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Differential regulation of gene expression in isolated tendon fascicles exposed to cyclic tensile strain in vitro

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Maeda E, Shelton JC, Bader DL, Lee DA. Differential regulation of gene expression in isolated tendon fascicles exposed to cyclic tensile strain in vitro. *J Appl Physiol* 106: 506–512, 2009. First published November 26, 2008; doi:10.1152/japplphysiol.90981.2008.—Mechanical stimulus is a regulator of tenocyte metabolism. The present study investigated temporal regulation of the expression of selected genes by tenocytes in isolated fascicles subjected to tensile strain in vitro. Cyclic tensile strain with a 3% amplitude superimposed on a 2% static strain was provided for 10 min, followed by either an unstrained period or continuous cyclic strain until the end of a 24-h incubation period. mRNA expression of selected anabolic and catabolic genes were evaluated with quantitative PCR at 10 min, 1 h, 6 h, and 24 h. The application of 6-h cyclic strain significantly upregulated type III collagen mRNA expression in strained fascicles compared with unstrained controls, but no alterations were observed in mRNA expression of type I collagen and *biglycan*. Significant downregulation in the expression of the decorin core protein was observed in fascicles subjected to 24-h cyclic strain. *MMP3* and *MMP13* expression levels were upregulated by the application of 10 min of cyclic strain, followed by a progressive downregulation until the end of the incubation period in both the absence and the presence of the continuing cyclic strain. Accordingly, alterations in the expression of anabolic genes were limited to the upregulation of type III collagen by prolonged exposure to cyclic strain, whereas catabolic genes were upregulated by a small number of strain cycles and downregulated by a prolonged cyclic strain. These findings demonstrate distinctive patterns of mechanoregulation for anabolic and catabolic genes and help our understanding of tenocyte response to mechanical stimulation.

cyclic strain; metabolism; mechanobiology

MECHANICAL LOADING is one of the regulatory factors influencing tendon metabolism. Tenocytes are responsive to mechanical stimuli through mechanotransduction processes (30) that regulate the expression of both anabolic and catabolic factors, which results in the maintenance of tendon structure and function or may lead to their alteration through remodeling. For example, the mechanical properties of tendon deteriorated when subjected to stress deprivation (33), a process associated with an upregulation of catabolic factors such as matrix metalloproteinases (MMPs) and interleukin-1β (10, 29) and an alteration of tissue ultrastructure (19).

Details of tenocyte response to mechanical stimuli have been studied in a two-dimensional (2D) model in which the cells are cultured on an elastic membrane and subjected to a variety of mechanical stimulation regimes (30). However, it has been demonstrated that tenocytes form a three-dimensional (3D) communicating network mediated by gap junctions throughout tendon (20) and are subjected not only to tensile strain but also to compressive and shear strains (25). Thus a 3D explant system has advantages over isolated tenocyte systems because it can ensure the maintenance of tenocyte morphology and intercellular network organization and provide a physiological local strain environment when subjected to mechanical stimulation.

The mechanoregulation of tenocyte catabolism has been examined with reference to the processes associated with tendon pathology. For example, the application of dynamic mechanical stimuli within a physiological range inhibits the expression of collagenase and other types of MMP (4, 15). By contrast, stimuli in excess of the physiological range induced an upregulation of tenocyte catabolism (5, 7, 9) or tenocyte apoptosis (24). However, relatively few studies have examined the mechanoregulation of anabolic processes. Tenocytes within tendon explants increased DNA synthesis and collagen production in response to cyclic tensile strain (6, 26) and sulfated glycosaminoglycan (GAG) content in response to static loading (1). The application of mechanical loading to tendon explants is also reported to be essential for the maintenance of their mechanical integrity (12, 32).

In our previous study (17), temporal modulation of collagen synthesis by tenocytes in tendon fascicles was demonstrated. In particular, the application of a small number of duty cycles of tensile strain over a 10-min period downregulated collagen synthesis, measured by proline incorporation, whereas incorporation was enhanced by the application of cyclic strain throughout a 24-h incubation period (17). In the present study, this effect was further investigated by testing the hypothesis that tensile strain influences the expression of selected anabolic and catabolic genes in a time-dependent manner. Specifically, it is predicted that prolonged cyclic strain is required for the upregulation of anabolic genes, with an associated downregulation of catabolic genes throughout the experimental period.

MATERIALS AND METHODS

Dissection of tendon fascicles. Fascicles, ~300 μm in diameter, were dissected from the tail tendons of male Wistar rats (5 mo old) under sterile conditions within 1 h of death. All relevant procedures were approved through the granting of appropriate licenses by the United Kingdom Home Office in accordance with the Animals (Scientific Procedures) Act 1986 and the Guidance Notes for this Act, specifically to cover maintenance and euthanasia of the animals according to Schedule 1 of the Act. The fascicles were kept moistened with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 150 mg/l ascorbic acid, 0.02 M L-glutamine, 1%

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nonessential amino acids, 0.01 M HEPES, 50 U/ml penicillin, and 0.05 mg/ml streptomycin (all from Sigma-Aldrich, Poole, UK) (DMEM + 10% FCS). The fascicles were incubated in DMEM + 10% FCS for 4 h to equilibrate the water content in the fascicles before the application of mechanical stimulation.

Free swelling studies. At the time of dissection, three fascicles, each trimmed to a length of 20 mm, were collected, briefly washed in ice-cooled RNase- and DNase-free PBS (Cambrex), immediately frozen in liquid nitrogen, and stored at −80°C. These samples served as postdissection controls (PDC). A further 15 fascicles were also collected, trimmed to 20 mm, placed in individual wells of six-well culture plates with 2 ml of DMEM + 10% FCS, and incubated at 37°C and 5% CO_2. Three of these free-swelling fascicles were collected at five time points: at the end of the preincubation period (0 h) and at 10 min, 1 h, 6 h, and 24 h, as indicated in Fig. 1. These time points are equivalent to those used in the tissue loading studies. The experiment was repeated three times on different days with fascicles dissected from different animals.

Tissue loading studies. Six fascicles were placed in a custom-made tissue loading system, which has been detailed in previous work (17, 26). Three fascicles were subjected to cyclic strain, whereas the other three served as unstrained controls. Cyclic strain, with a 3% amplitude superimposed on a 2% static strain, was applied to experimental fascicles for 10 min, followed by either an unstrained period (10-min cyclic strain group) or a continued strain period until the end of the 24-h incubation period (24-h cyclic strain group), as illustrated schematically in Fig. 1. The two strain regimes were selected on the basis of findings of a previous study by our group (17): the application of 10 min of cyclic strain with the same amplitude and frequency as used in the present study resulted in a downregulation of collagen synthesis during a 24-h incubation period, whereas the application of 24 h of cyclic strain upregulated synthesis. Accordingly, the former regime was considered to represent anti-anabolic stimulation and the latter pro-anabolic stimulation.

Both strained and unstrained control fascicles were collected at 10 min, 1 h, 6 h, and 24 h, quickly washed in RNase- and DNase-free phosphate buffer solution (Cambrex), immediately frozen in liquid nitrogen, and stored at −80°C. The unstrained samples differed from the free-swelling samples because the unstrained samples were manually transferred from culture dishes to tissue loading chambers at the end of the preincubation period in a manner identical to that used for the strained samples. By contrast, the free-swelling samples were not manipulated throughout the prescribed incubation period.

Quantitative PCR. Total RNA was extracted from the fascicles with Tri-reagent (Sigma-Aldrich). Total RNA was purified with RNeasy minicolumns and reagents (RNeasy Mini Kit, Qiagen, Crawley, UK) in accordance with the protocol provided by the manufacturer, in conjunction with a RNase-free DNase I digestion kit (Qiagen) to remove genomic DNA from the preparation. cDNA was synthesized from 100 ng of the total RNA with a commercially available kit (Protoscript first-strand cDNA synthesis kit, New England Biolabs, Hitchin, UK). The coapplication reverse transcription (Co-RT) method (35) was adopted to transcribe both mRNA and 18S rRNA (18S) after modifications; the latter was transcribed with a PCR reverse primer (Table 1).

Ten genes of interest were selected for quantitative PCR (qPCR) assays: three candidate reference genes (*18S*, *GAPDH*, and *β-actin*) and seven target genes including four anabolic genes (collagen types I and III, *decorin*, and *biglycan*), two catabolic genes (*MMP3* and *MMP13*), and the gene associated with transforming growth factor-β1 (*TGFβ1*). Primers and probes were designed and purchased from a commercial source (Sigma-Genosys, Poole, UK) (Table 1), except for the sequences for *β-actin*, which were taken from a previous study (11). Each cDNA was assessed in duplicate for each of the 10 genes with iTaq supermix with ROX (Bio-Rad Laboratories, Hemel Hempstead, UK). qPCR was performed with a thermal cycler (Mx3000P, Stratagene) according to the prescribed protocol, namely, 95°C for 3 min, followed by 40 cycles of 95°C for 30 s and 59°C for 60 s.

Data analysis and statistical analysis. For data analysis, a relative quantification method was used. First, the three candidate reference genes were evaluated in terms of changes in the threshold cycle (C_t), determined by qPCR, at which the fluorescence intensity, corresponding to the number of the copy of amplified sequence, exceeds the background level. The gene that demonstrated the smallest fluctuation was selected as the reference gene and used as a normalizer in the calculation of relative expression ratios. The relative expression ratio of strained samples to unstrained controls was determined by the method proposed by Pfaffl (23). The ratio R was calculated by the equation $R = E_{target}^{C_{t(control)}-C_{t(sample)}}/E_{reference}^{C_{t(control)}-C_{t(sample)}}$, where E_{target} and $E_{reference}$ are qPCR reaction efficiency determined by a standard curve with five serial dilutions of the cDNA of target and reference genes, respectively, and $C_{t(control)}$ and $C_{t(sample)}$ are C_t values of the unstrained controls and strained samples, respectively. The calculated ratio was logged to base 2 and used for statistical analysis. The relative expression level of a target gene to the reference gene in a sample was also determined by calculating $E_{reference}^{C_{t(reference)}}/E_{target}^{C_{t(target)}}$ and termed the sample expression level in the present study. For data presentation of sample expression levels in Figs. 2–5, the levels of the unstrained controls from the two strain regimes were combined and presented as a single group.

One-way analysis of variance (ANOVA) was performed to test significance in temporal changes in the relative expression ratios, followed by Ryan's multiple comparison methods if statistical significance was found. For comparison of the ratio between strained and unstrained data, one-sample *t*-tests were employed. Student's *t*-tests were also performed to analyze differences in the ratio between the two strain regimes. For all comparisons, statistical significance was prescribed at a 5% level ($P < 0.05$).

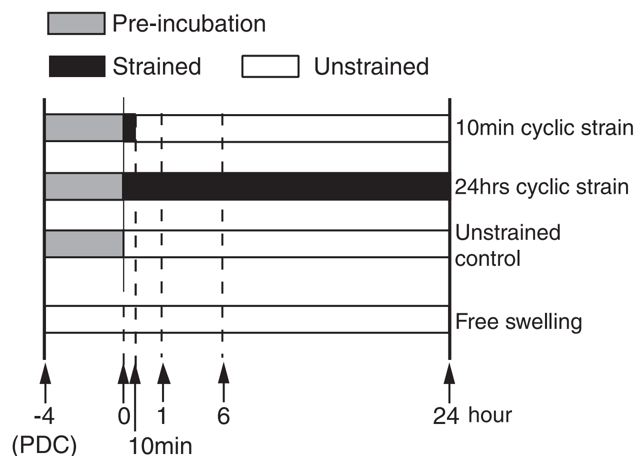

Fig. 1. Schematic representing the 3 test groups. Arrows indicate time points of the sample collection for subsequent gene expression analysis. PDC, postdissection controls.

RESULTS

18S was selected as the reference gene because it demonstrated the least fluctuation in C_t among the three candidates (Table 2). The expression levels of type I collagen mRNA at 24 h for both 10-min cyclic strained samples and unstrained controls were not greatly changed compared with the level of free-swelling samples at −4 h (PDC) (Fig. 2*A*). However, a reduction in the expression level was observed for samples at the end of the 24-h cyclic straining regime. Type III collagen mRNA expression for free-swelling samples demonstrated an approximately sevenfold change at the end of the 24-h incu-

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Table 1. *Forward and reverse primers and probes used for qPCR analysis*

Gene/GenBank Accession No.	Primer/Probe Sequences	nM	bp
Collagen I (α1)	F: AGACGTGGAAACCTGATGTATGC	200	97
NM_053304	R: TGGGACAGTCCAAGTCTTCTTTG	300	
	P: ATAGCACGCCATCGCTCTCTGCCG	100	
Collagen III (α1)	F: TCCTCTGTGATGACATAATGTGTG	200	100
NM_032085	R: GTAGAAGGCTGTGGACATATTGC	300	
	P: TGAGCCACTAGACTGCCCCAACCC	200	
Decorin	F: GACCCTGACAATCCCCTGATATC	300	133
NM_024129	R: GCAGGTCTAGCAATGTTGTGTC	300	
	P: TGTGCCCCTACCGATGCCAATGCC	200	
Biglycan	F: ATGAACTTCACTTGGACCACAAC	300	134
NM_017087	R: CAGAAAACTCAGGCTCCCATTC	200	
	P: CGGATCTGATTGTGTCCTAAGCCCAGCC	100	
TGFβ1	F: CTACCAGAAATATAGCAACAATTCCTG	200	150
NM_021578	R: CTGAAGCGAAAGCCCTGTATTC	300	
	P: TCTCCTTGGTTCAGCCACTGCCGGA	100	
MMP3	F: CTCTCTCAAGATGATGTAGATGGTATTC	200	124
NM_133523	R: AGCTACACATTGGTAAGGTCTCAG	200	
	P: ATCCCTCTATGGACCTCCCACAGAATCCC	100	
MMP13	F: ACCCTAAGCACCCCAAAACAC	200	115
XM_001072242	R: TGCAGACGCCAGAAGAATCTG	200	
	P: TGACCCAGCCCTATCCCTTGATGCCA	100	
GAPDH	F: TGTTCTAGAGACAGCCGCATC	300	75
BC059110	R: TCACACCGACCTTCACCATC	300	
	P: TTGTGCAGTGCCAGCCTCGTCTCA	200	
β-Actin	F: AGCCATGTACGTAGCCATCCA	200	81
NM_031144	R: TCTCCGGAGTCCATCACAATG	200	
	P: TGTCCCTGTATGCCTCTGGTCGTACCAC	100	
18S rRNA	F: CATTAAATCAGTTATGGTTCCTTTG	300	142
M11188	R: GTTGGTTTTGATCTGATAAATGC	300	
	P: CGCTCGCTCCTCTCCT	200	

qPCR, quantitative PCR; F, forward primer; R, reverse primer; P, probe.

bation period compared with the PDC level (Fig. 2*B*). Although the expression level of samples in both strain regimes and unstrained controls demonstrated variable trends during the 24-h incubation period, the 10-min cyclic strain and unstrained control groups exhibited similar expression profiles.

When expression levels were compared between strained fascicles and unstrained controls, the expression of type I collagen in strained fascicles was not significantly altered for either strain regime at any time point ($P > 0.05$, Fig. 2*C*). By contrast, a significant upregulation in type III collagen expression, by approximately threefold, was observed at 6 h compared with unstrained control samples ($P < 0.05$). An overall downregulation of both collagen type I and type III expression at the end of the incubation period was also observed with both strain regimes, although no significant difference from the unstrained control level was observed ($P > 0.05$ for all comparisons).

Table 2. *C_t number profiles of three candidate reference genes*

	18S	*β-Actin*	*GAPDH*
Max	9.22	24.88	28.37
Min	6.94	18.81	20.21
Mean	8.25	21.02	23.99
$D_{Max-Min}$	2.28	6.07	8.16

C_t, threshold cycle; Max, maximum; Min, minimum; $D_{Max-Min}$, Max − Min difference.

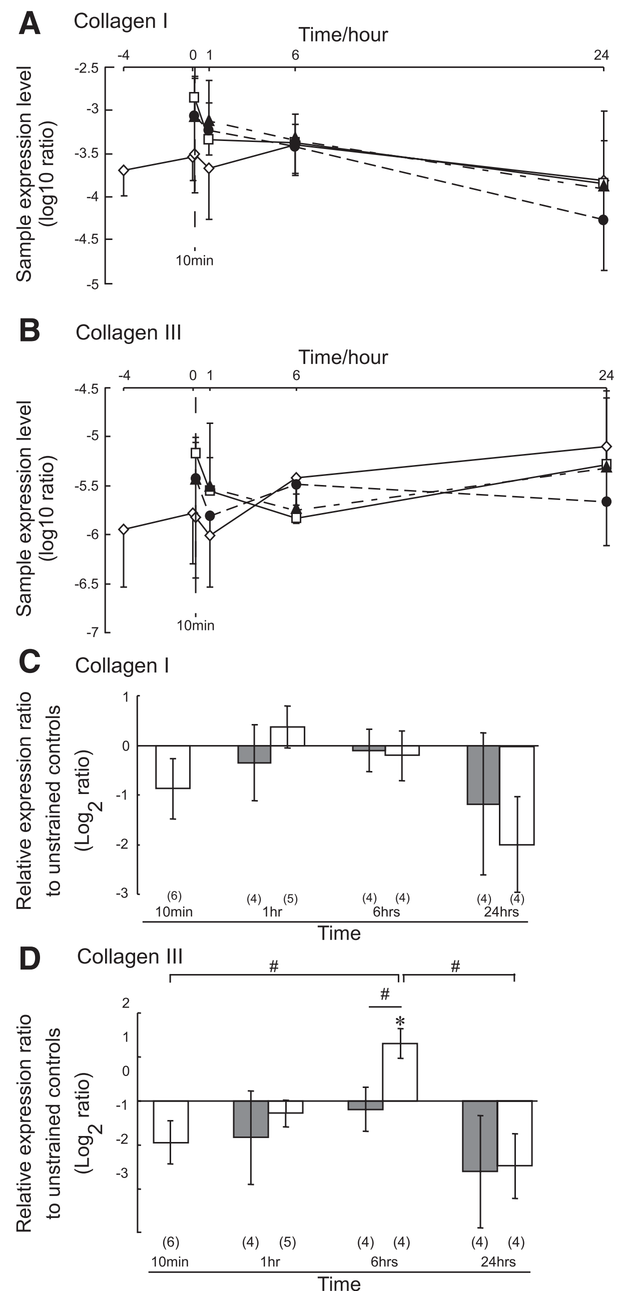

Fig. 2. Sample expression profiles of all experimental groups and relative expression ratio of strained fascicles to unstrained controls for collagen types I (*A* and *C*) and III (*B* and *D*). Symbols in *A* and *C* represent data of each group as follows: ◇, free swelling; □, unstrained control; ▲, 10-min cyclic strain regime; ●, 24-h cyclic strain regime. In *C* and *D*, gray and open bars indicate 10-min and 24-h cyclic strain regimes, respectively. Data are presented as means ± SD in sample expression profiles and means ± SE in relative expression ratio. Results of statistical analysis are indicated as follows: *$P <$ 0.05 to unstrained controls, #$P < 0.05$ across time within a group and between 2 strain regimes. Numbers of experiments are indicated in parentheses.

Significant changes in *MMP3* and *MMP13* expression levels were evident during the incubation period (Fig. 3, *A* and *B*, respectively). During the preincubation period, the free-swelling samples exhibited a >10-fold increase in expression for both genes. However, whereas the level of *MMP13* was further elevated at the end of the incubation period, the corresponding level of *MMP3* was equivalent to that at 0 h. Both *MMP3* and

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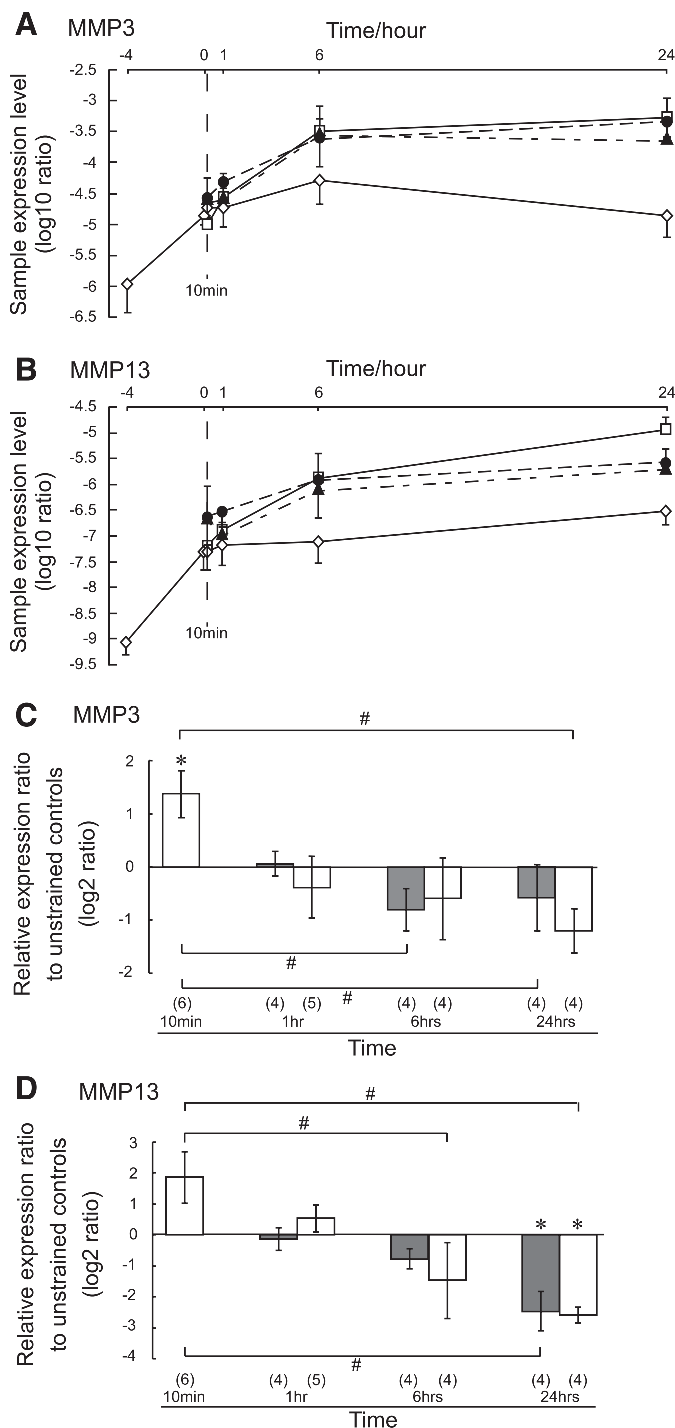

Fig. 3. Sample expression profiles of all experimental groups and relative expression ratio of strained fascicles to unstrained controls for *MMP3* (*A* and *C*) and *MMP13* (*B* and *D*). Symbols in *A* and *C* represent data of each group as follows: ◇, free swelling; □, unstrained control; ▲, 10-min cyclic strain regime; ●, 24-h cyclic strain regime. In *C* and *D*, gray and open bars indicate 10-min and 24-h cyclic strain regime, respectively. Data are presented as means ± SD in sample expression profiles and means ± SE in relative expression ratio. Results of statistical analysis are indicated as follows: *$P < 0.05$ to unstrained controls, #$P < 0.05$ across time within a group. Numbers of experiments are indicated in parentheses.

MMP13 mRNA exhibited a tendency to increase their expression levels in both strained and unstrained samples throughout the 24-h incubation period. However, the increase was less marked in strained samples at 24 h for *MMP13* (Fig. 3*B*).

The relative expression ratios of *MMP3* and *MMP13* in strained fascicles were significantly altered by both strain regimes, with a common trend of downregulation during the 24-h incubation period ($P < 0.05$ by ANOVA for 10-min and 24-h cyclic strain groups for *MMP3*, respectively, and $P < 0.01$ and $P < 0.05$ for *MMP13*, respectively; Fig. 3, *C* and *D*). Both catabolic genes were upregulated to approximately threefold by the application of 10-min cyclic strain compared with unstrained control level, although the difference was only found to be significant for *MMP3* ($P < 0.05$). At 24 h, *MMP3* expression for both regimes was downregulated by approximately twofold, although the differences were not significant ($P > 0.05$). *MMP13* expression for both regimes was significantly downregulated at 24 h, by more than fivefold, compared with unstrained control level ($P < 0.05$ and $P < 0.01$ for 10-min and 24-h strain regimes, respectively).

Only small changes were observed for *decorin*, *biglycan*, and *TGFβ1* in samples subjected to tissue loading experiments (Figs. 4 and 5). The levels for *decorin* from the free-swelling samples exhibited an approximately threefold increase from the PDC level at 6 h (Fig. 4*A*). The unstrained control level was not altered until 6 h, which was followed by an upregulation at 24 h. For *biglycan*, both groups of strained samples as well as unstrained samples demonstrated only small changes (Fig. 4*B*). By contrast, *biglycan* expression in free-swelling samples exhibited an approximately fivefold continuous increase by the end of the incubation period. Analysis of relative expression ratio revealed a small but significant reduction in the expression of *decorin* in the 24-h cyclic strain group compared with the unstrained controls at 24 h ($P < 0.05$, Fig. 4*C*). Little change was observed in the expression of *biglycan* in both strained groups ($P > 0.05$ for all comparisons, Fig. 4*D*).

The expression levels for *TGFβ1* from the free-swelling samples demonstrated an approximately sixfold increase from the PDC level at 6 h (Fig. 5*A*). *TGFβ1* expression was significantly upregulated at 6 h in the 10-min cyclic strain group, compared with the level of unstrained controls ($P < 0.05$, Fig. 5*B*). By contrast, no significant alterations were observed in the 24-h cyclic strain group ($P > 0.05$ for all comparisons).

DISCUSSION

mRNA expression of selected genes by tenocytes within tendon fascicles subjected to cyclic tensile strain was examined in the present study to test the hypothesis that tensile strain influences the expression of these anabolic and catabolic genes in a time-dependent manner. The findings revealed regulation of gene expression by mechanical stimulation, although it was evident that each gene demonstrated mechanoresponsiveness in a distinctive manner.

The small number of RNA samples examined in each group may provide a limitation to the present study, although each sample was prepared from three fascicles in each condition. However, power analysis demonstrated that observed statistical significances were detected with sufficient power, ranging between 0.69 and 0.99, except for two cases. Accordingly,

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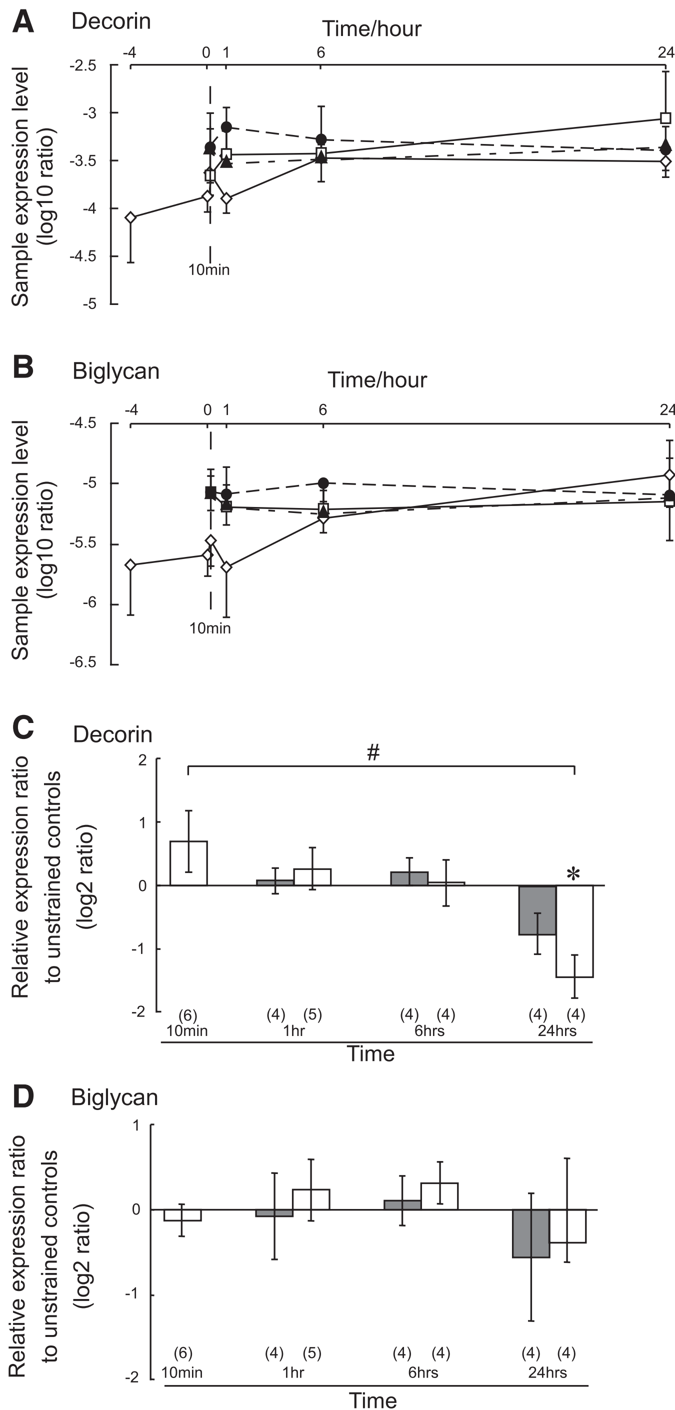

Fig. 4. Sample expression profiles of all experimental groups and relative expression ratio of strained fascicles to unstrained controls for *decorin* (*A* and *C*) and *biglycan* (*B* and *D*). Symbols in *A* and *C* represent data of each group as follows: ◇, free swelling; □, unstrained control; ▲, 10-min cyclic strain regime; ●, 24-h cyclic strain regime. In *C* and *D*, gray and open bars indicate 10-min and 24-h cyclic strain regimes, respectively. Data are presented as means ± SD in sample expression profiles and means ± SE in relative expression ratio. Results of statistical analysis are indicated as follows: $*P < 0.05$ to unstrained controls, $\#P < 0.05$ across time within a group. Numbers of experiments are indicated in parentheses.

these differences are deemed to be sufficiently robust in statistical terms. It may also be argued that the 4-h preincubation period adopted in the present study is insufficient to equilibrate fascicles to the culture conditions before the application of cyclic strain. Indeed, the substantial increase in MMP expression during the preincubation period may suggest that a longer equilibration period is required for the samples to achieve a steady state. However, equilibration to a new state associated with marked variation in expression levels compared with the PDC specimens may be of limited relevance to the physiological state.

The expression profiles of unstrained control samples and free-swelling samples varied depending on the gene. The profiles were broadly similar for collagen types I and III, *decorin*, *biglycan*, and *TGFβ1* (Figs. 2 and 4). By contrast, *MMP3* and *MMP13* demonstrated a similar level of expression at 10 min in free-swelling and unstrained samples, but showed markedly higher expression levels in the unstrained controls compared with the free-swelling samples during the remainder of the incubation period (Fig. 3). These differences might be attributed to the experimental protocols used in the loading experiment. Fascicles were handled with forceps, transferred from a culture dish to culture chambers, and attached to grips. These procedures may therefore induce alterations in the expression of the specific catabolic genes.

Minimal changes in both type I and III collagen mRNA relative expression ratio were induced by the application of the 10-min cyclic strain over the 24-h incubation period. This regime was demonstrated previously to inhibit proline incorporation into newly synthesized collagen over the 24-h period (17). By contrast, an upregulation of type III collagen expression at 6 h was observed during the 24-h strain regime in the

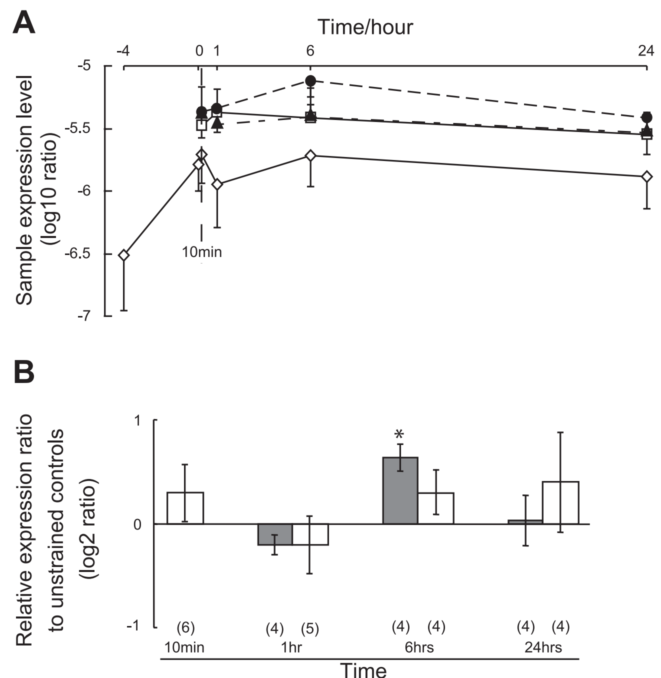

Fig. 5. Sample expression profiles of all experimental groups (*A*) and relative expression ratio of strained fascicles to unstrained controls (*B*) for *TGFβ1*. Symbols in *A* represent data of each group as follows: ◇, free swelling; □, unstrained control; ▲, 10-min cyclic strain regime; ●, 24-h cyclic strain regime. In *B*, grey and open bars indicate 10-min and 24-h cyclic strain regimes, respectively. Data are presented as means ± SD in sample expression profiles and means ± SE in relative expression ratio. Result of statistical analysis is indicated as follows: $*P < 0.05$ to unstrained controls. Numbers of experiments are indicated in parentheses.

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present study (Fig. 2*D*). These findings may be correlated to the upregulation of collagen synthesis by 24 h of cyclic strain reported in the previous study (17). These data indicate that an extended stimulation, associated with a large number of total duty cycles, is necessary to induce an upregulation of collagen gene expression (21,600 cycles) and protein synthesis (86,400 cycles). No data were collected between 6 h and 24 h for gene expression in the present study, or for collagen synthesis in the previous study. Thus an optimal number of strain cycles for an upregulation of each level of collagen production may exist between these time points and may depend on the original mechanical environment of the tenocyte. Indeed, the upregulation of type I collagen gene expression and protein synthesis has been reported in human patellar tendon fibroblasts after 4 h of cyclic stretching (7,200 cycles, 4 h of stimulation at 0.5 Hz + 4 h of rest) (34). By contrast, cells from human ligamentum flavum were reported to require 48 h of cyclic stimulation (10-s stimulation + 10-s recovery) to induce an upregulation of type III collagen mRNA expression (21).

The data obtained also suggest a selective mechanoregulation of collagen gene expression. The upregulation of type III collagen has been observed in cells subjected to cyclic stimulation both in vivo and in vitro. For example, chronic repetitive loading in vivo of rabbit Achilles tendons for 11 wk induced a significant upregulation of type III collagen gene expression, with no associated change in type I collagen (2). In addition, rat plantaris tendons loaded in vivo exhibited a significant upregulation of type III collagen mRNA expression after only 2 days of loading, whereas type I collagen was significantly upregulated only after 8 days of loading (22). The mechanical loading regimes adopted in these two previous studies did not induce either inflammation or degeneration. These findings may indicate that the initial response by tenocytes in vivo to repetitive loading within a physiological range mainly involves the enhancement of type III collagen production, suggesting a reparative-type response. Indeed, type III collagen has been shown to be produced in granulation tissues in healing tendons (8, 18, 31). However, these healing tissues were found to exhibit inferior mechanical properties compared with normal healthy tendons (28), and thus the production of type III collagen in tendon may lead to impaired mechanical integrity. This could, in turn, lead to microtears within the structure, which may ultimately lead to tendon rupture or tendinopathy.

MMP gene expressions from free-swelling fascicles were broadly similar to recent findings (16) that reported a gradual increase in *MMP3* mRNA expression during the 12 h after dissection to ~100-fold of the level of the fresh sample. In addition, a 100-fold elevation of *MMP13* at 8 h was reduced to less than a 10-fold increase at 24 h (16). In the present study, *MMP3* was markedly upregulated at 4 h after dissection (0 h), and this level remained stable throughout the subsequent 24-h culture (Fig. 3*A*). However, the level of *MMP13* continued to rise throughout the culture period, reaching an ~500-fold increase at the 24 h time point compared with the level of the PDC (−4 h) (Fig. 3*B*), in contrast to the study by Leigh et al. (16). It was also previously reported that *MMP13* mRNA expression by tenocytes in tendon fascicles is downregulated by the application of static or dynamic mechanical strain in a dose-dependent manner (3, 15). *MMP3* mRNA expression has also been reported to be inhibited by the application of cyclic loading (4). In the present study, an inhibitory regulation of both types of MMP mRNA expression levels was clearly observed at the end of the incubation period in both groups of strained samples, involving a relatively small number of duty cycles (Fig. 3). It is interesting to note that the disruption of the tensional homeostasis of the cytoskeleton in tenocytes, by releasing cell-seeded gels from the constraint imposed by the base of a culture dish, was associated with an upregulation of *MMP13* expression (14). Thus this mechanism may account for the present observations; stress deprivation induced by free swelling might induce the disruption of the cytoskeleton in tenocytes, whereas mechanical stimulus may maintain tensional homeostasis in such structures, even if provided during only a short period.

No alterations were observed in *decorin* and *biglycan* expression profiles by the application of cyclic strain, except for a significant downregulation of decorin at 24 h in strained fascicles from the 24-h cyclic strain regime (Fig. 4*C*). These findings are broadly consistent with a previous study from our group (26) reporting that continuous cyclic strain for 24 h did not induce alteration in GAG synthesis by tenocytes in isolated tendon fascicles. However, static loading has been reported to increase the GAG content of rat tail tendon fascicles (1). Accordingly, static and dynamic strain regimes may induce different effects on proteoglycan synthesis. An upregulation of *TGFβ1* mRNA expression was observed only in the 10-min cyclic strain group at 6 h (Fig. 5*B*). Mechanical strain-induced upregulation of *TGFβ1* by tendon/ligament cells has been well documented in the literature (21, 27, 34). Coregulation of *TGFβ1* with collagen genes has also been reported previously for tendon cells (34), ligament cells (21), and dermal fibroblasts (13). However, these studies utilized 2D model systems, which generate different local strain fields around tenocytes compared with 3D systems.

In our previous study using the same mechanical strain regime (17), we suggested a magnitude-related adaptation mechanism of tenocyte metabolism, in addition to temporal modulation associated with the total number of strain cycles. The present findings support the mechanism. During the initial cycles of the 24-h continuous cyclic strain regime, strained fascicles were subjected to a relatively high level of mechanical loading, which was reduced because of stress relaxation to ~30% of the initial peak amplitude at 10 min and subsequently to ~10% with the application of extended strain cycles (17). The upregulation of *MMP3* and *MMP13* without alteration in the expression of anabolic genes at 10 min may indicate that the initial strain cycles are catabolic in nature. The 5% strain level (2% static strain + 3% amplitude) may correspond to the upper range of physiological strain and thus may produce a relatively high level of associated load. By contrast, the lower magnitude of load, associated with an extended number of duty cycles up to 24 h after stress relaxation, may reflect a more physiological and anabolic stimulus, linked to a significant upregulation of type III collagen expression at 6 h and a significant downregulation of *MMP13* expression at 24 h. Therefore, the adaptation of tendon tissue may involve the alteration of the balance of anabolism/catabolism of tenocytes, which are dependent on both the magnitude and the strain cycles (duration). The details of the change in anabolism/catabolism balance will be studied in future work using a gene microarray.

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In summary, type III collagen was significantly upregulated from unstrained control levels by the application of cyclic strain at 6 h. The catabolic genes *MMP3* and *MMP13* exhibited similar temporal profiles, characterized by an upregulation induced by 10-min cyclic strain with a downregulation at later time points in the presence or absence of further strain cycles. These findings suggest the presence of different mechanoregulatory pathways for tenocyte anabolism and catabolism. Accordingly, this study enhances understanding of the role of cyclic strain in regulating matrix turnover in tendon and may be applied, ultimately, to the development of repair strategies that are based on the application of defined loading regimes in vivo or ex vivo as part of regenerative therapies.

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