

ANALYSIS OF COLLAGENASE, COLLAGEN, AND GLYCOSAMINOGLYCAN CONTENT OF CYCLICALLY LOADED TENDON EXPLANTS IN CULTURE

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INTRODUCTION

Overuse injuries comprise a significant portion of tendon injuries. These injuries are a combination of simple material fatigue damage as well as the cells' matrix remodeling response to the load stimulus. To better understand the role of the cellular response in overuse injuries, tendon explants were cyclically loaded across one or twelve days with loading regimens of two magnitudes. The sulfated glycosaminoglycan content, collagen content (by measuring hydroxyproline) and the collagenase content were measured.

METHODS

Avian flexor digitorum profundus tendons were isolated and placed in a custom built load-controlled tissue loading device. Tendons were cyclically loaded to a fixed number of cycles at 3 or 12 MPa (Low, High) across 1 or 12 days (Short, Long). Media was exchanged before and after the full regimen as well as every 3rd day.

Media samples were analyzed for collagenase content by measuring the release of a red dye (Azocoll) impregnated in collagen [1]. Samples were activated with APMA to evaluate total potential collagenase activity. Data was normalized by the day 0 value for each sample. Positive and negative controls were included with collagenase injected and freeze-killed trials, respectively.

Tissue segments were digested with papain, and then analyzed for collagen content [2] and sGAG content [3]. Collagen content was measured by a spectrophotometer from hydrolyzed digests introduced to Chloramine T with an aldehyde dye, DAB and quantified using hydroxyproline standards. sGAG was measured by a spectrophotometer in a DMMB (blue) buffer and quantified using chondroitin sulfate (shark cartilage) standards. All data was normalized by dry weight of the original tendon segment prior to digest.

Table 1: Tissue content of cyclically loaded tendons explants ($\mu\text{g}/\text{mg}$ dry weight). Groups without a common letter are significantly different ($P < 0.05$).

	Hydroxyproline	Sulfated GAG
High Long (HL)	91.86 \pm 0.661 A	6.725 \pm 0.89 A
High Short (HS)	102.0 \pm 18.92 A	6.628 \pm 1.48 A,C
Low Long (LL)	92.71 \pm 16.25 A	4.914 \pm 0.94 B,C
Low Short (LS)	82.08 \pm 8.882 A	4.203 \pm 0.76 B

RESULTS AND DISCUSSION

Sulfated GAG concentrations were found to be in the range of macroscopically normal cadaver tendons [3]. A significant difference in the main effect of magnitude was seen. Both high groups, HL and HS, were statistically larger than the low groups, LL and LS, respectively (Table 1), as measured by a two-way ANOVA. While elevated GAGs were expected in

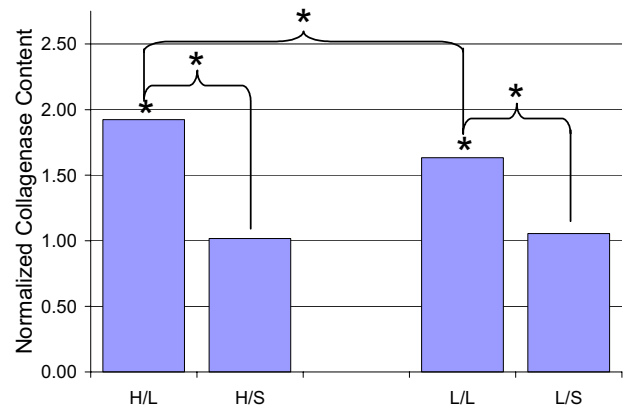


Figure 1: Collagenase content of cyclically loaded tendons, normalized by time 0.

the HL [4], the elevated levels in the HS was not expected because a duration dependence (response time) was expected.

Collagen content, measured by hypro content, was found to be in the range of macroscopically normal, cadaver supraspinatus tendons [2]. However, no differences were seen between any of the groups (Table 1). Though collagen content did not differ, collagenase content did. A significant increase in the collagenase content was seen in the HL and the LL groups relative to time zero. This difference was significant by day 9 for the HL group and day 12 for the LL group. Both long groups were significantly larger than their respective short groups indicating a duration dependence of collagenase release. Magnitude also affected the collagenase content as the HL group was found to be significantly larger than the LL group. This difference was not seen between the short groups however, and could be due to a lack of response time. The high levels of collagenase with a concurrent lack of differences in collagen content potentially suggest a high collagen turnover. However, as active and inactive collagenase was measured with the performed assay, it cannot be concluded with certainty how much collagen turnover occurred.

CONCLUSIONS

Cyclically loaded in vitro tendon explants exhibited significant magnitude dependant GAG increases, as has been observed in overuse tendinopathy lesions. Furthermore, significant differences in collagenase content with a concurrent lack of differences in collagen content was observed, suggesting collagen turnover.

REFERENCES

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