CONSIDERATIONS FOR MECHANICAL TESTING OF MOUSE LONG BONES

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INTRODUCTION

The ability to manipulate murine embryonic stem cells has led to the development of a wide variety of transgenic and knockout mouse models. These animals are valuable tools for skeletal research, allowing the in vivo role of different mutations to be investigated. Assessing the integrity of the skeletons of these mice is important to understanding the functional impact of the genetic manipulations. Skeletal integrity is measured by testing whole bones to failure in a loading mode such as bending, torsion or compression. Long bones such as the femur are commonly tested in bending or torsion, each test having distinct advantages and disadvantages. Torsion and four-point bending have been shown to measure similar failure and stiffness relationships with age [1]. Whether different mechanical tests of the same bones measure similar characteristics is unknown. Therefore, we compared paired whole mouse bones tested in three-point bending and torsion to determine whether the data produced by each test were similar.

One of the advantages of torsion is the insensitivity to cross-sectional orientation [2]. On the other hand, bending depends on the orientation of the cross section (anterior-posterior or medial-lateral). However, the impact of orientation within a given loading plane is unknown. Therefore, we also asked whether within a particular test plane, such as medial-lateral bending, the direction of loading impacted the measured whole bone strength and stiffness of paired mouse long bones.

METHODS

Two series of paired mechanical tests were performed (1) to compare bending and torsion results, and (2) to compare anterior-posterior and posterior-anterior bending. In each test series paired femora and humeri were loaded, each in one configuration, and the data were compared.

Three point bending vs. torsion:

Paired femora and humeri of two strains of 4 month old mice (C3H, n=5; Balb/C-DBA/1 hybrid cross, n=5) were tested in three-point bending and torsion. Left and right bones were assigned randomly to either loading mode. For three point bending, the whole bone was loaded to failure at 0.05 mm/second. A 7-mm span was used for all tests. In the humerus, the central load was applied to the anterior (cranial) surface with the deltoid tuberosity along the bending plane. In the femur, the load was also applied to the anterior surface. Maximum and failure bending moments (Mf, M) and displacements (Df, D) and bending stiffness (EI) were calculated from the load-displacement data. Different maximum and failure deformations indicate postyield deformation. For torsion, the bone ends were potted in polymethylmethacrylate and the exposed gage length measured prior to testing. The torqued bones were tested to failure in torsion at 1 degree/sec. Maximum torque to failure (Tf) and twist to failure and torsional rigidity (GI) were calculated from the torque-angle data. The data were qualitatively compared and statistically analyzed for the effect of mouse strain.

Bending loading direction:

Paired femora and humeri of 2 month old mice (NZB-SWR hybrid cross, n=5) were tested in three-point bending as described above. One bone of each pair was loaded in the anterior to posterior direction (AP bending) and the other bone in the posterior to anterior direction (PA bending). All other parameters were identical between tests. The data were analyzed by paired t-tests (Statview, SAS Institute). p<0.05 was considered significant.

RESULTS

Substantial differences were noted between three-point bending and torsion tests when paired femora and humeri were tested. The torsion tests were nearly linear whereas the bending tests demonstrated a nonlinear toe-region at the beginning of the test as well as a nonlinear postyield region (figure). Postyield deformation was present in all bending tests, but not in any torsion tests. These findings were similar for both the femur and humerus and for the two genotypes.

For three-point bending, the loading direction had significant, but different effects in both the humerus and femur (Table). In the humerus, the bending stiffness was significantly lower and the deformation to maximum moment and to failure were significantly greater when loaded on the anterior surface (AP bending). In the femur, the maximum bending moment was significantly lower when loaded on the anterior surface (AP bending). All other bending parameters were not significantly different between the paired tests.

TABLE: Effect of loading direction on whole bone bending strength of paired long bone, *p<0.05 for AP vs. PA bending

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AP Bending Mean ±SD</th>
<th>PA Bending Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humerus:</strong></td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Mf, N-mm</td>
<td>20.3 ± 2.21</td>
<td>19.7 ± 0.311</td>
</tr>
<tr>
<td>Mi, N-mm</td>
<td>18.4 ± 4.39</td>
<td>18.2 ± 0.635</td>
</tr>
<tr>
<td>EI, N-mm²</td>
<td>2232 ± 713</td>
<td>3727 ± 494</td>
</tr>
<tr>
<td>Df, mm</td>
<td>0.568 ± 0.107</td>
<td>0.321 ± 0.063*</td>
</tr>
<tr>
<td>Df, mm</td>
<td>0.677 ± 0.123</td>
<td>0.411 ± 0.049*</td>
</tr>
</tbody>
</table>

DISCUSSION

Assessing the load-bearing function of murine long bones is critical to understanding the impact of genetic background on the development and maintenance of skeletal integrity in vivo. Bending and torsion have well-established differences [2]. In three-point bending, the failure location is predefined by the central load application point and depends on orientation. The stresses induced include axial tension and compression, shear, as well as compression at the loading points. In torsion, failure occurs at the weakest point along the exposed bone length independent of specimen orientation. The stresses induced are shear and off-axis tension and compression. The presence or absence of postyield deformation in whole bone tests appears to be a characteristic of the loading mode and end conditions, not the bone of interest. The torsional response may not capture the postyield behavior seen in bending. However, bending may produce local crushing and slipping or rotation artifacts that would contribute to the nonlinear behavior. In cortical bending material tests, local surface yielding is present, and could also contribute to the whole bone bending behavior measured here [3]. Further tests are required to answer these important questions.

When performing bending tests, maintaining and reporting consistent orientation is critical to the test outcome. Simply inverting the loading orientation resulted in significant differences between paired bones and produced different effects in the femur and humerus. The differences measured by bending in two different orientations cannot be explained by cross-sectional geometry. In both cases, the bending plane is similar and the appropriate moment of inertia is identical. Whole bone curvature and the microstructure within the cortex likely contribute to the mechanical property differences measured.

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