TECHNICAL NOTE

IN VITRO LAXITY-TESTERS FOR KNEE JOINTS OF MICE*

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Abstract—The knee joints of mice can be used as a model for studying the effects of interventions on knee laxity. The goal of this study was to quantify knee joint laxity *in vitro*. Three devices were developed: a positioning- and cementing device, an anterior–posterior (AP) laxity tester and a varus–valgus (VV) laxity tester. The positioning- and cementing device was used to position the joint in a reproducible way and to attach clamping pins to the proximal femur and distal tibia using PMMA. The clamping pins were used to fix the joint to the AP- and VV-testers. In both testers the load was applied by means of a spindle-actuated spring while load and displacements were measured simultaneously. The load–displacement data were used to calculate displacement and compliance parameters. The performance of the testers was evaluated by testing 5 normal knee joints of 5 mice. Total AP-translation at ± 0.8 N was $0.43 (\pm 0.16 \text{ S.D.})$ mm with compliances of $0.14 (\pm 0.05 \text{ S.D.}) \text{ mm N}^{-1}$ and $0.12 (\pm 0.05 \text{ S.D.}) \text{ mm N}^{-1}$ at 0.8 N posterior and anterior force, respectively. Total VV-rotation at ± 4 Nmm was $17.2 (\pm 2.6 \text{ S.D.})$ degrees with compliances of $0.9^\circ \text{ Nmm}^{-1} (\pm 0.2^\circ \text{ Nmm}^{-1} \text{ S.D.})$ and $1.0^\circ \text{ Nmm}^{-1} (\pm 0.4^\circ \text{ Nmm}^{-1} \text{ S.D.})$ at 4 Nmm valgus and varus moment, respectively. The contributions of the deformations of the bones and the fixtures to the rotations were negligible in the VV-test. In the AP-test they account for approximately $0.07 (\pm 0.03 \text{ S.D.})$ mm of the total AP-translation. This will not affect the utilization of the device for comparative analysis. It is concluded that *in vitro* evaluation of AP- and VV-laxity in knees of mice is feasible with sufficient accuracy for evaluation of changes after ligament damage. Copyright © 1996 Elsevier Science Ltd.

Keywords: Mouse; Knee joint; Laxity tester.

INTRODUCTION

The knee joints of mice can be used as a model for studying the effects of interventions on knee laxity. For example, interventions aimed at inducing osteo- or rheumatoid arthritis may have an effect on knee laxity, which in its turn influences the arthritic process (Brandt *et al.*, 1991; Felson, 1990; Kannus and Järvinen, 1988; McDevitt *et al.*, 1977). The goal of the present study was to quantitatively evaluate knee joint laxity in mice. Standardized instrumented knee laxity tests have been successfully performed on animal knees *in vitro*: rabbits (e.g. Ballock *et al.*, 1989), dogs (e.g. Woo *et al.*, 1987a), sheep (e.g. Amendola and Fowler, 1992) and goats (e.g. Oster *et al.*, 1992). Two different tests are common, the anterior–posterior test to examine the function of the cruciate ligaments (Amendola and Fowler, 1992; Ballock *et al.*, 1989; Oster *et al.*, 1992) and the varus–valgus test to determine the function of the collateral ligaments (Oster *et al.*, 1992; Woo *et al.*, 1987a).

The purpose of this study was to develop devices for evaluation of the laxity characteristics of murine knee joints *in vitro*. The correct position and orientation of the knee relative to the

loading direction has proven to be very important for the laxity test (Woo *et al.*, 1987b, 1991). The main problem in this study was the small size of the joint. Because of its small size, manual positioning of a murine knee joint in the testers is difficult to perform in a reproducible way. Therefore, morphometric studies were performed to determine anatomic landmarks for reproducible positioning. Another difficulty of testing murine knee joints was that very small forces and displacements had to be measured, stressing the importance of minimization of the effects of friction forces. The devices developed were an AP-tester for evaluating anterior–posterior laxity, a VV-tester for evaluating varus–valgus laxity and a separate device to assure standardized positioning of the knee joints in both devices.

MATERIALS AND METHODS

The mouse strain used in this study is C57Black (Van der Kraan *et al.*, 1990). The weight of the adult mouse is about 25 g and its total length without the tail is about 90 mm. The procedures for care and use of the mice comply with the recommendations of the declaration of Helsinki and the IASP.

Only the proximal part of the femur and the distal part of the tibia could be used for positioning and clamping the joint in the testers, because the tissues surrounding the knee had to be kept intact in order to prevent dehydration of the knee joint (Fig. 1).

Consistent and reproducible positioning of the knees required an evaluation of the variations in size and geometry and the determination of the anatomical landmarks for positioning. The contours of the silhouettes of the bones of the right and left femurs of 10 mice (age 10–14 weeks) as projected on the sagittal and frontal plane were measured using a profile projector equipped with a digitizer (NIKON 6C 7545, Nippon Kogaku

Received in final form 21 August 1995.

*Presented in part at the 40th Annual Meeting of the Orthopaedic Research Society, 21–24 February 1994, New Orleans LA, U.S.A.

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Fig. 1. Two murine leg preparations with cleaned proximal femur and distal tibia and fibula. All soft tissues around the knee are kept intact. A indicates the head of the femur and C indicates the third trochanter.

Fig. 2. Tracings of the contours of the silhouettes of 10 murine femurs as projected on the sagittal plane (top) and on the frontal plane (bottom). The head of the femur (A), the mid-shaft diaphysis (B) and the third trochanter (C) are at fixed positions resulting in small variations of position and orientation at the level of the knee joint. Relative to the mean positions the standard deviations are: $\sigma_1 = 0.20$ mm, $\sigma_2 = 0.22$ mm, $\sigma_3 = 0.11$ mm.

k.k., Tokyo). By using different sets of anatomical landmarks for realigning the femoral contours, it was found that when using the head of the femur, the third trochanter and the mid shaft diaphysis 7 mm from the center of the head, as anatomical

landmarks, it was possible to position the femurs with a variation between the mice of 0.2 mm standard deviation (S.D.) in the sagittal plane (σ_1 and σ_2 in Fig. 2) and 0.1 mm S.D. in the frontal plane (σ_3 in Fig. 2) at the level of the joint line.

Fig. 3. Positioning and cementing device opened after cementing the bone ends to the clamping pins which are connected to a bridge. A: knee specimen; B: PMMA; C: clamping pins; D: bridge connecting the two clamping pins; E: cementing channels; F: cementing syringes.

Fig. 4. AP-laxity tester. A: spindle; B: displacement rig; C: load application spring; D: load transducer; E: displacement transducer; F: low-friction linear bearing; G: fixation clamps; H: knee specimen; I: bridge.

A special device was developed for reproducible positioning and fixation of the limbs to clamping pins. The clamping pins were used to fix the limbs in the testing devices. Cams and set screws, manipulated by micrometer spindles were used to position the tibia and femur prior to cementing the bones and the pins. A bridge (Fig. 3) was fixed between the pins before cementing. This bridge was not removed until the pins had been

clamped in one of the testing devices. This procedure facilitated manipulation, prevented premature loading of the knee joint and enabled standardization of the position of the joint in the test devices.

The positioning and cementing device was machined from aluminum (Fig. 3). Before use, it was greased to prevent sticking of PMMA-cement to the device. The clamping pins were not

Fig. 5. VV-laxity tester. A: spindle; B: rotation rig; C: load application spring; D: load transducer (load times moment arm gives the applied moment); E: rotation transducer (shaft encoder); F: low-friction bearings; G: fixation clamps; H: knee specimen.

greased. Using the anatomical landmarks on the femur, the limb was positioned in the device. The tibia was allowed free rotation around its long axis with the knee in 60° flexion. The femur and tibia were plotted in PMMA (Fastacryl®) to within approximately 8 mm from the joint line. The PMMA was injected with a syringe in the direction along the long axis of the bone to prevent transverse forces on the knee joint. With this procedure no loads were applied to the tibia which could force the knee into a non-anatomical position. Together with femur and tibia, the clamping pins were potted in the PMMA, perpendicular to the long axis of each bone.

The choice was made to perform the laxity tests at a flexion angle of 60° and to allow for only two degrees of freedom of motion. The flexion angle of 60° was selected as being a slightly flexed position as compared to the fully extended murine knee which is approximately 45° . With this, the posterior capsule of the murine knee was assumed to be relaxed and should not influence the laxity tests. In order to keep the tests as simple as possible, it was decided to test in one direction with only one additional degree of freedom of motion, without allowing for other coupled motions (Oster *et al.*, 1992). It was expected that in one-degree-of-freedom tests contact of the articulating surfaces could not be prevented. Therefore, as an additional degree of freedom, proximal-distal translation along the axis connecting the knee and the ankle was allowed. During the test, this prevented additional restraints caused by articular contact, which could possibly mask the laxity test.

In the testers the loads were gradually applied through a spindle-actuated spring whereby the load and the displacement in the test direction were measured simultaneously. The testing procedure was as follows. The limb was clamped firmly in the AP-testing device (Fig. 4). After this, the bridge was removed and the tibia was free to move in the AP-direction while the femur could translate in the direction of the long axis of the tibia. By rotating a spindle manually, a spring was loaded, generating a force in anterior or posterior direction relative to the tibia. Using this spring (a wire of 0.6 mm diameter), a smooth application of AP forces was obtained, whereby torsional and shear loading of the bearings and the force transducer were prevented. The forces were transmitted through the clamp holding the tibia-pin and through a strain-gauge-type force transducer (Sensotec type 8432-2,5N) mounted on a linear bearing. The usable range of the forces was from 1 N anterior to 1 N posterior. The kinetic friction force in the linear bearing was 0.02 N, as was evaluated by measuring the forces when moving the AP-tester with no limb mounted. The AP displacement between distal femur and proximal tibia, with a maximum total usable range of 2.54 mm, was measured with a LVDT-type translation transducer (Trans-Tek type 87240-000/87c04-000).

The clamps of the VV-tester (Fig. 5) held the knee in the same position as in the AP-tester. The tibia was free to rotate in the VV-direction and the femur free to translate in the direction of the long axis of the tibia. Moments ranging from 5 Nmm varus

Fig. 6. Anterior-posterior and varus-valgus laxity curves of a normal murine knee. The vertical dashed lines indicate the intervals used to calculate the laxity and compliance values.

to 5 Nmm valgus were applied to the tibia through a spring (0.55 mm diameter) by rotating a gear wheel through a spindle. The kinetic friction moment in the system was 0.1 Nmm. Rotations of the tibia were measured with a rotary encoder (Heidenhain ERO 1450) connected to a PC-interface (Heidenhain IK 120). The main design problem was the unavailability of a low-load moment transducer with sufficient accuracy. Therefore use was made of a strain-gauge-type force transducer (Sensotec type 8432-0.5N) placed on a moment arm of 22 mm. Because the joint was not loaded in the positioning- and cementing device, the output of the load transducers in the testers was set zero before clamping a joint in one of the testers. The zero-load level was not changed after removing the bridge from the clamping pins.

In each tester the knee is preconditioned by applying five full loading cycles. Then, during three full loading cycles, the load and displacement are measured at a sample rate of 1 Hz. The total test of three cycles was completed in about 100 s. Because the loads were applied through a manually driven spindle and spring, there were small variations in the loading rate, but they did not have a marked effect on the load-displacement data. After testing in one device, the limb was moved to the other device. For this purpose the metal bridge between the pins was reconnected prior to removing the joint from one tester and before moving it to the other tester.

The load-displacement curves obtained were non-linear, representing a relatively high compliance of the knee around zero load and a decreasing compliance with increasing load in both directions (Fig. 6) (compliance is the inverse of the stiffness). Due to energy dissipation within joint structures (hysteresis), a difference existed between motion from anterior to posterior, or varus

to valgus, represented by the upper branch, and motion from posterior to anterior, or valgus to varus represented by the lower branch (Fig. 6). In a similar fashion as was done previously by Edixhoven *et al.* (1987) for AP-laxity testing of human knees, each load-displacement curve was characterized by the total displacement between two load levels, the displacement and the compliance in each direction for the prescribed load levels (Table 1). These values are calculated from three complete cycles of anterior-posterior or varus-valgus loading. The values are obtained from linearization of the curve segments with respect to intervals of ± 0.15 N around the force values of ± 0.8 N and of ± 0.55 Nmm around moment values of ± 4 Nmm (Fig. 6) by using distribution-free estimation methods (Dawson-Saunders and Trapp, 1990; Theil, 1950). The load ranges were chosen such as to cover enough data points around the selected load levels and to cover a more-or-less linear region in the upper and lower branch in the curve. The same intervals were used around zero load to determine the zero compliance. The laxity- and compliance values represent the averages of the upper and lower branches of the curves.

Before the execution of the laxity tests, murine hind limbs were carefully isolated by disarticulation of the hip joint. Care was taken not to load the knee joint during the dissection. The foot was removed at the ankle joint and all soft tissues were removed from the femoral and tibial diaphyses to approximately 5 mm from the joint line (Fig. 1). Special attention was given to cleaning of the anatomical landmarks used for positioning. During positioning and cementing, the joint was covered by cloth tissue soaked in saline, which was removed just before the laxity tests.

To determine the variation in compliance and laxity parameters between mice, five normal knee joints, three right and two left joints randomly selected from five mice, were tested and the above described laxity parameters were calculated from the load-displacement data. The procedure of moving the limb between the testers was verified by alternately testing the five knees in the AP- and the VV-tester two times. A Wilcoxon signed rank test was used to determine the significance of the differences between the first and second test, with an α of 0.05.

The testers were meant to measure the displacement of the tibia relative to the femur at the level of the knee joint. However, the deformation of the bones, the PMMA and the bone pins contribute to the displacement measured. In order to quantify this effect, the laxity tests were performed on five isolated tibias and femurs. For this purpose the tibia was in its original position and the proximal part of the tibia was fixed relative to the base of the tester. For the femur the same test was performed, except that the femur was placed in the tibia clamp. The displacement as functions of the applied load would be due, for the major part, to the deformations of the bone as caused by bending.

RESULTS

Normal knees showed a relatively high AP-compliance of the knee around zero force, decreasing to low compliance at high anterior and posterior forces. The average total AP-translation of a normal murine knee joint between 0.8 N anterior and 0.8 N posterior force was 0.43 mm (± 0.16 mm standard deviation, S.D.). The zero compliance was high ($0.58 \text{ mm N}^{-1} \pm 0.36 \text{ mm N}^{-1}$ S.D.) and showed a relatively high interindividual variation as compared to the posterior and anterior compliances, 0.14 mm N^{-1} ($\pm 0.05 \text{ mm N}^{-1}$ S.D.) and 0.12 mm N^{-1} ($\pm 0.05 \text{ mm N}^{-1}$ S.D.), respectively (Table 2a).

The average total varus-valgus rotation of a normal murine knee joint between 4 Nmm varus and 4 Nmm valgus moment was 17.2° ($\pm 2.6^\circ$ S.D.). The VV-compliance around zero moment was relatively high, $7.5^\circ \text{ Nmm}^{-1}$ ($\pm 2.6^\circ \text{ Nmm}^{-1}$ S.D.), and showed a high variation between the knees. At high varus and valgus moments the compliance was about seven times lower, $0.9^\circ \text{ Nmm}^{-1}$ ($\pm 0.2^\circ \text{ Nmm}^{-1}$ S.D.) for valgus and $1.0^\circ \text{ Nmm}^{-1}$ ($\pm 0.4^\circ \text{ Nmm}^{-1}$ S.D.) for varus (Table 2b).

Table 1. List of laxity parameters characterizing the load-displacement data for the AP-test and the VV-test

AP-test		
<i>Shift</i>		
$S_{-0.8}$	(mm)	the average shift (lower and upper branch together) for a posterior force of 0.8 N, relative to the lower branch position at zero force
$S_{0.8}$	(mm)	as $S_{-0.8}$, but at a force of 0.8 N anterior
$TS_{0.8}$	(mm)	the total shift between 0.8 N posterior force and 0.8 N anterior force
<i>Compliance</i>		
$^{ap}C_0$	(mm N ⁻¹)	the average compliance (slope) of lower and upper branch at zero force
$^{ap}C_{-0.8}$	(mm N ⁻¹)	the average compliance (lower and upper branch together) at a force of 0.8 N posterior
$^{ap}C_{+0.8}$	(mm N ⁻¹)	as $^{ap}C_{-0.8}$ but at a force of 0.8 N anterior
VV-test		
<i>Rotation</i>		
R_{-4}	(°)	the average rotation (lower and upper branch together) for a valgus moment of 4 Nmm, relative to the lower branch position at zero moment
R_{+4}	(°)	as R_{-4} , but at a moment of 4 Nmm varus
TR_4	(°)	the total between 4 Nmm varus moment and 4 Nmm valgus moment
<i>Compliance</i>		
$^{vv}C_0$	(° Nmm ⁻¹)	the average compliance (slope) of the upper and lower branch at zero moment
$^{vv}C_{-4}$	(° Nmm ⁻¹)	the average compliance (lower and upper branch together) at a moment of 4 Nmm valgus)
$^{vv}C_{+4}$	(° Nmm ⁻¹)	as $^{vv}C_{-4}$ but at a moment of 4 Nmm varus

Table 2. *In-vitro* laxity characteristics of 5 normal murine knees (2 right and 3 left knee joints, randomly selected from 5 mice). a. Anterior-posterior laxity. b. Varus-valgus laxity. Values are given of mean and standard deviation (S.D.)

a. AP-test	mean ± S.D.	
$S_{-0.8}$	0.18 ± 0.06	(mm)
$S_{+0.8}$	0.25 ± 0.11	(mm)
$TS_{0.8}$	0.43 ± 0.16	(mm)
$^{ap}C_0$	0.58 ± 0.36	(mm N ⁻¹)
$^{ap}C_{-0.8}$	0.14 ± 0.05	(mm N ⁻¹)
$^{ap}C_{+0.8}$	0.12 ± 0.05	(mm N ⁻¹)
b. VV-laxity	mean ± S.D.	
R_{-4}	4.5 ± 1.1	(°)
R_{+4}	12.7 ± 3.1	(°)
TR_4	17.2 ± 2.6	(°)
$^{vv}C_0$	7.5 ± 2.6	(° Nmm ⁻¹)
$^{vv}C_{-4}$	0.9 ± 0.2	(° Nmm ⁻¹)
$^{vv}C_{+4}$	1.0 ± 0.4	(° Nmm ⁻¹)

Alternate testing of limbs in both testers did not influence the laxity parameters of the AP-laxity (Table 3a). The mean difference between the alternate tests was less than 5% for the translation values and less than 17% for the compliance values. Although not significantly different from zero, the VV-rotation parameters were lower in the second test (Table 3b), which was mainly caused by a 20% lower valgus laxity. The mean differences of compliance values were 8% for

Table 3. Mean differences of the laxity parameters between the first and second test after repositioning the joint. Mean and standard deviation (S.D.) of 5 joints

a. AP-laxity	mean ± S.D.	
$S_{-0.8}$	0.00 ± 0.02	(mm)
$S_{+0.8}$	0.01 ± 0.03	(mm)
$TS_{0.8}$	0.01 ± 0.03	(mm)
$^{ap}C_{-0.8}$	0.02 ± 0.02	(mm N ⁻¹)
$^{ap}C_{+0.8}$	0.02 ± 0.01	(mm N ⁻¹)
b. VV-laxity	mean ± S.D.	
R_{-4}	- 0.89 ± 1.04	(°)
R_{+4}	- 0.13 ± 1.12	(°)
TR_{-4}	- 0.93 ± 0.49	(°)
$^{vv}C_{-4}$	0.07 ± 0.24	(° Nmm ⁻¹)
$^{vv}C_{+4}$	- 0.21 ± 0.42	(° Nmm ⁻¹)

valgus and 21% for varus, but were not significantly different from zero.

In the AP-tester, the total deformation of the tibia and fixture was 0.30 mm (± 0.012 mm S.D.) at an anterior force of 0.8 N and 0.026 mm (± 0.009 mm S.D.) at a posterior force of 0.8 N. The deformation of the femur and the fixture was 0.048 mm (± 0.020 mm S.D.) to anterior and 0.030 mm (± 0.012 mm S.D.) to posterior, when the load was applied perpendicular to the shaft. According to the specifications of the load cell, about 0.008 mm in each direction was explained by the deflection of

the load cell which was located between the leg specimen and the displacement transducer. If correcting for this and taking into account that in the actual test the angle between the femoral and tibial axis is not 90° but 60°, the effect during the test of femoral bending could be calculated. Then in the AP-test of the intact joint, the contribution of femoral bending in anterior or posterior direction was small: 0.016 mm (± 0.008 mm S.D.) relative to the total contribution of tibial bending to the total AP displacement. Hence, a part of the motion measured in the intact limbs was not due to translation of the tibia relative to the femur at the level of the knee. This effect will not influence the relative laxity variations between joints when tested in the same device.

In the VV-tester the motion due to deformation of the bones was negligible, less than 0.01°, indicating that practically all measured rotation represented that at the joint level. In the VV-tester, the deflection of the load cell did not affect the rotation measurement because the shaft encoder was located between the load transducer and the leg specimen.

DISCUSSION

Measurement of laxity of murine knee joints *in vitro*, implying small loads and displacements, was shown to be possible with sufficient accuracy. The variation of the laxity parameters was small by virtue of the careful positioning of the limbs, using the special positioning- and cementing device. The positioning could be performed in a standardized way, because the variation in morphometry in mice with age 10–14 weeks proved to be small. Also the use of inbred strains could have had a positive effect on the homogeneity of the morphology of the bones, which is essential for reproducible joint positioning.

As described in Materials and Methods, the testers allow for only two degrees of freedom of motion, the primary motion being either AP-translation or VV-rotation, and the secondary, coupled motion being translation along the tibial long axis. It should be recognized that the primary displacements will be different if other coupled motions are allowed to occur (Oster *et al.*, 1992). However, when ligaments are damaged, the coupling between the primary motion and the secondary motions is affected. That, in itself, may have a marked effect on the resulting displacements for the primary motion. The change of coupling makes the interpretation of the changes in displacement due to ligament damage complicated if the coupled motions during the tests are not measured. Allowing for more unconstrained degrees of freedom of motion and introducing additional displacement transducers would make the testers more complex and will not add much to the value of the testers in comparative analyses.

The bone and fixture deformations during testing were negligible for the VV-test. For the AP-test however, the values for the bone and fixture deformations indicated that the laxity values were slightly overestimated by the tester. If assuming that these deformations were similar for all specimens, then the comparative effects of interventions on joint laxity can be evaluated by the AP-tester. In view of this, no attempt was made as yet to correct for the bone deformations.

The choice of the zero points for analysis of AP translation and VV rotation is arbitrary. For the AP-tests, the translation at the point where the force was zero when moving the tibia from posterior to anterior was chosen as a reference. For VV rotation the zero moment when moving the tibia from valgus to varus direction was chosen as a reference point. The total laxity was not affected by the zero point, but the absolute values in each direction were. This means that care should be taken when comparing the absolute laxity values in each direction. For example, one cannot conclude that varus laxity is greater than valgus laxity in the murine knee joint, because with another choice of the zero rotation, this might be reversed. The variation of laxity for the separate varus and valgus directions is higher than for the total varus–valgus laxity because of the necessarily arbitrary selection of zero points. The zero point is sensitive to

small deviations from zero load since the compliance around zero load is high.

The compliance values around zero showed higher variations as compared to the compliance values at the extreme load levels. This could be attributed to the non-linearity of the curve segments around zero, in combination with the zero load shift between specimens. In the AP- and VV-curves around zero load, there was actually no linear region. Therefore, a small shift of the zero load curve segment will have a marked effect on the compliance as calculated from the data points within the interval around zero load. Reducing the size of the interval will not reduce this problem. Therefore, the zero load compliance is not a very good parameter to use in a comparative study.

With these devices it is possible to study more precisely the effects of interventions on knee joint laxity characteristics when the murine knee joint is used as a model. This includes the effects of chemicals to initiate pathogenetic processes (e.g. Van Osch *et al.*, 1994), surgical interventions, pharmaceutical interventions and immobilization of the joint.

Acknowledgements—The authors would like to thank Jan de Haard and Henk Hoving, who realized the mechanical parts of the devices, and Marco van der Sluijs, who was responsible for the electronics and the data-acquisition software. We are grateful to Willem van de Wijdeven and Huub Peeters for technical assistance and Rik Huiskes, Wim van den Berg and Peter van der Kraan for their critical comments during the developments.

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