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Research article

EFFECTS OF FUNCTIONAL ELECTRIC STIMULATION CYCLE ERGOMETRY TRAINING ON LOWER LIMB MUSCULATURE IN ACUTE SCI INDIVIDUALS

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ABSTRACT

The effect of early intervention using functional electric stimulation cycle ergometry (FES-CE) on skeletal muscle morphology was evaluated in traumatic spinal cord injured (SCI) patients 4 – 6 weeks after injury. Motor complete SCI patients (n = 10) were assigned to either a SCI control group (IC) or FES-CE group (IE) and compared to uninjured controls (UIC) matched for age, activity, and gender. Training via FES-CE was performed 3 days/week for 13 weeks. In the FES-CE trained group, power output increased from $2.4 \pm .88$ Watts to 24.5 ± 3.2 Watts. Muscle biopsies were taken from the vastus lateralis muscle at pre- and post-training for subsequent morphological analysis. Without intervention, muscle fiber cross sectional area (CSA_f) decreased 38% and 65% at 6 and 19 weeks post-SCI, respectively. The loss of CSA_f had no impact on myonuclear density. Following 13 weeks of FES-CE training, CSA_f increased was 63% greater when compared to the IC group. Results of the present investigation suggest that the initiation of FES-CE in first weeks after traumatic SCI attenuates the loss of muscle mass and power output.

KEYWORDS: Muscle atrophy, myosin heavy chain, myonuclei.

INTRODUCTION

The functional consequence of Spinal Cord Injury (SCI) are associated with changes in the physiologic, histologic, and morphologic properties of the affected skeletal muscle. Changes include muscle atrophy, decreased muscle fiber cross sectional area (CSA_f), decreased force of contraction in response to

electrical stimulation, decreased oxidative enzymes, decreased fatigue resistance and a transformation of Type I, slow-twitch muscle fiber to Type II_x fast-twitch muscle fibers (Baldi et al., 1998; Burnham et al., 1997; Castro et al., 1999).

It has been reported that CSA_f decreases more than 60% by 24 weeks post-injury (Castro et al., 1999) with a 21% decrease in lower extremity lean

body mass 36 weeks post injury (Baldi et al. 1998). Transition from Type I to Type II_x muscle fibers appears to begin 24 weeks post-injury with the initial changes being from Type II_a to II_x (Castro et al. 1999) with a complete transition occurring one to two years post injury (Burnham et al. 1997). Remodeling of muscle to faster isoforms has also been demonstrated in a number of other disuse models such as spinal contusion (Hutchinson et al., 2001) and short-term space flight (Day et al., 1995). The shift to faster muscle isoforms may have some effect to conserve power output by shifting the force-velocity curve (Faulkner et al., 1995), however, when normalized to fiber area (N·cm⁻²), force production is reportedly not affected by fiber type (Brooks and Faulkner, 1988). Therefore, decreased force and power following SCI appears primarily to be due to decreased CSA_f, while a shift towards Type II isoforms would decrease fatigue resistance of muscle.

One modality used to try to attenuate or reverse changes in skeletal muscle as a result of SCI is electrical stimulation, however to date the affects have been modest. In chronic SCI survivors, more than 2 years post-injury, exposure to prolonged low frequency electrical stimulation resulted in a reversal of Type II muscle fibers towards Type I muscle fibers and no change in CSA_f (Martin et al., 1992). Other researchers reported increased fatigue resistance and an improvement in oxidative capacity (Rochester et al., 1995a; 1995b) with similar protocols. Other more modest low frequency stimulation protocols documented an increase in fatigue resistance, no change in MHC isoform content, but in situ hybridization documented that mRNA for the Type I MHC isoforms were up regulated and the Type II_x MHC isoforms were down regulated (Harridge et al., 2002).

Protocols utilizing functional electrical stimulation cycle ergometry (FES-CE) to train chronic SCI survivors have been documented to alter the effects of SCI on muscle morphology and histology. An FES-CE protocol of one year duration reported a significant shift in MHC composition from an overwhelming predominance of Type II_b to a predominance of Type II_a fibers with no discernable change in Type I MHC isoforms (Mohr et al., 1997). Others have reported an increase in muscle mass (Mohr et al., 1997; Scremin et al., 1999) and a modest increase in CSA_f (Chilinbeck et al., 1999). Significant improvement in fatigue resistance (Gerrits et al., 2000) and power output (Faghri et al., 1992; Hooker et al., 1992) have also been reported in similar studies using FES-CE training. One study trained acute SCI (14-15 weeks post-SCI) using FES-CE, reported prevention of the loss of lean body mass after 3 month of training and

hypertrophy of leg muscles after 6 months of training (Baldi et al., 1998).

These data then support the potential for electrical stimulation to reverse the plasticity observed in skeletal muscle after SCI and thereby improve the strength and endurance properties of that muscle for potentially functional purposes. The magnitude of the strength and endurance changes in response to electrical stimulation of chronic SCI survivors have been relatively modest despite the utilization of varied stimulation protocols and parameters under isometric contraction, contraction against no load or FES-CE conditions. Previous work in our laboratory (Baldi et al., 1998) demonstrated attenuation of loss in lean body mass muscle mass when FES-CE training was initiated within weeks of SCI. However, conservation of muscular power or changes to CSA_f or fiber type in response to early intervention with FES-CE is unknown. Therefore, the primary purpose of this study was to determine if intervening 4 to 6 weeks post injury using FES-CE can attenuate the change in muscle fiber cross sectional area and MHC composition that is otherwise observed in response to SCI. It was hypothesized that 13 weeks of FES-CE training starting 4 to 6 weeks post injury would increase average cyclic power output and attenuate decreases in CSA_f seen post SCI.

METHODS

Subjects

A total of ten SCI subjects participated in this study and were recruited from the acute spinal cord injury (SCI) in-patient rehabilitation center. Subject demographic information can be found in Table 1. Criteria for selection were cervical or thoracic motor complete SCI (American Spinal Injury Association [ASIA] A or B) individuals 17-50 years of age (Maynard et al., 1997). All subjects were clinically stable before entering the study and continued their normal rehabilitation programs throughout the period of the study. Subjects were required to be able to sit upright for greater than thirty minutes before they were allowed to start training. Exclusion criteria include the presence of pressure ulcers, recurrent urinary tract infection, and transportation problems, prescribed medications that directly affect bone and muscle metabolism, lower extremity fracture at the time of the SCI, prolonged bleeding times, full dose anticoagulation, a history of clotting abnormalities, or known bleeding diathesis. All prospective subjects were given a thorough neurological and physiological exam including range of motion of lower limbs, resting electrocardiograph, and X-rays of lower extremities before participating in the study. Subjects were randomly assigned to

Table 1. Subject characteristics.

Group	Gender	Age in years	ASIA rating	Level of injury
UIC				
1	M	31	NA	NA
2	M	29	NA	NA
3	M	24	NA	NA
4	F	23	NA	NA
5	M	24	NA	NA
Mean (\pm SD)		26.2 (3.0)		
IC				
1	M	17	A	C5
2	M	24	B	C5
3	M	18	A	T6
4	M	47	A	C7
5	F	46	A	T8
Mean (\pm SD)		30.4 (14.0)		
IE				
1	M	18	A	C5
2	M	20	A	C4-C5
3	F	17	A	T4
4	M	24	B	T6
5	M	26	A	T7
Mean (\pm SD)		22.2 (5.0)		

UIC; uninjured control, IC; injured control, IE; injured exercise trained.

either SCI-control (IC) or SCI- Functional Electric Stimulation Cycle Ergometry (FES-CE) (IE) groups. Non-injured age and gender-matched individuals were recruited from the faculty and student population of the Ohio State University to serve as uninjured controls (UIC). Following explanation of the procedures; purposes, benefits and risks of the study each subject provided written informed consent. This study was approved by the Institutional Review Board.

Equipment and training protocol

The FES-cycle ergometer training system is composed of a Stimaster Clinical Ergometry system (Electrologic of America, Inc., Dayton, OH), which was connected to an electrically braked cycle ergometer (Therapeutic Alliance, Inc., Fairborn, OH). As described previously (Baldi et al. 1998) training sessions were held 30 minutes \cdot day $^{-1}$, 3 days \cdot week $^{-1}$ for 13 weeks. Before each FES-CE training session 12 reusable electrodes were placed over appropriate motor points on the quadriceps, hamstring, and gluteal muscle groups. Each training session began with a one-minute warm-up, during which stimulation was delivered to all muscles at 50% of the threshold value (the minimum required to produce a palpable, tetanic contraction). Pedaling motion was assisted by an able-bodied operator throughout the warm-up and for the first 10-30 seconds of full stimulation, until the subject's legs created enough power to maintain the cycling motion independently. All subjects began at 2 watts with the goal of completing 30 minutes of

continuous cycling. As subject's legs fatigued, and after delivered stimulation increased to the maximum (140 mA), pedaling cadence would slow. The stimulator is programmed to terminate the stimulation once cadence decreased to 35 rpm. Post ride, each subject went through a two-minute passive pedaling cool-down. Upon completion of 3 consecutive 30-minute training sessions, subjects' resistance was increased by 6.1 watts.

Subjects that were unable to ride at 2.4 watts for any length of time were trained to increase their quadriceps strength by performing electrically stimulated knee extensions. The leg was stimulated for 5 seconds with a 30 seconds rest before the next leg extension. When the subject was able to perform 30 repetitions of 45° knee extension with 1 kg, they were placed on the cycle ergometer and followed the training regimen as described above.

Functional changes

Functional changes in power output of the IE group were determined by changes in average weekly power output during FES-CE training. Average weekly power output was determined from the average power output of each ride as calculated by the Stimaster Clinical Ergometry system and the number of training sessions per week.

Tissue Collection

Needle biopsies of the vastus lateralis (VL) muscle were taken initially 4-6 weeks post-SCI and after 13 weeks of training. Initial biopsies in SCI subjects varied between 4 and 6 weeks post-injury when

subjects could sit upright for 30 minutes without orthostatic hypotension. Biopsies were taken from the non-injured control subjects 13 weeks apart. Biopsies for all subjects were taken from the non-dominant leg with a 25-gauge biopsy needle. Tissue was rapidly coated with Tissue-Tek OCT compound (Fisher 15-183-13), placed in a microcentrifuge tube, rapidly frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

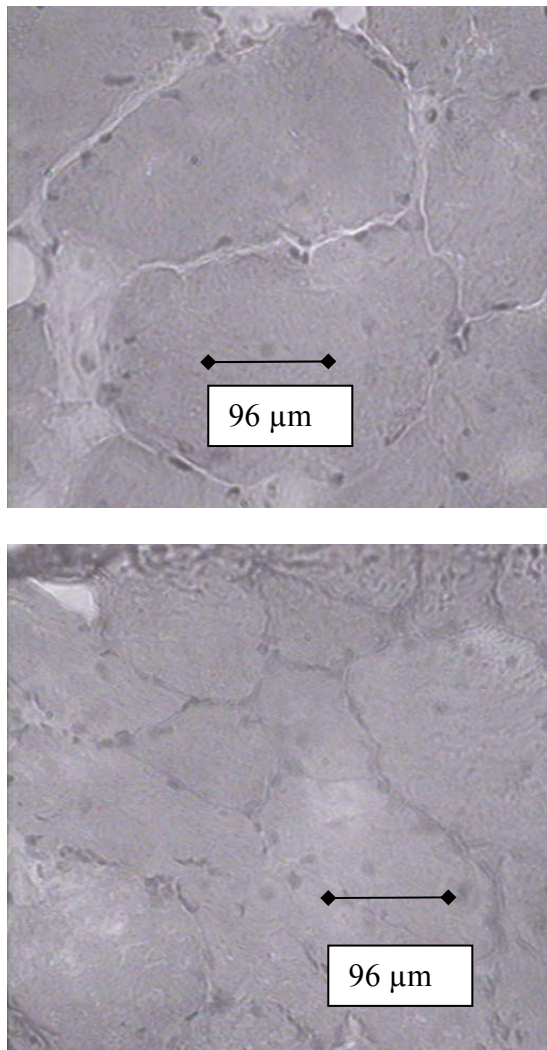


Figure 1. Cross sectional area (CSA) as seen with Hemotoxin and Eosin stain of initial biopsy uninjured control (top) and injured control (bottom) 5 weeks post injury.

Muscle histology and morphology

Ten-micrometer serial cross-sections were cut from the muscle biopsy sample using a Reichert Histostat Microtome cryostat (-20°C), placed onto coverslips and stained using hematoxylin and eosin for identification of nuclear density and muscle fiber cross sectional area (CSA_f) (Luna 1968). Slides were viewed under a Leitz Weitcher microscope equipped with a top mounted video camera. Images were sent to a Pentium 1 computer with Bioquant Classic 95

software package (R&M Biometrics, Inc.) for analysis.

Fiber CSA was determined by calculating the CSA of 100 fibers using computer software (Bioquant 95, R&B Biometrics, Inc.). The muscle fiber cross-sections were captured and traced on a video monitor using a hand held mouse. The software was calibrated to determine the area in μm^2 . Average fiber CSA was determined for each muscle from the mean of 100 fibers quantified for each muscle. To reduce experimental bias in the selection of fibers for measurement, all of the fibers on randomly selected slides were quantified. Quantification of CSA_f was practiced until a coefficient of variance of less than 5% was repeatedly achieved (See Figure 1).

Myonuclear density was defined as the number of myonuclei $\cdot\text{mm}^{-2}$ of CSA and was determined by counting the number of nuclei in a set area and dividing the number by the area in square millimeters (Allen et al., 1997). Five counts were taken from different regions of each muscle section and the average of these values was used to determine nuclear density. To reduce experimental bias in the selection of nuclei for measurement, the slide was randomly selected for performing myonuclei counting.

Myosin heavy chain (MHC) composition was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Talmadge and Roy, 1993). The SDS-PAGE gels were stained with rapid coomassie blue and scanned with a Pharmacia LKB Ultrascan laser scanning densitometer for quantification of MHC composition (See Figure 2).

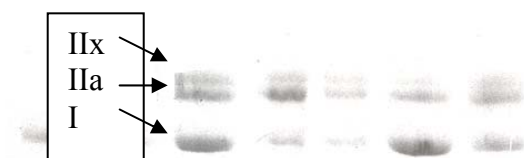


Figure 2. SDS-PAGE Myosin Heavy Chain (MHC). Representative picture of MHC gel.

Statistical analyses

A one-way ANOVA with repeated measures was used to determine changes in functional power output for the SCI exercise group. A two-way ANOVA was used for statistical analysis of nuclear density, fiber CSA, and changes in MHC composition. Level of significance was set a priori at $p < 0.05$. The Student-Newman-Keul method was used to determine specifically where the significant difference occurred.

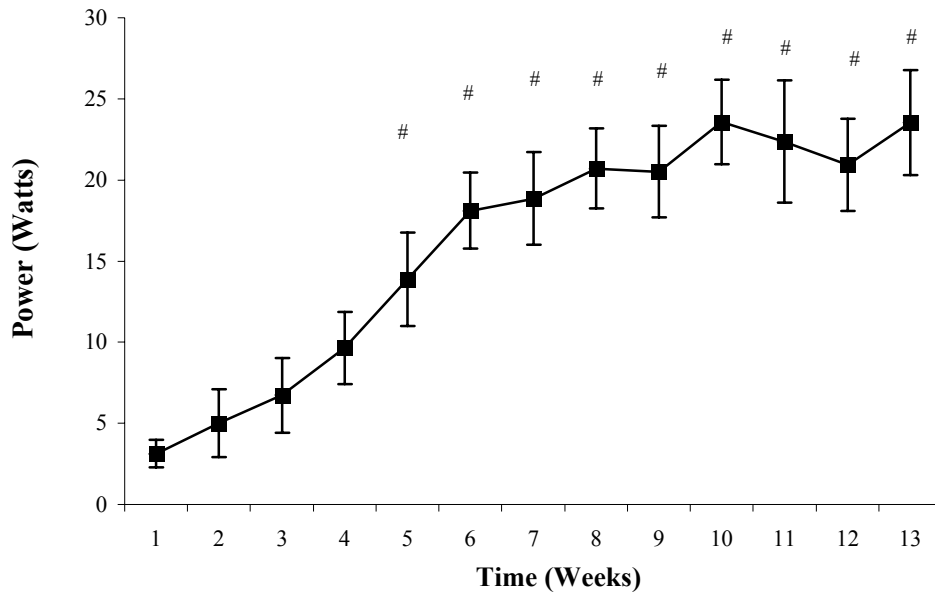


Figure 3. Effect of FES-CE (Functional electric stimulation cycle ergometry) training on power output in acute SCI (spinal cord injured) patients. All values are mean \pm SEM, $n = 5$. # denotes significant difference from weeks 1-4 ($p < 0.05$).

RESULTS

Functional changes

At the onset of training, subjects average power output was 2.4 ± 0.88 watts (mean \pm SEM). Average weekly power output increased in the IE group by week four. Power output continued to increase with training and reached a maximum of 24.5 ± 3.2 watts (Figure 3).

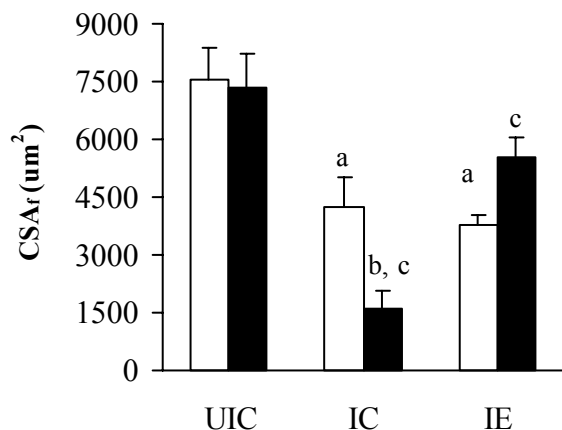


Figure 4. Effect of SCI (spinal cord injury) and FES-CE (functional electric cycle ergometry) on CSA_f (muscle fiber cross sectional area; μm^2) pre-treatment (empty); vs. post-treatment (black). UIC; uninjured control, IC; injured control, IE; injured exercise trained. a = significantly different than UIC; b = significantly different than baseline in same group; c = significant difference between IC and IE. Level of significance was ($p < 0.05$).

Muscle morphology

There was no difference in CSA_f between SCI groups at time of initial biopsy. However, compared with uninjured control subjects, SCI resulted in a 36% decrease in CSA_f in both injured groups (Figure 4; $p = 0.009$). There was a continued decrease (72%) from initial biopsy after 13 weeks in the IC subjects ($p < 0.001$). In contrast, FES-CE training increased CSA_f 63% in IE subjects following 13 weeks of training (3376 ± 625 vs. $5518 \pm 1205 \mu\text{m}^2$). However, the difference was not significant ($p = 0.172$). After 13 weeks of FES-CE training the CSA_f of the IE groups was 171% greater than the CSA_f in the IC group ($p = 0.05$).

There was no significant difference in nuclear density in SCI subjects compared to UIC at baseline. In addition FES-CE had no effect on nuclear density (Figure 5). Similarly, MHC composition of the muscle was unaffected by SCI and FES-CE (Table 2).

DISCUSSION

There is a paucity of data related to understanding the training consequences of functional electric stimulation cycle ergometry (FES-CE) on the maintenance of skeletal muscle form, structure, and function after acute spinal cord injury (SCI). To date, the effect of FES-CE in acute SCI subjects has been limited to measures of lean body mass and not focused on potential alterations to the morphological and physiological characteristics of skeletal muscle. Therefore, the primary aim of the present study was

Table 2. Effects of SCI and FES-CE on myosin heavy chain composition (%) pre-treatment vs. post-treatment. Values are mean (\pm SEM).

	UIC		IC		IE	
	Pre	Post	Pre	Post	Pre	Post
Type I	41.4 (13.8)	43.3 (11.9)	38.8 (14.9)	36.8 (11.1)	42.5 (13.5)	45.4 (26.2)
Type IIa	43.9 (9.2)	35.3 (8.8)	38.2 (11.0)	32.6 (18.6)	39.0 (17.5)	36.5 (10.8)
Type IIx	19.2 (10.1)	21.4 (8.4)	23.8 (5.8)	28.9 (6.6)	18.5 (5.6)	26.1 (12.3)

UIC; uninjured control, IC; injured control, IE; injured exercise trained.

to determine the affect of FES-CE on muscle fiber cross-sectional area (CSA_f), as well as to characterize changes in myosin heavy chain (MHC) composition, myonuclear density, and power output on a bicycle ergometer in acute SCI participants.

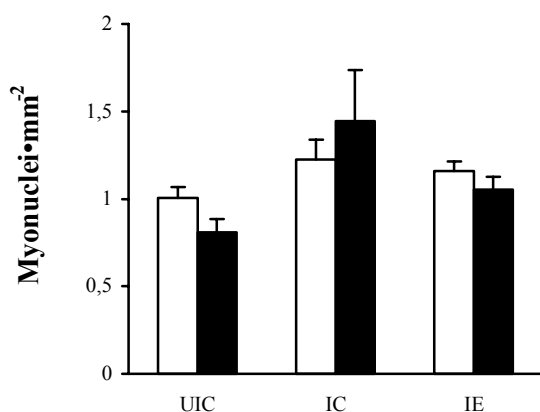


Figure 5. Effect of SCI (spinal cord injury) and FES-CE (Functional electric stimulation cycle ergometry) on myonuclear density (myonucle \cdot mm $^{-2}$) pre-treatment (empty) vs. post treatment (black). All values are mean \pm SEM, n = 5, IC-post n = 4. UIC; uninjured control, IC; injured control, IE; injured exercise trained.

Previous studies utilizing FES-CE training on chronic SCI survivors have reported increases in power output, defined as force input of a particular skeletal muscle or muscle group multiplied by velocity, that range from 5.1-17.1 watts following 8-52 weeks of FES-CE training (Chilinbeck et al., 1999; Faghri et al., 1992; Hooker et al., 1992; Mohr et al., 1997; Scremin et al., 1999). In contrast, data from the present study demonstrate that the weekly power output averaged 24.5 ± 3.2 watts, which was achieved with 13 weeks of FES-CE training. Thus, the increase in power output found in the present study is substantially greater than seen in previous studies using chronic SCI subjects, suggesting that early intervention utilizing FES-CE training more effectively conserves power output when compared with other electrical stimulation training protocols using chronic SCI patients.

A potentially salient adaptation known to result in an increase in power output is an increase in

force development and, force production of a given muscle is directly related to its cross-sectional area (CSA). Previous work in chronic SCI subjects indicated the increases in power output were due to increases in either whole muscle CSA (Mohr et al., 1997; Scremin et al., 1999) or CSA_f (Chilinbeck et al., 1999). In the present study, there was a 3 times greater increase in power output than reported by Mohr et al. (1997) suggesting that the more substantial attenuation of muscle atrophy observed in this study as a result of the acute intervention, contributed to diminishing the loss of power output (Figure 3).

In the present study, the CSA_f in SCI subjects decreased by 36% by 4-6 weeks post-injury, and 72% at 17-19 weeks post-injury relative to uninjured control subjects. These data are in close agreement with those of Castro et al. (1999) who reported decreases in CSA_f of 37% and 62% 6 and 24 weeks post-injury, respectively. Previous reports state that the CSA_f of chronic SCI subjects was 53% to 68% less than the CSA_f of uninjured control subjects (Castro et al., 1999; Martin et al., 1992). Therefore, the present data demonstrate a similar progressive loss in CSA_f and underscores the observation that the majority of changes in CSA_f occur within the first 24 weeks following a spinal cord injury event.

The CSA_f in the SCI exercise group (IE) of the present study, increased by 63% above pre-training levels after 13 weeks of FES-CE training and was 171% greater than the SCI control group at the same time point post-SCI. Previously, Scremin et al. (1999) and Mohr et al. (1997) reported increases of 31% and 12% in vastus lateralis and thigh CSA respectively; with 1 year of FES-CE training in chronic SCI patients while Chilinbeck et al. (1999) reported a 23% increase in CSA_f with 8 weeks of training. However, the increases in both CSA_f and whole muscle CSA seen in training chronic SCI subjects are relative to the pre-training CSA, but when the training effect are compared with the CSA_f of the uninjured control subjects, there is still an approximate 55% deficit. Thus, the training-induced increases in CSA_f in the present study are greater in magnitude than seen in chronic SCI training studies (68% vs. 23%), and occurred following a much shorter training period. Also the CSA_f post training in the acute SCI group was only 24% less than the

uninjured control group CSA_f versus approximately a 55% deficit seen in chronic SCI studies. Collectively, these observations again strongly support early intervention using FES-CE training as more effective in increasing CSA_f following SCI.

Following SCI muscle decreased power associated with decreases in whole muscle CSA and CSA_f , may be partially compensated for through a shift of MHC isoforms from slow to fast (Castro et al., 1999; Burnham et al., 1997). Mohr et al. (1997) reported that in chronic SCI subjects, the MHC composition of the vastus lateralis muscle adapted to 5%, 33%, and 62% for Type I, Type II_a, and Type II_x MHC, respectively. However, in spite of the large decrease in power MHC composition in SCI subjects was not different than control at either 6 or 19 weeks post-injury. Similarly, others (Castro et al., 1999) have reported no change in muscle MHC up to six months post-SCI. Therefore, changes in MHC in SCI subjects appears to occur at a slower time course than in other disuse models, such as spaceflight (Day et al., 1995) and further, decreased muscular power in SCI subjects appears to be largely due to decreased CSA_f .

The present study also showed no changes in MHC composition due to FES-CE training, whereas, studies utilizing FES-CE training with chronic SCI subjects reported changes in MHC isoforms from Type II_x to Type II_a, but no changes in Type I MHC composition (Mohr et al., 1997). One possible reason for no training effect of FES-CE on MHC in the present study was that no shift in MHC had occurred pre-training, therefore no stimulus for a reversal adaptation was present. Another possibility is that while the training stimulus was not great enough to cause shifts in MHC isoform profile, *in situ* hybridization could have potentially documented an up-regulation of the Type I MHC and down-regulation of Type II_x MHC isoform mRNA as noted by Harridge, et al in their electrical stimulation study involving chronic SCI survivors. The precise stimulus parameters needed to maximally elicit adaptation of MHC isoforms using electrical stimulation are not currently known.

One potential cause for lack of a training effect in response to FES with chronic SCI subjects compared to that observed in this study utilizing acute SCI subjects could be a change in the adaptability of the muscle. Muscle adaptations are related to the number of myonuclei present within fibers (Allen et al., 1997). To date no data exist that report alterations in myonuclear density in either acute or chronic SCI patients. Previous studies in non-SCI subjects reported increases in the number of myonuclei with increases in CSA_f (Hikida et al., 2000). In the present study, with acute SCI subjects, the loss of CSA_f had no effect on myonuclear density. Therefore our data suggest that sufficient

myonuclei are retained in acute SCI survivors to facilitate the increase in CSA_f and a subsequent increase in power output, with FES-CE training. Thus, our findings on myonuclear density suggest that the large leg muscles of acute SCI patients retain their potential to adapt to functional demands.

Although these data demonstrate an enhanced response to FES training in acute SCI subjects relative to chronic SCI subjects, the CSA_f had already decreased by 36% at our baseline assessment of 4-6 weeks post-SCI. These data would then suggest that to maximally conserve the cross-sectional area of the muscle, training should start immediately post-injury. However, the time required to recover from surgical stabilization, secure hemodynamic stability, and recover from spinal shock sufficiently to observe muscle contractions in response to stimulation realistically precludes the initiation of a FES-CE training program sooner than our baseline 4-6 weeks post-injury.

A potential limitation of this study is the sample size and therefore the applicability of the study to the general SCI population. The small sample size ($n = 5$ per group) occurred despite recruitment of individuals with SCI from a large Midwestern rehabilitation program with approximately 100 admissions for SCI rehabilitation per year. The recruitment pool was primarily limited by the inclusion criteria that potential subjects must have a motor complete injury and therefore fall within an ASIA A or B (American Spinal Injury Association) injury severity category due to the concern that the active exercise through volitional movement during the concurrent rehabilitation of patients with ASIA C or D type injuries would confound our outcome variables (Maynard et al., 1997). The second largest category of subjects excluded from the study involved injury related issues such as lower extremity fractures precluding FES-CE training, peripheral nerve injuries resulting in an inadequate response to electrical stimulation and thromboembolic disease requiring full dose anticoagulation, thereby precluding a muscle biopsy. Finally, the third significant barrier to subject recruitment involved transportation difficulties to training sessions.

CONCLUSIONS

In conclusion these data indicate that early intervention after SCI with FES-CE attenuates the changes in both the CSA_f and the loss of power output more effectively than that seen with chronic SCI and the maintenance of myonuclei suggests a preservation of the muscle's potential to adapt to functional demands. In view of the fact that the medical and neurologic consequences of the acute SCI precludes earlier intervention with FES than the

4-6 weeks after injury seen in this study, the rehabilitation and performance implications of these observations suggest that further development of training and stimulation protocols that enhance muscle strength and endurance for functional purposes such are needed. Future studies may therefore consider utilizing a combination of isotonic extension against resistance with FES-CE. Additionally, modifying the FEC-CE protocol once weekly to incorporate "sprint" training with repeated short bouts of high relative resistance followed by a rest period could be explored as mechanism to elicit a greater training effect.

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KEY POINTS

- Muscle fiber cross sectional area (CSA_f) decreased 38% following spinal cord injury (SCI).
- Early intervention with functional electric stimulation cycle ergometry (FES-CE) prevented further loss of CSA_f in SCI patients and increased power output.
- Muscle myosin heavy chain (MHC) and myonuclear density were unaffected by SCI or FES-CE.

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