

# ADAMTS5 is Required for Biomechanically-Stimulated Healing of Murine Tendinopathy

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**ABSTRACT:** A recently developed murine model of tendinopathy, induced by TGF- $\beta$ 1 injection, has been used to examine the reparative capacity of tendinopathic Achilles in *Adamts5*<sup>-/-</sup> mice. After TGF- $\beta$ 1 injection and 2 weeks of treadmill exercise, the Achilles from *Adamts5*<sup>-/-</sup> mice exhibited a reduction in maximum tensile stress of approximately 60%. However, in contrast to wild type mice previously characterized by this model, *Adamts5*<sup>-/-</sup> mice subjected to further treadmill exercise were unable to reverse this biomechanical deficit. This nonreparative phenotype was accompanied by a major deficiency, relative to wild-type, in expression of *Col1a1* and *Col3a1* and an abnormally elevated expression of a wide range of integrins. In addition, the tendinopathic *Adamts5*<sup>-/-</sup> mice showed a persistent accumulation of chondrogenic cells in the tendon body and an aggrecan-rich fibrocartilaginous matrix within disorganized collagen fiber bundles. Moreover, consistent with the compromised biomechanical properties of the Achilles in the *Adamts5*<sup>-/-</sup> mice, *in vivo* gait analysis revealed a strong trend ( $p = 0.07$ ) towards increased swing time of the injected limb in *Adamts5*<sup>-/-</sup> relative to wild-type mice. These findings demonstrate that a deficiency in ADAMTS5 promotes a chondrogenic response to TGF- $\beta$ 1 injection that is not reversed by treadmill exercise. Hence, repair of biomechanically compromised tendons exhibiting midsubstance chondroid accumulation requires ADAMTS5. © 2013 Orthopaedic Research Society Published by Wiley Periodicals, Inc. *J Orthop Res* 31:1540–1548, 2013

**Keywords:** tendinopathy; gene expression; aggrecan; biomechanics; gait; ADAMTS5

Human tendinopathies (e.g., Achilles, rotator cuff, epicondylitis) have been characterized histologically by the variable presence of features such as increased cellularity, collagen disorganization, mucoid degeneration, lipid accumulation and calcific deposits.<sup>1</sup> In contrast to the fibrocartilage that forms in adaptation to normal mechanical compression,<sup>2</sup> mucoid deposits appear to be a pathologic response to an abnormal biochemical and/or biomechanical environment within the tendon body. Data on the mechanisms underlying generation of mucoid deposits and its likely pathogenic effects in tendinopathy have not been forthcoming, in part due to the unavailability of models in which abundance of these deposits can be experimentally manipulated.

We have recently shown that uninjured mice deficient in ADAMTS5 (TS5) exhibit pericellular aggrecan accumulation, an increased collagen fibril density, and compromised tendon mechanical properties relative to wild type mice.<sup>3</sup> Indeed, other *in vivo* studies have suggested a requirement for TS5 in the deposition of fibrous tissues in general. Thus, joint wounds in ADAMTS5-knockout (TS5<sup>-/-</sup>) mice are protected against degenerative fibrosis<sup>4</sup> and excisional dermal wounds in these mice do not scar.<sup>5</sup> Moreover, an apparent deficiency in active TS5 accompanies degeneration of the suspensory ligaments of horses.<sup>6</sup> Importantly,

in joints, dermis and ligament, the absence of fibrogenesis is associated with the accumulation of aggrecan-rich deposits (ARDs).

We have recently developed a murine model of Achilles tendinopathy in which TGF- $\beta$ 1 injection into the tendon body results in a chondrocytic cell morphology with abundant ARDs, accompanying a marked decrease in biomechanical strength.<sup>7</sup> The use here of TGF- $\beta$ 1 as a pathogenic agent, when it is commonly thought to be reparative for extracellular matrix, was motivated by the known range of potential effects of TGF- $\beta$ 1 on cell activities. For example, TGF- $\beta$ 1 can have multiple downstream effects in a wound healing environment because it can induce a reparative fibrogenic response via Smad2/3 signaling, or a chondrogenic response via Smad1/5/8 signaling.<sup>5,8–10</sup> It is clear that in this new model of tendinopathy we have established conditions where the response to TGF- $\beta$ 1 is primarily chondrogenic and therefore pathogenic.

## METHODS

**Animals:** C57Bl6 mice were bred in-house and all studies received IACUC approval. TS5<sup>-/-</sup> mice were generated by excision of exon 2 to delete the catalytic site,<sup>11</sup> and phenotypic traits of this colony have been described in relation to mechanical allodynia,<sup>11</sup> joint cartilage repair,<sup>4</sup> dermal repair,<sup>5</sup> and tendon structure–function properties.<sup>3</sup> **Tendinopathy induction:** As described previously,<sup>7</sup> mice were injected into the mid-portion of the right Achilles tendon with 100 ng hrTGF- $\beta$ 1 (Active Form, PeproTech Inc., Rocky Hill, NJ) in 6  $\mu$ l of sterile saline containing 0.1% ultrapure BSA (Sigma–Aldrich, St. Louis, MO). Mice were sacrificed at 48 h (acute response), 2 or 4 weeks following TGF- $\beta$ 1 injection; a separate group of noninjured control (i.e., naïve) mice was included for comparison. The number of mice used in each

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experimental group, for each assay, is provided in the respective figure legends. **Mechanical stimulation:** Mice were subjected to uphill (17°) running on a Stoelting/Panlab treadmill (TM) at 32 cm/s for 20 min/day for 5 days/week, starting 1 day after TGF- $\beta$ 1 injection.<sup>7</sup> A control group of cage (i.e., no TM) activity mice was examined at 4 weeks post-injection. **Biomechanical testing:** of Achilles tendons was performed as described.<sup>3,7</sup> **Gait analysis:** of TS5<sup>-/-</sup> and wild type (WT) mice was conducted at baseline (3 days prior to injection) and at 2, 3, and 4 weeks post-injection using a TreadScan system (CleverSys Inc., Reston, VA). For each mouse, gait parameters were normalized to its baseline value.<sup>12</sup> **Quantitative PCR:** Tendons ( $n = 20$  pooled per experimental group) were harvested and stored at -20°C in RNALater (Qiagen, Valencia, CA). RNA was isolated and primers for the Taqman assay were from Life Technologies (Grand Island, NY) as previously described<sup>7</sup>; primers were also obtained for *Itga1* (Mm01306375\_m1), *Itga2* (Mm00434371\_m1), (*Mm01309565\_m1*), *Itga5* (Mm00439797\_m1), *ItgaV* (Mm00434506\_m1), *Itgab1* (Mm01253230\_m1), *Itgab3* (Mm00443980\_m1), and *Itgb5* (Mm00439825\_m1). **Histology and immunohistochemistry (IHC):** Lower hind limb samples were prepared as described previously.<sup>7,13</sup> Antibodies to integrin  $\alpha$ V,  $\alpha$ 2,  $\beta$ 3,  $\beta$ 5 and collagen type II were from Abcam (Cambridge, MA); aggrecan was detected with anti-DLS as described.<sup>3,7</sup> **Statistical analyses:** Biomechanical properties and gene expression data were compared across time points using a one-way ANOVA (SPSS 17; IBM). Temporal gait results were assessed using one-way ANOVA with repeated measures. Post-hoc Tukey's tests were used for pairwise comparisons, and significance was assumed for  $p < 0.05$ .

## RESULTS

### Absence of TS5 Prevents Treadmill-Induced Recovery of Achilles Biomechanical Strength

Tendon maximum load, stiffness, maximum stress, and tensile modulus all exhibited reductions, relative to naïve mice, following TGF- $\beta$ 1 injection of TS5<sup>-/-</sup> mice. Maximum stress and modulus exhibited significant differences relative to uninjured tendons at each healing time point (Fig. 1), with sustained impairment of these tendon properties observed up to 4 weeks post-injection. Tendon cross-sectional area increased significantly at 2 weeks post-injection and then returned to naïve levels at 4 weeks. Comparison of results for TM and cage activity mice at 4 weeks post-injection revealed no differences ( $p > 0.28$  for all mechanical and geometric outcomes).

### The Non-Reparative Phenotype of TS5<sup>-/-</sup> Mice Is Accompanied by a Major Deficiency in Expression of Tendon Collagens

Comparison of gene expression levels in Achilles tendons of naïve WT and naïve TS5<sup>-/-</sup> mice (Table S1) showed that the order of transcript abundance was similar in each genotype (*Col1a1* > *Fn1* > *Col3a1* > *Col2a1* > *Acan*). However, there were major differences in absolute values, with *Col3a1* and *Col1a1* more abundant in WT (~20- and ~4-fold respectively,  $p \leq 0.001$ ) and *Col2a1* and *Acan* more abundant in TS5<sup>-/-</sup> (~60- and ~5-fold respectively,  $p < 0.01$ ). This difference in naïve mice is consistent with the finding

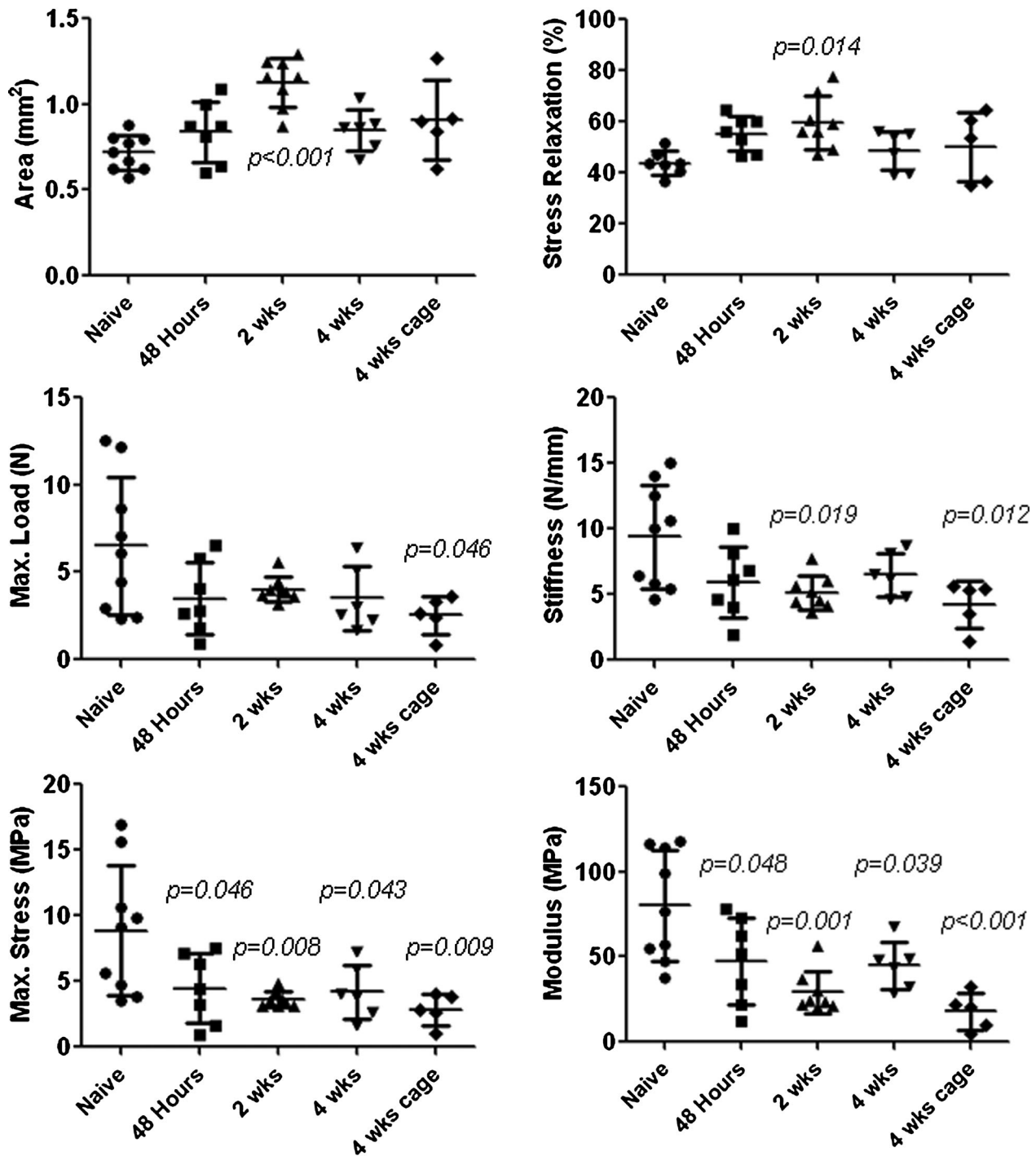
that the flexor digitorum longus (FDL) and Achilles tendons of naïve TS5<sup>-/-</sup> mice contain ARDs, which adversely affect their biomechanical properties.<sup>3</sup>

Another genotypic difference was in the time course of the response of individual genes to TGF- $\beta$ 1 injection (Table 1). In WT mice, all genes showed maximum expression at 2 weeks, except for *Col2a1* which peaked at 4 weeks. However, for TS5<sup>-/-</sup> mice the maximum expression was generally earlier; for *Col2a1* it was in naïve mice, for *Col3a1* and *Acan* at 48 h, and for *Col1a1* and *Fn1* at 2 weeks, suggesting that the absence of TS5 resulted in a more rapid response to TGF- $\beta$ 1 injection overall. However, the major distinction between WT and TS5<sup>-/-</sup> mice was in the extent of the change in transcript abundance for each gene. It was found (Fig. 2) that for both *Col1a1* and *Col3a1* the response in the TS5<sup>-/-</sup> mice was markedly lower, particularly for *Col3a1* which was about 100- and 1,000-fold lower at 2 and 4 weeks, respectively. Given that the absolute expression levels in naïve mice for *Col3a1* were markedly lower for TS5<sup>-/-</sup> relative to WT (Table S1), tendinopathy was associated with a severe deficiency in *Col3a1* expression in TS5<sup>-/-</sup> tendons. For *Fn1* the fold-change was similar between genotypes, although greater in TS5<sup>-/-</sup> mice at 2 weeks, and for *Acan* and *Col2a1*, both of which had higher naïve values in the TS5<sup>-/-</sup> mice, the fold-change was relatively minor for both genotypes and the response pattern was similar for both genes (Fig. 2). Since the tensile properties of tendons are largely attributable to the abundance, cross-linking and linear organization of collagens type I and type III, the inability of TS5<sup>-/-</sup> mice to reverse the tendinopathy appears to be at least partly explained by the very low expression of *Col3a1* at 2–4 weeks.

### The Non-Reparative Phenotype of TS5<sup>-/-</sup> Mice Is Accompanied by an Abnormally Elevated Expression of Integrins

Since the lack of repair in TS5<sup>-/-</sup> tendons was accompanied by abnormally low levels of expression of *Col1a1* and *Col3a1*, we next examined the expression of integrins which promote cell binding to collagens (integrins  $\alpha$ 1,  $\alpha$ 2, and  $\beta$ 1), fibronectin ( $\alpha$ 5,  $\alpha$ V,  $\beta$ 1,  $\beta$ 3,  $\beta$ 5), and laminin ( $\beta$ 1).<sup>14</sup> The expression level ( $\Delta$ CT) of integrin genes in naïve WT tendons was in the order  $\beta$ 1 >  $\alpha$ V >  $\alpha$ 5 >  $\alpha$ 1 >  $\alpha$ 2 >  $\beta$ 3 >  $\beta$ 5, which was similar to naïve TS5<sup>-/-</sup> ( $\beta$ 1 >  $\alpha$ V >  $\alpha$ 1 >  $\beta$ 5 >  $\alpha$ 5 >  $\alpha$ 2 >  $\beta$ 3) (Table 2). However, the expression of all integrins, except  $\beta$ 5, was about 10-fold reduced in naïve TS5<sup>-/-</sup> relative to WT mice, which is consistent with the notion<sup>5</sup> that TS5 is required for cell-matrix interactions involved in fibrogenic wound healing.

The relative fold change in transcript abundance (TS5<sup>-/-</sup> relative to WT) for each integrin gene (Fig. 3) was determined from the data in Table 2 (as described above for Fig. 2). All integrins showed a similar positive relative fold-change, which despite the lower naïve values in TS5<sup>-/-</sup> resulted in a markedly higher



**Figure 1.** Effect of TGF- $\beta$ 1 with TM on Achilles tendon mechanical properties in TS5<sup>-/-</sup> mice. The scatter plots show data for individual tendons within each experimental group (naïve,  $n = 9$ ; 48 h,  $n = 7$ ; 2 weeks,  $n = 8$ ; 4 weeks,  $n = 6$ ; 4 week cage,  $n = 5$ ).  $p$ -Values correspond to the comparison (post-hoc Tukey's test) between means of the respective experimental group and naive mice. For each time point, horizontal lines denote mean  $\pm$  1 standard deviation.

absolute transcript abundance for all integrins in the TS5<sup>-/-</sup> tendons, at both 2 and 4 weeks. The greater responsiveness of TS5<sup>-/-</sup> tendons to stimulation of integrin expression by TGF- $\beta$ 1<sup>15</sup> is consistent with the presence of an altered TGF- $\beta$ 1-signaling pathway in dermal fibroblasts from TS5<sup>-/-</sup> relative to WT mice.<sup>5,8</sup>

### Immunohistochemistry of Tendons From TS5<sup>-/-</sup> Mice Illustrates the Association of Fibrocartilage Formation With Poor Repair

The most marked histologic difference between normal and tendinopathic tissue was in the morphology and pericellular matrix of the tendon cells. When Achilles tendons from naive TS5<sup>-/-</sup> mice were stained for

**Table 1.** Effect of TGF- $\beta$ 1 Injection and TM Exercise on Matrix Gene Expression in Achilles Tendons of TS5 $^{-/-}$  Mice

	Col1a1		Col2a1		Col3a1		Fn1		Acan	
	WT <sup>a</sup>	TS5 $^{-/-}$	WT <sup>a</sup>	TS5 $^{-/-}$	WT <sup>a</sup>	TS5 $^{-/-}$	WT <sup>a</sup>	TS5 $^{-/-}$	WT <sup>a</sup>	TS5 $^{-/-}$
Naive	-0.14 (0.16)	1.86 (0.60)	16.65 (0.64)	10.93 (0.75)	4.04 (0.07)	8.27 (0.32)	1.38 (0.03)	1.13 (0.57)	15.91 (0.61)	13.79 (0.49)
Acute, $p^b$	-1.61 (0.38), 0.015	-1.93 (0.06), 0.0001	16.95 (0.64), 0.625	15.69 (0.23), <0.00001	2.08 (0.19), 0.037	1.28 (0.21), <0.00001	0.35 (0.11), 0.003	-0.23 (0.15), 0.047	10.56 (0.08), 0.004	8.14 (0.84), 0.002
2 weeks $p^b$	-4.47 (0.10), <0.00001	-4.32 (0.32), <0.00001	9.96 (0.02), 0.001	12.72 (0.36), 0.003	0.94 (0.08), <0.00001	6.00 (0.13), <0.00001	-3.19 (0.23), 0.001	-3.67 (0.33), 0.001	7.33 (0.29), 0.001	8.76 (0.36), <0.00001
4 weeks $p^b$	-0.11 (0.15), 0.290	-0.44 (0.19), 0.002	15.66 (0.18), 0.034	ND	8.96 (0.04), <0.00001	16.08 (0.73), <0.00001	-1.04 (0.47), 0.012	1.56 (0.15), 0.319	13.44 (0.42), 0.011	11.44 (0.66), 0.019

Data presented are mean  $\Delta$ CT values, with standard deviation in parentheses.

<sup>a</sup>WT data are from Bell et al.<sup>7</sup>

<sup>b</sup> $p$ -Values represent comparisons to  $\Delta$ CT of naive samples within each genotype.

aggrecan or collagen type II (Fig. 4), some cells were arranged in linear rows along the collagen fibers, much as seen for naive WT mice. However, as previously noted with naive TS5 $^{-/-}$  FDL tendons,<sup>3</sup> some cells also had a rounded morphology and appeared to reside within a disorganized collagen matrix. In contrast to WT mice, in the TS5 $^{-/-}$  Achilles tendons a large number of cells with the rounded morphology persisted even at 4 weeks post-injection and TM exercise, and all cells stained intensely for aggrecan and collagen type II. Whereas aggrecan was restricted to the immediate pericellular space, col II staining was seen both with cells and diffusely within the fibrillar matrix. The increased staining for these chondrocytic matrix molecules was consistent with the early activation of *Acan* and *Col2a1* gene expression (Table 1). In addition, similar to our prior work on WT mice,<sup>7</sup> TS5 $^{-/-}$  mice in the current study showed a persistent increase in cell density in response to TGF- $\beta$ 1 injection (data not shown).

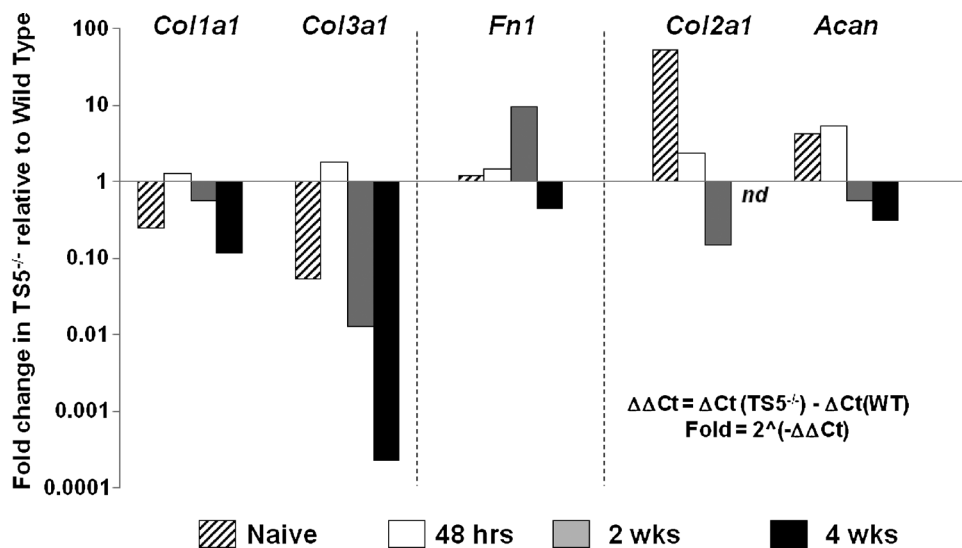
Integrin staining (Fig. 5) and gene expression changes (Fig. 2) provided strong evidence for a change to a fibrochondrocyte phenotype (expressing aggrecan and collagen II) in tendinopathic TS5 $^{-/-}$  mice. This change was particularly well illustrated on staining for integrin  $\alpha$ V and  $\beta$ 3 for the affected cells, as these showed a similar staining pattern as seen in native fibrocartilage in the Achilles tendon-bone insertion site (Fig. 5). Non-immune controls were essentially negative for both antibodies.

#### Gait Analysis after TGF- $\beta$ 1 With TM Shows Abnormal Swing Times in TS5 $^{-/-}$ Mice

TGF- $\beta$ 1 injection with TM exercise in WT mice resulted in no effects on gait parameters in the affected limb and a small reduction in pawprint area ( $p = 0.058$ , right vs. left, ANOVA) at 4 weeks. By comparison, for TS5 $^{-/-}$  mice at 4 weeks, in addition to a minor reduction in pawprint area, swing time of the injected limb increased ( $p = 0.07$ , Fig. 6). Neither genotype exhibited alterations in the swing time of the contralateral limb. Of note, the pawprint area, indicative of limb loading, was the same for both healed and nonhealed Achilles tendons, indicating that this parameter may not be a reliable outcome measure for healing efficacy in our model.

#### DISCUSSION

We have previously shown in WT mice<sup>7</sup> that tendinopathy generated by TGF- $\beta$ 1 injection can be healed by treadmill exercise, wherein tendon tensile properties were restored to those of uninjured mice, demonstrating a therapeutic role of biomechanical stimulation. However, in the present study, when the same model was applied to TS5 $^{-/-}$  mice, mechanical loading was ineffective in healing the tendinopathy. Moreover, the persistently impaired mechanical properties of TS5 $^{-/-}$  tendons (Fig. 1) was accompanied by an abundance of chondrocytic shaped, aggrecan/collagen II-enriched



**Figure 2.** Effect of TGF- $\beta$ 1 injection and TM on matrix gene expression in Achilles from WT relative to TS5<sup>-/-</sup> mice. Data for each experimental group were obtained from a pool of 20 tendons. The  $\Delta Ct$  values (Table 2) obtained from TS5<sup>-/-</sup> tendons were reduced by the  $\Delta Ct$  values for the equivalent WT samples to generate a  $\Delta\Delta Ct$  value, and the fold difference was calculated as  $2^{-\Delta\Delta Ct}$ , where the WT value was set at unity. Values above and below unity indicate a higher and lower fold-increase in the TS5<sup>-/-</sup> mice respectively.

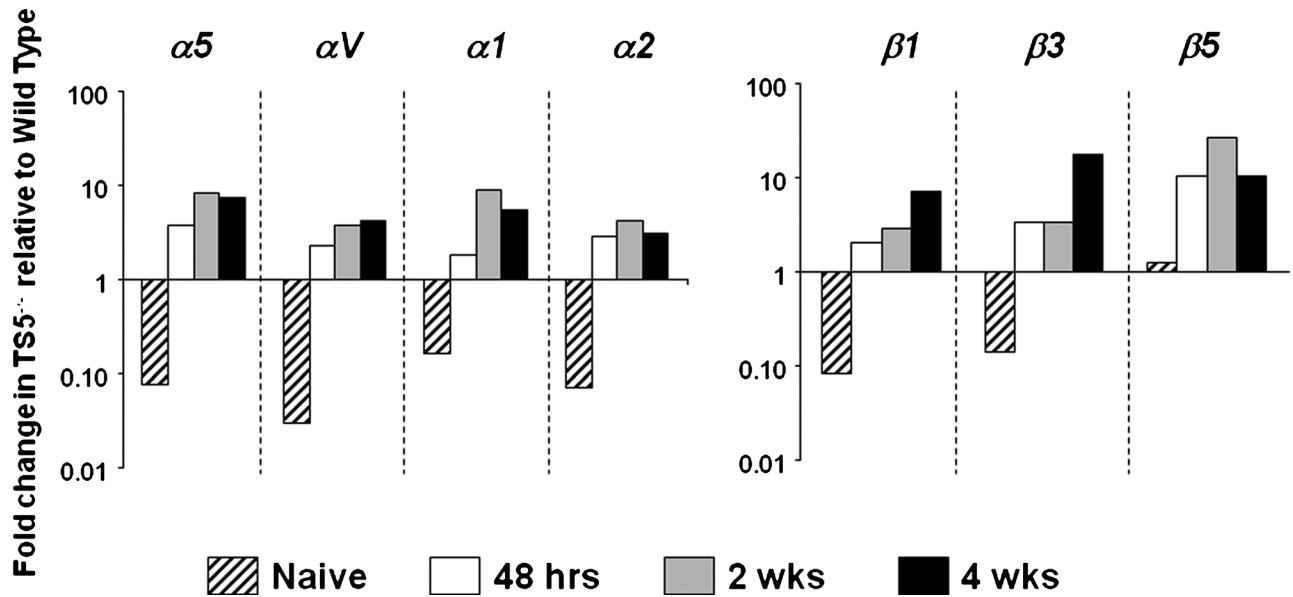
cells (Fig. 4) and markedly decreased fibrogenic gene expression relative to WT tendons (Fig. 2). These differences are consistent with an inappropriate chondrogenic response in TS5<sup>-/-</sup> mice during healing of fibrous connective tissues.<sup>5,7,16</sup> Either removal of TS5 by genetic ablation (current study) or replacement of TM exercise with cage-only activity<sup>7</sup> were found to prevent the repair process. Hence, collectively these results indicate that healing of tendinopathy in our murine model requires both mechanical loading and TS5. Moreover, the results strengthen the concept<sup>3,7</sup> that recovery of biomechanical properties in this model requires the removal of ARDs from the tendon body.

Since human tendinopathies are commonly accompanied by chondroid regions of tendon matrix,<sup>17-19</sup> it becomes important to determine whether such deposits are a by-product of, or a major pathogenic factor in, the human disease. As shown in the present study (Figs. 2 and 4), the persistence of an aggrecan-rich pericellular matrix can lead to the development of fibrocartilaginous regions and associated disruption of collagen fiber organization, within the body of the tendon. These changes may result in impaired tensile properties (Fig. 1) of such tissue regions. This is also consistent with the change in gait which might result from the loss of tendon tensile properties per se, or

**Table 2.** Effect of TGF- $\beta$ 1 Injection and TM Exercise on Integrin Gene Expression in Achilles Tendons of WT and TS5<sup>-/-</sup> Mice.

Group	Genotype	Beta1	Beta3	Beta5	Alpha5	AlphaV	Alpha1	Alpha2
Naïve	WT	1.69 (0.15)	11.17 (0.21)	11.47 (0.13)	7.44 (0.14)	5.28 (0.15)	8.42 (0.15)	9.48 (0.08)
	TS5 <sup>-/-</sup>	5.30 (0.06)	14.02 (0.23)	11.13 (0.18)	11.16 (0.33)	10.36 (0.20)	11.02 (0.13)	13.31 (0.45)
Acute	WT	3.25 (0.04)	10.90 (0.57)	12.73 (0.67)	6.19 (0.06)	6.62 (0.19)	8.75 (0.12)	12.33 (0.26)
	<i>p</i>	0.002	0.622	0.078	0.001	<0.001	0.041	0.001
	TS5 <sup>-/-</sup>	2.20 (0.30)	9.17 (0.21)	9.37 (0.30)	4.29 (0.43)	5.40 (0.15)	7.86 (0.05)	10.81 (0.42)
	<i>p</i>	0.002	<0.001	0.039	0.004	<0.00001	<0.001	0.002
2 weeks	WT	1.19 (0.28)	8.90 (0.17)	10.40 (0.56)	6.50 (0.13)	5.27 (0.26)	8.75 (0.23)	9.68 (0.27)
	<i>p</i>	0.070	<0.001	0.073	0.011	0.961	0.115	0.325
	TS5 <sup>-/-</sup>	-0.36 (0.27)	7.18 (0.29)	5.66 (0.50)	3.43 (0.37)	3.34 (0.02)	5.61 (0.17)	7.61 (0.29)
	<i>p</i>	<0.001	<0.00001	0.001	<0.00001	<0.001	<0.00001	<0.001
4 weeks	WT	2.58 (0.08)	12.06 (0.17)	9.52 (0.41)	8.76 (0.06)	6.34 (0.69)	8.87 (0.12)	10.43 (0.24)
	<i>p</i>	0.00294	0.131	0.07743	<0.001	0.111	0.016	0.013
	TS5 <sup>-/-</sup>	-0.25 (0.04)	7.90 (0.05)	6.17 (0.17)	5.85 (0.09)	4.29 (0.39)	6.41 (0.20)	8.82 (0.13)
	<i>p</i>	<0.00001	<0.001	<0.00001	<0.001	<0.001	<0.00001	0.002

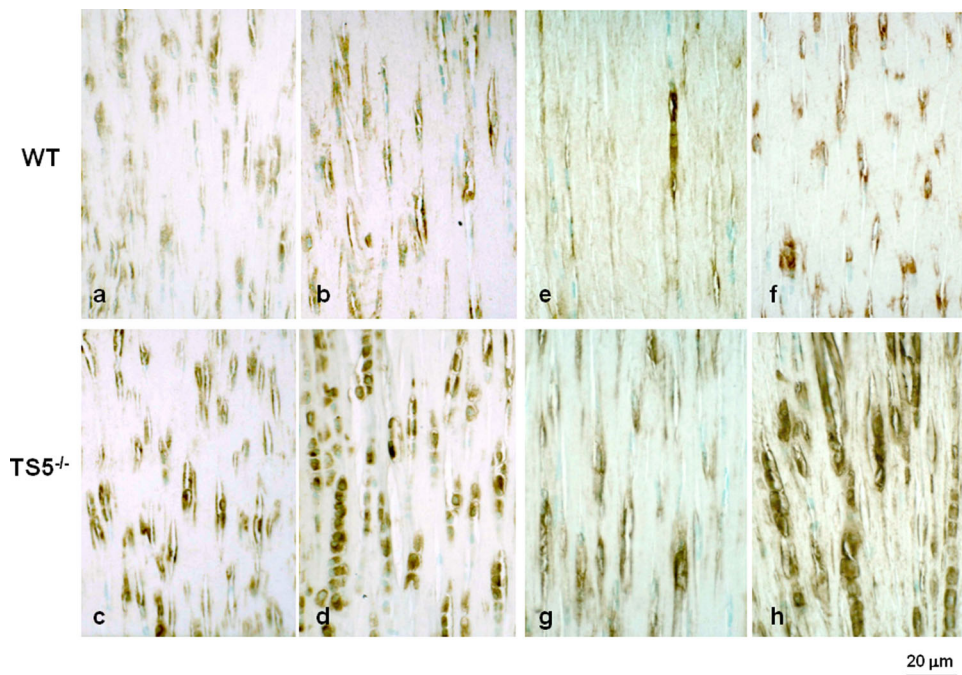
Data presented are mean  $\Delta Ct$  values, with standard deviation in parentheses. *p*-Values represent comparisons to  $\Delta Ct$  of naïve samples within each genotype.



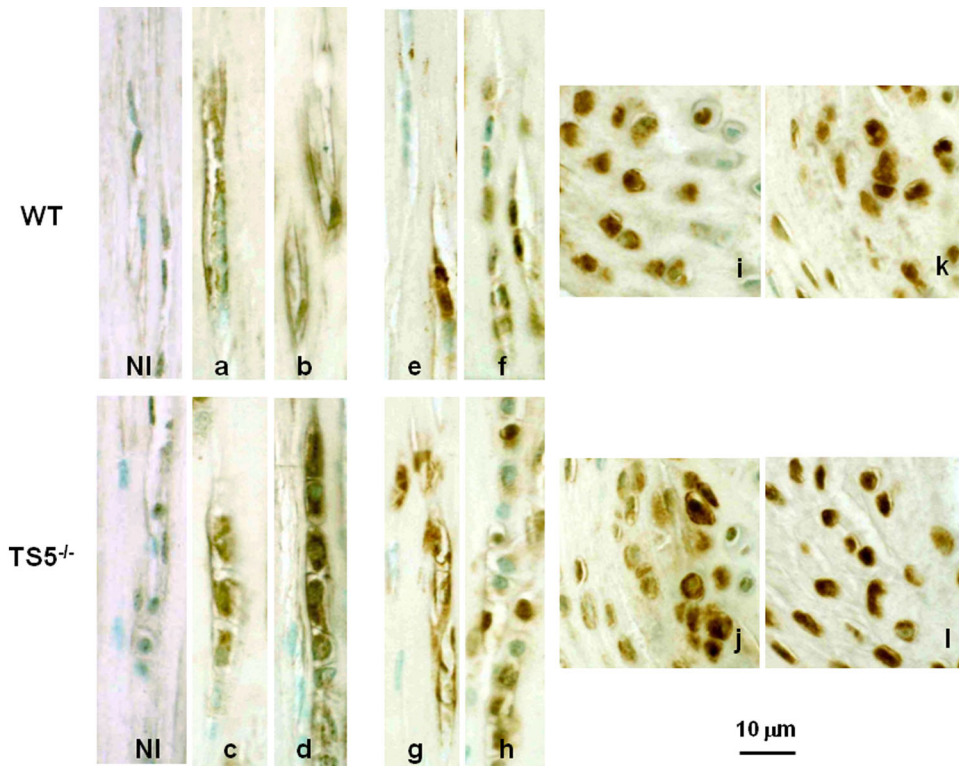
**Figure 3.** Effect of TGF- $\beta$ 1 injection and TM on fold-activation of integrin gene expression in Achilles from WT relative to TS5<sup>-/-</sup> mice. Data for each experimental group were obtained from a pool of 20 tendons. As described in the legend to Figure 2, values above and below unity indicate a higher and lower activation in the TS5<sup>-/-</sup> mice respectively.

from adaptive gait changes secondary to those of the tendon. In addition, in a series of human Achilles samples,<sup>20</sup> quantitative analysis of aggrecan IHC and histopathological scoring exhibited a strong positive correlation consistent with a causative relationship between aggrecan accumulation and tendinopathy. If,

as suggested here, aggrecan-rich and/or fibrocartilaginous deposits in the body of tendons are pathogenic, the mechanism by which they induce collagen disorganization and loss of tensile properties becomes a central question. In this regard, the matrix volume occupied by fully extended aggrecan suggests that it



**Figure 4.** Immunohistochemistry for aggrecan and collagen type II in midportion of Achilles tendons in WT and TS5<sup>-/-</sup> mice. Sections were immunostained with anti-aggrecan (a–d) and anti-collagen II (e–h) antibodies. Typical sections from naive (a, c, e, and g) and 4 week treated (b, d, f, and h) mice are shown. In naive tendons, TS5<sup>-/-</sup> mice contained a greater number of cells staining for both aggrecan and collagen type II in the pericellular matrix relative to WT tendons. Following TGF- $\beta$ 1 injection and 4-week TM exercise, enhanced pericellular staining was observed for both genotypes. However, TS5<sup>-/-</sup> tendons contained a larger number of rounded chondrocytic cells with robust cell associated aggrecan and collagen type II staining.

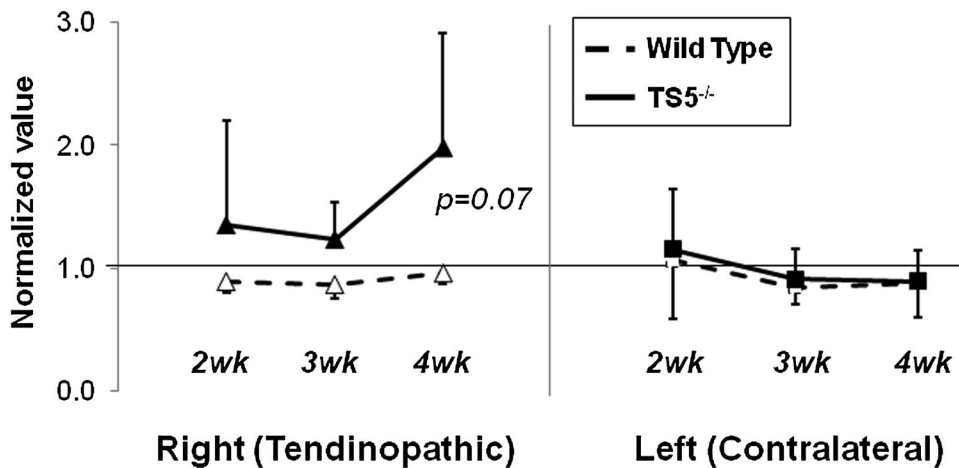


**Figure 5.** Immunohistochemistry for integrins  $\alpha V$  and  $\beta 3$  in midportion (a–h) and bone insertion sites (i–l) of Achilles tendons of WT and  $TS5^{-/-}$  mice. Sections were immunostained with anti-integrin  $\alpha V$  (a–d, i, j) and anti-integrin  $\beta 3$  (e–h, k, l) antibodies. A typical non-immune (NI) control section from each genotype is also provided. Typical sections from naive (a, c, e, and g) and 4 week treated (b, d, f, and h) mice are shown. Note that the cell-associated staining patterns for both integrins in groups of rounded chondrocytic cells at 4 weeks in  $TS5^{-/-}$  tendons (d and h) closely resemble those seen in cells at the tendon-bone insertion sites of both genotypes.

would most likely disrupt the organization of the extra-fibrillar space, consistent with its deposition and retention by cells which are known to populate the surface of the fibers.

The mechanism by which the marked differences between WT and  $TS5^{-/-}$  tendons in integrin expression regulate their respective capacities for repair is presently unknown. Indeed, since the activity of

integrins in tissues including tendon is determined by the combined effects of expression, membrane insertion, activation, endocytosis, and recycling,<sup>21</sup> additional work is needed to examine the relationship between mRNA levels and downstream control of cell matrix interactions. In this context, data shown here, where genotypic differences in expression of integrins  $\alpha 1$  and  $\beta 5$  (Fig. 3) were not accompanied by similar changes



**Figure 6.** Effect of TGF- $\beta 1$  injection and TM exercise on swing time of WT ( $n = 4$ ) and  $TS5^{-/-}$  ( $n = 5$ ) mice. Gait parameters of each mouse were normalized to their baseline (pre-injection) values and the mean (with standard deviation plotted as error bars) of the normalized values are presented.

in protein staining (Fig. 5), are therefore most likely related to the multi-step, post-translational control of those integrins. Nonetheless, the low expression levels in naïve TS5<sup>-/-</sup> tendons might be accompanied by a loss in integrin-mediated attachment to the fibrous matrix, consistent with the inferior biomechanical properties of the TS5<sup>-/-</sup> tissue.<sup>3</sup> Similarly, the higher induction of integrin expression by TGF-β1 in the TS5<sup>-/-</sup> tendons (to levels similar to WT) would be consistent with a failed repair response, since the tensile properties of the TS5<sup>-/-</sup> tendons do not recover to those of uninjured tendons, even after treadmill exercise.

Since the formation of a cartilage-like ECM in the tendon body appears to be pathogenic, it becomes important to establish the cellular source of this abnormal matrix. Such cells could be derived from populations of mature tendon fibroblasts, tendon fibroblasts transdifferentiating into fibrochondrocytes, or tendon stem cells differentiating into fibrochondrocytes. Given the current understanding of stem cell niches in wound repair<sup>22</sup> and specific tendon repair mechanisms,<sup>23</sup> it seems likely that maintenance of fibrillar tendon structure over long durations of biomechanical challenge in vivo requires continuous recruitment of adult stromal progenitor cells and their differentiation into fibrogenic tenocytes. In contrast, the appearance of fibrocartilaginous deposits presumably results from an increased differentiation of progenitors into fibrochondrocytes, rather than into mature tendon fibroblasts.<sup>24</sup> When taken together with our previous studies on dermal and cartilage repair<sup>4,5</sup> the new data presented here indicates that differentiation of progenitors into fibrochondrocytes can result directly from a lack of TS5 in any of these tissue types. In summary, our studies suggest that stimulating the pathway which facilitates removal of aggrecan from tendon progenitor cells, and also eliminates ARDs from the tissue, should be effective in promoting the healing of those tendinopathies which involve excessive mucoid deposition. Since it appears that elimination of ARDs can be induced by controlled mechanical loading in mice and humans,<sup>25,26</sup> we propose that appropriate exercise, together with local delivery of chondrolytic biologics<sup>27,28</sup> may markedly improve therapeutic outcomes for patients with tendinopathies in which cartilage-like matrix deposits are present in the body of the tendon.

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### Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website.

**Table S1.** Comparison of matrix gene expression in naïve Achilles tendons of WT and TS5<sup>-/-</sup> mice.