Torn Human Rotator Cuff Tendons Have Reduced Collagen Thermal Properties Which Are Quantifiable Using Differential Scanning Calorimetry

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INTRODUCTION:

Larger rotator cuff tendon (RCT) tears are associated with poorer scores and function, and are more likely to re-rupture after surgical repairs compared to smaller tears [1]. It is hoped that a greater understanding of the structural changes and pathophysiology underlying different sized RCT tears will help to identify effective and appropriate treatment strategies.

As collagen is the primary component of RCT, it is possible that the structure and mechanical properties may be affected by any collagen conformational changes that occur in tears. Changes in mechanical strain and cross-linking have been shown to alter the thermal properties of collagen, suggesting that these are useful parameters to measure. Differential scanning calorimetry (DSC) is a useful technique that measures thermal properties and allows quantification of subtle conformational and chemical changes in tendon collagen integrity [4]. Another advantage is that it can be used to study small intraoperatively obtained human specimens. Most histological analysis is qualitative and thus subject to interpretation errors. It has been previously demonstrated that mechanical overloading of bovine tendon reduced collagen thermal stability [2]. Dermis with increased collagen cross-linking was shown to be associated with stronger mechanical properties such as increased tensile strength and strain modulus [3]. During heating with DSC, collagen molecules undergo a sudden and specific triple helical to amorphous random coils transition, at a defined temperature [5]. DSC has been previously used to study other heterogenous biological materials such as muscle, cartilage and vertebral discs.

It was hypothesized that massive rotator cuff tendon tears will have different collagen structural compositions and thus altered thermal properties compared to normal RCTs. We also hypothesized that using DSC massive tendon tears can be differentiated from smaller tendon tears, as well as normal tendons. This study aimed to address whether the thermal properties of rotator cuff tendons vary between different sized RCT tears when compared to normal RCTs. We also aimed to quantify if differences in collagen structural integrity existed between normal, small and massive RCT tears.

METHODS:

27 human RCT biopsies were collected intra-operatively from the edges of rotator cuff tears during surgical repair and classified according to tear size. All specimens were obtained from the same position to reduce sampling error, from the bursal side, as close to the insertion point as possible. 8 small (< 1 cm) and 9 massive tears (>5 cm) were obtained. 11 normal control tendons were also obtained. All specimens were immediately frozen in liquid nitrogen. 3 samples were taken from each patient, weighing 0.5 mg each (+/-0.01 mg). Approval for the study was obtained from our institutional review board.

During the denaturation experiments the specimens were subjected to a modulated temperature ramp between 20-80^oC at a rate of 2^oC per minute and amplitude of 0.318C, using a TA instruments Q2000 DSC. The denaturation temperature, TD, reportedly corresponds to the temperature at which a protein falls out of solution (water-protein hydrogen bond breakage). The denaturation enthalpy, ΔH, was calculated as the area under the heat absorption curve and is thought to correspond to the distribution of protein structures in the sample. The melting temperature (TM) is proposed to represent breaking of the amide-amide hydrogen bonds, resulting in an increase in the mobility of the protein backbone.

All data analysis was run using the GraphPad Prism 5 software package. The study had 90% power to detect a 10% difference in denaturation and melting temperature between groups with alpha = 0.05.

1 specimen per patient was taken and cryosectioned for quantitative validation. In order to confirm that changes were occurring in the collagen composition, we used performed polarised light microscopy. This allowed quantitative validation of changes in collagen organisation. The collagen structural composition of tendon sections was determined using a pico-syrius red stain for collagen.

RESULTS:

This study has demonstrated that normal and torn RCTs have altered thermal properties which can be detected using DSC. The denaturation and melting temperature were reduced in torn tendons. The denaturation temperature was significantly lower in small (mean TD = 62.13^oC, p=0.026, unpaired t-test) and massive RCT tears (mean TD = 62.08^oC, p=0.001) when compared to small tears and normal RCT (mean TD = 63.84^oC). Similarly the melting temperature was also lower in both the small (mean TM = 65.11, p=0.005) and massive RCT tears (mean TM = 64.83^oC, p =0.001) relative to normal tendons (mean TM = 67.26^oC). No significant difference was detected between the denaturation enthalpy, ΔH, of the different RCT groups. Histology of small and massive rotator cuff tendon tears demonstrated increased structural disruption of collagen fibres compared to normal tendons ($p < 0.05$).

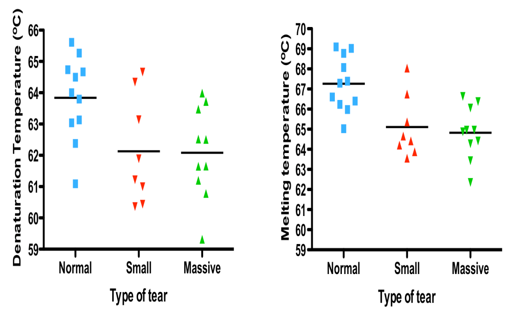

Figure 1: *Graph comparing the a) Denaturation temperature and b) Melting temperature of small and massive rotator cuff tendon tears compared to normal tendons. Tendon samples taken from small and massive tendons had a significantly reduced denaturation and melting temperature compared to healthy intact tendons*

DISCUSSION:

The thermal properties of rotator cuff tendons are clearly altered in tendon tears. It is assumed that this is primarily due to alterations in collagen thermal stability which reflect alterations in collagen structural arrangements. This prospective non-randomized case study indicates that the onset of RCT tear pathology is mainly due to an alteration of the collagen structural arrangements. The lack of change detected in the denaturation enthalpy suggests that the amount of collagen structures did not vary between different sized tears and normal RCT. This technique allows a unique perspective on the fact that tendon tears are associated with changes in collagen structure rather than the amount of collagen.

This unique study has demonstrated greater collagen thermal instability and structural disruption in torn rotator cuff tendons, compared to intact healthy tendons. However no differences were detected in the thermal properties of small and massive RCTs, suggesting that collagen thermal stability does not vary between these groups. This unique study has demonstrated differences in some of the thermal properties of normal and torn rotator cuff tendons. These are likely due to collagen structural changes. A decrease in the denaturation temperature, TD, of torn tendons, suggests that the material is intrinsically less stable. It is likely that torn tendons cannot withstand changes in temperature or stress as well as a perfect material could. This study offers insight into possible mechanisms for, or adaptation to, RCT failure and weakness.

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