Semitendinosus Muscle Fatty Infiltration Following Tendon Resection in Rabbits

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Introduction

The current gold standard for anterior cruciate ligament (ACL) reconstruction is to use either a bone-patellar tendon-bone (BPTB) autograft or a hamstring tendon (HT) autograft. Each has advantages and disadvantages but it is unclear which results in the best outcome. Reviews do not show consistent differences between the two graft types [1]. Excellent functional results are reported for patients who had a BPTB graft.[2,3]. However, the BPTB graft is associated with multiple morbidities including postoperative patellofemoral pain, chondrosis, patellar fractures, patellar tendon ruptures, and quadriceps weakness[4,5]. Many surgeons prefer the HT graft because of the morbidity associated with the BPTB graft. In addition, previous studies show the HT graft has superior biomechanical stability[6], and better approximates the anatomy and functionality of the normal ACL[7]. In follow-up studies of patients who have undergone ACL reconstruction with a HT graft, patients regain near normal hamstring strength within 3 years [8,9]. However, a number of recent studies demonstrated that hamstring strength deficits persist even after 3 years, especially in deep knee flexion and internal rotation[10]. Many recent reports have described in detail the morphological and histological regeneration of the hamstring tendon after resection [11]. However, relatively little research has been done to elucidate similar information about the hamstring muscles.

Studies in the rotator cuff demonstrate that after transection the muscles become infiltrated with fat. No studies have examined the microstructural changes that occur in hamstring muscles following tendon harvest. We hypothesize that following tendon transection there will be fatty infiltration of the muscles and that this infiltration will progress with time.

Materials and Methods

The study was approved by the Wright State University Laboratory Animal Care and Use Committee. Fifteen mature female New Zealand white rabbits weighing an average of 3.6 ± 0.07 kg were randomized into three groups of five rabbits each. Unilateral semitendinosus tendon removal was performed on all rabbits with the surgical side randomized to control for leg dominance. The contralateral limb served as the control group. Rabbits in Group I were euthanized at three months, Group II were euthanized at six months and Group III were euthanized at 12 months postoperatively. Following euthanasia muscle fat percentage was measured.

Operative Technique: Rabbits were anesthetized with ketamine (20 mg/kg), xylazine (2.5 mg/kg) and glycopyrrolate (0.1 mg/kg) and maintained on isoflurane. The skin overlying the posterior knee was shaved, sterile prepared, and draped. A medial parapatellar incision was made and the semitendinosus tendon was dissected. A whipstitch was placed in the semitendinosus tendon proximal to its insertion and the distal tendon was dissected from its insertion on the tibia. A tendon stripper was then passed over the tendon proximally to the tendon-muscle junction. The tendon was cut at its origin and discarded. The wound was cleansed with sterile saline and closed with absorbable suture. The rabbits were housed in 0.48 meter cages and allowed full activity. Postoperative pain was managed with a fentanyl patch for six days.

Each rabbit was euthanized at the scheduled time by anesthesia followed by whole body perfusion. While deeply anesthetized the rabbit legs were held in full extension at the knee and then perfused at 100mm Hg with cold phosphate buffered saline solution (1.5 L), followed by 1 L McDowell-Trumps fixative. Muscles were stored in McDowell-Trumps fixative until histological examination.

Fat Percentage Measurement: Muscle specimens taken from the most proximal portion, the midsubstance and at the musculotendinous junction were stained with osmium tetroxide, processed through a gradient of alcohols, embedded in paraffin, and sectioned at 4 μm (Histo-Path of America, Inc., Millersville, MD). A DP72 Olympus camera was used to take digital images of each specimen at 10x magnification and image J software was used to examine the specimens. Fat percentage was calculated by using the ratio of fat area to the total area of the fat and muscle for all tissue on the slide (multiple pictures were analyzed per slide). Samples were randomized for two blinded individuals to measure each specimen twice. Given the small sample sizes and non-normality of the data the non-parametric Mann-Whitney test was used to compare groups. Inferences were made at 0.05 significance level.

Results

Fatty infiltration at the musculotendinous junction was significantly greater in the experimental muscle (7.8%) compared with the control muscle (2.7%) for all 15 specimen (p=0.05). The fat to muscle ratio was marginally significant at three months (p=0.056). At six and 12 months the two groups did not differ for the musculotendinous junction (6 months p=0.69; 12 months p=0.31). There was no significant difference in fatty infiltration between the control and experimental muscle in the midsubstance or muscle origin at any time point (p values ranged from 0.056 to 1.0). Histologically fatty infiltration in the semitendinosus muscle was found around muscle bundles and did not infiltrate into the fascicles (Figure 1).

Figure 1. Osmium tetroxide staining of fat (black). A) Three month transected semitendinosus muscle B) Control semitendinosus muscle C) Three month transected supraspinatus muscle D) Control supraspinatus muscle

Discussion

Harvesting the semitendinosus tendon in rabbits increases fatty infiltration at the musculotendinous junction but not in the midsubstance or origin of the muscle. The fatty infiltration was not progressive in the musculotendinous junction, but rather was normalizing as the injury resolved. In addition, the injury pattern is less infiltrative compared with a rotator cuff injury model. Fatty infiltration in the supraspinatus extended into the muscle fascicles (Figure 1) whereas it was limited to around the muscle in the semitendinosus. The fatty infiltration was also greater in the rotator cuff averaging 19% at 3 months compared to 6.2% in the semitendinosus at the same time point. This suggests that the reparative ability of the semitendinosus muscle is likely different.

A limitation of this study is that the ACL was not repaired with the graft material. The rabbits were allowed full range of motion following the tendon removal. However, in human’s range of motion is usually limited by the surgical repair after the procedure. Thus, healing parameters may be different when considering an actual ACL repair. In conclusion, use of the semitendinosus tendon for ACL repair may not lead to irreversible fatty infiltration of the muscle. Further studies in humans may show the use of the semitendinosus tendon in ACL repair to be beneficial compared to patellar tendon use because of a reduction in the morbidity of the graft harvest site. Future studies to examine this potential should be performed.

References