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Tunnel Widening After Anterior Cruciate Ligament Reconstruction

An Experimental Study in Sheep

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Background: A common clinical concern after anterior cruciate ligament reconstruction is the expansion of the bone tunnels as seen radiographically. The etiology and clinical relevance of this phenomenon remain unclear.

Hypothesis: Tunnel widening results in an increased anteroposterior translation, and there are specific histologic changes due to osteoclastic bone resorption associated with this phenomenon.

Study Design: Controlled laboratory study.

Methods: Thirty sheep (age, 4 months) underwent an anterior cruciate ligament reconstruction using a soft tissue graft. Graft fixation was achieved using the EndoButton and Suture Washer. Six animals each were sacrificed at 0, 3, 6, 12, and 24 weeks after surgery. Each anterior cruciate ligament–reconstructed knee was examined by computed tomography. Anteroposterior translation was determined using a universal force-moment sensor robot. The bone surrounding the tunnel was evaluated histologically.

Results: The prevalence of tunnel enlargement on the femoral side was 77.3%. Animals with tunnel widening did not demonstrate increased anteroposterior translation. Widening of the femoral tunnel was significantly associated with a higher stiffness of the graft ($P < .05$) and hypertrophy of the graft throughout the remodeling process. The histologic evaluation of the bone tunnel walls demonstrated an increase of bone volume in animals with tunnel enlargement. No statistically significant correlation could be found between the number of osteoclasts and the presence of tunnel widening.

Conclusion: In this large animal model of anterior cruciate ligament reconstruction, animals with significant tunnel widening did not suffer increased anteroposterior translation. Tunnel widening was associated with a high stiffness of the graft, graft hypertrophy, and an increase in bone volume of the tunnel wall.

Clinical Relevance: The present data correspond to the current opinion in humans that tunnel widening is not associated with knee instability. Further research is needed to understand the role of graft stiffness, graft hypertrophy, and the increase in bone volume in this phenomenon.

Keywords: anterior cruciate ligament (ACL) reconstruction; sheep; tunnel widening; tunnel enlargement; knee; biomechanics; histology

The phenomenon of tunnel enlargement (TE) was first described in 1990 in association with freeze-dried, ethylene oxide–sterilized bone–patellar tendon–bone allografts used for ACL reconstruction.[12] Nineteen years later, the exact cause of TE after ACL reconstruction remains unknown. Mechanical and biological theories have been proposed to explain this phenomenon. Today, it seems that this process is most likely multifactorial, arising from both mechanical and biological causes.[9] The current perception of the etiologic factors arises from clinical observations predominantly of retrospective studies. Mechanical factors include motion of the graft within the tunnel, fixation methods and devices, stress shielding of the graft, improper

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graft placement, and accelerated rehabilitation. Graft swelling during the remodeling process, the use of allograft tissue, synovial fluid propagation within bony tunnels, and increased cytokine levels within the knee are thought to be biologic factors inducing osteolysis and eventual radiographic evidence of TE.[26]

The clinical relevance of tunnel widening is 2-fold; whereas most clinical studies failed to demonstrate an association between TE and instability, recent data suggest that tibial TE might be associated with anterior knee laxity.[23] Moreover, TE in the revision setting presents a difficult technical challenge with possible compromise of graft placement, fixation, and ultimately graft healing within the tunnels themselves. Because of these difficulties in revision surgery, attempts to avoid tunnel widening should be continued.[13] In contrast to other fields of ACL reconstruction in which a wide variety of basic research studies have been performed, few such models have been developed for studying TE. Because of the increasing numbers of ACL revision surgeries, it seems important to study the underlying mechanisms of tunnel widening using a standardized model.

It was the aim of the present study to measure TE in the early course after ACL reconstruction in a large animal model. After a radiographic evaluation of the ACL-reconstructed knees, the tunnel diameters were put in context to biomechanical and histologic data obtained from the same knees. We hypothesized that (1) the presence of significant tunnel widening is associated with an increased anteroposterior (AP) translation. We further tested the hypothesis that (2) the bone of the enlarged tunnel would demonstrate histologic signs of osteolysis.

MATERIALS AND METHODS

Experimental Design

All procedures were performed with permission of the local governmental animal rights protection authorities (Ref. No. 05/933) and in accordance with the National Institutes of Health guidelines for the use of laboratory animals. Thirty black headed sheep, aged 4 months, underwent a reconstruction of their right ACLs. The left knee served as a control.

Surgical Procedure

The right knee joint was exposed through an anteromedial incision with release of the medial parapatellar retinaculum. The patella was displaced laterally, the anterior fat pad sharply separated, and the ACL exposed and removed. Through an extra 3-cm incision, the ipsilateral gastrocnemius tendon and flexor digitorum tendon were exposed. Each of the 2 tendons was split longitudinally, and a half portion of each tendon was harvested, measuring about 65 mm in length and about 2.5 mm in diameter. Both strips of tendon were sewn together side-to-side at each end with baseball stitches using a polyester suture (Ethibond Excel No. 0, Ethicon Inc, Johnson & Johnson, Somerville, New Jersey) (Figure 1). The graft diameter consistently measured 4.5 mm. The tibial and femoral tunnels were created in a standardized technique. The tibial tunnel was created using a drill guide that entered 10 mm medially and distally of the most prominent point of the tibial tuberosity and was aimed toward the posterior part of the original ACL footprint. The tunnel was created using a 4.5-mm drill. The femoral tunnel was created using a transtibial technique. The drill guide was positioned in the posterior part of the notch in the 11-o'clock position and aimed toward the lateral cortex of the distal femur. Again, a 4.5-mm drill was used to create the tunnel. Graft fixation was achieved via the EndoButton (Smith & Nephew, Andover, Massachusetts) proximally and the Suture Washer (Smith & Nephew, Andover, Massachusetts) distally. For postoperative care, 5 mg/kg procaine-benzylpenicillin (long-acting penicillin and dihydrostreptomycin) was given as an antibiotic. Six and 24 hours after the initial injection of buprenorphine, the sheep received further doses. Carprofen was given in a dose of 2 mg/kg for 3 postoperative days. The animals were followed for weight gain. Five groups with 6 animals each were assigned and sacrificed 0, 3, 6, 12, and 24 weeks after surgery by a dose of barbiturates, administered intravenously.

Radiographic Evaluation

The evaluation of the CT scans with a slice thickness of 1.0 mm was done using the software Advantage Workstation, version 4.4 (GE Healthcare, Fairfield, Connecticut). An oblique multiplanar reformation was calculated using the 4-quadrant view (axial, sagittal, coronal, and the desired oblique view displayed on 1 screen at the same time). This oblique multiplanar plane showed the femoral tunnel in its full expansion (Figure 2A). Length measurements were taken from the articular entrance of the femoral tunnel to its exit on the lateral femoral cortex. Along this femoral tunnel axis, cross sections were defined perpendicular to the tunnel axis (Figure 2B). The diameter and cross-sectional area of the corresponding tunnel portion were determined using a measurement tool for distances (0.1-mm precision) and a software tool for calculating areas by defining a polygonal figure (eg, the tunnel wall). All measurements were done on a "class A" high-resolution screen approved for diagnostic radiology. The tunnel data were set in relation to the initially created tunnel diameter of 4.5 mm. The widening of the tunnels was then classified according to a modified grading system described by Nebelung et al.[16] No TE was present if the widening was within 10% compared with the time zero measurement; a grade 1 or mild TE was defined as widening of 10% to 25%, a grade 2 or moderate TE as a widening of 25% to 50%, and a grade 3 or excessive TE if the widening exceeded >50%. Finally, the different types of tunnel widening were determined on AP and lateral radiographs and classified according to the system of Klein et al.[13] This system describes different shapes of TE and tries to refer this shape to the type of fixation. A linear type of expansion is thought to be a sequel of a bungee effect; a cone type would be expected in the windshield wiper mechanism. According to Klein et al, a cross-pin fixation may cause a cavity or cystic type of TE.

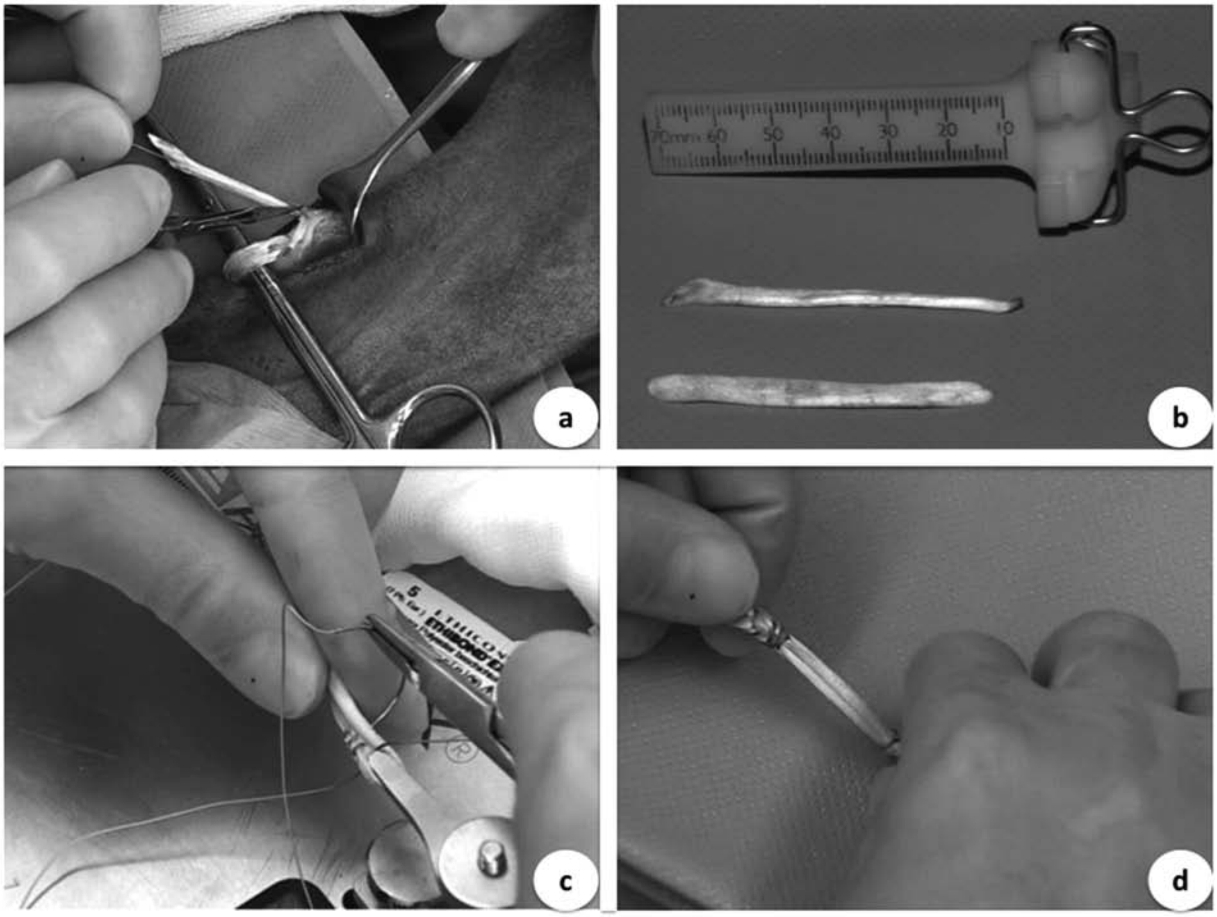

Figure 1. Exposure of the flexor digitorum tendon through an extra incision of 3 cm. A, the tendon was split longitudinally, and a portion of the tendon was harvested. B, a portion of the gastrocnemius tendon was then harvested through the same incision. C, the 2 strips of tendon were then sewn together side-to-side at each end. D, the resulting graft measured 65 mm in length and had a diameter of 4.5 mm, which was confirmed using a graft-sizing device.

Histology and Histomorphometry

The distal femur that contained the femoral tunnel with the ACL graft was harvested, trimmed, and fixed in 3.7% commercial formalin for 7 days at 4°C. After embedding and polymerization in methyl-methacrylate (Technovit 9100 New, Heraeus-Kulzer, Hanau, Germany) according to established protocols, the tissue blocks were sectioned perpendicular to the bone tunnel using a RM 2155 microtome (Leica, Bensheim, Germany). Five-micrometer-thin sections were cut and placed onto poly-L-lysine–coated glass slides. Before the different staining procedures, the sections were first dried for 2 days at 37°C, then deacrylated in xylol and 2-methoxyethyl acetate, cleared through a decreasing ethanol series (2 × isopropyl alcohol, 2 × 96% ethanol, 2 × 70% ethanol, 2 minutes each), and rehydrated to distilled water (2 minutes).

Bone mineral depositions were detected by the von Kossa staining method. Sections were placed for 30 minutes in a solution of 5% silver nitrate and exposed to a 100-W lamp. Sections were then washed in distilled water, placed in a solution of 1% pyrogallol for 1 minute, washed again, and fixed by finally placing the sections in a solution of 5% sodium thiosulphate for 5 minutes. Sections were again washed in distilled water, dehydrated in ethanol, and mounted in Aquatex (Merck, Darmstadt, Germany).

Osteoid surfaces were detected using toluidine blue staining. Sections were incubated in 0.1% toluidine blue O (Sigma, Taufkirchen, Germany) for 20 seconds, washed in distilled water, dehydrated in ethanol, and mounted in Eukitt (Labonord, Mönchengladbach, Germany).

Tartrate resistant acidic phosphatase (TRAP) staining was used to detect osteoclasts.[15] Sections were first placed for 20 minutes in 0.2 M acetate buffer (pH 5.0) and then for 120 to 180 minutes in freshly prepared TRAP staining solution containing naphthol-AS-MX phosphate sodium salt as enzyme substrate and fast red TR salt as azo dye in 0.2 M acetate buffer (all from Sigma). After staining, the sections were washed in distilled water and mounted in Aquatex. Control sections were incubated in incubation solution that did not contain the enzyme substrate. No staining developed

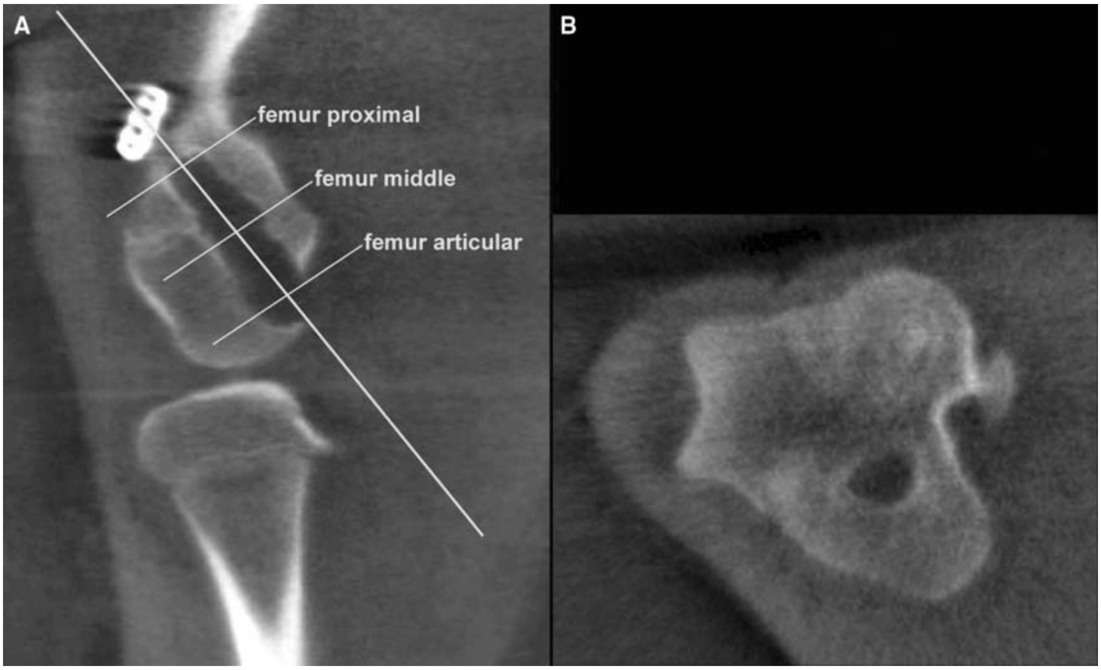

Figure 2. Multiplanar reformation (MPR) of a CT scan 24 weeks after surgery, right knee. A, the fixation device and tunnel orientation are visible. B, CT scan of the same animal through the femoral midportion, demonstrating the bone tunnel and a sclerosis of the tunnel wall. These sections were used for measuring the tunnel diameter and tunnel area.

in these control sections. As a positive control, young bone from New Zealand White rabbits was used.

Photomicrographs were taken with a Zeiss Imager Z1 microscope equipped with a scanning stage, a Zeiss AxioCam Mrc digital camera, and Zeiss AxioVision software (all from Zeiss, Oberkochen, Germany).

The histologic evaluation was done in the midportion of the femoral bone tunnel. All sections contained the intact bone tunnel and the surrounding bone. The examiner was blinded to the group assignment. For each stain, a series of 3 sections with 100-µm distance to each other was evaluated using standardized scripts of AxioVision software. In each section, a 500-µm broad seam around the bone tunnel was defined as region of interest. However, physis cartilage and cortical regions were selectively excluded from data analysis. Within the region of interest and using the von Kossa staining, the parameters bone volume, tissue volume, and bone surface were determined. Osteoid surface was measured using toluidine blue staining, and the number of osteoclasts was determined using TRAP staining. The index bone volume/tissue volume showed the amount of bone tissue surrounding the femoral tunnel, the index osteoid surface/bone surface showed the amount of bone formation, whereas the index osteoclast number/bone surface showed the amount of bone resorption within the tunnel wall. All parameters were measured according to the system of Parfitt.[17,18]

Biomechanical Testing

The ovine knee was mounted in a sensor-guided robot (KUKA KR 15/1, KUKA Robotics, Augsburg, Germany) in a specially designed testing fixture. The tibia was mounted to the force-moment sensor (IpeA, Berlin, Germany) and the femur to a fixed mounting block. Laxity of the specimens was tested by applying an AP displacement at 90° of flexion according to a protocol used by Weiler et al.[25] During this anterior-posterior motion, the tibia was allowed to freely translate about the remaining 2 axes and rotate in the varus-valgus and internal-external directions, thus allowing coupled secondary motions to occur. Anteroposterior displacement was applied until a force of 50 N in both directions was detected, and the direction of motion was reversed. Loading was applied over 3 complete anterior-posterior motion cycles at a displacement rate of 0.1 mm/s. The third hysteresis curve was taken for the calculation of AP range of motion. Stiffness was calculated from the ends of the loading portion of the hysteresis curve of the drawer testing as a measure of the rate of load take-up of the reconstruction. To quantify the enlargement of the graft throughout the remodeling process, the cross-sectional area of each graft was measured using a laser micrometer (Takikawa Engineering, Tokyo, Japan). The midportion of the intra-articular graft was measured and compared to the time zero animals. The midportion of the graft was chosen as this zone is the most representative to measure the diameter. The means of 3 measurements of each specimen were used for statistical analysis.

Statistical Methods

All data were tested for normal distribution using the Shapiro-Wilk test and the Kolmogorov-Smirnov test.

Comparisons between different time points were performed by 1-way analysis of variances, followed by post hoc tests for pairwise comparisons and independent *t* tests in the case of 2 samples. These differences were tested for significance at the .05 level.

The bone tunnel area was correlated with the AP translation, the graft cross-sectional area, and the histomorphometric parameters (bone volume, osteoid surface, and osteoclast number) using the Pearson correlation coefficient. The level for significance was set at $P < .05$. The data were analyzed using SPSS software (SPSS 11.5, SPSS Inc, Chicago, Illinois).

RESULTS

Postoperative Course

Two animals died of pneumonia (3- and 6-week groups) and had to be excluded from the study, leaving 28 animals for the final evaluation. Within 4 weeks after surgery, all animals had returned to a normal gait pattern. At the time of sacrifice, all grafts were in place, tensioned, and covered by a synovial sheath as early as 3 weeks after surgery.

High Prevalence of TE in the CT Scans. The analysis of the CT scans demonstrated an increase of the femoral tunnel diameter from 4.52 ± 0.71 mm at time zero to 7.91 ± 1.67 mm at 24 weeks ($P = .017$). The corresponding values for the tunnel cross-sectional area at time zero were 20.18 ± 6.86 mm^2 and increased to 36.96 ± 8.09 mm^2 at 24 weeks ($P = .015$) (Figure 3, A and B). According to the modified grading system of Nebelung et al,[16] 5 animals (22.7%) demonstrated no TE; 4 animals (18.2%), a grade 1 TE (enlargement of 10%-25%); 10 animals (45.5%), a grade 2 TE (enlargement of 25%-50%); and 3 animals (13.6%), a grade 3 TE (enlargement of >50%). The overall prevalence of TE in our animals was 77.3%. The analysis of the tunnel shapes according to the system of Klein et al[13] of the 17 animals demonstrating a TE indicated a linear type in 11 animals, the cavity type in 2 animals, the cystic type in 3 animals, and the cone type in 1 animal (Figure 4).

Good Restoration of Graft Stiffness Due to Graft Hypertrophy. The AP translation of the intact (left) control knees was constant at the different time points. The AP translation of the ACL-reconstructed knees increased from 7.47 ± 0.71 mm at time zero to 8.52 ± 1.04 mm at 3 weeks and finally decreased to 6.72 ± 1.80 mm at 24 weeks (Figure 5A). The differences of the AP translation were not significant ($P > .05$). The stiffness of the operated knees at time zero was 16.0 ± 2.20 N/mm and significantly increased to 30.9 ± 1.46 N/mm at 24 weeks ($P = .03$) (Figure 5B). There was also a significant increase of the graft's cross-sectional area from 16.99 ± 3.92 mm^2 immediately postoperative to 51.07 ± 12.71 mm^2 at 24 weeks, representing a graft enlargement during the remodeling process ($P = .001$) (Figure 3C).

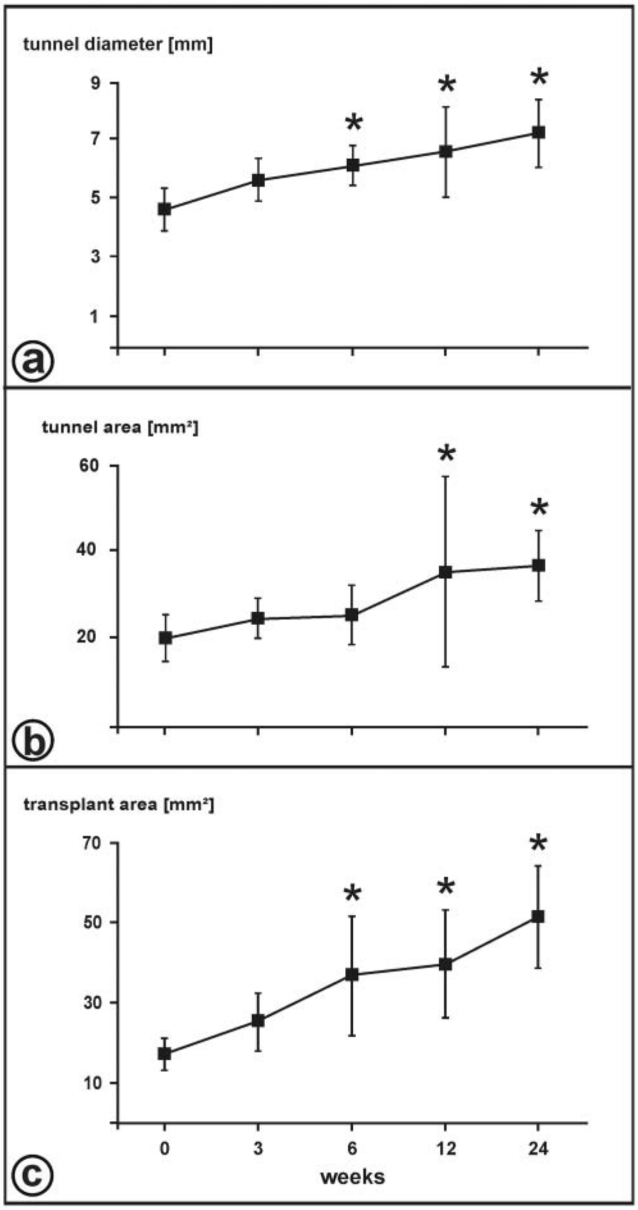

Figure 3. There was an increase in tunnel diameter, tunnel cross-sectional area, and transplant cross-sectional area throughout the time of observation. Tunnel diameter (A) steadily increased concomitantly with the tunnel area (B). This increase had statistically significant values at 6, 12, and 24 weeks. The transplant area (C) also steadily increased as measured with a laser micrometer, indicating a graft hypertrophy throughout the remodeling process. For the sake of clarity, the individual time points were connected by a line.

A Sclerotic Ring Emerges and Accompanies the Widening of the Tunnel. The histologic evaluation revealed the spatiotemporal dynamics of the formation of the tunnel wall healing and of its progressive enlargement. Immediately after surgery, the tunnel wall was dominated by remnants of broken bone, and it was rough and irregular (Figure 6, A and B). After 12 weeks, the tunnel increased in diameter and cross-sectional area. The soft tissue graft claimed the entire space of enlarged tunnel as it had hypertrophied. The bone of the tunnel wall considerably thickened, now forming a solid ring with a smooth surface, interrupted only by few remaining gaps (Figure 6, C and D). After 24 weeks,

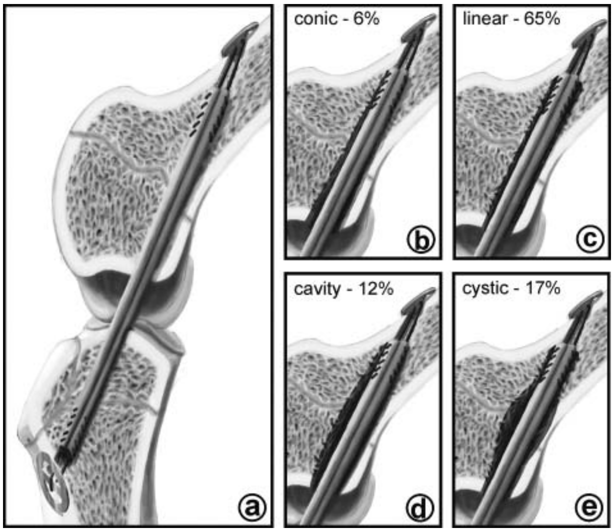

Figure 4. A, schematic diagram showing the spatial orientation of the ACL graft and the tunnels in a parasagittal view without any signs of tunnel widening. If tunnel enlargement (TE) is present, it may appear polymorphic and can be classified in 4 different types according to Klein et al.[13] The percentages of occurrence in the present study are shown: (b) conic (6%), (c) linear (65%), (d) cavity (12%), and (e) cystic (17%).

the tunnel wall had further thickened and appeared as a sclerotic ring enclosing the soft tissue graft. Numerous trabeculae were seen in the intimate vicinity of the tunnel, fixing and stabilizing it (Figure 6, E and F).

"Forming" Rather Than "Resorbing" Activities at the Tunnel Wall. Histomorphometrically, the development of this sclerotic ring was based on an increase of bone volume due to excessive osteoblastic activity. The bone volume surrounding the tunnel (indexed as bone volume/tissue volume) at time zero was 35.70% ± 7.1% and significantly increased to 70.98% ± 13.86% after 24 weeks (Figure 7A) (P = .001). The osteoid surface (indexed as osteoid surface/bone surface) at time zero was 4.94% ± 1.90% and significantly increased to 25.56% ± 8.19% at 3 weeks after surgery (P = .001). At the end of the observation period at 24 weeks, the osteoblast surface was still significantly increased with 18.43% ± 6.20% (P = .03) (Figure 7B). The corresponding data for the osteoclast number (indexed as osteoclasts/bone surface) were 0.028% ± 0.02% at time zero, 0.057% ± 0.02% at 3 weeks, and 0.056% ± 0.04% at 24 weeks after surgery. This increase was not statistically significant (Figure 7C).

No Correlation With Stability but Significant Correlation With Graft Hypertrophy. No correlation was detected between the diameter and the cross-sectional area of the tunnel and an increased AP translation. Even when correlating the data of animals with an excessive (grade 3) TE, there was no correlation to the AP translation. There was a statistically significant positive correlation between the tunnel area and the graft stiffness, the cross-sectional area of the graft, and the bone volume of the tunnel wall.

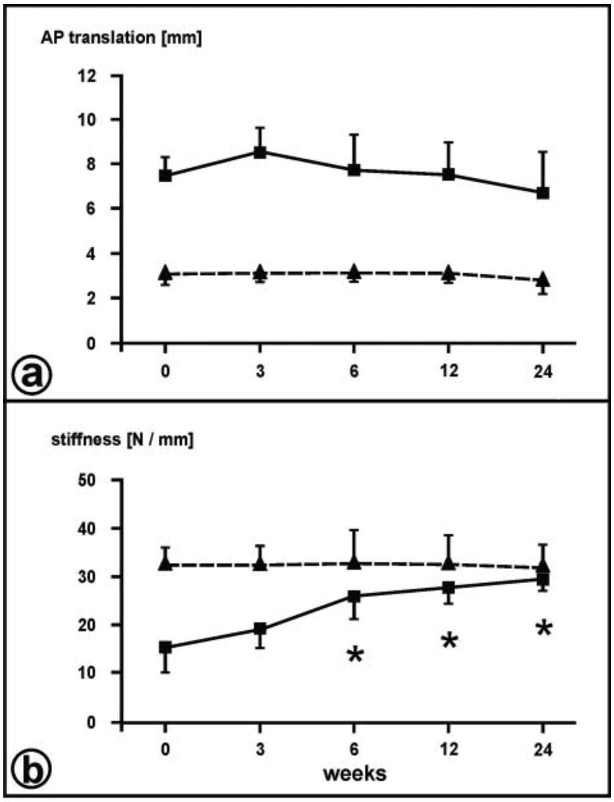

Figure 5. The biomechanical data show that the anteroposterior (AP) translation of the ACL-reconstructed knees did not reach the values of the intact, left knees but that the graft stiffness significantly increased to nearly normal values. A, the data for the AP translation in the operated knees (squares, continuous line) demonstrated a slow approach toward the data of the control knees (triangles, dashed line). However, even after 24 weeks, the values for the control knees are not reached. B, the stiffness data of the operated knees (squares, continuous line) demonstrated a progressive, statistically significant improvement. Finally, the graft stiffness approximated the values of the control knees (triangles, dashed line). For the sake of clarity, the individual time points were connected by a line.

DISCUSSION

To our knowledge, this is the first experimental model on TE that correlates the tunnel diameter to biomechanical and histologic data. This lack of basic science data on the interrelationship of tunnel widening and stability as well as graft hypertrophy has been emphasized in a recent review article.[26]

In a first step, we identified the presence of tunnel widening after ACL reconstruction by means of a CT scan. The radiographic data of our animal study are in line with human studies in terms of prevalence, timing, and grading of tunnel widening. The incidence of TE after ACL reconstruction reported in the literature varies from 37% to 75%.[14,16,21] Several studies in humans have demonstrated that TE is seen in the first 6 to 24 weeks after ACL

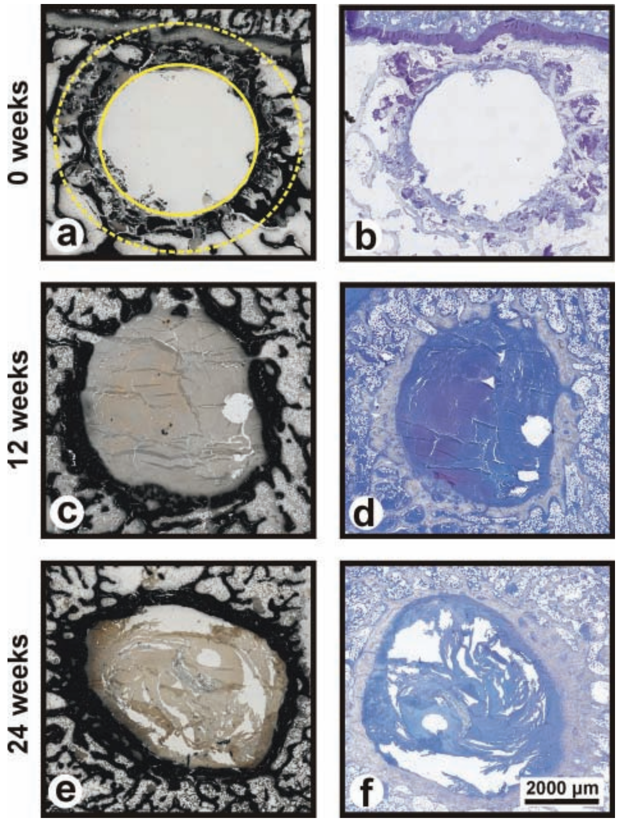

Figure 6. Bone-specific von Kossa staining (a, c, e) and toluidine blue staining (b, d, f) after 0 (a, b), 12 (c, d), and 24 weeks (e, f). For the sake of clarity, the time points 3 and 6 weeks were omitted. After 0 weeks, the wall of the tunnel was still rough and irregular (a, b). After 12 weeks, the tunnel increased in area and diameter, and the wall of the tunnel was solid and significantly thickened. The transplant has hypertrophied and filled the entire space inside the tunnel (c, d). After 24 weeks, the wall of the tunnel further thickened and was now surrounded by numerous trabeculae that crossed through the bone marrow. The solid yellow line marks the inner boundary and the dashed yellow line the outer boundary of the 500-µm broad region of interest, which was used for histomorphometry (Bar, 2000 µm).

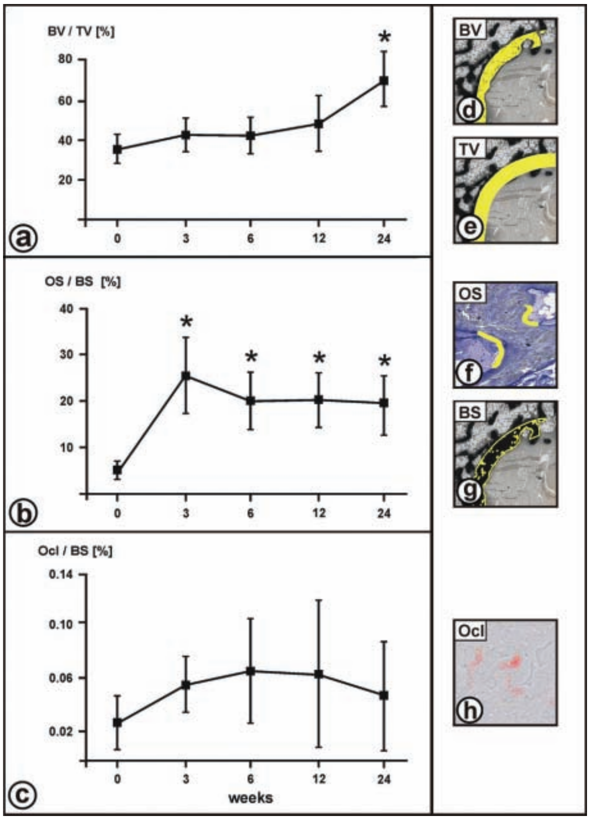

Figure 7. The histomorphometric analysis clearly revealed that the widening of the tunnel is accompanied by an increase in bone volume. A, the amount of bone tissue, indicated by the bone volume index (BV [d])/tissue volume (TV [e]), slightly increased during the whole time of observation and demonstrated a relatively steep increase from 12 weeks to 24 weeks. B, this increase in bone volume was caused by an activation of osteoblasts as determined by the osteoid surface, indicated by the osteoid surface index (OS [f]/bone surface BS [g]). The osteoid surface increased 5-fold and statistically significantly within 3 weeks and then slightly decreased. C, the number of osteoclasts (Ocl [h]/bone surface BS [g]) only demonstrated a slight increase up to 6 weeks that was not significant. For the sake of clarity, the individual time points were connected by a line.

reconstruction.[4,7,26] Consequently, it is supposed to be a phenomenon that parallels the tendon graft-to-bone healing process.[20] We further determined the joint laxity by use of a universal force-moment sensor robot. In this context, it has to be considered that ACL reconstructions in ovine and caprine models always result in biomechanically inferior situations when compared with that of humans.[5,6] This has to be kept in mind when analyzing the apparently excessive side-to-side differences in our study. We further did a thorough histologic workup to define bone-forming and bone-resorbing processes at the tunnel wall by use of established methods. We finally correlated the radiographic findings to the biomechanical and histologic data.

The main finding in the present study was that TE is not associated with an increased AP translation. This is in line with all recent clinical studies that investigated the interrelationship of form and function in patients with TE.[1,2,4,8,14,19,22,24] So far, only one study has postulated a correlation between the tibial tunnel widening and an increased instability as long as measurement errors in interpreting the radiographs were considered.[23] However, the authors did not find a correlation between a femoral tunnel widening and an increased instability in the same patients. What we found is a statistically significant correlation between a widening of the tunnel and the stiffness of the graft. Stiffness is a measure of the ability of the grafted tendon to take up load. This increase of stiffness is known to be associated with a hypertrophy of the graft that occurs during the remodeling process. This graft hypertrophy was found in the present study and has been described in previous studies.[3,11,25] It has also previously been associated with the phenomenon of tunnel widening.[3,11,26] It can be speculated that the hypertrophy of the graft throughout the remodeling process may not only occur in the intra-articular portion of the graft but also within the bone tunnel.[10] It is reasonable to hypothesize that this graft enlargement causes the tunnel to widen.[26] In the present study, the tunnel wall consisted of a sclerotic ring as soon as 3 weeks after surgery. This sclerotic ring expanded and consisted of newly formed bone that demonstrated a high bone volume as measured histomorphometrically. We found a statistically significant association between the presence of TE and the bone volume: the knees that radiologically demonstrated a TE in our study were significantly associated with a high bone volume surrounding the tunnel wall. A recently published study advocates a predominance of bone-resorbing processes responsible for tunnel widening, mediated by osteoclasts.[20] In that study, Rodeo et al hypothesized that an excessive osteoclastic activity may contribute to tunnel widening. New Zealand White rabbits underwent an ACL reconstruction with a soft tissue graft. A locally applied antiresorptive agent inhibited the osteoclastic activity and resulted in an increased amount of bone surrounding the tendon graft at 4 and 8 weeks. They theorized that tunnel widening may be owing to osteoclastic bone resorption. However, in the present study, the osteoclast number as a measure of bone-resorbing processes was low at any time point and lacked a statistically significant increase as seen in the osteoblast activity. Moreover, there was no statistically significant correlation between a widening of the tunnel and the osteoclast number. It is important to keep in mind that the results of this animal model are not transferable to the human situation. It has been well documented that ACL reconstructions in quadruped animals always result in an increased AP laxity when compared with humans.[5,6]

In summary, we can conclude that the widening of the bone tunnel throughout the first 6 months after ACL reconstruction is not associated with an increased AP laxity in this animal model. The TE is related to a high stiffness and an enlargement of the graft. We therefore postulate that TE is mediated at least to some extent through a graft enlargement due to the remodeling process. The graft enlargement within the bone tunnel may result in a significant increase of bone volume in the surrounding wall. We could not identify any signs of osteoclast-mediated bone resorption associated with tunnel widening in the present study.

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