The Effect of Anterior Cruciate Ligament In Situ Graft Force on Graft-Tunnel Incorporation Following Reconstruction

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Disclosures:

Introduction: A number of orthopaedic procedures require secure healing of tendon autografts to bone in order to achieve a successful clinical outcome. This includes soft-tissue anterior cruciate ligament (ACL) reconstruction where technical factors, such as graft position and pre-tension, alter the mechanical forces experience by the graft and the graft-bone tunnel interface during knee motion. A clinical example where this may be relevant is the recent technical shift from the traditional transtibial ACL reconstruction to a more “anatomic” ACL reconstruction. While existing studies suggest these newer anatomic techniques improve knee rotatory stability, there is also evidence that these ACL grafts experience higher in situ forces with knee motion when compared to traditional transtibial ACL grafts [1]. It is not well understood how these elevated in situ graft forces may affect ACL healing following reconstruction.

Previous studies have demonstrated that mechanical loading plays a critical role in maintaining the homeostasis of native musculoskeletal tissues. A similar relationship likely exists for the healing tendon-bone enthesis following placement of a tendon graft into a bone tunnel for an ACL reconstruction. Poor healing along the ACL graft-tunnel interface has been characterized by decreased biomechanical properties, decreased bone mineralization, as well as increased osteoclastic activity [2]. Existing preclinical studies on ACL healing have been limited by an inability to quantify or control the loads applied to the ACL graft and its tendon-bone interface in a clinically relevant in vivo model. The objective of this study is to evaluate the effects of elevated in situ ACL graft force on graft-bone tunnel incorporation. We utilized a novel rat ACL reconstruction model where the in situ ACL graft force at the time of surgery and ACL load following surgery were defined and controlled. Our hypothesis is that high ACL in situ graft force will delay graft-bone tunnel incorporation as evident by biomechanical, radiographic, and histologic analyses following reconstruction.

Methods: Male Sprague-Dawley rats (n=76) underwent unilateral ACL resection followed by reconstruction using flexor tendon autograft. The animals were allocated into one of three groups during surgery: (1) ACL reconstruction followed by knee immobilization for the entire duration of the study, (2) ACL reconstruction with a "high-tension" ACL graft and daily knee motion, or (3) ACL reconstruction with an "isometric" low-tension ACL graft and daily knee motion. For the "high-tension" ACL graft, the femoral tunnel is oriented so that there is an increase in ACL in situ graft force with knee flexion (Fig. 1). This is in contrast to an "isometric" graft where the tunnel is orientated such that graft forces remain relatively constant with knee motion. All grafts were pre-tensioned to 5N with the knee in 40 degrees of flexion. An external fixator was applied to eliminate ACL graft load during cage activity. For animals randomized into the knee motion groups, daily range of motion (ROM) was started on post-operative day #3. Animals were sedated, external fixators were removed, and the knees were ranged using a computer-controlled motion device (ROM 0-90 degrees, 50 complete cycles daily) (Fig. 2). Outcomes measured included biomechanical, micro-CT, and histologic analyses at 3 and 6 weeks following reconstruction. Comparison was performed using ANOVA. All data are presented as mean ± standard error of the means (SEM). A p-value <0.05 was considered to indicate significant differences. The study was approved by the local Institutional Animal Care and Use Committee.

Results: ACL grafts that experienced high in situ graft forces demonstrated inferior biomechanical properties at the 3-week time point. At 3 weeks, the load-to-failure of the femur-ACL graft-tibia complex was significantly lower for high-tension ACL grafts (5.50 ± 2.30 N) than isometric ACL grafts (9.91 ± 3.36 N, p<0.05) and stress-deprived ACL grafts (knee immobilization) (10.9 ± 2.78 N, p<0.05) (Fig. 3). At 6 weeks, isometric ACL grafts (24.16 ± 5.72 N) demonstrated superior load-to-failure properties when compared to high-tension (17.84 ± 4.84 N, P<0.05) and stress-deprived ACL grafts (12.51 ± 4.58 N, P<0.05). In terms of stiffness of the femur-ACL graft-tibia complex, high-tension ACL grafts demonstrated lower mean stiffness at 3 weeks when compared to stress-deprived or isometric ACL grafts, but this did not reach statistical significance. At 6 weeks, however, stress-deprived ACL grafts in immobilized knees had significantly lower stiffness when compared to grafts that experienced graft load. The mode of failure for all tested ACL grafts was graft-tunnel pullout from either the femoral (41%) or tibial (59%) tunnel. Micro-CT analyses demonstrated less bone volume per tissue volume (BV/TV) in the femoral tunnels of high-tension ACL grafts when compared to isometric ACL grafts at 3 weeks (0.28 ± 0.07 vs. 0.49 ± 0.05, p<0.05) and 6 weeks (0.16 ± 0.05 vs. 0.4 ± 0.06, p<0.05). No significant differences were seen in the tibial graft tunnels at either time point. Histologically, a higher prevalence of osteoclasts was seen on TRAP-stained sections in high-tension ACL graft. This may be indicative of greater osteoclastic activity.
was seen along the graft-bone tunnel interface in high-tension ACL grafts when compared to immobilized or isometric ACL grafts.

**Discussion:** The biologic consequences of elevated ACL graft forces on graft-tunnel incorporation are relatively unknown. Existing preclinical studies on ACL healing have been limited by an inability to quantify or control the loads applied to the ACL graft while utilizing a clinically relevant in vivo model. We developed a novel small animal ACL reconstruction model where the in situ ACL graft force at the time of surgery and subsequent ACL load following surgery were defined and controlled. Our results suggest that ACL grafts that experience high in situ forces post-operatively had inferior biomechanical properties as evident by the lower load-to-failure forces when compared to isometric ACL grafts. The inferior biomechanical properties may be related to the decrease mineralization and increased osteoclastic activity seen along the graft-bone tunnel interface. Interestingly, our results also suggest that prolonged stress deprivation of the ACL graft with limb immobilization may not be necessarily advantageous. At 6 weeks, ACL grafts in immobilized knees demonstrated both inferior load-to-failure and stiffness properties. Our data therefore suggests there is likely an optimal level of mechanical load during the post-operative period where graft-tunnel incorporation is optimized.

**Significance:** Contemporary anatomic ACL reconstructions likely result improved knee stability at the expense of higher in situ graft forces. While the clinical consequences of higher in situ ACL graft force seen is far from clear, our results does provide some preclinical evidence that ACL grafts that experiences early high in situ forces may not heal as well as more isometric grafts. This raises the issue whether our traditional ACL rehabilitation regimen should be modified in light of more contemporary reconstruction techniques that place more demand on the ACL graft.

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**References:**
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Figure 2. Custom daily knee motion device where rats’ knees were ranged daily following reconstruction. Post-operative motion was only allowed for high-tension and isometric ACL graft groups.

Figure 3. ACL load-to-failure properties (left graph) and stiffness (right graph) following ACL reconstruction.

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