# ORIGINAL ARTICLE

# A longitudinal study of skeletal muscle following spinal cord injury and locomotor training

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**Study design:** Experimental rat model of spinal cord contusion injury (contusion SCI).

**Objective:** The objectives of this study were (1) to characterize the longitudinal changes in rat lower hindlimb muscle morphology following contusion SCI by using magnetic resonance imaging and (2) to determine the therapeutic potential of two types of locomotor training, treadmill and cycling.

**Setting:** University research setting.

**Methods:** After moderate midthoracic contusion SCI, Sprague–Dawley rats were assigned to either treadmill training, cycle training or an untrained group. Lower hindlimb muscle size was examined prior to SCI and at 1-, 2-, 4-, 8-, and 12-week post injury.

**Results:** Following contusion SCI, we observed significant atrophy in all rat hindlimb muscles with the posterior muscles (triceps surae and flexor digitorum) showing greater atrophy than the anterior muscles (tibialis anterior and extensor digitorum). The greatest amount of atrophy was measured at 2-week post injury (range from 11 to 26%), and spontaneous recovery in muscle size was observed by 4 weeks post-SCI. Both cycling and treadmill training halted the atrophic process and accelerated the rate of recovery. The therapeutic influence of both training interventions was observed within 1 week of training and no significant difference was noted between the two interventions, except in the tibialis anterior muscle. Finally, a positive correlation was found between locomotor functional scores and hindlimb muscle size following SCI.

**Conclusions:** Both treadmill and cycle training diminish the extent of atrophy and facilitate muscle plasticity after contusion SCI.

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**Keywords:** spinal cord injury; skeletal muscle; locomotor training

## Introduction

One of the most common clinical problems after spinal cord injury (SCI) is muscle atrophy and loss of muscle function.[1] Disruption of the neural input as well as decreased loading lead to rapid loss in muscle mass in persons with SCI.[2] This significantly impacts the care and lifestyle of patients and also increases their risk to develop secondary health problems such as cardiovascular disease and diabetes.[3] Therefore, a major area of interest is the prevention or attenuation of muscle atrophy associated with SCI. Therapeutic interventions that focus on locomotor training have shown the potential to promote muscle plasticity in lower extremity muscles.

One way to evaluate the effects of therapeutic interventions is to use an animal model of SCI.[4,5] Transection of the

spinal cord has been widely used to study SCI, because it allows for a reproducible (complete) injury. However, contusion injury of the spinal cord seems to more closely reproduce the histopathological features observed in human trauma cases.[6,7] Although contusion injuries have been performed in a variety of animal species, electrophysiologic, behavioral and imaging studies have indicated that SCI in rats reasonably models events that occur after human SCI.[8,9] Therefore, contusion SCI in the rat appears to be a clinically relevant model to use for the assessment of intervention therapies following SCI.

Rehabilitation strategies that utilize repetitive locomotor activity, such as treadmill and stationary bicycle training, have yielded encouraging results.[5,10] Recent evidence indicates that individuals with incomplete SCI observe an improvement in ambulatory ability, gain functional independence and demonstrate increased muscle size following 9–12 weeks of body weight-supported treadmill training.[11–14] However, the implementation of this intervention in the clinic may be particularly challenging due to the cost of the

equipment/infrastructure and intensive personnel requirements. On the other hand, a motor-driven stationary bicycle is spatially compact, and cycle locomotor training may be performed with fewer facilitators.[15] Additionally, since bicycle exercise can be performed in a seated or recumbent position, it may offer the feasibility of initiating a training program earlier following injury. Since there are significant practical differences in the performance of treadmill versus cycle locomotor training, the relative efficacy of each type of training modality in reversing muscle atrophy induced by SCI should be addressed.

The purpose of this study was twofold. The first objective was to quantify the changes in muscle morphology of rat lower hindlimb muscles following midthoracic contusion SCI using magnetic resonance (MR) imaging. The use of MR imaging provides a noninvasive approach, which allows longitudinal repeated measures of muscle morphology. A second objective was to compare the effects of cycling and treadmill training on the attenuation of muscle atrophy following spinal cord contusion injury.

## Materials and methods

### Experimental animals
Twenty-four Sprague–Dawley rats (female, 228–260 g; Charles River, NJ, USA) were used in this study. The rats were housed in a temperature-controlled room at 21 °C and were provided unrestricted access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

### Spinal cord contusion injury
Spinal cord contusion injuries were produced using the protocol described previously.[16] Briefly, a 10-g weight (New York University impactor) was dropped from a 2.5-cm height onto the T8 segment of the spinal cord, which was exposed by laminectomy. Animals were given buprenophine (0.05 mg kg$^{-1}$) and ketoprofen (5.0 mg kg$^{-1}$, s.c.) for pain and inflammation over the first 36 h after SCI. Manual expression of bladders was performed two to three times daily until spontaneous voiding returned. Animals were subsequently assigned (randomly) to either treadmill training, cycle training or an untrained group.

At postoperative day 7, open-field locomotion was assessed using the Basso, Beattie, Bresnahan (BBB) Locomotor Scale to determine if the animals met inclusion criteria for this study. Because of potential variability in the severity of the injury, animals that did not fall within a preset range (3–7) were regarded as too mildly or severely injured and were excluded from the study.

### Magnetic resonance imaging
Magnetic resonance imaging was employed to measure the cross-sectional areas (CSAs) of four different hindlimb muscles. Rats were anesthetized with 1–2% gaseous isoflurane in oxygen and placed on a cradle in the prone position with the right leg secured in the center of the coil. All imaging procedures were performed in a horizontal, 4.7-T
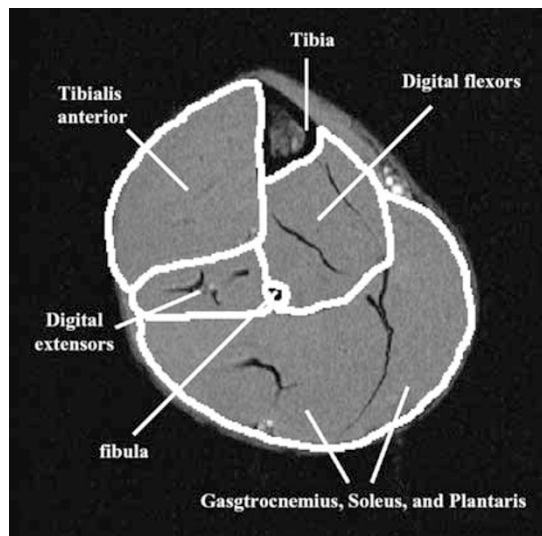


**Figure 1** Outline of tibialis anterior, triceps surae, extensor digitorum and flexor digitorum muscles on a representative transaxial magnetic resonance image of the rat lower hindlimb. Data were acquired with a slice thickness of 1 mm and field of view of 2.5 × 2.5 cm.

magnet with Paravision 2.1.1 software (Bruker Medical, Ettlingen, Germany).

Three-dimensional proton MR images were obtained prior to injury and at 1, 2, 4, 8 and 12 weeks post injury. The three-dimensional data were acquired with an encoding matrix of 516 × 256 × 64, field of view of 2.5 × 2.5 × 4 cm, slice thickness of 1 mm, pulse repetition time of 100 ms and an echo time of 6.4 ms. The total region imaged extended from the knee to the calcaneus. Owing to the lack of a clear division (thin fascia) between individual muscles, muscle functional groups were segmented. Specifically, we determined the CSA of the tibialis anterior, triceps surae, extensor digitorum and flexor digitorum muscles for each 1-mm slice and recorded the maximal CSA (CSA$_{max}$) of each muscle group (Figure 1). Image analysis was performed using a custom-designed interactive computer program, EXTRACTOR.[17]

### Treadmill and cycling locomotor training
In both types of training, animals were trained continuously for 3 months (5 days per week, two sessions per day, 20 min per session), starting on postoperative day 8.

Treadmill training consisted of quadrupedal treadmill stepping. Body weight support was provided manually by the trainer when necessary. The level of body weight support was adjusted to make sure that the hindlimbs of the animals did not collapse and was gradually removed as locomotor capability improved. Typically, the rats started stepping when they experienced some small load on their hindlimbs. In addition, during the first week of training, when all rats had profound paraplegia, assistance was provided to place the rat hindpaws in plantar-stepping position during training.

The design of the motor-driven cycle trainer used in these studies was adapted from the one developed by Houle et al.[18]

The bicycle is composed of a direct drive gear box, adjustable foot pedals and a support harness. The animal was suspended in the harness, and the hindlimb feet were strapped onto the pedals of the motorized cycle. The exercise consisted of a circular pedaling motion, which produced flexion of one limb and extension of the other. During each cycle, the ankle joint was dorsiflexed 45–90°. The pedaling rate was set at 31 rotations $min^{-1}$ as it approximates the self-selected cadence at which animals walk on the treadmill. During the first week of training, the rat tail was attached to an aluminum support beam to maintain the trunk stability during exercise and approximately 70% of body weight was supported. Gradually, the load on the hindlimbs was increased by positioning the body harness toward the chest and allowing the hind portion of the body to fall over the pedal ($\sim$30–40% body weight support).

*Open-field locomotor function*
Open-field locomotion was assessed by using the BBB Locomotor Rating Scale as a measure of general locomotor function.[19] The BBB scale is an operationally defined ordinal scale, which measures locomotor behavior. Each of the components of the 21-point scale is based on specific features of locomotor recovery after spinal cord contusion including joint movement, trunk posture, stepping and weight support ability, paw position and tail position.

*Statistical procedures*
Research hypotheses were tested at an α-level of 0.05. One-way analysis of variance for repeated measurements was performed to test changes in $CSA_{max}$ following contusion SCI. Training effects on the $CSA_{max}$ of the hindlimb muscles were assessed using two-way repeated measures analysis of variance (group $\times$ time). *Post hoc* tests were performed using Bonferroni–Dunn procedure for multiple pairwise comparisons.

## Results

*Effects of spinal cord contusion injury on hindlimb muscle size*
The effects of spinal cord contusion injury on muscle size was determined in four different hindlimb muscle groups (Table 1). The injury resulted in significant atrophy in all lower hindlimb muscles studied. The degree of atrophy appeared to be muscle specific, with the posterior muscles (triceps surae and flexor digitorum) showing greater atrophy than the anterior muscles (tibialis anterior and extensor

digitorum). The most extensive amount of atrophy was observed at 2 weeks post injury. At this time, atrophy was greatest in the triceps surae and least in the extensor digitorums. Starting between the second and fourth week post-SCI, there was a reversal in muscle atrophy as indicated by a steady increase in the $CSA_{max}$ of each muscle. By 12 weeks post-SCI, the $CSA_{max}$ of all muscles studied had recovered to values that were no longer statistically different from preinjury control values.

*Effect of treadmill and bicycle locomotor training on muscle size*
Both types of locomotor training resulted in increased muscle size at all time points (Figure 2). It is interesting to note that a significant increase in muscle size was observed as early as 1 week after the onset of training. For instance, at 2 weeks post-SCI, the $CSA_{max}$ of the triceps surae was $73.4 \pm 4.1\%$ of preinjury values in the untrained SCI group and $88.8 \pm 1.4$ and $91.2 \pm 0.9\%$ in the cycling and treadmill training groups, respectively (Figure 2a). As shown in Figure 2b, a similar acute response to training was observed in the tibialis anterior muscle as well as the extensor and flexor digitorum muscles (data not shown). Consequently, by 2 weeks post-SCI, the total CSA of the rat hindlimb muscles was 10–13% greater in the locomotor-trained groups compare with the untrained group (Figure 3).

The therapeutic effect of locomotor training was observed not only at this early time point but also throughout the remaining training period. The total CSA in both cycling and treadmill locomotor training animals was significantly greater than that of untrained SCI animals at both 4 and 8 weeks post-SCI. Finally, a direct comparison between the cycling- and treadmill-trained animals showed no significant difference between the two locomotor training interventions for all muscles studied, except the tibialis anterior muscle. The $CSA_{max}$ of the tibialis anterior muscle was fully recovered at 4 weeks post-SCI in the treadmill training group, whereas in the cycling training group preinjury values were reached at 8 weeks post-SCI.

*Relationship between hindlimb muscle size and locomotor function*
To determine if the recovery of muscle size is associated with an improvement of animal locomotor function, we examined the relationship between muscle size and locomotor function. Both types of locomotor training resulted in improved locomotor function, with significantly higher BBB scores at all time points (4, 8 and 12 weeks). At 12

**Table 1** Maximal cross-sectional area ($CSA_{max}$, mm$^2$) of individual muscle

| | Preinjury | Post-SCI | | | | |
|---|---|---|---|---|---|---|
| | | *1 week* | *2 week* | *4 week* | *8 week* | *12 week* |
| Extensor digitorums | 14.5 ± 0.1 | 13.2 ± 0.3 (90.6%)* | 12.9 ± 0.4 (88.9%)*,§ | 13.9 ± 0.4 (95.9%)§ | 14.5 ± 0.3 (99.9%) | 14.8 ± 0.3 (102.2%) |
| Flexor digitorums | 32.1 ± 0.7 | 27.8 ± 0.7 (86.8%)* | 26.9 ± 1.1 (84.6%)*,§ | 28.6 ± 1.1 (90.3%) | 31.1 ± 0.1 (98.1%) | 32.3 ± 0.9 (102.1%) |
| Tibialis anterior | 41.8 ± 0.9 | 36.9 ± 1.4 (88.3%)* | 33.3 ± 1.7 (79.5%)*,§ | 35.9 ± 1.8 (87.5%)* | 38.8 ± 1.4 (94.9%) | 40.3 ± 1.5 (98.3%) |
| Triceps surae | 106.4 ± 3.3 | 88.7 ± 3.5 (83.4%)* | 78.1 ± 4.4 (73.7%)* | 91.5 ± 2.6 (86.3%)* | 98.5 ± 3.3 (92.9%)* | 101.3 ± 3.5 (95.4%) |

Abbreviation: SCI, spinal cord injury.
Values are means ± s.e.m.* Significantly different with preinjury value ($P < 0.05$). §Significantly different with triceps surae ($P < 0.05$).
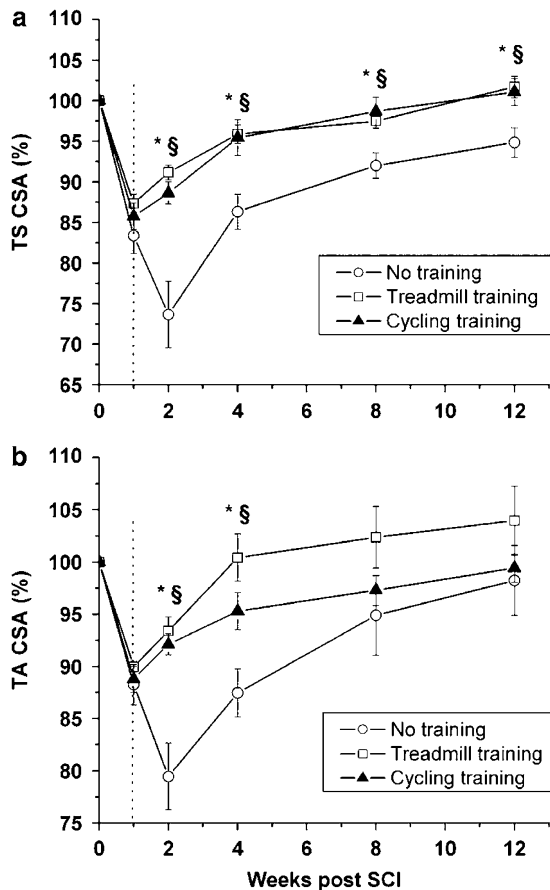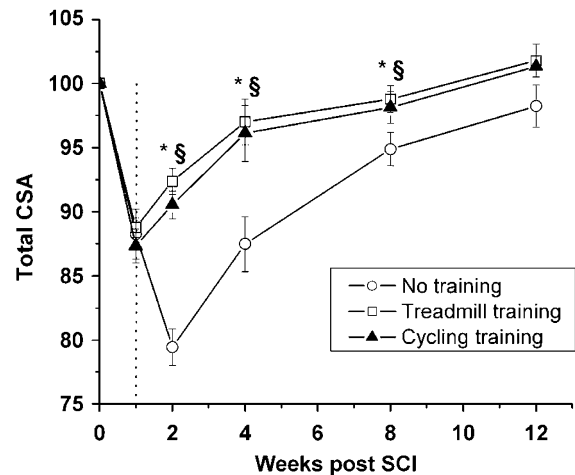
Figure 2 Relative change in the maximal CSA (CSA$_{max}$) of the triceps surae (TS) (**a**) and tibialis anterior (TA) (**b**) in the no training group, cycling training group and treadmill training group. Data are expressed as a percentage of the CSA$_{max}$ measured at preinjury. Training started at 1 week post injury (dashed line). *Statistically significant differences between no training group and treadmill training group ($P<0.05$). §Statistically significant differences between no training group and cycling training group ($P<0.05$). CSA, cross-sectional area.



Figure 3 Relative change in total maximal CSA (CSA$_{max}$) in the no training group, cycling training group and treadmill training group. Data are expressed as a percentage of the CSA$_{max}$ measured at preinjury. Training started at 1 week post injury (dashed line). *Statistically significant differences between no training group and treadmill training group ($P<0.05$). §Statistically significant differences between no training group and cycling training group ($P<0.05$). CSA, cross-sectional area.



Figure 4 Relationship of rat locomotor function (Basso, Beattie, Bresnahan (BBB) scale) and total low hindlimb limb maximal CSA (CSA$_{max}$; $r=0.71$, $P<0.001$). Data of all the rats at each time point were pooled together. Note that the line is drawn for visual purposes only and not to indicate a linear function. CSA, cross-sectional area.
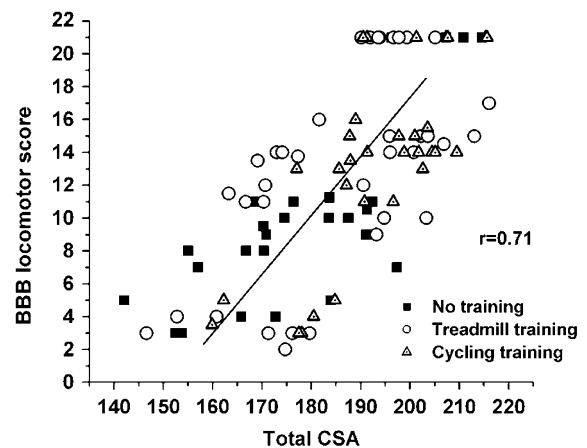
weeks post-SCI, the untrained SCI animals showed an average BBB score of 11.13, while bicycle- and treadmill-trained SCI animals showed an average BBB score of 14.64 and 15.09, respectively. Figure 4 provides a plot of the hindlimb total CSA versus the respective BBB scores of the animals. All groups of animals, uninjured, treadmill-trained, cycling-trained and injured with no training were included. When a correlation was performed with these data, there was a significant positive relationship between scores on the BBB locomotion rating scale and rat hindlimb total CSA$_{max}$ ($r=0.71$, $P<0.001$). However, it should be pointed out that even though in the locomotor-trained animals muscle size reached normal values during the course of training, locomotor function did not fully recover.

## Discussion

Many investigators have reported a rapid loss of muscle mass following SCI.[2,20–22] A large number of these studies were performed in spinal cord-transected or -isolated studies.

However, only a limited number of studies examined the effects of spinal cord contusion injury on skeletal muscle morphology.[16,23] Hutchinson et al.[23] reported a significant decrease in muscle wet weight (20–25%) in all lower hindlimb muscles, except the EDL, at 1week following moderate contusion injury. They also reported that muscle atrophy occurred in flexor as well as in extensor muscle groups and that the severity was similar in fast (tibialis anterior 21%) and slow muscles (solues (SOL) 22%). In contrast, a pattern of differential amounts of atrophy was noted in the present study. At 2 weeks post spinal cord contusion injury, when muscle CSA measures revealed the greatest degree of atrophy, MR quantitative assessments

showed the following atrophic hierarchy: triceps surae > tibialis anterior > flexor digitorums > extensor digitorums. This differential muscle response may possibly be attributed to the higher neuromuscular activity and loading in extensor muscles relative to flexor muscles during normal ambulation.[24] As a consequence, the relative loss in neuromuscular activity post-SCI may lead to more extensive muscle atrophy in the antigravity extensor muscles such as the triceps surae.

One interesting finding in this study is that there is spontaneous reversal of muscle atrophy following contusion injury of moderate severity. Recovery of muscle atrophy in untrained animals was first noted by 4 weeks post-SCI. The rate of spontaneous recovery ranged from 0.1 to 6.3 mm$^2$ per week. A similar pattern was reported by Hutchinson et al.,[23] who found that muscle atrophy was attenuated starting at 3 weeks post spinal cord contusion injury. However, patients with incomplete SCI do not show spontaneous recovery as seen in the animal model. Individuals with incomplete SCI still have significantly smaller (24–31%) muscle CSA in affected lower extremity muscles 5–30 months after injury.[2] In contrast, models of SCI involving transection of the spinal cord have shown little spontaneous recovery in muscle size.[25] This is probably due to the fact that communication between the supraspinal centers and caudal region of the spinal cord is eliminated. In addition, the restoration in muscle size in the moderate contusion injury model appears to mirror the spontaneous improvement in locomotor function. Basso et al.[7] showed that after a moderate thoracic spinal cord contusion injury, animals demonstrate hindlimb paralysis until 7 days post injury, which is followed by a progressive recovery in locomotor function over the next 5 weeks. In the present study, we observed a significant positive correlation between locomotor rating scale and hindlimb total $CSA_{max}$, supporting the contention that changes in muscle size contribute to gains in locomotor function and vice versa.

Repetitive locomotor training has been shown to promote neural plasticity and facilitate motor recovery following SCI.[5,26] Treadmill locomotor training takes advantage of the phasic, peripheral sensory information and loading that are associated with a rhythmic stepping pattern to provide critical activity in the remaining spinal cord circuits and facilitate neural recovery. Bicycle locomotor training uses a somewhat different strategy and is accomplished by simple circular movements of the hindlimbs on a bicycle-type device driven by a motorized belt.[27] Electromyographic data acquired in previous studies show that during cycling, the left and right hindlimb muscles are stretched in an alternating pattern, which results in alternating bursts of muscle activity in SCI animals.[18] Thus, cycling training also initiates sensory input to the spinal cord, and subsequently influences the firing pattern of the motoneurons that innervate the hindlimb muscles. Houle et al.[18] found that cycle locomotor-trained animals showed an increase in hindlimb muscle size following SCI. Additionally, cycling training has been found to normalize hyperreflexia in spinal rats[28] as well as in humans with SCI.[15] In the present study, we performed a direct comparison between bicycle and treadmill locomotor training and showed that both training interventions

effectively halt the atrophic process and accelerate the rate of muscle recovery. Interestingly, in both interventions significant muscle plasticity (6.3–7.1 mm$^2$ per week) was observed as early as 1 week after initiation of training. Finally, no significant differences were noted between the two locomotor training interventions in all muscles, except for the tibialis anterior muscle that showed slightly lower values in the cycling group between 4 and 12 weeks post-SCI. One possible explanation for the apparent smaller response in the tibialis anterior muscle is that during cycling revolutions the ankle was kept in a dorsiflexed (45–90°) position on the pedal, minimizing the stretch reflex in the tibialis anterior muscle.

Although there are some limitations in using animal models to understand human SCI recovery with locomotor training, these models do potentially offer a more careful analysis of the functional properties of complex systems. The results of the present study suggest that both treadmill and cycle training diminish the extent of atrophy and facilitate muscle plasticity after SCI. Since muscle atrophy is associated with a myriad of secondary health problems, the potential of maintaining muscle mass with repetitive training is exciting and warrants further research.

## Acknowledgements

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