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THE EFFECT OF VARIABLE RELATIVE INSERTION ORIENTATION OF HUMAN KNEE BONE–LIGAMENT–BONE COMPLEXES ON THE TENSILE STIFFNESS

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Abstract—In order to evaluate the contribution of the knee ligaments to restrain joint motions, knowledge about their structural properties is required. Due to the variable relative insertion orientation of the ligaments during knee motion, however, different fiber bundles are recruited, each with their specific mechanical properties. Hence, the structural properties vary as a function of knee motion. For this reason, a relationship between the structural tensile properties and the relative insertion orientation is required in order to define the role of the ligaments in knee mechanics. In the present study, this relationship is determined by performing a series of tensile tests in which the relative orientations of the insertion sites of human knee bone-ligament-bone complexes were varied systematically.

The experimentally obtained stiffness was significantly affected by the relative orientation of the insertion sites, but more profoundly for the anterior and posterior cruciate ligaments (ACL and PCL) as compared to the medial and lateral collateral ligaments (MCL and LCL). The average decreases in stiffness per 5° tilt of the insertion sites were estimated at $-11.6 \pm 3.5$ N mm$^{-1}$ (ACL), $-20.9 \pm 2.7$ N mm$^{-1}$ (PCL), $-2.6 \pm 0.9$ N mm$^{-1}$ (MCL) and $-3.7 \pm 0.3$ N mm$^{-1}$ (LCL). For the PCL and the MCL these changes in stiffness with tilt were rather insensitive to the side of the femoral insertion site which was lifted. The ACL and the LCL, conversely, displayed significant differences in stiffness changes between the different tilt directions.

It is shown that the results of ligament tensile tests, particularly of the cruciate ligaments, are extremely sensitive to the orientation of the specimens in the loading apparatus. Since there is bound to be a variation in ligament orientations in earlier tensile studies, the variation in properties reported can be explained. It is concluded that the functional roles of the ligaments, particularly the cruciate ligaments, vary considerably during knee motions. Hence, functionally speaking, there is no such thing as 'the ligament stiffness'.

INTRODUCTION

Knowledge of the tensile properties of human knee ligaments is a prerequisite for the kinematic analyses of the human knee in order to understand the role the ligaments play in joint function. Moreover, these data are needed for the selection, design and evaluation of ligament replacements. For these purposes, the tensile properties of human knee ligaments have been evaluated by tensile tests by many authors, particularly of the cruciate ligaments (Claes et al., 1987; Noyes and Grood, 1976; Prietto et al., 1988; Trent et al., 1976; Wasmer et al., 1987). These studies have reported a high variability. This can be partially explained by differences in age and activity level of the specimen donors and by technical causes (Butler et al., 1978; Vidik, 1973). Another potential cause of variability between the different studies is the bone-to-bone angle of the ligaments relative to the tensile direction. In more recent studies it has been demonstrated that the orientation of the femur relative to the tibia, and the direction of the loading axis relative to the ligament axis, are important factors in the determination of the tensile properties for the anterior cruciate ligament (ACL) (Figgie et al., 1986 (dog); Hollis et al., 1987 (human and pig); Lyon et al., 1989 (pig); Rogers et al., 1990 (sheep); Roux et al., 1986 (rabbit); Woo et al., 1987 (rabbit); Woo et al., 1991 (human)).

If the ACL was loaded along the ligament axis, higher stiffnesses were found than if loads were applied along the tibial axis. The stiffnesses tended to reduce with increasing flexion angle. This was explained by the nonuniform loading patterns of the fibers as the fibers of the ACL are arranged in an anatomically complex fashion. No variations in tensile properties due to variations in the geometric orientation have been reported for the collateral ligaments, nor for the posterior cruciate ligament (PCL).

During knee motions the configurations of the knee ligaments change (Butler et al., 1988; Girgis et al., 1975; Pollicic Prose et al., 1988). As a result, the ligament structural properties vary as well. In order to estimate the ligament forces from their deformations in different relative insertion orientations occurring during knee motions, a relationship between the relative insertion orientation and the ligament structural properties is thus required. The purpose of the present study was to determine the tensile stiffness of human knee ligaments as a function of the relative orientation of their insertion sites. Two additional questions were addressed. First, whether the susceptibility of tensile stiffnesses to insertion rotations vary with the type of ligament and, second, whether they depend on the direction of the rotations. In order to answer these questions, human bone–ligament–bone preparations of the medial collateral ligament (MCL), the lateral collateral ligament (LCL), the anterior cruciate ligament and the posterior cruciate ligament were subjected to a series of subultimate tensile tests in
which the tilt of the femoral insertion site relative to the tibial one was varied systematically.

MATERIAL AND METHODS

Five fresh cadaveric knee-joints were obtained from five human donors (aged 63–81 yr, average 69). The high age of these donors does not influence the relationship between specimen orientation and tensile stiffness, because the effects of age and specimen orientation on tensile stiffness were shown to be independent (Woo et al., 1991). All knee-joints appeared free from signs of osteoarthritic changes on bone abnormalities at radiography. The knee specimens were stored at −20°C in plastic bags. At the time of usage, the knee-joints were thawed at room temperature in the bags. This procedure does not markedly affect the mechanical properties of fibrous tissues (Woo et al., 1986).

After removal of the skin, muscles and joint capsule, the bones were exposed to the insertion sites of the MCL (defined as the parallel fibers of the superficial part of the medial collateral ligament), the LCL, the ACL and the PCL were marked with at least five tantalum pellets (0.5 mm diameter). These pellets were used to determine the relative rotations and translations of the femoral and tibial/fibular insertion sites with Röntgen stereophotogrammetric analysis (RSA, Selvik, 1974) in the tests. The pellets were placed in small holes, made with a dentist’s drill in the bone, and covered with a small dot of tissue glue (Histoacryl, B. Brown AG, Melsungen, FRG). Subsequently, the ligaments were isolated as bone–ligament–bone units (BLB), using a reciprocating saw (Micro-aire, Valencia, California). Care was taken that each bone block contained enough tantalum pellets to locate the bone in space (a minimum of three).

In order to improve the grip of the specimens during experimental testing, each bone block was embedded in polymethylmethacrylate (PMMA), using a two-tier mold (Fig. 1). This mold was specially designed to position the insertion sites of the ligament as best as possible in the mold center which corresponds to the intersection point of the tilting axes in the experimental setup, and to orient the bones such that, by visual examination, as many ligament fibers were tensed as possible. Note that this is not necessarily the anatomical orientation of the ligament. Holes were drilled in the bones and the bone blocks were trimmed, insuring good mechanical interlock with the PMMA. After un-molding, some of the ligaments were immediately refrozen at −20°C in separate plastic bags. Others were used directly for experimental testing.

The BLB preparations were positioned in a specially designed loading rig (Fig. 2) in a material testing machine (MTS, Berlin, Germany). The PMMA blocks were fixed by screws in steel pots (a). The parts of the PMMA block containing the bones (b) were free from the steel pots to insure the free transmission of the Röntgen beams for the RSA measurements of the three-dimensional kinematics of the bone blocks during experimental testing. The tibial fixation pot was attached to a load cell (c). Alignment of the ligament with the tensile direction was achieved by moving the load cell in the anterior–posterior and medial–lateral directions, perpendicular to the loading axis, with the aid of an X–Y table (d). The femoral and tibial bone blocks were visually aligned by axial rotation of both pots and fixed in this relative position. The initial relative orientation of the bones (init. Fig. 3) was created in this way. A device (e), attached between the pot containing the femoral bone-block and the actuator of the testing machine (f), enabled tilting of this bone-block around axis g. By a same axial rotation of both pots, tilting around other axes was also possible. All these axes intersected in the center of the femoral insertion site of the ligament. In this study only two perpendicular axes, a medial–lateral axis and an anterior–posterior axis, were used. By tilting around the medial–lateral axis up to 25° maximally, in increments of at least 5°, the anterior and posterior sides of the femoral insertion sites were lifted up, which was called the anterior (A1–A5, Fig. 3) and posterior tilt direction (P1–P5, Fig. 3), respectively. By a similar stepwise tilt around the anterior–posterior axis, the medial and lateral sides of the femoral insertion site were lifted up, which was called the medial (M1–M5, Fig. 3) and lateral tilt direction (L1–L5, Fig. 3), respectively. A conductor (h) prevented the device from axial rotation during tensile testing.

For each ligament, with the exception of the MCL of knee specimen 1, tensile tests were performed in a number of the tilted positions, starting every tilt direction with a newly adjusted initial orientation of the bones (Fig. 3, center), with the aid of the X–Y table. We varied the order of tilt directions between the different ligaments to reduce bias. A selection of the tilted positions had to be made for most ligaments, because of time and physical restrictions. Finally, the first test was reproduced with the aid of the X–Y table settings. In this way, it was possible to detect possible failures of fibers (which occurred in two ligaments), or failure of bone (which was found in three cases). In each test, a preload of 2 N was applied on the bone–ligament–bone unit. This was called the zero force position. Subsequently, loading cycles were performed to obtain reproducible load–displacement characteristics (pre-conditioning). For the cruciate ligaments, the maximal actuator displacement was about 10% of the initial length, which was approximated with the aid of vernier calipers, and for the collateral ligaments about 7% of the initial length. These moderate displacement levels were chosen to prevent irreversible damage to the ligament in certain orientations (Butler et al., 1992; Claes et al., 1987). The strain rate was about 66% s⁻¹. The precise relative orientations and translations of the bones were measured with RSA. For that purpose, two double exposures of the BLB preparations were taken of the last cycle of each test, one at the zero force position and one at the maximal force position (Fig. 3). The testing process takes about 2 h per ligament. Because the
ligaments were kept moist by frequently spraying with 0.9% saline solution during the testing process and the ligaments were wrapped in saline-moistened gauze between the tensile tests, as well as during the preparation, tissue degradation was not expected in this time period (Vidik and Lewin, 1966).

Because of registration errors for two ligaments, 12 ligaments remained for data processing: three ACLs, four PCLs, three LCLs and two MCLs. In order to determine the stiffnesses of these ligaments in a specific relative orientation of the insertion sites, the sample-force registrations of the five last cycles were synchronized into one force–displacement curve, using the RSA displacement data of the last cycle, the sample frequency and the signal frequency. In this force–displacement curve, the stiffness $K$ was determined by
means of linear regression of the data between 6 and 7% strain, in which strain is the displacement divided by the zero force length of the ligament. This zero force length is defined as the maximal distance between the geometric centers of the insertion sites for all zero force positions of the ligament. To determine the three-dimensional positions of these geometric centers, the outlines of the insertion sites were marked with additional tantalum pellets after tensile testing and RSA was performed. The strain rates and strain levels of each ligament were recalculated using the more precisely defined zero force lengths.

To test if the stiffness is affected by varying the tilt of the femoral insertion site, a one-way analysis of covariance was performed for each ligament separately, in which the stiffness is the dependent variable, the tilt is the covariate and the tilt direction is the independent classification variable. Both log-transformed and untransformed stiffness data were analyzed. However, because transformation of the data did not change the conclusions obtained, only the results of the analyses of the untransformed data were presented. Estimates for the increase or decrease in tensile stiffness per tilt step of 5° were obtained for each ligament and for each tilt direction of each ligament. The level of statistical significance was set at $p = 0.05$.

RESULTS

The zero force lengths of the ACL, PCL, MCL and LCL were 35.5 ± 4.5 mm, 39.2 ± 3.3 mm, 102.3 ± 2.3 mm and 67.6 ± 6.5 mm, respectively (Table 1). The RSA evaluations showed that the maximal strain levels were higher for the cruciate ligaments as compared to the collateral ligaments, while for the strain rates the opposite was true. Both were lower than initially estimated with the aid of calipers and actuator displacements.

The stiffnesses measured in the initial orientations of the bones were higher for the cruciate ligaments than for the collateral ligaments, with the exception of the ACL of knee specimen 1 (Fig. 4). The standard deviations of the stiffnesses in the initial orientations were the highest for ACL and higher for the PCL than for the collateral ligaments (Fig. 4). However, the accuracy with which the initial orientation was reproduced was similar for all ligaments: the standard deviations of the stiffnesses measured varied among the different initial orientations.

The tensile stiffnesses were affected by the relative orientations of the insertion sites for the cruciate ligaments [Fig. 5(a) and (b)] and the collateral ligaments [Fig. 6(a) and (b)]. For the cruciate ligaments, for example, the highest effect of tilt on the tensile stiffness was found for the ACL of knee specimen 1, for which the stiffness value ranged from 0.66 to 1.10 times the initial stiffness value [Figs 4 and 5(a)]. For the collateral ligaments the highest variation was found for the LCL of specimen 5, for which the stiffness value ranged from 0.66 to 1.10 times the initial stiffness value [Figs 4 and 6(b)].

An average decrease in tensile stiffness per tilt step of 5° was estimated at $-11.6 ± 3.5$ N mm$^{-1}$ for the ACL, $-20.9 ± 2.7$ N mm$^{-1}$ for the PCL, $-2.6 ± 0.9$ N mm$^{-1}$ for the MCL and $-3.7 ± 0.3$ N mm$^{-1}$ for the LCL, which are significantly different from a zero decrease (ANOVA; Table 2). Furthermore, the analysis of covariance revealed that the changes in tensile stiffness per tilt step were not identical for the four tilt directions (Table 2). For the ACL and the LCL the decreases in tensile stiffnesses were significantly higher for the posterior tilt direction as compared to the other tilt directions. For the LCL the tensile stiffness decrease was significantly higher for the medial tilt direction than for the anterior and lateral tilt directions. For the MCL the changes in tensile stiffnesses per tilt step were not significantly different among the four tilt directions.

Table 1. Zero force lengths (mm), maximal strain levels (%) and strain rates (%) s$^{-1}$ for each ligament

<table>
<thead>
<tr>
<th>Ligament</th>
<th>Zero force length (mm)</th>
<th>Max. strain level (%)</th>
<th>Strain rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL1</td>
<td>32.9</td>
<td>9.1</td>
<td>60.7</td>
</tr>
<tr>
<td>ACL2</td>
<td>41.2</td>
<td>7.3</td>
<td>48.5</td>
</tr>
<tr>
<td>ACL4</td>
<td>32.3</td>
<td>9.3</td>
<td>61.9</td>
</tr>
<tr>
<td>PCL2</td>
<td>41.1</td>
<td>7.3</td>
<td>48.6</td>
</tr>
<tr>
<td>PCL3</td>
<td>41.3</td>
<td>7.3</td>
<td>48.4</td>
</tr>
<tr>
<td>PCL4</td>
<td>40.0</td>
<td>7.5</td>
<td>50.0</td>
</tr>
<tr>
<td>PCL5</td>
<td>34.3</td>
<td>8.7</td>
<td>58.3</td>
</tr>
<tr>
<td>MCL1</td>
<td>102.3</td>
<td>6.8</td>
<td>71.3</td>
</tr>
<tr>
<td>MCL4</td>
<td>105.6</td>
<td>6.9</td>
<td>68.6</td>
</tr>
<tr>
<td>LCL2</td>
<td>73.6</td>
<td>6.8</td>
<td>54.4</td>
</tr>
<tr>
<td>LCL4</td>
<td>68.5</td>
<td>6.6</td>
<td>63.7</td>
</tr>
<tr>
<td>LCL5</td>
<td>60.7</td>
<td>6.6</td>
<td>65.9</td>
</tr>
</tbody>
</table>

The tensile stiffnesses were affected by the relative orientations of the insertion sites for the cruciate ligaments [Fig. 5(a) and (b)] and the collateral ligaments [Fig. 6(a) and (b)].}

DISCUSSION

In the present study, it was demonstrated that tilt of the femoral insertion site affects the tensile stiffness of knee ligaments significantly. Even when the tensile test in the same orientation of the bones was repeated, a variation in tensile stiffness was observed. This was due to small relative translations of the bones, which could be concluded directly from
Fig. 5. The absolute stiffness change (N mm⁻¹) from the initial orientation (init.) to the tilted positions 1–5 for each tilt direction (Fig. 3) of the anterior cruciate ligaments (a) and the posterior cruciate ligaments (b). A: anterior; P: posterior; M: medial; L: lateral; init.: initial orientation of the bones.

Table 2. The estimated decrease in tensile stiffness per tilt step of 5° [β(N mm⁻¹ per 5°)] ± the standard error (S.E.) for the ACL, PCL, MCL and LCL (total) and specified for the posterior (P), anterior (A), medial (M) and lateral (L) tilt directions of these ligaments (ANCOVA). The brackets indicate tilt directions for which β is not significantly different (p > 0.05)
Fig 6. The absolute stiffness change (N mm⁻¹) from the initial orientation (init.) to the tilted positions 1–5 in each tilt direction (Fig. 3) for the medial collateral ligaments (a) and the lateral collateral ligaments (b). Note that the scale of the y-axis has been changed in comparison to Fig. 5. A: anterior; P: posterior; M: medial; L: lateral.

The effect of tilt on the tensile stiffness is higher for the cruciate ligaments as compared to the collateral ligaments. The main explanation for this phenomenon is the difference in the length-to-width ratios. For the collateral ligaments, which are long and thin, tilting of the femoral insertion site has a less shortening or lengthening effect on the outer fibers as compared to the cruciate ligaments which are relatively wide and short. A second explanation is that the geometry of the cruciate ligaments is more complex, involving different lengths and orientations of the fibers, than the collateral ligaments, which are rather parallel-fibered structures (Butler et al., 1985). Furthermore, it has been shown that the tangent modulus of the fiber bundles vary within the anterior cruciate ligament (Butler et al., 1992) as well as within the posterior cruciate ligament (Race and Amis, 1992). These aspects also explain that the standard deviations of tensile
The relationship between the stiffnesses and the relative insertion orientations is not expected to be biased by time-dependent effects. For the purpose of preconditioning, five loading cycles were applied at the beginning of each tensile test. Only the latter five cycles of each tensile test were processed for stiffness calculations. The load–displacement curves revealed no significant changes after about three loading cycles.

The mechanical properties of human knee bone–ligament–bone preparations were determined in a number of studies (Table 3). Most of the stiffness values reported are near the lower limit of the range of stiffnesses found in the present study (Figs 4–6), in spite of the high aged donors used in the present study, for which lower stiffness values are to be expected (Butler et al., 1978). From the results of the present study it became clear, however, that the observed differences between ligaments cannot be explained by interindividual differences only, but that also the geometric orientation of the ligament has to be taken into account. Apparently, the ligaments in the present study were oriented such that more uniform loading patterns of the fibers were achieved. Race et al., (1992) created test configurations with extreme non-uniformity in fiber loading by varying the rotation angle of the femoral insertion site. The variations described in measured structural properties by varying the rotation angle of the femoral insertion site are much more precise than the use of vernier calipers (Blankenvoort et al., 1988; de Lange et al., 1985), which suffer from ligament accessibility and are only suitable for superficial measurements.

Only in those orientations of the femoral insertion sites, in which all fibers are tensed between 6 and 7% strain, the term ‘linear’ stiffness can be used for the stiffness parameter. Otherwise, the stiffness still increases with progressive displacement of the femoral bone, due to loading of the lax ligaments function. The insіпIon sites of the ligaments are known to rotate and translate during knee flexion (Butler et al., 1988; Poliacu Prose et al., 1988; van Dijk et al., 1983). As has become clear from the present study, these rotations have less effect on the mechanical response of the collateral ligaments as compared to the cruciate ligaments. In other words, the way in which the ligament can perform its function is less dependent on knee position for the collateral ligaments than for the cruciate ligaments. For the cruciate ligaments, different portions are alternating in bearing loads during knee movement and thus in functional performance. For the ACL, for example, the anteromedial bundle is tense in flexion and the posterolateral bundle is tense in extension of the knee joint.

In conclusion, the mechanical response of a ligament, particularly the cruciates, is extremely sensitive to the relative orientations of the ligament insertions in the loading apparatus. Hence, a single unidirectional tensile test is insufficient to define the stiffness characteristics of a ligament. Furthermore, because the relative orientations of the insertion sites of the ligaments vary during knee motions (Butler et al., 1988; Girgis et al., 1975; Poliacu Prose et al., 1988), it is concluded that the functional role of the ligaments also varies.

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