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Controlled treadmill exercise eliminates chondroid deposits and restores tensile properties in a new murine tendinopathy model

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ABSTRACT

Tendinopathy is a widespread and disabling condition characterized by collagen fiber disruption and accumulation of a glycosaminoglycan-rich chondroid matrix. Recent clinical reports have illustrated the potential of mechanical loading (exercise) therapies to successfully treat chronic tendinopathies. We have developed a new murine tendinopathy model which requires a single injection of TGF-β1 into the Achilles tendon midsubstance followed by normal cage activity for 2 weeks. At this time, tendon maximum stress showed a dramatic (66%) reduction relative to that of normal controls and this persisted at four weeks. Loss of material properties was accompanied by abundant chondroid cells within the tendon (closely resembling the changes observed in human samples obtained intra-operatively) and increased expression of *Acan*, *Col1a1*, *Col2a1*, *Col3a1*, *Fn1* and *Mmp3*. Mice subjected to two weeks of daily treadmill exercise following TGF-β1 injection showed a similar reduction in tendon material properties as the caged group. However, in mice subjected to 4 weeks of treadmill exercise, tendon maximum stress values were similar to those of naive controls. Tendons from the mice exercised for 4 weeks showed essentially no chondroid cells and the expression of *Acan*, *Col1a1*, *Col2a1*, *Col3a1*, and *Mmp3* was significantly reduced relative to the 4-week cage group. This technically simple murine tendinopathy model is highly amenable to detailed mechanistic and translational studies of the biomechanical and cell biological pathways, that could be targeted to enhance healing of tendinopathy.

1. Introduction

Since effective treatment options for human tendinopathies are limited (Maffulli et al., 2010), the development of reliable *in vivo* animal models can improve our understanding of the disease process, thereby facilitating identification of novel therapeutic strategies. Three primary criteria in assessing the utility of such models for future clinical translation are (1) a reduction of tendon mechanical properties, as occurs in human tendinopathy (Arya and Kulig, 2010), (2) histopathologic features mimicking those of the human disease and (3) the amenability of the model for physiotherapy or biologic intervention.

A consistent feature of human tendinopathy is the accumulation of a chondroid matrix within the tendon body, as previously demonstrated by biochemical analysis (Corps et al., 2012; Samiric et al., 2009), histopathology (Kannus and Jozsa, 1991; Khan et al., 1999), and immunohistochemistry (Scott et al., 2008). Importantly, it appears that the chondroid accumulation is an injury response, rather than an adaptation to compression (Ralphs et al., 1992; Vogel and Peters, 2005), since there is marked upregulation of aggrecan expression in individuals with painful Achilles tendinopathy (Corps et al., 2006; de Mos et al., 2009). Furthermore, using a rat supraspinatus overuse model, Attia et al. (2012) observed an increase in glycosaminoglycans (GAGs) in the tendon mid-portion following 8 weeks of downhill running. Hence, we hypothesize that strategies to remove GAG deposits from tendons, or retard their formation, represent clinically relevant therapies.

There is increasing evidence that eccentric loading protocols can effectively treat human tendinopathies (Ohberg et al., 2004; Silbernagel et al., 2011; van der Plas et al., 2012; Visnes and Bahr, 2007). Animal studies (Eliasson et al., 2012; Gelberman et al., 1986; Palmes et al., 2002) have also demonstrated benefits of mechanical stimulation for tendon healing. While results from both clinical and bench studies highlight the importance of mechanobiologic stimulation, very little is known regarding the mechanisms through which this may occur (Sussmilch-Leitch et al., 2012).

The objectives of the current study were firstly, to develop a non-surgical murine tendinopathy model characterized by chondroid

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accumulation and loss of tensile properties, and secondly, to determine the efficacy of controlled mechanical loading to reverse progression of tendinopathy. We utilized TGF-β1 injection to induce tendinopathy, as this growth factor has both been demonstrated to stimulate chondrogenesis in numerous tissue and cell culture models (Shintani and Hunziker, 2007; Diederichs et al., 2012; Lorda-Diez et al., 2009; Morales, 1991) and is a critical biological factor translating mechanical overuse injury of tendon cells into a biological response (Maeda et al., 2011). We hypothesized that firstly, a single injection of TGF-β1 into the Achilles tendon results in tendinopathic changes which mimic human pathology and secondly that tendinopathic mice subjected to controlled exercise exhibit a reduction in chondroid deposits and a restoration of tensile properties.

2. Methods

2.1. Human studies

Intraoperative tendon specimens (IRB #11122301) were obtained from the proximal origin of the extensor carpi radialis brevis (ECRB) and distal origin of the biceps brachii tendons from patients undergoing surgical debridement for painful tendinopathy.

2.2. Induction of murine tendinopathy

C57Bl6 male mice (12 weeks old) were injected (28 G needle) into the mid-portion of the right Achilles tendon with 100 ng active TGF-β1 (PeproTech Inc) in 6 µL of 0.1%(v/w) BSA in saline. The study design included naïve (uninjured) controls, an acute response group (48 h post-injection), and mice which received cage or treadmill activity (Fig. 1).

2.3. Mechanical stimulation

At 24 h post-injection, mice began uphill (17°) treadmill running (Stoelting/Panlab) at 32 cm/s, 20 min/day, 5 days per week (Li et al., 2011) for 2 or 4 weeks.

2.4. Geometry and mechanical testing

The Achilles tendon–calcaneus complex was dissected and the calcaneus potted in methyl methacrylate. Tendon cross-sectional area (CSA) was measured using a precision caliper (for width) and a laser displacement sensor (for thickness), assuming a rectangular geometry (Wang et al., 2012). Material testing was conducted at a plantar flexion angle of 45° (Wang et al., 2006) with the specimen in an isotonic saline bath at 37 °C. Each tendon was preloaded to 0.05 N, followed by preconditioning (0.05–0.55 N at 0.1 N/s for 20 cycles), a five minute recovery in an unloaded state, a static stress relaxation test (5% strain at 2.5%/s, held for 600 s), and a load to failure test at 0.5%/s.

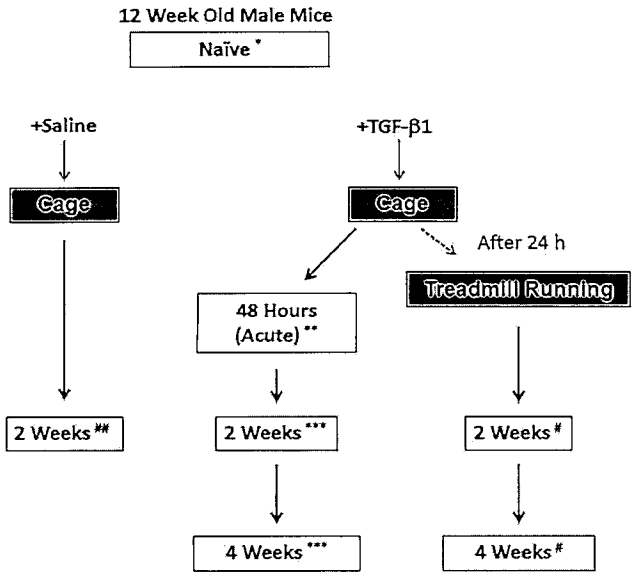

Fig. 1. Schematic of experimental design. * n=10 mice (20 tendons) for PCR, n=5 mice (8 tendons) for biomechanics, n=3 mice for histology; ** n=10 for PCR, n=7 for biomech, n=3 for histo; *** n=10 for PCR, n=6 for biomech, n=3 for histo; # n=10 for PCR, n=5 for biomech, n=3 for histo; ## n=3 for histo.

2.5. Histology and immunohistochemistry (IHC)

Following fixation, decalcification, and paraffin embedding, the Achilles tendon-bone complex was sectioned longitudinally and stained with Safranin O (Wang et al., 2012). The number of cells per 350 x 300 µm^2 field was counted using ImageJ (NIH), for each of four stained images per tendon specimen, by two investigators blinded to the treatment group. For IHC, deparaffinized sections were incubated with the following primary antibodies (10 µg/ml) overnight at 4 °C: high molecular weight aggrecan core protein (anti-DLS, Plaas et al., 2007), ADAMTS5 (anti-KNG, Plaas et al., 2007), collagen I (Abcam, ab-34710) and collagen III (Abcam, ab-7778). Sections were counter-stained with methyl green.

2.6. Quantitative PCR (QPCR)

The tendon proper (i.e. excluding calcaneal insertion and proximal myotendinous junction) was dissected fresh and placed in RNALater™ (Qiagen) at −20 °C. For RNA isolation, 20 tendons, pooled for analysis of each experimental group, were combined in liquid nitrogen, fragmented in a Bessman Tissue pulverizer, and extracted in 1 ml of Trizol™ (Life Technologies) by vortexing for 60 s. RNA was purified with an RNeasy Mini Kit™ (Qiagen) and yields of RNA were approximately 50 ng per tendon. cDNA was synthesized using the SuperScript First-Strand Synthesis Kit™ (Life Technologies) using 1 µg of RNA. All primers were from Life Technologies, Inc.: *Acan* (Mm00545794_m1); *Adamts5* (Mm01344180_m1); *Gapdh* (Mm99999915_g1); *Col1a1* (Mm00801666_g1); *Col2a1* (Mm01309565_m1); *Col3a1* (Mm00802331_m1); *Mmp3* (Mm00440295_m1); and *Fn1* (Mm01256744_m1). Amplifications were performed in triplicate with an Applied Biosystems 7300 Real-Time PCR System as follows: 50 °C, 2 min; 95 °C, 10 min; 95 °C, 15 s; 60 °C 1 min; repeated 39 times (Velasco et al., 2011). Data was processed as ΔCt (relative to *Gapdh*) for each gene at each time point, to provide relative transcript levels and fold-change was calculated as $2^{-\Delta\Delta Ct}$ relative to the ΔCt of the comparison group specified.

2.7. Statistical analysis

Biomechanical, cell counting, and gene expression results were compared across time points using one-way ANOVA with Tukey's post-hoc tests (SPSS 17, IBM, Armonk, NY). To test our study hypothesis, at each time point, an unpaired, two-tailed Student's t-test was used to compare data from the cage and treadmill groups.

3. Results

3.1. Striking histopathologic similarities between human and murine tendinopathy

Images from naive murine Achilles (Fig. 2A), 48 h following TGF-β1 injection (Fig. 2B), and normal human patellar tendon (Fig. 2I) exhibited the same, essentially GAG-free, linear organization of collagen fibers and cells. Typical images from TGF-β1 injected murine tendons illustrate that at both 2 (Fig. 2D) and 4 weeks (Figs. 2E and F), tendons showed pericellular and interfibrillar accumulation of GAG, an increase in chondrocyte-like cells, and a loss of parallel arrangement of collagen fibers in and around GAG-enriched areas. Of particular note, the development of these pathological features required the injection of TGF-β1, since injection of saline/BSA did not result in any marked changes in cell morphology or matrix appearance at 2 weeks (Fig. 2C). Histopathologic features of the affected 4-week murine tendons were also seen in tendinopathic human ECRB and biceps samples (Fig. 2G and H).

3.2. Decreased tensile properties following TGF-β1 injection and cage activity

At all times (48 h, 2 and 4 weeks) post-TGF-β1 injection, significant reductions in stiffness (~43%), maximum stress and

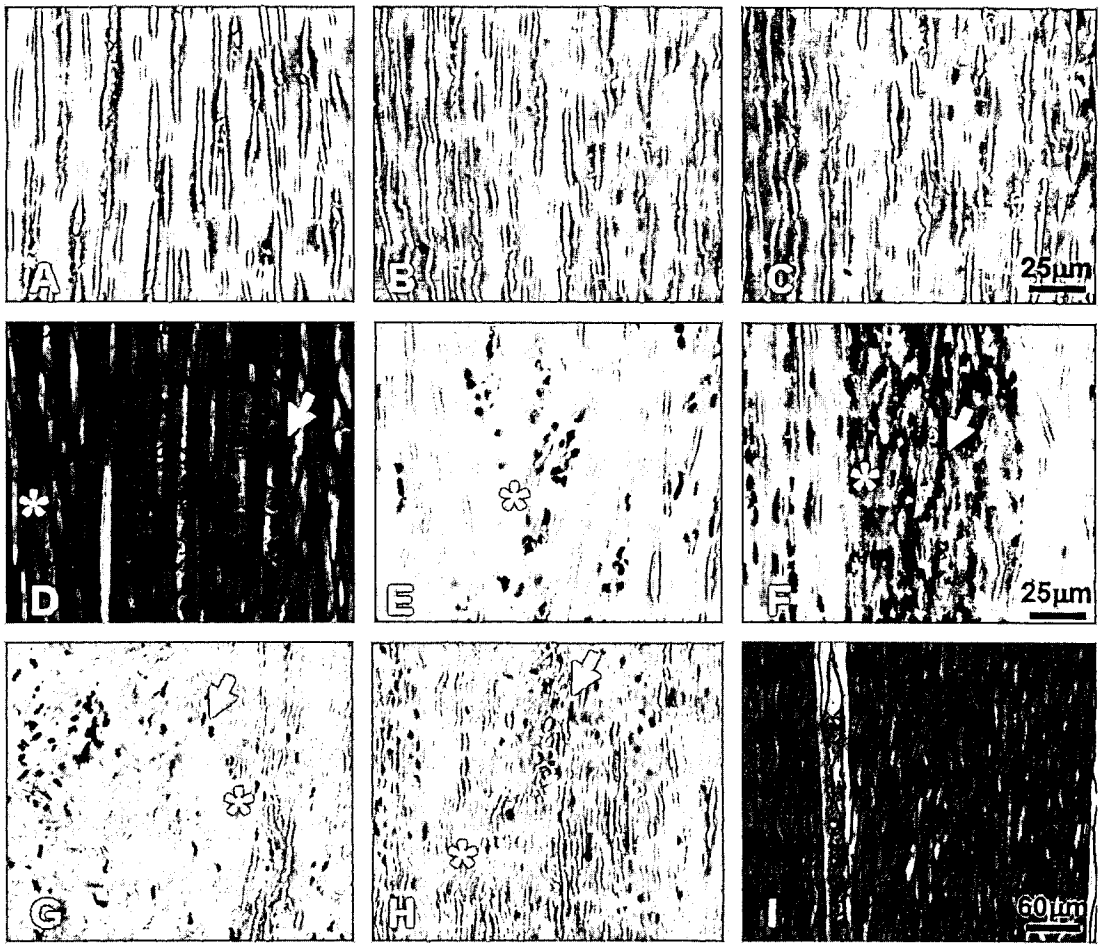

Fig. 2. Similar histopathological features of murine and human tendinopathy. In contrast to naive mouse Achilles tendon (A), 48 h post-TGF-β1 injection (B) and 2 weeks post saline-injected mouse Achilles tendon (C), accumulation of GAG, increased numbers of rounded chondrocytic cells (white arrows), and disorganization of the collagen fibers both within and adjacent to chondroid areas (white asterisk) were observed at 2 weeks (D) and 4 weeks post-TGF-β1 injection (E,F). Similar histological features were seen in tendinopathic human extensor carpi radialis brevis (G) and long head of the biceps (H) samples, with a normal human patellar tendon section shown in (I).

tensile modulus (greater than 53%) were seen, along with an increase in CSA of ~86% at 2 weeks (Fig. 3 and Table S1). There were no significant differences in stress relaxation or maximum load, despite a trend towards an increase in stress relaxation at 4 weeks ($p=0.07$), and a trend towards a decrease in maximum load at 48h and 4 weeks ($p=0.09$).

3.3. *Effect of TGF-β1 injection and cage activity on gene expression*

The ΔCt values for naive mice (Table 1) indicate that the order of transcript levels is *Col1a1* > *Fn* > *Col3a1*»*Acan* > *Col2a1* which appears to be consistent with the matrix composition of normal midsubstance tendon. Furthermore, consistent with known effects of TGF-β1 on both chondrogenic and fibrogenic signaling in dermal fibroblasts (Velasco et al., 2011), the expression of matrix genes at all time points (except for *Col3a1* at 4 weeks and *Col2a1* at 48h), were significantly elevated ($p<0.05$) relative to naïve tendons.

The fold-change in expression of fibrogenic genes (Fig. 4) showed that the patterns for *Col1a1*, *Col3a1 and Fn1* were similar, with limited activation by TGF-β1 at 48 h, peak activation at 2 weeks and a trend toward reduction to naïve levels at 4 weeks. However, the activation of chondrogenic genes appeared to be relatively delayed, with a progressive increase in *Col2a1* expression up to 4 weeks post-injection, and the fold-activation of *Acan* expression being markedly higher than that for fibrogenic genes at both 2 weeks and 4 weeks.

3.4. *Treadmill exercise following TGF-β1 injection increases tensile properties and alters gene expression*

Two weeks following injection, similar mechanical properties were noted between cage and treadmill exercise groups (Fig. 5 and Table S2). Notably, however, exercise for 4 weeks was effective in restoring biomechanical properties (Table S2). Specifically, it led to recovery in maximum load, stiffness, maximum stress and tensile modulus ($p<0.04$ in all cases, relative to 2 weeks).

At 2 weeks, relative to no exercise, there was a minor, but significant ($p<0.05$) reduction in the expression of *Col1a1* and *Col3a1*, an increase ($p<0.05$) of *Col2a1* and *Fn1* expression, and no significant change in *Acan* expression (Fig. 6). Following 4 weeks of exercise, there were further marked changes in gene expression. Firstly, relative to cage activity, at 4 weeks there was a 10-fold reduction in expression of *Col1a1* ($p=0.014$) and a 200-fold reduction in *Col3a1* ($p<0.0001$). Moreover, the high expression of *Col2a1* and *Acan* seen with exercise at 2 weeks was reduced by ~25-fold ($p=0.0004$) and ~12-fold ($p<0.001$) respectively at 4 weeks. The effect of treadmill exercise was also evident in the cellularity of the injected tendons. Naive tendons had 305 ± 33 cells per unit area, which was increased by approximately 1.7-fold (519 ± 9) and 2.1-fold (652 ± 95) at 2 weeks and 4 weeks, respectively, in mice maintained at cage activity. However, with treadmill exercise the corresponding increases were significantly lower at 1.3-fold (396 ± 18, $p<0.0001$) at 2 weeks and 1.6-fold (495 ± 56, $p=0.008$) at 4 weeks.

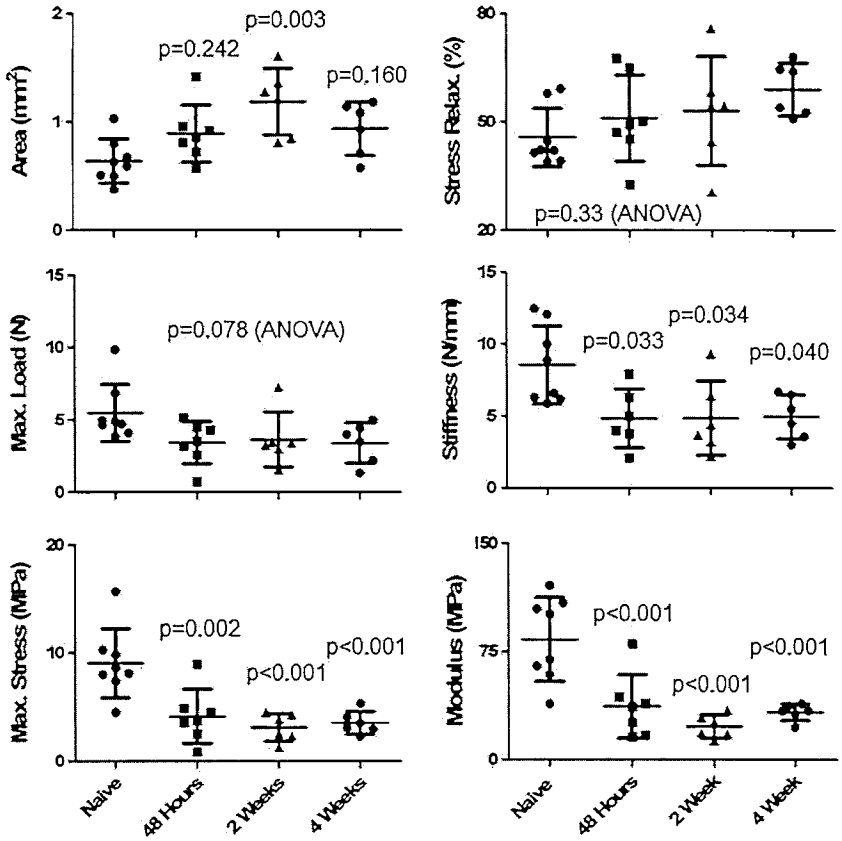

Fig. 3. Effect of TGF-β1 injection and cage activity on Achilles tendon mechanical properties. The scatter plots show data for individual tendons in each group, while the p-values correspond to the comparison between means of the experimental and naive mice. For each time point, horizontal lines denote mean ± one standard deviation.

Table 1
Effect of TGF-β1 injection and cage activity on gene expression.

TREATMENT	*Col1a1* ΔCT	*Col2a1* ΔCT	*Col3a1* ΔCT	*Fn1* ΔCT	*Acan* ΔCT	*Adamts5* ΔCT	*Mmp3* ΔCT
Naïve	−0.14(0.16)	16.65(0.39)	4.04(0.07)	1.38(0.03)	15.91(0.61)	7.26(0.07)	11.35(0.20)
Acute p^*	−1.61(0.38) 0.015	16.95(0.64) 0.625	2.08(0.19) 0.037	0.35(0.11) 0.0026	10.56(0.08) 0.0036	8.44(0.14) 0.027	6.58(0.14) 0.0003
2 weeks p^*	−5.13(0.11) <0.00001	12.28(0.15) 0.0009	−0.32(0.42) 0.0028	−0.43(0.43) 0.0183	7.92(0.37) 0.0004	7.27(0.16) 0.9445	4.70(0.31) <0.00001
4 weeks p^*	−3.52(0.51) 0.022	10.90(0.38) <0.00001	3.99(0.16) 0.215	0.36(0.16) 0.0066	9.78(0.45) 0.00025	7.83(0.03) 0.0014	4.50(0.31) <0.00001

Data presented are mean values, with standard deviation in parentheses.

* p-values represent comparisons to ΔCT of naïve samples.

3.5. Effects of TGF-β1 injection and treadmill exercise on Adamts5 and Mmp3 expression

The exercise-dependent reduction in gene expression seen between 2 and 4 weeks (Fig. 6) was also evident for *Adamts5* and *Mmp3* (Fig. 7). Without exercise, *Adamts5* expression was not markedly affected at any time, whereas *Mmp3* was activated ~25-fold at 48 h and further activated to ~110-fold after 2 and 4 weeks. Exercise for 2 weeks had no major effect on expression of either gene, however exercise for 4 weeks decreased *Adamts5* ($p=0.003$) and *Mmp3* expression ($p=0.003$).

3.6. Effects of TGF-β1 injection and treadmill exercise on the abundance of collagens I and III, aggrecan and ADAMTS5

The major increase in *Col1a1*, *Col3a1* and *Acan* expression following 2 weeks of cage activity was reflected in increased cell-associated staining (Fig. 8, Figs. S2–S4). The absence of change in *Adamts5* expression at 2 weeks was nonetheless accompanied by a marked increase in protein staining. Conversely, the decreased expression of these genes seen after 4 weeks of exercise (Figs. 6 and 7) did not appear to markedly alter the abundance of these components (Fig. 8 and Fig. S5).

3.7. Effects of TGF-β1 injection and treadmill exercise on cell shape and distribution of collagens I and III, aggrecan and ADAMTS5

The staining for all components except collagen I was primarily in the pericellular space, suggesting that the IHC procedure detects newly synthesized molecules (Li et al., 2012) (Fig. 8 and Fig. S2–S5). For collagen I, staining was also widely distributed throughout the matrix (Fig. 8 and Fig. S2), indicating that both new and resident molecules were detected. In the naive tissue, collagen I staining was robust throughout the matrix and in the vicinity of individual cells, whereas aggrecan was detected as a diffuse pericellular coat surrounding groups of tenocytes. It is

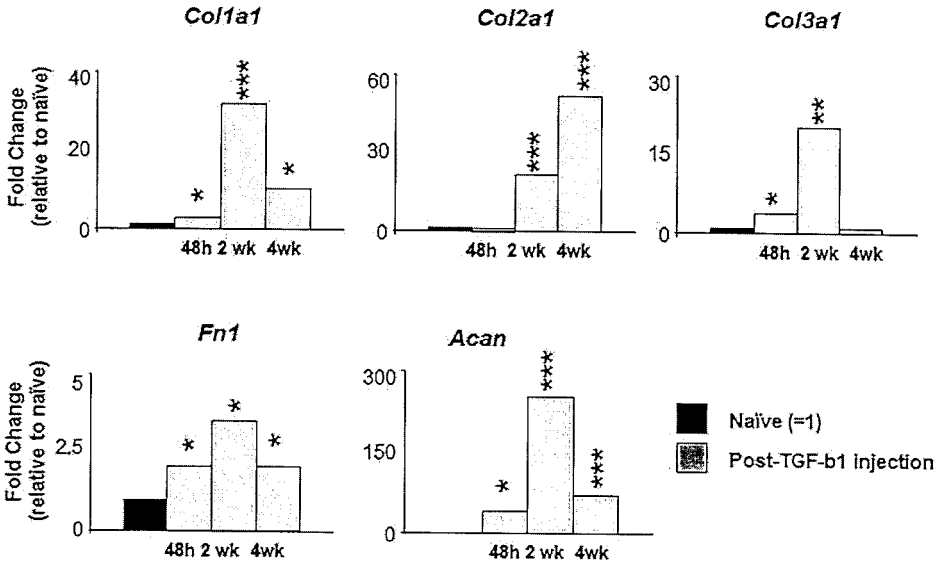

Fig. 4. Effect of TGF-β1 injection and cage activity on expression of genes for matrix proteins. The fold-change in expression of *Col1a1*, *Col2a1*, *Col3a1*, *Fn1*, and *Acan* relative to naive tendons. * denotes $p<0.05$, ** denotes $p<0.001$, and *** denotes $p<0.0005$.

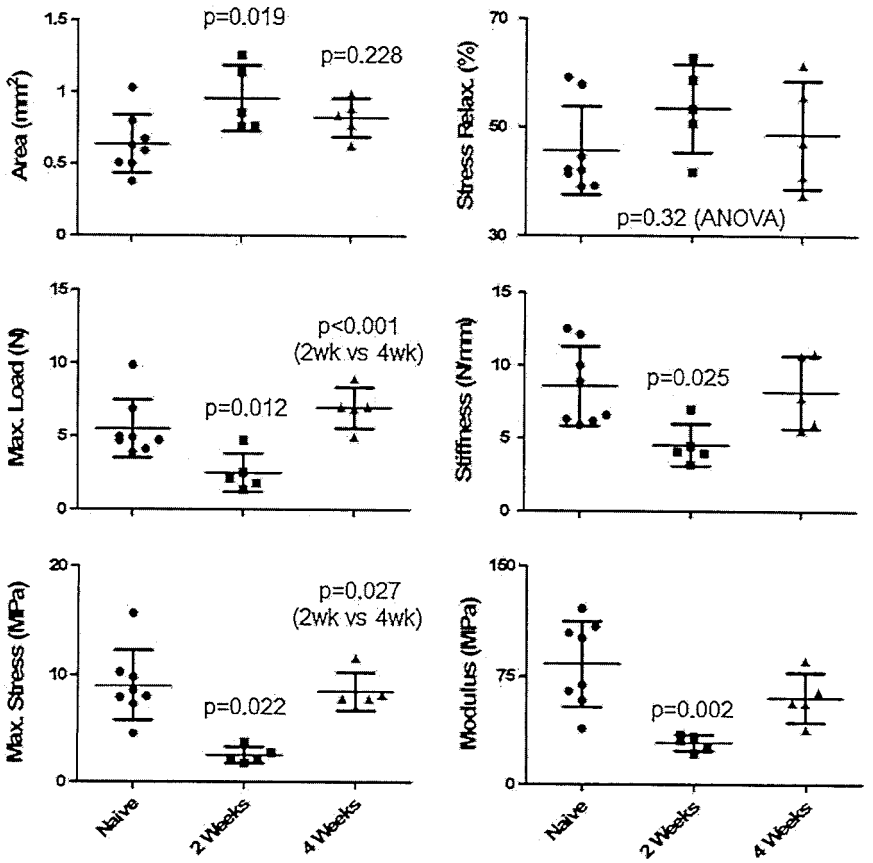

Fig. 5. Effect of treadmill exercise following TGF-β1 injection on tendon mechanical properties. The scatter plots show data for individual tendons in each group, while the p-values correspond to the comparison between means of the experimental and naive mice. For each time point, horizontal lines denote mean $\pm$ one standard deviation.

notable that there is staining for aggrecan in naive tendons, despite the very low, but detectable, transcript levels. Aggrecan is also present in naive mature mouse FDL tendon, as shown by Western analysis (Wang et al., 2012) suggesting that it largely represents molecules synthesized and retained during development and maturation of the tendon.

Conversely, collagen III and ADAMTS5 were not detected in naive tendons. At 48 h after TGF-β1 injection there was a robust

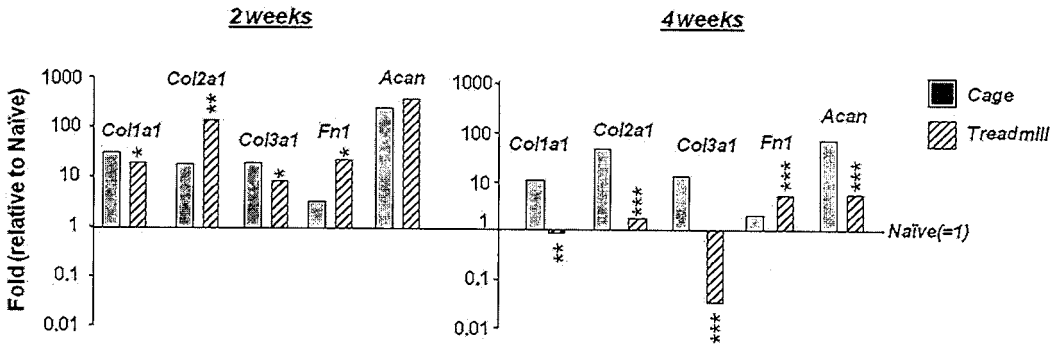

Fig. 6. Effect of treadmill exercise following TGF-β1 injection on gene expression. The fold-change in expression of *Col1a1, Col2a1, Col3a1, Fn1 and Acan* relative to naive tendons. *P*-values correspond to the comparison between means of caged and treadmill-run mice for each gene. * denotes $p < 0.05$, ** denotes $p < 0.001$, and *** denotes $p < 0.0005$.

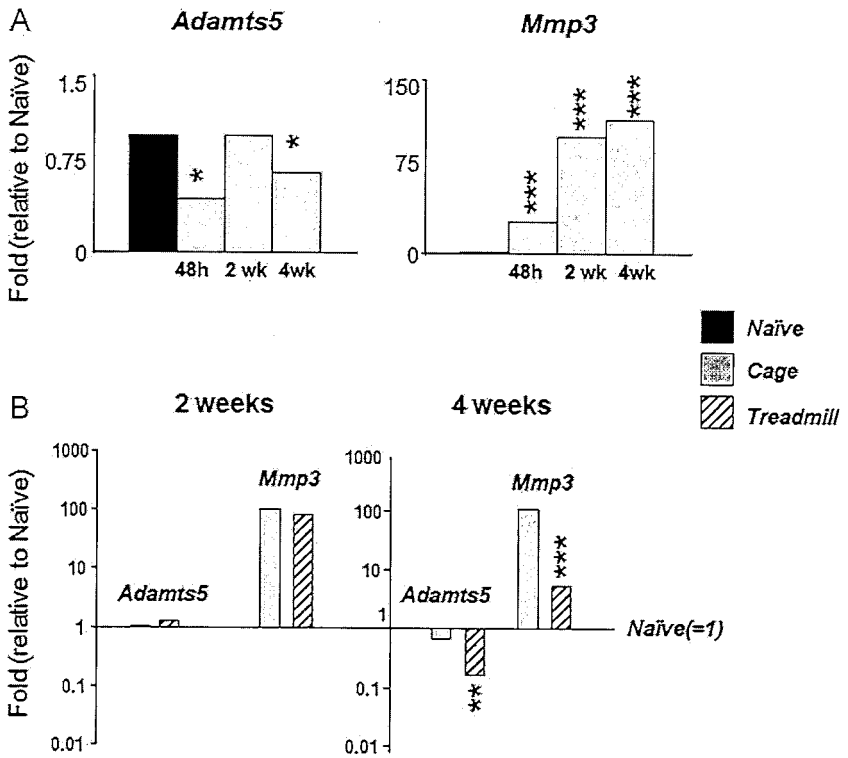

Fig. 7. Effect of treadmill running following TGF-β1 injection on gene expression of *Adamts5* and *Mmp3*. (A). The fold-change in expression of *Adamts5* and *Mmp3* relative to naive tendons, following TGF-β1 injection and cage activity. * denotes $p < 0.05$ and *** denotes $p < 0.0005$. (B) The fold-change in expression of *Adamts5 and Mmp3* relative to naive tendons. *P*-values correspond to the comparison between means of caged and treadmill-run mice. ** denotes $p < 0.001$ and *** denotes $p < 0.0005$.

increase in pericellular staining for both collagen III and ADAMTS5, but no clear change for either aggrecan or collagen I. Of interest, the increase in ADAMTS5 staining occurred in the absence of a change in expression, suggesting that post-translational events control its tissue abundance.

After 2 weeks of cage activity, collagens I and III, aggrecan and ADAMTS5 all showed increased staining in the cell-associated matrix (Fig. 8 and Figs. S2–S5), which was less evident after 2 weeks of treadmill exercise. The most notable difference between cage and exercised groups was the shape and organization of the cells (Fig. 8). Cage activity for 2 or 4 weeks resulted in groups of rounded cells, with enlarged and rounded nuclei, and with each cell surrounded by its own organized pericellular matrix. In contrast, treadmill exercise prevented the appearance of such chondrogenic groups at both times, such that resident cells exhibited the flat nuclei and elongated tenocyte morphology seen in naïve tendons. Moreover, due to the lack of an organized pericellular matrix, these elongated cells appeared to be interconnected and directly associated with the adjacent collagen fibers. The overall abundance and distribution of ADAMTS5 protein under each condition (Fig. S5), was compared to staining for the aggrecanase product, G1-NITEGE (Fig. S6). Both antigens showed an increased abundance in TGF-β1-injected tendons, and while the ADAMTS5 was confined to the cells, the G1-NITEGE was also abundant in the tendon matrix. However, many more cells stained for G1-NITEGE than ADAMTS5, suggesting the activity of other aggrecanases in the tendon.

4. Discussion

We have developed a murine model of tendinopathy that closely mimics the human pathology, and is also amenable to testing new therapeutic interventions. Firstly, the impairment of

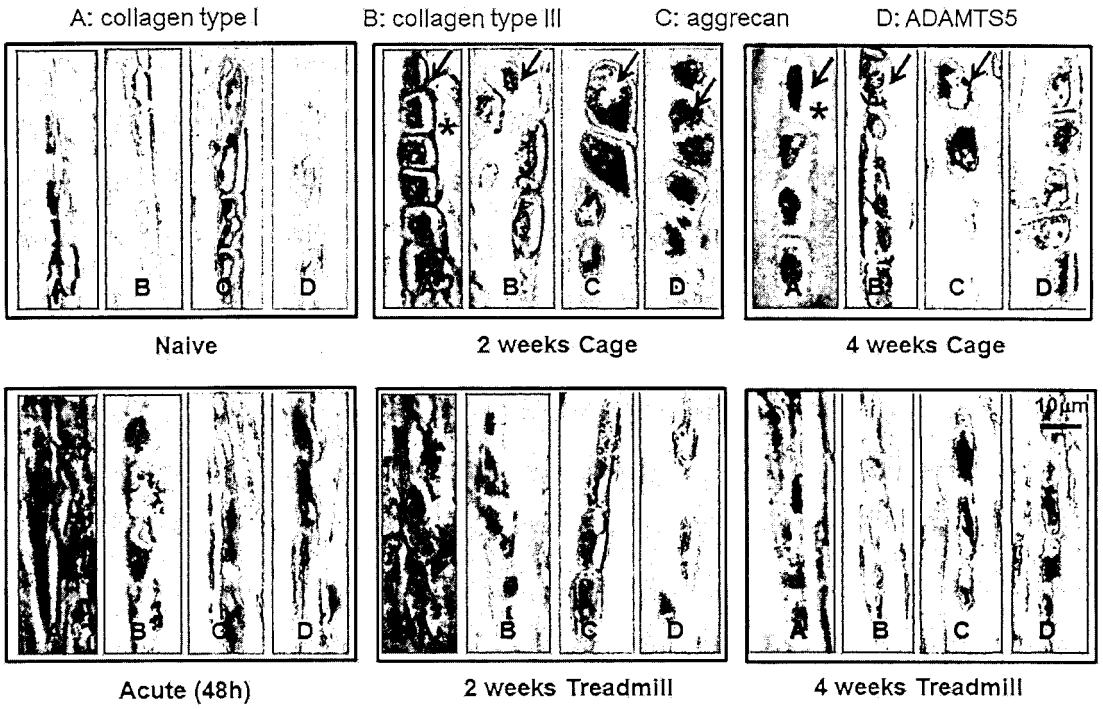

Fig. 8. Effect of treadmill running following TGF-β1 injection on immunolocalization of matrix proteins and ADAMTS5. IHC of typical cell groupings in tendons from naive, 2-week caged and 4-week caged mice are shown in the top three panels. The corresponding images for 48 h, 2-week treadmill and 4-week treadmill mice are shown in the bottom three panels. (A) collagen type I, (B) collagen type III, (C) aggrecan and (D) ADAMTS5. Cage activity resulted in the continuous presence of groups of rounded cells, with enlarged and rounded nuclei (indicated by arrows), and with each cell surrounded by its own pericellular, chondron-like matrix (labeled with asterisks). In contrast, tendon cells in the exercised groups exhibited the flat nuclei and elongated tenocyte morphology seen in the naïve tendons.

mechanical properties (Fig. 3) is consistent with the finding that human Achilles tendinopathy results in inferior mechanical properties (Arya and Kulig, 2010). Secondly, histopathologic findings such as hypercellularity, chondroid deposition and collagen disorganization, strongly resemble human tendinopathies (Fig. 2). Thirdly, activation of aggrecan gene expression in the model (Figs. 4 and 6) has also been reported in human Achilles tendinopathy (Corps et al., 2006; de Mos et al., 2009). Perhaps the most important authentication of the model is the finding that controlled exercise can reverse over-expression of *Acan*, *Col1a1*, *Col2a1*, *Col3a1* and *Mmp3*, and essentially eliminate chondroid accumulation. Moreover, this is accompanied by full recovery of tendon tensile properties, which supports clinical outcomes of exercise regimens (Silbernagel et al., 2011) and permits future quantitative therapeutic studies.

One advantage of this novel model is that tendinopathy is induced simply by injection of TGF-β1 into the midsubstance. The rationale for this derives from both clinical and bench studies indicating that excess active TGF-β1 in tendons is pathogenic. Analysis of tendinopathic Achilles (Pingel et al., 2012) showed increased expression of TGF-β1 associated with increased cellularity and collagen disorganization. Further, treatment of repair sites with antibodies to TGF-β1 (Chang et al., 2000) potentiated repair, whereas TGF-β1 generated by tendon transection was pathogenic (Maeda et al., 2011).

In our model, tensile properties correlated with changes in cell morphology, gene expression and abundance and localization of matrix proteins and ADAMTS5 (Fig. 8). The relationship between these parameters and tendinopathy was most apparent when comparing tendinopathic with exercised mice. At 2 weeks, the lowest values of maximum stress were observed (Fig. 3), and coincided with high abundance of chondroid cells (Fig. 8) and highest expression of *Acan*, *Col1a1*, *Col3a1* and *Fn* (Fig. 4). Further, in the absence of exercise these abnormalities remained largely unchanged. However, in mice exercised for 4 weeks, maximum stress recovered to naive levels, chondroid cells were reduced (Fig. 8) and expression of *Acan*, *Col1a1*, *Col2a1* and *Col3a1* essentially returned to levels of naive tendon (Fig. 6). Moreover, exercise reduced the expression of *Mmp3* to near naive levels and expression of *Adamts5* to levels well below naive (Fig. 7). We conclude that it is the reduction in expression between 2 and 4 weeks, of all genes examined, which appears to be responsible for the reparative effect of 4 weeks of exercise.

Our findings are consistent with the beneficial effects of loading on healing of transected rodent Achilles (Andersson et al., 2009; Palmes et al., 2002; See et al., 2004; Virchenko and Aspenberg, 2006). In addition, 3–12 months of exercise has been shown to eliminate pain and immobility in Achilles tendinopathy patients for up to 3–5 years (Ohberg et al., 2004; Silbernagel et al., 2011; van der Plas et al., 2012), with one study (Verrall et al., 2011) noting that only 6 weeks of exercise was required.

Our data strongly suggest that loss of tendon mechanical properties results directly from the presence of chondrogenic cells, and their formation of aggrecan-rich deposits. This is supported by our finding (Wang et al., 2012) that flexor digitorum longus tendons of *Adamts5*$^{-/-}$ mice are enriched in pericellular aggrecan and exhibit inferior material properties relative to wild-type controls. Moreover, equine suspensory ligaments which have lost mechanical strength are populated with chondroid cell groups in an aggrecan-rich matrix (Plaas et al., 2011). This general theme is also consistent with observations made on mid-portion Achilles tendinotic lesions, which were found to have increased glycosaminoglycan staining, an increase in rounded cell nuclei and a gene expression pattern characteristic of fibrochondrocytes (*de Mos* et al., 2009).

Finally, if accumulation of a chondroid matrix in the tendon midsubstance is responsible for the pathologic loss of tensional

properties, it seems logical to develop therapies which can quickly remove the abnormal matrix and also prevent its reformation in the long-term. The demonstration here that controlled exercise can rapidly achieve chondroid matrix removal, should stimulate further research into the specific biomechanical transduction mechanisms involved.

Conflict of interest statement

All authors acknowledge that they have nothing to disclose with regard to financial or personal relationships with other individuals or organizations that could inappropriately influence the work presented herein.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jbiomech.2012.10.020.

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