Bone Mineral Analysis through Dual Energy X-Ray Absorptiometry in Laboratory Animals

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(Received 20 April 2009/Accepted 17 July 2009)

ABSTRACT. To determine how to eliminate species difference in animal bone experiment, bone mineral content (BMC) was measured using dual energy X-ray absorptiometry (DXA) on the femurs of laboratory mice (Mus musculus) and rats (Rattus norvegicus), and common marmosets (Callithrix jacchus). Measures were taken on femurs in situ, detached from the body, skinned and defleshed, or dried completely. When the BMC of the bone measured in the intact limb attached to the trunk was set at 100%, the actual BMC of the dry bone was 58.7 ± 11.5% in mice and 103.2 ± 3.2% in rats. Similarly, the bone area (Area) and bone mineral density (BMD) of the dried femur was significantly lower in the mouse femurs than intact limb. Thus, soft limb tissue such as skin and muscle modified the BMC, Area, and BMD only in mouse but not in those from rats or marmosets. The bone mineral ratio (BMR; BMC divided by dry bone weight) was nearest to the human bone value in the rat femurs, whereas the mouse femur BMR was the most different. The BMR was proved completely. When the BMC of the bone measured in the intact limb attached to the trunk was set at 100%, the actual BMC of the dry bone was significantly lower in the mouse femurs than intact limb. Thus, soft limb tissue such as skin and muscle modified the BMC, Area, and BMD only in mouse but not in those from rats or marmosets. The bone mineral ratio (BMR; BMC divided by dry bone weight) was nearest to the human bone value in the rat femurs, whereas the mouse femur BMR was the most different. The BMR was proved to be a practical index in evaluating bone characteristics in laboratory animals, but the mouse femur might not be suitable as an animal model for research into the aging of human bone.

KEY WORDS: bone mineral content, bone mineral density, bone mineral ratio, dual energy X-ray absorptiometry, femur.

Longevity sciences which consist of gerontology, geriatrics and sociogerontology require practical animal models of basic aging processes. Many animal models (e.g., Caenorhabditis elegans [4], Drosophila [17], rodents [15] and primates [6]) have been used for such studies. However, species-specific factors sometimes produce results that cannot be extrapolated to human beings, and although experimental animals are bred under special conditions, researchers sometimes forget this limitation. Therefore, we need to categorize the species-specific physiology of animals.

The temporal measurement of human bone mass is very important for the diagnosis of osteoporosis [5, 7, 8]. Dual X-ray absorptiometry (DXA) is widely used in the world for the analysis of bone mineral content (BMC). The advantage of DXA is that it is noninvasive and uses low radiation dosage. Therefore, many bone analyses have been performed on experimental animals using DXA. However, bone characteristics and size differ between animals and humans. Above all, the bone mineral density (BMD) of the femur measured by DXA varies widely from about 30 mg/cm² in mice to about 830 mg/cm² in humans [14].

The present study was conducted to evaluate such differences and to test whether different preparation methods modify bone measurements. We report here on variations in animal bone content characteristics and on new methods for evaluating bone composition.

MATERIALS AND METHODS

A total of five C57BL/6CrSlc mice (Mus musculus) (9.8–12.8-month-old male) and five F344/NSlc rats (Rattus norvegicus) (12.7–13.2-month-old male) were used. Mice and rats were established at the Aging Farm of National Center for Geriatrics and Gerontology (NCGG) (Aichi, Japan), and were housed in conventional temperature- and humidity-controlled animal facilities [15]. Additionally, two common marmosets (Callithrix jacchus) (a 12.5-year-old male and a 6.5-year-old female), laboratory simian, reared at Clea Japan, Inc. (Tokyo, Japan) were used. The animals used here were died by natural death (abnormality was not observed in the bone) during housing. The bodies were frozen immediately and stored at ~80°C.

All bodies were thawed gently, and the BMC of the right...
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femur was analyzed using a DXA scanner (DCS600EX-IIIR, ALOKA Co., Ltd., Tokyo, Japan). The DXA scanner set up for the analysis of small animals. The BMC, bone area (Area), and BMD measures were performed on four different preparations of the legs as follows: 1) attached to the trunk (At), positioned dorsoventrally, in situ, except for common marmoset; 2) detached from the trunk (Dt); 3) free of skin and muscle (Fsm) and 4) as completely dried bone (Db) (Fig. 1). All analyses were conducted using the same specimens using high-precision mode DXA at 1 mm scan pitch. The region of interest (ROI) for analysis was the whole femur.

For preparing dry bone specimens, the right hind limb was autoclaved at 121°C for 5 min. The soft tissue was removed from the femur by incubating it in 0.5% papain solution (ED 3.4.22.2, Merck KGaA, Darmstadt, Germany) at 37.0°C overnight. After washing and drying, the bone specimen was weighed.

For statistical analysis, the mean and standard deviation (SD) were calculated. Differences among different preparations were compared using Scheffe’s post hoc test after one-way repeated measures ANOVA. A null hypothesis probability of \( P<0.05 \) was considered statistically significant.

The bone mineral ratio (BMR) was calculated by dividing the BMC by the dry bone weight, as described [12, 13]. Differences between mouse and rat measures were analyzed using Student’s \( t \) test after an F test. A null hypothesis probability of \( P<0.05 \) was considered statistically significant.

All experimental procedures and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of the NCGG.

RESULTS

**BMC measures:** The result of BMC was shown in Table 1. The mean BMC of the At mouse femur was 28.1 ± 2.9 mg. The Dt, Fsm and Db BMC values were significantly lower than the At measure (At/Dt; \( P<0.05 \), At/Fsm, At/Db; \( P<0.01 \)). And the Db BMC was significantly lower than Dt BMC value (Dt/Db; \( P<0.01 \)). The BMC of the mouse femur decreased greatly among preparations. In the rat, the mean At BMC was 384.4 ± 15.9 mg. The BMC was not significantly changed among At, Fsm and Db measures, unlike the mouse. The significant difference was detected between Dt and Db BMC (\( P<0.01 \)). In the common marmoset, the Dt BMC was 414.9 mg. The Fsm and Db BMC were 381.6 and 390.4 mg, respectively. Although the common marmoset was only the mean of two examples, the BMC did not change among different preparations in contrast to the mouse case. In order to identify whether the surrounding soft tissue will be measured, the DXA analysis was performed only on skin and muscle additionally. As a result, the BMC was not detected from the skin and muscle of mouse.

**Comparison of femur BMC among three animal species:** When the At BMC was assumed to be 100% in the mouse,

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**Table 1. The bone mineral content (BMC) analysis of the femur in three animal species**

<table>
<thead>
<tr>
<th></th>
<th>Attached to the trunk</th>
<th>Detached from the trunk</th>
<th>Free of skin and muscle</th>
<th>Dried bone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse (n=5)</strong></td>
<td>28.1 ± 2.9</td>
<td>22.9 ± 3.4( a)</td>
<td>19.2 ± 2.5( b)</td>
<td>16.9 ± 3.3( c)</td>
</tr>
<tr>
<td><strong>Rat (n=5)</strong></td>
<td>384.4 ± 15.9</td>
<td>377.6 ± 18.4</td>
<td>385.7 ± 20.6</td>
<td>396.7 ± 13.1( c)</td>
</tr>
<tr>
<td><strong>Common marmoset (n=2)</strong></td>
<td>–</td>
<td>414.9</td>
<td>381.6</td>
<td>390.4</td>
</tr>
</tbody>
</table>

Mouse and rat data are shown as the mean ± SD. Data for the common marmoset are means. 
\( a) P<0.05 \) compared with the value for “Attached to the trunk”. 
\( b) P<0.01 \) compared with the value for “Attached to the trunk”. 
\( c) P<0.01 \) compared with the value for “Detached from the trunk”. 

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Fig. 1. The four methods of preparing the femur for dual energy X-ray absorptiometry (DXA) analysis. The dotted line shows the location of the femur. A, Attached to the trunk (At, only for mouse and rat). B, Detached from the trunk (Dt). C, Free of skin and muscle (Fsm). D, Dried bone (Db).
of the mouse femur was decreased by removing surrounding tissue. In the rat, the significant difference was only detected between At (2.528 ± 0.043 m²) and Db (2.707 ± 0.084 cm²) (P<0.01). The Area value of the common marmoset femurs was stable regardless of the preparation (Dt; 3.843 cm², Fsm; 3.566 cm², Db; 4.052 cm²).

Comparison of femur Area among three animal species: When the At Area was set at 100% in mouse and rat, the Fsm (71.7 ± 4.8%) and Db (71.3 ± 6.8%) Area of mouse were significantly lower than At Area (P<0.01) (Table 2). In contrast, the At Area of rat was significantly lower than Db Area (107.1 ± 3.3%) (P<0.01). In the common marmoset, when the Dt Area assumed to be 100%, the Fsm and Db Area were 92.8% and 105.5%, respectively.

BMD: The result of BMD was shown in Table 3. The BMD of the Db preparation in the mouse (34.3 ± 3.5 mg/cm²) was the smallest of the preparative methods (P<0.01). The rat BMD values did not change significantly among preparations. The common marmoset BMD was 107.8 mg/cm² at Dt, 106.8 mg/cm² at Fsm, and 96.3 mg/cm² at Db.

Comparison of femur BMD among three animal species: When the At BMD value was positioned at 100% in the mouse and rat, the mouse BMD percentages of each preparation were 81.4 ± 12.2% at Dt, 68.2 ± 8.7% at Fsm, and 58.7 ± 11.5% at Db (Table 3). The mouse BMD showed significant decrease among preparations (At/Dt, At/Fsm, At/Db, Dt/Db; P<0.01). In contrast, the rat BMD only showed slightly changes among preparations (Dt; 98.2 ± 4.8%, Fsm; 100.3 ± 5.4%, Db; 103.2%). When the Dt BMD assumed to be 100% in the common marmoset, the Fsm BMD was 99.1% and Db BMD was 89.3%.

BMR: The result of BMR was shown in Table 4. The mean BMR of the mouse femur was 0.435 ± 0.021, whereas for the rat it was 0.501 ± 0.017. The mice BMR was significantly lower than that of the rat (P<0.01). The BMR of the common marmoset was 0.506. Thus, the BMR of the mouse showed low value in contrast to that of other species.

Table 2. The bone area (Area) of the femur in three animal species

<table>
<thead>
<tr>
<th></th>
<th>Attached to the trunk</th>
<th>Detached from the trunk</th>
<th>Free of skin and muscle</th>
<th>Dried bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (cm²)</td>
<td>Area (cm²)</td>
<td>Area (cm²)</td>
<td>Area (cm²)</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.685 ± 0.084</td>
<td>0.591 ± 0.043</td>
<td>0.491 ± 0.033</td>
<td>0.489 ± 0.047</td>
</tr>
<tr>
<td>(n=5)</td>
<td>100.0 ± 10.4%</td>
<td>86.3 ± 6.3%</td>
<td>71.7 ± 4.8%</td>
<td>71.3 ± 6.8%</td>
</tr>
<tr>
<td>Rat</td>
<td>2.528 ± 0.043</td>
<td>2.609 ± 0.075</td>
<td>2.571 ± 0.149</td>
<td>2.707 ± 0.084</td>
</tr>
<tr>
<td>(n=5)</td>
<td>100.0 ± 1.7%</td>
<td>103.2 ± 3.0%</td>
<td>101.7 ± 5.9%</td>
<td>107.1 ± 3.3%</td>
</tr>
<tr>
<td>Common marmoset</td>
<td>3.843</td>
<td>3.566</td>
<td>4.052</td>
<td></td>
</tr>
<tr>
<td>(n=2)</td>
<td>100.0%</td>
<td>92.8%</td>
<td>105.5%</td>
<td></td>
</tr>
</tbody>
</table>

Mouse and rat data are shown as the mean ± SD. Data for the common marmoset are means. The percentage of mouse and rat shows the comparison value when setting the “attached to the trunk” at 100.0%. The common marmoset sets 100.0% at detached from the trunk a) P<0.01 compared with the value for “Attached to the trunk”. b) P<0.05 compared with the value for “Attached to the trunk”.

Fig. 2. Comparison of femur BMC values as percentages. Mean ± SD. ‘Attached to the trunk’ is shown by white bar. ‘Detached from the trunk’ is shown by black bar. ‘Free of skin and muscle’ is shown by dotted bar. ‘Dried bone’ is shown by slashed bar. The value for ‘Attached to the trunk’ is taken as 100% in mouse and rat. In the common marmoset, ‘Detached from the trunk’ is set at 100%. The mouse BMC value decrease when soft tissue is removed from around the femur.
DISCUSSION

In the present study, BMC analysis using DXA was performed on different preparation methods of the femurs from three laboratory animal species. DXA is a quantitative method for analysis of BMC using two different X-ray energies and is widely used in diagnosis and research. However, there has been little research performed on different preparations of the same bone [1, 3, 8, 9]. We investigated whether the method of preparation would modify the bone measurements.

Using DXA for measuring bone composition, it is known that the BMC and BMD are affected by the Area, the size of ROI, the apparatus being used, and observer-related factors [10, 16]. Therefore, in this research, the ROI size was set to cover the whole femur as far as possible. However, the measured BMC, Area and BMD of the mouse femur decreased gradually with preparations in spite of the identical bone. In contrast, the measurement of rat femur was barely affected (Fig. 2). We considered at first that the surrounding soft tissue of the mouse femur was measured as BMC with bone and the results of rat were within the limits of the error of measurement. However, the BMC analysis of only skin and muscle of the mouse showed no value of BMC. These results suggest that the confusing problem exists in the mouse. The BMC of the DXA is calculated by finite difference of X-ray absorption ratio in between hard tissue and soft tissue. The mouse is too small and surrounding tissue of the bone is poor. Moreover, the lower limit of detectable of DXA we used was 22 mg/cm^2 in BMD. The accuracy and precision of DXA in mice may be impaired by the very thin soft tissue and the measure limitation. The similar opinion about the limitation of DXA in mice has been reported by Dickson et al. [1]. Moreover, highly precision and accuracy between in situ bone and in vitro bone in DXA has been reported in rat [3] and guinea pig [2]. Lochmüller et al. demonstrated in the human femur that the in situ BMC correlated with in vitro chemical analysis of the ash weight, calcium, and phosphorus [9]. Also, Svendsen et al. reported that, except for the lateral spine and greater trochanter, the slopes of the linear regressions of in vivo BMC against in vitro BMD were not significantly different in human bones [11]. Thus, no difference between in vivo and in vitro bone measures has been observed in rat size species and over. The difficulty in obtaining accurate data from the mouse femur may have a serious disadvantage for any extrapolation to human tissues.

Using DXA, BMD is calculated by dividing the BMC by the cross-sectional bone area in cm^2. The BMD depends on the bone size and therefore is unreliable for interspecies comparisons of bone characteristics of the animal species. The BMR is a measure in which the BMC is divided by the bone weight. This evaluation can compare the bone composition among species regardless of body size. Tanaka et al. reported using this new evaluation that the F344 rat substrains have clearly different mandibular bone characteristics in terms of BMR [12, 13]. The BMR of the human femur is about 0.557 [14]. In this study, the BMR of the femur was 0.435 ± 0.021 in mice, 0.501 ± 0.017 in rats, and 0.506 in common marmosets. The mouse BMR was the

Table 3. The bone mineral density (BMD) of the femur in three animal species

<table>
<thead>
<tr>
<th></th>
<th>Attached to the trunk</th>
<th>Detached from the trunk</th>
<th>Free of skin and muscle</th>
<th>Dried bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMD (mg/cm^2)</td>
<td>BMD (mg/cm^2)</td>
<td>BMD (mg/cm^2)</td>
<td>BMD (mg/cm^2)</td>
</tr>
<tr>
<td>Mouse (n=5)</td>
<td>41.3 ± 4.0</td>
<td>38.6 ± 3.3^a</td>
<td>38.9 ± 2.3</td>
<td>34.3 ± 3.5^a</td>
</tr>
<tr>
<td></td>
<td>100.0 ± 9.8%</td>
<td>93.4 ± 8.1%</td>
<td>94.2 ± 5.6%</td>
<td>82.9 ± 8.5%</td>
</tr>
<tr>
<td>Rat (n=5)</td>
<td>152.0 ± 4.0</td>
<td>144.7 ± 3.4</td>
<td>150.0 ± 2.5</td>
<td>146.6 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>100.0 ± 2.6%</td>
<td>95.2 ± 2.3%</td>
<td>98.7 ± 1.7%</td>
<td>96.4 ± 3.6%</td>
</tr>
<tr>
<td>Common marmoset</td>
<td></td>
<td>107.8</td>
<td>106.8</td>
<td>96.3</td>
</tr>
<tr>
<td>(n=2)</td>
<td>100.0%</td>
<td></td>
<td>99.1%</td>
<td>89.3%</td>
</tr>
</tbody>
</table>

Mouse and rat data are shown by mean ± SD. Data for the common marmoset are means.

The percentage of mouse and rat shows the comparison value when setting the “attached to the trunk” at 100.0%. The common marmoset sets 100.0% at “detached from the trunk”.

a) P<0.05 compared with the value for “Attached to the trunk”.
b) P<0.01 compared with the value for “Attached to the trunk”.
c) P<0.01 compared with the value for “Detached from the trunk”.
d) P<0.01 compared with the value for “Free of skin and muscle”.

Table 4. Bone weight and bone mineral ratio (BMR) of the femur in three animal species

<table>
<thead>
<tr>
<th></th>
<th>Bone weight (mg)</th>
<th>BMR (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (n=5)</td>
<td>38.7 ± 6.3</td>
<td>0.435 ± 0.021a</td>
</tr>
<tr>
<td>Rat (n=5)</td>
<td>792.6 ± 33.7</td>
<td>0.501 ± 0.017</td>
</tr>
<tr>
<td>Common marmoset</td>
<td>774.1</td>
<td>0.506</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mouse and rat data are shown as the mean ± SD. Data for the common marmoset are means.

BMR=BMC divided by dry bone weight.
a) P<0.01 compared with the value for rat BMR.

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The smallest among three species and was completely dissimilar to human bone. In contrast, the BMR value of the rat and a common marmoset femur was alike, and close to that of human. Similar results have been reported elsewhere [14]. For mice which have small body and weight, high value of the BMR like other species may be not necessary. This result suggests that the mouse femur is not an appropriate model for human bone, and the rat femur is better.

In conclusion, the mouse femur has a species-specific disadvantage as an animal model for aging research on bone characteristics. The BMR may be a practical index in evaluating the bone characteristics in laboratory animals.

REFERENCES