INTRODUCTION:
By 2050, 22% of Americans will be over 65 years of age [1]. The people in this demographic have decreases in musculoskeletal function and mobility. To compensate for these losses and to promote cardiovascular fitness, the elderly have been encouraged to exercise [2]. This increased activity, coupled with age-related declines in soft tissue biomechanics [3] increases the risk of injury to connective tissues such as ligaments and tendons. Damaged ligaments and tendons in the young adult regain 20-30% of normal, uninjured strength [4]. Tendon strength is most closely correlated with the size of the type I collagen fibril, larger fibrils providing greater strength. In healing tendons, collagen fibril diameter is smaller than the normal tissue, which is consistent with the decreased strength. In a previous study, we found that there was a decrease in the strength of normal patellar tendon (PT) with age and a correlating decrease in fibril diameter [3]. It is generally believed that as we age there is a decline in healing ability. We speculated that because of this and the decrease in normal tissue strength, there would be a similar decrease in the repaired tendon biomechanical properties. We thus hypothesized that collagen fibril diameter in aging adult rabbit PT repairs, would be smaller than fibrils from young adult rabbits.

METHODS:
Animal Model
Full-length, full-thickness, central-third PT injuries were created in one-year old (n=12) and four-year old (n=11) New Zealand White rabbits in accordance with protocols approved by the University of Cincinnati and Veterans Affairs Medical Center Animal Care and Use Committees. One-year old rabbits have closed epiphyses and are considered to be young adults, while four-year old rabbits represent a senescent age group. During surgery suture markers were placed in the adjacent normal tendon. These marks were utilized subsequently during dissection to identify the limits of the wound site. The skin was closed and after recovery from anesthesia the rabbit was returned to its cage. All rabbits were ambulatory on the same day following surgery, and showed no obvious negative effects of the surgical procedure. The contralateral tendon served as an unoperated control. 

Ultrastructural Analysis
Patellar tendons were harvested from the 1- and 4-year old rabbits and a small section (1mm³) was collected from the patellar end, center, and tibial end of each tendon for ultrastructural analysis 12 and 26 weeks post-surgery. The tissue was fixed in 2% glutaraldehyde for four hours and then transferred to 100mM cacodylate buffer (pH 7.4). The tendon was then post-fixed with osmium tetroxide and thin sections (70-80Å) were stained with lead acetate. Cross-sections were sampled and photographed (Hitachi H600 microscope) from five randomly selected grid areas at 30,000 X magnification. Developed negatives were digitally photographed and minimum fibril diameter was computed using The Image Processing Tool Kit, an Adobe Photoshop compatible plug-in from Reindeer Graphics. 

Statistical Analysis
Four-factor mixed model ANOVA analyses were conducted corresponding to each of the 5 quintiles, as well as the median, maximum, minimum, and mean observations. The models fit correspond to a split-split plot design in which the fixed whole plot factors are Age and Time, the split plot factor is Injury, and the split-split plot factor is Location. SAS PROC MIXED was used for the analysis. A subsequent pair-wise contrast analysis was also performed on the main effect of each factor as well as the various combinations of these 4 factors’ levels. 

RESULTS:
Between 84 and 1366 fibrils were analyzed for each tendon (average=724). Histograms were created from the pooled samples (Figure 1). A significant reduction in the size of the fibrils was seen in the repairing tendons at all time points compared with normal controls. There was a significant reduction in the control tendon fibril size in the middle of the tendon in the 4-year old rabbit compared to the 1-year old rabbit, however there was no difference at the patellar and tibial ends. Significant differences were observed between different locations in the control tendon, with the middle portion being significantly larger than the patellar and tibial ends. There was no significant difference with location in the repairing tendon. There was no significant difference at 12 and 26 weeks post-injury in the repair tissue collagen fibril diameters between 1- and 4-year old rabbits.

DISCUSSION:
As expected, there was a decrease in the size of the collagen fibrils in the repairing tissue compared to normal tendons in both the 1- and 4-year old rabbits. However, the fibril diameter in the repairing tendon was not significantly different between 1- and 4-year old rabbits. We have also previously reported the biomechanical properties of repairing patellar tendons of 1 and 4-year old rabbits [5]. There was no significant difference in the biomechanical properties between 1- and 4-year old rabbits at 6 and 12 weeks post-injury, and at 26 weeks there was an increase in the modulus and maximum stress of the aging tendon [3]. Our research challenges a decreased healing potential in aging animals, despite decreases in normal tendon strength and fibril diameter with age [5]. Differences in repair failures in the elderly compared to the young may thus reflect a change in the normal tendon surrounding the injury site, instead of a change in the repair tissue itself. 

A second finding was the absence of a difference between the healing tendon at the patellar, tibial and middle portions of the tendon, suggesting that the collagen fibrils in these three locations are the same diameter. This is in contrast to the normal tendon in which the fibrils are significantly smaller at both ends of the tendon. The normal collagen fibrils start splitting at the end of the tendon and integrate into the bone as Sharpey’s fibrils. This is not occurring in the repairing tendon. Further studies to improve tendon integration to bone are ongoing.

REFERENCES:
2. Kannus et al. (1992) JAMA. 28:1

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