ABSTRACT INTRODUCTION:
The relationship between collagen fibril morphology/distribution and the functional behavior of tendon has been investigated in numerous experimental studies. Several suggest that larger fibril diameter is a primary determinant of higher tendon stiffness and strength [1], others have shown that factors apart from fibril diameter (such as fibril-fibril interactions) may be critical to improved tendon strength [2].

In the present study, we evaluated the ultrastructure and mechanical properties of the Achilles tendon in the two inbred mouse strains C3H/HeJ (C3H) and C57BL/6J (B6). Adult B6 and C3H mice are similar in body size and weight and their skeletons are similarly sized, but they show morphologically distinct traits [3]. Despite differences in adult peak bone density and whole bone cross-sectional area, they have similar mechanical properties at the structural level [4].

We hypothesized that, as with bone, these two genotypes would show differences in tendon architecture but exhibit similar macroscopic mechanical behavior. Our aim was to compare mechanical and structural characteristics of these two genomic groups, and to establish a baseline for examining which tendon architecture parameters might relate to the tensile mechanical properties. For this purpose Achilles tendons from both groups were tested to failure in tension. Tendon cross-sections were analyzed using transmission electron microscopy (TEM).

METHODS:
Achilles tendons from female 19-week-old B6 and C3H mice were used. For the mechanical tests, twenty Achilles tendons per group were dissected with the calcaneous and the intramuscular tendon fibers to the midsubstance. The tendon was partially stained with indigo ink to facilitate relative movement of the fibrils through the matrix.

Synchronized with the mechanical test, a video was recorded with a high-speed digital camera (VT Cam, AOS Technologies AG, Switzerland), in order to quantify the local strain in the tendon midsubstance. The tendon was partially stained with indigo ink to improve image contrast.

For the morphological investigation with TEM, seven freshly dissected Achilles tendons from each mouse strain were fixed and stained according to the method of Reynolds [4]. Regions of interest (ROI) were identified at low magnification. Micrographs of seven ROI were taken per tendon at a final magnification of x40'000 making a total of 98 micrographs. A custom, automatic script was used to segment and component label the collagen fibrils as shown in Figure 1.

RESULTS SECTION:
The C3H group exhibited a median of 385MPa in apparent modulus while B6 266MPa (p<0.005), no statistically significant difference in either mean failure strain (C3H = 41±33%, B6 = 36±17%) or mean ultimate stress (C3H = 33±9MPa, B6 = 28±11MPa). Image analysis results from the transmission electron micrographs are summarized in Table 1. Here it can be seen that C3H mice have a larger mean fibril radius than B6 (p<0.005), while the collagen area fraction of the extracellular matrix and the lateral spacing between fibrils are the same for both groups. Total fibril perimeter (surface area) was higher in the B6 (p<0.005), but effective contact area between fibrils was again similar.

DISCUSSION:
Given comparable macroscopic tendon architecture in the two evaluated mouse strains, classic composite theory suggests that similar values of collagen area fraction in both groups would yield a similar mechanical response (stiffness and strength) in tensile testing. The present study suggests that tendon structure-function is not adequately described by this theoretical framework. Our mechanical and morphological comparison between B6 and C3H showed that despite very similar collagen area fractions, C3H tendons are considerably stiffer, possibly due to larger fibril size and/or less inter-fibril gliding promoted by fibril-hydrated ECM interactions; collagen fibrils are densely linked to hydropilic proteoglycan chains where their surface is in contact with the matrix, and these chains may facilitate relative movement of the fibrils through the matrix.

Consistent with composite theory, no statistical differences were found in maximal stress or strain at failure. Furthermore, the close similarity in both mean interfibrillar distance and fibril-fibril contact area suggests there would be no relative advantage in either group with regard to fibril-fibril interactions (lateral force transfer between fibrils) that might promote a higher apparent modulus of the tendon.

This study provides useful insight into the influence of collagen fibril morphology on tendon mechanical properties. It establishes a baseline for quantifying natural differences in tendon structure and function in two inbred mouse populations. This information will aid in the interpretation of studies that employ selective chemical degradation of tendon ECM components, and transgenic animal models used to understand how tendon derive its mechanical characteristics from its basic architecture and biochemical composition.

REFERENCES:

Poster No. 1427 • 55th Annual Meeting of the Orthopaedic Research Society