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W. T. Stauber & M. E. T. Willems

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FORCE DURING STRETCHES OF RAT SKELETAL MUSCLES AFTER HYPERTONIA AT SHORT AND LONG LENGTHS

M.E.T. Willems and W.T. Stauber

Department of Physiology, West Virginia University, Morgantown, USA

ABSTRACT

Following injection of tetanus toxin into rat gastrocnemius muscle to produce hypertonia, plantar flexor muscles were allowed to shorten (S, $n = 5$) without restraint or held lengthened (L, $n = 3$) by splinting. Saline injected rats served as control ($n = 5$). One week after injection, peak forces during 3 stretches with passive muscles and acute isometric force deficits produced by 15 stretches of electrically stimulated muscles were examined under pentobarbital anesthesia. Isometric force and mass of plantar flexors were similar in S rats but 16% lower in L rats compared to control. Peak passive forces were highest in S rats but not different between L rats and control. At the end of the stretch protocol, isometric force deficits were 26% larger in S rats compared to L rats and 17% smaller in L rats compared to control. Acute isometric force deficits produced by stretches of active skeletal muscles were dependent on the muscle length maintained during hypertonia. Our animal model could be used to test rehabilitation interventions during hypertonia of skeletal muscles.

KEYWORDS: Injury, contracture, muscle length, eccentric contraction, stretch, hyperactivity, rat, *in vivo*, dysfunction.

INTRODUCTION

Tetanus toxin, when injected into skeletal muscles, ascends the peripheral motor nerve and impairs the action of inhibitory neurons on alpha motor neurons (Brooks et al., 1957) resulting in the continuous firing of some alpha motor neurons. The continuous firing of alpha motor neurons results in hypertonia of the skeletal muscles innervated by the alpha motor neurons. Without constraints on joint positions, the tetanus toxin-induced hypertonia produces an active immobilization of skeletal muscles in a shortened position (Huet de la Tour et al., 1979) resulting in anatomically shorter muscles within 1 week (Ranson & Dixon, 1928; Huet de la Tour et al., 1979). Tetanus-toxin induced hypertonia of skeletal muscles could be used as a potential model for some features of hypertonia such as activity in the shortened position leading to contractures.

Address correspondence to: William T. Stauber, Department of Physiology, West Virginia University, Morgantown, WV 26506-9229, USA. Fax: +1 304 293 3850; E-mail: wstauber@hsc.wvu.edu

Skeletal muscles held in a shortened position by immobilization are known to become anatomically shorter by removal of sarcomeres in series (Heslinga et al., 1995). However, the adaptation process during immobilization is muscle length-dependent (Williams & Goldspink, 1978; Järvinen et al., 1992). Skeletal muscles held in a lengthened position became anatomically longer (Williams & Goldspink, 1976). In an intact animal, longer muscle lengths protect against the acute force deficits produced by stretches of active skeletal muscles (Morgan & Allen, 1999). For example, in skeletal muscles of rabbits where muscle length was decreased by tendon shortening, smaller force deficits were observed by a single stretch in muscles which had the least shortening (Best et al., 1998).

In the present study, tetanus-toxin induced hypertonia was used to test the prediction that the acute force deficits by repeated stretches of active plantar flexor muscles following 1 week of local tetanus toxin injection were larger for muscles chronically active and held in a shortened position than muscles chronically active and held in a lengthened position. The stretches were performed in the same range of ankle motion. Isometric

forces were measured following the repeated stretches to reveal force deficits due to acute injury (McCully & Faulkner, 1986).

MATERIALS AND METHODS

Female Sprague Dawley rats (3–4 months, $n = 13$) were used. Animal use complied with Animal Welfare Act P.L. 91-579 and DHHS Guidelines governing the care and use of laboratory animals. All procedures were approved by and followed the guidelines of the West Virginia University Animal Care and Use Committee (WVU-ACUC #9511-05).

Injection of tetanus-toxin and experimental groups

In eight rats, the gastrocnemius muscle of the left hindlimb was injected with a single dose of tetanus toxin (24 mg) using a fine needle (gauge 27.5). Entrance point of the needle through the skin was halfway between knee and achilles tendon.

Next day, the left hindlimb of three rats was set in dorsiflexion at an ankle angle (i.e., the angle between the tibia and the plantar surface of the foot) at least under 50° (i.e., plantar flexor muscles in a lengthened position) by using two light-weight plastic rings attached to the foot and shank and connected by a metal wire to form a shackle.

There were no constraints on joint positions in five tetanus-toxin injected rats resulting, within 1–2 days after injection, in a sustained plantarflexion of the ankle (i.e., plantar flexor muscles in a shortened position). When moving around, these rats ambulate on three limbs. The injected hindlimb was held in the air with the ankle angle at about 140°. Five saline-injected rats served as controls.

General treatment of rats

Details on dissection procedure for nerve cuff placement, animal positioning and dynamometer are described elsewhere (Cutlip et al., 1997; Willems & Stauber, 1999). Briefly, an incision was made in the posterior aspect of the hindlimb. Blunt dissection through the popliteal fossa exposed the tibial nerve. Connective tissue and adipose tissue surrounding the tibial nerve were removed and the common peroneal and sural nerves were cut. A bipolar cuff electrode, made of silastic tubing (Dow Corning, Midland, MI, USA) and steel wires (Cooner wire AS632, Chatsworth, CO, USA), was put around the tibial nerve just before the point where tibial and sural nerve adjoin. Steel wires were connected to an electrical stimulator (Grass SD9 stimulator, Grass Medical Instruments, Quincy MA, USA). The knee was held in flexion (90°) in a knee holder. Figure 1 shows the position of the rat's foot on aluminum plate and knee holder. The foot of the rat was held on the aluminum plate and this plate was connected to a dynamometer (Cutlip et al., 1997). During contractions, the foot was kept firmly positioned on the aluminum plate using two cross-bars. The

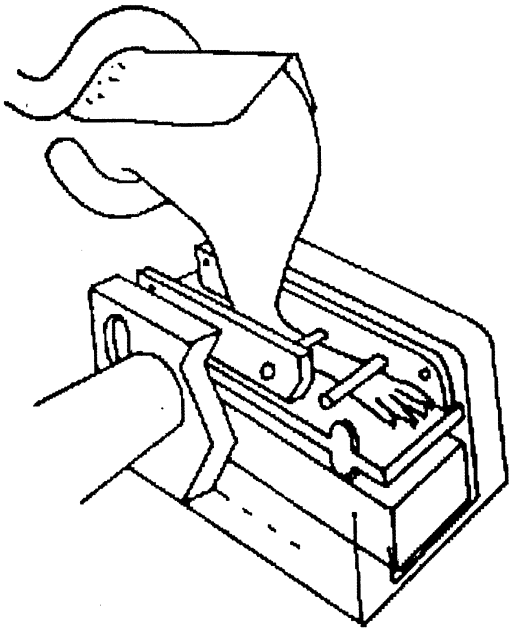

Fig. 1. Position of the rat's foot in load cell fixture and knee holder (from Cutlip et al., 1997, reprinted with permission from *Med Biol Eng Comput 35:* 541).

dynamometer consists of a DC permanent magnet servomotor (Model 1410C) and Unidex 1 single axis motion controller (Aerotech Inc, Pittsburgh, PA, USA). A Z-11 / 5 kg load cell (HBM Inc, Marlboro, MA, USA) is located under the aluminum plate. The axis of rotation of the rat's ankle and dynamometer were visually aligned. Forces were recorded under the sole of the foot of the rat (Willems & Stauber, 1999). Acute experiments were performed on plantar flexor muscles (i.e., soleus, plantaris, and gastrocnemius muscles) in anaesthetized rats (sodium pentobarbital: 75 $mg \cdot kg^{-1}$ i.p.) with electrical stimulation of the tibial nerve, one week after adaptation to an injection of tetanus toxin. Sodium pentobarbital abolishes the hypertonia induced by the tetanus toxin injection (Muchnik & Rubinstein, 1967). In rats with hypertonia in short position, we observed an eradication of hypertonia by a decrease in ankle angle during anaesthesia. Five to 7 short duration isometric contractions (600 ms, rest periods 2 min) at an ankle position of 120° were used to establish stimulus parameters to fully recruit the muscles for high force (>90% of maximal force) muscle stimulation (200 μs pulse duration; 80 Hz, 4.0 ± 0.4 V (mean ± SEM)) with minimal fatigue.

Functional testing one week after injection of tetanus toxin

Stretches of passive skeletal muscles. In each group, 3 acute stretches without muscle activation (i.e., muscles were passive) were produced by ankle movement from an ankle position of 90° to 40° (range of motion 50°) at an angular velocity of $50° \cdot s^{-1}$ with the return of the foot performed 2.0 s after the start of the movement. After the series of 3 stretches with passive skeletal muscles, stretches of active skeletal muscles were performed.

Stretches of active skeletal muscles. In each group, 15 acute stretches with active skeletal muscles were initiated 600 ms after the onset of stimulation from an ankle position of 90° (isometric preload) by movement to an ankle position of 40° (range of motion 50°) at an angular velocity of $50° \cdot s^{-1}$. The passive return of the foot was performed after the end of stimulation. Total stimulation time of one repetition (i.e., isometric preload, stretch, and hold-phase following the stretch) was 1.9 s with rest periods of 3 min. Stretches of active skeletal muscles with a stimulation frequency of 80 Hz guarantee substantial force deficits with only a few contractions (Willems & Stauber, 2000). Using our stimulation protocols, muscle fatigue is negligible because isometric contractions of 1.9 s with rest periods of 3 min resulted in a force decline of about 0.5% per contraction (Willems & Stauber, 2000). Following a recovery of 2 h, the isometric force deficits caused by the stretches were taken as indirect evidence of muscle injury. At the end of the experiments, the rats were euthanized by an intracardial injection of sodium pentobarbital.

Data collection and analysis

Data collected were (1) force during stretches with passive muscles, (2) the isometric force at an ankle position of 90° [IF90° (isometric preload), calculated by subtracting the average force 100 ms before stimulation from the average total force between 500 and 600 ms after stimulation (i.e., on the isometric plateau)] before each stretch and 2 hours after the series of 15 stretches, and (3) peak force at 40° (PSF40°) for each stretch with active muscles. One-way analysis of variance (ANOVA) was done to test for differences between the groups for (1) body weight; (2) muscle masses of plantar flexor muscles of left and right hindlimb; (3) peak forces of comparable stretches with passive muscles; (4) IF90° before the first stretch and 2 hours after the stretches with active muscles; (5) PSF40° of the first stretch with active muscles. Two-way ANOVA was used to test, for all groups, stretch number and (1) the decline in absolute IF90°; (2) the decline in relative IF90°; and (3) the decline in absolute PSF40°. Post-hoc testing for differences was done with a Bonferroni test. Values were presented as means ± SEM. Significance was accepted at $p < 0.05$.

RESULTS

Data on body mass, mass of plantar flexor muscles of the left and right hindlimb, isometric force at 90° before the first stretch of active muscles and peak force of the first stretch of active muscles for control rats and rats adapted to hypertonia and held in long and short positions, respectively, are given in Table 1. Among the three groups, no differences were observed for body weight and mass of the right hindlimb plantar flexor muscles. Both mass and IF90° of left hindlimb plantar flexor muscles of rats with hypertonia held in long positions were 16% smaller than control rats but PSF40° was not changed. For left hindlimb plantar flexor muscles of rats with hypertonia held in a short position, PSF40° was 20% higher, respectively, than control rats even though with similar muscle mass (Table 1). IF90° of left hindlimb plantar flexor muscles of rats with hypertonia held in a short position was unchanged.

Table 1. Body mass, mass of plantar flexor muscles of left and right hindlimb, and muscle forces of plantar flexor muscles of the left hindlimb for control and tetanus-toxin injected rats.

Parameter	C (n = 5)	S (n = 5)	L (n = 3)
Body mass, g	248.1 ± 2.0	253.8 ± 4.2	251.0 ± 8.7
Left muscle mass, mg	2255.1 ± 76.3	2078.4 ± 22.7	1894.1 ± 78.5*
Right muscle mass, mg	2169.0 ± 86.3	2152.1 ± 28.0	2197.8 ± 98.3
IF90°, N	26.2 ± 1.0	28.8 ± 0.5	22.1 ± 1.3*#
PSF40°, N	31.1 ± 1.5	37.4 ± 1.6*	32.0 ± 0.8

Values are means ± SEM. C = saline injected rats. L = tetanus toxin-induced hypertonia with plantar flexor muscles of the left hindlimb held in a lengthened position. S = tetanus toxin-induced hypertonia with plantar flexor muscles of the left hindlimb held in a shortened position. IF90° = isometric force at an ankle position of 90° before the first stretch with active muscles of the left hindlimb. PSF40° = peak force of the first stretch with active muscles of the left hindlimb at an ankle position of 40°. *Significant difference compared with C rats, $p < 0.05$. #Significant difference compared with S rats, $p < 0.05$.

Stretches with passive skeletal muscles

In Figure 2A, the exponential increase of force during a movement of the ankle from an ankle position of 90° to an ankle position of 40° with passive plantar flexor muscles is illustrated. For three consecutive passive stretches of the plantar flexor muscles, the peak force at the end of movement was higher in rats with hypertonia held in a short position but similar in rats with hypertonia held in a long position compared to control rats (Fig. 2B).

Stretches with active skeletal muscles

After 1 acute stretch of active plantar flexor muscles, the isometric preload forces were similar for all groups and showed identical decline for the remaining 14 stretches (Fig. 3A). Two hours following the stretching protocol, recovery of the isometric force was incomplete for all three groups. During the stretching protocol, the decline in relative isometric force for in rats with hypertonia held in a long position was smaller compared with rats with hypertonia held in a short position and reached significance for stretches 3 to 15 (Fig. 3B) (two-way ANOVA). The decline in relative isometric force was also smaller for in rats with hypertonia held in a long position compared with control rats for stretches 2 to 9 (Fig. 3B) (two-way ANOVA). The decline in relative isometric force between rats with hypertonia held in a short position and control rats did not reach significance for any stretch. The peak stretch forces (i.e., PSF40°) were higher for rats with hypertonia held in a short position for stretch 1 compared to control rats. Peak stretch forces were not different between rats with hypertonia held in a long position and control rats (Fig. 4).

DISCUSSION

Hypertonia of the plantar flexor muscles can be observed within 1–2 days after unilateral injection of tetanus toxin into gastrocnemius muscle of rats. Plantar flexors muscles become active and immobilized in a shortened position (Huet de la Tour et al., 1979). Immobilization of skeletal muscles in a shortened position by casting is known to decrease muscle mass (Booth, 1977; Savolainen, 1987; Järvinen et al., 1992). In contrast, maintaining muscles in a shortened position during hypertonia did not change muscle mass (Table 1). An absence of a change in muscle mass was also reported for soleus muscle 1 week (Mizuno & Chou, 1990) and 6 weeks (Stauber et al., in press) after tetanus-toxin-induced shortening. However, muscle mass was reduced when plantar flexor muscles were held in a lengthened position for 7 days following tetanus toxin-induced hypertonia. Apparently, tetanus toxin-induced hypertonia of skeletal muscles in a shortened position but not in a lengthened position maintained muscle mass. The decrease in muscle mass occurred within 1 week and makes any decrease in mass of connective tissue highly unlikely. Even after 6 weeks, connective tissue did not increase significantly (Stauber et al., in press). The decrease in muscle mass is expected to be due to a decrease in mass of myofibres, as indicated by the comparable decrease in isometric force (Table 1), and would result in a relative increase in amount of connective tissue in muscles immobilized in a lengthened position during tetanus toxin-induced hypertonia.

The active immobilization of skeletal muscles is a stimulus for structural and functional adaptation of muscles (Huet de la Tour et al., 1979). For gastrocnemius muscle, 5 to 7 days following injection with tetanus

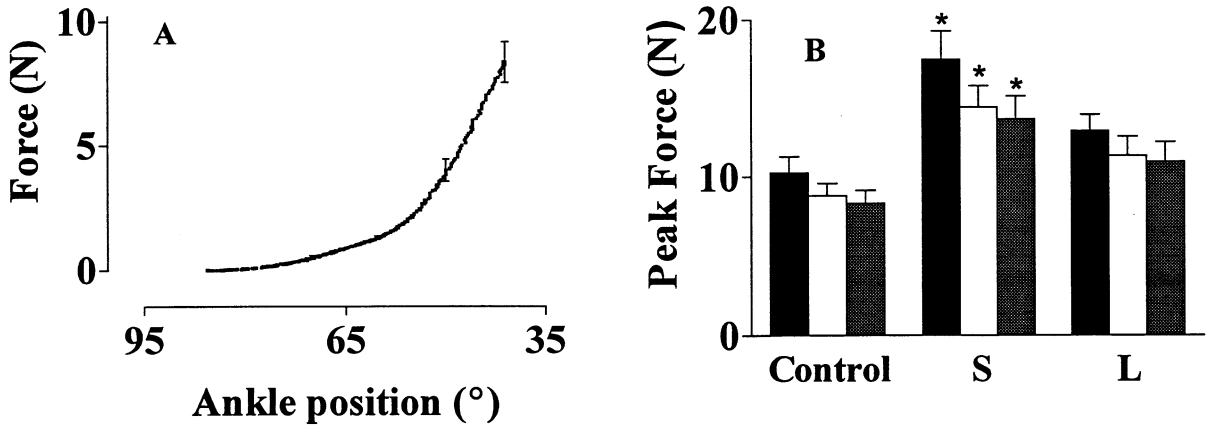

Fig. 2. (A) The relationship between ankle position and force during plantarflexion movement with passive stretch of the plantar flexor muscles (force data are mean ± SEMs of the third stretch for control rats, for illustrative purposes only the error bars at 90°, 80°, 70°, 60°, 50° and 40° are given). (B) Peak forces for three consecutive movements with passive stretches of the plantar flexor muscles for control rats, rats with hypertonia held in a short position (S), and rats with hypertonia held in a long position (L). Black bars indicate the first stretch, blank bars indicate the second stretch, blocked bars indicate the third stretch. *Significant difference compared with comparable stretch in control, $p < 0.05$.

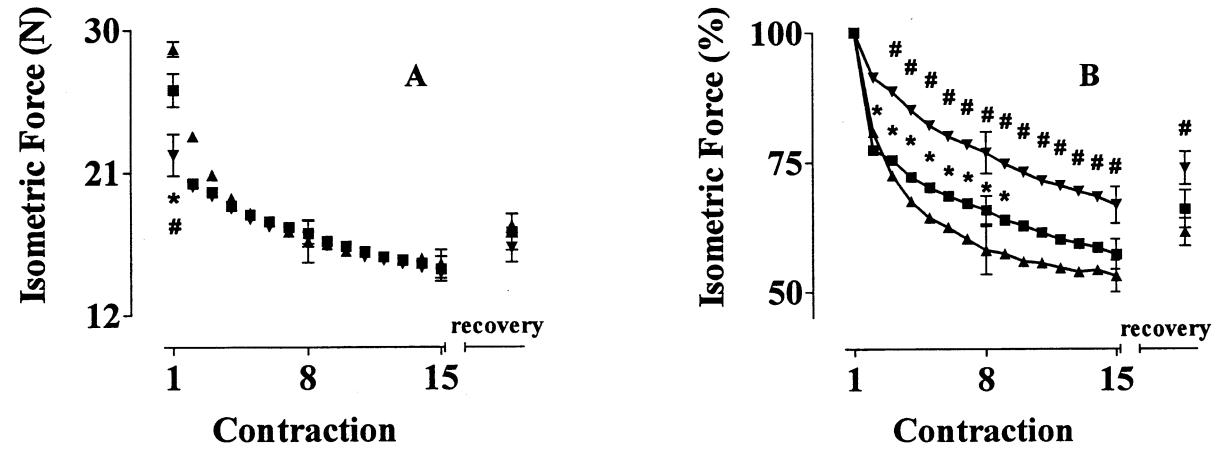

Fig. 3. The absolute isometric force (A) and relative isometric force (B) of the rat plantar flexor muscles as a function of contraction number during the stretch protocol and after 2 hours of recovery for control rats (C) (■), rats with hypertonia held in a short position (S) (▲), and rats with hypertonia held in a long position (L) (▼). Data are presented as mean ± SEM. *Significant difference compared with comparable stretch between L rats and C rats (Figs. A and B), $p < 0.05$. #Significant difference compared with comparable stretch between L rats and S rats (Figs. A and B), $p < 0.05$. For clarity of the figures only error bars for contractions 1, 8, 15 and following 2 h of recovery are plotted.

toxin but with tenotomy of the patellar tendon, the length of muscle fibres was reduced between 7% and 68% (Ranson & Dixon, 1928). In guinea pig, soleus muscles became shorter by reducing sarcomere number (by 45%) 1 week after injection of the gastrocnemius muscle (Huet de la Tour et al., 1979). In addition, higher forces during movements with passive stretches were observed for soleus muscles in anaesthetized guinea pig 1 week after injection (Huet de la Tour et al., 1979). A similar observation was seen in the present study for rat plantar flexor muscles 1 week after tetanus toxin-induced hypertonia in the shortened position.

Some activity of muscles by spontaneous firing of the motor neurons while under anaesthesia would have influenced forces observed during movements with passive skeletal muscles. However, such influence can be excluded because muscle activity was reduced following anaesthesia with sodium pentobarbital due to inhibition of spontaneous motor neuron firing (Muchnik & Rubinstein, 1967) – a decrease in plantar flexion angle was observed. In addition, in one rat with hypertonia held in a short position, cutting the tibial nerve did not alter the peak forces during movements with passive skeletal muscles before and after nerve transection. If functional shortening of skeletal muscles after tetanus toxin-induced hypertonia was the only factor contributing to a change in passive force, one would expect to see a decrease in passive force for muscles in

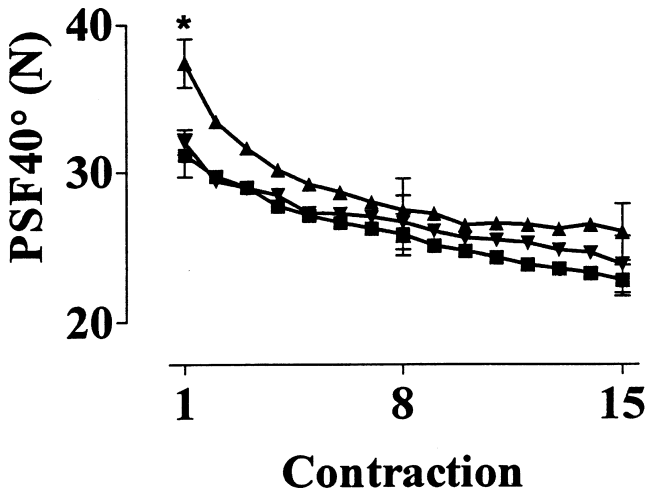

Fig. 4. The absolute peak force at an ankle position of 40° (PSF40°) during stretches of active rat plantar flexor muscles as a function contraction number during the stretch protocol for control rats (C) (■), rats with hypertonia held in a short position (S) (▲), and rats with hypertonia held in a long position (▼). Data are presented as mean ± SEM. *Significant difference between S rats and C rats for comparable stretch, $p < 0.05$. For clarity of the figures only error bars for contractions 1, 8, and 15 are plotted.

the lengthened position. However, peak forces during movements with passive muscles were not higher in rats with plantar flexor muscles being held in a lengthened position. This lack of effect of length could be due to relative increases in connective tissue; the increase in passive force compensates for the expected decrease in passive force due to functional lengthening. It is concluded that higher peak forces during movements with passive muscles in rats with hypertonia held in a short position provide indirect evidence that stretches of the active plantar flexor muscles in these rats were initiated at longer muscle lengths compared to muscles of control rats and rats with hypertonia held in a long position due to shorter fibre lengths in the individual plantar flexor muscles.

ACUTE FORCE DEFICITS BY STRETCHES OF ACTIVE SKELETAL MUSCLES

One week after injection of tetanus toxin in gastrocnemius muscle of rats, the plantar flexor muscles, which were hypertonic and held in a shortened position by the tetany, were more susceptible to acute strain injury (i.e., larger isometric force deficits following repeated stretches of active muscles) than muscles held in a lengthened position. Morgan (1990) predicted that the amount of injury (i.e., force deficits) by stretches of active muscles which occur on the descending limb of the force-length relationship of skeletal muscles (i.e., long muscle lengths) would be larger than for stretches involving relatively short muscle lengths. Any muscle length change would alter the amount of injury (i.e., force deficits) from stretches of active skeletal muscles. For example, single acute stretches of TA muscles in rabbits in which tendon shortening had been produced resulted in larger force deficits 48 h after the stretching (Best et al., 1998). In contrast, smaller force deficits by stretches of active skeletal muscles occurred for muscles containing a greater number of sarcomeres in series (Lynn et al., 1998). However, besides the initial and final muscle lengths of the active stretches as strong predictors of isometric force deficits by acute stretching of active skeletal muscle (Hunter & Faulkner, 1997; Talbot & Morgan, 1998), peak forces during active stretches also need to be considered (Warren et al., 1993). For gastrocnemius medialis muscle *in situ*, it was shown that active stretches initiated from muscle optimum length had higher peak forces than stretches with similar strains ending at muscle optimum length (Ettema et al., 1990). If we assume that the plantar flexor muscles had shortened in the present study, the stretches would occur at longer muscle lengths (more on the descending limb), than control rats. Thus, the larger isometric force deficits produced by acute stretches of active plantar flexor muscles held in a shortened position by hypertonia were due to probable adaptations of longer initial muscle lengths and higher peak stretch forces. Apparently, plantar flexor muscles held in a lengthened position by hypertonia with splinting were able to produce similar peak stretch forces than control but that did not result in similar acute force deficits by the active stretches. It appears that muscle length is more critical to the production of force deficits

than peak stretch force and that splinting of hypertonic muscles at long muscle length is protective.

Caution is needed to characterize tetanus-toxin induced shortening and hypertonia of skeletal muscles as a valuable model for spasticity. Spasticity in humans is a complex disorder which requires weeks or months to develop. Our animal model is acute and not permanent- effects of tetanus-toxin injection disappear four to six weeks after injection. However, our model shows increased resistance to passive movements and could potentially by used to examine intervention procedures during hypertonia like daily intermittent stretching or progressive casting.

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