Right–Left Differences in Knee Extension Stiffness for the Normal Rat Knee: In Vitro Measurements Using a New Testing Apparatus

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Knee stiffness following joint injury or immobilization is a common clinical problem, and the rat has been used as a model for studies related to joint stiffness and limitation of motion. Knee stiffness measurements have been reported for the anesthetized rat, but it is difficult to separate the contributions of muscular and ligamentous restraints to the recorded values. in vitro testing of isolated rat knees devoid of musculature allows measurement of joint structural properties alone. In order to measure the effects of therapeutic or surgical interventions designed to alter joint stiffness, the opposite extremity is often used as a control. However, right–left stiffness differences for the normal rat knee have not been reported in the literature. If stiffness changes observed for a treatment group are within the normal right–left variation, validity of the results could be questioned. The objectives of this study were to utilize a new testing apparatus to measure right–left stiffness differences during knee extension in a population of normal rat knees and to document repeatability of the stiffness measurements on successive testing days. Moment versus rotation curves were recorded for 15 right–left pairs of normal rat knees on three consecutive days, with overnight specimen storage in a refrigerator. Each knee was subjected to ten loading–unloading cycles, with the last loading curve used for analysis. Angular rotation (AR), defined here as the change in flexion–extension angle from a specified applied joint moment, is commonly used as a measure of overall joint stiffness. For these tests, ARs were measured from the recorded test curves with a maximum applied extension moment of 100 g cm. Mean rotations for testing days 2 and 3 were 0.81–1.25 deg lower ($p < 0.001$) than for day 1, but were not significantly different from each other. For each testing day, mean rotations for right knees were 1.12–1.30 deg greater ($p < 0.001$) than left knees. These right–left stiffness differences should be considered when interpreting the results of knee treatment studies designed to alter knee stiffness when using the opposite extremity as a control. [DOI: 10.1115/1.4032693]

Keywords: rat, knee stiffness, biomechanics

Introduction

Knee stiffness due to trauma, prolonged immobilization, or surgery is an important clinical problem. It is commonly observed after anterior cruciate ligament (ACL) reconstruction [1–3], patellar realignment or stabilization surgery [1,4], and total knee replacement [1,5]. Such cases are often difficult to manage conservatively and often require surgical lysis of adhesions and manipulation under anesthesia [1,3,6–9]. Use of a suitable animal model to evaluate novel therapeutics for the treatment of joint fibrosis would have direct clinical applicability.

Clinically, a knee with increased resistance to flexion–extension movement is often described as stiff or inflexible. In biomechanical terms, a common method to measure stiffness of a joint is to record its applied moment versus rotation response curve. This curve is normally nonlinear, and joint stiffness can be determined at any point on the curve by computing the slope of a tangent drawn at a specified level of applied moment or rotation. Alternatively, a more simplified measure of overall knee joint stiffness is often defined as the amount of AR that occurs when a specified level of flexion–extension moment is applied. The less the rotation produced, the greater the overall stiffness of the joint.

Due in part to its relatively low procurement and housing costs, the rat knee has been utilized as an animal model for prior biomechanical studies related to ACL reconstruction [10], cartilage repair [11], and joint immobilization [12,13]. However, there are challenging technical problems in recording AR data for a rat knee, most of which are related to its small size and fragile nature. AR data have been recorded for anesthetized rats using finger pressure [14,15] and mechanized loading frames [10,11].

When performing AR measurements on anesthetized animals, there are two separate factors that can influence the recorded values: a myogenic component (caused by muscles spanning the joint) and an arthrogenic component (produced by ligaments, joint capsule, and intra-articular adhesions). In order to determine the arthrogenic contribution alone, the ability to perform AR measurements in a cadaveric specimen is desirable. Postmortem AR measurements for the rat knee have not been reported in the literature.

In order to measure the effects of therapeutic or surgical interventions designed to alter joint stiffness, the opposite extremity is often used as a control. However, variations in normal right–left stiffness differences for the normal rat knee are unknown. If stiffness changes observed for a treatment group are within the normal right–left variation, significance of the results could be open to

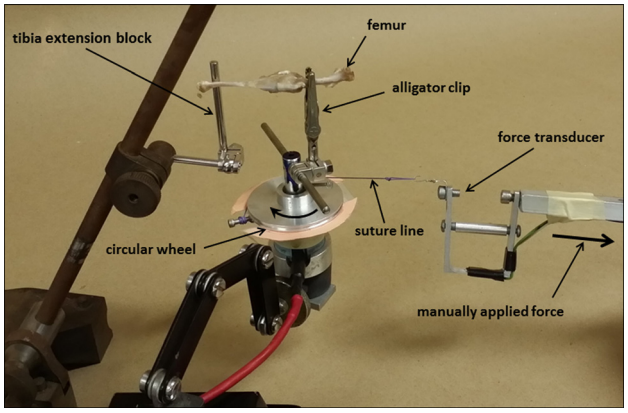

Fig. 1 The test apparatus used to record moment versus rotation response curves during extension of a cadaveric rat knee

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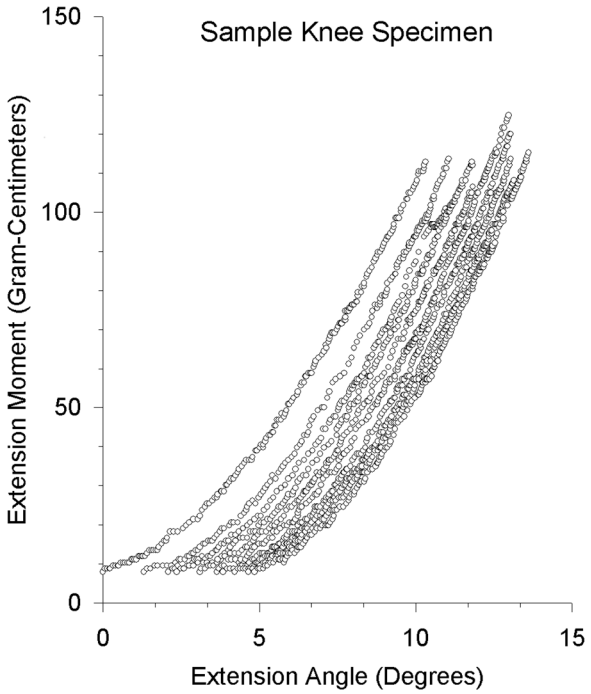

Fig. 2 Raw data recoded for sample rat knee, showing ten successive loading curves for knee extension

challenge. The objectives of this study were to (1) describe a new apparatus for measuring stiffness of the cadaveric rat knee during extension, (2) measure side to side differences in stiffness for a normal population, and (3) document repeatability of the stiffness measurements on successive testing days after refrigerated specimen storage.

Methods

Fifteen male Sprague-Dawley rats, 10 weeks of age and 300–350 g in weight, were used for this study. Approval for use of these animals was granted by our Institutional Review Board. Each animal was immediately frozen after sacrifice for storage. During preparation of the thawed knees for testing, all the muscle tissues were removed from the tibia and femur, leaving the joint capsule and collateral ligaments intact. Each knee was then placed in a polyethylene bag containing normal saline and refrozen.

A custom apparatus, designed to be operated on a flat table, was used for all the testing (Fig. 1). The femur was gripped by a serrated alligator clip that was attached, through an adjustable clamp, to a circular wheel fixed to the shaft of a rotary transducer (Schavetz R30A, Pennsauken, NJ). This transducer (accurate to 0.05 deg) recorded flexion–extension angle of the knee in the plane of the table. A suture line, fixed at the edge of the wheel and seated within a groove at its periphery, was connected to a custom-built and calibrated hand-held force transducer (accurate to 0.2 g). The applied suture force was converted to knee moment by multiplying it by the radius of the wheel (2.5 cm). As extension moment was applied to the femur, the accompanying movement of the tibia was blocked by a vertical rod grounded to the table through an adjustable mounting arm. This configuration resisted the applied extension moment while allowing free internal–external rotation of the tibia and free displacements of the tibia in the proximal–distal and medial–lateral directions.

Alignment of the knee flexion–extension axis with the rotary transducer axis was accomplished by a trial and error procedure. The position of the femur within the alligator clip (and of the clip relative to the wheel) was adjusted until flexion–extension motion of the clamped femur occurred in the plane of the table, and there was no proximal–distal or medial–lateral displacement of the tibia relative to the blocking rod. Lack of these coupled motions indicated that the knee's flexion–extension axis was coincident with the axis of the rotary transducer and the tibia remained stationary in space during testing. An extension moment of slightly more than 100 g cm was found to produce consistent response curves without producing knee dislocation or cruciate ligament rupture. Knee flexion testing was not possible for reasons presented in the "Discussion" section.

Due to the viscoelastic nature of the joint tissues, measured rotations increased for successive testing cycles (Fig. 2). The tenth loading cycle was selected for analysis, based upon the experience of Trudel et al. [15]. The knee extension angle analyzed (AR) was defined as that recorded from the loading curve between 7.5 g cm and 100 g cm of applied moment. Use of a baseline moment of 7.5 g cm eliminated any small variations in rotation output due to frictional effects within the potentiometer and slight initial alignment motions of the tibia and femur within the alligator clips. Unloading curves were not analyzed because they were of less interest clinically.

After the specimen had been aligned within the test apparatus (as described above), the knee extension moment was applied and removed manually ten times in succession at an approximate loading rate of 2.5 g cm/s. The applied moment and knee extension angle were recorded at a sampling rate of 100 Hz using a personal computer containing an A/D board with data-acquisition software.

On the first day of testing, all the right–left pairs were thawed to room temperature and tested for ten cycles. The knees were returned to their individual saline moistened bags for storage overnight in a refrigerator at 40 °F. The following morning, the knees were returned to room temperature and tested again. They were again stored overnight, and on the third day tested a final time.

In order to determine stiffness of the testing apparatus, a special series of tests was performed using a 3.5 mm steel rod mounted in the alligator clip. The distances from the center of joint rotation to the alligator clip and blocking rod were equal to those for a mounted knee specimen. These tests were performed to simulate a knee of "infinite stiffness," which in theory would produce zero rotation for the applied moment levels used in these tests. We found that 100 g cm produced a rotation of 0.1307 deg, due to inherent flexibility of the testing apparatus. Since the rotations recorded for rat knees at this applied moment level were on the order of 6–8 deg, measurement errors due to stiffness of the apparatus were considered negligible.

A two-way repeated measures analysis of variance was used to determine the significance of differences in AR between test conditions; the fixed effects were limb side and day of testing. Multiple pairwise comparisons were made using the Bonferroni procedure. It was determined that a difference of 1 deg in knee extension angle could be detected between testing conditions with 90% power using 15 animals.

Results

There were statistically significant right–left AR differences ($p < 0.001$) for all the testing days (Table 1). There were no significant interactions between the day of testing and limb side ($p < 0.181$). The mean right–left AR differences were 0.99 ± 1.13 deg (day 1), 1.12 ± 1.05 deg (day 2), and 1.30 ± 0.36 deg (day 3).

Mean AR values for days 2 and 3 were not significantly different from each other, but each was significantly less ($p < 0.002$) than the mean for day 1 (Table 1). On a right–left scatter graph (Fig. 3), individual data points for day 3 were more tightly grouped than those for days 1 and 2, and the regression line for day 3 was closer to the ideal slope of 45 deg (which represents right–left equivalence).

Discussion

This apparatus could be potentially useful in testing the effects of therapeutic agents designed to alter tissue properties or reduce the formation of intra-articular adhesions that restrict

postoperative joint motion. It has significant advantages when compared to the hand-held protractor device described by Trudel et al. [15], as it is capable of recording a complete moment versus rotation response curve for an isolated cadaveric rat knee. For this study, a measure of the overall joint stiffness was determined by measuring AR values from the recorded response curves between moment levels of 7.5 g cm and 100 g cm. However, a more detailed analysis would also be possible by computing stiffness from the slope of the test curve at a specified level of applied moment. We chose to report AR measurements for ease of interpretation and consistency with prior published stiffness studies.

There were several limitations to this study. This apparatus was only suitable for testing cadaveric specimens with removed knee musculature. It could not be used to perform in situ measurements on an anesthetized or euthanized animal. Our apparatus provides no means for supporting the weight of the animal during testing and secure clamping the tibia and femur with the alligator clip through intervening skin and musculature would not be possible.

We utilized a hand-held transducer to apply tensile force to the suture line, which in turn produced an extension moment to the knee specimen about the center of rotation. By visualizing a direct readout of the moment versus response curve on a computer monitor, it was possible to control the loading rate in real time. Although this manual control was not as precise as would have been possible with a servomotor, every attempt was made to provide a consistent loading rate to each specimen. We do not believe that variations in loading rate would be a significant source of error in these experiments because any variations in loading rate would have been distributed equally among testing groups.

We were only able to measure moment versus rotation response of the knee during knee extension. This limitation was not due to the test apparatus itself, as the test procedure with knee flexion was exactly the same as for knee extension. The critical problem was inability of the isolated specimen to tolerate knee flexion moment without sustaining knee damage. During preliminary trials, we found that an applied flexion moment of only 50 g cm caused the knee to hyperflex, producing nonphysiological contact between the tibial and femoral shafts and posterior dislocation of the tibia in several specimens. In an intact lower limb, compression of intervening muscle tissue surrounding the tibia and femur limits the amount of joint flexion possible. This muscle tissue had been removed from our specimens, thereby permitting excessive knee flexion and the associated knee damage. Moment levels less than 50 g cm were difficult to apply manually, and the test curves at lower moment levels were erratic and inconsistent.

The greatest source of experimental error with our device was the method for clamping the femur. This clamping system was quick, simple, and practical in terms of aligning the specimen's flexion–extension axis with the rotary potentiometer axis, and there was no visible movement of the femur within the alligator clip during testing. However, when the femur of an individual specimen was removed and reclamped, there was no means for assuring that the femur was gripped in exactly the same position as before. This test–retest reproducibility was difficult to quantify because the recorded curves were also affected by viscoelastic changes in the knee tissues as testing cycles accumulated. That is to say, we had no unchangeable standard that could be used to accurately evaluate clamping errors alone during repeat tests with the same knee specimen.

Table 1 Knee extension angle (deg; between 7.5 and 100 g cm of applied extension torque)

	Day 1	Day 2	Day 3
Right knees	8.29 (±1.46)	7.48 (±1.32)[b]	7.35 (±0.77)[b]
Left knees	7.30 (±1.00)[a]	6.36 (±0.66)[a,b]	6.05 (±0.65)[a,b]

[a]Significantly different from right knees.
[b]Significantly different from day 1.

We found that the mean rotations recorded for days 2 and 3 were approximately 1 deg less than day 1 (Table 1), indicating an approximate 13% change after the first round of cyclic testing and overnight storage. The right–left scatter in knee extension angle for day 3 was noticeably less than for days 1 and 2 (Fig. 3), indicating more consistent and repeatable measurements for both right and left groups with continued cyclic testing. This suggests mechanical stabilization of the viscoelastic tissues with accumulated testing cycles.

Prior biomechanical testing on the human medial collateral ligament [16] and patellar tendon [17] has documented that there were no significant effects of freeze–thaw cycles on the tensile properties of these tissues. It is reasonable to expect that these findings would also hold for rat ligamentous tissues. Therefore, we believe that it is unlikely that overnight storage alone was responsible for the observed reductions in mean extension angle between the first day and successive days, because the mean extension angles for days 2 and 3 were very similar in magnitude (Table 1). Based upon these results, we believe that at least 30 flexion–extension cycles should be performed before recording a final test curve for analysis.

We found that, on average, right knees had significantly greater AR than left knees, indicating less overall joint stiffness. The reasons for this finding are unclear. One possible explanation would be the fact that the femurs of left knees were rotated 180 deg in the alligator clips compared to right knees, meaning that the direction of rotation of the rotary transducer shaft was reversed. However, the output of the rotary potentiometer transducer was highly linear ($r^2 = 0.999$) for either direction of rotation, and we have discounted this factor as an explanation for our findings.

It is also possible that rats have a dominant side for in vivo activities and that increased use of the dominant limb could make it less stiff compared to the opposite extremity. We have been unable to find any prior studies directly related to right–left differences in hindlimb loading patterns or hindlimb dominance in the rat. A study by Fox et al. [18] was able to demonstrate that the combined weights of bones in the forelimbs of rats were significantly greater on the left side compared to the right, and it is possible that there might be hindlimb differences in bone mass as

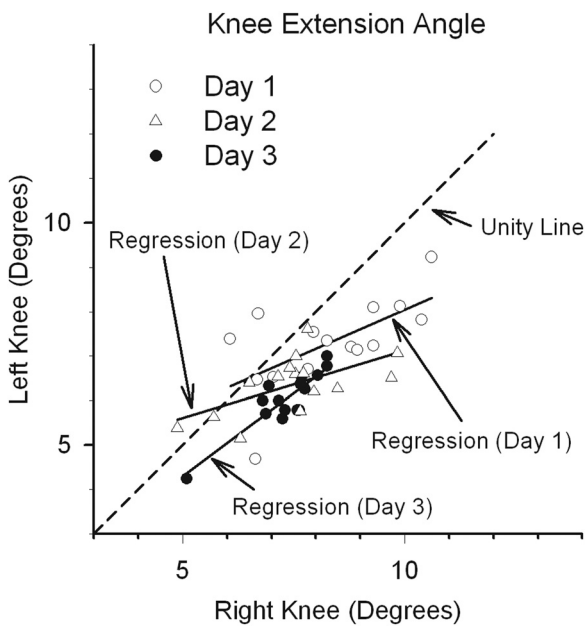

Fig. 3 Right–left scatter plots for testing days 1–3. The unity line represents the ideal situation, where knee extension angles for right knees and left knees are equal. The linear regression lines of data for all the testing days are shown.

well. However, any right–left differences in bone mass may be unrelated to hindlimb stiffness.

We do not believe that our findings indicate an inherent right–left stiffness difference in the rat knee nor do they represent any systematic artifact related to our testing procedure. We are simply reporting that for this group of knees undergoing testing with this particular apparatus, the right knees were significantly less stiff than the left. It is quite possible that there would be no significant right–left stiffness difference or that the right–left findings could have been reversed for a different group of rat knees. The important point to be made is that if knee testing with an apparatus similar to ours was to be used to evaluate the effects of a treatment modality on knee stiffness, our study suggests that a mean rotation difference between treated limb and untreated control would need to be greater than of approximately 1 deg to have high confidence that the observed difference is meaningful and outside the range of normal right–left scatter.

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