INTRODUCTION:
A number of orthopaedic procedures require secure healing of bone to tendon to obtain a successful outcome, including ACL reconstruction using a soft-tissue graft. Healing of a tendon graft within a bone tunnel requires the ingrowth of new bone at the bone-tendon interface. In this study, modulation of the mechanical environment was achieved by subjecting a tendon graft to cyclic axial loading. Understanding how the graft microenvironment influences healing has critical implications for the design of postoperative rehabilitation protocols following ACL reconstruction. The purpose of this study was to determine the effect of magnitude, timing, and duration of mechanical stimulus on bone formation and osteoclastic activity.

METHODS:
I. Surgical Procedure. After obtaining IACUC approval, 72 skeletally mature male Sprague-Dawley rats underwent surgical placement of an Achilles tendon autograft with calcaneal bone block in a femoral tunnel (Fig 1A). A wire was placed in the calcaneal bone block, passed through the skin, and attached to a custom-designed motorized loading device. An external fixator was placed on the operative extremity to minimize loading of the graft during ad-libitum cage activity (Fig 1B). Prior to surgery, each animal was assigned to one of ten groups depending on the initiation of loading (immediate, delayed, or immobilized), the magnitude of loading (low 3N or high 6N), and time to sacrifice (3 weeks or 6 weeks). The daily loading regimen included application of 50 cycles of uniaxial load from 0.15N to targeted tensile load (3N or 6N) at 0.7 mm/sec with the animal under light anesthesia (Fig 1C).

II. Micro-CT Analysis. Trabecular architecture, bone formation, and bone remodeling along the tendon-bone interface were assessed with use of micro-CT (4-5 specimens per group). A global threshold, based on the histogram of CT attenuation values derived from the Otus discriminant, was used for each specimen to distinguish bone voxels in the images. Six outcome measures were evaluated: total bone volume (mm³), bone volume (mm³), tissue mineral density (mg/mL), trabecular thickness (μm), trabecular number, and trabecular spacing (μm) to assess new bone formation at the tendon-bone interface at the apature, middle, and exit aspects of the tunnel (Fig 2A).

III. Histological Analysis. The distal femur with attached autograft was prepared for routine histologic analysis. Osteoclast activity was evaluated by chemical staining for tartrate-resistant acid phosphatase (TRAP). Histologic measurements were carried out using Bioquant Osteo II software (BIOQUANT OSTEO II, Nashville TN). Measurements to quantify osteoclast activity were made at 6 different regions along the bone-tendon interface within the femoral tunnel (2 at proximal end, 2 at middle, and 2 in the distal end). These regions were 750μm long and 200μm wide and aligned so that the width was half way between the tendon-bone interface (Fig 2B).

RESULTS:
Total bone volume at the proximal aspect of the tunnel was higher in the immediate 3N group (7.0±0.1mm³) when compared to the immobilized group (6.7±0.1mm³) at 3 weeks (p<0.05). Total mineral density at the middle aspect of the tunnel was higher in the immobilized group (423.5±1.8 mg/mL) compared to the delayed 6N loading group (418.7±2.6 mg/mL) at 3 weeks. At 6 weeks, bone volume was slightly higher in the immobilized group (0.3±0.05mm³) compared to immediate 3N group (0.2±0.05mm³, p<0.05). We observed a greater trabecular number in the immobilized group (4.4±0.3) when compared to all other loading groups (p<0.05). With regards to trabecular spacing, the immediate 3N group (0.5±0.1μm) was greater than the immobilized group (0.2±0.02μm, p<0.05). All of the differences within the 6 week groups were at the proximal aspect of the tunnel.

Our histological data displayed greater osteoclastic activity at the proximal aspect of the tunnel from 2.2±2.4 cells per HPF in the immobilized group to 15.1±14.1cells per HPF (p<0.05) in the immediate 6N group at 3 weeks. A significant increase in activity was also found in the delayed 6N group (4.6±5.3cells per HPF) compared to the immediate 6N group (5.2±5.6 cells per HPF, p<0.05). At 6 weeks, we observed a significant difference at the distal end of the tunnel between the immediate 3N (0.8±1.2cells per HPF) and immediate 6N groups (14.0±7.1cells per HPF, p<0.01), as well as between the immediate 3N and delayed 3N groups (0.8±1.2cells per HPF vs 7.5±7.0cells per HPF, p<0.01).

DISCUSSION:
This new model allows direct and controlled application of load of a healing tendon graft. Immediate application of low magnitude (3N) cyclic axial loading in this tendon-bone rat model resulted in increased total new bone formation at the proximal aspect of the enthesis as compared to prolonged immobilization at 3 weeks postoperatively. However, by 6 weeks, the bone volume, trabecular number, and trabecular spacing were superior in the immobilized animals. This is supported by greater osteoclast activity in the animals that received loading. Immobilization appears to allow better new bone formation at the healing tendon-bone interface over time. These results support prior studies from our laboratory using a rat model of ACL reconstruction (Bedi et al).

SIGNIFICANCE:
Greater understanding of the effect of the mechanical stimulus on tendon-to-bone healing may allow surgeons to improve healing by modifying the post-operative rehabilitation regimen, resulting in more stable reconstructions and repairs.

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