Neuroscience Letters 492 (2011) 170–174

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Treadmill step training-induced adaptive muscular plasticity in a chronic paraplegia model

Jocemar Ilha[a,b], Núbia B. da Cunha[a], Mariane Jaeger[b], Daniela F. de Souza[c], Patrícia S. do Nascimento[a,b], Simone Marcuzzo[a,b], Micheli Figueiró[a], Carmem Gottfried[a,c], Matilde Achaval[a,b,*]

[a] Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS, Brazil
[b] Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS, Brazil
[c] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 30 November 2010
Received in revised form 1 February 2011
Accepted 1 February 2011

Keywords:
Spinal cord injury
Treadmill training
Soleus muscle
Muscular trophism
BDNF expression

ABSTRACT

The purpose of this study was to provide evidence that treadmill step training is capable of attenuating muscle atrophy and may regulate brain derived neurotrophic factor (BDNF) in soleus muscle after complete spinal cord transection (SCT) at T8–T9 in rats. Five days after SCT, spinal animals started a 9-week step-training program on a treadmill with partial body weight support and manual step help. The muscular trophism was studied by analyzing muscle weight and myofiber cross-sectional area of the soleus, while Western blot analysis was used to detect BDNF expression in the same muscle. Step training, initiated immediately after SCT in rats, may partially impede/revert muscular atrophy in chronic paralyzed soleus muscle. Moreover, treadmill step training promoted upregulation of the BDNF in soleus muscle, which was positively correlated with muscle weight and myofiber cross-sectional size. These findings have important implications for the comprehension of the neurobiological substrate that promotes exercise-induced effects on paralyzed skeletal muscle and suggests treadmill training is a viable therapeutic approach in spinal cord injuries.

Skeletal muscle is a dynamic tissue that can adapt to mechanical stimulus such as reduced neural activity after nervous system injuries and/or muscular training [24]. Spinal cord injury (SCI) is a devastating neurological condition that produces muscular pareses/paralyses caudal to the lesion level, leading to a pronounced loss of muscle mass and severe muscle atrophy [9,31]. This paraplegia-induced muscle atrophy increases the risk of developing secondary health problems such as cardiovascular disease and diabetes in paraplegic patients [7].

Though the muscle to body weight ratio, muscle and myofiber cross-sectional area (CSA) and muscle function are reduced in several muscles after SCI in animal models [9,22,23,25,31], the greatest damage is mainly seen in postural muscles composed predominantly of type 1 fibers [5,22].

Activity-based restorative strategies have been used in attempts to restore muscle mass and preserve some muscle functions after SCI. Functional electrical stimulation employed after SCI has been shown to capable of improving muscular trophism [12]. Body weight-supported treadmill training (BWSTT) has been shown to be effective in restoring muscle mass and function. Using this procedure, muscle atrophy was partially reversed and greater muscular activation promoted [1,10,11]. Though these studies have shown the promising beneficial effects of BWSTT in muscular trophism after SCI, further research is needed in order to establish the biological mechanisms involved in this rehabilitation strategy [12].

Among other factors, muscular disuse severely reduces the expression of brain-derived neurotrophic factor (BDNF) protein and mRNA levels in both lumbar spinal cord and soleus muscle in acute and chronic stages after SCI [15,33]. This trophic factor can activate the rapamycin (mTOR), the protein that participates in mammalian cell size control and plays an important role in muscular trophism [6]. Furthermore, paraplegia-induced muscle atrophy in rats has been associated with downregulation of the mTOR signaling pathway [8].

Studies have shown that repetitive motor activity, such as cycling exercise training accelerates muscle size restoration after complete SCI in rats [9,25,26]. Furthermore, treadmill training has been shown to diminish the extent of muscle atrophy [23,31] and restores BDNF levels in both the lumbar spinal cord and soleus muscles [15] in moderate SCI models. In contrast to the well reported effects of treadmill training on several growth factors and muscular trophism in intact and after moderate SCI models, few studies have attempted to examine the effectiveness of this activity-based therapy on the neuromuscular system after complete SCI. Since, mTOR

* Corresponding author at: PPG Neurociências, ICBS, Universidade Federal do Rio Grande do Sul, R. Sarmento Leite 500, CEP 90050-170 Porto Alegre, RS, Brazil. Tel.: +55 51 33163624; fax: +55 51 33163092.
E-mail address: achaval@ufrgs.br (M. Achaval).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.02.004

is the downstream effector of the action of BDNF on cell size, the correlation of the regulation of this growth factor and trophism in paralyzed muscle may shed some light on the mechanism by which treadmill step training affects muscular tissue and promotes maintenance/restoration of the muscle mass in spinal subjects. In this context, the main aim of this study was to test the hypothesis that treadmill step training is capable of attenuating the loss of muscle mass and myofiber atrophy and modifying the BDNF content in soleus muscle after SCT in rats.

Experiments were performed on 30 adult male Wistar rats (2.5 months old) from a local breeding colony (ICBS, UFRGS, Brazil). The rats were housed in standard plexiglass boxes (2 per cage), under a 12:12-h light/dark cycle, in a temperature-controlled environment ($22 \pm 1\,^{\circ}C$), and given free access to food and water.

Animals were randomly divided into the following groups: (a) rats without spinal cord transection, sham-operated (control, $n = 10$); (b) untrained rats with spinal cord transection (untrained SCT, $n = 10$); and (c) step-trained rats with spinal cord transection (trained SCT, $n = 10$).

Procedures were in accordance with Brazilian laws and the recommendations of the Brazilian Society of Neurosciences and the International Brain Research Organization. This study was approved by the Ethics Committee of our institution (Nr. 2007738).

Animals were anesthetized using pentobarbital (40 mg/kg, i.p., Cristália, Brazil) and subjected to a vertebral laminectomy at thoracic levels T8–T9. Spinal cord transection was performed using microscissors, and the completeness of the transection was ensured by passing a sickle probe (no. 3, White, Brazil) through the lesion site. The same surgical procedure, though without SCT was performed in the uninjured group (control). The surgical procedure was concluded by suturing the muscle plane and skin (6–0 and 4–0 nylon sutures, respectively; Somerville, Brazil). The skin surface was then disinfected with 2% iodine solution.

Following the surgery, rats were kept in a warm environment and monitored until they recovered from anesthesia. Animals were then returned to standard conditions. All animals were treated for 14 days with Baytril (Enrofloxacin 2.5 mg/kg, subcutaneously; Bayer S.A., Brazil) to prevent urinary tract infections. Furthermore, bladders were manually expressed twice a day until the bladder was no longer distended and palpable, indicating that the animal had developed an automatic bladder voidance reflex (10–14 days). Inspection for general health, skin irritation, decubitus ulcers or evidence of autophagia, was carried out daily throughout the post-injury survival period.

The training program was performed on a treadmill designed for human use (Runner, Brazil) and modified for use by rats. Before the SCT, the animals were familiarized with the treadmill apparatus at 5 m/min for 5 min a day on three consecutive days and at post-operative day 6 the trained SCT animals started a 9-week step-training program. The training program consisted of step training on a treadmill (band speed 6–7 m/min) with partial body weight support (BWS), once a day and 5 sessions per week. The first training day began with 5 min of step training. The training time was progressively increased every day up to 20 min on the second week and 30 min over the following 7 weeks. The design of this treadmill training regime took into account a previously published study using complete SCT in rats [34].

The step training was carried out using a manually adjustable weight-supporting counterbalance system to provide weight support assistance. Each rat was fitted with a Lycra vest that was closed with Velcro, and placed into a BWS harness, thereby supporting the thorax, while the head, forelimbs and hindlimbs had full range of movement. For step training, rats were placed in a quadripedal position, bearing ~15% of their body weight on their hindlimbs (i.e., ~85% BWS). Each spinal animal was individually trained and the hindlimbs were manually moved in a step pattern by the researcher holding the ankle region (as previously performed [20]). During the step training, special care was taken to place the rats' feet in a plantar stepping position and to keep the toes extended to ensure the footpad made contact with the treadmill band during the stance phase.

The day after the last training session, five animals from each group were deeply anesthetized with pentobarbital (100 mg/kg, i.p.; Cristália, Brazil) and the right soleus muscles were carefully dissected from the surrounding tissue and rapidly weighed. Results are presented as a percentage of total body mass and were calculated by dividing the weight of the soleus muscle by the weight of the animal. Additionally, samples of the central part of each muscle were excised under ice and stored at $-70\,^{\circ}C$ until processed for biochemical analysis.

The soleus muscle was chosen for the purposes of this study of muscular adaptations because it is predominantly composed of slow-twitch muscle fibers (type 1), which makes it more vulnerable to disuse atrophy after SCI (see review [5]). Moreover, the rat soleus muscle is used during standing and locomotion and intensively recruited during treadmill training [2].

Other five animals from each group were deeply anesthetized with pentobarbital and transcardially perfused with 300 mL of saline solution, followed by 400 mL of 0.5% glutaraldehyde (Merck, Germany) and 4% paraformaldehyde (Reagen, Brazil) in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature. The right soleus was carefully dissected from the surrounding tissue. Small samples (~2 mm × 1 mm) from the central part of each muscle were selected and post-fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in PB solution at room temperature for 1 h and at $4\,^{\circ}C$ until processed. The samples were washed in PB and post-fixed in 1% OsO_4 (Sigma, USA) in PB for 2 h. They were then washed with PB and dehydrated in a graded series of alcohol and propylene oxide (Electron Microscopy Sciences, USA), embedded in resin (Durcupan, ACM-Fluka, Switzerland), maintained in vacuum for 24 h, and, afterwards, polymerized for 72 h at $60\,^{\circ}C$. Serial transverse-semithin sections (1 μm) were obtained using an ultramicrotome (MT6000-XL, RMC, USA).

Images of the muscles were captured (20×) using a Nikon Eclipse E-600 microscope (Japan) coupled to a digital camera and Image Pro Plus Software 6.0 (Media Cybernetics, USA). For soleus myofiber morphometric evaluation, a set of 6 images was obtained for each muscle using random sampling of one slice and the transverse sectional areas of 120 muscle fibers, randomly chosen from the 6 digitalized images, were estimated. The area of each individual myofiber was estimated using a point-counting technique [24] using grids with a point density of one point per 58.56 μm^2 and the equation: $\hat{A} = \sum p \cdot a/p$. Where $\hat{A}$ is area, Σp is the total of counted areas/point and a/p is the area/point value (58.56 μm^2). This is an unbiased estimate of the area. The average of the cross-sectional areas of each individual rat was based on the mean obtained for the soleus myofiber areas measured per animal.

The muscle samples stored at $-70\,^{\circ}C$ were homogenized and equal amounts (30 μg) of proteins from each sample were boiled in sample buffer (0.0625 M Tris–HCl, pH 6.8, 2% (w/v) sodium dodecyl sulfate (SDS), 5% (w/v) β-mercaptoethanol, 10% (v/v) glycerol, 0.002% (w/v) bromphenol blue) and electrophoresed in 10% (w/v) SDS-polyacrylamide gel. The separated proteins were blotted onto a nitrocellulose membrane. Equal loading of each sample was confirmed with Ponceau S staining (Sigma, USA). Anti-BDNF antibody (Santa Cruz Biotechnology, USA) was used at a dilution of 1:200. After incubating with the primary antibody for 2 h at room temperature, membranes were washed and incubated with peroxidase-conjugated anti-rabbit immunoglobulin (IgG, Sigma, USA) at a dilution of 1:2000 for 1 h. The chemiluminescence signal was detected using an ECL kit (Amersham, USA). The films were digitally scanned and the optical density measured using Image Pro

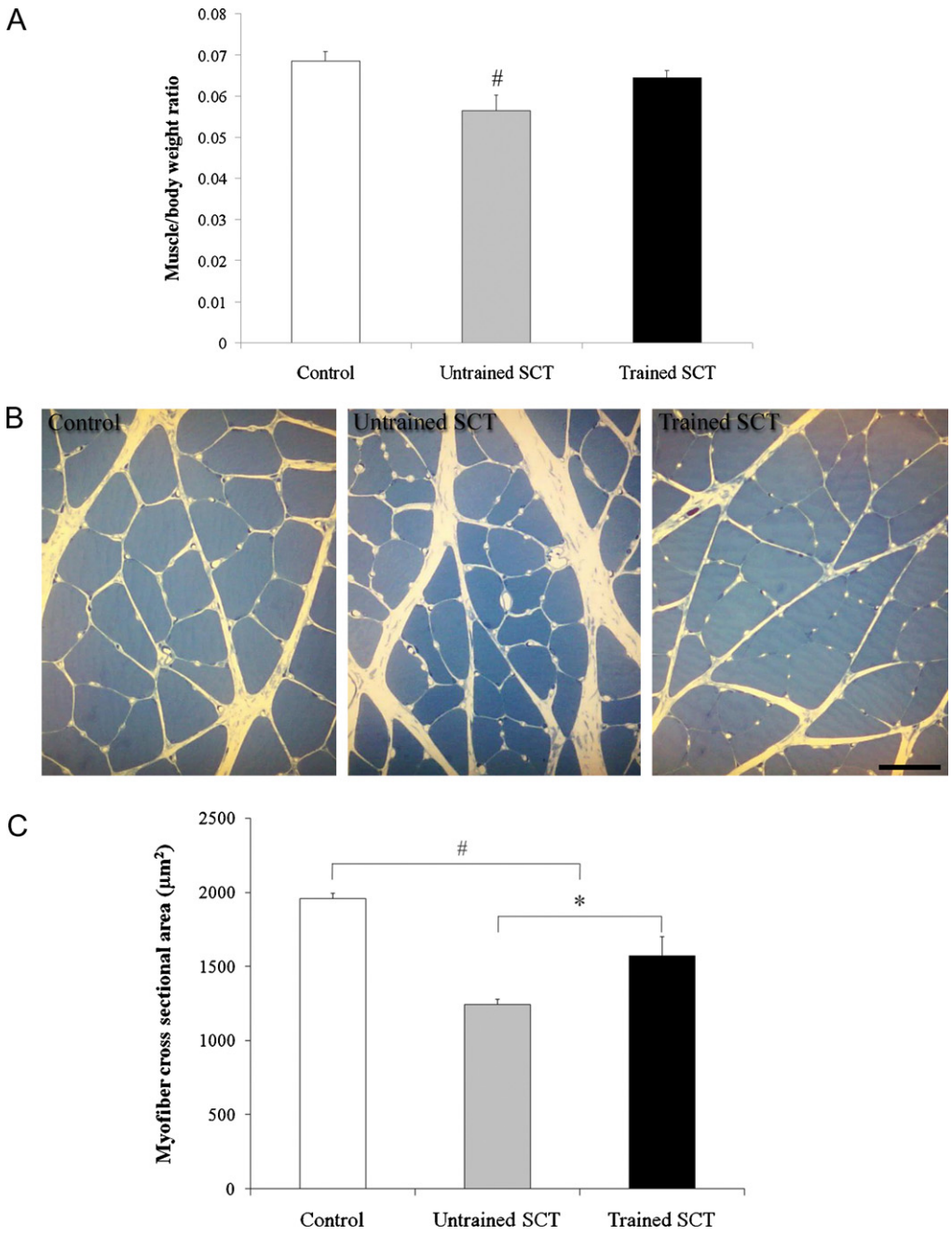

Fig. 1. Effects of treadmill step training on soleus muscle trophism. (A) Soleus muscle to body weight ratio. Soleus to body weight ratio was significantly reduced in the untrained group when compared to controls. There was no significant difference between the step-trained spinal animals and controls. (B) Digitalized images showing soleus muscle myofibers of the control and experimental groups. Untrained spinal animals showed myofiber atrophy compared with controls. Note that in the step-trained animals, the soleus myofibers were larger when compared with the untrained rats. Toluidine blue-stained semithin sections. Scale bar = 50 μm. (C) Soleus myofiber cross-sectional areas. Myofiber size was significantly reduced in the untrained and trained group when compared to control rats. In the step-trained animals the myofiber sizes were larger when compared with untrained rats. Values in graphs (A) and (C) are expressed as means ± S.E.M. Symbol "#" marks significant differences ($P < 0.05$) when compared with the control group and asterisk (*) marks significant differences ($P < 0.05$) when compared with the step-trained group.

Plus Software 6.0 and shown as a percentage of the control values. The protein content was measured using Lowry's method using bovine serum albumin (BSA) as a standard.

To test for statistically significant differences, morphological and biochemical data were analyzed using one-way ANOVA and, in the case of significant differences, the Tukey *post hoc* test was applied. Statistical significance was assumed at $P < 0.05$. Data were expressed as means ± S.E.M. Pearson's Correlation was used to determine the relationship between BDNF expression with both the soleus to body weight ratio and soleus myofiber size.

Analysis of the soleus muscle to body weight ratio revealed that SCT significantly reduced the soleus muscle mass in untrained animals compared with controls (0.056 ± 0.003 and 0.068 ± 0.002; respectively; $P < 0.05$; Fig. 1A). There was no difference in terms of the soleus muscle to body weight ratio between the step-trained group (0.064 ± 0.001) and the controls (Fig. 1A).

In order to assess changes in the cross-sectional myofiber size, transverse-semithin sections of the soleus muscle for each experimental group are shown in Fig. 1B. Analysis of the morphometric data revealed that soleus cross-sectional myofiber area was signif-

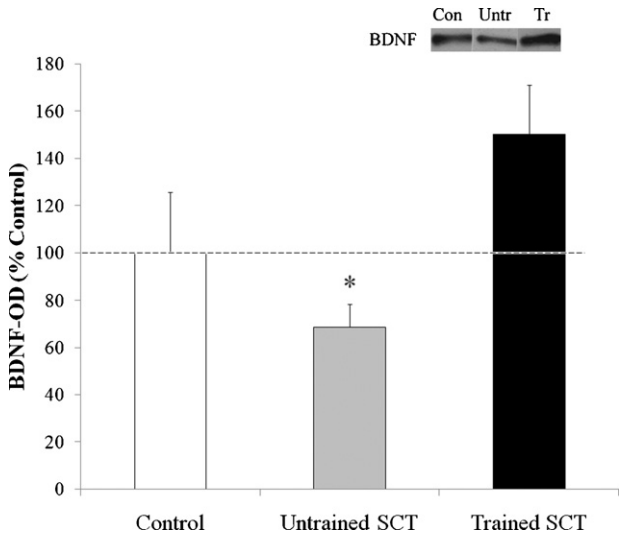

Fig. 2. Effects of treadmill step training on soleus BDNF expression. Western blot analysis with BDNF-stained bands to illustrate changes in muscular expression of this protein in the trained SCT (Tr) compared to the untrained SCT (Untr) and control (Con) groups are shown at the top of figure. Graph showing the quantification of the BDNF in the control and experimental groups. BDNF expression was significantly enhanced in the step-trained when compared with untrained spinal animals. Values are shown as a percentage of the control values and expressed as mean ± S.E.M. Asterisks (*) mark significant differences ($P < 0.05$) when compared with the trained SCT group.

icantly reduced with SCT in both the untrained and step-trained animals ($1245.47 \pm 34.08\ \mu m^2$ and $1573.06 \pm 131.69\ \mu m^2$, respectively) compared with controls ($1960.23 \pm 33.69\ \mu m^2$; $P < 0.05$; Fig. 1C). The step-trained group showed an enhanced myofiber cross-sectional area in the soleus muscle when compared with the untrained SCT group ($P < 0.05$).

Representative Western blot analyses with BDNF-stained bands that facilitate the examination of the changes in the expression of this protein in the soleus for each experimental group are shown at the top in Fig. 2. Although optical densitometry (OD) analysis revealed that SCT reduced BDNF expression by about 31% in soleus muscle, there was no statistically significant difference between the untrained SCT and control groups (Fig. 2). In the step-trained group, soleus BDNF expression was enhanced by between/about 50% and 80% when compared respectively with control and untrained spinal animals, and this value was significantly different from the untrained SCT group (Fig. 2).

In order to study the relationship between muscular expression of BDNF and soleus trophism, we examined the correlation between the expression of this protein and the soleus to body weight ratio and myofiber cross-sectional area in spinal animals. Pearson's Correlation showed that there is a strong positive relation between muscular BDNF expression with the muscle to body weight ratio ($r = 1$; $P < 0.001$) and with the myofiber size ($r = 0.959$; $P < 0.001$) in soleus muscle.

Muscle atrophy as detected by a reduction in muscle mass caudal to the site of the spinal lesion is an important hallmark of spinal cord injury. In this study, the SCT caused a severe decrease in soleus muscle weight and myofiber size. The untrained spinal animals showed a greater loss of soleus weight compared with controls. However, the treadmill step training prevented and/or reverted this muscle loss. Moreover, SCT lead to a reduction of ~37% in soleus myofiber size in the untrained spinal rats when compared with controls. Additionally, the step training was effective in partially maintaining and/or restoring muscle myofiber size, given that the average cross-sectional area of the soleus myofibers in the trained spinal animals was ~17% greater than that in the untrained group.

Severe muscle atrophy caused by the complete or incomplete SCI has been well documented in experimental studies [21,23,25,26]. Five days of locomotor training, starting one week after midthoracic contusion SCI, resulted in significant enlargement of the soleus cross-sectional myofiber area, with the trained animals having muscle fiber sizes 23% larger than the untrained [31]. Additionally, magnetic resonance imaging has shown that long-term locomotor training enhances the cross-sectional area and accelerates soleus muscle recovery in spinal cord contusion injured rats [22,23]. While few studies have evaluated the effects of treadmill training in fully spinalized rats, other exercise paradigms, such as cycling exercises, when started 5 days after midthoracic SCT restored skeletal muscle to body mass ratio and cross-sectional myofiber area in soleus muscle to control values [25,26].

Skeletal muscle is known to be an important secretor of growth factors. In our study, step training led to an increase of 80% in soleus BDNF expression in the trained spinal animals when compared with the untrained spinal animals. Other studies have shown that motor training has an intrinsic potential to enhance the production of neurotrophins. The expression of BDNF, neurotrophin-3 (NT-3), and their tyrosine kinase receptors (TrkB and TrkC, respectively) in both the spinal cord and soleus muscle of rats increases with locomotor exercise training in the intact spinal cord [14,30,32]. Moreover, locomotor training has been shown to restore BDNF levels in both the lumbar spinal cord and soleus muscle, which were severely reduced in the acute and chronic stages after SCI [15,33].

Muscular trophism in the soleus is correlated with muscle BDNF expression in our study. We showed that both soleus muscle weight and soleus myofiber size had a positive correlation with muscular expression of the BDNF protein in step-trained and untrained spinal animals 10 weeks after SCT. BDNF activates mTOR, the protein that participates in mammalian cell size control, and the down-regulation of this protein is associated with the muscle atrophy after SCI [8,17,27,29]. Skeletal muscle trophism is controlled by the regulation of cellular signaling pathways that involve muscle protein synthesis, breakdown and cellular proliferation [13]. The mTOR signaling pathway is capable of regulating translation initiation and cellular protein synthesis [18,19]. In this context, the level of mRNA translation is the primary muscle protein synthesis regulator [18,19]. Therefore, activation of mTOR by BDNF signaling may be involved in beneficial exercise-induced effects that may underlie the partial maintenance and/or recovery of the muscular trophism seen in the soleus from trained spinal animals in our study.

Stepping-based rehabilitation programs, such as wheel running, stationary bicycle or treadmill training, may activate a neural network located within the lumbar spinal cord, the central pattern generator (CPG), which is capable of generating rhythmic locomotor activity without descending control. The sensorial stimulation provided by Ia and Ib fiber groups during step training could play an important role in the normalization of motoneuron electrophysiological properties after SCI and reinforce the efficacy of specific sensorimotor pathways in promoting neuromuscular activity [3,4,28]. This plasticity could result in a more selective and stable network of neurons capable of controlling the limb muscles activated during locomotion in spinal rats [16].

In summary, our results provide evidence that step training in rats, when started immediately after SCT, may partially impede and/or revert the muscular atrophy in chronic paralyzed soleus muscle. Moreover, the treadmill step training promoted upregulation of BDNF in the soleus muscle, which was positively correlated with muscle weight and myofiber cross-sectional area in our study. We believe that the beneficial effects of treadmill training on soleus muscle trophism could be promoted by BDNF-induced mTOR activation.

References

[1] M.M. Adams, D.S. Ditor, M.A. Tarnopolsky, S.M. Phillips, N. McCartney, A.L. Hicks, The effect of body weight-supported treadmill training on muscle morphology in an individual with chronic, motor-complete spinal cord injury: a case study, J. Spinal Cord Med. 29 (2006) 167–171.

[2] R.B. Armstrong, H. Laughlin, Is rat soleus muscle recruited during swimming? Brain Res. 258 (1982) 173–176.

[3] E. Beaumont, J.D. Houlé, C.A. Peterson, P.F. Gardiner, Passive exercise and fetal spinal cordtransplant both help to restore motoneuronal properties after spinal cord transection in rats, Muscle Nerve 29 (2004) 234–242.

[4] E. Beaumont, S. Kaloustian, G. Rousseau, B. Cormery, Training improves the electrophysiological properties of lumbar neurons and locomotion after thoracic spinal cord injury in rats, Neurosci. Res. 62 (2008) 147–154.

[5] B. Biering-Sorensen, I.B. Kristensen, M. Kjaer, F. Biering-Sorensen, Muscle after spinal cord injury, Muscle Nerve 40 (2009) 499–519.

[6] S.C. Bodine, T.N. Stitt, M. Gonzalez, W.O. Kline, G.L. Stover, R. Bauerlein, E. Zlotchenko, A. Scrimgeour, J.C. Lawrence, D.J. Glass, G.D. Yancopoulos, Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo, Nat. Cell Biol. 3 (2001) 1014–1019.

[7] A.P. Buunk, R. Zurriaga, P. Gonzalez, C. Terol, S.L. Roig, Targets and dimensions of social comparison among people with spinal cord injury and other health problems, Br. J. Health Psychol. 11 (2006) 677–693.

[8] H.C. Dreyer, E.L. Glynn, H.L. Lujan, C.S. Fry, S.E. Di Carlo, B.B. Rasmussen, Chronic paraplegia-induced muscle atrophy downregulates the mTOR/S6K1 signaling pathway, J. Appl. Physiol. 104 (2008) 27–33.

[9] E.E. Dupont-Versteegden, R.J.L. Murphy, J.D. Houle, C.M. Gurley, C.A. Peterson, Mechanisms leading to restoration of muscle size with exercise and transplantation after spinal cord injury, Am. J. Physiol. Cell Physiol. 279 (2000) 1677–1684.

[10] G.F. Forrest, S.A. Sisto, H. Barbeau, S.C. Kirshblum, J. Wilen, Q. Bond, S. Bentson, P. Asselin, C.M. Cirnigliaro, S. Harkema, Neuromotor and musculoskeletal responses to locomotor training for an individual with chronic motor complete AIS-B spinal cord injury, J. Spinal Cord Med. 31 (2008) 509–521.

[11] L.M. Giangregorio, A.L. Hicks, C.E. Webber, S.M. Phillips, B.C. Craven, J.M. Bugaresti, N. McCartney, Body weight supported treadmill training in acute spinal cord injury: impact on muscle and bone, Spinal Cord 43 (2005) 649–657.

[12] L. Giangregorio, N. McCartney, Bone loss and muscle atrophy in spinal cord injury: epidemiology, fracture prediction, and rehabilitation strategies, J. Spinal Cord Med. 29 (2006) 489–500.

[13] D.J. Glass, Signalling pathways that mediate skeletal muscle hypertrophy and atrophy, Nat. Cell Biol. 5 (2003) 87–90.

[14] F. Gómez-Pinilla, Z. Ying, P. Opazo, R.R. Roy, V.R. Edgerton, Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle, Eur. J. Neurosci. 13 (2001) 1078–1084.

[15] K.J. Hutchinson, F. Gómez-Pinilla, M.J. Crowe, Z. Ying, D.M. Basso, Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats, Brain 127 (2004) 1403–1414.

[16] R.M. Ichiyama, G. Courtine, Y.P. Gerasimenko, G.J. Yang, R. van den Brand, I.A. Lavrov, H. Zhong, R.R. Roy, V.R. Edgerton, Step training reinforces specific spinal locomotor circuitry in adult spinal rats, J. Neurosci. 28 (2008) 7370–7375.

[17] N.N. Johnson-Farley, K. Patel, D. Kim, D.S. Cowen, Interaction of FGF-2 with IGF-1 and BDNF in stimulating Akt, ERK, and neuronal survival in hippocampal cultures, Brain Res. 1154 (2007) 40–49.

[18] L.D Kapp, J.R. Lorsch, The molecular mechanics of eukaryotic translation, Annu. Rev. Biochem. 73 (2004) 657–704.

[19] S.R. Kimball, Interaction between the AMP-activated protein kinase and mTOR signaling pathways, Med. Sci. Sports Exerc. 38 (2006) 1958–1964.

[20] A.S. Laird, P. Carrive, P.M.E. Waite, Effect of treadmill training on autonomic dysreflexia in spinal cord-injured rats, Neuroreabil. Neural Repair 23 (2009) 910–920.

[21] Y.-S. Lee, C.-Y. Lin, V.J. Caiozzo, R.T. Robertson, J. Yu, V.W. Lin, Repair of spinal cord transection and its effects on muscle mass and myosin heavy chain isoform phenotype, J. Appl. Physiol. 103 (2007) 1808–1814.

[22] M. Liu, P. Bose, G.A. Walter, D.K. Anderson, F.J. Thompson, K. Vandenborne, Changes in T2 relaxation properties following spinal cord injury and locomotor training, Eur. J. Appl. Physiol. 97 (2006) 335–361.

[23] M. Liu, P. Bose, G.A. Walter, F.J. Thompson, K. Vandenborne, A longitudinal study of skeletal muscle following spinal cord injury and locomotor training, Spinal Cord 46 (2008) 488–493.

[24] S. Marcuzzo, M.F. Dutra, F. Stigger, P.S. do Nascimento, J. Ilha, P.I. Kalil-Gaspar, M. Achaval, Beneficial effects of treadmill training in a cerebral palsy-like rodent model: walking pattern and soleus quantitative histology, Brain Res. 1222 (2008) 129–140.

[25] R.J.L. Murphy, E.E. Dupont-Versteegden, C.A. Peterson, J.D. Houlé, Two experimental strategies to restore muscle mass in adult rats following spinal cord injury, Neurorehabil. Neural Repair 13 (1999) 125–134.

[26] J.-M. Nothias, T. Mitsui, J.S. Shumsky, I. Fischer, M.D. Antonacci, M. Murray, Combinated effects of neurotrophin secreting transplants, exercise, and serotonergic drug challenge improve function in spinal rats, Neurorehabil. Neural Repair 19 (2005) 296–312.

[27] S. Oldham, E. Hafen, Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control, Trends Cell Biol. 13 (2003) 79–85.

[28] J.C. Petruska, R.M. Ichiyama, D.L. Jindrich, E.D. Crown, K.E. Tansey, R.R. Roy, V.R. Edgerton, L.M. Mendell, Changes in motoneuron properties and synaptic inputs related to step training after spinal cord transection in rats, J. Neurosci. 27 (2007) 4460–4471.

[29] T. Schmelzle, M.N. Hall, TOR, a central controller of cell growth, Cell 103 (2000) 253–262.

[30] M Skup, A. Dwornik, M. Macias, D. Sulejczak, M. Wiater, J. Czarkowska-Bauch, Long-term locomotor training up-regulates TrkB receptor like proteins, brain-derived neurotrophic factor, and neurotrophin 4 with different topographies of expression in oligodendroglia and neurons in the spinal cord, Exp. Neurol. 176 (2002) 289–307.

[31] J.E. Stevens, M. Liu, P. Bose, W.A. O'Steen, F.J. Thompson, D.K. Anderson, K. Vandenborne, Changes in soleus muscle function and fiber morphology with one week of locomotor training in spinal cord contusion injured rats, J. Neurotrauma 23 (2006) 1671–1681.

[32] Z. Ying, R.R. Roy, V.R. Edgerton, F. Gómez-Pinilla, Voluntary exercise increase neurotrophin-3 and its receptor TrkC in the spinal cord, Brain Res. 987 (2003) 93–99.

[33] Z. Ying, R.R. Roy, V.R. Edgerton, F. Gómez-Pinilla, Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury, Exp. Neurol. 193 (2005) 411–419.

[34] Y. Zhang, S.R. Ji, C.Y. Wu, X.H. Fan, H.J. Zhou, G.L. Liu, Observation of locomotor functional recovery in adult complete spinal rats with BWSTT using semiquantitative and qualitative methods, Spinal Cord 45 (2007) 496–501.