Time to recovery of sciatic function index after induced tibialis anterior strain in rats

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Summary

***Background*: Muscle strain is a common injury with a high recurrence rate. Due to the heterogeneity of strain injuries, experimental animals provide controlled and reproducible models to investigate such injuries. Sciatic Function Index (SFI) is a clinically feasible method to assess hind limb recovery in rodents after induced injuries.**
***Objectives*: To investigate time to recovery of SFI after induced-strain in tibialis anterior (TA) muscle in rats.**
***Methods*: Sixteen adult male Wister rats were randomly and equally divided to a normal control group that received no intervention, and TA induced muscle strain group. Muscle strain was induced using an external weight that corresponded to 150% of the animal body weight. SFI was tested only once in the control group. For the muscle strain group, SFI was tested on the 1st, 2nd, 3rd, 4th, 7th, 11th, 20th and 24th days after strain induction.**
***Results*: Comparisons between group showed significant difference in SFI on the 1st, 2nd, 3rd and 4th days (p= 0.012, 0.012, 0.012 and 0.028, respectively).**
***Conclusions*: In a rat animal model of TA induced muscle strain, functional recovery measured by SFI is evident on the 7th day post-injury, which corresponds to the sub-acute phase of injury.**
Level of evidence: V.

KEY WORDS: sciatic function index, muscle strain, functional recovery, rats.

Introduction

Walking track analysis is a commonly used assessment tool to quantify functional recovery after hind limb injuries in rodent animal models. Many indices have been used to quantify changes in walking pattern after nerve injury, for example, the Sciatic Function Index (SFI), peroneal function index and tibial function index[1]. SFI is a popular functional test as it reflects the whole function of the hind limb. It has been used after both nerve and strain muscle injuries[2,3]. However, SFI has never been validated to assess functional recovery following strain induced muscle injury.
Muscle strain is very common and has a high recurrence rate, long recovery time and a significant economic impact, in terms of days lost in sports and work[4,5]. Strain usually occurs when the muscle is subjected to a load beyond its failure point. Injuries usually occur at the myotendinous junction[6]. It is manifested clinically by immediate onset of disabling pain that limits the function, followed by weakness and decreased range of motion[5,7]. Basic research in a controlled experimental setting may clarify structural and functional changes associated with strain injury, which can help in better understanding and treating strain injuries. In lower limbs, locomotion is one of the most important functional outcomes to consider. It is crucial for researchers and clinicians to have scientific evidence supporting whether lower limb returns back to function during the acute, subacute or chronic stages of myotendinous healing. Thus, exercise prescription and assessment can be tailored accordingly. Therefore, this study investigated the natural history of functional recovery following a controlled induced strain injury.

Objective

The objective of this study was to investigate the time taken to restore normal SFI values after induced-strain of Tibialis Anterior (TA) myotendinous in a rodent animal model.

Methods

Sixteen male Wister rats, weighing 169 ± 23 grams, were enrolled in this study. Animals were housed in standard cages, and were kept under a controlled

temperature and a balanced day/night cycle of 12 hr. All animals were fed regular plates and were given water ad libitum. The experiment design and housing condition of animals were approved and done in accordance to the animal care guidelines of the Institutional Animal Care and Use Committee (IACUC) of Kasr AL Ainy medical school, Cairo University, Egypt, and in accordance to ethical standards of the international guidelines[8].

Animals were randomly divided into two equal groups using the Excel software random generation function as follows: (I) a normal control group that did not receive any interventions and (II) a TA myotendinous strained animal (experimental) group. Following randomization, all animals were color coded and numbered.

Strain induction

The right TA muscle was strained using a simple valid and non-invasive protocol[2], whereas the left limb served as an internal control.

First, each rat was weighed using a commercially available weighing scale. Then, each rat was anaesthetized by intramuscular injection of ketamine/xylazine (100 and 20 mg kg^{-1} respectively)[3]. The right hind limb was positioned with the knee extended using a 20 x 12.5 x 7.5 cm costumed made wooden frame (Fig. 1). The right foot was strapped to a moveable hinged footplate. Each footplate had a metal hock fixed to its outer surface so that external weights could be hanged and dangled freely.

To induce myotendinous strain, an external total weight corresponding to 150% of the animal body weight was attached gradually to the footplate. A weight corresponding to 25% of animal's body weight was attached first, and then the weight was gradually increased with 25% increments every one minute until the total weight was attached. The full weight was kept for 20 minutes. The total procedure was repeated twice; with a 3 minute interval in between[2].

SFI measurement

To calculate SFI, animals were made to walk in a 1-meter length wooden walkway that was covered with squared paper sheet using adhesive plaster. First, animal paws were dipped in blue ink. Then, the animal cage was placed at the end of the walkway to motivate the animal to walk in this direction.

Animals were allowed several conditioning trials before data were collected. A total of three trials were collected and the clearest print was selected for measurement of SFI by a trained observer[9]. If the paw print was unclear or distorted, for example by tail dragging, smearing of the print, too much or dry ink, or contamination of the front limbs, print data were

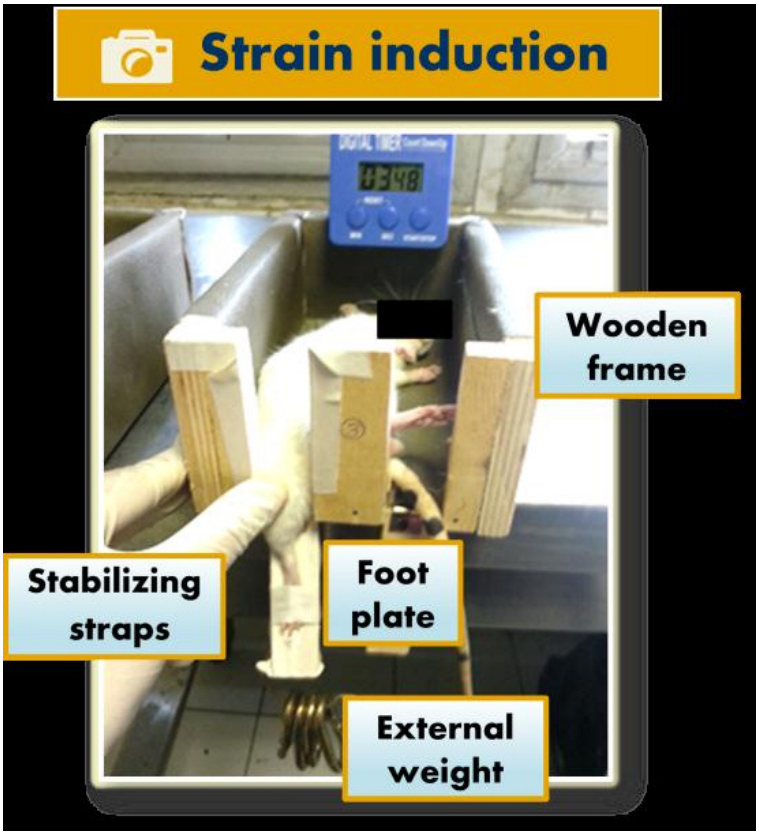

Figure 1. Strain induction set up.

discarded and data collection was repeated[1,10].
The SFI of control animal group was measured once, whereas that of the experimental animal group was measured on the 1st, 2nd, 3rd, 4th, 7th, 11th, 20th and 24th days after injury (Fig. 2).

Outcome measures

The main outcome measure was the SFI. This is a valid and reliable index that ranges between -11 or +11; according which limb is involved. Values exceeding this range indicates impaired or lost hind limb function[11].
SFI is calculated using the following parameters: *Print length (PL):* this refers to the distance from the center of heel to the tip of the third toe; *Toe spread or Total spread (TS)* represents the linear distance from the first to the fifth toe; and *Intermediate toe spread (ITS)* which is the linear distance from the second to the fourth toe.
All these measurements were taken from the experimental (injured) and normal (uninjured) sides, as following, NPL (normal print length), EPL (experimental print length), ETS (experimental toe spread), NTS (normal toe spread), EIT (experimental intermediary toe spread) and NIT (normal intermediary toe spread). The SFI is then calculated using the following equation

$$[\mathrm{SFI} = -38.3\frac{\mathrm{EPL}-\mathrm{NPL}}{\mathrm{NPL}} + 109.5\frac{\mathrm{ETS}-\mathrm{NTS}}{\mathrm{NIT}} + 13.3\frac{\mathrm{EIT}-\mathrm{NIT}}{\mathrm{NIT}} - 8.8]^{1}.$$

First, essential landmarks were marked on the feet print sheet using a black ink pin. Then, each distance was measured and recorded manually using microsoft excel.
A video recording of animal walking pattern was concomitantly used to assist deciding on unclear traces as needed[10,11]. If the assessor was unable to measure any parameter of interest due to, for example, toe drag or non-weight bearing status of the injured limb, a fixed value was then used as follows; EPL = 80 mm, ETS = 6 mm, and EIT = 6mm[11].

Statistical design

The main outcome measure was SFI values during the first 3 weeks following TA induced muscle injury in a rat model. This period was selected to reflect the

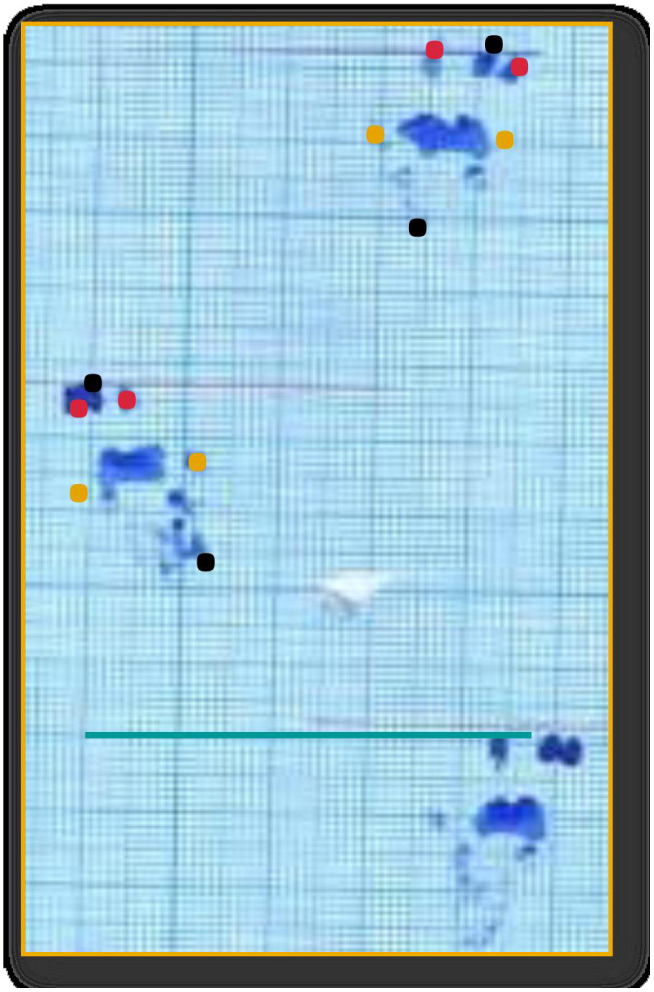

Figure 2. SFI measured parameters.
1. (●) Total spread; 2. (●) Print length; 3. (●) Intermediary spread.

acute, subacute and chronic phases of injury.
Between group comparisons at different time points were done using the non-parametric Mann-Whitney U test. All statistical analyses were done using the SPSS; with the significance level set at $p<0.05$.

Results

For the control group, the median SFI was (-9) and ranged between (-29) and (-2). For the experimental group, the median was (-46) at the 1st day post injury and ranged between (-169) and (-14).

Between-groups comparisons showed significant deterioration of SFI in the experimental group compared to the control animal group on the 1st, 2nd, 3rd and 4th days (p-value= 0.002, 0.002, 0.005 and 0.02, respectively). For the remaining time points, no significant differences were found between the two animal groups ($p\geq0.05$). SFI was not significantly different between groups starting from the 7th day post injury, with the median SFI was (-24) and ranged between (-83 and 14) (Tab. I, Fig. 3).

Further, analysis of SFI individual parameters showed significantly higher EPL compared to NPL on the 1st and 2nd days (p-value= 0.01, and 0.003, respectively), whereas ETS showed significantly lower values compared to NTS only on the 1st post injury (p-value= 0.038). On the other hand, EIS and NIS

Table I. The SFI values for control and experimental animal models. The median, minimum and maximum for each group at various time points tested.

		SFI **Median [** (min) - (max) **]**
Control		**-9** [(-29) – (-2)]
Experimental group	**1st**	**-46** [(-169) – (-14)]
	2nd	**-90.5** [(-152) – (-19)]
	3rd	**-32** [(-144) – (-10)]
	4th	**-33**. [(-112) – (-9)]
	7th	**-24** [(-83) – (14)]
	11th	**-27** [(-96) – (-2)]
	20th	**-26.5** [(-43) – (6)]
	24th	**-22** [(-66) – (4)]

SFI median, minimum and maximum for each group and time point.

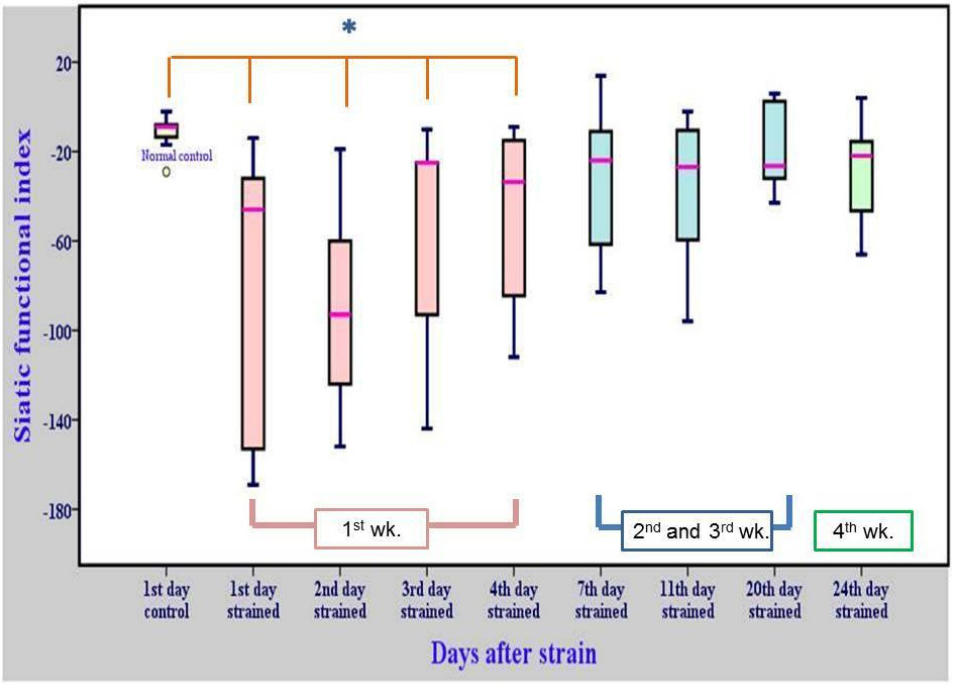

Figure 3. Boxplots showing SFI values in the control and experimental animal groups at different time points during healing stages: 1. (●) acute stage; 2. (●) sub-acute; 3. (●) chronic. (*) indicates significant differences between the two animal groups.

Table II. Print length, total spread and intermediary spread for control and experimental animal models. The median, minimum and maximum for each group at various time points tested.

Group	Print length **Median** (Min-Max) mm	Total spread **Median** (Min-Max) mm	Intermediary spread **Median** (Min-Max) mm
Control	**2.7** (2.2 - 3)	**1** (0.6 - 1.7)	**0.75** (0.5 - 0.8)
Experimental			
1st	**3.25** (2.5 - 8.0)	**1.0** (0.6 - 1.7)	**0.65** (0.5 - 0.8)
2nd	**3.10** (2.7- 8.0)	**0.70** (0.5 - 1.9)	**0.70** (0.5 - 0.9)
3rd	**2.80** (2.4 - 8.0)	**1.20** (0.3 - 1.9)	**0.80** (0.3 - 0.9)
4th	**2.65** (2.2 - 2.9)	**1.45** (0.4 - 2.0)	**0.55** (0.4 - 1.0)
7th	**2.25** (1.3 - 3)	**1.55** (0.5 - 2.0)	**0.75** (0.5 - 0.9)
11th	**2.45** (1.1 - 2.9)	**1.50** (0.3 - 1.9)	**0.70** (0.3 - 0.9)
20th	**2.50** (1.3 - 2.9)	**1.60** (1.1 - 2.0)	**0.75** (0.6 - 1.0)
24th	**2.55** (1.3 - 3.1)	**1.45** (0.6 - 1.9)	**0.75** (0.4 - 1.0)

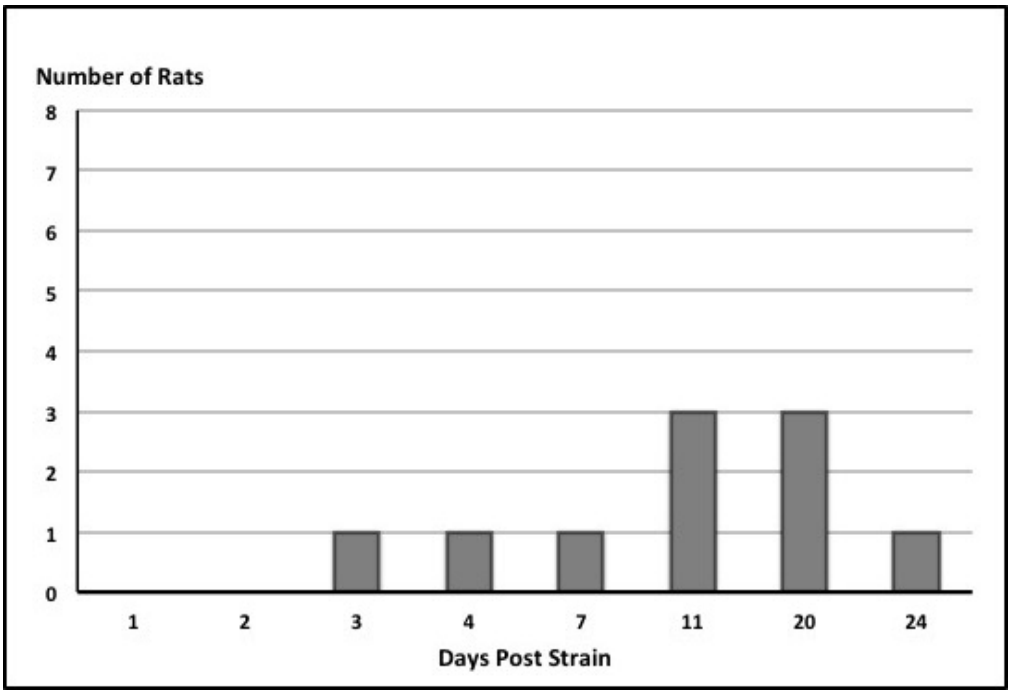

Figure 4. Number of rats achieving the normal SFI value (±11) at different time points.

showed no statistical significant differences at any time point ($p>0.05$) (Tab. II).

The maximum number of animals that achieved the normal SFI (ranging between 11 and -11), was 3 animals out of 8 (37.5%) on the 11th and 20th days (Fig. 4).

Discussion

The main purpose of this study was to investigate time to recovery of SFI after induced-strain injury of TA muscle in a rat animal model. This muscle was selected for strain induction due to its easy accessibility[12]. Further, it is a parallel muscle with all fibers arranged longitudinally[13], thus, the externally applied force can be assumed to act perpendicular on all fibers[12]. Additionally, the TA muscle has been widely used and tested in animal models. For example, it was used in contraction induced muscle injury in rodent animal models[14-17]. It was also used in testing the effect of passive induced muscle injuries[2,3,18].

Controlled muscle strain was first introduced by Nikolaou et al.[18] who exposed and detached TA distal end then strained it under tension using the Instron testing machine. This model was later modified by Ramos et al.[2] to allow non-invasive induction of a consistent muscle injury pattern similar to that observed in humans following excessive stretching[2,3].

In this study, a controlled passive non-invasive second degree myotendinuous strain was induced[2,18]. The SFI was the main outcome measure to assess functional recovery of hind limbs. Altered locomotion pattern is usually the first reported sign following induced strain injury[2,19]. The SFI has the advantage of assessing animals as they voluntarily walk, which provides a scenario similar to that seen in humans after strain injury. Alternatively, muscle performance recovery could be assessed using electric stimulation[18]. This requires muscle to contract involuntarily and does not consider the influence of pain on functional recovery. The results of the current study showed a significant reduction in SFI value in the experimental compared to control animal groups. This implies a deteriorated hind limb function. Previous studies have reported a reduction in various animal models of muscle injury. For example, Paiva Carvalho et al.[3] observed the deterioration in function six hours post strain induction, with SFI values significantly deteriorated to 30 compared to the control. Also, Ramos et al.[2] measured the SFI at 6, 12 and 24 hours post strain induction. The three time points were significantly differed from normal control. Deterioration in SFI peaked at 12 hours (40.99± 10.81), yet differences in SFI in strained muscle animals were not substantial between 6 and 12 hours[2]. In the current study, 24-hour time point was selected as a start point to assess SFI following injury, as earlier animals showed an obvious avoidance behavior to bear weight on the affected limb. The reduction of SFI was significant from first point measurement and then started to improve after the 2nd or the 4th day post strain.

Immediate functional deterioration may be attributed to reduced muscle performance and fatigue resistant[3,20]. This could have been accentuated by the presence of histopathological changes[18] that may directly interfere with force generation capabilities of the muscle[2] or indirectly through muscle inhibition by pain[18]**.** In animal models of arthritis, pain affected muscle function and interfered with the ability to bear weight on the affected side[21]. A similar response to pain might occur from TA induced strain. These changes are characteristics of post traumatic inflammation that lasts between the 1st and 7th day[18].

By the 7th day, the SFI values were not different between the two animal groups indicating restoration of hind limb function. This is in agreement with the findings of Nikolaou et al. who attributed similar recovery to ending of the inflammatory stage of injury and beginning of the sub-acute stage. The later stage is characterized by marked reduction in inflammation and leukocytes number, resolution of edema, and absence of hemorrhage. By this stage, fibroblasts mature into elongated fibrocytes and localized fibrosis could be seen[18].

By the 24th day the number of rats within the normal range started to decrease again which may be attributed to the formation of adhesions accompanied by the chronic stage resulted in decreasing range of motion and eventually affect SFI.

TA muscle injury resulted in increasing print length due to rat inability to dorsiflex its foot[1]. This was supported by the current findings that print length was the most affected parameter and needed a longer time to return normal.

In contrast, results showed that the intermediary spread has not been affected by the TA injury and did not differ significantly from normal control group despite the injury. Whereas, total spread parameter returned to normal values from the second day post injury and this could be attributed to the pain or the difficulty to weight bear on the affected limb that is accompanied the acute stage of the injury, which is relieved as soon as the stage passed.

It should be emphasized that the normal SFI ranges between 11 and -11[11], however, in the current study two control animals showed exceeding values (-17 and -29). A couple of studies have reported similar ranges in normal animals[22,23].

This study has a few strengths such as the use of a valid, non-invasive simple and feasible method to induce strain and to assess its natural functional recovery. However, a few limitations exist. In this study, animals were tested for 24 days, which represents the acute, sub-acute until the early beginning of chronic stage of myotendinuous recovery. A longer follow-up period into the chronic stage is recommended. Furthermore, although this method is simple and valid, a more detailed kinematic and kinetic analysis using laboratory instruments may give more detailed information on functional recovery. Validation of SFI recovery against biomechanical and histological analyses could add more prospective in understanding this animal model.

Conclusion

In a rat animal model of TA induced muscle strain, functional recovery measured by SFI is evident on the 7th day post-injury, which corresponds to the sub-acute phase of injury.

Conflict of interest

Nothing to disclose.

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