Laxity characteristics of normal and pathological murine knee joints in vitro

Citation for published version (APA):

Osch, van, G. J. V. M., Blankevoort, L., Kraan, van der, P. M., Janssen, B., Hekman, E., Huiskes, H. W. J., & Berg, van den, W. B. (1995). Laxity characteristics of normal and pathological murine knee joints in vitro. *Journal of Orthopaedic Research*, 13(5), 783-791. <https://doi.org/10.1002/jor.1100130519>

DOI:

[10.1002/jor.1100130519](https://doi.org/10.1002/jor.1100130519)

Document status and date:

Published: 01/01/1995

Document Version:

Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.tue.nl/taverne

Take down policy

If you believe that this document breaches copyright please contact us at:

openaccess@tue.nl

providing details and we will investigate your claim.

Laxity Characteristics of Normal and Pathological Murine Knee Joints *In Vitro*

*†Gerjo J. V. M. van Osch, *Leendert Blankevoort, †Peter M. van der Kraan,
‡Bernard Janssen, ‡Edsko Hekman, *Rik Huiskes, and †Wim B. van den Berg

*Biomechanics Section, Institute of Orthopaedics, †Department of Rheumatology, and ‡Engineering Department,
University of Nijmegen, Nijmegen, The Netherlands

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 $17.4 \pm 3.3^\circ$. 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, overloading, 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-

Received February 16, 1994; accepted October 13, 1994.

Address correspondence and reprint requests to G.J.V.M. van Osch at Department of Rheumatology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

FIG. 1. Tester for anterior-posterior laxity. A = Spindle for load application, B = load transducer, C = displacement transducer, D = low-friction linear bearing, E = fixation clamps, and F = knee specimen.

ally one knee is injected and the contralateral knee is used as a control. For practical purposes, it often is necessary to store limbs until the time of measurement. Therefore, the effect of freezing at -70°C and thawing was investigated. Finally, the effects on joint laxity of acute inflammation induced by injection of zymosan, by chronic antigen-induced arthritis, and by injection of collagenase were compared to determine whether these agents are likely to produce osteoarthritis indirectly, through the insufficiency of ligamentous restraints.

MATERIALS AND METHODS

Male C57 Black mice (age 10-14 weeks, weight 25 g) were used. The animals were kept in cages, with wood chip bedding, in an air-conditioned room. They were fed a standard laboratory diet (Hope Farms, Deventer, The Netherlands) and acidified water *ad libitum*.

The hindlimbs were carefully isolated by disarticulation of the hip joint, thereby preventing overloading of the knee joint. The foot was removed at the ankle joint, and all soft tissues were removed from the femoral and tibial diaphyses, except around the knee joint (approximately 5 mm from the joint line in both directions) where tissue was left to prevent dehydration of the joint structures. The limbs were stored in airtight tubes at 7°C when stored for no longer than 2 days or at -70°C when stored for a longer period of time. After thawing, the joints were tested within 2 days.

Laxity Tests

The limbs were fixed in a specially designed positioning device. To achieve reproducible positioning, the head of the femur and the third trochanter were used as anatomical reference points. The tibia was allowed free rotation with the knee in 60° of flexion. The proximal femur and distal tibia were potted in fast acryl to a point approximately 8 mm from the knee joint line. Together with the femur and tibia, pins were potted perpendicular to the diaphyses to facilitate positioning of the limb in the testing devices.

In the anterior-posterior test (Fig. 1), forces were applied perpendicular to the tibia in the anterior and posterior directions by manual rotation of a spindle. The force of friction in the system was about 0.02 N. Freedom of motion existed in the direction along the tibial axis to prevent relative motions and contact loads of the surfaces from influencing the anterior-posterior measurement. The forces applied ranged from 1 N anterior to 1 N posterior and were measured with a highly sensitive and accurate strain-gauge type force transducer (Burster type 8432-2,5N; Multitronics, Amersfoort, The Netherlands). Anterior-posterior displacement between the distal femur and proximal tibia was measured with a linear variable differential transducer-type translation transducer (Burster type 87240-000 with kern ausführung type 87c04-000; Multitronics). The limbs were preconditioned by performance of five anterior-posterior cycles. In the following two full anterior-posterior cycles, force and displacement were measured continuously, amplified, converted from analog to digital, sampled at 100 points (1 Hz), and stored in a personal computer for further analysis.

The anterior-posterior tester measures linear displacements, whereas the varus-valgus tester determines rotations (Fig. 2). Moments ranging from 5 Nmm of varus to 5 Nmm of valgus were applied to the tibia through a spring (0.55 mm) by rotation of a spindle. The friction in the system was about 0.1 Nmm. Freedom of motion existed in the direction along the tibial axis to prevent restraints when the femur forced the tibia to move distally or proximally. Rotations of the tibia were measured with a shaft encoder (type ero 1450; Heidenhain, Veenendaal, The Netherlands) and a personal computer interface (type IK 120; Heidenhain). Applied moments were measured with a strain-gauge type force transducer (Burster type 8432-0.5N; Multitronics) placed at an arm of 22 mm. After the limb had been preconditioned for five full varus-valgus cycles, moments and rotations were measured continuously during two varus-valgus cycles, amplified, converted from analog to digital, sampled at 100 points (1 Hz), and stored in a personal computer for further analysis.

The curves obtained (Figs. 3-4) were nonlinear, representing relatively low stiffness of the knee around zero load; stiffness increased progressively with loads in both directions. Due to dissipation of energy within the joint structures (hysteresis), there was a difference between motion from anterior to poste-

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 Edixhoven 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 $^{ap}C_0$ —the average compliance (slope) of the branches (lower and upper branches together) at zero force; $^{ap}C_{-0.8}$ —the average compliance at 0.8 N of posterior force; and $^{ap}C_{0.8}$ —the same as $^{ap}C_{-0.8}$ but at 0.8 N of anterior force. The parameters for varus-valgus laxity for rotation (in degrees) were R_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_4 —the same as R_4 but at a varus moment of 4 Nmm; and TR_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 $^{vv}C_0$ —the average compliance (slope) of the branches (lower and upper branches together) at zero moment;

$^{vv}C_4$ —the average compliance at a valgus moment of 4 Nmm; and $^{vv}C_4$ —the same as $^{vv}C_4$ 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_4 , and C_{-4} can be considered as estimates of the endpoint compliances, the inverses 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 uninjected 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.

FIG. 3. A typical, representative anterior-posterior laxity curve of a normal murine knee joint. The lower curve represents displacement from posterior to anterior and the upper curve shows displacement from anterior to posterior due to applied loads.

FIG. 4. A typical, representative varus-valgus laxity curve of a normal murine knee joint. The lower curve represents rotation from valgus to varus and the upper curve shows rotation from varus to valgus due to applied moments.

for measurements to be made at a later time. To study the effects of freezing and thawing on murine knees in our system, we compared the laxity parameters of eight fresh knee joints with those of eight frozen ones.

Induction of Inflammation and Osteoarthritis

The effect of inflammation was investigated with use of two models: an acute model induced by a single injection of zymosan and a chronic antigen-induced arthritis model induced by injection of methyl bovine serum albumin into immunized mice (30). For the zymosan model, 180 µg of zymosan was injected intra-articularly into eight knee joints. For the antigen-induced arthritis model, 18 mice were immunized with methyl bovine serum albumin in 0.1 ml of Freund's complete adjuvant emulsion by injection into the flank skin and the footpads of the forelimbs. Heat-killed *Bordetella pertussis* organisms (2×10^9) (National Institute of Public Health, Bilthoven, The Netherlands) were administered intraperitoneally as an additional adjuvant. After 1 week, booster injections were given subcutaneously in the neck region. Four weeks after the first injections, arthritis was induced by intra-articular injection of 60 µg of methyl bovine serum albumin in 6 µl of phosphate buffered saline.

Osteoarthritis was induced by intra-articular injection of 10 U of bacterial collagenase (type VII; Sigma Chemical, St. Louis, MO, U.S.A.), dissolved in 6 µl of saline, into the right knee joints of 16 mice. In a pilot study (data not shown), it was shown that laxity of

the joint can best be measured 3 days after collagenase is injected. After day 1, changes in laxity may not be complete; after day 7, osteophyte formation may obscure the measurements. The effect of inflammation also was measured on day 3. Because antigen-induced arthritis is a model for chronic arthritis, and damage to the ligament may be induced by the bulk of mediators released during a prolonged period of time, laxity also was measured on days 14 and 28.

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 1. Laxity characteristics (mean $\pm$ SD)

Parameter	Normal murine knees	Left minus right knee joint ^a (n = 8)
Anterior-posterior laxity		
Shift (mm)		
Posterior (-0.8 N)	0.19 ± 0.05	0.00 ± 0.03
Anterior (0.8 N)	0.28 ± 0.05	0.01 ± 0.05
Total (± 0.8 N)	0.47 ± 0.10	0.01 ± 0.06
Compliance (mm/N)		
Neutral (0 N)	0.72 ± 0.32	
Posterior (-0.8 N)	0.16 ± 0.04	0.00 ± 0.01
Anterior (0.8 N)	0.16 ± 0.03	0.01 ± 0.02
Varus-valgus laxity		
Rotation (°)		
Valgus (-4 Nmm)	5.5 ± 1.6	-0.5 ± 1.9
Varus (4 Nmm)	11.8 ± 2.9	0.8 ± 6.4
Total (± 4 Nmm)	17.4 ± 3.3	0.4 ± 5.0
Compliance (°/Nmm)		
Neutral (0 Nmm)	5.3 ± 2.6	
Valgus (-4 Nmm)	1.0 ± 0.3	-0.2 ± 0.5
Varus (4 Nmm)	1.1 ± 0.7	-0.3 ± 0.8

^aThere 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*

	Anterior-posterior laxity				Varus-valgus laxity			
	Shift		Compliance		Rotation		Compliance	
	-0.8 N	0.8 N	-0.8 N	0.8 N	-4 Nmm	4 Nmm	-4 Nmm	4 Nmm
Fresh (n = 8)	0.20 ± 0.02	0.29 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	5.0 ± 1.8	9.9 ± 2.0	0.70 ± 0.21	0.70 ± 0.16
Frozen/thawed (n = 8)	0.22 ± 0.03	0.30 ± 0.04	0.17 ± 0.02	0.16 ± 0.02	4.9 ± 0.8	11.8 ± 1.5	0.77 ± 0.17	0.72 ± 0.12

as high as the variation between knees of different inbred animals.

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

TABLE 3. *Comparison of laxity parameters among joints (mean ± SD)*

	Anterior-posterior laxity				Varus-valgus laxity			
	Shift		Compliance		Rotation		Compliance	
	-0.8 N	0.8 N	-0.8 N	0.8 N	-4 Nmm	4 Nmm	-4 Nmm	4 Nmm
Control (n = 25)	0.19 ± 0.05	0.28 ± 0.05	0.16 ± 0.04	0.16 ± 0.03	5.5 ± 1.6	11.8 ± 2.9	1.0 ± 0.3	1.1 ± 0.7
180 µg of zymosan								
Day 3 (n = 8)	0.13 ± 0.04 ^a	0.22 ± 0.03 ^a	0.09 ± 0.01 ^a	0.10 ± 0.02 ^a	4.8 ± 1.2	11.8 ± 5.3	1.3 ± 0.5	1.8 ± 0.3
Antigen-induced arthritis								
Day 3 (n = 6)	0.21 ± 0.02	0.32 ± 0.04	0.19 ± 0.04	0.19 ± 0.04	6.0 ± 1.7	11.3 ± 1.5	0.9 ± 0.1	0.8 ± 0.10
Day 14 (n = 6)	0.22 ± 0.02	0.33 ± 0.03	0.22 ± 0.05	0.19 ± 0.02	4.6 ± 0.9	10.9 ± 2.1	0.7 ± 0.2	0.8 ± 0.2
Day 28 (n = 6)	0.22 ± 0.03	0.34 ± 0.09	0.20 ± 0.06	0.19 ± 0.06	3.0 ± 0.8 ^a	9.2 ± 1.8 ^a	0.6 ± 0.1 ^a	0.9 ± 0.4
10 U of highly purified bacterial collagenase								
Day 3 (n = 16)	0.47 ± 0.30 ^a (0.29)	0.73 ± 0.36 ^a (>1)	NC	NC	29.1 ± 17.4 ^a (>45)	25.2 ± 14.5 ^a (24.3)	NC	NC

For collagenase treatment, median values are presented in parentheses.

Maximal displacement in the anterior or posterior direction was 1 mm; maximal rotation in the varus or valgus direction was 45°. Compliance values after collagenase injection could not be calculated (NC).

^aP < 0.05.

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 col-

lateral 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

FIG. 6. Examples of varus-valgus laxity curves. **A:** A normal knee joint. **B:** A joint showing increased valgus laxity and unchanged varus laxity. **C:** A joint showing a major increase in valgus laxity and a slight increase in varus laxity.

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 de-

sign; 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 joints. In this study, it was shown that inflammatory conditions did not induce changes in laxity, whereas injection of collagenase affected stability of the ligament. Further study is now possible to investigate more precisely the effects of injection of collagenase on laxity and the correlation with damage to cartilage.

Acknowledgment: The authors would like to thank Jan de Haard and Henk Hoving for the mechanical part of the devices and Marco van der Sluijs, who was involved with the electronics. We are grateful to Willem van de Wijdeven, Huub Peeters, and Huub van der Heide for technical assistance.

REFERENCES

- Arsever CL, Bole GG: Experimental osteoarthritis induced by selective myectomy and tendotomy. *Arthritis Rheum* 29:251-261, 1986
- Ballock RT, Woo SL-Y, Lyon RM, Hollis JM, Akeson WH: Use of patellar tendon autograft for anterior cruciate ligament reconstruction in the rabbit: a long-term histologic and biomechanical study. *J Orthop Res* 7:474-485, 1989
- Blanckevoort L, van Osch GJVM, Hekman E, Janssen B: In vitro laxity testers for knee joints of mice. Unpublished data
- Brandt KD, Braunstein EM, Visco DM, O'Connor B, Heck D, Albrecht M: Anterior (cranial) cruciate ligament transection in the dog: a bona fide model of osteoarthritis, not merely of cartilage injury and repair. *J Rheumatol* 18:436-446, 1991
- Colombo C, Butler M, O'Byrne E, Hickman L, Swartzen-druber D, Selwyn M, Steinetz B: A new model of osteoarthritis in rabbits. I. Development of knee joint pathology following lateral meniscectomy and section of the fibular collateral and sesamoid ligaments. *Arthritis Rheum* 26:875-886, 1983
- Dahlkvist NJ, Seedhom BB: Objective measurement of knee laxity and stiffness with reference to knee injury diagnosis. Part I: Design considerations and apparatus. *Proc Inst Mech Eng [H]* 204:75-82, 1990
- Dorlot J-M, Ait Ba Sidi M, Tremblay GM, Drouin G: Load elongation behaviour of the canine anterior cruciate ligament. *J Biomech Eng* 102:190-193, 1980
- Edixhoven Ph, Huiskes R, de Graaf R, van Rens ThJG, Slooff TJ: Accuracy and reproducibility of instrumented knee-drawer tests. *J Orthop Res* 5:378-387, 1987
- Edixhoven P, Huiskes R, de Graaf R: Anteroposterior drawer measurements in the knee using an instrumented test device. *Clin Orthop* 247:232-242, 1989
- Feder SM, Butler DL, Holden JP: Short communication. A technique for the evaluation of the contributions of knee structures to knee mechanics in the knee that has a reconstructed anterior cruciate ligament. *J Orthop Res* 11:448-451, 1993
- Gillquist J: Knee stability: its effect on articular cartilage. In: *Articular Cartilage and Knee Joint Function: Basic Science and Arthroscopy*, pp 267-271. Ed by JW Ewing. New York, Raven Press, 1990
- Grood ES, Walz-Hasselfeld KA, Holden JP, Noyes FR, Levy MS, Butler DL, Jackson DW, Drez DJ: The correlation between anterior-posterior translation and cross-sectional area of anterior cruciate ligament reconstructions. *J Orthop Res* 10:878-885, 1992
- Hede A, Svalastoga E, Reimann I: Articular cartilage changes following meniscal lesions: repair and meniscectomy studied in the rabbit knee. *Acta Orthop Scand* 62:319-322, 1991
- Inoue M, McGurk-Burleson E, Hollis JM, Woo SL-Y: Treatment of the medial collateral ligament injury. I: The importance of anterior cruciate ligament on the varus-valgus knee laxity. *Am J Sports Med* 15:15-21, 1987
- Johnson RG, Poole AR: The early response of articular cartilage to ACL transection in a canine model. *Exp Pathol* 38:37-52, 1990
- Kannus P, Järvinen M: Osteoarthritis in a knee due to chronic posttraumatic insufficiency of the medial collateral ligament: nine-year follow-up. *Clin Rheumatol* 7:200-207, 1988
- Kannus P: Osteoarthritis in a knee due to chronic posttraumatic insufficiency of the lateral ligament compartment: eight-year follow-up. *Clin Rheumatol* 7:474-480, 1988
- Kannus P, Järvinen M: Age, overweight, sex, and knee instability: their relationship to the post-traumatic osteoarthritis of the knee joint. *Injury* 19:105-108, 1988
- Kannus P, Järvinen M: Posttraumatic anterior cruciate ligament insufficiency as a cause of osteoarthritis in a knee joint. *Clin Rheumatol* 8:251-260, 1989
- Markolf KL, Mensch JS, Amstutz HC: Stiffness and laxity of the knee—the contributions of the supporting structures: a quantitative in vitro study. *J Bone Joint Surg [Am]* 58:583-594, 1976
- Markolf KL, Graff-Radford A, Amstutz HC: In vivo knee stability: a quantitative assessment using an instrumented clinical testing apparatus. *J Bone Joint Surg [Am]* 60:664-674, 1978
- Markolf KL, Kochan A, Amstutz HC: Measurement of knee stiffness and laxity in patients with documented absence of the anterior cruciate ligament. *J Bone Joint Surg [Am]* 66:242-253, 1984
- Marshall JL, Olsson S-E: Instability of the knee: a long-term experimental study in dogs. *J Bone Joint Surg [Am]* 53:1561-1570, 1971
- Martin JW, Whiteside LA: The influence of joint line position on knee stability after condylar knee arthroplasty. *Clin Orthop* 259:146-156, 1990
- Pond MJ, Nuki G: Experimentally-induced osteoarthritis in the dog. *Ann Rheum Dis* 32:387-388, 1973
- Sandy JD, Adams ME, Billingham MEJ, Plaas A, Muir H: In vivo and in vitro stimulation of chondrocyte biosynthetic activity in early experimental osteoarthritis. *Arthritis Rheum* 27:388-397, 1984
- Seering WP, Piziali RL, Nagel DA, Schurman DJ: The function of the primary ligaments of the knee in varus-valgus and axial rotation. *J Biomech* 13:785-794, 1980
- Shapiro MS, Markolf KL, Finerman GAM, Mitchell PW: The effect of section of the medial collateral ligament on force generated in the anterior cruciate ligament. *J Bone Joint Surg [Am]* 73:248-256, 1991
- Steinbach HL, Jansen PS: Roentgenographic changes in the arthrides (part II). *Semin Arthritis Rheum* 5:203-246, 1976

30. van den Berg WB, Kruijsen MWM, van de Putte LBA, van Beusekom HJ, van der Sluis-van der Pol M, Zwarts WA: Antigen-induced and zymosan-induced arthritis in mice: studies on in vivo cartilage proteoglycan synthesis and chondrocyte death. *Br J Exp Pathol* 62:308-316, 1981
31. van der Kraan PM, Vitters EL, van Beuningen HM, van de Putte LBA, van den Berg WB: Degenerative knee joint lesions in mice after a single intra-articular collagenase injection: a new model of osteoarthritis. *J Exp Pathol* 71:19-31, 1990
32. van Osch GJVM, van der Kraan PM, van den Berg WB: Site-specific cartilage changes in murine degenerative knee joint disease induced by iodoacetate and collagenase. *J Orthop Res* 12:168-175, 1994
33. Woo SL-Y, Orlando CA, Camp JF, Akeson WH: Effects of postmortem storage by freezing on ligament tensile behavior. *J Biomech* 19:399-404, 1986
34. Woo SL-Y, Gomez MA, Inoue M, Akeson WH: New experimental procedures to evaluate the biomechanical properties of healing canine medial collateral ligaments. *J Orthop Res* 5:425-432, 1987
35. Woo SL-Y, Hollis JM, Roux RD, Gomez MA, Inoue M, Kleiner JB, Akeson WH: Effects of knee flexion on the structural properties of the rabbit femur-anterior cruciate ligament-tibia complex (FATC). *J Biomech* 20:557-563, 1987
36. Woo SL-Y, Hollis JM, Adams DJ, Lyon RM, Takai S: Tensile properties of the human femur-anterior cruciate ligament-tibia complex: the effects of specimen age and orientation. *Am J Sports Med* 19:217-225, 1991