

# Subacromial Impingement of Aged Murine Tendons Leads to Functional Impairments and Inferior Humeral Bone Quality

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**INTRODUCTION:** Rotator cuff tendinopathies are among the most common upper extremity musculoskeletal conditions, with a significantly higher incidence in the aging population<sup>1,2</sup>. While studies evaluating other injured aging tendons have shown more degeneration and slower healing than young tendons<sup>3</sup>, most preclinical murine studies evaluating rotator cuff pathology utilize young adult mice, limiting the translational potential of these studies to human patients. Additionally, rotator cuff repair is the mainstay of surgical treatment. This is commonly performed with suture anchors placed in the humeral head, making adequate bone strength critical<sup>4</sup>. Therefore, the objectives of this study were to evaluate the differences in biology, functional outcomes (gait), and humeral epiphysis bone properties due to supraspinatus tendinopathy in aged mice versus young mice utilizing a subacromial impingement model<sup>5</sup>. We hypothesized that impinged aged mice will have impaired gait, inferior humeral bone morphometrics, and inferior biologic potential.

**METHODS:** Nine C57BL/6 male mice from each age group (72 weeks old - Aged group and 12 weeks old - Young group) were assigned to 3 different subgroups: controls (n=3), sham surgery (n=3), and impingement surgery (n=3). Gait analysis (DigiGait™) was performed at a constant speed (18 cm/s) at 3 weeks and 6 weeks post-surgery to assess several parameters, including stride frequency and maximal paw area at contact of each forelimb (n = 6). All animals were sacrificed at 6 weeks; whole shoulder joints (n=3) were processed for paraffin embedded histology, supraspinatus tendons (n=3) were collected for bulk RNA-seq, and humeri (n=3) were scanned via microCT. Two-way ANOVA was used for gait and microCT outcomes. RNA-seq significance was set at false discovery rate (FDR) < 0.05; other tests used p < 0.05.

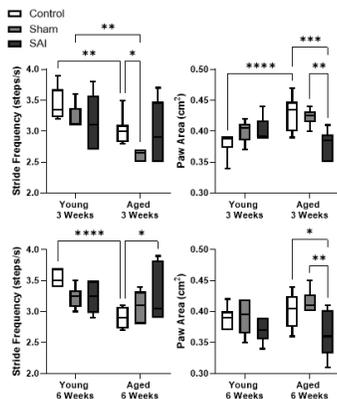
**RESULTS:** Following impingement, aged mice exhibited increased stride frequency at 6 weeks and decreased paw area at both 3 and 6 weeks compared to aged controls, whereas there were no gait differences in young mice regardless of surgery. Sham groups largely resembled controls, except for reduced stride frequency in aged sham mice at 3 weeks (Fig.1). Histological analysis revealed that impinged tendons were hypercellular with disorganized ECM for both age groups. Aged tendons appeared less cellular compared to young tendons in all groups (Fig. 2A). For RNASeq evaluation, baseline differences were determined for unoperated old vs young tissues and there were 43 (20 up and 13 down) differentially expressed genes (DEGs) for tendons but no pathways were differentially regulated via QuSAGE<sup>6</sup>. The top 10 DEGs are shown in Fig.2B. There were no differentially expressed genes between the aged impinged and young impinged tendons. MicroCT scans demonstrated that unoperated aged mice had a lower bone volume fraction (BV/TV), lower trabecular number (Tb.N), and a higher trabecular separation (Tb.Sp) compared to the unoperated young mice. With impingement, there was a further reduction in BV/TV, Tb.N, bone mineral density (Tb.BMD) and an increase in Tb.Sp which was not observed in young impinged mice except for BV/TV (Fig.3).

**DISCUSSION:** This study investigated the effect of age on supraspinatus tendinopathy, gait, and changes in humeral head bone structure after subacromial impingement. Consistent with our hypothesis, we found that aged mice with subacromial impingement showed more impaired gait than younger mice. Young mice seemed to recover quicker as they showed no differences in gait after 3 weeks post-surgery. On the other hand, the stride frequency of impinged aged mice increased at 6 weeks and paw area decreased at 3 and 6 weeks, indicating shorter steps and reduced contact, suggesting delayed pain resolution and less resilience to impingement with aging. The impaired gait, however, was not associated with transcriptional changes as RNA sequencing did not reveal any differences between operated aged and young tendons. This contrasted with our hypothesis and prior literature<sup>3</sup>. It is possible that in this model, the age-related transcriptional changes due to injury are resolved in 6 weeks and evaluation at earlier timepoints in addition to proteomics analysis might provide further insight. However, there were differences in the unoperated tendons, where the aged tendon demonstrated an aging phenotype (increase in senescence marker Cdkn2a and oxidative gene Nox4) and a decrease in ECM genes (Scx, Cnmd, Fbn2) which suggests a baseline deficit in the aging tendon. Histology did not reveal a significant difference between young and aged tendons except for aged tendons being slightly less cellular which might also be one of the reasons for impaired healing. We also found differences in the humeral epiphysis bone of the aged impinged mice (lower BV/TV, Tb.N, BMD, and increased Tb.Sp). This may be due to the reduced mechanical stimulus to the humeral bone, also suggested by gait analysis. A limitation of this study is that due to cost prohibitive nature of aged mice, we performed the pilot study with a small sample size of 3 mice in each treatment group and we did not study the effect of sex in our model, but this will be explored in future studies. Overall, our study suggests that subacromial impingement leads to functional impairments (worse gait) and inferior humeral bone quality in the aged mice. Additional studies are needed to understand the biological reasons for these deficits.

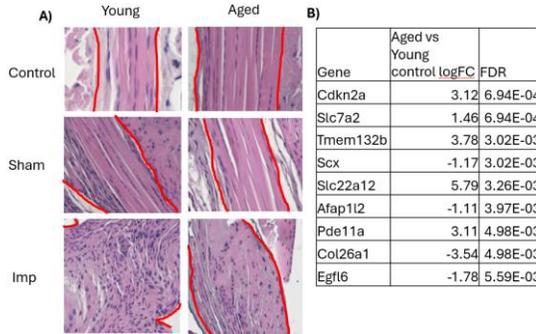
**SIGNIFICANCE/CLINICAL RELEVANCE:** Rotator cuff injuries become more prevalent in the aged population, and our study suggests that aged mice respond differently to subacromial impingement than young mice. Therefore, additional studies should be conducted to study the effect of aging on rotator cuff disease in our preclinical models.

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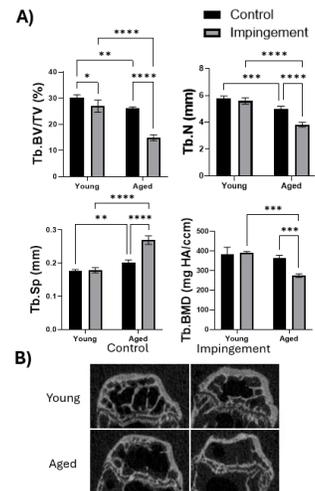
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**Fig.1.** Gait analysis displays impaired gait parameters in older mice at 3 weeks (top row) and 6 weeks (bottom row)



**Fig.2.** Biological changes via A) histology showed lower cellular density in aged tendons. Red line outlines the boundaries of tendon. B) Top 10 DEGs in aged control vs young control via RNA sequencing.



**Fig.3.** MicroCT A) demonstrated reduced bone properties in humeral head as shown by B) representative images