

Gene Expression Changes in Response to Severe Burn Injury in Rat Achilles Tendon

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Background

- Severe burn injury (SBI), defined as a full-thickness burn of greater than 30% of total body surface area, occurs at a rate of 5/100,000 per year and can result in many serious sequelae such as infection, shock, and direct organ damage.¹
- It has previously been shown that SBI results in severe systemic inflammation for up to 3 years^{2,3}, as well as local inflammation in muscle^{4,5} and in bone⁶ distant to the burn site.
- However, we are unaware of any work that investigates inflammatory effects in tendon, which connects muscle and bone.

Purpose/Aims

- To determine whether changes occur in gene expression, protein synthesis, and biomechanical properties in rat Achilles tendon after distant severe burn injury

Methods

- Burn:** Rats were subjected to thoracolumbar full-thickness severe burn injury under anesthesia by dorsal submersion into a hot water bath.
- Sacrifice & Tissue Harvest:** Rats were sacrificed and Achilles tendons were harvested at time point of 6h, 1d, 3d, 7d, and 14d. Unburned rats were used for control.
- qPCR:** RNA expression of collagen I (Col1), collagen III (Col3), MMP9, MMP13, TNF, IL-1, IL-6, and Tenomodulin were measured by qPCR
- WB:** Quantitative Western Blot analysis was performed for Col1, Col3, and MMP9
- Biomechanics:** Stress testing performed on separate study group (n = 8) 14d vs. control

Results

- Gene Expression:** MMP9 was upregulated on the order of 20-fold at 14d ($p = 0.0016$) and 10-fold at 3d ($p = 0.0255$) and MMP13 showed a trend toward 12-fold increase at 3d ($p = 0.079$). A significant increase in expression of IL-1 β and a trend toward significance IL-6 were also observed at 3d ($p < 0.01$, and $p = 0.059$, respectively). Differences in expression of Col1, Col3, TNF- α , and tenomodulin were not significant compared to control.
- Protein Expression:** Preliminary data from Western blots showed 5-fold decrease of collagen 1 at 7d ($p < 0.01$) and 2-fold increase of collagen 3 at 14d ($p = 0.016$). Significant changes were not found in MMP9.
- Biomechanics:** Preliminary biomechanical data shows a trend toward a 4-fold decrease in stiffness in the burn group.
- All p values are by t-test. All figures show mean with positive error bars for error propagation (qPCR) or standard deviation (WB, stiffness).

References

- Evers, et al. 2010
- Gauglitz, et. al. 2008
- Jeschke, et. Al. 2011
- Song, et. al. 2015
- Quintana, et. al. 2015
- Klein, 2006

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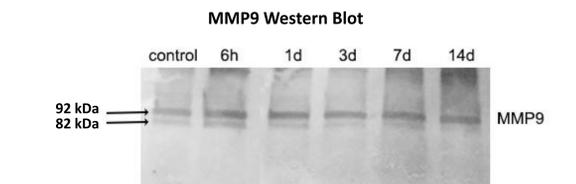
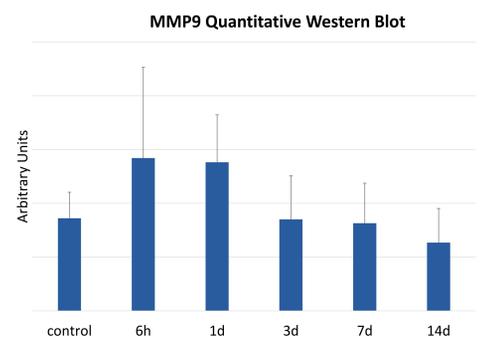
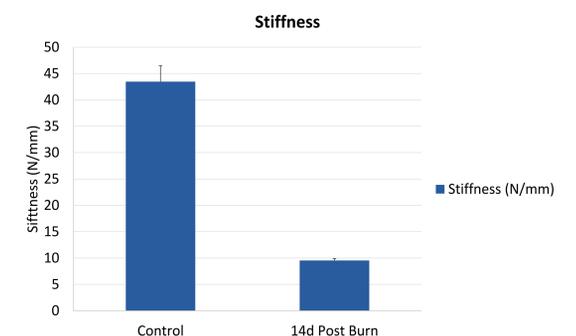
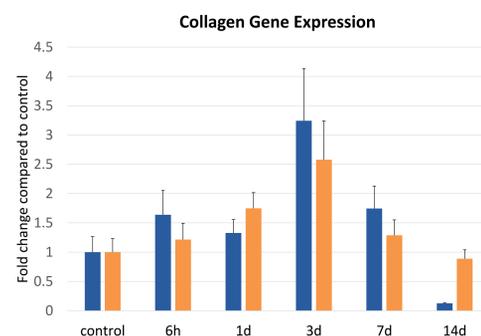
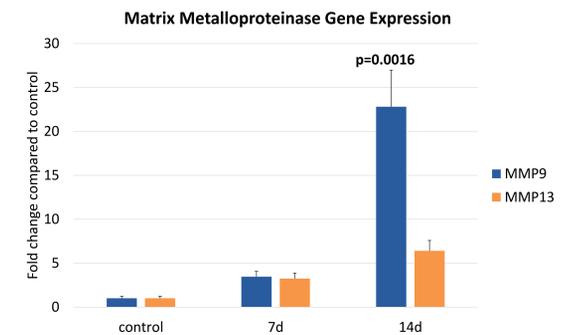
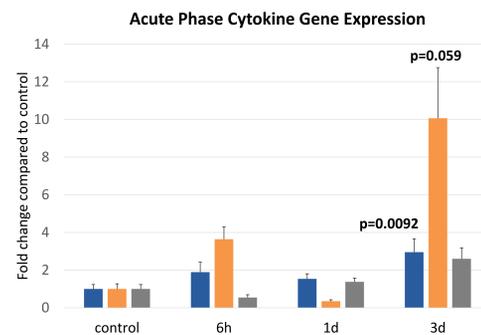
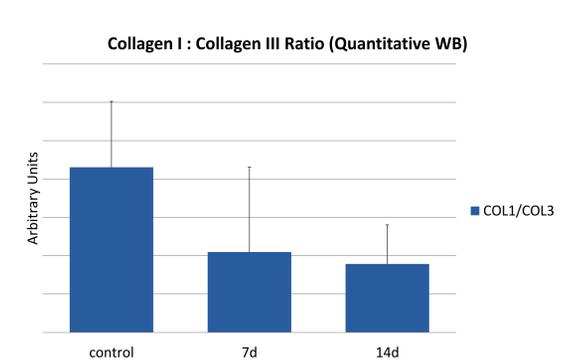
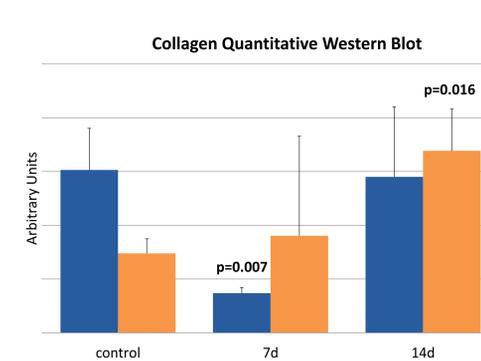


Figure 1. The 82kDa band indicates the cleaved, active form of MMP9. Interestingly, the active form subjectively shows increased expression at 6h. We plan in the future to further quantify & characterize protein expression specific to the active form.



Conclusions

These results newly confirm the existence of inflammation in tendon distant from the burn site after severe burn injury. Specifically, these changes could indicate initial matrix remodeling as carried out by the MMPs leading up to 14d, concurrent with collagen deposition with a decreased Col1:Col3 ratio, resulting in decreased tissue stiffness. These findings are consistent with the repair processes known to occur in other tissues after inflammation. Additionally, IL-6 and IL-1 β may have a more significant role in post-burn acute-phase inflammation in the Achilles tendon than other acute-phase reactants like TNF- α .

Future Directions & Contact Info

- Short-Term
 - Further biomechanical studies
 - Histological studies
 - Manuscript preparation
- Long-Term
 - Therapeutic rat studies

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