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Laxity Characteristics of Normal and Pathological
Murine Knee Joints In Vitro

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Summary: The aim of this study was to validate a device developed previously to measure laxity of murine knee joints and to investigate whether experimentally induced pathological conditions result in measurable laxity. The laxity characteristics of normal murine knee joints were derived from measurements of 25 left knees of normal mice. Reproducible, nonlinear s-shaped load-displacement curves were determined, and parameters of anterior-posterior translation, varus-valgus rotation, and compliance were calculated from the curves. No differences were found between the left and right knee joints of eight mice. The average displacement between 0.8 N of anterior force and 0.8 N of posterior force was 0.47 ± 0.10 mm. The endpoint compliances for anterior and posterior displacements were 0.16 ± 0.03 and 0.16 ± 0.04 mm/N, respectively. The average rotation between a 4 Nmm valgus moment and a 4 Nmm varus moment was 17.4 ± 3.3 °. The endpoint compliances for varus and valgus rotations were 1.1 ± 0.7 and 1.0 ± 0.3 °/Nmm, respectively. Storage of the joints at -70°C had no effect on laxity. We also studied the parameters of laxity after pathology of the knee joint was induced. Zymosan-induced or antigen-induced arthritis did not increase laxity of the joint. In an osteoarthritis model induced by injection of collagenase, laxity was markedly increased. In conclusion, laxity in the knees of mice can be measured reproducibly, and changes in the characteristics of laxity due to pathological conditions can be quantified.

Osteoarthritis is a progressive degenerative disease of the joints, characterized by focal loss of cartilage, formation of new bone at the margins of the joint, fibrosis of the joint capsule, and sclerosis of the subchondral bone. The etiology of the disease is thought to be multifactorial. Joint laxity is well known to be involved in the development of osteoarthritis in humans as well as in animal models (1,4,5,11,13,15-19,23,25,26). Laxity of the joint can be caused by damage to ligaments or menisci. In humans, overdrawing, as may occur in traumatic events, can lead to ligamentous lesions. Furthermore, it is possible that the bulk of mediators and enzymes released in inflammatory conditions lead to damage of the ligament or meniscus.

Recently, van der Kraan et al. (31) developed a model for osteoarthritis of the knee in mice. A single intra-articular injection with highly purified bacterial collagenase caused histological changes, after a month, that mimicked those in osteoarthritis in humans. In an early stage, histology showed damage to ligaments but not to cartilage. We hypothesize that osteoarthritic changes develop due to joint laxity caused by degradation of structures containing type-I collagen, such as ligaments. In humans and in animal models in mice, osteoarthritic changes commonly are found in the late stages of chronic inflammation of the joint (29,30). The osteoarthritis-like changes may be due to laxity of the joint induced by mediators released in inflammatory arthritis.

These hypotheses of increased joint laxity due to injection of collagenase or induction of arthritis can be tested by measurement of joint laxity in murine knees after pathology is induced. Standardized instrumented tests of instability have been successfully performed on human knees in vivo and in vitro and on lapine, canine, and caprine knees in vitro (2,6,9,12,14,20,21,24,34). In an analogous study, an anterior-posterior and a varus-valgus tester were developed for the murine knee (3). The present study was carried out to examine the reliability of this device and to investigate the possibility of quantifying changes in laxity in normal and pathological murine knee joints. Therefore, the amount and variation of anterior-posterior laxity and varus-valgus laxity of 25 normal murine knee joints in vitro were examined. The variability between left and right knees was studied, because usu-
There was a difference between motion from anterior to posterior, in the anterior portion of the experiment. The results obtained from the 4-mm anterior portion (Fig. 1) were compared with those obtained from the 4-mm posterior portion (Fig. 2). The anterior portion showed a higher percentage of movement, whereas the posterior portion showed a lower percentage. This difference was statistically significant, as determined by a t-test.

Materials and Methods

The experiments were conducted using a mouse model. The animals were housed in standard laboratory conditions. The experiments were divided into two portions: anterior and posterior. The anterior portion was defined as the first 4-mm section, whereas the posterior portion was defined as the second 4-mm section. The movement of the mouse was recorded using a high-speed camera.

Results

The results obtained from the anterior portion showed a higher percentage of movement compared to the posterior portion. This difference was statistically significant. The anterior portion showed a higher percentage of movement, whereas the posterior portion showed a lower percentage. This difference was statistically significant, as determined by a t-test.
rior (and varus to valgus), represented by the upper branches of the curves, and motion from posterior to anterior (and valgus to varus), represented by the lower branches. As suggested by Edhoven et al. (8,9), different parameters were defined to describe the curves. The parameters used to describe anterior-posterior shift values (in millimeters) were $S_{0.8}$—the average shift (lower and upper branches together) for a posterior force of 0.8 N, relative to the lower branch position at zero force; $S_{0.8}$—the same as $S_{0.8}$ but at an anterior force of 0.8 N; and TS$_{0.8}$—the total shift between 0.8 N of posterior force and 0.8 N of anterior force. The anterior-posterior compliance values (in millimeters per newton) were $wC_{0.8}$—the average compliance (slope) of the branches (lower and upper branches together) at zero force; $wC_{0.8}$—the same as $wC_{0.8}$ but at 0.8 N of anterior force. The parameters for varus-valgus laxity for rotation (in degrees) were $R_{0.4}$—the average rotation (lower and upper branches together) for a valgus moment of 4 Nmm, relative to the lower branch position at zero moment; $R_{0.4}$—the same as $R_{0.4}$ but at a varus moment of 4 Nmm; and TR$_{0.4}$—the total rotation between 4 Nmm of varus and 4 Nmm of valgus moment. The varus-valgus compliance values (in degrees per newton-millimeter) were $wC_{0.8}$—the average compliance (slope) of the branches (lower and upper branches together) at zero moment.

$wC_{0.4}$—the average compliance at a valgus moment of 4 Nmm; and $wC_{0.4}$—the same as $wC_{0.4}$ but at a varus moment of 4 Nmm.

The parameters were calculated on the basis of two complete measurement cycles of anterior-posterior or varus-valgus laxity. The values were obtained from linearization of the curve segments with respect to certain intervals around the force values concerned, by use of distribution-free methods of estimation (8). $C_{0.8}$, $C_{0.8}$, $C_{0.4}$, and $C_{0.4}$ can be considered as estimates of the endpoint compliances, the inversions of the endpoint stiffnesses.

For statistical analysis of the results, we used the Wilcoxon signed rank test. A p value less than 0.05 indicated a statistically significant difference.

Reproducibility

To determine the variation in laxity parameters between mice, the left knee joints of 25 untreated mice were tested and the parameters just described were calculated. With animal models, often a chemical is injected in the right knee joint, and measurements performed on the right knee joint are compared with those of the un.injected or saline-injected left knee joint. For statistical analysis, the amount of variation between left and right knees is important. Therefore, laxity parameters for both left and right limbs of eight mice were calculated.

For practical purposes, limbs often must be frozen and stored.

**FIG. 2.** Tester for varus-valgus laxity. A = spindle for load application, B = load transducer (load multiplied by moment arm gives the applied moment), C = rotation transducer (shaft encoder), D = low-friction bearings, E = fixation clamps, and F = knee specimen.
RESULTS

Normal Knee Joints (Table 1)

Normal knees showed relatively high compliance around zero force (0.72 ± 0.32 mm/N), progressively decreasing to lower compliance at high anterior force (0.16 ± 0.03 mm/N at 0.8 N) and posterior force (0.16 ± 0.04 mm/N at -0.8 N) (Fig. 3). The average total anterior-posterior shift of a normal murine knee joint between 0.8 N anterior force and 0.8 N posterior force was 0.47 ± 0.10 mm.

The compliance around zero moment was high (5.3 ± 2.6 °/Nmm), progressively decreasing to lower compliances at high varus loads (1.1 ± 0.7 °/Nmm at 4 Nmm) and valgus loads (1.0 ± 0.3 °/Nmm at -4 Nmm) (Fig. 4). The average total varus-valgus rotation of a normal murine knee joint between 4 Nmm of varus moment and 4 Nmm of valgus moment was 17.4 ± 3.3°.

There was no significant difference between left and right knees, as is shown in Table 1. The variation in laxity parameters between left and right knees was

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal murine knees</th>
<th>Left minus right knee joint* (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior-posterior laxity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior (-0.8 N)</td>
<td>0.19 ± 0.05</td>
<td>0.00 ± 0.03</td>
</tr>
<tr>
<td>Anterior (0.8 N)</td>
<td>0.28 ± 0.05</td>
<td>0.01 ± 0.05</td>
</tr>
<tr>
<td>Total (±0.8 N)</td>
<td>0.47 ± 0.10</td>
<td>0.01 ± 0.06</td>
</tr>
<tr>
<td>Compliance (mm/N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral (0 N)</td>
<td>0.72 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Posterior (-0.8 N)</td>
<td>0.16 ± 0.04</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td>Anterior (0.8 N)</td>
<td>0.16 ± 0.03</td>
<td>0.01 ± 0.02</td>
</tr>
<tr>
<td>Varus-valgus laxity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotation (°)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valgus (-4 Nmm)</td>
<td>5.5 ± 1.6</td>
<td>-0.5 ± 1.9</td>
</tr>
<tr>
<td>Varus (4 Nmm)</td>
<td>11.8 ± 2.9</td>
<td>0.8 ± 6.4</td>
</tr>
<tr>
<td>Total (±4 Nmm)</td>
<td>17.4 ± 3.3</td>
<td>0.4 ± 5.0</td>
</tr>
<tr>
<td>Compliance (°/Nmm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral (0 Nmm)</td>
<td>5.5 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Valgus (-4 Nmm)</td>
<td>1.0 ± 0.3</td>
<td>-0.2 ± 0.5</td>
</tr>
<tr>
<td>Varus (4 Nmm)</td>
<td>1.1 ± 0.7</td>
<td>-0.3 ± 0.8</td>
</tr>
</tbody>
</table>

*There were no significant differences between left and right knees. Positive values indicate that the parameter had a higher value in the left limb than in the right limb. Negative values indicate that the parameter is higher in the right limb.
TABLE 2. Laxity parameters of frozen and thawed joints compared with fresh joints

<table>
<thead>
<tr>
<th></th>
<th>Anterior-posterior laxity</th>
<th>Varus-valgus laxity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shift</td>
<td>Compliance</td>
</tr>
<tr>
<td></td>
<td>−0.8 N</td>
<td>0.8 N</td>
</tr>
<tr>
<td>Fresh (n = 8)</td>
<td>0.20 ± 0.02</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Frozen/thawed</td>
<td>0.22 ± 0.03</td>
<td>0.30 ± 0.04</td>
</tr>
</tbody>
</table>

Frozen and Thawed Joints (Table 2)

Joints stored for as long as 1 month showed no effects of freezing and thawing on anterior-posterior or varus-valgus laxity. Therefore, storage of the limbs at −70°C appears allowable. Study of the effect of freezing joints with increased laxity is difficult because of the high variation in response between animals. Our general impression is that freezing does not influence the parameters of laxity of pathological knees.

Inflammation and Osteoarthritis Models (Table 3)

The injection of zymosan led to swelling of the joint and a prominent inflammatory reaction but did not increase laxity. Although zymosan-injected knees seemed stiffer in the anterior-posterior direction when compared with the group of 25 normal mice, comparison of the results from the knees injected with zymosan with those of untreated knees of mice of the same group (data not shown) showed no significant differences. Those untreated knees did not appear to be significantly different from our control group. This showed, however, that in spite of the small variation in laxity parameters, differences between experiments sometimes did exist. No explanation for this variation was found. In general, the deviation from the values for the 25 normal mice was not large and usually was not significant. In order to prevent false positive results, we defined a 95% confidence interval. According to this definition, none of the knees injected with zymosan was significantly different from normal.

Antigen-induced arthritis showed no effects on anterior-posterior laxity on day 3, 14, or 28. The value for varus-valgus rotation was lower on day 28, perhaps because of the presence of osteophytes and calcification of collateral ligaments. However, the significance of these measurements was doubtful because all the results were within the 95% confidence interval of the values for the normal knees.

Collagenase caused minor inflammation but did have pronounced, significant effects on the laxity of the injected joints. It did not have any effect on the contralateral joint. Considerable variation between animals was noted. In general, laxity was increased as high as the variation between knees of different inbred animals.
FIG. 5. Examples of anterior-posterior laxity curves. A: A normal knee joint. B: A joint showing increased anterior laxity and unchanged posterior laxity. C: A joint showing a large increase in anterior laxity and a slight increase in posterior laxity.

more in the anterior direction than in the posterior direction, and valgus laxity was affected more than varus laxity. This is more obvious when, instead of the average values, the median values (which are more accurate, because after injection of collagenase the results are not distributed normally) were compared. Gross increases in varus-valgus laxity were never observed without gross increases in anterior-posterior laxity; gross increases in anterior-posterior laxity were seldom observed without changes in laxity in the varus-valgus direction.

Some typical examples are shown in Figs. 5 and 6. Figure 5B demonstrates an increase in anterior laxity compared with Fig. 5A, which represents a normal knee joint. This increase probably indicates loss of function of the anterior cruciate ligament. Posterior laxity was unchanged. Injection of collagenase sometimes led to destruction of both cruciate ligaments. In Fig. 5C, an increase in posterior laxity in combination with greatly increased anterior laxity is shown. Severe damage to the ligaments, shown by a major increase in laxity, indicates total loss of function of that ligament. Collagenase also can cause damage to the collateral ligaments. Slightly increased laxity in the valgus direction with unchanged varus laxity is shown in Fig. 6B. It also happens that only varus laxity or both varus and valgus laxity are increased (Fig. 6C). Different combinations of damage to collateral and cruciate ligaments were found.

DISCUSSION

Measurement of the laxity of murine knee joints requires detection of small forces and displacements; however, this was shown to be possible with sufficient accuracy. The variations in translational and rotational laxity parameters of a group of normal mice of an inbred strain appeared to be small. Parameters of compliance showed more variation. The good reproducibility, due in part to limited genetic variation, probably was mainly due to the careful positioning of the limbs (24,35,36), which was made possible by the use of the special device. The positioning can be performed in a standardized way because the variation in skeletal morphology in mice 10-14 weeks of age appears to be small (3).

The shapes of the curves very much resemble those
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found for human knees by Edixhoven et al. (8,9). This confirms the reliability of our measurements of laxity. In human knees, considerable scatter is found in left-right differences in normal subjects (22). Left-right differences appeared to be distributed normally in normal subjects but not in injured subjects (6,21,22). In our population of mice, the left and right knees of any one particular mouse appeared to be as similar as knees of different mice. The differences between left and right knees of normal mice showed a normal distribution, with the mean near zero.

In the literature, the reported effects of freezing on properties of the ligament are contradictory. Woo et al. (33) did not find any significant effect of freezing. Only the first cycles showed a decreased hysteresis loop, the importance of which is not clear. According to Dorlot et al. (7), however, ligaments become stiffer after being frozen. In our system, storage at -70°C did not alter the laxity parameters. Storage for longer periods is expected to be acceptable too, because any damage probably will occur mainly during the processes of freezing and thawing. We did not find any effects of freezing and thawing. This would allow storage of limbs at -70°C until they are used for anterior-posterior or varus-valgus testing.

Significant increases of laxity were never seen after injection of the highly inflammatory agent zymosan or in mice with antigen-induced arthritis. These results indicate that inflammation does not evoke significant changes in laxity. Although the possibility of the existence of small changes in laxity, unmeasurable with our device, cannot be completely ruled out, it is concluded that changes in cartilage seen in the chronic phase of antigen-induced arthritis are caused not by changes in laxity but by a direct effect of inflammatory mediators on the cartilage. Changes in laxity were measurable in the osteoarthritis model, however. Induction of instability of the joint by collagenase therefore can be seen as a specific process. This makes it highly likely that osteoarthritic changes due to injection of collagenase are predominantly induced by instability of the knee joint. We showed earlier that in vitro incubation of intact patellae with collagenase had no effects on the metabolism of cartilage (32). After collagenase is injected, the differences between the injected right knee and the uninjected left knee are no longer normally distributed. The extent of the changes in laxity in anterior-posterior and varus-valgus directions was variable, which indicates that the effect of injection of collagenase is not always identical. This may explain the variations in damage that can occur in this model.

Exactly what happened to the ligaments after collagenase is injected remains uncertain. The results of studies concerning the contribution of joint structures to translational and rotational parameters differ. This can be explained by variations in experimental design; differences in angle of knee flexion or degrees of freedom can lead to variable contributions of the structures to laxity of the joint (14,24,27,28,35). To provide more insight into the meaning of increased laxity in our experimental setting, ligaments were sectioned to study their contribution to joint stability in the anterior-posterior and varus-valgus directions. It was quite possible to section the collateral ligaments. Sectioning of the posterior cruciate ligament was less reproducible, and sectioning of the anterior cruciate ligament could be performed with acceptable accuracy only in combination with sectioning of the posterior cruciate ligament. These experiments showed us that, in this experimental setting, anterior-posterior laxity is determined mainly by the mechanical properties of the cruciate ligaments. This also was found for humans, although sectioning of the medial collateral ligament, posterior capsule, or menisci was shown to have little influence on anterior-posterior laxity (10,20). Varus-valgus laxity is affected by sectioning of collateral ligaments. Although transection of the medial collateral ligament caused an increase in valgus rotation in humans and in mice (about 10° in mice), transection of the lateral collateral ligament (alone or in combination with the popliteal tendon) caused an increase in varus rotation in humans but had no effect in our setting (6,20,27). Varus-valgus rotation is not determined only by the mechanical function of the collateral ligaments but also may be influenced by the function of the cruciate ligaments and menisci, although results from the literature are contradictory. Markolf et al. (20) found that varus-valgus laxity was relatively unaffected by transection of the anterior cruciate ligament, whereas Seering et al. (27) and Dahlkvist and Seedhom (6) observed that valgus laxity can be increased after transection of the posterior cruciate ligament and that varus laxity can be increased after transection of the anterior cruciate ligament. We also found that elimination of the cruciate ligaments can increase varus-valgus laxity. In our investigation, sectioning of only one ligament never induced total laxity of more than 1 mm in the anterior or posterior direction or more than 45° in the varus or valgus direction. Gross laxity can only be due to combined insufficiency of several ligaments and the joint capsule.

The choice of the reference points for analysis of anterior-posterior translation and varus-valgus rotation is arbitrary. For anterior-posterior translation, the position where the force is zero while the tibia is moved from posterior to anterior is chosen as a reference point. For varus-valgus rotation, the zero loading position when the tibia is moved from valgus to varus direction is used. Therefore, caution is needed for statements about comparison of laxities in different directions. When comparison is necessary, it would
be better to consider different points of zero force for the various directions. This means that the point of zero force of the lower graph in Fig. 3 (motion from posterior to anterior) should be used for laxity in the anterior direction, and the zero force point on the upper graph in Fig. 3 (motion from anterior to posterior) should be used for laxity in the posterior direction. Application of this principle to our data shows that the amount of laxity in the anterior and posterior directions becomes equal (approximately 0.30 mm in both directions). Varus laxity remains higher than valgus laxity in normal murine knee joints, but the difference becomes much smaller (10.7° of varus laxity and 9.0° of valgus laxity). Although the choice of the reference point is arbitrary, the absolute amount of laxity can be used confidently for comparisons of changes in laxity.

We conclude that it is possible to measure laxity of murine knee joints reproducibly with the device developed. Increased laxity in pathologic knees can be quantified. For correct judgment of laxity of the knee, pathological knees should be compared either with the contralateral knee or by application of the 95% confidence interval as a definition for normal knee laxity. Further study is now possible to investigate more precisely the effects of injection of collagenase on laxity and the correlation with damage to cartilage.

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