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Doublet stimulation protocol to minimize musculoskeletal stress during paralyzed quadriceps muscle testing

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Abstract

With long-term electrical stimulation training, paralyzed muscle can serve as an effective load delivery agent for the skeletal system. Muscle adaptations to training, however, will almost certainly outstrip bone adaptations, exposing participants in training protocols to an elevated risk for fracture. Assessing the physiological properties of the chronically paralyzed quadriceps may transmit unacceptably high shear forces to the osteoporotic distal femur. We devised a two-pulse doublet strategy to measure quadriceps physiological properties while minimizing the peak muscle force. The purposes of the study were 1) to determine the repeatability of the doublet stimulation protocol, and 2) to compare this protocol among individuals with and without spinal cord injury (SCI). Eight individuals with SCI and four individuals without SCI underwent testing. The doublet force-frequency relationship shifted to the left after SCI, likely reflecting enhancements in the twitch-to-tetanus ratio known to exist in paralyzed muscle. Posttetanic potentiation occurred to a greater degree in subjects with SCI (20%) than in non-SCI subjects (7%). Potentiation of contractile rate occurred in both subject groups (14% and 23% for SCI and non-SCI, respectively). Normalized contractile speed (rate of force rise, rate of force fall) reflected well-known adaptations of paralyzed muscle toward a fast fatigable muscle. The doublet stimulation strategy provided repeatable and sensitive measurements of muscle force and speed properties that revealed meaningful differences between subjects with and without SCI. Doublet stimulation may offer a unique way to test muscle physiological parameters of the quadriceps in subjects with uncertain musculoskeletal integrity.

Keywords

spinal cord injury; electrical muscle stimulation; potentiation; contractile speed

SCIENTISTS are vigorously pursuing therapeutic methods to maintain the musculoskeletal properties of limbs after spinal cord injury (SCI). It is widely accepted that the loss of routine muscle contractions removes a powerful stimulus for the maintenance of bone mineral density (BMD). One method to exert stress to the paralyzed extremities is through electrical activation of key paralyzed muscles in individuals with SCI.

The quadriceps femoris is considered a prime muscle to electrically activate and train after SCI because of its two-joint role with standing and applying loads across the hip and knee. In order to elicit desirable adaptations in muscle force-production output and fatigue resistance, electrical stimulation training protocols must deliver an “overload” stimulus to the trained muscle. To accomplish this, the electrical stimuli delivered during training must be sufficient

to induce strong contractions (45) [the principle of stress adaptation (32)]. Ideally, this same population of motor fibers should be recruited during testing of physiological adaptations to training. To accomplish this, a testing protocol must use a sufficient stimulus intensity to recruit the trained population of motor fibers; this will cause high contractile forces to be developed during test sessions. However, assessing the physiological properties of the chronically paralyzed quadriceps muscles may transmit high shear forces to the distal femur (11,22). BMD of the distal femur declines by ~50% in the first 3–8 years post-SCI (10). Lifetime fracture risk for people with SCI is approximately double the risk experienced by the general population (54). Several unpublished communications and one published report (22) support that fracture of the distal femur may occur during tetanizing contractions of the paralyzed quadriceps femoris in a seated position. As such, our ability to quantify the effects of reduced use and electrical stimulation training on the paralyzed quadriceps muscle is limited.

Force-generating capacity and contractile speed are important physiological properties of paralyzed muscle. These muscle physiological parameters provide insights into the extent that paralyzed muscle shows fast vs. slow adaptations. In general, human muscle testing is conducted with the muscle near its optimal position on the torque-angle curve (14,41). In the case of the quadriceps muscle, optimal length, and the greatest torque, are achieved by placing the knee in approximately 90–100° of flexion during sitting. However, within this position, shear forces across the femur increase with muscle activation and may present a fracture risk to subjects with SCI (22). Rittweger and colleagues (36) recently assessed the paralyzed quadriceps muscle with an electrical stimulation protocol consisting of three pulses (triplet), preventing full tetanic force development. However, they did not determine whether this method is suitable to measure quadriceps muscle physiological properties (force, rate, fusion, etc.) as a function of reduced use or training. Another study by Gerrits and colleagues (16) explored quadriceps force-frequency behavior with triplet stimulation but did not examine contractile speed characteristics. We have observed that as much as 80–90% of the fully tetanized force may still be generated by just three stimulus pulses in chronically paralyzed muscle (44). It is questionable whether triplet stimulation [particularly at high frequencies: 150 Hz (16) to 300 Hz (36)] offers adequate subject-safety advantages over tetanic stimulation.

Recently, we developed a protocol to assess muscle physiological properties of the quadriceps while limiting peak muscle force. We used a two-pulse doublet to minimize peak force but still reveal important physiological properties of the skeletal muscle. The first pulse is presumed to “take up the slack” (contractile filament nonoverlap, muscle passive elastic structures, tendon compliance, etc.) while the second pulse is believed to be less affected by any confounding influence of muscle/tendon compliance (35). The overall summated force developed by the second doublet pulse is only a fraction of the maximum tetanic force capacity of the muscle (42). This feature is particularly appealing because the stress placed on the skeletal system remains low.

Accordingly, the purposes of this study are to determine the 1) repeatability of a novel doublet stimulation protocol specifically designed to minimize quadriceps muscle force in individuals with SCI, and 2) to compare this protocol among individuals with and without SCI.

METHODS

Subjects

Six men with complete motor and sensory SCI (American Spinal Injury Association ASIA-A) participated in the study(1). Four additional individuals without SCI (1 woman) served as a control cohort. This protocol was approved by the University of Iowa Human Subjects Office institutional review board. All subjects provided written informed consent before participation. Exclusion criteria were lower motor neuron injury to the lumbar-sacral spinal segments,

musculoskeletal injury to the paralyzed limbs, systemic illness, and history of fracture to the lower extremity. Demographic data for the subjects appear in Table 1.

The subjects with SCI comprised a sample of convenience designed to survey a range of SCI durations (Table 1). No paralyzed quadriceps muscles studied had previously received any training before this study. Two of the subjects (*SCI 3* and *SCI 4*, Table 1) participated in a unilateral quadriceps training protocol. Their untrained limbs only are included in group mean values in this report.

Instrumentation

Subjects sat in a wheelchair or a standard chair with the ankle positioned against a force transducer apparatus. The transducer consisted of a padded, semicircular metal plate that cupped the anterior surface of the leg. This plate was connected to a force transducer (1500ASK-200, Interface, Scottsdale, AZ) that was mounted on a rigid aluminum backplate. Measured accuracy, hysteresis, repeatability, and resolution error of the force transducer were all <1%. The force transducer could be adjusted vertically to suit the height of the subject's ankle. Two padded straps passed behind the ankle to draw the tibia securely into the force transducer system. An experimenter measured the subject's knee and hip angles with a goniometer and then measured the distance from the force transducer to the subject's lateral femoral condyle. This distance was considered to be the moment arm for quadriceps force (Table 1). These values were used during repeated measurements to replicate the initial subject position.

The experimenters affixed self-adhesive carbon electrodes (7×13 cm) to the skin over the quadriceps muscles. The distal margin of the distal electrode was aligned with the distal-most palpable border of the vastus lateralis. The proximal border of the proximal electrode was positioned as close as possible to the inguinal crease. Medial-lateral position of both electrodes was replicated by palpation of the adductor musculature; the medial border of each electrode was placed just lateral to the adductor group. Stimulation was controlled by digital pulses from a data-acquisition board (Metrabyte DAS 16F, Keithley Instruments, Cleveland, OH) housed in a microcomputer under custom software control. The microcomputer output was conveyed via shielded cabling to a muscle stimulator unit (Digitimer model DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK). The stimulator was set to deliver 500- μ s pulses at 400 V, at intensities up to 200 mA. All testing in both groups was conducted at intensities that elicited maximal twitch forces.

Non-SCI subjects tolerated levels of stimulus intensity that were comparable to those used for maximal contractions in SCI subjects. For all subjects, we determined the stimulus intensity that elicited a maximal twitch response. Testing was then conducted at 1.2 times the maximal twitch intensity. We never had a need to exceed 200 mA (Table 1). Because individuals with SCI could not feel the stimulation, we routinely increased the stimulation greater than the 1.2 times the maximal intensity.

Quadriceps force signals were amplified and input to a 12-bit-resolution analog-to-digital converter with a sampling rate of 2,000 samples/s. The digitized signals were analyzed with Datapac 2K2 software (RUN Technologies, Mission Viejo, CA).

Test procedure

Ten stimulus pulses were given at progressively increasing intensities up to 200 mA to familiarize the experimenters with the subject's quadriceps force characteristics. The pulses were widely spaced (10 s between each) to avoid potentiating the muscle before the test protocol began (47). The test protocol consisted of three phases (Fig. 1). First, twitches and trains were

given to delineate the twitch, tetanus, and potentiation properties of the muscle. Next, doublets were given at incrementally increasing frequencies to establish the doublet force-frequency relationship. Finally, twitches were again given to determine whether the doublet testing protocol induced fatigue or further potentiation.

The first phase of the protocol consisted of five twitches at 1 Hz, three 10-pulse stimulus trains at 5 Hz, and another five twitches at 1 Hz. In Fig. 1 these are denoted “1t,” “5t,” and “1t2,” respectively. Next, three doublets (separated by 2 s each) were given at each of the following frequencies: 6, 7, 8, 9, 10, 15, 20, and 30 Hz. Finally, after completing the doublet recruitment curve, five more twitches were collected at 1 Hz, denoted as “1t3” (Fig. 1). Force and contractile speed-dependent variables were obtained from the mean value of all events at each stimulus condition.

Data analysis

We identified five dependent variables related to muscle physiological properties (Fig. 2). Peak force was defined as the maximum peak amplitude of the force signal, averaged for all twitches/trains/doublets at a given stimulus frequency (1t, 5t, 8d, etc.). At low doublet frequencies where summation did not occur, the peak force in any given event could occur after either the first or the second doublet pulse. At higher doublet frequencies, summation of force caused the peak force to occur after the second doublet pulse. To allow comparisons of the quadriceps force-frequency relationship among subjects, we expressed the peak force at each frequency as a percentage of the maximum peak force generated for the entire protocol.

We analyzed contractile speed properties for the 1-Hz single twitches only (denoted as 1t, 1t2, and 1t3, as depicted in Fig. 1). Twitch speed properties are known to vary according to fiber type (4). We examined twitch speed properties between 20% and 80% of the peak force for each contraction (Fig. 2, *top*). The lower percentage constraint was employed to eliminate the portion of the force record most heavily influenced by muscle and tendon compliance (0–20%). The upper constraint was used to eliminate the “turning” region of the force record, where calcium transients begin to decline and progressively more cross bridges become quiescent. By using these constraints, we focused on the portion of the twitch force record most representative of the muscle's true contractile speed properties.

The rate of force rise was defined as the quotient of the force developed in the above-mentioned region of interest and the elapsed time of this region (Fig. 2, *top*). The rate of force rise has been shown to differ between functionally fast and functionally slow muscle (43,45). Because it is also influenced by the peak force (21), we normalized the rate of rise for each twitch to the peak force for that twitch.

The rate of force fall was likewise determined between 80% and 20% of peak twitch force on the descending portion of the twitch response (Fig. 2, *top*). Rate of force fall was defined as the quotient of the force decay between 80% and 20% and the elapsed time of this interval. For each twitch response, the rate of force fall was normalized to the peak force for that twitch.

Finally, we desired an estimate of the contractile fusion present within the doublet force record because this factor may also vary according to muscle physiological properties. We subtracted the force attributable to doublet *pulse 1* from the peak force, which was invariably associated with doublet *pulse 2*. This difference value reflects the degree of twitch fusion within the doublet force response (the “twitch difference”) (Fig. 2, *bottom*). A higher twitch difference indicates greater fusion between twitches. Twitch difference would be expected to differ between functionally fast and functionally slow muscles. However, because it is mathematically dependent on peak force, we normalized the twitch difference by peak force to yield a “fusion ratio” that can be compared across subjects. In essence, the fusion ratio

represents the percentage of the total force contributed by *pulse 2*, the pulse which should be more or less summated depending on the contractile speed properties exhibited during the first doublet pulse. A higher fusion ratio indicates a larger contribution of *pulse 2*, and hence greater summation between the two doublet force events.

To obtain fusion ratio values, the two force events in each doublet were demarcated according to the onset and offset times of the doublet stimulus pulses (Fig. 2, *bottom*). At 5-Hz through 10-Hz doublet frequencies, the force contributed by the first doublet pulse was declining when the second doublet pulse arrived. Two distinct force “peaks” are therefore identifiable at these doublet frequencies. However, summation of force at 15, 20, and 30 Hz obscured the division between the portions of the force record contributed by *pulse 1* and *pulse 2*. Moreover, at 20 Hz and 30 Hz (50- and 33-ms interpulse intervals), very little force had developed by the time the second doublet pulse arrived (Fig. 2, *bottom, inset*). (That is, the interpulse interval was only slightly longer than the muscle electromechanical delay.) Fusion ratio values at 15, 20, and 30 Hz are therefore not reported.

Between- and within-day variation

We desired an estimate of the between- and within-day variability of the force-frequency relationship using this doublet protocol in the SCI group. On the first day of testing, subjects underwent the test protocol and then repeated the protocol after 5 min of rest. Comparisons between the 1st and 2nd test bouts for this day provide an estimate of within-day repeatability. Subjects returned for a second day of testing after at least 3 days. Stimulus intensity on the second day was identical to the first day. Only one bout of the protocol was collected on the second day. Comparisons between *day 1* and *day 2* provide an estimate of between-day repeatability.

Statistical analysis

A two-way ANOVA (repeated measures) was used to determine whether differences existed between the peak force for the first and second bouts (within-day repeatability) and between the first and second days (between-day repeatability) of testing. We computed the difference between repeated days and bouts as a percent error and calculated a Pearson product-moment correlation coefficient.

We next established the variation about the force-frequency curve for both the SCI and non-SCI groups. We used a two-way split plot repeated-measures ANOVA to determine if the peak force for the non-SCI group responded similarly to the SCI group for each stimulation frequency (interaction; $P < 0.05$). In the event of a significant interaction, we carried out simple effects analysis (t -tests) to determine where differences existed between the two groups and among frequencies. We adjusted the P value (Bonferroni) to compensate for multiple comparisons.

Independent t -tests were used to determine if the twitch speed properties were different between the SCI and non-SCI groups. Regression analysis was used to calculate the slopes for the twitch difference and fusion ratios for each group.

RESULTS

Peak force

The repeatability of this doublet testing protocol was high for both within-day and between-day assessments. Mean (SE) within-day absolute error for repeated testing was 5.17% (0.47%) across all frequencies (Pearson correlation: $r = 0.870$). Mean between-day absolute error was 5.18% (0.37%) across all frequencies (Pearson correlation: $r = 0.884$).

Peak quadriceps force for both subject cohorts appears in Table 1. A representative example of the force response to various doublet activations is presented in Fig. 3. The normalized force-frequency graphs (Fig. 4) demonstrate that the plots are different between the SCI and non-SCI groups across frequencies (significant interaction: $P < 0.05$). For single twitches and for doublet frequencies below 20 Hz, SCI subjects generated a higher relative force than the non-SCI subjects ($P < 0.05$) (Fig. 4). The normalized force-frequency relationship for the SCI group was therefore shifted leftward compared with the non-SCI group.

Potentialiation of twitch force occurred after the 5-Hz tetanic train (5t) for the SCI subjects; the posttetanic twitch (1t2) was higher than the pretetanic twitch (1t) ($P < 0.05$) (Fig. 4). Mean force at 1t2 was 20.09% (SE 6.04%) higher than force at 1t. A smaller (7.29%) but significant ($P < 0.05$) degree of posttetanic potentialiation occurred in the non-SCI subjects. No potentialiation or fatigue occurred as a result of the series of doublet pulses; 1t2 and 1t3 did not differ for the SCI subjects ($P = 0.62$) (Fig. 4) or for the non-SCI subjects ($P = 0.12$).

Rate of force rise

The rate of force rise was analyzed for the single-twitch data (1t, 1t2, and 1t3) (Fig. 5, *top*). For all twitches, normalized rate of force rise was lower for the non-SCI subjects than for the SCI subjects (all $P < 0.05$) (Fig. 5, *top*). For the non-SCI subjects, rate of force rise was significantly higher for the postdoublet (1t3) than the pretetanic (1t) or posttetanic (1t2) twitches ($P < 0.05$). Among the SCI subjects, rate of force rise was significantly higher for the posttetanic (1t2) and postdoublet (1t3) twitches than for the pretetanic twitches (1t) ($P < 0.05$). Thus while both subject groups demonstrated increased rate of force rise, the significant increase only emerged after completion of the doublet protocol for the non-SCI subjects.

Rate of force fall

Rate of force fall was analyzed for the single-twitch data (1t, 1t2, and 1t3) (Fig. 5, *bottom*). No significant differences in fall rate emerged at any twitch condition for the SCI group. At 1t and 1t2, rate of force fall was lower for the non-SCI subjects than for the SCI subjects. The non-SCI group demonstrated a significant increase in fall rate after the tetanic trains (1t vs. 1t2) and another significant increase after the doublet protocol (1t2 vs. 1t3) ($P < 0.05$).

Fusion index

Figure 6 depicts mean (SE) peak force values for events “A” and “B” of the doublet protocol, subdivided as described in Fig. 2. No summation of force was expected to occur in the first twitch of each doublet pair. As such, *event A* force values should be relatively constant across frequencies, as is depicted in Fig. 6, *top*. When mean values for all A events are plotted separately, they fall along a line with slope = 0.134 for the SCI group, confirming that force for the 1st twitches of the doublets changes very little across frequency. Similarly, plotting non-SCI A-event data yields a slope of 1.189, suggesting that in these subjects, 1st-twitch force increased slightly across frequencies.

Summation of force was expected to occur during the second event of each doublet. A shorter interpulse interval would allow less force decline after *pulse 1* before the start of *pulse 2*, yielding greater between-pulse fusion and higher *twitch 2* force. Plotting all B events separately for the SCI group yields a line with slope = 3.600, confirming that *twitch 2* force did indeed increase across frequencies (Fig. 6, *top*). Similarly plotting all non-SCI B-event data yields a slope of 6.127, suggesting that the augmentation of *twitch 2* force across frequencies for this cohort was even steeper than for the SCI cohort.

To more adequately categorize the contribution of *twitch 2* to the total force, we calculated a fusion ratio as described in Fig. 2. Fusion ratio is, in essence, the individual contribution of

twitch 2 as a percentage of the peak force. Because *twitch 2* is subject to between-pulse summation of force, a higher fusion ratio at any given frequency is indicative of greater force summation, a hallmark of functionally slower muscle. At all frequencies (5–10 Hz), fusion ratio was significantly higher for the non-SCI subjects than for the SCI subjects ($P < 0.05$), suggesting that between-pulse fusion was more prevalent in the non-SCI subjects (Fig. 6, *bottom*).

DISCUSSION

The purpose of this study was to determine if a lower force-generating stimulation protocol (doublet stimulation) could reliably discriminate between muscle with different physiological properties (SCI and non-SCI). We found that this testing strategy had sufficient sensitivity to reveal differences between subjects with and without SCI, to characterize the quadriceps force-frequency relationship, and to reveal posttetanic potentiation of quadriceps force. The protocol had sufficient within- and between-day repeatability to be a useful measurement strategy in studies of quadriceps adaptation to electrical stimulation training.

Rationale for the doublet protocol

The essential function of the quadriceps muscle in gait makes it a prime candidate for therapeutic electrical stimulation training. Moreover, its orientation and large cross-sectional area allow it to deliver high compressive loads to the skeletal system, hinting at its usefulness for protocols designed to prevent neurogenic osteoporosis after SCI (45,46). Modeled patellofemoral contact force increases dramatically as the knee moves from 0 to 90° of flexion and as quadriceps force increases (29). High patellofemoral contact force during quadriceps stimulation in 90° of knee flexion was suspected to be the cause of the lateral femoral condyle fracture previously reported in the literature (22). Testing chronically paralyzed quadriceps muscle properties without clear knowledge of the underlying skeletal integrity may be a risky undertaking. Therefore, there is a need for a low force-generating protocol to establish physiological effects of reduced and increased use following SCI.

During physiological testing, force could be limited by reducing the stimulus intensity of the tetanizing trains. Sub-maximal activation with trains, however, tends to elicit spasm activity that compromises the quality of force recordings. An alternative strategy is to limit the number of stimulus pulses, thus avoiding full tetanic force development. Rittweger and colleagues (36) used a triplet stimulation protocol to describe the tendon forces and bone strains that develop during quadriceps stimulation. However, they did not document the extent to which their protocol could characterize physiological parameters of the quadriceps in subjects with and without SCI. Gerrits and colleagues (16) described quadriceps force-frequency behavior in response to triplet stimulation but did not explore the usefulness of such a protocol for delineating between-twitch fusion or other speed-related parameters. Because we have observed that chronically paralyzed muscle may develop 80–90% of full tetanic force after just three stimulus pulses (44), we opted to explore the usefulness of doublet stimulation for quadriceps physiological testing. Low-shear test protocols will be critical as novel rehabilitation intervention strategies are developed and tested in the future (9,44,45).

Repeatability of the doublet protocol

Force values (normalized to the peak force of the bout) demonstrated excellent between-day (5.18%) and within-day (5.17%) repeatability. Among the various stimulus conditions, the poorest repeatability occurred during the 10-pulse, 5-Hz trains (7.15% error between days, 11.4% error within day). Subjects routinely demonstrated quadriceps spasm activity during the 5-Hz train, usually beginning after the fifth stimulus pulse. The appearance of spasm was unpredictable, resulting in less consistent peak force values between bouts and between days.

Repeatability values for the doublet and twitch stimulus conditions were superior to the 5-Hz train, underscoring the utility of using doublet stimulation in lieu of tetanic trains for physiological testing of paralyzed muscle.

Peak force

Single-twitch force values for the SCI cohort (mean 126.35 N) were only 35% lower than twitch forces elicited in the non-SCI subjects (mean 195.85 N) (Table 1). Due to post-SCI muscle atrophy, it may seem counterintuitive that subjects with SCI generate high muscle forces. However, high evoked forces have previously been observed in paralyzed muscle, even in the absence of electrical stimulation training (17,41,45). Proliferation of inelastic tissue after SCI may alter the force-length relationship of paralyzed muscle (27,55). The test position may have placed the noncontractile and contractile elements of the paralyzed quadriceps in a more advantageous position compared with the non-SCI muscles. However, other authors have suggested that force-length adaptations in the quadriceps may be minimal after SCI, likely due to knee positioning during long-term wheelchair use (16).

Second, numerous previous studies have shown that paralyzed muscle functions as a composite of fast fatigable muscle fibers (43,45). Histological studies confirm that the quadriceps fiber-type profile shifts from slow oxidative to fast fatigable gradually after SCI (8,28,38,49). Several studies suggest that fast fatigable muscle fibers possess a higher specific tension than slow fibers (31). Thus compared with the non-SCI state, higher force per fiber may be generated in the muscles of SCI subjects, predisposing this group toward higher force values.

Quadriceps force increased with each progressive frequency increase in the non-SCI group. In contrast, summation of force for the SCI group showed greater variability, peaking in most subjects at 15 Hz but at 10, 20, or 30 Hz in other instances. Reflex-mediated contractions do not seem to explain this variation because any spasms that were observed occurred after the peak force from the doublet had declined.

The quadriceps force-frequency relationship varied markedly between groups. At each frequency below 20 Hz, SCI subjects generated a higher percentage of maximum force than non-SCI subjects (Fig. 4). This leftward shift of the force-frequency relationship is normally indicative of functionally slow muscle. However, this explanation would certainly not be supported by the force rate of rise and rate of fall data (Fig. 5), which characterized the nonparalyzed muscles as functionally slower than the paralyzed muscles. This phenomenon does not appear to be unique to the present study; a leftward shift of the force-frequency relationship of paralyzed muscle was also observed in a protocol involving triplet stimulation (16) and in others involving tetanic stimulus trains (12,14). An elevated twitch-to-tetanus ratio has previously been reported for paralyzed quadriceps muscle (16,41). This suggests that doublets in paralyzed muscle may “outperform” similar doublet twitches in nonparalyzed muscle at any given stimulus frequency (17), yielding the observed leftward force-frequency shift. Previous authors (16) speculated that the leftward force-frequency curve may be artifactual; if SCI yields loss of quadriceps muscle length (as has been intimated for other neurological conditions such as stroke), calcium sensitivity in the paralyzed quadriceps may be superior because its sarcomeres would be relatively more lengthened during testing (48). However, optimum angle of force development did not differ between subjects with and without SCI, suggesting that SCI did not yield loss of quadriceps length (16) (as would be reasonably expected in long-term wheelchair users). The observed leftward force-frequency shift during triplet stimulation in paralyzed muscle was not attributable to relative differences in muscle length (and by extension, calcium sensitivity) during testing. The explanation for this paradoxical but well-attested phenomenon remains unclear. One animal study suggests that the explanation may lie in fiber-type transformation after SCI. Fast-twitch motor units in rats possessed an inherently higher responsiveness to changes in doublet interpulse interval

than slow-twitch motor units (19). If this phenomenon also occurs in human muscle, post-SCI fiber-type adaptations may enhance the sensitivity of paralyzed muscle to increases in stimulation frequency, skewing the force-frequency relationship leftward.

Another important difference between the SCI and non-SCI groups was the emergence of differing degrees of posttetanic potentiation (PTP) (33) (20% in the SCI group vs. 7% in the non-SCI group). The differing degree of potentiation between the subject groups is congruent with fiber-type differences expected to be present in subjects with and without SCI. PTP is more strongly demonstrated by fast-fatigable muscle fibers (20,30), which the SCI subjects would be expected to possess in greater abundance. The lower degree of potentiation demonstrated by the non-SCI subjects may reflect the more mixed population of fast and slow fibers normally present in nonparalyzed muscle. Alternatively, the muscles of the non-SCI subjects may have only minimally demonstrated potentiation because they entered the test protocol in a relatively more potentiated state. The mechanism of PTP is believed to be phosphorylation of myosin regulatory light chains (30,51-53), which enhances the sensitivity of the actin-myosin complex to Ca^{2+} released by subsequent action potentials. Volitional activity by these subjects while entering the test apparatus likely maintained the phosphorylation state of the myosin light chains at a higher level than in the paralyzed subjects. Minimal further phosphorylation was elicited by the 5-Hz trains, yielding less PTP in the subsequent 1-Hz trains.

Fatigue is often conceptualized as a process that competes with potentiation; whereas phosphorylation of myosin light chains increases Ca^{2+} sensitivity of the contractile filaments, fatigue tends to restrict calcium availability via derangement of the sarcolemmal excitation-contraction coupling system. We observed no evidence that fatigue developed during the course of this protocol; twitch force, rate of force rise, and rate of force fall were the same before the doublets (1t2) and after the doublets (1t3) for both subject groups. This finding supports the viability of this test protocol for measuring quadriceps muscle physiological parameters after SCI.

Contractile speed

As observed in previous studies (14,43), paralyzed muscle in the present study demonstrated characteristics of functionally fast fibers. While without histochemical assays we cannot comment on fiber-type transformation, the correlations between fast fatigable fibers and faster contractile speed properties are well typified.

By normalizing twitch contractile rates to peak force, we are able to compare contractile speed properties across subjects with widely varying peak force capacities. Contractile speed was slower in the non-SCI subjects, as demonstrated by a lower rate of force rise and lower rate of force fall (Fig. 5). This is consistent with a number of previous studies that show that paralyzed muscle shifts toward a faster profile (14,43). A variety of adaptations may underlie this shift, including changes in myosin light and/or heavy chains (MLC, MHC) (5,18,38,40,49), including hybrid MHC isoforms (2,39,49,50), and sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) iso-forms (49). These adaptations may all occur according to different time courses after SCI. As with MHC hybrid iso-forms, these adaptations may also be transient (5) or may reach a steady state (50). In the present study, the normalized rate of twitch force rise was higher after repetitive stimulation (Fig. 5), a phenomenon known as “rate potentiation” (as opposed to potentiation of force, or PTP). Both subject groups demonstrated rate potentiation in the present study (Fig. 5), although rate potentiation did not achieve significance in the non-SCI group until completion of the doublet protocol. Possible physiological mechanisms for rate potentiation are still under investigation.

The two subject cohorts in the present study were not age matched, and, as such, age-related changes in contractile speed could have contributed to the observed between-group differences. Compared with the large influence of SCI, however, age-related contractile slowing does not likely contribute a considerable degree of variability between subject groups. The contractile speed properties of young men and octogenarians have been observed to differ by 10–23% (6,37). Other work suggests that contractile speed changes may be most notable only after age 50 (34), applicable to just one subject in the non-SCI cohort. In contrast, normalized rate of twitch force rise differed by 32–34% between SCI and non-SCI subjects in the present study (Fig. 5). Normalized rate of twitch force fall differed by 33% between the groups overall. This difference rose to 59.6% when considering the 1t and 1t2 conditions alone (Fig. 5). Thus, although age-related effects may have contributed to the slower contractile speed properties exhibited by the non-SCI subjects, the magnitude of the difference between groups cannot solely be explained by this factor.

Doublet fusion

To estimate the degree of contractile fusion between the doublet pulses, we calculated a fusion index that describes the relative contribution of the second pulse to the overall force. A high fusion index indicates that the second pulse contributed a considerable portion of the total force (up to ~40%, Fig. 6), suggesting that summation of force occurred between the doublet pulses. Summation occurs most readily in muscles with a low rate of force decline, a hallmark of a slowly contracting muscle. A high fusion index would therefore be associated with functionally slow muscle. As expected, the force contribution of the first doublet pulse varied little across frequencies (Fig. 6, *top*). However, congruent with our previous characterization of the non-SCI muscles as functionally slower, they demonstrated a significantly higher fusion index than the SCI group (Fig. 6, *bottom*).

Evidence also emerged that the non-SCI group experienced a greater increase in force due to the second doublet pulse at each frequency than did the SCI group. When plotting the force contributed only by *pulse 2* (the “B” events), the slope of the regression for the non-SCI group (6.127) exceeded the slope of the SCI group (3.600). This result may appear paradoxical with the observed leftward force-frequency shift for the SCI group, which suggested that doublet stimulation is instead particularly effective in paralyzed muscle (Fig. 4). We believe that the observed leftward force-frequency shift in paralyzed muscle may primarily be attributable to the well-documented increase in twitch-to-tetanus ratio after SCI (16,41). Other authors have held the same viewpoint (12). Although fusion between the doublet pulses was superior for the non-SCI subjects due to their slower contractile properties, this responsiveness to doublet stimulation could not offset the elevated twitch response characteristic of paralyzed muscle. Thus at the lowest tested frequencies, paralyzed muscle force output was a higher percentage of the maximum force value (a leftward force-frequency shift), but at higher frequencies, the enhanced fusion capabilities of the nonparalyzed muscle counteracted this force-frequency offset between groups. We speculate that the contractile speed alterations that limited doublet fusion in the SCI group may reflect possible post-SCI adaptations in myosin regulatory light chains, SERCA isoforms, or other calcium regulatory molecules such as parvalbumin, phospholamban, ryanodine, and dihydropyridine. Although isoform expression of some of these molecules is known to vary according to fiber type (3,23–26,28,49), the adaptations of these molecules to SCI have not yet been extensively investigated.

Maximizing recruitment while minimizing stress

In an ideal situation, the entire quadriceps muscle could be recruited during studies of paralyzed muscle physiology, to most accurately characterize the adaptations that occur after SCI. If the full muscle were activated in tetany, however, total forces across the femur could be dangerously high. Previous studies using tetanic stimulation have not yielded dangerous forces

because they recruit only 25–35% of the quadriceps muscle fibers (14,15,41,42). These studies have employed low stimulus intensities (7,13,14). In contrast, the present doublet stimulation strategy activated the muscle with enough stimulation intensity to recruit maximal twitch forces. However, by limiting summation, the doublet protocol yielded quadriceps force values no higher than those reported by previous studies using tetanizing contractions and submaximal stimulation (75–180 N) (7,13,14,41,42). In particular, the forces developed in this doublet testing protocol were only one-third as high as the forces reported to have caused a femoral condyle fracture during testing with tetanic stimulation (22). We believe that doublet stimulation at high stimulation intensities provides a novel way to sample force and speed properties from nearly the entire muscle while minimizing the peak forces placed upon the femur.

Conclusion

Physiological properties including force, contractile speed, potentiation, and fusion were measured with a doublet stimulation protocol and differed, as expected, between the quadriceps of individuals with and without SCI. This protocol offered a safe and efficient method to assess the quadriceps muscles of individuals with uncertain skeletal integrity. With long-term electrical stimulation training, paralyzed muscle can serve as an effective load delivery agent for the skeletal system (45). Muscle adaptations to training, however, will almost certainly outstrip bone adaptations (22). Mismatches between muscle force and bone strength expose participants in training protocols to an elevated risk for fracture. In such studies, it is imperative that we are mindful of the stresses bones experience during routine physiology tests of the quadriceps. Based on the findings from this study, a force-limiting stimulation strategy (doublets) provides repeatable and sensitive measurements of physiological differences in SCI and non-SCI muscle.

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Fig. 1.

Schematic representation of the stimulation protocol. (Interpulse intervals are to scale, but rest periods between the conditions are truncated.) Five repetitions were performed of each single-twitch condition (1t, 1t2, 1t3). Three repetitions were performed of each train (5t, bracket) and doublet condition (5d, 6d, 7d, etc). Each repetition was separated by 1 s (1t, 1t2, and 1t3) or by 2 s (train and doublet conditions) to ensure force returned to baseline before the next stimulation.

Fig. 2.

Method of determination of dependent variables. *Top*: a single twitch is depicted. On the ascending limb of the twitch, the rate of force rise was defined as the quotient of 1) the force developed in the rectangular region (80% of peak minus 20% of peak), and 2) the elapsed time of the rectangular region (ms). On the descending limb of the twitch the rate of force fall was defined as the quotient of 1) the force decay in the rectangular region (80% of peak minus 20% of peak), and 2) the elapsed time of the rectangular region (ms). For each twitch response, rate of force rise and rate of force fall were divided by the peak force of that twitch to yield normalized rates in ms^{-1} . *Bottom*: a 10-Hz doublet response, with stimulus pulses at 0 and 100 ms, is depicted. To analyze twitch difference, the force record was partitioned into 2 events based on the onset/offset of the stimulus pulses (denoted “A” and “B”). Twitch difference was defined as the maximum force developed in *event B* minus the maximum force developed in *event A*. The twitch difference was normalized by peak force to yield the fusion ratio. *Bottom, inset*: a doublet at 30 Hz is depicted. Note that the short interpulse interval causes *event A* to contain almost no force data, undermining the validity of the fusion ratio. Fusion ratio is therefore not reported for doublets with small interpulse intervals (15, 20, and 30 Hz).

Fig. 3.
Representative example of force response to doublet stimulation at several frequencies (*subject* SCI 2, Table 1). SCI, spinal cord injury.

Fig. 4. Mean (SE) normalized quadriceps force elicited by twitches (1t, 1t2, 1t3), 5-Hz trains (5t), and various doublet stimulation frequencies (6d, 7d, etc.). SCI and non-SCI refer to subjects with and without spinal cord injury, respectively.

Fig. 5. Contractile speed properties for pretetanic (1t), posttetanic (1t2), and postdoublet (1t3) twitches. *Top*: normalized rate of force rise. *Greater than 1t ($P < 0.05$); **Greater than 1t and 1t2 ($P < 0.05$). *Bottom*: normalized rate of force fall. *Less than 1t2 and 1t3 ($P < 0.05$); **Less than 1t3 ($P < 0.05$).

Fig. 6.

Twitch difference (*top*) demonstrates the relative contribution of the two doublet pulses (A and B) to the overall force. Note that the force contributed by the A pulse varies little across frequencies, while the force contributed by the B pulse is subject to summation of force. The fusion ratio (*bottom*) demonstrates the specific contribution of the B pulse, normalized to peak force. A higher fusion ratio denotes a greater relative influence of summation of force between doublet pulses.

Table 1

Subject demographics

Subject	Age, Yr	Sex	Moment Arm, cm	SCI Level	SCI Years	Intensity, mA	Peak Force, N
SCI 1	36	M	30	T9	7.3	200	72.4
SCI 2	27	M	35	T4	5.2	200	120.1
SCI 3	39	M	34	T8	1.6	200	150.4
SCI 4	20	M	35	T8	0.8	200	157.7
SCI 5	28	M	40	T7	0.8	200	101.5
SCI 6	25	M	34	T4	4.0	200	156.0
NON 1	41	M	34			200	196.8
NON 2	34	M	30			167	209.9
NON 3	30	F	31			144	160.4
NON 4	52	M	32			200	216.3

Subjects with and without spinal cord injury are denoted by SCI and NON, respectively. M, men; F, women; T, thoracic.