Asynchronous Muscle and Tendon Adaptation After Surgical Tensioning Procedures

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Background: Donor muscles are often highly stretched in tendon transfer surgery. Despite literature reports that showed adaptation of the serial sarcomere number to moderate stretch, little is known regarding adaptation to stretch outside of the physiological range (commonly seen in clinical tendon transfer). This study was performed to evaluate muscle-tendon-unit adaptation to tendon transfer surgery in an animal model.

Methods: Thirty-seven male New Zealand White rabbits were used for muscle analysis, and twenty-five of those rabbits were also used for biological analysis of the tendons after the experiment. The extensor digitorum muscle of the second toe was transferred at a specific sarcomere length of 3.7 μm, chosen to be near the end of the descending limb of the rabbit sarcomere length-tension curve. Animals were killed at five time points, at which complete muscle architectural analysis as well as measurements of tendon dimension, tendon water content, and tendon cytokine transcript levels were performed.

Results: As expected, a rapid increase in the serial sarcomere number (mean and standard error of the mean, 4658 ± 154 in the transferred muscle compared with 3609 ± 80 in the control muscle) was found one week after the surgery. From this time point until eight weeks, this increased serial sarcomere number paradoxically decreased, while the sarcomere length remained constant. Eventually, at eight weeks, it reached the same value (3749 ± 83) as that in the control muscle (3767 ± 61). Tendon adaptation was delayed relative to muscle adaptation, but it was no less dramatic. Tendon length increased by 1.43 ± 0.74 mm over the eight-week time period, corresponding to a strain of 15.55% ± 4.08%.

Conclusions: To our knowledge, this is the first report of biphasic adaptation of the serial sarcomere number followed by tendon adaptation, and it indicates that muscle adapts more quickly than tendon does. Taken together, these results illustrate a complex and unique interaction between muscles and tendons that occurs during adaptation to stretching during tendon transfer.

Clinical Relevance: Understanding the time course of muscle-tendon-unit adaptation can provide surgeons with information to guide postoperative care following tendon transfers as well as guidelines for tensioning muscles during tendon transfer.

Skeletal muscles have been shown to adapt to chronic length change by altering the serial sarcomere number when subjected to joint immobilization[1-5] and in retinaculum transection models[6-8]. In one of the most widely cited models of change in the serial sarcomere number, the serial sarcomere number in the stretched soleus increased by approximately 20% in just four weeks and generated maximum force at the angle at which the immobilization occurred[2,4]. These results have been interpreted as illustrating the general principle that the serial sarcomere number in muscles adjusts to reset sarcomere length to its optimal length. Proof of this concept would be advantageous clinically because several orthopaedic surgical procedures such as tendon transfers and joint replacements create chronic changes in muscle length that may be functionally relevant. Since changes in muscle length result in concomitant changes in sarcomere length and sarcomere length directly regulates force generation, it is important to understand the nature of muscle ad-

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aptation to predict the functional outcomes of the surgical procedures.

One experimental study demonstrated that muscle adaptation may not be "typical" when a muscle-tendon unit is stretched to the extra-physiological point[9]. In this case, greater stretch was accompanied by less muscle adaptation for an unknown reason. It has been demonstrated that, in clinical tendon-transfer surgery, muscles are overstretched, a condition in which muscles generate much less force[10], which may correlate with the observation that donor muscles lose at least one strength grade after tendon transfer[11,12]. One feasible reason for this adverse outcome is that overstretched muscles may not adapt correctly. Currently, the basic mechanical and biological factors that affect sarcomerogenesis have not been well described and it is very difficult to create general rules regarding muscle adaptation after surgical procedures.

To address these issues, we developed a high-resolution rabbit model of serial sarcomere number adaptation. In this model, a distal tendon is surgically relocated to another place at a predetermined stretched sarcomere length measured intraoperatively with use of laser diffraction[13]. We believe that this study is the first of its kind to provide insight into the time course of changes in muscle sarcomere length, since sarcomere length was measured intraoperatively. We also measured the dimensions and gene-expression profile of the tendons to try to understand some of the interactions between muscles and tendons during muscle-tendon-unit adaptation. Here, we report that the muscle synthesizes thousands of serial sarcomeres within a week after a transfer and then, over the next several weeks, removes these sarcomeres as tendon length increases in an asynchronous fashion.

Materials and Methods

All experimental methods were approved by the institutional Animal Care and Use Committee of the University of California and Veterans Affairs Medical Center, San Diego. The surgical procedures were performed as previously described[13]. Thirty-seven male New Zealand White rabbits were used for muscle architectural analysis, and twenty-five of those rabbits were used for biological study of tendons after the tendon transfer. An additional four rabbits were used as controls to validate muscle and tendon-length measurements with use of suture markers, as described below.

Rabbit Experimental Model of Tendon Transfer

The extensor digitorum muscle of the second toe was chosen as the donor for transfer since it has a long and large tendon that can be manipulated easily surgically and the muscle is thin enough for laser diffraction to be performed without the need for tissue dissection. All muscle fibers of the extensor digitorum muscle of the second toe originate from the medial surface of the tibia. The animals were anesthetized with isoflurane (2.5% in 2 L/min of oxygen), and the right ankle and knee joints were secured in 90° of flexion in a custom-made jig. Under sterile conditions, the full length of the extensor digitorum muscle was exposed to enable measurement of muscle length with digital calipers as the distance between the origin of the most proximal fibers and the insertion of the most distal fibers after placement of suture markers (5-0 Ethilon; Ethicon, Somerville, New Jersey) on both ends. The extensor digitorum tendon was released at the level of the metatarsal for transposition, passed deep to the ankle extensor retinaculum, looped back onto itself, and sutured with 5-0 Ethibond (Ethicon) (Fig. 1). The extensor digitorum was intentionally transferred at the specific muscle length at which sarcomere length was 3.7 μm. This sarcomere length was chosen to be near the end of the descending limb of the rabbit muscle sarcomere length-tension curve[14] and to be representative of the sarcomere length that is seen in clinical tendon transfer surgery[10]. Sarcomere length was measured intraoperatively in the most distal fiber bundle of the extensor digitorum (Fig. 1, circle) with use of a laser diffraction device. In order to permit stress relaxation of the muscle and tendon, the measurement was performed one minute after the applied stretch. The diffraction pattern was analyzed and converted to sarcomere length with use of the grating equation: $n\lambda = L_s \sin\theta$, where L_s is the sarcomere length, λ is the laser wavelength (632.8 nm), θ is the diffraction angle, and n is the diffraction order[15]. After completion of the transfer, the tendon length was measured as the distance from the marker at the muscle-tendon junction to the distal end of the extensor retinaculum. These markers were also used to measure tendon length after the animals were killed to calculate changes in tendon length over the course of the experiment. Finally, the fascia, subcutaneous tissue, and skin were carefully closed. Buprenorphine (0.03 mg/kg) was administered for pain management twice a day for three days after the surgery, and the animals were allowed to run freely in their cages. None showed any visible signs of distress.

Muscle Architectural Examination

Animals were killed with pentobarbital at five different time points: one day and one, two, four, and eight weeks after the surgery (Table I). We measured the following muscle architectural properties: muscle length, muscle mass, raw fiber length, and sarcomere length. We then calculated the serial sarcomere number and physiological cross-sectional area, as described by Sacks and Roy[16]. Both hindlimbs were skinned, amputated at the level of the femur, and fixed in 10% buffered formaldehyde for seventy-two hours with the ankle and knee joints fixed in 90° of flexion and the toes extended. The hindlimbs were rinsed in physiological phosphate-buffered saline solution to prepare samples for architectural measurement. After the extensor digitorum muscle of the second toe was detached from the tibia, the surface pennation angle was measured at three regions (proximal, middle, and distal) with use of image analysis software (ImageJ; National Institutes of Health, Bethesda, Maryland). The extensor digitorum was then weighed and was digested in 15% H_2SO_4 for twenty minutes to facilitate microdissection of the muscle fiber bundles. The left, untreated extensor digitorum of each animal served as a control.

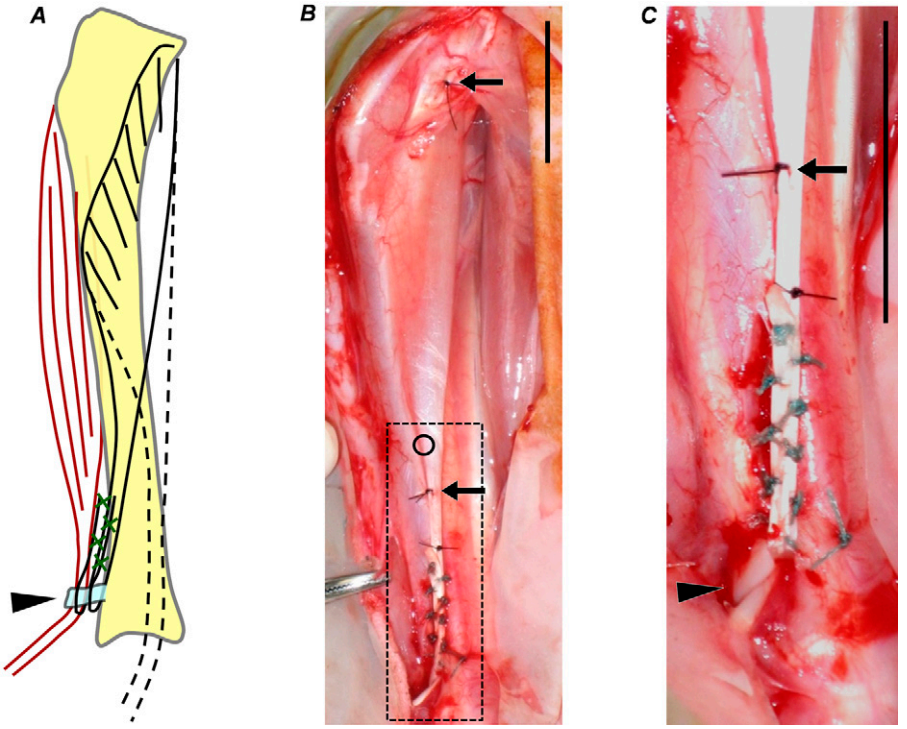

Fig. 1

A: Schematic diagram of the surgical procedure in this experiment. The tendon of the extensor digitorum muscle of the second toe (broken lines) was translocated (black solid lines) into the extensor retinaculum (arrowhead). *B:* An inclusive view of the surgical field after completion of the transfer. A laser diffraction device was applied intraoperatively to the most distal fiber bundle (circle) of the extensor digitorum to measure sarcomere length. Suture markers (arrows) were placed at the proximal end of the muscle and at the myotendinous junction to track the changes in the lengths of both the muscle and the tendon. *C:* Close-up view of the suture site shown by the broken line in *B*. The tendon was sutured to itself with at least seven knots after it was looped back around the extensor retinaculum (arrowhead). The scale bars represent 2 cm in *B* and *C*.

Each extensor digitorum was examined in the same three regions to yield architectural fiber-length and sarcomere-length values across the entire muscle. The full length of small fiber bundles (consisting of five to fifteen fibers) was teased from each region under a dissecting microscope. Raw fiber length was measured with use of digital calipers under the dissecting microscope in two different bundles from each of three regions. Sarcomere length was measured with laser diffraction in three regions of each bundle. Thus, muscle sarcomere length was calculated as the average of eighteen sarcomere-length measurements (three regions per muscle × two bundles per region × three sarcomere-length measurements per bundle) obtained across the entire muscle. The serial sarcomere number was calculated by dividing the raw fiber length by the sarcomere length for each fiber.

The physiological cross-sectional area (in square centimeters) was calculated according to the equation[16]: $(M \times \cos\theta)/(\rho \times L_{f[N]})$, where M represents muscle mass (in grams), θ represents the fiber pennation angle, ρ represents muscle density (1.056 g/cm^3), and $L_{f(N)}$ is the raw fiber length (in centimeters) normalized when sarcomere length is optimal (2.4 μm). The physiological cross-sectional area and serial sarcomere number are important muscle parameters because the physiological cross-sectional area is directly proportional to the maximum tetanic tension and the serial sarcomere number is proportional to muscle excursion and contraction velocity[17,18].

Tibial Length Measurement

Tibial lengths were measured to define tibial growth, which could affect muscle and tendon adaptation. Radiographic images of both hindlimbs with a calibration scale were obtained with use of a Faxitron machine (Faxitron X-Ray, Wheeling, Illinois) with the joints perpendicular to the x-ray beam and explicitly corrected for magnification. The image was scanned, and the tibial length was measured between the tibial plateau and the ankle mortise with use of image analysis software (ImageJ).

Tendon Water Content

The tendons, between the muscle-tendon junction and the extensor retinaculum, were harvested at one day and at two

TABLE I Sample Numbers, Body Mass, and Amount of Stretch Imposed on Extensor Digitorum Muscle During Transfer Surgery to Obtain a Sarcomere Length of 3.7 μm

	1 Day	1 Week	2 Weeks	4 Weeks	8 Weeks
Muscle architectural study					
No. of animals	7	7	8	8	7
Initial body mass* *(kg)*	2.62 ± 0.39	2.61 ± 0.36	2.58 ± 0.44	2.46 ± 0.26	2.46 ± 0.23
Final body mass* *(kg)*	Not measured	2.68 ± 0.39	2.83 ± 0.34	3.10 ± 0.17	3.22 ± 0.39
Applied muscle stretch* *(mm)*	2.98 ± 0.58	3.03 ± 0.47	3.21 ± 0.48	2.81 ± 0.53	3.03 ± 0.68
Tendon biological study					
No. of animals	7		9	9	
Initial body mass* *(kg)*	2.54 ± 0.50		2.60 ± 0.38	2.54 ± 0.47	
Final body mass* *(kg)*	2.47 ± 0.52		2.69 ± 0.34	2.96 ± 0.29	
Applied muscle stretch* *(mm)*	3.00 ± 0.72		3.21 ± 0.54	2.84 ± 0.47	

*The values are given as the mean and standard deviation.

and four weeks after the surgery (Table I). The contralateral tendons from the corresponding region were also harvested as controls. The tendons were rinsed in saline solution and frozen in liquid nitrogen immediately after removal and were stored at –80°C. Approximately one-third of each tendon sample was thawed to determine water content while special care was taken to keep each sample away from any exposure that might alter water content. Wet weights of the samples were measured on a microbalance, and the samples were then dried under vacuum to constant weight[19]. The water content of each sample was calculated by subtracting the dry weight from the wet weight and dividing by the wet weight.

Tendon RNA Extraction and Reverse Transcriptase-Polymerase Chain Reaction

RNA extraction from the tendons was performed with use of the TRIspin method[20]. Frozen samples were pulverized in liquid-nitrogen-cooled vessels with use of a Braun Mikro-Dismembrator (B. Braun Biotech International, Melsungen, Germany), thawed in 1 mL of TRIzol reagent (Invitrogen, Carlsbad, California), and mixed for five minutes. After addition of chloroform (0.3 mL), samples were centrifuged, and ethanol (70%, 0.6 mL) was added to the upper aqueous phase. Isolation of total RNA was performed with use of the RNeasy Mini RNA Kit (QIAGEN; Mississauga, Ontario, Canada), and DNase was added to eliminate potential DNA contamination. Fluorometric quantification of the isolated RNA was performed with use of the SYBR Green II reagent (Mandel; Guelph, Ontario, Canada). Samples were stored at –80°C until further analysis.

Reverse transcription of 1 μg of total RNA from each sample into cDNA was performed with random primers and the Omniscript RT Kit (QIAGEN). Polymerase chain-reaction analysis was subsequently performed with rabbit-specific primers for molecules relevant to synthesis and degradation of tendon matrix (see Appendix), as described previously[21-23]. Absence of DNA contamination was confirmed with a negative control of non-reverse-transcribed total RNA. The protocol was developed so that products were within the linear range of the polymerase chain-reaction amplification and the image analysis system. Gels were stained with ethidium bromide and were photographed for analysis with densitometry. Values for each gene from each sample were normalized to housekeeping gene 18S rRNA values[24] to yield a semiquantitative assessment.

Statistical Analysis

All data except body mass and degree of muscle stretch are expressed as means and the standard error of the mean. Paired t tests were used to compare variables between the transfer and control sides for each time-point group. One-way analysis of variance followed by post hoc tests was used to test for differences among the five time points for the muscle parameters and among the three time points for the tendon parameters. Because of variability in mass and physiological cross-sectional area across animal subjects, the transferred-muscle values were normalized to the contralateral, control side for statistical analysis by dividing the experimental by the control value. The significance level (α) was defined as 0.05 for all analyses. Because of the multiple comparisons that were made, p values between 0.05 and 0.01 should be interpreted with caution.

Source of Funding

The funding sources used for this study were grants (HD048501 and HD050837) from the National Institutes of Health and support from the Department of Veterans Affairs.

Results

It was necessary to stretch muscles by 3.01 ± 0.54 mm over in vivo muscle length to achieve a sarcomere length of 3.7 μm (~0.6 μm beyond the physiological range) during surgery. Neither the initial body mass nor the amount of stretch applied to the muscles differed among the groups ($p > 0.9$; Table I).

Raw Fiber Length

The raw fiber length of the control muscles (i.e., the fiber length as directly measured in the tissue without sarcomere-length correction) was consistent throughout the course of the study (Fig. 2, *A;* expressed by the horizontal line). Within the first week, the raw fiber length of the transferred muscles increased by ~2 mm compared with the values for the control muscles ($p < 0.05$). The raw fiber length of the transferred muscle then continuously decreased over the period of the experiment and reached a final length of 8.48 ± 0.20 mm. At the latest two time points, the raw fiber length of the transferred muscles was significantly shorter than that of the control muscles ($p < 0.05$ at four weeks and $p < 0.001$ at eight weeks).

Sarcomere Length

The sarcomere length of the control muscles was consistently ~2.75 μm throughout the course of the experiment (Fig. 2, *B;* expressed by the horizontal line). The sarcomere length in the transferred muscles rapidly decreased to 3.28 ± 0.09 μm one day after the surgery, presumably as a result of tendon stress relaxation. The sarcomere length rapidly and significantly ($p < 0.001$) decreased to 2.56 ± 0.04 μm at one week after transfer, at which time it was significantly shorter than the control-muscle sarcomere length (2.78 ± 0.04 μm; $p < 0.01$). This rapid decrease within the first week corresponded to a rapid increase in serial sarcomere number, as discussed below. Eventually, the sarcomere length plateaued to a value of ~2.2 μm four to eight weeks after the surgery. It was notable that the sarcomere length remained lower than the control-muscle values and even lower than the optimal sarcomere length four weeks after the surgery.

Serial Sarcomere Number

A rapid and significant (~30% [~1000]) increase ($p < 0.001$) in serial sarcomere number was observed one week after the surgery (Fig. 2, *C*) (4658 ± 154 serial sarcomeres in the transferred muscle compared with 3609 ± 80 in the control muscle). After this time point and until eight weeks, this increased serial sarcomere number paradoxically decreased, with sarcomere length remaining constant over the same period. Up to four weeks after the surgery, the serial sarcomere number in the transferred muscle remained significantly greater than both the number in the transferred muscle one day after the surgery and the number in the control muscle. By eight weeks, the serial sarcomere number (3749 ± 83) reached the same value as that found in the control muscle (3767 ± 61). The serial sarcomere number in the control muscles was consistent throughout the period of the experiment (Fig. 2, *C;* expressed by the horizontal line).

Mass and Physiological Cross-Sectional Area

The control-muscle mass increased over the course of the experiment, reflecting normal growth (Table II). The transferred-muscle mass was ~30% greater than the control mass as early as one day after the surgery. This rapid increase was presum-

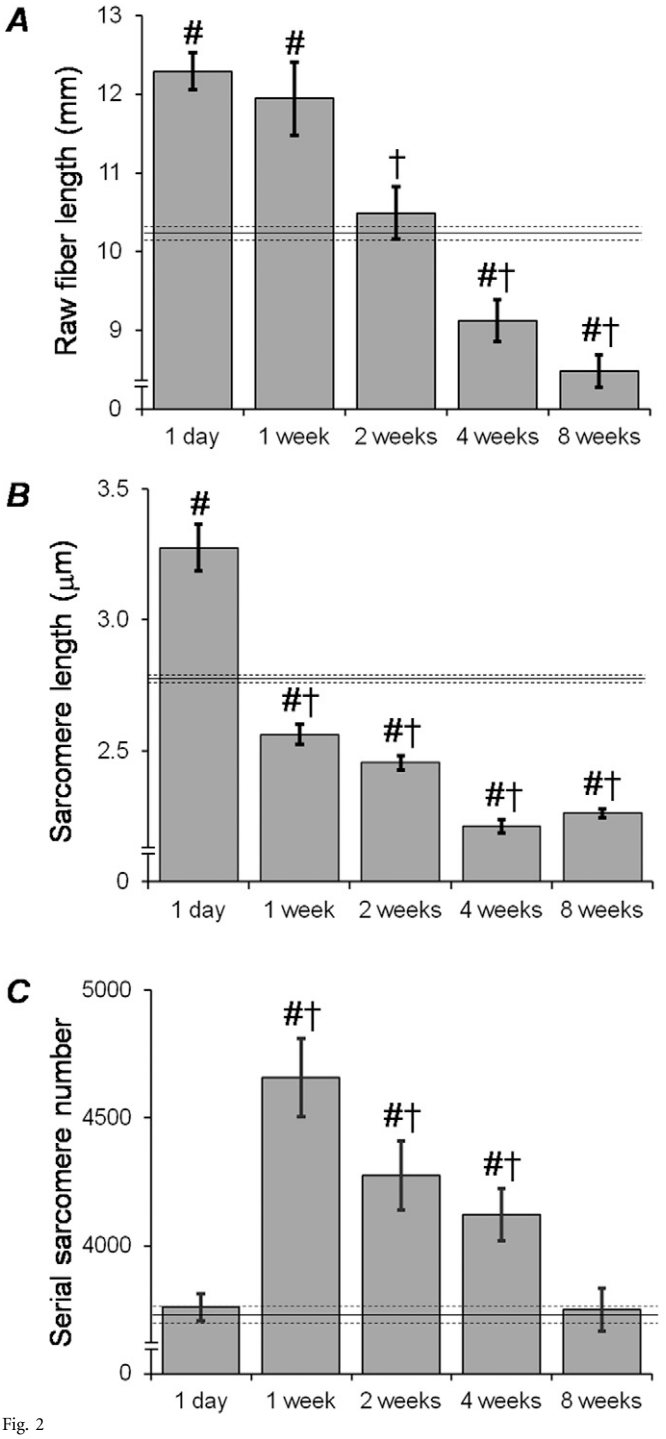

Fig. 2

Raw fiber length (A), sarcomere length (B), and serial sarcomere number (C) of the transferred muscle over the course of the experiment. The x axes denote the period after the operation. The horizontal solid and dashed lines represent the grand mean and the standard error of the mean, respectively, for the control-muscle values. #Significantly different ($p < 0.05$) compared with the corresponding control-muscle value. †Significantly different ($p < 0.05$) compared with the value in the one-day group.

TABLE II Mass and Normalized Physiological Cross-Sectional Area of Control and Transferred Muscles Measured in Muscle Architectural Analysis*

	1 Day	1 Week	2 Weeks	4 Weeks	8 Weeks
Mass					
Control muscle *(g)*	0.451 ± 0.023	0.480 ± 0.025	0.485 ± 0.023	0.542 ± 0.022	0.533 ± 0.019
Transferred muscle *(g)*	0.573 ± 0.025	0.612 ± 0.036	0.566 ± 0.042	0.568 ± 0.020	0.523 ± 0.024
Fold change relative to control value†	1.278 ± 0.044†	1.281 ± 0.054†	1.162 ± 0.049§	1.051 ± 0.017	0.984 ± 0.036
Normalized physiological cross-sectional area					
Control muscle *(cm^2)*	0.449 ± 0.020	0.511 ± 0.020	0.521 ± 0.021	0.562 ± 0.021	0.545 ± 0.014
Transferred muscle *(cm^2)*	0.591 ± 0.025	0.511 ± 0.028	0.504 ± 0.027	0.531 ± 0.017	0.541 ± 0.032
Fold change relative to control value†	1.326 ± 0.052#	1.000 ± 0.035	0.970 ± 0.035	0.950 ± 0.024	0.993 ± 0.051

*The values are given as the mean and standard error of the mean. †The fold changes were calculated by dividing the transferred-muscle value by the control-muscle value. ‡Significantly different ($p < 0.01$) from the four and eight-week groups. §Significantly different ($p < 0.01$) from the eight-week group. #Significantly different ($p < 0.01$) from the other time-point groups.

ably due to edematous change caused by surgical intervention and did not imply a bona fide increase in contractile protein within the muscle. The increased mass was maintained until one week after the surgery, at which time the physiological cross-sectional area returned to the control value, suggesting that the increase in mass at this time point reflected the increase in serial sarcomere number. The mass of the transferred muscle relative to that of the control muscle gradually decreased in the subsequent period.

Changes in Muscle Length and Tendon Length

Elongation of the muscle length and tendon length on the transfer side was measured directly as the distance between the suture markers. This method was validated in separate control experiments (four animals) by placing muscle and tendon suture markers at the same levels as described above but without the performance of other surgical procedures. Animals were permitted normal cage activity and growth for eight weeks. The increases in muscle and tendon length were 4.08 ± 0.52 and 1.43 ± 0.74 mm, respectively, which were considered to reflect the natural growth over the eight-week time period.

Tendons secured side by side could no longer be distinguished individually at four weeks after the transfer. The muscle and tendon lengths immediately after tendon transfer were 53.3 and 29.6 mm, respectively, after which the muscle-tendon-unit length increased over time (Fig. 3, *A*). When expressed as strain (the length change divided by the initial length at the time of the surgery), tendon elongation (15.55% ± 4.08%) was greater than muscle elongation (10.25% ± 2.05%) over the eight-week period, although absolute muscle elongation (5.40 ± 1.01 mm) was greater than absolute tendon elongation (4.47 ± 1.09 mm). Neither muscle elongation ($p = 0.37$) nor tendon elongation ($p = 0.08$) over the eight-week period was significantly different from that observed during normal growth. Interestingly, an increase in muscle length was accompanied by a decrease in serial sarcomere number beginning at one week after the surgery, and muscle length was not ultimately affected by the early rapid increase in the sarcomere number.

Estimation of Bone Growth

The tibial lengths on both the transfer and the untreated side increased by ~7 mm over approximately eight weeks. Thus, the results of this study should be considered in the context of an acute, early length increase of ~3 mm followed by a slower, more consistent stretch over the subsequent eight weeks.

Tendon Water Content

The water content of the transferred extensor digitorum tendon did not differ from that of the contralateral tendon one day after the transfer, but it did increase significantly two to four weeks after the transfer, as compared with the water content of the control tendon ($p < 0.01$) and as compared with that of the transferred tendon one day after the transfer ($p < 0.01$ for the comparison with both the two and four-week values).

mRNA Expression of Collagen and Collagen-Inducing Factors in the Tendon

Neither type-I nor type-III collagen mRNA was altered in the transferred tendon throughout the experimental period. Consistent with these results, expression of tenomodulin and basic fibroblast growth factor (bFGF) was also unchanged in the transferred tendon. In contrast, expression of insulin-like growth factor-1 (IGF-1) in the transferred tendon increased twofold, as compared with that in the control tendon, four weeks after the transfer ($p < 0.01$), but it was not sig-

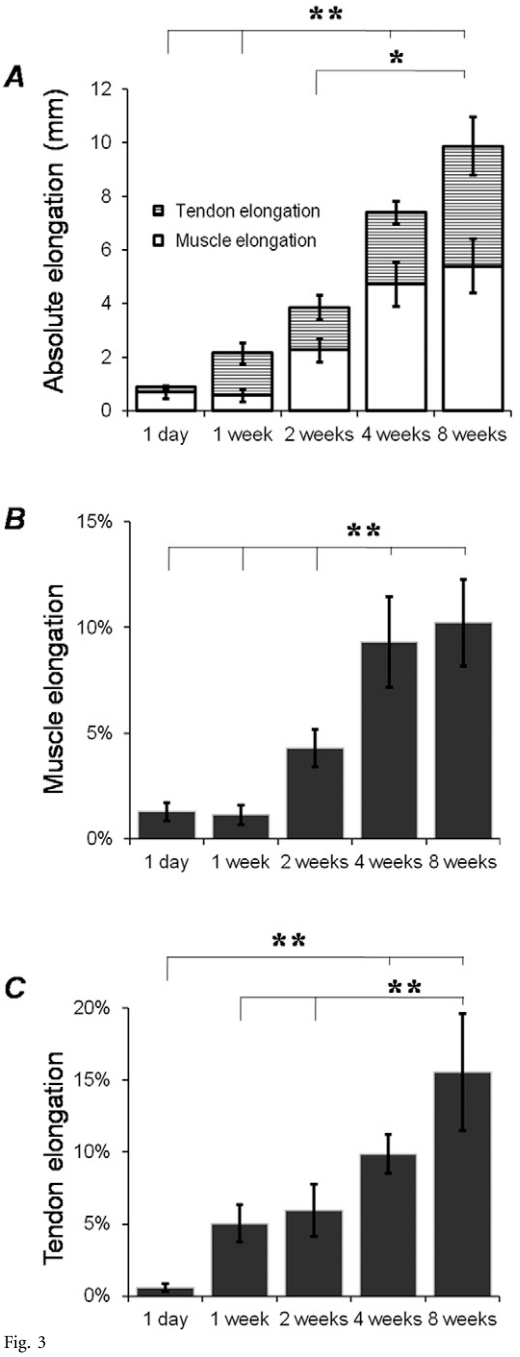

Fig. 3

Elongation of the muscle-tendon unit expressed as absolute values (millimeters) (A) and elongation of the muscle (B) and the tendon (C) expressed as strain values (the length change divided by the initial length at the time of the surgery) over the course of the experiment. In *A*, the muscle length change (white bars) and the tendon length change (hatched bars) are stacked in one bar. The x axes denote the period after the operation. *P < 0.05. **P < 0.01. There were no differences between the values expressed by the absolute scale and those expressed by the strain scale.

nificantly changed either at one day or at two weeks (Fig. 4, *A*). Expression of transforming growth factor-β1 (TGF-β1) (Fig. 4, *B*) in the transferred tendon was consistently elevated (2.5 to fivefold) compared with that in the corresponding control tendon throughout the period of the experiment ($p < 0.01$).

mRNA Expression of Inflammatory Cytokines and Matrix Metalloproteinases in the Tendon

Levels of interleukin-6 (IL-6), IL-8, and cyclooxygenase-2 (COX-2) were significantly elevated in the transferred tendon at one day ($p < 0.05$ for all of the objectives), but all were reduced by two weeks, suggesting that these inflammatory cytokines were induced only during the acute postoperative phase (Fig. 5, *A, B,* and *C*). Levels of IL-1β, a major cytokine that induces an immune response, were not significantly altered ($p > 0.05$).

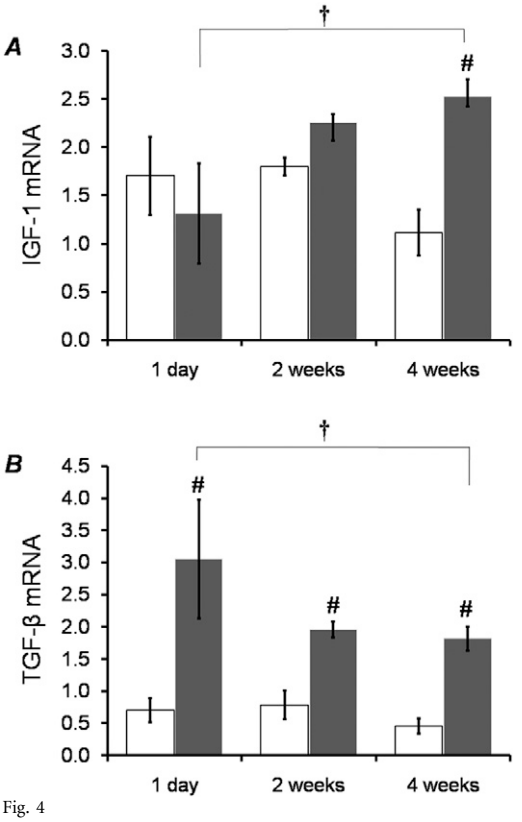

Fig. 4

Expression levels of insulin-like growth factor-1 (IGF-1) (A) and transforming growth factor-β1 (TGF-β1) (B) mRNAs in the contralateral, control tendons (white bars) and the transferred tendons (gray bars) are presented as fold changes relative to 18S rRNA values. The x axes denote the period after the operation. #Significantly different ($p < 0.05$) compared with the corresponding control-tendon value. †A significant difference ($p < 0.05$) between the one-day and four-week groups of transferred tendons.

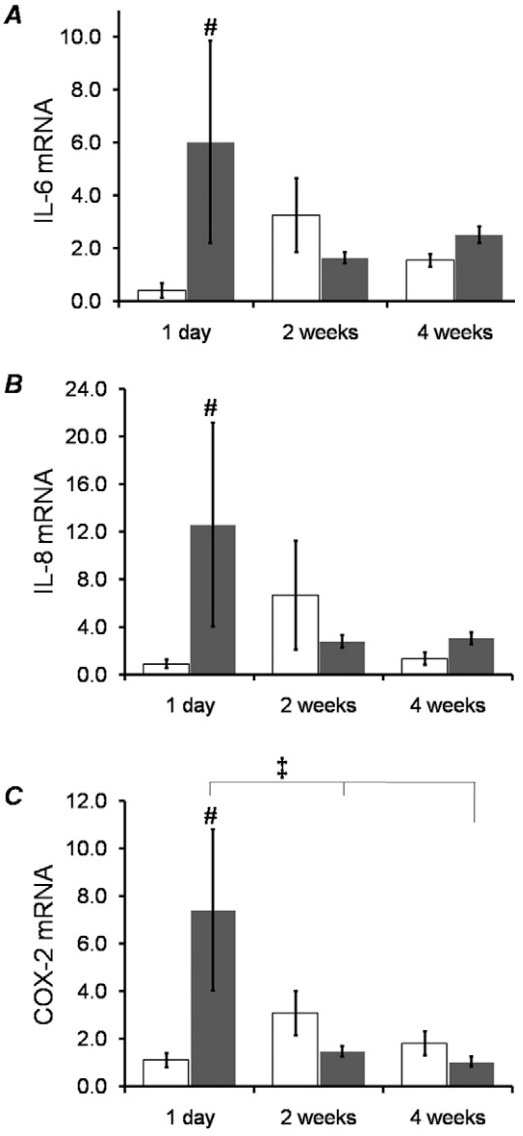

Fig. 5

Expression levels of interleukin-6 (IL-6) (A), IL-8 (B), and cyclooxygenase-2 (COX-2) (C) mRNAs in the contralateral, control tendons (white bars) and the transferred tendons (gray bars) are presented as fold changes relative to 18S rRNA values. The x axes denote the period after the operation. #Significantly different ($p < 0.05$) compared with the corresponding control-tendon value. ‡A significant difference ($p < 0.05$) between the one-day group and the other groups of transferred tendons.

The matrix metalloproteinase-1 (MMP-1) transcript level was elevated in the transferred tendon at one day ($p < 0.05$) but not later (Fig. 6, *A*). Both MMP-3 and MMP-13 mRNA expression (Fig. 6, *B* and *C*, respectively) were significantly increased in the transferred tendon, as compared with the control tendon, throughout the experimental period except at one day, when MMP-13 expression was not significantly increased. Expression of TIMP-1 (tissue inhibitor of matrix metalloproteinase type 1), a specific inhibitor of MMPs[25], did not significantly differ between the control and transfer sides throughout the course of the experiment.

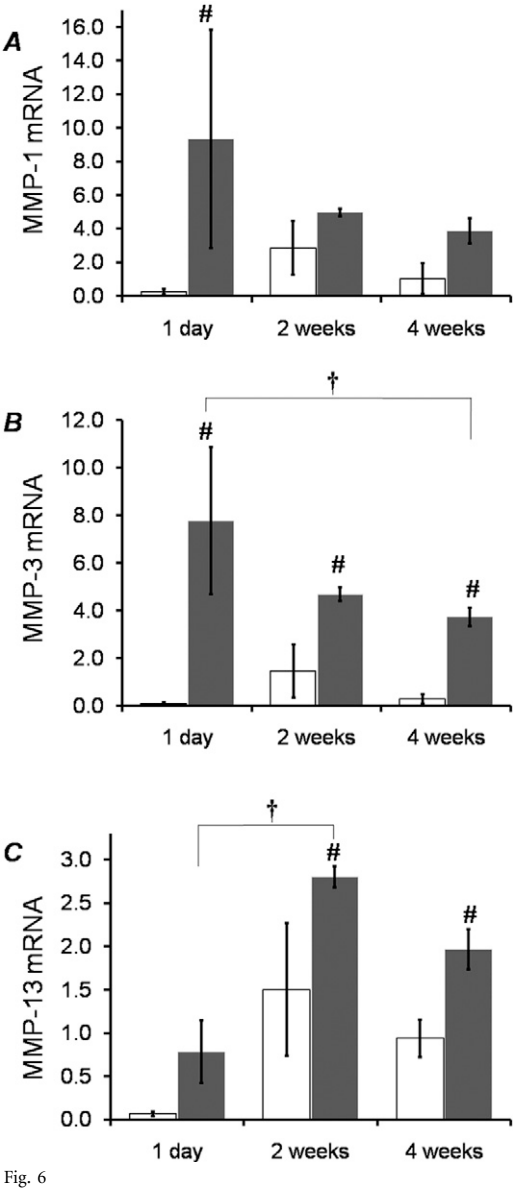

Fig. 6

Expression levels of matrix metalloproteinase-1 (MMP-1) (A), MMP-3 (B), and MMP-13 (C) mRNAs in the contralateral, control tendons (white bars) and the transferred tendons (gray bars) are presented as fold changes relative to 18S rRNA values. The x axes denote the period after the operation. #Significantly different ($p < 0.05$) compared with the corresponding control-tendon value. †A significant difference ($p < 0.05$) between the transferred tendons at the two different time points.

Discussion

The main finding of this study is that both muscle and tendon adapt dramatically to a chronic length increase after tendon transfer, albeit with asynchronous timing. The net result is a transient increase in serial sarcomere number that appears to be reversed after the delayed adaptive response of the tendon. In this model, the muscle first adapted to the imposed stretch by adding a large number of sarcomeres in series. This increase was relatively rapid, peaking within the first week. However, the tendon was slightly elongating as early as one day after the transfer and was substantially elongated by one week after the transfer. Apparently, the muscle adaptation does "predict" any tendon elongation, and, as a result, the muscle "readjusts" by subtraction of sarcomeres. As the tendon finally reaches its ultimate length, additional subtraction of sarcomeres occurs until the serial sarcomere number reaches the original value. Clearly, this represents a tremendous "waste" of synthetic activity over the course of the adaptation in order to possibly keep the muscle functional by maintaining near optimal sarcomere length values. Ancillary studies ongoing in our laboratory are defining these functional properties.

To our knowledge, this is the first study in which sarcomere adaptation was evaluated to define the sarcomere length at which the muscle was to be set by the procedure itself. Our pilot experiments demonstrated that the physiological sarcomere length range of the extensor digitorum muscle of the second toe is between 2.6 and 3.1 μm. Therefore, the sarcomere length of 3.7 μm that was chosen in this experiment was outside of the anatomical range. We are aware of only one study in which muscle adaptation was examined after intentional extra-physiological stretch[9], and this study was based on clinical reports that such lengths are often achieved during upper-extremity surgery[10]. Three weeks after transfer at various sarcomere lengths ranging from ~3.5 to ~5.5 μm, the serial sarcomere number in that study was negatively correlated with the sarcomere length at the time of transfer. Nevertheless, it was clear in the current study that the muscle was still able to adapt to stretch even at this relatively long length. The transferred muscles had a larger mass and sarcomere number through four weeks, while stretch did not substantially influence the physiological cross-sectional area since the mass increase occurs largely in series. The increased serial sarcomere number predicts that the adapted muscle would produce higher excursion and contraction velocity, and of course more power. Unfortunately, these potentially positive effects of muscle architecture are canceled by eight weeks after a transfer.

Tendon length adaptation over the course of natural growth consisted of two phases: (1) initial elongation during the first week and (2) subsequent elongation over the two to eight-week time period. The initial elongation of the tendon was thought to be promoted by stress relaxation of tendon material and/or suture loosening, which may occur on the hour time scale under the considerable tension produced by the stretch, although water content (which is directly related to relaxation parameters in tendons[26,27]) did not increase one day after the transfer. The other cause, described below, may exist for the late tendon elongation. By four weeks, the presence of abundant fibrous tissue at the suture site suggests intrinsic tendon healing. Our results demonstrated that MMP-1, 3, and 13, all of which could degrade tendon matrix through cleavage of collagen molecules and proteoglycans[28-30], were increased during most of the course of the experiment without any detectable changes in their inhibitor, TIMP-1. Since MMPs are known to be regulated after expression by several factors such as TIMP-1[31], these results suggest that transcript expression levels probably led to collagen catabolism. MMP expression is thought to be induced by inflammatory cytokines already up-regulated at one day. Growth factors that potentially stimulate collagen synthesis and the other tendon matrix proteins[32-35] were also up-regulated, although levels of collagen mRNA on the transfer side did not differ significantly from those on the control side. On the basis of these results, we anticipated that the late elongation of the tendon after the surgery would be enhanced by extracellular matrix remodeling in which degradation predominated. It is interesting to contrast this negative balance of extracellular matrix remodeling with the finding in several experiments that exercise promoted collagen, MMP, and TIMP expression in tendons[36-40]. Surgical intervention involving tendons may induce a distinct reaction compared with traditional physiological modulation. Additional investigations are needed to determine whether, in the long run, surgical procedures themselves are actually beneficial to induce tendon adaptation.

A limitation of this study is that the model reflects conditions in which immature animals are still experiencing substantial growth. Thus, the actual muscle-tendon-unit deformation consists of a step increase in length followed by a slow ramp increase in length. Interestingly, this is not unlike what has been reported in most of the previous reports in the literature in this field[1-3,5-8], and it highlights the importance of defining both the age of the model species and the growth rate when interpreting muscle-tendon-unit adaptation data. This may also thus be relevant to pediatric orthopaedic procedures or Ilizarov procedures, which involve chronic lengthening. The classic studies by Williams and Goldspink[4] clearly demonstrate this point, as they present results of mature and young animals that are in stark contrast to one another. Additional studies are needed to compare the adaptations in mature animals with the current results.

In summary, this study has demonstrated that both muscle and tendon adapt, in an asynchronous fashion, to tendon transfer. The asynchronous adaptation was likely due to differences in the properties of muscle and tendon and, in part, to surgical intervention. Muscle adapted more quickly than tendon did, which presumably encourages the muscle-tendon unit to remain functional. Tendon adaptation consisted mainly of a catabolic response that is often considered unnecessary for muscle-tendon-unit function. Unwanted tendon elongation has already been demonstrated after clinical tendon transfer[41].

Loss of strength after tendon transfer could result from the tendon elongation that often exceeds the applied stretch and following muscle adaptation rather than disadaptation of muscle. The control of tendon matrix degradation might be required for a preferable outcome of tendon transfer surgery.

Appendix

eA A table listing the primer sequences used in the polymerase chain-reaction analysis is available with the electronic version of this article on our web site at jbjs.org (go to the article citation and click on "Supporting Data"). ■

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