-old geldings	$y = 3{,}480.4 + 0.1270z + 0.001864t - 0.00007g - 0.00952r + (4.11 \times 10^{-11} \bullet g^2) - (4.14 \times 10^{-7} \bullet t \bullet z) + (2.284 \times 10^{-6} \bullet r \bullet z)$
		Right limb of 3-year-old fillies	$y = -1{,}122.72 + 0.262z$
		Right limb of 3-year-old colts	$y = 1{,}153.64 - 0.3157z$
		Left limb of 3-year-old geldings	$y = -39.7875 + 0.000125g - (337 \times 10^{-13} \bullet g^2)$
		Right limb of 3-year-old geldings with MCIII failure	$y = 12.6768$
7	0.13	Right limb of 3-year-old geldings	$y = 3{,}290.57 + 0.1701z - 0.001185t - 0.000202g - 0.00005r + (3.62 \times 10^{-10} \bullet t^2) + (8.1527 \times 10^{-13} \bullet g^2)$
		Right limb of 3-year-old fillies	$y = -50.009 - 0.00006r$
		Right limb of 3-year-old colts	$y = -86.0572 + 0.000682r$
		Right limb of 2-year-old geldings	$y = -113.59 + 0.000156g - (54 \times 10^{-12} \bullet g^2)$
		Right limb of 3-year-old geldings with MCIII failure	$y = -1{,}556.31 + 0.3945z - 0.000775t + 0.00024g + (691 \times 10^{-12} \bullet t^2) - (8.15 \times 10^{-11} \bullet g^2)$
8	0.25	Right limb of 3-year-old geldings	$y = 1{,}464.51 + 0.6136z + 0.003244t - 0.00014g + (4.5 \times 10^{-10} \bullet t^2) + (3.9 \times 10^{-11} \bullet g^2) - (9.42 \times 10^{-7} \bullet z \bullet t)$
		All 2-year-old horses	$y = -24.4010 + 0.000043g$
9	0.18	Right limb of 3-year-old geldings	$y = 3{,}183.08 + 0.193z + 0.00043t - 0.00017g + 0.000983r - (417 \times 10^{-12} \bullet t^2) + (6.43 \times 10^{-11} \bullet g^2) - (7.17 \times 10^{-9} \bullet 9r^2)$
		Right limb of 3-year-old fillies	$y = 10.988 + 0.000649t - 0.00335r - (908 \times 10^{-12} \bullet t^2) + (1.67 \times 10^{-8} \bullet r^2)$
		Right limb of 3-year-old colts	$y = 270.61 - 0.00104t - 0.0022r + (7.74 \times 10^{-10} \bullet t^2) + (1.238 \times 10^{-8} \bullet r^2)$
		Right limb of 2-year-old geldings	$y = 3.7985 - 0.00088t + 0.000275g + (1.043 \times 10^{-9} \bullet t^2) - (108 \times 10^{-12} \bullet g^2)$

$g = CWI_{gallop}$. $r = CWI_{race}$. $t = CWI_{trot}$.

See Table 3 for remainder of key.

Table 5—Width of the 95% tolerance intervals for SOS of each of the 9 sites in the MCIII of 62 racing and training Thoroughbreds.

Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Models of CWI_{total}	668.8	515.3	511.1	401.6	459.8	442.6	733.7	626.2	751.6
Models for each gait-related CWI	637.9	505.8	505.8	398.0	455.5	449.5	739.3	605.8	736.8

Values reported are m/s.

and coefficient for $CWI_{total}{}^2$ in the left limb; thus, the initial SOS was lower but increased more in the left limb than in the right limb. All these findings indicated that SOS progressed differently as workload accumulated depending on the limb and that these differences were consistent within aspects (ie, medial, dorsal, and lateral).

The association of the gait-related CWIs with SOS differed between the right and left limbs at 5 sites (sites 1, 4, 5, 6, and 7). This indicated that they were not more sensitive than CWI_{total} for detecting the effect of limb on SOS progression.

Effect of MCIII failure—Failure of the MCIII (dorsal metacarpal disease, metacarpal disease in the second or fourth metacarpal bones, or any type of metacarpal fracture) had a significant effect on SOS in each of several models. In the CWI_{total} model at site 6, SOS in horses with MCIII failure followed the same progression, but with a slightly higher intercept, than the progression in horses without MCIII failure. Therefore, the predicted SOS values for horses with signs of MCIII failure were slightly higher than the predicted SOS values for horses with clinically normal MCIIIs.

Significant associations were detected for SOS progression in horses with MCIII failure, compared with the progression in horses without MCIII failure, in the separate analyses of the gait-related CWI models at 5 sites (*P* values: site 3 = 0.01, site 4 = 0.04, site 5 = 0.04, site 6 = 0.03, and site 7 = 0.01). At site 1, the association was not significant (*P* = 0.052). At 4 sites (sites 1, 3, 4, and 5), there was a higher intercept, so the initial SOS was higher. The $\mathrm{CWI_{trot}}$, which was significantly associated with MCIII failure at sites 3 and 7, had a lower slope and a higher coefficient for $\mathrm{CWI_{trot}}^2$. Therefore, as $\mathrm{CWI_{trot}}$ accumulated, SOS increased less in horses with MCIII failure than in horses with MCIII that did not fail. The $\mathrm{CWI_{gallop}}$ (significantly different at site 7) had a negative slope and a negative coefficient for $\mathrm{CWI_{gallop}}^2$ in horses with MCIII failure; thus, in these horses, SOS decreased as $\mathrm{CWI_{gallop}}$ accumulated (**Figure 5**). The $\mathrm{CWI_{race}}$ was associated with MCIII failure at site 1 and had a lower coefficient for MCIII failure, with a higher coefficient for $\mathrm{CWI_{race}}^2$. Therefore, overall, SOS increased less in MCIII that failed than in MCIII without failure.

Discussion

For the group of horses in the study reported here, mean $\mathrm{CWI_{total}}$ and the means of the CWI for the various gaits increased linearly with time. Galloping and trotting were the main gaits used during training, whereas race speed (workouts and races) was performed once weekly or less often for short distances (rarely farther than 1,200 m). Therefore, it was not surprising that $\mathrm{CWI_{race}}$, although being the index for the fastest gait, accounted for minimal amounts of $\mathrm{CWI_{total}}$. The longer gallop distances, together with the higher speed of this gait, explained the reason that $\mathrm{CWI_{gallop}}$ accounted for approximately 75% of $\mathrm{CWI_{total}}$. Therefore, the dorsal aspect of MCIII is exposed daily to tensile forces as a result of training at a trot and a gallop, whereas compressive forces act on the MCIII only sporadically during races and workouts. In light of results of another study[6] and the results of our study, the dorsal aspect of the bone adapted to routine tensile forces and it was not prepared for the high compressive forces that acted on it during races and workouts.

In our population of horses, we detected the highest overall SOS on the lateral aspect of MCIII, which is in agreement with results reported in another study.[21] In that study,[21] investigators attributed differences among aspects (lateral to medial) to the different mechanical properties found for each of the aspects of MCIII. The lateral aspect of MCIII has a higher monotonic strength, whereas the dorsal aspect is more resistant to cyclic fatigue.[23] Furthermore, it has been reported that osteons on the various aspects of the MCIIIs of racehorses have regional variations in size and structure, which has mechanical implications.[24] These differences most likely also contribute to the differences found in SOS.[25] In addition, we found that SOS progressed differently for the various regions of the bone because SOS for the proximal region typically increased as workload accumulated, whereas SOS varied slightly for the middle region and decreased in the distal region. These differences may only be following an anatomic pattern, but the finding that SOS diverged after the beginning of the study may be evidence that the metacarpus adapts to exercise differently in each region.

Overall, $\mathrm{CWI_{total}}$ and SOS were significantly associated. However, SOS was not significantly associated with $\mathrm{CWI_{total}}$ at site 7, which corresponds to the medial aspect of the distal region of MCIII. This is a site where the surface of the bone is irregular, which makes it difficult to consistently place the probe to obtain good sound transmission.[g] Therefore, probe placement during measurements at this site may have been less consistent than at other sites. That fact, combined with the variability of 8% in our QUS system and the low number of horses that completed the 6 SOS measurements, may have been the reason that a significant association was not detected at this site.

With the aforementioned exception, all CWI-SOS relationships had a positive slope, which indicated an overall increase in SOS as workload accumulated. This increment was most likely attributable to the bone's response to exercise as an increase in the apparent BMD and bone volume, as has been reported in other studies.[10,26,27]

The CWIs for the various gaits appeared to be better predictors of SOS in the MCIII. When CWI was considered independently for each gait, an association with SOS was found at all 9 sites, and the R^2 values were higher. In another study,[6] investigators characterized tensile forces that act on the dorsal aspect of MCIII during trotting and galloping and compressive forces at race speed, which elicit a different adaptive response from the bone. Presumably, there will also be differences in the forces acting on the bone as well as other aspects of MCIII. Therefore, it would appear logical that when the CWI is evaluated separately for each gait, it is associated with SOS at more sites.

Race speed is used only sporadically, which results in compressive forces that differ from the usual pattern of forces acting on the bone (tensile forces secondary to trotting and galloping). Thus, it could be expected that this gait would elicit a pronounced adaptive response and that $\mathrm{CWI_{race}}$ would be associated with SOS at most of the sites. However, $\mathrm{CWI_{race}}$ was only associated with SOS at 4 sites, which indicated that racing may have had little impact on SOS because of differences in loading modes or a limited number of loading cycles.

The coefficient of the quadratic equation for work index differed (positive vs negative values) for horses of different ages. The positive sign of the squared term indicated an initial decrease in SOS followed by an increase as $\mathrm{CWI_{total}}$ increased. The negative sign reflected an initial more pronounced increase of SOS followed by a decrease in the rate of increase of SOS per CWI unit and finally a decrease in SOS, although this decrease was small. The positive coefficient for the squared term for most of the sites in the 2-year-old horses could have been a reflection of a pronounced response to exercise in horses that have not been trained previously. In these situations, there is an initial deposition of new bone to adapt the bone's geometry to the mechanical necessities imposed by the exercise. This bone is deposited at a high rate, which incites a deposition of preferentially nonorientated woven bone.[6] This bone has higher porosity than mature bone and could have been the reason that SOS decreased initially. This agrees with the hypothesis proposed in another study[6] that inertial properties in MCIII have a more pronounced initial increase as horses undergo training. This increase is intended to reduce strain and deformation as determined on the basis of the fact that the most substantial changes to the MCIII's second moments of inertia were detected in Thoroughbreds at 1 to 2 years of age, and the change continued (although to a smaller degree) until horses were 4 years old.[6] When this bone is remodelled into mature bone with higher BMD, there is a consequent increase in SOS. As work continues to accumulate, SOS continues to increase, but as in most biological processes, subsequent increments will reach a maximum. In this case, the maximum will probably be limited by the maximum apparent BMD that bone can achieve under physiologic conditions, which could be the reason that the 3-year-old horses had primarily a negative coefficient for the squared term. Because these horses had been in training for 1 year prior to the study and had already made adaptations of the metacarpal bones in response to exercise, they had a less pronounced response to exercise than did the 2-year-old horses. Bone remodelling in the 3-year-old horses was less intense and reached a plateau late during the racing season. The low number of horses that remained in the study for the last SOS measurements may have been the reason that SOS at some sites appeared to decrease, and it explains the negative coefficient for the squared term.

Sex affected the association between CWI_{total} and SOS at 5 sites, with consistencies evident among sites. Differences in SOS among horses on the basis of sex also have been reported by others,[21,28] and they were explained as an effect of sex hormones on bone metabolism. Therefore, sex should be considered when assessing SOS progression of a specific horse.

At most of the sites, limb (right vs left) had a significant effect on the association between CWI_{total} and the CWIs of the various gaits with SOS. This was most likely attributable to different forces in the limbs. In North America, racehorses race and train by running in a counterclockwise direction; this results in differences in loading patterns on the right and left limbs. These differences in loading patterns are also the reason for differences in the type of fractures seen most commonly in each limb. Lateral condylar fractures affect the right forelimb twice as often as they affect the left forelimb, and the right forelimb is more likely to sustain a complete, displaced fracture, whereas the left forelimb is more likely to sustain an incomplete, nondisplaced fracture.[8,29]

Although the study reported here was not specifically designed for the investigation of SOS and bone failure, we attempted to investigate differences in SOS progression as CWI accumulated between horses that sustained bone failure and those that did not. Bone failure had a significant effect on the association between SOS and CWI_{total} in the middle lateral site, where the model for MCIIIs with and without bone failure had a similar curve. However, there was a higher intercept for horses that had bone failure, so SOS was slightly higher in them. That contrasts with results of an unpublished postmortem study conducted by our laboratory group in which it was found that fractured MCIIIs of racehorses had a lower SOS (10% lower) than was found in nonfractured MCIIIs of racehorses. The reason for that finding is not clear, but it may have been attributable to the grouping of periosteal reactions (dorsal metacarpal disease and metacarpal disease in the second or fourth bones) and fractures listed under the term bone failure. The fact that only 2 horses had a fracture in the forelimbs (1 condylar fracture and 1 incomplete, nondisplaced [ie, saucer] fracture) prevented independent analysis of the effect of this condition on the association between SOS and CWI_{total}.

Models accounting for the work indexes for each gait were better at identifying differences between MCIIIs with signs of bone failure and MCIIIs without failure. Failure of MCIII affected the association between CWI_{trot} and SOS, CWI_{gallop} and SOS, and CWI_{race} and SOS at 5 sites.

From a clinical point of view, it would be desirable to find thresholds that allow identification of horses at risk for bone failure. In this study, we evaluated the CWI-SOS association and how this association was affected by MCIII failure and other variables (ie, age, sex, and limb). The identification of such thresholds requires the establishment of tolerance intervals that would include most of the horses without signs of MCIII failure (high sensitivity) but the least number of horses with MCIII failure (high specificity). The data collected in this study were highly variable, most likely secondary to the variability of the QUS system and intrinsic variability among horses. This resulted in overlapping of SOS for horses with and without MCIII failure, which prevented the effective establishment of a threshold. Nevertheless, we found that the CWI-SOS association at various sites was altered by MCIII failure, which suggested that QUS may allow discrimination of horses at risk for MCIII failure, provided a slightly more complex algorithm is used.

Three investigators collected SOS measurements during the study. The interoperator coefficient of variation for SOS measurements was calculated to be 0.78% to 2.70% in 1 study.[20] In other studies, investigators examined variability in the precision after repositioning the probe and calculated values of 0.94% to 1.3% at the dorsal aspect, 0.66% to 1.79% at the medial aspect, and 0.62% to 1.82% at the lateral aspect of the middle region of the MCIII[20] and between 0.25% to 2.08%.[h]

In the study reported here, we identified an association between the workload and SOS in the MCIII of active racehorses. This association was stronger when the CWIs for each gait were used. Models that accounted for the workload accumulated at various gaits provided a stronger basis for discriminating between clinically normal horses and horses with MCIII failure. The variability of our data, loss of horses from the study, and small population sample impeded the establishment of simple thresholds to identify horses at risk for MCIII failure, but the association between SOS and CWI will allow a predictive algorithm to be used. Future studies conducted with QUS should focus on additional quality-assurance procedures for the QUS system and larger cohorts of horses to ensure a sufficient number with bone failure. Quantitative ultrasonography may be a useful tool to reduce catastrophic musculoskeletal injuries in racehorses through early identification of horses at risk.

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a. Polytrack, Martin Collins Surfaces & Footings, Lexington, Ky.
b. Omnisense, Sunlight Ltd, Rehovot, Israel.
c. CM-probe, Sunlight Medical Ltd, Rehovot, Israel.
d. Ecogel 200, Eco-Med Pharmaceutical Inc, Mississauga, ON, Canada.
e. Perspex, Sunlight Ltd, Rehovot, Israel.
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