Genetic Response of Rat Supraspinatus Tendon and Muscle to Exercise

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Introduction

Muscle and tendon beneficially adapt to non-injurious exercise. Previous studies suggest that inflammation plays an important role in the regeneration of muscle and tendon following acute injury; e.g.,[1] however, the mechanisms governing the roles of inflammation in the adaptation of muscle and tendon to beneficial loading have not been identified. The objective of this study was to screen for acute and chronic inflammatory, as well as ECM genes involved in the beneficial adaptation of rat supraspinatus (supra) tendon and muscle to non-injurious loading. Our global hypothesis was that a mild inflammatory response is a normal, physiologic requirement for muscle and tendon to adapt to load. Specifically, 1) a mild inflammatory response (changes in arachidonic acid cascade) would present in the tendon and muscle after a single bout of loading, and 2) the tissue will show adaptive matrix changes (increased collagen expression and MMP/TIMP changes indicating turnover) with chronic loading.

Methods

20 male, Sprague-Dawley rats (400-450g) were divided into cage activity (CA) or acute or chronic exercise (EX) groups (IACUC approved). Acute groups were divided into 12 or 24 hour euthanasia time points following a single exercise bout, and chronic groups were divided into 1 or 8 weeks of repeated exercise (n = 4 each group). EX animals walked on a flat treadmill using a previously validated protocol.[2] Control CA animals maintained cage activity for 5 weeks. Supra tendon and muscle were harvest, RNA was extracted, and a custom Panomics QuantiGene 2.0 Multiplex array was used to detect 48 target genes for inflammation, ECM components, matrix turnover, and factors associated with tissue adaptation or degeneration. Target gene signal was normalized by the geometric mean of 3 housekeeping genes and $\log_2$ transformed. Principal components analysis (PCA) was used to visualize global similarities among the 40 samples and for the 4 separate categories of interest: chronic tendon, chronic muscle, acute tendon, and acute muscle. For each category, a 1-way ANOVA (3 levels) with pairwise contrasts was used to compare CA and EX genes. Because this was a screening experiment, an inclusive analysis was conducted. Significance was set at $p \leq 0.05$ and genes with a positive or negative fold change ≥ 1.25 were included.

Results

PCA confirmed distinctions between tissues and time points supporting the study design (not shown). Supporting our hypotheses, acute exercise caused an altered inflammatory response in muscle and tendon, indicated by changes in arachidonic acid cascade components and MMP/TIMPs (Table 1). As expected, inflammatory genes were more changed acutely than chronically. Chronic tissue had more matrix-related gene changes, suggesting tissue adaptation (Table 2). Several growth factors also significantly changed with acute and chronic exercise (not shown).

Discussion

Tendon and muscle showed time-dependent responses to exercise. More chronic gene changes were found at 1 than 8 weeks, indicating that this adaptive process begins soon upon

Table 1. Inflammatory and matrix genes changed with acute exercise.

		Chronic Tendon	Chronic Muscle
Arachidonic Acid Cascade	**CA-EX12**	*Alox5ap*	*Ptgfr*
	CA-EX24	*Alox5ap*	*Ptgfr*
	EX12-EX24	—	—
Matrix Turnover	**CA-EX01**	*Mmp14, Timp1, Timp3, Col1a1, Col3a1*	*Mmp14, Col1a1, Col3a1*
	CA-EX08	*Mmp14, Timp1, Col1a1*	*Mmp14*
	EX01-EX08	*Mmp14, Timp3, Col1a1, Col3a1*	Col3a1

Table 2. Inflammatory and matrix genes changed with chronic exercise.

		Acute Tendon	Acute Muscle
Arachidonic Acid Cascade	**CA-EX12**	*Ptger4*	*Ptges*
	CA-EX24	*Ptges*	*Ptger4, Ptgfr*
	EX12-EX24	*Ptges, Ptger4*	*Ptges, Ptgfr*
Matrix Turnover	**CA-EX12**	—	*Timp4, Col1a1*
	CA-EX24	*Mmp14, Timp3*	*Timp3, Col1a1*
	EX12-EX24	*Mmp14, Timp3*	*Timp3, Timp4*

initiation of an exercise routine. Results suggest that tendon response to chronic, beneficial exercise is distinct from overuse. Unlike overuse, we did not find increased expression of cartilage markers (*Sox9, Acan, Col2a1*), heat shock proteins (*Hspa2, Hspb1*), or nitric oxide synthases (*Nos2, Nos3*) in tendon. In conclusion, this study suggests a role of physiologic inflammatory processes and matrix turnover in the response of supra muscle and tendon to acute and chronic beneficial load. Future studies can use these results to distinguish beneficial and detrimental loading effects, identify tissue recovery, and develop new treatments.

References

1. Connizzo BK, Yannascoli SM, Tucker JJ, et al. The detrimental effects of systemic ibuprofen delivery on tendon healing are time-dependent. *Clin Orthop Relat Res,* 472(8):2433-2439 (2014).

2. Rooney SI, Loro E, Sarver JJ, et al. Exercise protocol induces muscle, tendon, and bone adaptations in the rat shoulder. *Muscles Ligaments Tendons J.* in press.