The Impact of Hypercholesterolemia on Tendon Injury Repair

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Background: Hypercholesterolemia (high blood cholesterol) is linked to tendon xanthomas (cholesterol deposits found on superficial tendons). Lipid accumulation in the tendon's extracellular spaces[1] may disrupt the tendon's substructures and affect its mechano-sensing and mechanical properties, which may lead to injury.

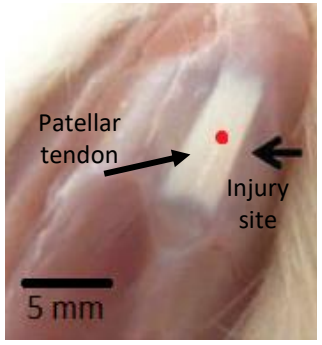
We hypothesised that tendon strength and metabolism would be inferior in a high cholesterol environment and attenuate the tendon's injury repair processes

Methods: 50 Sprague-Dawley (SD) and 50 apolipoprotein E knock-out rats (ApoE -/-; Envigo, IL, USA) were given a unilateral patellar tendon (PT) injury via 0.75mm biopsy punch at 12 wks. old (Fig. 1); the uninjured limb was used as the control.
Animals were euthanized at 3-, 14- or 42-days post-injury (Table 1) and assigned to (SD/ApoE per timepoint):

- Gene expression (n=4/4)
- Tissue histology (n=6/6)
- Mechanical testing (n=10/10; 14 + 42 days only)

Table 1	SD M/F	ApoE M/F
3 days	4/6	8/5
14 days	9/11	5/10
42 days	10/10	10/11

Fig. 1. Red dot represents (to scale) biopsy punch hole



Results: ApoE total cholesterol was over double that of SD rats (Fig. 3, mean 2.12 vs 0.99 mg/ml, $p<0.0001$).

Histology: No evidence of PT lipid content (oil red-O staining)
Biomechanics: No differences in PT mechanical properties (stiffness, hysteresis, strain, stress, modulus, strength).
qPCR: Group differences in gene expression (Fig. 4) suggest differences in injury repair at the cellular level.

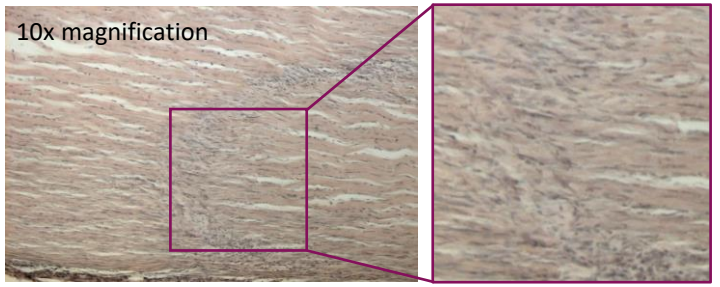


Fig. 2. H&E staining of injury; enlarged square shows hypercellular area.

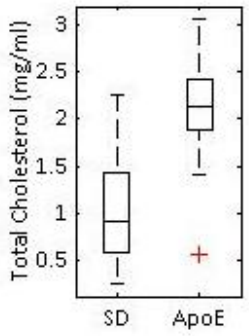


Fig. 3. Box-whisker plot; total blood cholesterol

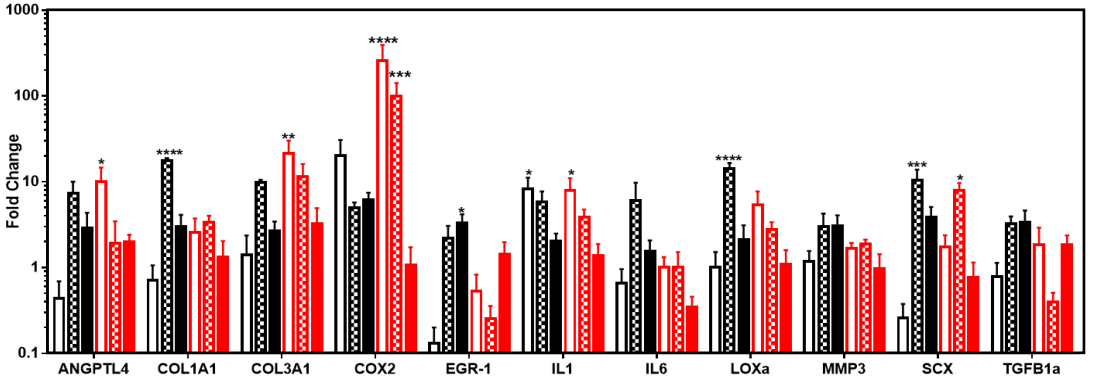


Fig. 4. Quantitative PCR results of injured PT (relative to uninjured PT in SD 42 days post-injury).

Discussion: We found the levels of expression and time-course of several genes to differ between SD and ApoE rats with injury repair. Of particular interest were a greater COX2 and collagen type III (COL3A1) and lesser collagen type I (COL1A1) response in ApoE compared to SD rats.

Despite a lack of lipid accumulation in the PT and recent evidence to suggest that the rat PT may be a poor model for examining our hypothesis[2], our results indicate that **high cholesterol modulates tendon inflammation and healing** even with a mild phenotype, as indicated by altered mRNA levels. These differences may contribute to the known consequences of tendon cholesterol on tendons in humans.

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[1]Grewal et al. (2014). *PLoS One* 9(12): e114214
[2]Rune et al., (2018). *Sci Rep* 8(1): 5416