INTRODUCTION

At the end of a growing long bone, the epiphysis adjacent to the articular cartilage is separated from the metaphysis and diaphysis by a cartilaginous disk known as the growth plate (physis). Growth plate physiology and timing of closure are complex events and vary between bones and among species. The choice of a research model for biomechanical experiments should account for the time of growth plate closure, particularly when dealing with joint structures such as ligaments and tendons. Kilborn et al (2002) compiled from a number of sources the age of sexual maturation and its correlation to the time of growth plate closure in different animal species. In mice, sexual maturity occurs at one month for females and one and a half-months for males. Based on histological criteria, Silbermann and Kadar (1977) reported that the closure of the proximal humeral physis occurs at five months, indicating that sexual maturity precedes cessation of longitudinal bone growth by a considerable time period.

In a previous study we assessed the tensile properties of the medial collateral ligament (MCL) in mice (Wright et al, 2003). We observed that failure in some intact specimens occurred at the femoral physis rather than at the MCL although the mice were sexually mature at the time of testing. The aim of our current study is to determine the age at which the femoral physis will cease to separate in our MCL load to failure model.

METHODS

Fifteen 129X1-SVJ male mice were used in this study. The mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) at three months of age. The animals were sacrificed in a carbon dioxide chamber at four, five and six months. Five animals were used at each time point. Following euthanasia, the mice were frozen at minus seventy degrees until the day of testing. The Animal Studies committee of Washington University approved all procedures.

We used the method described by Wright et al (2003). Prior to testing, both hind limbs from each mouse were disarticulated at the hip. Skin and muscles were then stripped from proximal and distal points of the limb to the knee. The femur and tibia were then potted in 6.4 mm diameter plastic tubes using polymethylmethacrylate (PMMA) to allow gripping of the knee during testing. The specimens were kept moist with saline solution until the time of testing. The specimens were clamped in a custom tensile testing machine with the tibia and femur at 22.5 degrees from horizontal resulting in a knee flexion angle of 45 degrees. The machine consists of a stepper-motor driven lead screw that distracts the proximal end of the specimen horizontally. A 22 N load cell (Transducer Techniques MDB-5, Temecula, CA) monitors the applied force. Force and displacement were recorded using a computerized data acquisition system (Labview 5.0 Austin, TX). Under a dissecting microscope all soft tissues structures of the knee except the medial collateral ligament were cut (LCL, ACL, PCL, and joint capsule). Specimens were then tested to failure in tension by displacing the tibia at 0.25 mm per second. The knee was observed under the microscope to determine whether the failure occurred at the physis or the ligament.
RESULTS

At four months, six (60%) of the ten specimens failed at the femoral physis with five/six (83%) occurring on the right side. At both five and six months, one (10%) of the ten specimens failed at the physis, also on the right side. Of note the 4 mice which failed at the MCL rather than the physis at five and six month of age demonstrated a trend that the right MCL failed at a higher load than the left MCL (table 1).

Table 1: Mean (± SD) ultimate force for the specimens that failed at the MCL.

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean ultimate force</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>5 months</td>
<td>8.93(±1.3)</td>
<td>7.17(±1.5)</td>
</tr>
<tr>
<td>6 months</td>
<td>11.64(±2.6)</td>
<td>7.57(±2.8)</td>
</tr>
</tbody>
</table>

DISCUSSION

Previously we had carried out MCL tension to failure experiments with the assumption that mice reach skeletal maturity and sexual maturity at the same time. Our observation that a high physeal failure rate occurred in 4 month old mice specimens led us examine at what age this ceases to occur as a mean of optimizing our MCL load to failure model. We noted a strong trend that the right MCL was stronger than the left MCL. This was not the focus of our study and future studies using larger numbers of mice would be necessary to demonstrate a greater significant difference. However, previous studies have suggested that limb dominance may contribute to increased ligament strength (Denti et al, 2000). Paw preference was investigated in 114 Sprague-Dawley rats. It was reported that 70.2 percent of rats were right-pawed, 19.3 percent were left-pawed and 11.9 percent were ambidextrous (Pence 2002). The results indicated that the distribution of paw preference in rats is similar to that of other animals and to human handedness. This may explain our results of the mouse MCL being stronger in the right knee than in the left knee.

SUMMARY

Biomechanical testing of the medial collateral ligament with load to failure can result in physeal failure rather than MCL failure in skeletally immature animals. This study examined the age at which a femoral physeal failure ceased to occur in a mouse model of MCL testing. Sixty percent of the knees tested at 4 months failed at the physis rather than at the ligament. However, only ten percent of the knees tested at 5 and 6 months failed at the physis. We now consider that 5 month old mice are functionally skeletally mature and old enough to be tested biomechanically with few failures at the physis.

REFERENCES


ACKNOWLEDGEMENTS

This study was funded by a grant from The National Football League (NFL) Charities, (RWW).