Understanding Soft Tissue Aging - the Effect of Cyclical Loading

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The importance of healthy living

- Life expectancy is increasing but healthy life expectancy is ‘levelling off’
- Greater importance of age-related diseases
  - Morbidity
  - Cost to health service
  - Need for prevention

‘Aging: Scientific Aspects’; House of Lords, Science and Technology Committee, 2005
Strain-induced tendinopathy in humans and horses

- Very common in human and equine athletes
  - Many similarities
- Achilles tendinopathy in humans
  - Increasing prevalence (Moller et al. 1996; Houshian et al. 1998; Maffulli et al. 1999)
- Superficial digital flexor tendinopathy in horses
  - Up to 43% of NH horses in training
  - 46% of limb injuries at racecourses
- Healing slow and poor
  - Re-injury common
  - Morbidity
Why do tendons fail?

- High risk tendons operating close to functional limit
- Cause of injury
  - Sudden over-extension
  - Preceding tendon degeneration
Evidence for preceding degeneration

- ‘Asymptomatic’ lesions at post mortem
- Bilateral nature of injuries
- Strong relationship between age and injury
- Aging induces a decline in mechanical competence
  - Onambele et al. 2006
- Cyclical loading *in vivo* drives cumulative fatigue damage in experimental exercise studies in the adult horse
Tendon degeneration – an inevitable consequence of aging and exercise

- Prevention strategies
  - Starting training early – tendon adaptive
    - Reduces risk of injury in racehorses
  - Post skeletal maturity - reduced degeneration will reduce risk of tendinopathy

- What are the mechanisms of tendon aging?
  - Failure of adaptation/functional repair
  - Degeneration processes
Failure of adaptation - ‘Aged’ tenocytes have reduced responsiveness

(Goodman et al. (2004) Biorheology, 41, 613-628)
Failure of adaptation - Reduced levels of anabolic growth factors (TGFβ) within the tendon after skeletal maturity

- TGFβ isoforms prominent in fascicles and endotenon septa during growth
- Decline in staining in fascicles after skeletal maturity

(Cauvin, Goodman, and Smith - unpublished)
Failure of adaptation - reduced intercellular communication

- Reduced gap junction numbers with age
  - Reduced co-ordinated response to load after skeletal maturity

(Stanley, Patterson-Kane – unpublished)
Mechanisms of degeneration

- Hypothesis – Ageing is a biologically active process in response to cyclical loading.
Cyclic loading reduces tensile strength of explants

- Cyclical strain (1 Hz, 5%) for 24 h reduces the ultimate tensile stress (UTS) of explants
- The reduction is greater in aged samples

* p≤0.05, n=20
Loss in UTS induced by cyclical load requires viable cells

- Changes in UTS prevented by
  - Freeze-thawing
  - Sodium azide

* p ≤ 0.05, n=12 for dead expts

**Ultimate tensile strength (MPa)**

- Live, non-stimulated
- Live, stimulated
- Dead, non-stimulated
- Dead, stimulated

**Cell viability after freeze thaw**

**Before strain**

**After strain**
Cyclical loading induces MMP expression and activity

- Cyclical strain induced the expression of total MMP-2 by 2 to 5-fold, and active form by up to 3-fold.
- Pro-MMP-2 induced to similar level at all ages, but active form induced more in the aged specimens.

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Pro MMP-9
Pro MMP-2
Active MMP-2
MMP activity is required for the loss in ultimate tensile strength induced by cyclical loading.

- The broad-spectrum MMP inhibitor (Ilomastat) reduced the magnitude of load-induced decrease in UTS.
- The inactive analogue of Ilomastat failed to modify strain-induced decreases in UTS.

* p<0.05, n= 4
Cyclic loading also induces the loss of a labile integral matrix constituent

- COMP is a labile component of tendon
  - Decreased with aging and exercise
- Cyclical loading promotes the release of COMP from tendon
  - Fragmented
  - No effect of age

![Graph showing change in COMP concentration over age and exercise status](image)

**In vitro**

**In vivo**
The action of matrix fragments ('matrikines') on tendon explants

- Matrix fragments induce a loss of COMP from tendon explants
  - 40kDa C-terminal fibronectin fragment (FNf-HepII)

![Graph showing the effect of matrix fragments on COMP levels](image)
Matrix fragments induce a loss of UTS in aged tendon explants

- BUT Fn-f Hep II did not induce loss of tensile strength of younger tissues
- Similar to the effects of applying strain
  - Mechanism? - FNf induces MMPs (Homandberg et al., 1996)

![Graph showing ultimate tensile strength (UTS) vs age (yrs) with control and Fn-f Hep II conditions.](image)
Conclusions – proposed mechanism for soft tissue aging

Cyclical load

Matrix protein fragmentation

AGED TENDON

Reduction of UTS Matrix resorption

‘Degenerative cycle’

Inhibit key ‘matrikines’

MMP production

Immature tendon

Matrix synthesis/repair

‘Anti-aging’ strategies for tendon

Inhibit key MMP activity
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