INTRODUCTION: The loading environment has a significant effect on muscle and tendon degeneration in the healthy and injured rotator cuff [1]. However, the molecular mechanisms of the corresponding patho-physiological processes have not been determined. The purpose of this study was to determine the effect of mechanical unloading on gene expression of healthy and injured supraspinatus tendon and muscle. We hypothesized that mechanical unloading using botulinum toxin would: 1) downregulate expression of myogenic transcription factors and markers in muscle, 2) upregulate expression of adipogenic transcription factors and markers in muscle, and 3) reduce the expression of collagen and collagen-inducing growth factors in healing tendon.

METHODS: The study protocol was approved by the Institutional Animal Studies Committee. Seventy-five Sprague-Dawley rats were divided into five groups. Thirty rats underwent a unilateral supraspinatus injury and repair as described previously [2]. Half were injected with botulinum toxin A in the supraspinatus (‘BTX Repair’ group). The other half received saline injections to serve as a control (‘SAL Repair’ group). Two additional groups of uninjured shoulders were injected with Botox (‘BTX Non-repair’ group) or Saline (‘SAL Non-repair’ group) in the supraspinatus muscle. Finally, a fifth group of uninjured rats without any injections were used as normal controls (’NL’ group). Animals were sacrificed at 3, 7 and 21 days (N=5 per timepoint per group). The supraspinatus muscles and tendons were dissected and frozen using liquid nitrogen. mRNA expression levels were examined in muscle for: 1) Myogenin, MyoD1, Myf5 and Myf6 (Myogenic transcription factors); 2) MHC IIB (fast twitch isofrom) and MHC IB (slow twitch isofrom); 3) C/EBPα and PPARγ (adipogenic transcription factors); and 4) Leptin (adipogenic marker). mRNA expression levels were examined in tendon for: 1) collagen I (COL1a1), collagen III (COL3a1) (extracellular matrix); 2) TGF-β1 and TGF-β3 (growth factors); and 3) scleraxis (tenogenic transcription factor). mRNA levels were quantified with qPCR using Sybr Green chemistry. Results were expressed as fold differences relative to GAPDH. Groups were compared using a multifactor ANOVA followed by a Fisher least-squares differences post-hoc test. An alpha level of p<0.05 was considered significant.

RESULTS: Myogenic transcription factors were significantly upregulated in the BTX Repair and Non-repair groups at all time points (Fig. 1). There was a significant upregulation of fast and slow twitch isoforms (MHC IIb & IIb) in response to unloading. C/EBPα and Leptin were significantly upregulated at 21 days with unloading (Fig. 2). PPARγ decreased significantly at 3 days in BTX Repair group and then gradually decreased. Injury and repair had little effect on muscle gene expression.

Unloading resulted in few significant changes in tendon mRNA expression. Only scleraxis showed a significant change due to unloading (Fig. 3). This gene also showed a significant increase over time, although TGF-β1 peaked at 7 days and decreased at 21 days.

DISCUSSION: Muscle unloading using botulinum toxin significantly upregulated expression of both myogenic and adipogenic genes. MyoD1 and Myf5 are early-acting factors, mainly involved in myoblast formation and in satellite cell proliferation during regeneration, while myogenin and MRF-4 are late-acting factors, expressed during muscle differentiation [3]. Therefore, the upregulation of myogenic transcription factors could indicate an early reparative response.

C/EBPα and PPARγ are critical transcriptional factors in adipocyte differentiation and leptin is produced by mature adipocytes. Upregulation of their expression corresponds to the accumulation of adipose tissue in muscle, as seen in chronic rotator cuff disease [4].

Unloading of uninjured tendon did not increase mRNA expression of collagen and TGF-β genes. However, injury and repair had a significant effect on tendon gene expression. As has been reported in other models, the healing process in this study was characterized by significant upregulation in growth factors and extracellular matrix genes. Interestingly, even though the natural healing response was scar mediated (characterized by TGF-β1 and collagen III expression), a tenogenic transcription factor (scleraxis) was also upregulated.

In conclusion, we showed that injury and repair had the greatest effect on tendon gene expression, while unloading had the greatest effect on muscle gene expression. Further studies will investigate the mechanism of this response. We will investigate the differentiation of progenitor cells in response to chronic mechanical unloading conditions.