The purpose of this investigation was to evaluate the clinical usefulness of the total radiographic bone aluminum equivalency (RBAE) method for the measurement of bone mineral content (BMC) in the third metacarpal bone (McIII) of the young Thoroughbred by examining: a) changes in BMC and bone metabolism marker levels in young Thoroughbreds being trained in the growth period; b) correlation of BMC and bone metabolism marker levels; and c) relationships of BMC and bone metabolism marker levels with the state of occurrence of bone disorders. The total RBAE method by the ortho system was chosen for this study. Ninety-one 2-year-old Thoroughbreds that showed no abnormalities on physical examination before the study were evaluated for BMC, and 39 were selected at random for evaluation of the bone metabolism marker levels. BMC of the normal horses showed no change between Days 0 and 90 of the study, but significantly increased on Day 180 (p<0.01). BMC showed no correlations with bone metabolism markers. In addition, there was a high incidence of bone disorders in Days 0–90 of the study when BMC did not change though training increased. These results suggest that the measurement of BMC by the total RBAE method in young Thoroughbreds is useful for providing information about skeletal development, and also for obtaining information on the predisposing period for bone disorders.

Key words: BMC, bone disorder, bone metabolism marker, horse
Concerning methods for the evaluation of bone remodeling, which determine BMC, the measurement of bone metabolism markers has been reported to be effective [7, 13, 36]. The concentrations of bone metabolism markers have also been reported to be related to bone disorders. Osteocalcin (OC) and type I collagen cross-linked C-telopeptide (ICTP) levels were higher in young Thoroughbreds that developed bucked shine [16], and a relationship between the occurrence of osteochondrosis and the OC level was reported in young Warmbloods [3]. Therefore, evaluation of changes in these parameters associated with training is considered to be important for the assessment of the training effect and the prevention of training-induced bone disorders. However, there have been no reports on measurement of BMC using the total RBAE method together with biochemical markers and incidence of bone disease in the young Thoroughbred.

In this study, BMC was measured by the total RBAE method in McIII of young Thoroughbreds to evaluate the clinical usefulness of the method for the assessment of the effect of training by examining: 1) changes in BMC and bone metabolism marker levels in young Thoroughbreds being trained in the growth period; 2) changes in bone metabolism marker levels in young Thoroughbreds; 3) correlation of BMC and bone metabolism marker levels; and 4) relationships of BMC and bone metabolism marker levels with the state of occurrence of bone disorders.

**Materials and Methods**

**Horses**

Ninety-one 2-year-old Thoroughbred horses (52 males and 39 females) that showed no abnormalities on physical examination at the start of the study were evaluated for BMC. Thirty-nine of them (24 males and 15 females) were selected at random for bone metabolism marker levels. Their mean age at the beginning of the study was 20 ± 0.8 months. All horses were trained in the training stable for races.

**Training and management of the horses**

The horses were broken-in riding for about 60 days prior to the beginning of this study. Their management methods had also been changed from pasturing to stabling with the beginning of the training and were released daily in a paddock 5 m × 5 m for 3–5 hr after training. They were trained 6 days a week, and a sand track course was mainly used.

Prior to the start of this study, all horses were exercised at a slow canter. The training intensity was modified depending on the fitness of each horse. The running distance (m) and velocity (m/min) were recorded as indices of the exercise intensity for 90 days prior to the beginning of this study.

**Measurements of the total RBAE**

The total RBAE was measured 4 times: at the beginning of this study (December, 2003; Day 0) and on Days 90, 180, and 270 of the study. BMC immediately below the nutritional foramen of the left McIII was calculated by the total RBAE method. A detailed description of the total RBAE method was reported in a previous study [19]. For radiography, a common portable X-ray generator (FPS-X, Flat Co., Kobe) and orthotype intensifying screen and film were used. Aluminum step wedges (Aluminum Type 2024 11 Step Wedge, X-ray Marketing Associates Inc., IL) were attached to each radiographic cassette, and dorsopalmar views were taken. The films were developed with an automatic Roentgen developing machine (X-Omat 1000 Processor, Kodak, Tokyo), and they were analyzed using a photodensitometer (GS700, BioRad, Tokyo).

**Measurements of the bone metabolism markers**

Blood samples were collected from the jugular vein at the same hour on Days 90 and 270 of the study, because the marker levels are known to show marked diurnal changes. They were immediately centrifuged (2,300 g × 20 min). The serum was stored by freezing at −80°C, and the concentrations of bone metabolism markers [OC, type I procollagen carboxyterminal propeptide (PICP) as bone formation markers and ICTP as a bone resorption marker] were determined. The measurements were performed by RIA (double-antibody method) using commercial kits, OC (DiaSorin Inc., Stillwater, MN) [29] and PICP/ICTP (Orion Diagnostica, Oulunsalo, Finland) [32]. Lepage et al. (1990) reported on the validity of the commercial kit for horse OC [20]. The intra- and inter-assay coefficients of variation were reported as 5.2% and 5.9%, respectively, for the OC commercial kit [15]. The measurement range of the OC commercial kit is 0.78–25 ng/ml. Price et al. (1995) reported on the validity of the commercial kit for horse PICP [32].
intra- and inter-assay coefficients of variation were reported as 4.5% and 6.6%, respectively, for the PICP commercial kit [15]. The measurement range of the PICP commercial kit is 6.25–500 ng/ml. Price et al. (1995) also reported on the validity of the commercial kit for horse ICTP [32]. The intra- and inter-assay coefficients of variation were reported as 4.2% and 5.4%, respectively, for the ICTP commercial kit [15]. The measurement range of the ICTP commercial kit is 0.25–50 ng/ml.

Statistical analyses
All statistical analyses were conducted using John’s Macintosh Product (JMP) 5J (SAS Institute Japan, Tokyo). The data for BMC, bone metabolism markers and exercise intensity are presented as mean ± S.D. Monthly changes in the values of BMC and bone metabolism markers were assessed by Tukey’s HSD test. The analysis of correlations between BMC and bone metabolism markers were determined by Spearman’s rank correlation coefficient (rs), and comparisons between the sexes were determined using the t-test for homoscedastic data and Welch’s test for non-homoscedastic data. All significance levels were set as 5% or 1%.

Results
Changes in exercise intensity
The running distance was 434 ± 670, 1776 ± 811, 2354 ± 536, and 2270 ± 423 m on Days –90–0, 0–90, 90–180, and 180–270, respectively (Fig. 1). Both the distance and velocity increased significantly (p<0.01) with time between Days –90–0 and 90–180, but no change was observed between Days 90–180 and 180–270. No sex difference was observed in the distance or velocity.

Changes in BMC of healthy horses
Twenty-two horses that needed rest of 1 week or longer due to injury or locomotive diseases during this study were excluded from the measurement of BMC of healthy horses, the horses that left the training stable to race were also excluded. Therefore, BMC was measured in 69, 68, 54, and 46 horses on Days 0, 90, 180, and 270 of the study, respectively. BMC of healthy horses significantly increased from Day 90 to Day 180 of
The BMC below the nutritional foramen of the left McIII was 984 ± 246, 974 ± 186, 1,146 ± 222, and 1,128 ± 141 mm²Al on Days 0, 90, 180, and 270 of the study, respectively, in the males and 898 ± 226, 897 ± 151, 1,106 ± 218, and 1,026 ± 157 mm²Al, respectively, in the females (Fig. 2). BMC was significantly increased on Days 180 and 270 of the study in the males, and on Day 180 in the females, compared with the levels on Days 0 and 90 (p < 0.01), but the differences between Days 0 and 90 and between Days 180 and 270 were not significant in the males or females. BMC was significantly higher in the males than in the females on Day 270 (p < 0.05).

Changes in bone metabolism marker levels of the healthy horses

Nine horses that had bone disorders during this study were excluded from the measurement of bone metabolism markers of the healthy horses. The values of bone metabolism markers significantly decreased (p < 0.01) with time except for OC in the females (Table 1). No sex difference was observed in the bone metabolism marker levels.

Correlation of BMC and bone metabolism marker levels

The BMC showed no significant correlation with any bone metabolism marker on Days 90 and 270 of the study (Table 2).

Relationships of the occurrence of bone disorders with BMC and bone metabolism marker levels

Twenty-two horses developed disorders and required a rest of 1 week or longer. Sixteen of them developed bone disorders (Table 3). Of the bone disorders, 56% occurred between Days 0 and 90, 25% occurred between Days 90 and 180, and 19% occurred between Days 180 and 270 (Fig. 3). No significant difference was observed in the BMC or bone metabolism marker levels between the horses that developed bone disorders and those that did not (Table 4).

Discussion

BMC has been reported to decrease by 0.45% during a 1-week rest [30]. Therefore, in the present study, BMC was measured after excluding 22 horses that required a rest of 1 week or longer and also those horses that left the training stable to race. After exclusions, BMC was measured 237 times in 69 healthy horses.

Table 1. Changes in the bone metabolism marker levels of the healthy horses

<table>
<thead>
<tr>
<th>Males (n=20)</th>
<th>Females (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 90</td>
<td>270</td>
</tr>
<tr>
<td>OC</td>
<td>16.42 ± 3.27</td>
</tr>
<tr>
<td>PICP</td>
<td>704.45 ± 397.5</td>
</tr>
<tr>
<td>ICTP</td>
<td>15.38 ± 1.72</td>
</tr>
</tbody>
</table>

OC: osteocalcin, PICP: type I procollagen carboxyterminal propeptide, ICTP: type I collagen cross-linked C-telopeptide (Unit: ng/ml). *: Data significantly different from Day 90 (p<0.01). The bone metabolism marker levels significantly decreased with time, except for OC in the females (p<0.01).

Table 2. Correlation of BMC with bone metabolism marker levels

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>rs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC vs OC</td>
<td>30</td>
<td>0.004</td>
<td>0.98</td>
</tr>
<tr>
<td>BMC vs PICP</td>
<td>30</td>
<td>-0.27</td>
<td>0.15</td>
</tr>
<tr>
<td>BMC vs ICTP</td>
<td>30</td>
<td>-0.15</td>
<td>0.44</td>
</tr>
<tr>
<td>Day 270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC vs OC</td>
<td>30</td>
<td>-0.03</td>
<td>0.89</td>
</tr>
<tr>
<td>BMC vs PICP</td>
<td>30</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>BMC vs ICTP</td>
<td>30</td>
<td>0.08</td>
<td>0.66</td>
</tr>
</tbody>
</table>

OC: osteocalcin, PICP: type I procollagen carboxyterminal propeptide, ICTP: type I collagen cross-linked C-telopeptide, rs: Spearman’s rank correlation coefficient. BMC showed no significant correlation with any bone metabolism marker on Days 90 and 270 of the study.
BMC and exercise intensity increased with time. However, between Days 0 and 90 of the study, no change was observed in BMC despite an increase in the exercise intensity. It has been reported that BMC of McIII diaphysis of the Thoroughbred racehorse increases linearly from 2 to 4 years of age [39]. On the other hand, in reports concerning racehorses in the growth period, decreases in the BMC immediately below the nutritional foramen of the McIII associated with the beginning of training have been described [26, 31]. In these reports, BMC continued to decrease for about 50–100 days after the beginning of training and did not return to the initial level even after 240 days. An increase in bone resorption associated with the beginning of training is considered to be the cause of these decreases in BMC.

Bone is known to develop a structure optimal for resisting mechanical stress imposed on it, and this property is known as Wolff’s law [5, 24]. Also, bone has been reported to adapt to mechanical stress applied to it [11]. Such adaptation of bone to stress is made through bone turnover implemented by osteoclasts and osteoblasts. Bone turnover is divided into 4 stages: the activation period, resorption period, reversal period, and formation period.

<table>
<thead>
<tr>
<th>Table 3. Bone disorders</th>
</tr>
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<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>Splint</td>
</tr>
<tr>
<td>Bucked shine</td>
</tr>
<tr>
<td>DOD</td>
</tr>
<tr>
<td>Sesamoiditis</td>
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<tr>
<td>Total</td>
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</table>

DOD: Developmental Orthopedic Diseases. The incidence of bone disorder was the highest between Days 0 and 90 of the study. ( ) shows the number of horses that had their bone metabolism markers measured.

<table>
<thead>
<tr>
<th>Table 4. Changes in the bone metabolism marker levels in uninjured and bone injury horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 90</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>U-i B-i</td>
</tr>
<tr>
<td>OC</td>
</tr>
<tr>
<td>PICP</td>
</tr>
<tr>
<td>ICTP</td>
</tr>
</tbody>
</table>

Uninjured: normal horses (n=30), Bone injury: horses in which bone disorders occurred. *: Data significantly different from Day 90 (p<0.05). **: Data significantly different from Day 0 and 90 (p<0.01). No significant difference was observed in the bone metabolism marker levels between the horses that developed bone disorders and those that did not.

Fig. 3. Relationships of the occurrence of bone disorders with BMC. Uninjured: normal horse (n=69, 68, 54 and 46 on Days 0, 90, 180 and 270 of the study, respectively). Bone injury: horses with bone disorders (n=16 on all days). % bone injury: incidence of bone disorders. *: Data significantly different from Day 90 (p<0.05). **: Data significantly different from Day 0 and 90 (p<0.01). There was a high incidence of bone disorders (56%) on Days 0–90 when BMC did not change though training increased.
and formation period. Exercise stimulates the bone promoting bone turnover; bone resorption is considered to be activated first [2]. In the equine McIII, it has been reported that bone resorption associated with the activation of bone remodeling at the beginning of training continues for 30 days and that about 90 days is necessary for BMC to return to the level before the beginning of training [28].

There has also been a report of decreases in BMC associated with management change from pasturing to stabling at the onset of training [14]. In the study by Nielsen and Porr et al. [26], horses were moved from pasture to stalls at the onset of training. The young horses used in the present study had also been trained for about 60 days before the beginning of the study, and the facility in which they were kept had been changed from pasture to a small paddock. Therefore, we consider the period between Days 0–90 of the study, in which no change in the BMC was observed, was a period of reversal of bone metabolism in which BMC was decreased by the change in animal management and increased by bone resorption induced by training. No significant change was observed in BMC between Days 180–270 of the study, probably because there was no change in the exercise level. Therefore, in young Thoroughbreds managed as described above during the early period of training, BMC is considered to increase with the training level for approximately 180 days after a reversal period, which continues for 90 days or longer.

BMC was significantly higher in the males than in the females on Day 270 (mean age 29 months) of the study. BMC has been reported not to differ between males and females in Quarter horses for 1 year after birth [34] or young Thoroughbreds aged about 20–25 months [31]. The results of BMC measurement in the diaphysis of human antebiachial bone by peripheral quantitative CT (pQCT), show that BMC is higher in males than in females [25]. No significant sex difference was noted in the BMC of horses [6], that the OC and ICTP levels are higher in males than in females [17], and that there is no sex difference in the OC or ICTP level in Warmbloods [21]. No significant sex difference was noted in the present study. The differences among these reports suggest that the bone metabolism markers depend on the bone growth stage [17], type of the horse [17], sex hormone [6], and exercise intensity [38, 40]. However, the reasons remain unclear and further study to elucidate them is necessary.

BMC showed no significant correlation with any bone metabolism marker on Days 90 and 270 of the study. We speculate that the reason for this is that the bone metabolism markers show the situation of the bone metabolism at present, while BMC shows the amount of the bone mineral accumulated every day.

During this study, 16 horses developed bone disorders and required a rest of 1 week or longer. Of these horses, 4 developed bucked shine, a percentage that was less than previously reported for racehorses [28]. This difference may be due to the training intensity in young horses being lower than that in racehorses. OC and ICTP levels were reported as being higher in young Thoroughbreds that developed bucked shine [16], and a relationship between the occurrence of osteochondrosis and the OC level was reported in young Warmbloods [3, 9]. Therefore, we compared BMC and bone metabolism marker levels between the horses that developed bone disorders and those that did not, but noted no significant difference. The absence of a difference may have been due to the relatively long interval of measurement (180 days), rarity of severe bone disorders such as fracture, and the mildness of the disorders, which allowed resumption of training after a short rest in most horses. In future studies, we consider the evaluation of factors such as the severity of disorders and the interval of measurement to be necessary. Of the bone disorders observed during this study, 56% occurred between Days 0 and 90 of the study, 25% occurred between Days 90
and 180, and 19% occurred between Days 180 and 270. In Quarter horses, bone disorders are reported to have occurred in 15 (28%) of 53 horses, with 50% of them occurring in the periods with reduced BMC [27], similar to the results of our present study. Therefore, the periods with an increased training level, during which BMC remains low due to reversal of bone turnover, should be regarded as risk periods for bone disorders in young Thoroughbreds.

The present results suggest that the measurement of BMC by the total RBAE method immediately below the nutritional foramen of the McIII in young Thoroughbreds is useful for providing information about the skeletal development, and for obtaining information on the predisposing period of bone disorders.

Acknowledgments

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