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Mechanically overloading collagen fibrils uncoils collagen molecules, placing them in a stable, denatured state

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ABSTRACT

Due to the high occurrence rate of overextension injuries to tendons and ligaments, it is important to understand the fundamental mechanisms of damage to these tissues' primary load-bearing elements: collagen fibrils and their constituent molecules. Based on our recent observations of a new subrupture, overload-induced mode of fibril disruption that we call discrete plasticity, we have sought in the current study to re-explore whether the tensile overload of collagen fibrils can alter the helical conformation of collagen molecules. In order to accomplish this, we have analyzed the conformation of collagen molecules within repeatedly overloaded tendons in relation to their undamaged matched-pair controls using both differential scanning calorimetry and variable temperature trypsin digestion susceptibility. We find that tensile overload reduces the specific enthalpy of denaturation of tendons, and increases their susceptibility to trypsin digestion, even when the digestion is carried out at temperatures as low as 4 °C. Our results indicate that the tensile overload of collagen fibrils can uncoil the helix of collagen molecules, placing them in a stable, denatured state.

1. Introduction

Collagen fibrils are the primary tensile load-bearing elements of tendons and ligaments (Hoffman et al., 1973; Screen et al., 2005; Svensson et al., 2011). Given the high occurrence rate of overextension injuries to these tissues (Bureau of Labor Statistics, 2009, 2011), it is important to understand the fundamental mechanisms of tendon and ligament overload damage at the nanoscale level of the collagen fibrils and their constituent molecules. In particular, it has remained unclear until now whether tensile overload can alter the helical structure of collagen molecules; that is, can tissue overload produce molecular damage?

The affect of tensile overload on collagen fibrils was originally studied using rat tail tendons and transmission electron microscopy. Several studies have documented the same phenomenon: tensile overload producing a progressive breakup of collagen fibrils into their subfibrillar components (Torp et al., 1975; Kastelic and Baer, 1980; Knorzer et al., 1986). More recently, though, Willett et al. (2007, 2008, 2010) explored whether tensile overload might affect collagen molecules. Using a bovine tail tendon model, those authors found that overload increased the susceptibility of collagen molecules to enzymatic cleavage by the serine proteases trypsin and chymotrypsin (Willett et al., 2007). These two proteases cannot in principle cleave regions of a collagen molecule that have triple-helical conformation (Bruckner and Prockop, 1981; Ryhanen et al., 1983; Bank et al., 1997), thus serving as probes for molecular conformation—and, in the case of collagen, as probes for denaturation.

When present as isolated monomers in solution, the triple helix of type I collagen molecules becomes unstable near body temperature causing molecules to transition into random coils: denaturation (Leikina et al., 2002). When packed in a fibril, however, the proximity of neighboring molecules restricts the conformational freedom of individual α-chains, substantially increasing the thermal stability of collagen molecules (Na, 1989; Tiktopulo and Kajava, 1998). This mode of stabilization is known as polymer-in-a-box constraint (Miles and Ghelashvili, 1999).

Taken together, polymer-in-a-box molecular stabilization combined with observations of overload-induced fibril dissociation appeared to provide adequate explanation for the increased enzymatic susceptibility of tendons following overload (Willett et al., 2007). If overload causes fibril dissociation, this would decrease the packing density of collagen molecules, thereby increasing their conformational freedom and reducing their thermal stability. In turn, this reduction in thermal stability might allow temporary unwinding of local regions of the collagen helix, an event known as micro-unfolding (Kadler et al., 1988). Micro-unfolding, then, might be enough to increase the susceptibility of collagen molecules to enzymatic cleavage by the serine proteases.

Recently, we re-examined the effects of tensile overload on the bovine tail tendon model developed by Willett et al. (2007, 2008, 2010). Using high magnification scanning electron microscopy to assess fibril damage, we made three novel observations. First, overloading bovine

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tail tendons does not result in the same fibril breakup as previously documented in rat tail tendons. Instead, fibrils develop discrete, repeating zones of pronounced distortion giving them a nanoscale kinked appearance (Fig. 1A) (Veres and Lee, 2012; Veres et al., 2013). We have termed this mode of fibril damage "discrete plasticity". Second, with repetitive overloading, fibrils develop an increasing number of discrete kink distortions, i.e. new discrete zones of plasticity develop between the pre-existing zones (Fig. 1B) (Veres et al., 2013). Third, the dissolution of the overloaded fibrils by serine proteases is predominantly at the location of the kink morphologies (Fig. 1C) (Veres and Lee, 2012).

Prompted by the striking difference between (i) the overload-induced, discrete plasticity fibril distortion and (ii) the earlier reports of fibril dissociation on which the molecular disruption theory of Willett et al. (2008) was based, we have now re-explored the mechanism of collagen molecular-level damage in overloaded tendons. Specifically, we have investigated whether the increased trypsin solubility of overloaded tendons is caused by an increase in thermally-driven micro-unfolding of collagen molecules. In order to cause significant discrete plasticity damage to collagen fibrils, we have subjected tendons to 15 cycles of repeated subrupture overload. We have then assessed the conformation of collagen molecules in the overloaded tendons using both differential scanning calorimetry (DSC), and serine protease (trypsin) digestion at decreasing temperatures (Fig. 2). Our results provide clear evidence that tensile overload does more than simply destabilize collagen molecules thereby promoting micro-unfolding: it places them in a stable, denatured state.

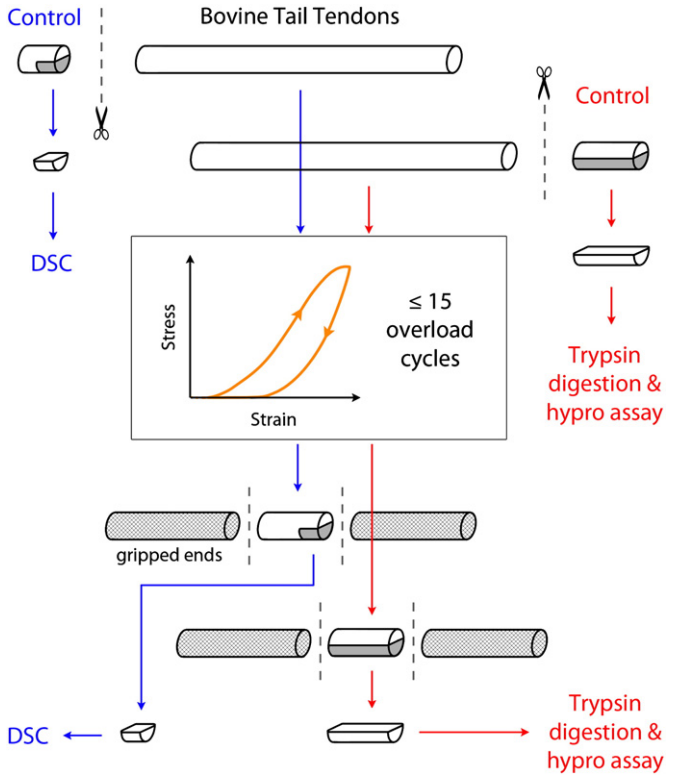

Fig. 2. The current study sought to investigate whether overloading collagen fibrils in tension can damage collagen molecules. Collagen fibrils were damaged by subjecting tendons to repeated, sub-rupture, tensile overload as done previously (Veres et al., 2013). Damage to collagen molecules was investigated using differential scanning calorimetry (DSC) and trypsin digestion susceptibility.

2. Results

2.1. Differential scanning calorimentry

The average thermal stabilities of collagen molecules within both overloaded and matched-pair control tendons were assessed using DSC. Representative endotherms from an overloaded tendon and its matched-pair control, including annotations showing the parameters measured, are presented in Fig. 3. Mechanical overload reduced the enthalpy of denaturation of tendons by over 50%. Similarly, mechanical overload reduced peak endotherm temperature by 2 °C and endotherm onset temperature by over 4 °C. The larger reduction in onset temperature compared to peak temperature increased the width of the

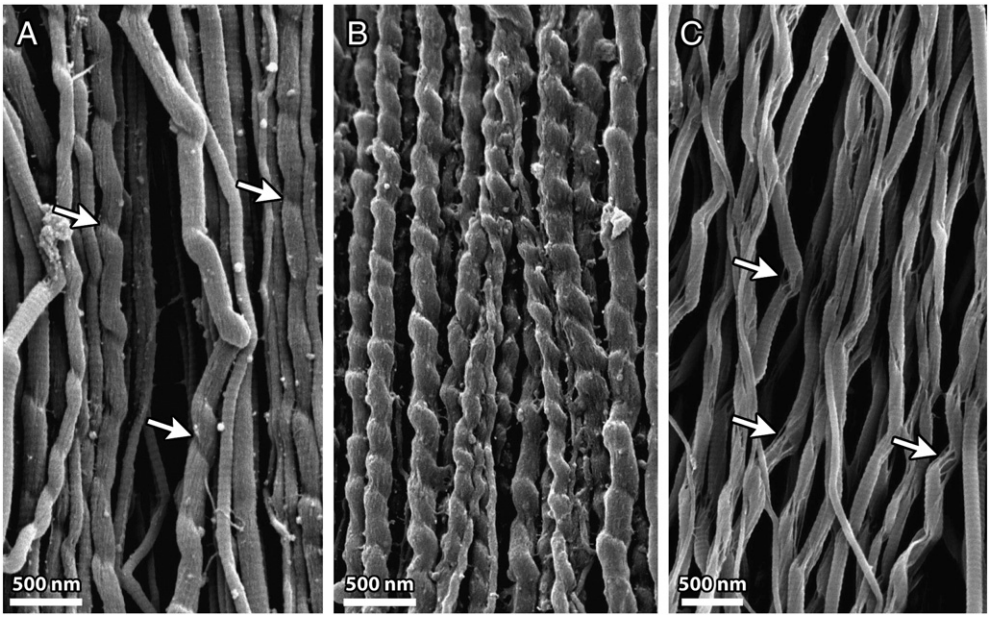

Fig. 1. Tensile overload causes discrete plasticity in collagen fibrils. A: Pulling tendons to rupture causes fibrils to develop kinks (arrows) that repeat every ~300–800 nm along their length. B: Repeated, subrupture overloading causes the number of kinks that fibrils have to increase. Note that the kinks in image B (tendon subjected to 5 subrupture overload cycles) are much closer together than the kinks in image A (tendon overloaded once to rupture). C: Exposing fibrils with discrete plasticity damage to proteases that can only digest non-helical collagen causes partial dissolution of the kinks. Note that the kinks marked by arrows in image C (tendon pulled to rupture and then digested at 30 °C using trypsin, as described previously (Veres and Lee, 2012)) are missing material compared to the kinks marked by arrows in image A (tendon pulled to rupture but not digested). Images A & B taken at 30,000×. Image C taken at 25,000×.