Tissue memory in healing tendons: short loading episodes stimulate healing

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Andersson T, Eliasson P, Aspenberg P. Tissue memory in healing tendons: short loading episodes stimulate healing. J Appl Physiol 107: 417–421, 2009. First published June 18, 2009; doi:10.1152/japplphysiol.00414.2009.—Intact tendons adapt slowly to changes in mechanical loading, whereas in healing tendons the effect of mechanical loading or its absence is dramatic. The longevity of the response to a single loading episode is, however, unknown. We hypothesized that the tissue has a “memory” of loading episodes and that therefore short loadings are sufficient to elicit improved healing. The Achilles tendon of 70 female rats was transected and unloaded by tail suspension for 12 days (suspension started on day 2 after surgery). Each day, the rats were let down from suspension for short daily training episodes according to different regimes: 15 min of cage activity or treadmill running for 15, 30, 60, or 2 × 15 min. Rats with transected Achilles tendons and full-time cage activity served as controls. The results demonstrated that full-time cage activity increased the peak force over three times compared with unloading. Short daily loading episodes (treadmill running) increased the peak force about half as much as full-time activity. Prolongation of treadmill running above 15 min or dividing the daily training in two separate episodes had minimal further effect. This mechanical stimulation increased the cross-sectional area but had no effect on the mechanical properties of the repair tissue. The findings indicate that once the tissue had received information from a certain loading type and level, this is “memorized” and leads to a response lasting many hours. This suggests that patients might be allowed early short loading episodes following, e.g., an Achilles tendon rupture for a better outcome.

hindlimb suspension; immobilization; Achilles tendon; tendon healing; mechanical stimulation

ALL ORGANISMS HAVE TO LIVE IN a world of mechanical stress. Because the ability to perceive and respond to mechanical perturbation is essential for survival, it has roots early in evolution. The response to mechanical stimuli can therefore be expected to be complex and function in many different ways on a cellular and biochemical level. This complexity could motivate a “black-box” approach, going directly from mechanical stimulation to resulting functional properties of whole tissues or organs, to get ideas about the clinical consequences of loading.

Because tendons are subject mostly to traction, their response to mechanical forces on an organ level can be measured as changes in tractiveal strength, which makes them an ideal tissue for this kind of study. Although tendons not only transfer traction but sometimes also store mechanical energy, this latter function is not further considered here. Many studies show that tendons adapt to changes in mechanical loading (1–3, 9, 14, 15). However, these adaptations are slow, due to the low remodeling rate, and somewhat controversial. In contrast, the healing of injured tendons comprises rapid proliferation and remodeling, and here the effects of mechanical loading are dramatic. Numerous animal studies show that immobilization of a healing tendon compromises the healing process (4–6, 12, 13, 17). Immobilization and unloading are not necessarily the same thing; when the experimental animals with immobilized limbs are limping about, they may load their tendons isometrically. In many of these models, the degree of unloading is therefore unknown.

So far, to our knowledge, no study has addressed the longevity of the response to a short episode of loading, with the reason probably being methodological difficulties with removing and reapplying any immobilization device. The question of this longevity or “tissue memory” is interesting not only from a biological point of view but also for clinical purposes. Patients with Achilles tendon injuries often have the injured limb immobilized for several weeks. Because animal data suggest that this harms healing in an otherwise unloaded limb, it is important to develop clinical loading regimes during healing. For designing such regimes, one needs to know the duration of the tissue memory. This necessitates an animal model, which allows discrete loading episodes in an otherwise unloaded limb. We have applied such a model, namely Achilles tendon healing in tail-suspended rats (10), and developed it by allowing short episodes of treadmill running.

Our intention with this study was to answer the following questions: Can short loading episodes improve the healing of otherwise unloaded healing tendons? If so, will this effect last for a long time, making a second episode within 8 h ineffective? Does the duration of the episode influence tendon healing?

MATERIALS AND METHODS

Ethical approval. The study was approved by the Regional Ethics Committee for Animal Experiments, and institutional guidelines for care and treatment of laboratory animals were adhered to.

Animals, treadmill, and unloading devices. One hundred female Sprague-Dawley rats were used, weighing mean 216 g (SD 15 g) at the time of surgery. Handling of the rats started 2 wk before the experiment. Because rats are active during the night, the 12-h light and dark cycle was changed so that the animals could be handled during their activity period. The animals were habituated to a treadmill apparatus (Exer 3/6, Columbus Instrument, Columbus, OH) for 5 days (speed 12 m/min, for 7–30 min/day) during these 2 wk. Special cages were used for hindlimb unloading by tail suspension. On top of the cage there was a metal rod attached to two wheels in each end allowing it to move back and forth on the cage walls. The rat’s tail was wrapped in adhesive tape connected to a fish-line swivel and a fish-line running to a wheel on the rod on top of the cage. This made it possible for the rat to move in all directions and turn around, using the fore legs while the hind limbs were lifted just above the cage floor. Hindlimb unloading by tail suspension is described more in detail elsewhere (10). The rats were kept one per cage and were housed in a room with temperature maintained at 24°C. Two days before surgery, 70 rats were acclimatized to the special suspension cages.
The remaining 30 rats were housed in ordinary cages throughout the study. All rats were given food and water ad libitum.

**Surgery and unloading.** On the day of surgery, the rats were anesthetized with 5% isoflurane gas (Forene, Abbot Scandinavia, Solna, Sweden) in a chamber and then with 3.5% isoflurane on a mask. Antibiotics (25 mg/kg, Oxytetracycline, Engemycin; Intervet, Boxmeer, Holland) and analgesics (0.045 mg/kg, Buprenorphine, Temgesic; Schering-Plough, Brussels, Belgium) were given subcutaneously preoperatively. The skin on the right hind limb was shaved and washed with chlorhexidine ethanol. The surgery was performed under aseptic conditions. A transverse incision was made in the skin lateral to the right Achilles tendon and the tendon complex was exposed. The plantaris tendon was removed to simplify the mechanical evaluation at the end of the experiment. Thereafter, the Achilles tendon was transversely cut, and a 3-mm-thickness segment was removed. The distal end of the defect was 3 mm from the calcaneal bone. The tendon was left with a gap between the tendon stumps, and the skin was closed. During the first 2 days after surgery, the rats were allowed full-time activity. Two days after surgery, the hind limbs were unloaded by tail suspension, and the rats were divided, by random, into groups for different training regimes. Two types of training were used: treadmill running at a speed of 9 m/min and unrestricted cage activity once daily where the rats were allowed to move around in the cage on their four legs. Treadmill “running” was essentially walking, although the rats kept an uneven pace, sometimes slowly running, sometimes sitting while the belt transported them back. This low speed was chosen to avoid stress, because the rats were limping. Regarding the unrestricted cage activity, once daily, the investigator watched the rats so that they were moving throughout the entire training sessions; if not, the rats were stimulated manually.

**Experiments.** This study consisted of three experiments, separated in time and including different groups (Table 1).

**Experiment 1** comprised suspension with 15 min of unrestricted cage activity once daily, suspension without exercise (unloaded control), and full-time cage activity in normal cages.

**Experiment 2** comprised suspension with 30 min of exercise on treadmill once daily, suspension with 15 min of exercise on treadmill twice daily (the second episode after 8 h), unloaded control, and full-time cage activity.

**Experiment 3** comprised suspension with 15 min of exercise on treadmill once daily, 60 min of exercise on treadmill once daily, and full-time cage activity.

All groups included 10 rats each, so in total 70 hind limb unloaded rats and 30 rats in normal cages with full-time cage activity were included in the study.

**Evaluation.** Fourteen days after surgery, the rats were anesthetized with a subcutaneous injection of dexamethasone (0.5 mg/kg, Dexamtor; Orion Pharma, Esbo, Finland) and ketamine (75 mg/kg, Ketaminol; Intervet, Boxmeer, Holland) while still suspended and then killed by an overdose of pentobarbital sodium. The Achilles tendon with the attached calcaneal bone was dissected free and harvested together with parts of the gastrocnemius and soleus muscle complex. Sagittal and transverse diameters of the callus tissue were measured with a caliper. This method has previously shown a good reproducibility (unpublished data), and bias was avoided by blinding. The old tendon stumps were visualized by transillumination without tension, and the distance between them was measured (gap distance).

For clamping, the muscle was carefully scraped off the tendon by blunt dissection to produce a fan of tendon fibers. These fibers were fixed between fine sandpaper in a metal clamp. The calcaneus was fixed in a custom-made clamp in 30° dorsiflexion relative to the direction of traction. The distance between the metal clamp and the calcaneus was used as an approximation of tendon length. Finally the tendons were mounted vertically in a materials testing machine (100R; DDL, Eden Prairie, MN) and were pulled until failure at a constant speed of 0.1 mm/s. Force at failure and stiffness were calculated by the software of the testing machine. The investigator marked a linear portion of the elastic phase of the curve for stiffness calculation. Cross-sectional area, elastic modulus, and ultimate stress were calculated. The investigator was blinded throughout the measurements.

**Statistical analysis.** The experiments were done in a sequence, where one result raised the hypothesis for the next experiment. Data were therefore analyzed in the same sequence, using one-way ANOVA (StatView 5.0.1 for Windows) for each experiment. Post hoc comparisons for difference between groups were made using Bonferroni-Dunn test. Accordingly, in experiments 1 and 2, P < 0.0167 was considered significant, and in experiment 2 P < 0.0083 was considered significant. A regression analysis including 15, 30, and 60 min of treadmill running was made to evaluate the influence of the duration of a loading episode.

**RESULTS**

**Exclusions.** In total, seven animals were excluded. In the first experiment, two rats with full-time cage activity were lost because they gnawed open their wounds. In the second experiment, one unloaded rat was excluded for the same reason. In the last experiment, two rats with full-time cage activity were excluded, one due to wound gnawing and one due to technical errors during the mechanical evaluation. One rat from 15 min and one from 60 min were also excluded because the adhesive tape on one occasion loosened from the tail, resulting in unwanted loading.

**Experiment 1.** Full-time cage activity increased the peak force in the healing tendons more than three times compared with unloading (P < 0.0001) (Fig. 1). The same pattern was seen for cross-sectional area, stiffness, ultimate stress, and gap distance (Figs. 2–4 and Table 2). The elastic modulus did not differ. Fifteen minutes of cage activity did not affect the healing process much; only stiffness was increased (P = 0.01) compared with the unloaded group.

**Experiment 2.** During experiment 1, there was a problem that the suspended rats with 15 min of free cage activity tended to sit still when they were let down. We therefore stimulated the rats manually so that they moved around in their cages during the entire 15-min period. To achieve more standardized loading episodes, a treadmill was used in the ensuing experiments. The total loading time was also doubled in experiment 2. The results revealed that 30 min, or 2 × 15 min, of daily treadmill running enhanced tendon healing. Peak force was almost doubled by 30 min and 2 × 15 min compared with the unloaded group (P = 0.0001 and P = 0.002). Importantly, the two exercise groups did not differ. The 95% confidence interval for the difference between group means ranges from 2 × 15

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Table 1. Experiment lay-out

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<tr>
<th>Experiment</th>
<th>Suspension</th>
<th>No Suspension, Full-Time Cage</th>
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<td>1</td>
<td>x</td>
<td>15 cage</td>
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<tr>
<td>2</td>
<td>x</td>
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Columns represent training regimes. 15 cage means stimulated cage activity for 15 min/day; 0, 15, 30, and 60 means treadmill exercise for 0, 15, 30, or 60 min once daily; 2 × 15 means treadmill running for 15 min twice daily; No Suspension, Full-Time Cage means full-time cage activity. Each x represents 10 rats.
min yielding 25% lower to 3% higher peak force than the
30-min group.

The group with full-time cage activity was still >50%
stronger than both exercise groups ($P<0.0001$ for both). The
same pattern was found for stiffness. The cross-sectional area
was larger for $2 \times 15\text{ min}$ compared with the unloaded group
($P = 0.0007$), but there was no difference between the exercise
groups. The gap distance was shorter for both exercise groups
compared with the free cage activity group ($P = 0.001$ and
$P < 0.0001$); the $2 \times 15\text{ min}$ group was even shorter than the
unloaded group ($P = 0.0077$). No difference was found be-
tween the exercise groups. There was no difference in material
properties (ultimate stress and elastic modulus) between any of
the groups.

**Experiment 3.** To study the influence of the duration of a
loading episode, a comparison between 15 and 60 min of
treadmill running was performed in experiment 3. Only the
estastic modulus and cross-sectional area differed between the
two exercise groups. For 60 min, elastic modulus was lower
and cross-sectional area greater compared with 15 min ($P = 0.015$ and $P = 0.014$). The exercise groups still did not reach
the values of the full-time cage activity group concerning peak
force and stiffness. Similarly, the cross-sectional area was
smaller for 15 min of exercise compared with free cage activity ($P < 0.0001$), but 60 min of exercise did not differ from free
cage activity. Both exercise groups had a smaller gap distance
than the free cage activity group (15 min, $P = 0.014$; 60 min,
$P = 0.0002$). Ultimate stress and elastic modulus did not differ
between the exercise groups and full-time cage activity. No
comparison with unloaded controls was done in this experi-
ment.

To further evaluate the influence of the duration of a loading
episode, a linear regression analysis was made including 15,
30, and 60 min of treadmill running. It demonstrated weak
correlations between loading time and peak force ($r^2 = 0.21$;
$P = 0.015$) and cross-sectional area ($r^2 = 0.33$; $P = 0.0013$).
However, this correlation does not mean that further increase
of the daily treadmill running time would yield as strong
tendons as normal cage activity. If one assumes a linear
relationship between running time and peak force, the rats
would have to run for more than 24 h each day, which is, of
course, impossible. Thus the difference in effect between daily

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**Fig. 1.** Box plot for peak force (N) 14 days after tendon transection. 15 min
cage is unloaded tendons with stimulated cage activity for 15 min per day. 15
min, 30 min, and 60 min are unloaded tendons subjected to treadmill exercise
for 15, 30, or 60 min once daily. $2 \times 15\text{ min}$ is unloaded tendons subjected to
treadmill running for 15 min twice daily.

**Fig. 2.** Box plot for cross-sectional area ($\text{mm}^2$) 14 days after tendon transec-
tion.

**Fig. 3.** Box plot for ultimate stress (MPa) 14 days after tendon transection.

**Fig. 4.** Box plot for gap distance (mm) 14 days after tendon transection.
cage activity and treadmill running lies not in time but in the character of the mechanical loading.

DISCUSSION

Full-time cage activity increased the peak force over three times compared with unloading, yet there was no effect on the mechanical properties of the repair tissue. Only the amount was affected and thereby also the peak force. Full-time cage activity had the strongest effect on peak force, but almost half of this effect was achieved by a short loading episode on a treadmill each day. Dividing the daily loading episode in two separate periods had no further effect. This indicates that the tissue “remembered” the stimulation from one loading episode for most of the day. Moreover, prolongation of the single loading episode had minimal effect (see detailed discussion in RESULTS), suggesting that, once the tissue had received information from a certain loading type and level, it led to a response that lasted many hours. However, the stimulus from treadmill running was not sufficient to make the tendon heal as well as the tendons subjected to the loading of free cage activity. This implies that the rats loaded their tendons during cage activity in a way that yielded a better stimulus. The nature of this more optimal stimulus is unknown, but it may be a reasonable guess that it has to do with higher strain rates. Despite the daily loading episodes, the repair tissue could contract as much as the unloaded tendons, or even more, and still gain strength. This implies that patients might be allowed early short training episodes with less risk of elongation compared with full-time activity.

As mentioned, numerous animal studies show that immobilization of an injured tendon compromises the healing process. Still, there is little work carried out on sequential immobilization and mobilization during the healing process. Enwemeka et al. have shown that denotomized Achilles tendons in rats become stronger if immobilized during days 1–5 of 8 days compared with tendons immobilized days 1–2 and 5–8 or all 8 days (5). To our knowledge, there are no experimental studies on the effect of short, intermittent loading episodes on the mechanical properties of otherwise unloaded healing tendons. Clinical trials of surgically repaired Achilles tendon ruptures suggest that clinical outcome, as measured by subjective function scores, may be improved with early mobilization compared with immobilization (11, 16). However, allowing active plantar flexion with weight-bearing did not increase the strength of the muscle (8). It is possible that many patients load their tendons also while immobilized (7). On intact tendons, Reeves et al. have performed a study on the influence of 90 days of simulated microgravity on human Achilles tendon mechanical properties, showing that resistive exercise episodes to some extent can prevent the detrimental effect of unloading (14).

Even though all treadmill exercise groups increased the strength dramatically compared with the unloaded group, there was still much more strength to gain compared with the cage activity group. There was only a weak correlation between loading time and peak force. Extrapolation of the regression line to, say, 9 h of daily treadmill running would still not reach the peak force of the free cage activity animals. This implies that treadmill running does not produce the necessary kind of stimulation, even if the rats had been running all day. What is the difference between treadmill running and full-time cage activity? Full-time activity rats might be able to adjust and gradually increase their loading in an optimal way, thereby giving the new tissue an appropriate mechanical stimulation. In contrast, treadmill running episodes were unchanged throughout the study. The difference may also originate from the fact

<table>
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<th>Table 2. Mechanical results including all three experiments</th>
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<td>Peak force, N</td>
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<td>Length, mm</td>
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<td>Cross-sectional area, mm²</td>
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<td>Gap distance, mm</td>
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<td>SD</td>
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<td>Ultimate stress, MPa</td>
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<td>Elastic modulus, MPa</td>
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15 min c.a. means unloaded tendons with stimulated cage activity for 15 min/day; 15 min, 30 min, and 60 min mean unloaded tendons subjected to treadmill exercise for 15, 30, or 60 min once daily; 2 × 15 min means unloaded tendons subjected to treadmill running for 15 min twice daily; Full-time c.a. means tendons from rats with full-time cage activity. *Differ significantly from unloaded. †Differ significantly from Full-time c.a. ‡Differ significantly from 60 min. Experiment 1: P < 0.0167; experiment 2: P < 0.0083; experiment 3: P < 0.0167.
that rats in cages get more varied mechanical stimulation compared with just running on a treadmill. However, because time had so little influence, the most likely explanation may be that the intratendinous mechanical signals, produced by treadmill running, were suboptimal. We do not know whether a higher or lower strain would be required or whether the clue lies in strain rate.

Cells can perceive strain via cytoskeletal deformation but also via fluid flow at their surface. If cellular deformation is the most important, training regimes for improved mechanical stimulation should optimize tissue strain. If fluid flow were more important, stain rate would be crucial, since it would influence fluid flow velocity.

We can only account for a biomechanical evaluation of the tendons in this study, and we are aware of this limitation. Gene expression analysis might have further explained the mechanisms behind the differences found between unloading, short episodes of loading, and full-time cage activity. The problems with animal “rights” puts a severe constraint on the number of animals that can be tail-suspended, but a new study, addressing the concept of tissue memory by gene expression measurements, is hopefully underway. In contrast, we believe the chances to find important information via histology are limited: because the mechanical properties of the tissue are unaffected, when corrected for size, it is likely that histological differences are subtle.

Our animal model differs from the clinical situation. Rats are quadrupeds and can choose how much to load the injured leg. It seems that humans tend to load their healthy tendons also while in a cast (7). We do not know to what extent this also occurs during healing of ruptured Achilles tendons. It might be that the difference between rats and people in this respect is less than one would think. The rat tendon was cut transversely, whereas human ruptures show frayed ends. However, a human less than one would think. The rat tendon was cut transversely, whereas human ruptures show frayed ends. However, a human would grow only if the difference found between unloading, short episodes of loading, and full-time cage activity. The problems with animal “rights” puts a severe constraint on the number of animals that can be tail-suspended, but a new study, addressing the concept of tissue memory by gene expression measurements, is hopefully underway. In contrast, we believe the chances to find important information via histology are limited: because the mechanical properties of the tissue are unaffected, when corrected for size, it is likely that histological differences are subtle.

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