O0844-R

NCT01970592

Skeletal Muscle Plasticity As An Indicator of Functional Performance Post-Stroke

July 14, 2011

PI Name: Chris Gregory, PhD, PT: PI
Aaron Embry, DPT, MSCR: Investigator
Patrick Morgan, MS: Coordinator

Study Title: Lower extremity power and locomotor function after stroke.

TABLE OF CONTENTS

A. SPECIFIC AIMS

Post-stroke motor control is characterized by greatly reduced muscle power generation. To date, it has yet to be established the extent to which muscle power limits walking performance or whether its remediation should be a primary component of locomotor rehabilitation. The potential efficacy of increasing muscle power is suggested by studies showing that its reduction, compared to other impairment level measures, is more strongly associated with risk of falls and slow gait speeds. In fact, impaired muscle power generation is the single strongest predictor of functional disability in an aging population. Further, while basic training to increase muscle strength (one component of power) has produced equivocal results, substantial evidence suggests that appropriately designed training can have potent effects on walking in those for whom it is appropriate.

The major hurdle preventing understanding of how muscle power generation contributes to functional walking ability after stroke, and thus the efficacy of its rehabilitation, is the lack of a theoretical framework that defines and measures the complex multifactorial relationships between neural activation, muscle force generation and functional task performance. Impaired neural activation of a muscle group is differentially related to walking performance depending on activation magnitude, independence of activation at the appropriate time in the gait cycle, leg kinematics during activation and overall coordination of other walking tasks by different muscle groups. To gain a better understanding of this complex interaction, we have developed a comprehensive theoretical framework that defines and measures relevant neural and muscular factors that contribute to walking function in order to control for potential confounds in determining intervention efficacy. We will apply this framework to ***Post-stroke Optimization of Walking using Explosive Resistance (POWER)*** training, a multi-joint, high-velocity resistance training paradigm that includes task-specific elements and individually targets muscle power dysfunction. We are uniquely well-suited to perform this work because we a) have developed key coordination measures on which the theoretical framework is based; b) have unique equipment to perform the novel intervention and c) have the imaging expertise to apply cutting-edge techniques for the first time in the stroke population. Through the use of our theoretical framework we will be able to explain for the first time why people did or did not respond and provide evidence establishing the

characteristics of those most likely to benefit (or not) from training. As such, we propose the following aims over a four-year period:

Aim 1: Determine the presence and magnitude of neural and muscular adaptations post-stroke as predictors of impaired muscle power generation. We will measure pre-training muscle function parameters (strength & power) as well as neural (central activation & muscle coordination) and muscular (morphology & *in-vivo* bioenergetics) adaptations in the paretic and non-paretic legs of *28* persons with chronic hemiparesis (*vs*. matched controls). We will use 3-dimensional magnetic resonance (MR) imaging to assess individual muscle cross-sectional area as well as ^{31}P-MR spectroscopy to measure *in-vivo* muscle oxidative capacity and resting concentrations of inorganic phosphate.

Aim 2: Quantify the effects of POWER training on the presence and magnitude of neural and muscular adaptations. POWER training consists of high-velocity resistive exercises that independently target the paretic and non-paretic limbs and will utilize a ZeroG^{TM} overground body-weight support (BWS) system to facilitate progressive loading and safe practice. Training also includes trials of fast overground walking (≥125% self-selected speed) with minimal BWS (or added mass as appropriate) to emphasize lower extremity power generation during walking as well as self-selected overground walking for task-specific practice.

Aim 3: Establish the relationship between neural and muscular adaptations, lower extremity power generation and functional (locomotor) improvements following POWER training. To determine the most relevant predictors of functional recovery we will measure neural and muscular contributors to muscle power generation, complete a battery of clinical and functional assessments, and perform full biomechanical analyses of walking at four times (at 0, 4 & 8 weeks of training and 8 weeks after training). Secondarily, we will associate changes in self-selected walking speed, muscle coordination and power generation during walking to our historical cohort following task-specific locomotor training (VA RR&D B3983).

B. BACKGROUND AND SIGNIFICANCE

Stroke is the leading cause of long-term disability in the United States, affecting approximately 795,000 people each year, with a surviving cohort of nearly 6.5 million (Lloyd-Jones et al., 2009). Seventy-three percent of those surviving stroke will have some degree of long term disability (Gresham, Duncan et al. 1995), and less than 50% of survivors progress to independent community ambulation (Perry, Garrett et al. 1995). Even among those who do achieve independent ambulation, significant residual deficits persist in balance and gait speed, with 60% of persons post-stroke reporting limitations in mobility related to walking (Perry, Garrett et al. 1995).

Hemiparesis, strictly defined as a muscular weakness or partial paralysis of half of the body, is seen in three-quarters of patients post-stroke. A primary disability associated with post-stroke hemiparesis is the failure to make rapid graded adjustment of muscle force within the context of purposeful complex movement patterns, such as are required during walking (i.e. muscle power). In walking, a primary impairment is a reduced ability to sufficiently activate muscles (Mulroy, Gronley et al. 2003). Since improving walking is the most often stated goal of patients following a stroke (Bohannon, Andrews et al. 1988), interventions aimed at improving functional walking status, are critical for improving quality of life for hemiparetic individuals and their caregivers.

Currently there are not established answers as to what extent muscle power generation deficits limit walking performance and whether their remediation should be a primary component of locomotor rehabilitation. We believe that much uncertainty arises from the complex multifactorial relationships between muscle force generation, neural activation and task performance. To

distinguish among these, we have developed the first comprehensive theoretical framework that defines and measures these different factors in order to control for potential confounds and to identify the neurological, biomechanical and biochemical reasons an intervention was successful or not.

Theoretical framework: Relationship between neural activation and task performance: The theoretical framework underlying the importance of impaired neural activation for walking performance is straightforward: acceleration of the mass of the body (or individual body segments) requires adequate force generation by the appropriate skeletal muscles coordinated to be produced at the appropriate time in the gait cycle. Impaired neural activation thus consists of two separate components: decreased neural activation magnitude (muscle weakness) and impaired neural activation coordination (independent muscle coordination), which is the ability to independently activate (not necessarily by itself, but with its usual synergists) a muscle at the appropriate time in the gait cycle. In addition, the specific muscle force generation patterns resulting from neural activation deficits will also differentially affect walking performance (e.g., speed) depending on the kinematic state of the leg at the time of activation and the overall coordination of other walking functions by different muscle groups (e.g., plantar flexor strength increases might not improve walking speed if a different muscle group is the weak link). Figure 1 provides an example specific to the plantar flexor muscle group how the different factors can influence walking performance and the measurements we will make to assess the influence of each factor.

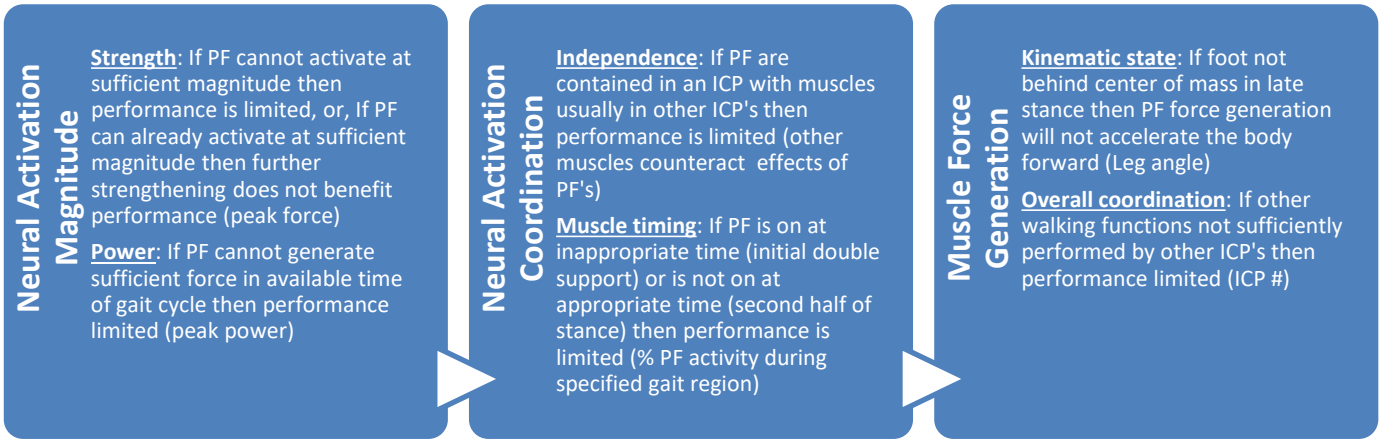

Figure 1: Theoretical framework of complex multifactorial relationships between neural activation, muscle force generation and task performance. Illustrated are examples of how task performance can be limited by the plantar flexor (PF) muscle performance relative to each factor and how we will measure each factor. ICP refers to "Independent Co-activation Pattern" as identified by factor analysis from EMG data.

Impaired neural activation magnitude (strength and power): Hemiparesis, strictly defined as (muscular) weakness affecting one side of the body, is seen in three-quarters of individuals following stroke. Weakness in this population results from both neural and muscular factors which include, respectively, the ability of the central nervous system to activate skeletal muscle as well as the force generating capacity of the muscle. Given the nature of neurological insult, the prevailing notion is that neural factors (i.e., central activation) are predominately responsible for weakness post-stroke. However, more recent studies confirm muscle atrophy as contributing mechanism underlying hemiparetic weakness and studies in clinical populations other than stroke suggest that *in-vivo* bioenergetic properties of skeletal muscle also contribute to weakness and explain strength deficits not accounted for by changes in muscle size and central activation. To date, the influence of these factors on hemiparetic weakness has not been explored.

Central activation: It is widely accepted that hemiparetic weakness results primarily from reduced central (cortical) activation of agonist muscles. Evidence of reduced central activation has been reported in the knee extensor muscles both during the early time period following stroke (i.e., first six months) as well as in subjects with more chronic injuries, indicating early and persistent bilateral disruption in the ability to volitionally activate skeletal muscle. Impaired central activation in these studies was greater in paretic vs. non-paretic muscles, yet could not completely explain the magnitude of weakness in either leg (Miller et al.;2009). Using a combination of superimposed electrical stimulation and surface electromyography (EMG), Klein et al. concluded that weakness of the plantar flexor muscle group in chronic stroke subjects mostly reflects central activation impairments, with small, but significant, contributions from muscle atrophy. Reduced muscle activity, indicated by diminished amplitude of EMG activity, is also indicative of impaired central activation and generally observed in paretic muscle during isolated joint strength testing as well as during functional movements (e.g. walking or reaching), with the extent of central activation impairment associated with functional disability. A "saturation" of EMG activity, reflected by an absence of modulation in response to increased task demands, is also a common phenomenon following stroke and further supports reduced central activation as a primary contributor to post-stroke muscle weakness. Despite reports of strong associations between impaired central activation, reductions in muscle strength and reduced functional performance, definitive evidence regarding the relationship between these outcomes is lacking because of the paucity of studies that simultaneously assess these and other variables that contribute to hemiparetic weakness. In the absence of studies quantifying central activation, muscle atrophy and other factors concurrently, the relative contributions of neural and muscular factors to hemiparetic weakness and the ensuing functional limitations remains largely unknown.

Muscle atrophy: The loss of skeletal muscle mass (i.e. atrophy) following stroke has negative implications for strength and functional performance and may also have significant health-related consequences. Although it is well established that inactivity or disuse results in muscle atrophy, early studies provide conflicting results regarding the magnitude of muscle atrophy post-stroke. Reports of muscle atrophy (Jorgensen et al, 2001; Metok et al, 2003; Ryan et al, 2002) are countered by evidence of preserved muscle mass at both the whole muscle (Hachisuka, 2007; Carin-Levy et al., 2006) as well the individual fiber level (Slager et al., 1985). Such conflicting data led Patten and Pak (2008) to conclude that there is limited evidence to either support or refute the incidence and severity of muscle atrophy following stroke. These authors do, however, point out that more recent studies afforded the use of high-resolution imaging techniques (as proposed herein) offer considerably more support for muscle atrophy as a mechanism underlying hemiparetic weakness, though its importance in explaining strength deficits (or recovery) is unknown. More recently, English et al. (2010) concluded that persons post-stroke have significantly less regional muscle mass than neurologically healthy individuals as well as in the paretic versus the non-paretic thigh, with reductions in thigh muscle mass measured using both magnetic resonance imaging (MRI) and computed tomography (CT). Interestingly, the extent of paretic muscle atrophy (as opposed to weakness) correlates strongly with gait speed and reduced fitness in individuals following chronic stroke (Ryan et al.), though the lack of available knowledge regarding changes in muscle mass and their relationship to weakness and function represents a significant deficiency in the scientific literature that must be addressed.

In-vivo bioenergetics: In addition to central activation and muscle atrophy, *in-vivo* bioenergetic properties of skeletal muscle can play a significant role in the development of muscle dysfunction. Specifically, an increase in inorganic phosphate concentration [P_i] measured using ^{31}-phosphorous magnetic resonance spectroscopy (^{31}P-MRS) is predictive of decrements in muscle function, both *in-vitro* and *in-vivo*. Increased [Pi] reduces calcium sensitivity in muscle and results in a rightward shift in the [Ca^{++}]/tension curve, thus requiring greater calcium release to activate skeletal muscle. Increase in [Pi] has been observed in a variety of disorders including primary

mitochondrial diseases, myopathies, muscle injury, cast immobilization and denervation, (McCully, Kent et al. 1988; Argov and Arnold 2000; Tartaglia, Chen et al. 2000; Pathare, Walter et al. 2005; Pathare, Vandenborne et al. 2007) while reductions in [P_i] correlate with functional improvements following rehabilitation. In addition to changes in [P_i], ^{31}P-MRS data have been utilized to identify abnormalities in skeletal muscle oxidative capacity, reflected by the rate of phosphocreatine (PCr) recovery following exercise, in various patient populations (Rossi and Lortet, 1996; Yoshida et al, 2001) as well as with normal aging (Waters et al, 2003). PCr recovery rates have been extensively used in both healthy and diseased muscles as estimates of muscle oxidative capacity (Meyer 1988; Paganini, Foley et al. 1997; McCully, Mancini et al. 1999) and findings from these studies suggest that recovery of function is directly related to the increases in muscle oxidative capacity (Yoshida et al, 2001). To our knowledge there are no published data available that provide information describing *in-vivo* bioenergetics properties of muscle following stroke.

Muscle power**:** Muscle power involves both strength and velocity components, and is determined by the force generating capacity of the muscle as well as its speed of shortening. Although the loss of muscle mass as well as the inability to activate muscle clearly contribute to the loss of muscle strength (and consequently power), these variables alone cannot account for the proportionally greater loss of muscle power than strength following stroke (Clark et al, 2006). Pronounced velocity dependent muscular deficits are present post-stroke and, in combination with substantial muscle weakness, significantly impact power generation when compared to neurologically healthy aged counterparts (Pohl et al, 2002; Clark et al, 2006). To a greater extent than muscle strength, muscle power is a significant predictor of functional ability and direct comparisons of power and strength demonstrate that muscle power consistently describes more of the variance in functional ability in mobility-limited elders (Bean et al, 2002). Deficits in lower extremity power generation are associated with increased levels of dependence, greater risk of falls and decreased walking speeds, (Bean et al, 2003; Chan et al, 2007; Kuo et al, 2006) though the relative importance of the various contributors to (reductions in) power generation post-stroke are largely unexplored. Our focus in this proposal is on the (in)ability of post-stroke muscle to generate power both in and away from functional task performance and to determine the effects of a novel intervention targeting lower extremity power generation in both the paretic and non-paretic legs on functional (locomotor) recovery.

Impaired neural activation coordination: What has often been incompletely addressed in previous research examining the role of weakness in impaired hemiparetic walking is the difference between what a muscle is capable of producing in a strength/power test (usually an isolated voluntary movement) and what it actually produces during the task of walking. Key issues to address are: 1) is voluntary strength/coordination representative of that during walking; and 2) can the muscle group being assessed/strengthened be independently activated during walking and is it being activated with the appropriate timing?

Walking specific activation: In spinal cord injured subjects it has been shown that the muscle activation during voluntary testing is much reduced relative to that in walking (Maegele et al, 2002). We (SK) have shown that evaluation using the Fugl-Meyer assessment, based on voluntary movement items meant to test independence from abnormal synergies, more poorly predicts walking function than does a prediction based on a factor analysis that determines independence from abnormal synergies during walking (Bowden et al., 2010). Thus, it is clear that voluntary strength testing outside of the context of walking may result in different force generation than seen during walking. We propose to make detailed biomechanical measures during walking to in part control for this issue.

Independent muscle coordination: It is well understood that if there is increased activity in an antagonistic muscle group then the net joint moment associated with the target muscle output is

reduced. However, it is typically not considered that activity in muscles other than the classical antagonists can also reduce the output of the target muscles. Forward dynamical modeling has revealed the counterintuitive finding that muscles can accelerate all joints, even those that are not directly spanned by the muscle (Zajac et al 2002 & 2003). Using the example of the plantar flexor group (PF), we (SK) have shown that a primary function of the PF during walking is to accelerate the trunk forward in late stance (Neptune 2001). We have also shown that activity in the vasti group, rectus femoris, and hamstrings can impede forward trunk acceleration during the same gait cycle period (Neptune et al., 2004). We (SK) have also shown that the PF are often co-activated with the vasti group, rectus femoris, and hamstrings in walking by more severely affected hemiparetic subjects (e.g., those with merged modules in Clark et al., 2010). An inescapable consequence of lost independence of the PF is that the effective trunk forward acceleration that would have occurred due to PF alone will be reduced due to the net backward trunk acceleration induced by the other coactive muscles. Thus, a substantial strength gain in PF may not have the desired effect on trunk acceleration if the subject co-activates the other muscles whenever they activate PF. Additionally, activity usually must occur at the proper time or else it can have a negative effect. Again using the example of the PF, activity during the initial double support phase (i.e. before PF is typically activated in healthy walking) actually acts to accelerate the trunk backward (Neptune 2001). Thus, the common complete stance phase co-activation pattern of PF with the uniarticular extensors and the hamstrings found by Clark et al. (2010) in the most impaired hemiparetic subjects would result in increased forward deceleration of the trunk in initial double support and decreased forward acceleration of the trunk in late stance as the positive PF effect is countered by the negative effect of the other coactive muscles. Note that increasing the PF activation (e.g., strengthening) in a subject locked in this co-activation pattern could even result in slower walking speed if the negative effects outweighed the positive effects.

Influence of individual muscle force generation on walking performance: A final often unappreciated aspect of the influence of muscle force production on walking performance is that it is dependent on the kinematic state of the leg (e.g., Zajac et al 2002 & 2003). For example, we (SK) have shown that the extension of the paretic leg in terminal stance is strongly related to the propulsion generated (Peterson et al., 2010). This can be particularly problematic in subjects with a “step-to” gait who do not advance the non-paretic leg beyond the paretic leg during non-paretic step. As a result the paretic leg may not extend beyond the trunk center of mass and PF force generation can act to raise the body center of mass instead of accelerating the trunk forward as would be the result if the leg were in its usual kinematic state. Again, note that increasing the PF activation (e.g., strengthening) in a subject with this type of kinematic pattern would not be expected to increase speed as the trunk would not be accelerated forward by the increased PF force generation.

Importance of plantar flexor function: Hemiparetic walking is associated with a reduced ability to generate sufficient muscle power, especially within a critical temporal window to execute the transient motor events encountered during the gait cycle. We posit that locomotor ability following stroke is causally related to a person’s success at producing sufficient muscle power (with the ankle plantar flexor muscle group having a primary role) to meet the task demands of bipedal walking. Recent studies have demonstrated the importance of the ankle plantar flexor power generation to locomotor ability, (Kim et al., 2003) with deficits in plantar flexor function reported to explain 67 to 72% of the variance in walking speeds post-stroke (Nadeau et al., 1999; Neptune et al., 2005). Decreased power generation means that the necessary mechanical energy for the trunk and legs may not be available, thereby negatively impacting walking performance (e.g. trunk forward progression and ipsilateral swing initiation) and decreasing functional independence (Mulroy et al., 2003; Chen et al., 2008). By training using a program designed to positively affect

muscle power generation, we aim to effectively attenuate impaired power generation, thereby improving locomotor ability post-stroke.

Impact of training on hemiparetic muscle function: Hemiparetic muscle weakness is a significant predictor of lower and upper extremity motor recovery in both acute and chronic stroke subjects (Sharp & Brouwer, 1997; Patten et al., 2003; Northrop et al., 2003). Given this observed relationship, it would seem logical to intervene by strengthening weak muscle groups. However, evidence regarding the efficacy of interventions aimed at attenuating muscle weakness and the ensuing functional consequences in the post-stroke population is equivocal and viable therapeutic options to remediate hemiparetic muscle weakness remain a pressing challenge.

Progressive resistance training is widely accepted as the most effective method for developing muscular strength and is currently prescribed by most major health organizations for improving health and fitness (National Institute on Aging, 1999). Resistance training is shown to improve lower extremity strength following stroke and, when delivered at appropriate intensities, can provide significant functional benefit (Teixeira-Salmela, 1999; Lee et al, 2010). A recent quantitative review,(Dickstein, 2008) concluded that even though prevailing clinical thought argues that functional improvements emerge only from task-specific training, measurable gains in lower extremity strength following resistance training are associated with functional improvements in the post-stroke population. It should be noted that studies describing functional outcomes following strengthening in the post-stroke population are historically equivocal, though consistency with regards to intensity of the intervention in recent studies supports the argument for strengthening this cohort. A unique aspect of the proposed training is the focus on high-velocity concentric and eccentric contractions, a characteristic seemingly most appropriate for targeting post-stroke muscular dysfunction (Clark et al, 2006). Interventions targeting muscle power (i.e. training at high-velocities) in the aged result in similar increases in muscle strength compared to slow velocity training, yet elicit an over two-fold greater improvement in peak power. The high-velocity component is critical to elicit these responses, as losses in muscle power with aging (as well as neurological injury) appear to be due to greater declines in the velocity rather than the force component of muscle power production (Fielding et al, 2002; Bean et al, 2004). In fact, recent studies have shown that muscle power explained more of the variability in functional performance than maximal strength (Bean et al, 2002).

Design of viable therapeutic options targeting hemiparetic muscle dysfunction and the ensuing functional consequences remains a pressing challenge for biomedical research, and is contingent upon reliable information describing the functional significance of target muscle(s) as well as the most important neural and muscular characteristics to address to maximize functional gains. Our long-term goal is to develop rehabilitation therapies that maximize improvements by addressing the physiological mechanisms that limit functional performance in each individual, thereby reducing disability and improving quality of life following stroke.

C. PRELIMINARY STUDIES

We believe that a theoretical framework based on the underlying causative mechanisms contributing to limitations in functional walking performance offers the greatest potential to develop best practice for walking recovery. Our proposal is based on the assertions that: 1) the ability to generate sufficient lower extremity muscle power is impaired and is in large part responsible for functional disabilities following stroke and 2) the proposed training protocol effectively attenuates impaired muscle power generation and improves locomotor function. These beliefs were developed based on findings from our preliminary studies as well as the studies of others. The following section outlines work to date quantifying adaptations in lower extremity skeletal muscle in the paretic and non-paretic legs that are known contributors to weakness and impaired locomotor function. In addition, we demonstrate preliminary support for the use of a

novel rehabilitation program herein referred to as Post-stroke Optimization of Walking using Explosive Resistance (POWER) training.

Mechanisms underlying deficits in muscle power generation: The impaired neural activation that characterizes hemiparesis results in a functional muscle weakness during walking as muscles often cannot generate sufficient force in the required amount of time. This rate dependent deficit is characterized by the reduced muscle power generation ability that is the focus of the proposed study. Muscle weakness is arguably the most prominent motor deficit in the post-stroke population and is strongly correlated with decreased functional abilities, including reduced gait speeds. To date, the relative importance of the various factors that contribute to muscle weakness (and subsequently to muscle power generation) in this population have not be adequately described. The following preliminary studies highlight significant reductions in muscle peak torque generation about the ankle and knee joints that is associated with reductions in muscle cross-sectional area (CSA) as well as the inability to voluntarily activate muscles in persons post-stroke. We also demonstrate preliminary findings suggesting that inorganic phosphate concentration [P_i] is elevated, a characteristic known to inhibit force production *in-vitro* and *in-vivo* following periods of disuse.

Muscle strength and power deficits are significant post-stroke: Figure 2 illustrates deficits in the ability to generate peak torque in the plantar flexor and knee extensor muscle groups in a group of subjects post-stroke (n=7 with moderate to severe motor impairments), relative to matched (gender, age, height and weight) neurologically healthy individuals. Specifically, peak torque production in the paretic and non-paretic muscles was 76% and 62% lower in the plantar flexors and 78% and 61% lower in the knee extensor muscle group, respectively. In addition, the average rate of torque development (ARTD), defined as the time integral necessary to generate from 20 to 80% of peak torque, was significantly reduced in person's post-stroke (~89% and 78% in paretic and non-paretic muscle, respectively), indicative of substantial deficits in muscle power generation in these muscle groups. Deficits in torque production in our subjects were associated with reduced voluntary activation in both the knee extensor and plantar flexor muscle groups (Figure 2). Central activation impairment is only ~5% in neurologically healthy individuals, whereas paretic central activation impairments are 66% and 64%; and non-paretic activation impairments are 40% and 52%; in the KE and PF muscle groups, respectively. Collectively, our data demonstrate significant muscle force generation dysfunction in both paretic and non-paretic limbs that is partially explained by reduced central activation of these muscles following stroke.

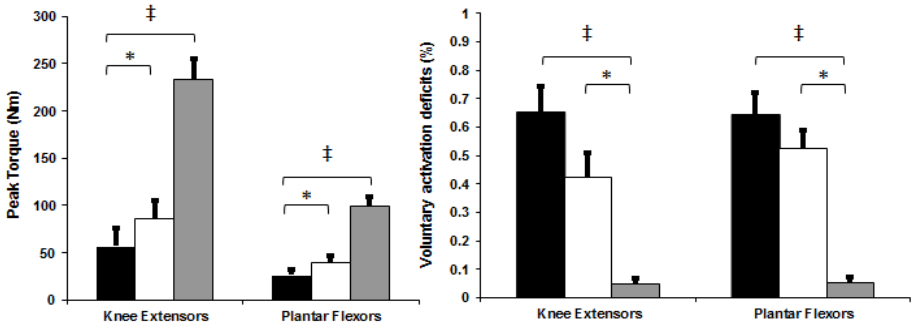

Figure 2: Peak torque and voluntary activation deficits in paretic (black bars), non-paretic (white bars) and control (grey bars) PF and KE muscle groups

Muscle morphology post-stroke: We collected MRI data from 17 hemiparetic subjects (6-60 months post-stroke) as well as from matched (gender, age, height and weight) controls. Individual muscle cross-sectional area (CSA) was measured in plantar flexor (soleus, med. & lat. gastroc), dorsi-flexor (tibialis anterior) and the quadriceps femoris muscle group (Figure 3). Muscle atrophy is defined as the % difference between control and hemiparetic limbs. Our data show a reduction in CSA following stroke that is muscle group dependent. Specifically, CSA was reduced in the paretic and non-paretic medial gastrocnemius (27% & 9%), lateral gastrocnemius (16% & 6%) and soleus 20% & 13%). Quadriceps muscle CSA was reduced 24% and 5% while the TA was 9% and 17% smaller in paretic and non-paretic muscle, respectively.

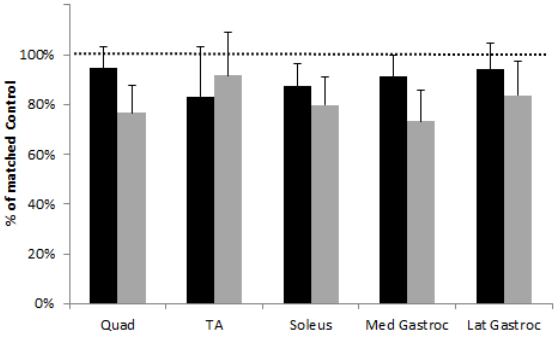

Figure 3: Relative differences in paretic (grey bar) and non-paretic (black bar) lower extremity muscles compared to controls. Dashed line represents average

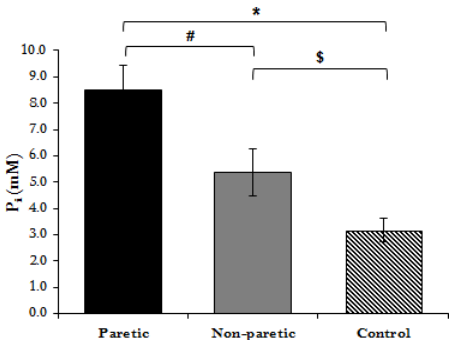

Figure 4: [Pi] in paretic, non-paretic and control PF muscles collected via ^{31}P-MRS showing significant differences between legs (paretic >

***In-vivo* bioenergetics are altered and associated with muscle dysfunction post-stroke:** We collected localized ^{31}P-MRS data at rest from the paretic and non-paretic limb from eight post-stroke participants as well as from the dominant limb of 8 neurologically healthy individuals matched on the basis of age, gender, height and weight. Data were acquired with a total averaging time of 5 minutes and a TR = 5 sec. Marked differences were found in resting [P_i] levels between the paretic limb and the control limb (5.43 vs. 3.01 mM P_i) as well as between the paretic and non-paretic limbs (5.43 vs. 4.46 mM P_i; Figure 4). These data demonstrate experience acquiring ^{31}P-MRS data in muscle from individuals post-stroke. In addition, the findings provide support for the contribution of *in-vivo* bioenergetics properties to be affected following stroke and negatively impact muscle function in this population.

Summary: The consequences of alterations in skeletal muscle post-stroke are far reaching and contribute to decreased motor control and overall fitness, development of functional limitations, and long term disability. We have highlighted several adaptive changes that occur that contribute to muscle dysfunction in persons post-stroke when compared to neurologically healthy individuals. To date, however, the relative importance of these factors as well as whether and how these impairments relate to existing functional deficits is yet unknown. Furthermore, the extent to which these alterations are amenable to rehabilitation training and the degree to which changes in these properties relate to functional improvements would be valuable information to help guide therapeutic decision making in the treatment of post-stroke locomotor dysfunction.

Plantar flexor muscle power generation is associated with locomotor function post-stroke: Individuals following stroke typically present with some degree of residual motor function. The extent of this residual function varies greatly from person to person, and significant locomotor impairments often persist. Although a number of factors likely contribute to slow walking, we examined the importance of muscle power generating capacity of the plantar flexor muscle group during dynamometric strength testing in predicting locomotor ability in individuals with chronic post-stroke hemiparesis (n=23). Ankle plantar flexor power was determined bilaterally. In addition, self-selected gait speed was measured during over-ground walking. Significant correlations were

found between self-selected gait speed and ankle power in the paretic (r = 0.78) and non-paretic (r = 0.46) legs (Figure 5). The strength of the correlation between the paretic PF muscle group and gait speed suggests the importance of ankle power in predicting locomotor ability post-stroke and suggests paretic PF function as a potential target for improving locomotor ability in this large clinical cohort.

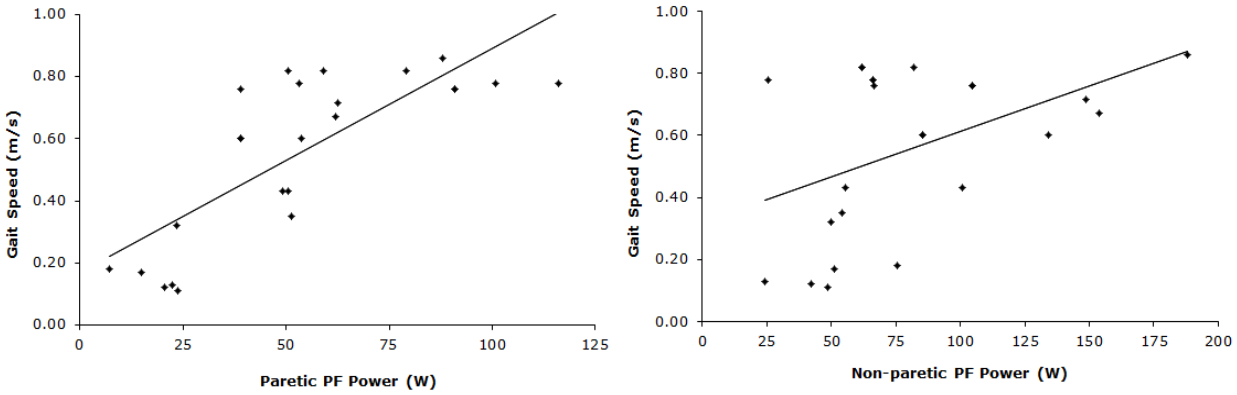

Figure 5: Correlations between self-selected gait speed and plantar flexor power in the paretic (left) and non-paretic (right) legs.

Paretic ankle powers relate to locomotor performance: We measured ankle power in the paretic limb during walking at self-selected walking speed in hemiparetic subjects (n=21; gait speeds 0.2–1.0 m/s) as well as a control group at matched speeds (Figure 6). Significant reductions in ankle power generation are present in participants classified as having mild and moderate strokes. Reduced power generation was also evident across the hip and knee joints at matched speeds, though the most prominent deficits are in the ankle and strongly correlate to hemiparetic severity. Additional data from this study of reveal that peak ankle power during walking in hemiparetic subjects (independent of severity) is highly correlated with self-selected walking velocities (r=0.77, p=0.0004), suggesting a significant functional relationship between paretic ankle power generation and gait speed post-stroke.

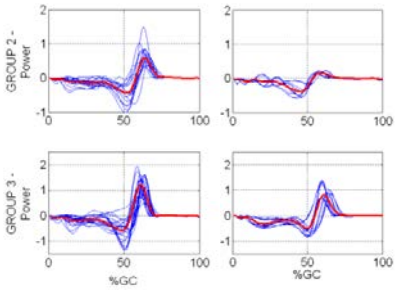

Figure 6: Ankle power during walking following moderate (top) and mild (bottom) stroke.

Understanding muscle coordination through independent muscle co-activation patterns. We have established a basis for studying muscle co-activation patterns consistent with the concept that stroke limits the ability to activate certain muscles, or patterns of muscles, independent of a mass extension and flexion co-activation. In addition, recovery of mobility occurs when independent muscle activation is regained allowing the critical biomechanical functions associated with mobility to be better executed (published in J Neurophysiology; Clark et al, 2010). Analyzing EMG recordings from healthy individuals, we found that a small set of independently timed patterns (n=4) of muscle co-activation accounted for step-to-step variability of muscle activity in eight muscles over a wide range of walking speeds (Figure 7). We hypothesized that the health of the locomotor control system depends on the number of independent co-activation patterns (ICPs) needed to account for the EMG signals. Indeed, we found that the muscle activity in many paretic legs could be accounted for with only two ICPs compared to control legs where four ICPs were needed (i.e., muscle activity was less independently controlled in the paretic legs; Figure 7). We further found that reduced ICPs was associated with poorer walking performance, including decreases in speed, step length asymmetry, propulsive force asymmetry and speed adaptability (Figure 8).

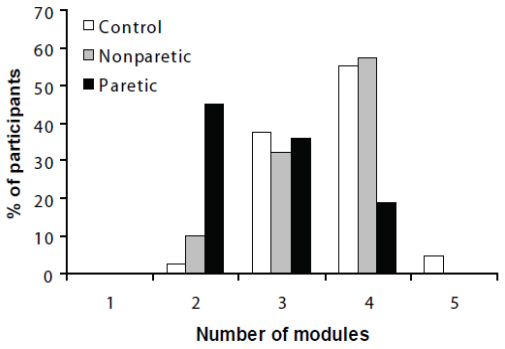

Figure 7: Number of ICP's during walking in control and hemiparetic subjects.

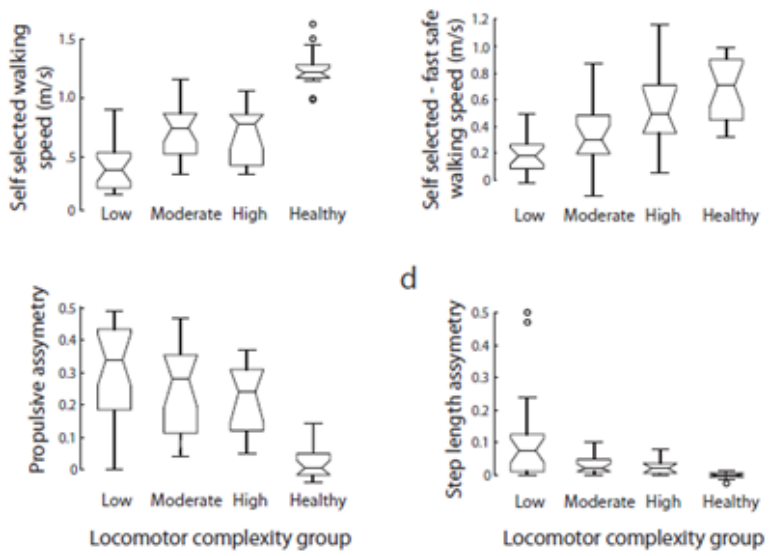

Figure 8: Locomotor output complexity (i.e., number of ICP's) and walking performance assessed via overground self-selected walking speed (top left), speed difference between self-selected and fast walking (top right), propulsive asymmetry (bottom left), and step length asymmetry (bottom right). Healthy control data are shown as a reference. The 3 horizontal bars representing each variable indicate, from *bottom* to *top*, the lower quartile, median, and upper quartile. Error bars indicate ±1.5 · interquartile range. Circles represent outlying data points. Low, moderate and high complexity represent two, three and four ICP's, respectively.

Effective locomotor training is able to improve the quality and independence of co-activation patterns. Two recent studies using rigorous quantitative EMG analysis have emphatically concluded that the recovery from acute stroke accompanying a non-locomotor-specific rehabilitation protocol occurs without improvement in the timing of EMG activity (Den Otter, Geurts et al. 2006; Buurke, Nene et al. 2008). In contrast, we find that improvement in timing does occur in recovery using a locomotor-specific training protocol. We tested locomotor output before and after an intensive locomotor training program, consisting of 36 sessions over 12 weeks. Twenty-seven participants walked on a treadmill with the minimum amount of body weight support needed to allow for 20 total minutes of walking per session. Using the same EMG analysis as in the previously mentioned studies we showed that EMG timing changes do occur.

We also demonstrated that the EMG changes were consistent with increased independence from a general stance-phase co-activation. The number of independent co-activation patterns needed to account for muscle activity of the paretic limb following locomotor training increased in fourteen of the nineteen participants who began with less than four patterns (nine subjects began with four patterns). No participants experienced a reduction in the number of patterns. These findings strongly suggest that post-stroke locomotor training (cf., general neurodevelopment approach) improves muscle activation differentiation during walking and that this differentiation is crucial for improved walking. Of interest is whether POWER training will similarly improve muscle activation differentiation, or will provide different benefits that may be enhanced if combined with other training approaches.

Subjects without ability to independently activate PF generate dramatically less propulsion with their paretic leg. Considering the hemiparetic subjects in our experimental study who had only three instead of four independent patterns: one subset, who merged the normal late stance pattern (e.g., soleus and gastrocnemius) with the normal early stance pattern (e.g., vastus medialis and gluteus medius), generated significantly less body forward propulsion with their paretic leg (mean = 73% less; Figure 9 left plot). However, another subset, who retained the independent late stance pattern (soleus and gastrocnemius) but instead merged the swing-to-stance pattern (e.g., hamstrings) with the early stance pattern (vastus medialis and gluteus medius), generated only slightly reduced propulsion (~11% less; Figure 9 right plot).

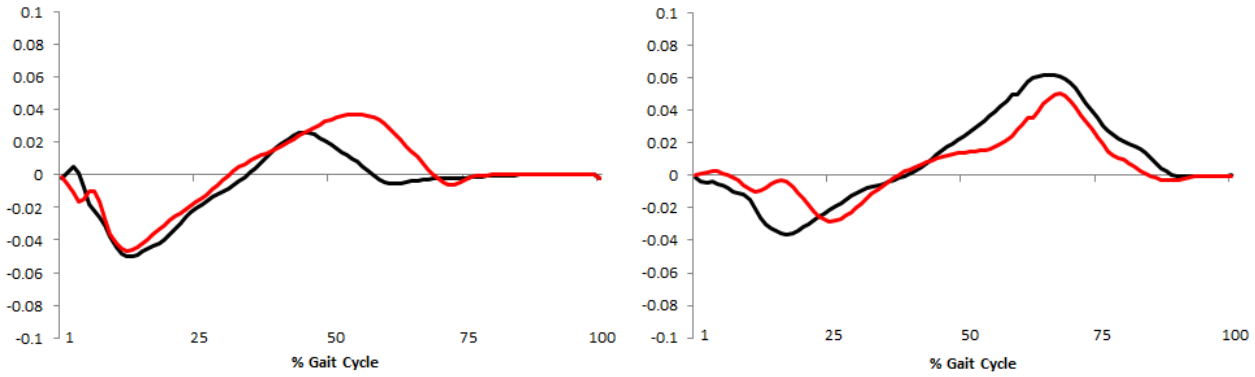

Figure 9: Examples of A/P ground reaction forces in post-stroke subjects with merged early and late stance modules (left) and with merged swing-to-stance and early stance modules but independent late stance pattern (right).

Effects of POWER training on persons post-stroke: We recently completed data collections on five individuals (4 males; 62.4 ±7.3 yrs; 45.2 ± 25.7 mos. post-stroke) who completed 24 sessions of POWER training. Training resulted in increases in self-selected and fastest comfortable walking speeds, improved walking endurance, as well as bilateral increases in muscle strength and power generation. Specifically, overground self-selected walking speed (SSWS) increased from 0.58 ± 0.24 m/s to 0.78 ± 0.28 m/s following training. Similarly, fastest comfortable walking speed increased on average from 0.91 ± 0.32 m/s to 1.35

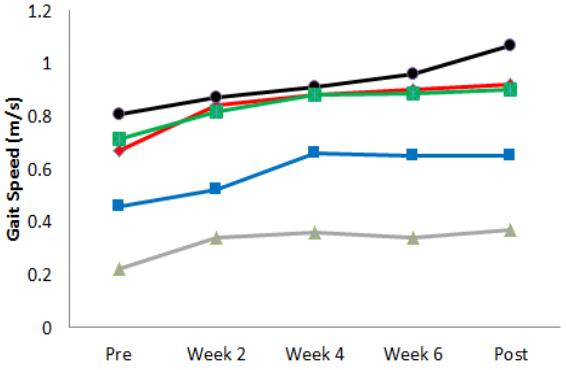

Figure 10: Bi-weekly gait speeds for individual subjects completing POWER training.

± 0.52 m/s. Bi-weekly measures of SSWS suggest continuous improvements over the 8 week period (Figure 10). Of note, the average increase in gait speed (i.e., 0.20 m/s) for our group (n=5) exceeds the clinically meaningful difference recently reported for participants in the recently completed LEAPS trial. Increases in gait speed for individual subjects were also accompanied by increases in paretic hip, knee and ankle joint powers. Of note, peak paretic ankle power increased by an average of ~31% during walking at self-selected speed between the pre- and post-training time points. This increase is important as it provides evidence of carry-over from training-induced improvements in muscle power generation to task-specific performance (Individual subject power profiles shown in *Figure 11*). Non-paretic ankle power during decreased ~12%, suggesting that compensatory strategies at the ankle were not associated with behavioral improvements. In addition to changes in gait speed, subjects realized average improvements in 6 minute walk test (6MWT) of (139.8 ± 47.6 ft.), a change shown to be clinically important.

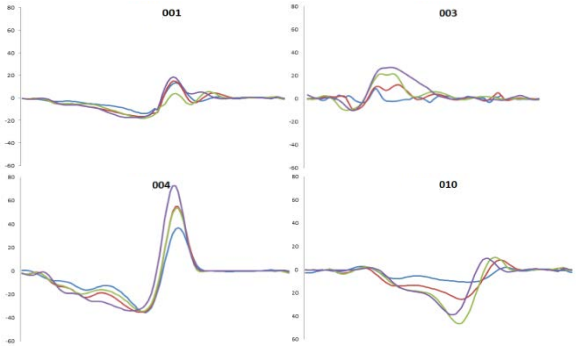

Figure 11: Paretic ankle power profiles for 4 subjects at self-selected speed at pre (blue), 4 week (red), 6 week (purple) and post (green) POWER training.

In addition to improved walking speeds, peak muscle power generation during strength testing increased in the paretic and non-paretic plantar flexor (64.9% and 52.5%; Figure 12) and knee extensor (62.3% and 68.7%) muscles. Contributing to increases in muscle power, peak torque production increased in paretic and non-paretic plantar flexor (39.7% and 47.7%) and knee extensor (23.3% and 32.6%) muscle groups. In addition, greater plantar flexor peak velocity of contraction in paretic (154±39 º sec^{-1} pre vs. 237±55 º sec^{-1} post) and non-paretic (213±44 º sec^{-1} pre; 306±69 º sec^{-1} post) legs as well as increases in paretic (12.8%) and non-paretic (9.1%) cross-sectional area also resulted from training. Similarly, increases in peak contractile velocity as well as muscle CSA and voluntary activation for the KE muscle group also resulted from training.

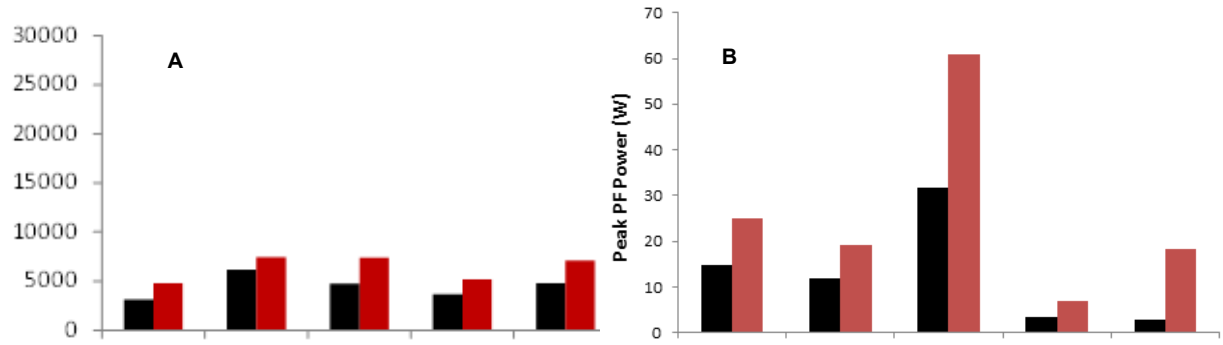

Figure 12: Individual subject peak paretic plantar flexor muscle power generation during dynamometric evaluation (A) as well as during self-selected walking speed (B) at pre (black bars) and post (red bars) POWER training time points.

D. RESEARCH DESIGN AND METHODS (including data analysis)

Procedures Relative to Specific Aims:

Aim 1: Determine the presence and magnitude of neural and muscular adaptations as

predictors of impaired muscle power generation in persons post-stroke.

Rationale: Our preliminary data indicate a significant reduction in peak torque generation in the plantar flexor and knee extensor muscle groups relative to matched (gender, age, height and weight) controls. In addition, reductions in muscle cross-sectional area (CSA) along with impaired central activation and altered *in-vivo* bioenergetic properties (e.g., increased [Pi]) are also characteristic of muscle post-stroke and likely contribute significantly to hemiparetic muscle dysfunction. To date, the physiological mechanisms that contribute to hemiparetic muscle dysfunction are largely unstudied.

Summary of Experiment & Research Design: We will perform comprehensive dynamometric, electrophysiological and magnetic resonance assessments of paretic and non-paretic musculature. Measurements will be performed on a total of 28 subjects at least six months following stroke and comparisons made to the dominant leg of a group of matched (age, gender, height & weight) neurologically healthy individuals (n=28). In doing so, we will determine if, and to what extent, neural and muscular factors differ between legs (paretic vs. non-paretic vs. control). In addition, we will determine the relationship between these factors and impaired muscle power generation. Our primary outcome measures in the ankle plantar flexor and knee extensor muscle groups will include 1) peak torque and power generation determined via isokinetic and isotonic dynamometry, respectively; 2) central activation measured using superimposed neuromuscular electrical stimulation; 3) muscle cross-sectional area (CSA) determined via MRI; and 4) muscle oxidative capacity (reflected by the rate of PCr recovery) and resting concentrations of inorganic phosphate [Pi] measured using ^{31}P MR spectroscopy.

Data Analysis Plan: The influence of neural and muscular adaptations (central activation, muscle CSA, in-vivo oxidative capacity and [Pi]) on peak power generation at baseline will be conducted using mixed effect regression models. The mixed models will be used with random subject effects to account for the dependent nature of the data collected on paretic and non-paretic legs within the same individuals. The baseline measure of peak power generation will serve as the dependent variable, with fixed effects for leg involvement (paretic vs. non-paretic) and for neural and muscular adaptation measures. Model fit will be assessed via Akaike Information Criteria (AIC). Partial correlations and standardized β coefficients will determine those factors with the strongest influence on muscle power generation. The models will also allow us to address secondary questions such as the differences between peak power generation and neural and muscular adaptation measures in paretic and non-paretic limbs.

Expectation: We hypothesize that the strongest predictor of impaired muscle power generation in the paretic and non-paretic legs will be central activation. Further, in-vivo oxidative capacity and [Pi] in both the paretic and non-paretic legs will be weakly associated with peak power generation, while CSA will only be significant in the paretic leg. We also expect significant reductions in paretic peak power generation, central activation, CSA and in-vivo oxidative capacity as well as an increase in [Pi], when compared to non-paretic and control legs. Further, differences in peak power, central activation and [Pi], but not CSA, will be present between the non-paretic and control legs.

Innovation: The proposed investigation combines contemporary, high resolution MR imaging and spectroscopic techniques concurrently with electrophysiological assessment of central activation impairment and dynamometric evaluations of muscle strength and power, which together will produce unequivocal evidence of the presence, magnitude and relative importance of neural and muscular factors as contributors to impaired hemiparetic muscle function.

Aim 2: Quantify the effects of POWER training on the presence and magnitude of neural and muscular adaptations in paretic and non-paretic muscle post-stroke

Rationale**:** To date, the physiological mechanisms that contribute to improvements in lower extremity muscle power generation in hemiparetic subjects are largely unstudied. Moreover, how these variables impact functional performance would provide a foundation for the development of effective rehabilitation interventions targeting functional recovery post-stroke.

Summary of Experiment & Research Design**:** Twenty-eight subjects will participate in a novel training paradigm targeting lower extremity muscle function. Training will take place over an 8-week period, three times per week on non-consecutive days. Exercises will be performed in the paretic and non-paretic limbs separately and progressed as tolerated across the 8-week period. Dynamometric evaluations of isokinetic strength and isotonic power will be performed prior to, after 4 and 8 weeks of training as well as 8 weeks following cessation of training (Figure 13). Assessments of muscle morphology (CSA) and in-vivo bioenergetics (PCr recovery and [Pi]) as well as full biomechanical assessments of walking will be performed at the same time points, allowing determination of the efficacy (and persistence) of training on the proposed outcomes. We will perform walking analysis over-ground and on the instrumented treadmill by collecting: (i) kinetics (3- dimensional ground reaction forces and moments), (ii) kinematics and iii) EMG from each leg. For each kinesiological measure, averages over the gait cycle for each leg will be calculated for each subject. Our primary outcome measures during biomechanical assessments will be peak ankle plantar flexion and knee extension power generation, with secondary variables of interest to include paretic step length ratio and paretic propulsion. A battery of clinical assessments to evaluate degree of function and disability will be performed prior to as well following training and at the 8 week post-training time point (see **Procedures-Specific**).

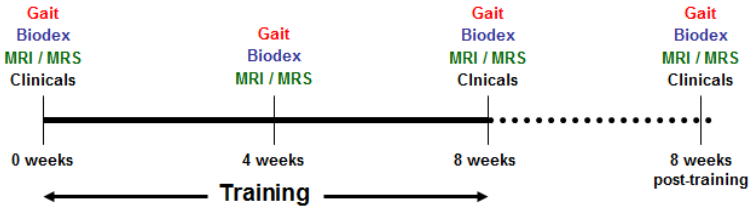

Figure 13: Timeline for measures during POWER training

Data Analysis Plan**:** To quantify the effects of our POWER training intervention on neural and muscular adaptations, we will utilize repeated measures mixed effects regression models to assess for significant changes in our outcomes over time. Neural and muscular adaptations (on both the paretic and non-paretic legs) will serve as dependent variables in separate models, with time serving as the key independent variable in each. Various error structures associated with the repeated measures over time will be considered (e.g. compound symmetry vs. auto-regressive), and final error structures will be selected based on AIC values. Having random subject effects in the models will account for correlation among measures within subjects over time. Secondary analyses, incorporating differences in the paretic and non-paretic legs as dependent variables, will examine whether changes over time are more pronounced in the paretic vs. non-paretic leg. These models will also incorporate random subject effects to account for within-subject dependence. Error correction will be set to maintain type one error at 5% with a desired power of 80%.

Expectation**:** We hypothesize that POWER training will result in significant increases in muscle peak power generation, central activation, muscle CSA and in-vivo oxidative capacity as well as a concomitant decrease in $[P_i]$. Further, we expect changes in these variables will be greater in the paretic compared to the non-paretic leg. We also expect improvements in Clinical Assessments as a result of training as well as increases in our measures of locomotor function (*see **Procedures-Specific***).

Innovation**:** Through conduct of the proposed experiments, we will generate a rich data set, capable of relating measures of lower extremity power generation (both in and away from the task of walking) with functional walking performance. The proposed neuromuscular assessment

techniques are state-of-the-art, and very few have even been applied to the post-stroke population, let alone used together to broadly assess changes in response to intervention. In addition, the unique biomechanical testing allows investigation of changes in steady-state bilateral walking, whereas most previous studies only evaluate the changes in outcomes (i.e., walking speed), with little (if any) information presented on changes in kinematics, kinetics or muscle coordination. The successful completion of this study will provide an entirely new level of evidence for the functional changes resulting from a novel rehabilitation intervention and the resulting framework for mechanisms underlying the adaptations in walking could lead to a more integrative approach in the development of novel neurorehabilitation strategies (e.g. adjunctive therapies incorporating multiple training modalities to maximize recovery of function). If our hypotheses prove to be correct, the data will emphasize the importance of lower extremity power generation for improving hemiparetic walking and provide a foundation upon which further novel rehabilitative programs can be developed, including application to populations other than stroke who present with reduced muscle function and impaired walking (e.g. aging, SCI, TBI, PD, MS).

Aim 3: **Establish the relationship between neural and muscular adaptations, muscle power generation and functional (locomotor) improvements in persons post-stroke following POWER training.**

Rationale: Given that in persons post-stroke, improving walking is 1) independently related to overall health status; 2) a strong predictor of functional recovery and 3) the most often stated goal during rehabilitation, interventions aimed at improving functional walking status are critical to increasing independence and improving quality of life for patients and their caregivers The advanced methodologies proposed herein and the novel theoretical framework we have developed allow for detailed investigation of the neural and muscular factors that contribute to (changes in) muscle power generation. In addition, the state-of-the-art assessments of walking provide a comprehensive evaluation of locomotor behavior and the biomechanical mechanisms underlying hemiparetic walking performance. Moreover, we seek to identify the muscular characteristic(s) that best predict functional improvement and provide evidence establishing the characteristics of those most likely to benefit (or not) from training.

Summary of Experiment & Research Design**:** We will determine the relationship between changes in neural and muscular factors and improved power generation following training. We will also assess functional improvements via the proposed clinical assessments as well as through full biomechanical evaluations of walking.

Data Analysis Plan**:** We will assess relationships for changes in central activation, muscle CSA and [Pi] (predictor variables) with improvements in muscle power generation (criterion variable) and self-selected walking speed by using mixed effects regression models, which, by incorporating random subject effects, will allow us to account for correlation of measures made on the same individual over time. We will also use measures of muscle power generation in the paretic and non-paretic knee extensor and plantar flexor muscle groups as predictor variables in a multivariable linear regression model to assess relationships with self-selected walking speed. Standardized β coefficients from these regression models will help us determine which factors have the strongest influence on our outcome variables (i.e. power generation and self-selected gait speed).

Secondary Questions: Although this proposal is not powered to examine differences in efficacy and mechanisms of response between POWER training and other intervention approaches (e.g., locomotor training), we intend to investigate these via exploratory post-hoc analyses to compare with an existing data set from a Locomotor Training trial in persons with chronic stroke (the same population we will be studying) recently completed by us (VA Merit Award B3983, Kautz PI). We will first compare the effect on self-selected and fastest comfortable walking speed for the two

interventions. Then we will conduct a detailed biomechanical assessment, from which we will calculate moments, powers, work, and kinematics, as well as the electromyography (EMG) data to measure muscle coordination. Although we do not present specific hypotheses related to differences between POWER training and the task-specific approach utilized in our previous trial, we believe this information will be extremely valuable in explaining the neural coordination associated with recovery of walking and provide insight on the emerging similarities and differences of these presumably distinct approaches to locomotor recovery. Dr. Kautz has a proven track record of performing successful quantitative analyses from biomechanical assessments and will direct the interpretation of these data. In addition, this proposal will leverage Dr. Kautz' currently funded R-01, which has already developed the tools to analyze the coordination of EMG activity during multiple locomotor tasks in even greater detail.

Expectation**:** We hypothesize that this type of training delivered separately to the paretic and non-paretic legs will result in improved functional performance that is associated with increased lower extremity muscle power generation (primarily the paretic plantar flexors). Further, we expect that post-hoc analyses of the characteristics of responders versus non-responders will be especially informative and will open the door for subsequent studies to study different adjunctive approaches to locomotor recovery.

Innovation**:** Three novel components make this study highly innovative: 1) state of the art magnetic resonance and biomechanical assessments of *in-vivo* bioenergetics and neural deficits; 2) development of a novel training intervention that includes task-specific components; and 3) a unique measure of the coordination of muscle activity to more fully quantify neural activation deficit (i.e., while activation deficit tells how completely the muscle is activated, our coordination measure tells whether the muscle can be independently activated at the appropriate point in the gait cycle). Detailed mechanistic studies, such as that proposed, allow for post-hoc analyses to determine for whom the intervention does and does not work, and can suggest why by providing scientific evidence of the underlying mechanisms contributing to post-stroke muscle dysfunction and, more importantly, how these mechanisms respond to therapeutic intervention.

Procedures (Specific):

Subjects: Subjects (male and female), ages 50-70, will be screened and recruited for the study 6-24 months following stroke. We will limit inclusion to subjects within this age range following stroke in an effort to control for differences in muscle power generation, coordination as well as responses to training that may associated with the aging process. The pool of post-stroke candidates for the study will be recruited from inpatient rehabilitation programs at the Ralph H. Johnson VAMC (see Dr. Hinson letter of support) as well as the Medical University of South Carolina, Charleston, SC. All participants will have had a new ischemic stroke. Diagnosis will be confirmed by a positive CT or MRI performed during stroke admission but not part of these study procedures. Eligible participants will be screened for study participation and, if appropriate, accepted into the study. In addition, a group of neurologically healthy individuals matched on the basis of gender, age (± 5 years), height (± 5 cm) and weight (± 5 kg) will also be recruited. **Inclusion criteria** will be: 1) age 50-70, 2) stroke within the past 6 to 24 months, 3) residual paresis in the lower extremity (Fugl-Meyer LE motor score <34), 4) ability to walk without assistance and without an AFO on the treadmill ≥ 30 seconds at speeds ranging from 0.3 - 0.8 m/s, and 5) provision of informed consent. In addition, all subjects who meet criteria for the training portion must complete an exercise tolerance test and be cleared for participation by the study cardiologist. **Exclusion criteria** will be: 1) Unable to ambulate at least 150 feet prior to stroke, or experienced intermittent claudication while walking; 2) rating on Modified Ashworth Scale ≥3 at the knee or ankle; 3) limited lower extremity range of motion of the knee (passive flexion ROM < 90); hip (inability to achieve neutral 0° hip extension); or ankle (inability to achieve 0° of active dorsiflexion); 4) history of congestive heart failure, unstable cardiac arrhythmias, hypertrophic

cardiomyopathy, severe aortic stenosis, angina or dyspnea at rest or during ADL's; 5) History of COPD or oxygen dependence; 6) Preexisting neurological disorders, dementia or previous stroke; 7) History of major head trauma; 8) Legal blindness or severe visual impairment; 9) history of significant psychiatric illness 10) Life expectancy <1 yr., 11) Severe arthritis or other problems that limit passive ROM; 12) post-stroke depression (PHQ-9 ≥10), 13) History of DVT or pulmonary embolism within 6 months; 14) Uncontrolled diabetes with recent diabetic coma, or frequent insulin reactions; 15) Severe hypertension with systolic >200 mmHg and diastolic >110 mmHg at rest; 16) Previous or current enrollment in a trial to enhance motor recovery; 17) Presence of non-MR compatible implants, pregnancy or severe claustrophobia.

Subject recruitment: The combination of the Ralph H. Johnson VAMC and the MUSC Stroke Center provide a unique environment from which to recruit participants (Veteran and non-veteran) for this study. These two centers consist of highly trained neuroscience nurses, stroke neurologists, diagnostic neuroradiologists, dedicated case managers and social workers, many of whom hold dual appointments (VA + MUSC). The MUSC program holds Joint Commission's *Certificate of Distinction for Primary Stroke Centers which* recognizes centers that make exceptional efforts to foster better outcomes for stroke care. The Stroke Center research team will assist with recruitment by reviewing previous patient rosters to identify potential participants**.** Potential participants will be given information while inpatient and again when they are seen for follow up in the outpatient clinic at the MUSC Stroke Center. The Stroke Center research team will establish communication with the potential participants and act as a liaison with the Principal Investigator to facilitate recruitment. The MUSC Stroke Center sees over 50 new stroke patients per month and routinely screens all patients for eligibility to our various research studies. Thus, with such a large database of stroke patients, we should have no difficulty in recruiting the hemiparetic subjects required to address our aims.

Subject screening and clinical assessments: After enrollment, participants will be thoroughly evaluated for functional and cognitive impairments as well as physical performance. Descriptive physical performance testing will include the lower extremity Fugl-Meyer, Stroke Impact Scale (SIS), Berg Balance Scale (BBS) and the NIH Stroke Scale**.** The lower extremity Fugl-Meyer is a 34-point scale assessing lower extremity function through a progression of items examining more complex movements, speed, and coordination. The SIS is a stroke-specific outcome measure that assesses physical function and other dimensions of health-related quality of life: emotion, communication, memory and thinking, and social role function. The physical functioning domain includes strength, hand function, mobility, and activities of daily living. The NIH Stroke Scale measures stroke related impairments including level of consciousness, ability to respond to questions, ability to follow simple commands, deviation of gaze, hemianopsia, facial palsy, limb movements of paretic and non-paretic limbs, limb ataxia, sensory loss, neglect, dysarthria, and aphasia. We will also assess passive ROM of the paretic and non-paretic ankle and knee joints, spasticity (Modified Ashworth Scale) and sensation (Fugl-Meyer sensory) so as to be able to retrospectively determine the potential impact of these measures on response to training. In addition, the Folstein Mini-Mental Status will be used as a screen of cognitive function. Specifically, we will use the three-step command item as a primary screen for study eligibility. Finally, subjects will be screened for depression using the PHQ-9. We will exclude subjects with major depression (PHQ-9 score ≥ 10). All clinical assessments will be performed by a research physical therapist employed by the Department of Health Sciences & Research and is not involved in any other aspects of the proposed study.

Exercise Tolerance Testing: For subjects enrolling in training, a bicycle ergometry protocol used successfully in the recently completed LEAPS trial (Duncan et al, 2007) will be used to assess exercise tolerance for inclusion in this study. The exercise protocol will commence with the subject seated on the bicycle ergometer with feet secured in toe clips. The subject will first sit quietly

without pedaling for two minutes. Exercise will begin with the subject pedaling at ~60 revolutions per minute (RPM) and 0 Watts (W) of workload. The workload will be increased by ~10 W every 3 minutes. If the subject's cadence drops below 50 RPM, additional reminders to pedal at 60 RPM will be given. Testing continues until maximal effort is achieved. Maximal effort is defined as the achievement of 90% maximal age-predicted heart rate (maximum heart rate = 220-age). The test will be terminated prior to achieving maximum heart rate for predefined symptomatic, clinical, and electrocardiographic criteria. Symptom-related reasons for termination include angina, dyspnea, and fatigue. Fatigue is defined as either voluntary exhaustion or inability to maintain a minimum cycling cadence of 40 RPM. Clinical criteria for termination include: 1) Hypertension: ≥ 220/120 mmHg, or 2) Hypotension: a drop in diastolic blood pressure >20 mmHg, and O_2 saturation <85%. Electrocardiogram criteria include: 1) ≥ 1 mm horizontal or down sloping ST segment depression, 2) sustained paroxysmal ventricular tachycardia (>30 beats), and 3) sustained paroxysmal supraventricular tachycardia (>30 beats). A 5 minute cool down period will immediately follow test termination. If the test is terminated because of electrocardiographic findings, the subject will be managed medically as needed, referred for care, and disapproved for participation. Brachial artery blood pressure from the subject's non-paretic upper extremity will be measured using an automated blood pressure monitor. Resting blood pressure and heart rate will be obtained prior to initiation of exercise as well as after the subject has been sitting on the stationary bicycle for 1 minute. During the graded exercise test, blood pressure readings will be obtained every 3 minutes. Heart rate will be obtained from the 12-lead EKG. Maximal heart rate will be recorded as the highest heart rate achieved during the exercise tolerance test.

Subject monitoring during testing and training: Blood pressure (BP) and heart rate (HR) will be monitored prior to, during, and at completion of each session. Subjects' resting diastolic BP must be <100 mmHg, systolic < 200 mmHg, and heart rate <110 beats/min to begin the testing (Mahler et al, 1995, Rimmer et al, 2000). Criteria for termination include subject complaints of shortness of breath, light-headedness, confusion, severe headache, or dyspnea; onset of angina; excessive blood pressure (systolic BP > 200mm Hg, diastolic BP >110 mm Hg), or drop in systolic BP >10 mm Hg and inappropriate bradycardia (drop in heart rate >15 bpm).

Post-stroke Optimization of Walking using Explosive Resistance (POWER) training: Training to improve muscle power (vs. strength) is shown to be effective for improving functional mobility the aged (Costello et al, 2008) as well as in stroke survivors, (Lubetzky-Vilnai et al, 2010) though no single intervention has been proven most beneficial for optimizing outcomes. To date, there is a paucity of information regarding the physiological mechanisms underlying improvements in muscle power generation as they relate to improved walking and to understand the differences in those who respond versus those who do not. Accordingly, there is a need for systematic investigation to identify the relevant mechanisms to determine whether and how they adapt to rehabilitation interventions and further, how significantly therapeutically-induced neuromuscular adaptations translate to improved functional performance. The novel theoretical framework we have developed will allow us to address these issues with a thoroughness not previously feasible. Individuals with chronic post-stroke hemiparesis will undergo training to improve muscle power generation for 24 sessions (3 times/week) that includes both resistive and task-specific elements. Session duration will be ~90 minutes/day (inclusive of rest intervals) although the total time spent in the laboratory will depend on the amount of rest needed for a given individual. Training will include five distinct resistance activities aimed at improving muscle power-- each previously reported to contribute to improved walking (Figure 14). Sit to stand and leg press exercises are elements of programs shown to be effective in improving strength and walking performance in the post-stroke population (Tung et al, 2010; Duncan et al, 2007). Repeated step-up training using a single riser targets both eccentric and concentric training in both the sagittal and frontal planes. As possible for individual subjects, we will utilize a step height higher than standard community steps (10") to further challenge neuromuscular requirements of

the task. Calf raises (i.e. plantar flexion exercise) will be performed on a small step so as to allow for adequate range of motion. In addition, our pilot data demonstrate that jump training, focused on lower extremity muscle power generation, is part of a comprehensive program that improves gait speed in people with post-stroke gait deficits. Sit to stand, step-up and calf raise activities will be performed within the novel overground body-weight support (BWS) environment enabled by the Zero-G (ZeroG, Bioness Inc., Valencia, CA), whether receiving support or not, to provide the maximum safety possible.

During the initial training session, subjects will perform the individual component tasks beginning with least amount of BWS (or highest load) required for successful completion of the required number of repetitions in order to match the participant's ability to the difficulty of the task (Guadagnoli and Lee, 2004). BWS will be progressively reduced so as to determine the minimum level of support necessary for subjects to complete each task. The level of support required for successful completion of each task will be recorded over time to monitor and document progression of the program. Participants will be progressed within each task by decreasing BWS by 5% of body mass (or adding 5% load for leg press) upon successfully meeting the goals for progression (see Figure 14). An adjustable weighted vest will be utilized to increase mass by 5% during training for a given task once support is not required for successful task completion. Subjects will not be permitted to use assistive devices and physical assistance will not be provided

Activity	Task Specifications	Goal for Progression
Sit to Stand	Standard straight back chair without arm rests	Stand upright from chair without use of upper extremities for support (2x10)
Leg Press	Standard leg press	Unilateral full ROM performed for all repetitions (2x10) at prescribed resistance
Calf Raise	* Forefoot on edge of step and heel off ground	Unilateral full ROM performed for all repetitions (2x10) at prescribed BWS resistance
Step-Up (Anterior / Lateral)	* Stationary step without hand rails (10" height)	Step up alternating leading foot (2x10 with each foot).
Jump Training	Shuttle Pro Jump Trainer (Shuttle Systems, Inc.)	Progress from bilateral to unilateral ground contacts (x60) followed by increases in resistance

Figure 14: Activities, task specifications and goals for progression during POWER training. * An alternative approach will be to perform these tasks using the ShuttlePro (i.e. in a supine position) if an individual cannot accomplish the task in an upright posture using the ZeroG.

during training. A given task will be considered unsuccessful if physical assistance or a mechanical stop to prevent a fall is required.

Sit to stand, leg press, step-up and calf raise activities will be performed under two conditions within each training session. Condition 1 will include performing two sets of ten repetitions with the targeted amount of resistance for the given session and based on rate of progression throughout training. Condition 2 will require the participant to perform as many repetitions as possible during a 20 second period utilizing either 80% of the resistance from Condition 1 or an increase in BWS of 20%. This condition is designed to encourage high velocity and hence high-power output contractions. As much rest as needed between sets will be allowed beyond the 3 minutes rest that will be required for all subjects. Given our focus on muscle power generation, all participants will be instructed to perform the concentric portion of each exercise at maximum velocity regardless of the prescribed condition, followed by a controlled eccentric contraction. This approach is adapted from programs specifically targeting power training in an aging population with mobility deficits and shown to result in significant increases in peak power generation when compared to slower velocity training, while achieving similar gains in strength

(Fielding et al, 2002; Bean et al, 2004).

For jump training, subjects will be familiarized with the equipment and, during the initial training session, will complete a total of 30 (3 sets of 10) bilateral ground contacts (jumps). We will begin the first session at a resistance of ~20% of body mass. Based on individual subject ability, external resistance will be increased or decreased accordingly and resistance documented as a reference for subsequent sessions. The number of jumps will be increased to 45 in week two and to 60 thereafter (the goal being to complete the prescribed # of jumps in 3 sets). Upon successful completion of at least 60 ground contacts (e.g. complete clearance from foot plate; Figure 15), resistance will be increased in increments of 5 lbs. or 5% body mass, whichever is greatest. As subjects feel comfortable, and no later than the beginning of week 4, unilateral jump training will be performed using each leg, the goal being to progress to a reciprocating jumping pattern (i.e. alternating legs each jump for a total of 60 jumps per leg). Throughout the training protocol, a minimum of two sessions at a given resistance will be required before load is increased and resistance will be held consistent between limbs throughout the training program. Session intensity will be systematically progressed and modified by changing either the applied resistance or the number of ground contacts.

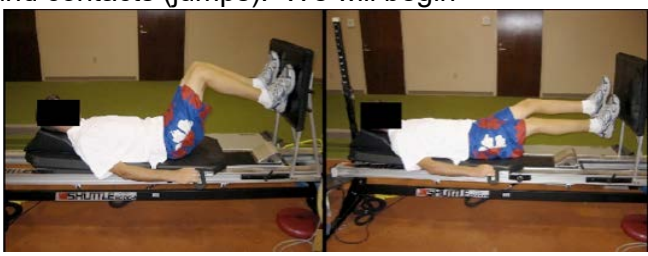

Figure 15: Exercise apparatus used for jump training task

Progressive loaded overground fast walking: As part of each training session, subjects will perform 10 trials of fast overground walking (10 meter distance per trial). These trials are intended to emphasize the task-specific lower extremity power generation required to accomplish increased walking speeds. Subjects will be asked to complete all trials without an assistive device or ankle-foot orthosis at speeds ≥ 125% of self-selected overground velocity. Although we will not allow the use of an AFO, subjects will be permitted to wear an ankle brace for medial-lateral support (AirCast®) that allows for plantarflexion and dorsiflexion voluntary movements. Subjects will walk over our instrumented walkway (GaitRite) so as to provide real time feedback of speed as well as spatio-temporal parameters of walking for each trial. These trials will also be performed in the ZeroG harness to protect the subject in the case of a loss of balance as well as to provide body-weight support as needed to facilitate walking at the required speeds. Subjects will begin at the level of BWS that allows them to successfully complete the 10 trials at an average of ≥125% of self-selected speed. After each training session the average speed will be calculated and compared to the self-selected speed. When the average speed exceeds 125% of self-selected speed, we will decrease BWS by 5% for the next training session. At the point when subjects are able to successfully exceed 125% of self-selected speed without body-weight support, a weighted vest will be worn and mass systematically added to allow progression. Note that in order to provide the optimal stimulus for increasing propulsion, we will provide an amount of BWS to offset to the added mass. This has been shown to engage the muscles most responsible for generating propulsion to a greater extent (McGowan et al., 2008) and should optimize the power training stimulus for the muscles generating propulsion.

It is important to point out that progression for muscle power training and overground stepping will be accomplished via reduction in BWS (or added mass) as tolerated, allowing for increased "resistance" and graded exercise. The overground permissive environment allows safe training without assistive devices or and allows determination of the precise ability level of the individual for a task. In creating a constant permissive environment, we are able to identify precisely how much support is required for independent completion of the task while simultaneously eliminating falls risk. This environment allows the participant to engage in therapy at a consistently more challenging level than is allowed in conventional rehabilitation. This approach is based on a

philosophy known as the “challenge point framework”, which theorizes that one’s ability level is defined by the level of difficulty at which one has an equal (50%) chance of success or failure (Guadagnoli and Lee, 2004), a level of difficulty that is rarely matched in clinical rehabilitation due to increased potential for falls.

Overground self-selected walking practice: In addition to progressive fast walking to emphasize task-specific power generation, subjects will also perform 10 min. of overground walking at a self-selected velocity in each session. The goal for this activity is continuous walking (as possible) and is designed to allow for regular, safe practice of newly acquired capacity. Rest as needed will be allowed. This training element is consistent with the amount of overground walking performed as part of our recently completed study of task-specific locomotor training (RR&D B3983-R; Kautz PI). Subjects will initially perform overground walking in the safety harness without bodyweight support but will progress to independent overground walking. We believe that progressive safe practice incorporating the increased power generating capacity (neural mechanisms could result in changes quite quickly) without initially needing to worry about the balance challenge is most appropriate. The distance walked during each session will be recorded to monitor improvements over the 8 week training period.

Dynamometric Assessment: Isometric and isotonic assessments will be performed at the hip (flexion only), knee (flexion/extension) and ankle (plantar flexion/dorsi-flexion) prior to, after 4 weeks and following 8 weeks of POWER training. Assessments will be performed using a Biodex isokinetic dynamometer (Biodex Corp., Shirley, NY). Prior to testing, each subject will go through a period of familiarization and warm-up consisting of 5 minutes of cycling and three sub-maximal contractions for each joint action. During isometric testing, maximum voluntary isometric contraction (MVIC) will be defined as the highest isometric torque achieved during 3 maximal contractions (~3 sec contractions separated by a minimum of 60 seconds rest). In the event that the MVIC values during the three trials differ by more than 5%, additional contractions will be performed. In addition to MVIC, values for the average rate of torque development (ARTD) will be calculated in the corresponding time interval between 20% and 80% of MVIC. (Gregory et al., 2007) This time interval was chosen to minimize potential errors in the determination of the precise onset and nadir of torque development while still representing a majority of the time necessary for achieving maximal torque production. ARTD is defined as the average increase in torque per unit time, and calculated by numerical differentiation as:

$$ARTD = \frac{1}{N}\sum_{i=1}^{N}\frac{\delta f_i}{\delta t}$$

Where, N is the total number of time slots for numerical differentiation, δf_i is the change in torque in the time slot **i** and δt is the unit time duration for a slot.

During isotonic testing, peak power will be assessed using 40% of MVIC. Differences in lower extremity maximal velocity are shown to occur at relatively low external forces (e.g. 40% 1 RM) and are most closely associated with gait velocity in older individuals. (Bean et al., 2002) To optimize reliability of the testing, each test will be repeated 3 times. If the coefficient of variation among the 3 highest powers is > 10%, additional contractions will be performed. Power-angle curves will be plotted and the peak powers recorded.

During all dynamometric testing, subjects will be instructed to (1) develop torque as fast as possible and (2) produce a maximal contraction. Subjects will be given an auditory cue and receive continuous visual feedback. All contractions will be performed with subjects positioned in the dynamometer and the axis of the dynamometer aligned with the joint axis of rotation. Proximal stabilization will be achieved with straps at the chest, hips, and knee as appropriate. In order to increase sensitivity, the force transducer signal will be amplified and fed into a data acquisition

unit (Lab View Inc.) and corrected for the effects of gravity.

Central Activation: Central activation will be determined in the plantar flexor and knee extensor muscles using the twitch interpolation method. Briefly, a single biphasic, supra-maximal electrical pulse will be delivered at rest and during maximal contractions. Activation deficits will be calculated using the ratio between the torques produced by the superimposition of a supra-maximal twitch on the maximal contraction and by the same stimulus in the potentiated resting muscle, as previously reported (Gregory et al., 2007). Electrical stimuli will be generated using a Grass S8800 stimulator and delivered through surface electrodes placed over the proximal and distal portions of the muscles. The stimulator and the dynamometer will be interfaced with a personal computer through a commercially available hardware system (MP150). Data will be sampled at 400Hz using commercially available software (AcqKnowledge 3.7.1).

Experimental protocol for MRI data collection: 3D-Magnetic Resonance Imaging will be implemented to quantify the maximal cross-sectional area of the flexor and extensor muscles about the hip, knee and ankle. Briefly, once subjects reach the MR facility, they will remain in a supine position for 30 minutes prior to imaging. All imaging will be performed in a 3.0 Tesla magnet (Siemens) using either a body coil or a standard quadriture extremity coil (ankle). 3D data will be acquired using a fast gradient-echo sequence with TR=100 ms, TE=10ms, flip angle=30°, an encoding matrix of 256x256, field of view of 16-24cm and 7mm slice thickness. Muscle and non-muscle tissue will be differentiated and CSA measured via a publicly available software (Osirix®).

Experimental protocol for MRS data collection: Figure 16 shows the five peaks, corresponding to inorganic phosphate (P_i), phosphocreatine (PCr), and the three phosphates of ATP, that can be quantitated in a ^{31}P spectrum. By integrating the individual peaks, the amount of each metabolite can be estimated. Further, the position of the Pi peak is pH dependent so monitoring its position allows us to estimate intracuscular pH. The concentration of these bio-energetically important compounds, as well as the intracellular pH, can thus be monitored continuously, at rest as well as during exercise and recovery. Specifically, data collected during recovery are used to determine the muscle's *in-vivo* oxidative capacity (Rossi et al, 1996; Kemp et al, 1994; Meyer, 1988). This capacity is commonly inferred from the rate of PCr resynthesis following exercise (Blei et al 1993; Kemp et al, 1994; McCully et al, 1992). Based on these observations, a decreased muscle oxidative capacity has been demonstrated in various patient groups, including those with mitochondrial myopathies (Argov & Arnold, 2000), peripheral vascular disease (McCully et al, 1992), multiple sclerosis (Kent-Braun, et al, 1994), and congestive heart failure (Rossi et al, 1996).

^{31}P-MRS data will be collected to determine the resting concentrations of inorganic phosphate [Pi] as well as the *in-vivo* oxidative capacity of the knee extensor and plantar flexor muscle groups. Oxidative capacity will be determined based on the rate of PCr resynthesis following repeated plantar flexor contractions at ~30% maximal voluntary contraction (MVC). An exercise device will be utilized for ^{31}P-MRS data collection that consists of an MR-compatible foot pedal attached to a force transducer and adjustable load. ^{31}P-MRS data will be collected using a standard pulse-acquire sequence on a 3.0 Siemens Trio scanner with a 4.5 cm circular transmit-receive surface coil. The voxel for localized spectra will be prescribed based on images obtained using a 3D gradient echo imaging sequence (field of view = 14x14 cm, 1 NEX, a minimum pulse repetition time (~20 ms), and an echo time of 2 ms).). In order to acquire accurate resting ^{31}P MRS spectra we will initially acquire spectra with 256 averages (TR = 5 s, spectral width of 6000 Hz, 2048 data points). ^{31}P-MRS kinetic data will be acquired with the same parameters but with only 2 averages at rest, during exercise, and recovery giving us a time resolution of 10 seconds.

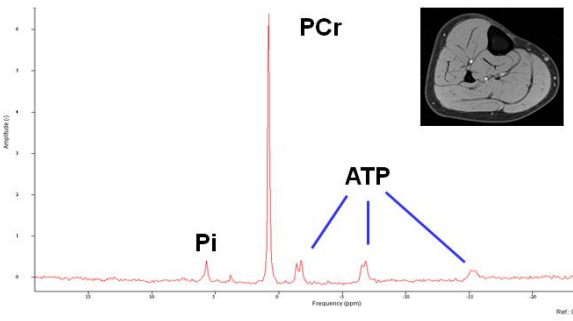

Figure 16: Illustration of phosphorous containing compound peaks during ^{31}P-MRS of the calf muscles.

Lower extremity power and lc

Data Processing: Resting spectra will be manually phased and the areas of the βATP, Pi, and PCr peaks integrated. PCr levels during exercise will be measured using complex principal component analysis of the entire time series, as previously described (Stoyanova, Brown et al., 2001). PCr recovery data will be fitted to a single exponential curve, and the psuedo-first-order rate constant (kPCr) determined as follows:

$PCr\ (t) = PCr_0 + \Delta PCr\ (1\text{-}e^{-kt})$

where PCr is the concentration of PCr at a given time t during recovery; PCr_0 is the PCr concentration at end of exercise and ΔPCr is the change in PCr concentration after recovery. The maximal rate of PCr resynthesis (V_{max}-lin) (a measure of mitochondrial ATP resynthesis) will be calculated based on kPCr and PCr rest (V_{max}-lin = kPCr [PCr] $_{rest}$).

Experimental protocol for walking data collection: At the beginning of the session, subjects will walk on a 14 ft. long gait mat (GaitRite) to measure self-selected and fastest comfortable walking speed and other spatiotemporal parameters. Treadmill speed will then be set to the subject's self-selected speed and fastest comfortable speed. Subjects will be permitted a practice trial, and then be asked to complete three trials at each speed during which kinematic, kinetic and EMG data will be captured. To optimize capture of steady state data, each subject will walk for ~10 sec prior to the 20 sec of data collection; therefore, each trial duration is 30 sec. This will allow capture of ~10 consecutive steady state gait cycles per trial (depending on cadence). A modified Helen Hayes reflective marker set will be recorded using a 12-camera motion analysis system (PhaseSpace) to capture bilateral 3D kinematics. A 16-channel EMG system (Motion Labs Systems) will be used to record bilaterally from: medial gastrocnemius, soleus, tibialis anterior, rectus femoris, vastus medialis, biceps femoris, semimembranosus, and gluteus medius. Bilateral ground reaction forces, moments and center of pressure will be captured during split-belt treadmill walking via two force plates embedded in the instrumented treadmill (Bertec). For all trials, subjects will wear their own shoes and be asked to walk without an assistive device or ankle-foot orthosis (see **Potential Limitations**). A safety harness mounted to the ceiling will protect the subject but will only support their body weight in the case of a loss of balance. A physical therapist will be present for all testing sessions as needed. Data processing: EMG and ground reaction force data will be collected at 1000 Hz. Raw EMG will be high-pass filtered using a fourth-order, zero-lag digital Butterworth filter (20 Hz cutoff) to eliminate movement artifact. We will then subtract the mean value, rectify and low-pass filter the data with a fourth-order, zero-lag digital Butterworth filter to produce linear envelopes. Kinetic and kinematic data will also be low-pass filtered with fourth-order, zero-lag digital Butterworth filter (20 and 9 Hz cutoff frequencies). Inter-segmental joint moments (normalized by body mass) and powers (product of moments and angular velocity) will be calculated using standard 3D inverse dynamics techniques and time normalized to 100% of the paretic leg gait cycle. The typical propulsive regions of the ipsilateral gait cycle will be divided into late single-leg stance (i.e., second 50% of single-leg stance) and pre-swing (i.e., double support region preceding toe-off) regions. Sagittal plane hip (flexor positive), knee (extensor positive) and ankle (plantarflexor positive) joint moment impulses and the AP GRF impulse (AP impulse) will be calculated for each region. A muscular utilization ratio (Milot et al, 2007) will also be calculated by dividing the peak power during walking by the peak power during dynamometric testing in PF and KE.

Measure of neural activation coordination: Neural activation coordination will be assessed from the eight channels of EMG collected in the paretic leg similar to our published work (Clark, 2010). Muscle activation signals (EMGs) will be demeaned, rectified, and smoothed with a zero lag fourth-order low-pass (4 Hz) Butterworth filter. To facilitate comparisons between subjects and among different walking speeds, the EMG from each muscle will be normalized to its peak value from self-selected walking and resampled at each 1% of the gait cycle. For each subject, leg, and walking speed, the EMGs will be combined into an *8* x *t* matrix (EMG_o), where *8* indicates the

number of muscles and *t* is the time base (*t* = no. of strides x 101). For each subject, an NNMF algorithm (Lee & Seung, 1999; Ting and MacPherson, 2005; Clark et al., 2010) will be applied to the *8* x *t* matrix corresponding to all gait cycles from SS trials. A priori, the number of independent co-activation patterns (ICPs), *n*, is specified, and the non-negative matrix factorization (NNMF) algorithm finds the properties of the ICPs by populating two matrices: an *8* x *n* matrix, which specifies the relative weighting of a muscle in each ICP, with each muscle weight invariant across all gait cycles, and an *n* x *t* matrix, which specifies the activation timing of each ICP over each of the gait cycles. When these two matrices are multiplied, an *8* x *t* matrix is produced that attempts to reconstruct the EMGs over all of the consecutive gait cycles. Of note, NNMF allows muscles to belong to more than one ICP and that a muscle's reconstructed EMG is the summed contributions from all the ICPs. The 8 x t matrix of the reconstructed EMGs (EMG_r) is compared with the original EMG matrix (EMG_o) and the agreement quantified by calculating the sum of the squared errors: $(EMG_o - EMG_r)^2$. Within this framework, the NNMF algorithm performs an iterative optimization until it converged on the muscle weights and the activation timings of the modules that minimize the error.

We make no a priori assumptions regarding the number of ICPs that will be required to adequately reconstruct the EMG signals. Therefore separate NNMF analyses are performed with the output constrained to one, two, three, or four ICPs. To determine the minimum number of ICPs needed to adequately reconstruct EMG_o in each leg of each subject, we calculate the variability accounted for (VAF) as the ratio of the sum of the squared error values to the sum of the squared EMG_o values [$VAF = 1 - (EMG_o - EMG_r)^2/EMG_o{}^2$]. VAF is calculated for each muscle across all gait cycles. To ensure that EMG was adequately reconstructed within each region of the gait cycle, VAF is also calculated as the cumulative of all muscles within each of six regions for all gait cycles. The regions are defined as 1) first double support, 2) first half of ipsilateral single leg stance, 3) second half of ipsilateral single leg stance, 4) second double support, 5) first half of ipsilateral swing, and 6) second half of ipsilateral swing. The analysis for each subject begins by assuming that only one ICP is needed for EMG reconstruction. If VAF was ≥ 90% for each of the eight muscles and six regions, it is determined that additional ICPs are not needed. Otherwise, the number of ICPs assumed is increased until all muscles and regions achieve 90% VAF or until adding an additional ICP does not increase VAF by > 5% for the muscle(s) and/or region(s) with the lowest VAF. This approach is conservative and ensures a strong agreement between the original and reconstructed EMG signals.

Independence of the plantarflexor (PF) and knee extensor (KE) muscle groups will be categorized as a true or false value based on the number and composition of ICPs. In healthy subjects soleus and gastrocnemius are the primary muscles in an ICP that is active during the second half of stance and the KE are active with gluteus medius as the primary components of an ICP that is active in early stance (Clark et al., 2010). Hemiparetic subjects with only two ICPs merge the PF and KE ICPs with a third ICP (that primarily includes the hamstrings and is active in late swing and early stance) into a mass extensor ICP that is active the entire stance phase (Clark et al., 2010). Thus, subjects with two ICPs do not have independence for either muscle group. Subjects with three or four ICPs will be classified as independent in PF if they have one ICP that includes primarily soleus and gastrocnemius and in KE if they have one ICP that includes primarily vastus medialis, rectus femoris and gluteus medius. **Timing** of neural activation will be assessed by quantifying the percent of time that a muscle is active during specified regions of the gait cycle based on normal activation patterns. Specifically, we will calculate these quantities during the region of primary activity when the muscle should be "on" and during a stance phase region when the muscle should be "off" (e.g., a region when the muscle group's primary ICF is not active during healthy gait but active when the subject has only two ICFs). For PF the "on" region is from mid-single leg stance to toe-off and the "off" region is during initial double support. For KE the "on" region is the initial double support phase and the "off" region is second double

support phase. **Overall coordination** of walking will be quantified by the number of ICFs found for the subject.

Clinical Assessments: The assessments are standard to most post-stroke intervention trials. Including these assessments strengthens our design by allowing us to make conclusions regarding the effects of our intervention that extend beyond the behavioral measures of walking. We feel that the proposed benefits of our training will be most significant if they are shown to impact behaviors other than that which is targeted as the primary outcome and relate to other domains of function suggested to put individuals post-stroke at risk for disability. All clinical assessments will be performed by a staff physical therapist within the Center for Rehabilitation Research in Neurological Conditions who will be blinded to responses to training.

- Dynamic Gait Index (DGI): The DGI evaluates the ability to adapt to changes in task demands. The DGI rates performance from 0 (poor) to 3 (excellent) on eight different gait tasks, including even surfaces, changing speeds, head turns in a vertical or horizontal direction, stepping over or around obstacles, and gait with pivot turns, and steps (Shumway-Cook et al, 2001). The DGI examines complex walking tasks (e.g. accelerating, turning, head turns) and provides valuable information regarding dynamic balance (as opposed to static balance assessed using the BBS) and often reveals deficits that otherwise would not be identified.
- Step Activity Monitor (SAM): The SAM is worn on the ankle to quantify daily step activity. The SAM is a microprocessor-driven accelerometer that permits the researcher to count strides and observe activity during predetermined time spans. The SAM is 98%-99% accurate and has a test-retest reliability post-stroke of 0.98. (Mudge et al., 2007) The SAM will be worn for two consecutive days at a 6-second epoch, per the LEAPS protocol (Duncan et al, 2007). Information about the effects of POWER training on the amount of community stepping are critical as we attempt to generalize any improvements beyond the laboratory.
- 6-Minute Walk Test (6MWT)**:** Timed walking tests are primarily a measure of functional capacity (Solway et al, 2001). The 6MWT tests populations who present with little functional walking capacity and has been validated against the Rivermead Mobility Index (r=0.75) and 10-meter walk time (r=-0.61).(Rossier et al, 2001) The 6MWT has a high intra- and interrater reliability (ICCs = 0.82-0.95) (Wade, 1992).
- Stroke Impact Scale (SIS): The SIS assesses physical function as well as other dimensions of health-related quality of life: emotion, communication, memory & thinking, and social role function. The SIS was created to assess changes in impairments, disabilities and handicaps following stroke, but has been validated for repeated administration over time, in clinical and research settings (Duncan et al., 2003 a,b,c).

Projected Timetable:

- Project Development: The first quarter (Months 1-3) of Year 1 will be devoted to finalizing imaging sequences for MRI and ^{31}P-MRS studies and establishing the data acquisition pipeline for dynamometric and biomechanical data collections.
- Subject Recruitment: Recruitment of participants will begin early in the first quarter of the project period and will continue through the third quarter of year three.
- Longitudinal Evaluations: Baseline evaluations prior to POWER training will take place beginning in Quarter 2 of Year 1 and continuing through the end of Year 3. We will evaluate muscular function and perform MR imaging and spectroscopy studies to determine muscle power, central activation, muscle cross-sectional area, [Pi] and *in-vivo* oxidative capacity of the plantar flexor and knee extensor muscle groups.
- Therapeutic Intervention: Beginning in Quarter 2 of Year 1 and continuing through Quarter 2 of Year 4, hemiparetic subjects will participate in 8-weeks of POWER training.

- Follow-up Evaluations: Post-training evaluations will be conducted 8 weeks following POWER training. Assessments performed at pre- and post-training will be repeated during follow-up evaluation.
- Data Analyses: Data analysis will begin beginning in Year 3 and throughout the remainder of the project.
- Dissemination: Data dissemination will take place as appropriate, but not earlier than in Year 3, via meeting abstracts as well as full publications.

Sample size determination and Statistical Approach: A total of 28 subjects will be recruited for this study. With baseline data from n=28 subjects, we will have at least 80% power to detect measures of association (i.e. partial correlations) as small as 0.5 between neural/muscular adaptations and peak power generation. Our preliminary data suggest that the intraclass correlation between measures of power on paretic and non-paretic legs is extremely high (i.e. Spearman rho = 0.81, intraclass correlation coefficient [ICC - from mixed model] ~100%). This power estimate for Aim 1 is based on 1 observation per subject; although our mixed model approach for analyses for Aim 1 will include 2 observations per subject (i.e. paretic and non-paretic leg), the detectable correlation may not be substantially lower due to the strong intraclass correlation. For Aims 2 and 3, analyses rely on longitudinal measures, and we assume (based on experience with our patient population) that no more than 15% will fail to complete all assessments. With 28 subjects enrolled and a 15% attrition rate over time (resulting in complete data on 24 subjects), we would have at least 80% power to detect changes in measures from baseline to the end of the study equivalent to 0.6 standard deviation units (i.e. effect sizes). This power estimate is based on a paired t-test framework (i.e. pre-post comparisons), and thus power is likely underestimated, since our models will also include data from mid-intervention and follow-up time periods. For example, assuming an ICC of 90% associated with within-subject responses over 4 time periods, the effective sample size (see Campbell et al., 2001) would be slightly higher (~n=26), as would be our power (~83%) to detect an effect size of 0.6. Treatment effect sizes of 0.6 are consistent with our preliminary data, in which effect sizes of 0.7 (95% CI 0.5 to 0.9), 1.5 (95% CI 1.1 to 1.9, and 0.5 (95% CI 0.1 to 1.0) were observed for improvement in gait speed, paretic leg power, and non-paretic power, respectively. With complete data on 24 subjects, we will also be able to detect whether as little as 40% of the variability in changes in gait speed can be explained by changes in the neural / muscular adaptation measures, assuming an ICC of 90% associated with within-subject responses over 4 time periods. All power estimates assume 2-sided hypothesis tests and an alpha level of 0.05 and were conducted by a biostatistician using PASS 2008 (NCSS, Kaysville, Utah).

E. PROTECTION OF HUMAN SUBJECTS

1. Risks to the subjects

a. Human Subjects Involvement and Characteristics: We will recruit twenty-eight subjects with persistent motoric disability following a single stroke or two strokes on the same side of the brain, the most recent no less than six months prior. Subjects may be between 50 and 70 years of age, of either sex, and of diverse ethnic background. Subjects who have experienced more than one stroke will be accepted only if all strokes are on the same side of the brain, there is no history of a clinical ischemic or hemorrhagic event affecting the other hemisphere, and there is no evidence of more than a lacune or minor ischemic demyelination affecting the other hemisphere. Subjects will be recruited to participate in 8-weeks of a high-velocity resistance training paradigm designed to target lower extremity power generation. We will perform comprehensive dynamometric and magnetic resonance assessments of the paretic and non-paretic hip, knee and ankle musculature. In addition, we will also perform analysis of over-ground walking on an instrumented treadmill. During walking we will collect: (i) kinetics (3-dimensional ground reaction forces and moments),

(ii) kinematics (3-D) and iii) EMG from each leg as well as self-selected and fastest comfortable walking velocities. We will also perform a battery of clinical assessments to evaluate degree of function and disability. In addition to our post-stroke participants, we will also recruit twenty-eight neurologically healthy controls matched on the basis of age, gender, height and weight. Control subjects will participate in dynamometric and magnetic resonance evaluations but will not complete the training.

Subjects (male and female), ages 50-70, will be screened and recruited for the study six months to two years post-stroke, allowing for natural recovery during the first 6 months post-stroke. The pool of candidates for the study will be recruited from rehabilitation programs at the Ralph H. Johnson VAMC; the Medical University of South Carolina; and Charleston area communities. Eligible participants will be screened for participation and if appropriate will be accepted into the study for training.

Inclusion criteria will be: 1) age 50-70, 2) stroke within the past 6 to 24 months, 3) residual paresis in the lower extremity (Fugl-Meyer LE motor score <34), 4) ability to walk without assistance and without an AFO on the treadmill ≥ 30 seconds at speeds ranging from 0.3 - 0.8 m/s, and 5) provision of informed consent. In addition, all subjects who meet criteria for the training portion must complete an exercise tolerance test and be cleared for participation by the study cardiologist. **Exclusion criteria** will be: 1) Unable to ambulate at least 150 feet prior to stroke, or experienced intermittent claudication while walking; 2) history of congestive heart failure, unstable cardiac arrhythmias, hypertrophic cardiomyopathy, severe aortic stenosis, angina or dyspnea at rest or during ADL's; 3) History of COPD or oxygen dependence; 4) Preexisting neurological disorders, dementia or previous stroke; 5) History of major head trauma; 6) Legal blindness or severe visual impairment; 7) history of significant psychiatric illness 8) Life expectancy <1 yr., 9) Severe arthritis or other problems that limit passive ROM; 10) post-stroke depression (PHQ-9 ≥10), 11) History of DVT or pulmonary embolism within 6 months; 12) Uncontrolled diabetes with recent weight loss, diabetic coma, or frequent insulin reactions; 13) Severe hypertension with systolic >200 mmHg and diastolic >110 mmHg at rest; 14) Previous or current enrollment in a clinical trial to enhance motor recovery; 15) Presence of non-MR compatible implants, pregnancy or severe claustrophobia.

b. Sources of Materials: All data will be acquired for purposes of research only and will be kept confidential. Data will be coded and not traceable to individuals in any publication. Files will be stored in locked offices or password-protected servers dedicated to Dr. Gregory and key study personnel only.

c. Potential Risks: The risks of the exercise tolerance tests are minimal but could include fainting, falling, irregular heartbeat, and very rarely heart attack, stroke, or death (less than 1 in 2500 cases). Professional staff (exercise physiologist, physical therapist, and cardiologists) will be present and available throughout and emergency treatment will be available if it becomes necessary.

There are no significant risks to the subjects in the proposed training methods. The risks to individuals participating in this study are no greater than the risks when providing conventional physical rehabilitation services to an individual after stroke. The same precautions and safety guidelines will be taken that are provided in patient care in rehabilitation settings. The rehabilitation program and the muscle and functional performance testing should not present a risk for the patient but could result in muscle soreness and/or joint stiffness but these symptoms should not persist more than a few days. There is a minimal risk for muscle strains during the testing and training. Under the conditions proposed for this project, MRI is not known to harm living systems. The electrical stimulation may be moderately uncomfortable at times, but is expected and typical for use of electrical stimulation and is not harmful. The discomfort is temporary and lasts only a few seconds. The locomotor assessments used in the proposed study are routine, clinical assessments of gait used in physical therapy clinics and rehabilitation

facilities. The GaitRite system has been used to assess walking performance with no adverse reactions or report of discomfort. The experimental protocol to be used in this portion of the proposal involves minimal risk, and is considered standard clinical practice. During all treadmill walking trials, a safety harness will be worn and a physical therapist will be present to provide assistance in the event of loss of balance. The harness will be designed to eliminate the consequences of falling as the device "catches" the subject should they trip or stumble. The presence of this device affords comfort and diminishes the "fear of falling" in subjects.

2. Adequacy of Protection Against Risks:

a. Recruitment and Informed Consent: A total of fifty-six subjects (male and female; n=28 post-stroke and n=28 controls), ages 50-70, will be screened and recruited for the proposed study. The pool of post-stroke candidates for the study will be recruited from the Ralph H. Johnson VAMC; the Medical University of South Carolina; and the Charleston, SC area communities. Before any tests are conducted, the protocol and tests to be used in this study and the potential risks and benefits of participation will be explained to each potential subject. All participants will review and sign an informed consent form approved by the local Institutional Review Board prior to initiating any portion of the study.

b. Protection Against Risk: A licensed physical therapist will be present during all treatment sessions. In addition, during all treadmill walking trials, a safety harness will be worn to provide assistance in the event of loss of balance. The research staff will closely monitor the subject to ensure their comfort. Any adverse events will be recorded and monitored as required by our Institutional Review Board. In the event of an adverse medical event, standard facility emergency procedures will be followed and proper personnel notified. The PI on this proposal and is a licensed physical therapist with several years of experience in the development and implementation of exercise interventions. Any adverse events will be recorded and monitored as required by the IRB. On-site medical services will be available in the event of adverse events to the subjects. Subjects will be able to terminate the training or testing sessions at their request at any time without prejudice. Minimization of risk will be accomplished by monitoring vital signs within prescribed criteria for termination of the training session. We will follow the American College of Sports Medicine criteria for terminating an inpatient exercise session which includes: subject complaints of light-headedness, confusion, or dyspnea; onset of angina; excessive blood pressure changes (systolic BP greater than 220 mmHg, diastolic BP greater than 110 mmHg); and inappropriate bradycardia (drop in heart rate >10 beats per minute).

Confidentiality: All records regarding participation in this study will be kept in locked file cabinets in the appropriate laboratories and/or offices, and stored on password-protected computers/servers in the offices and laboratories of the PI's research team. There will be no direct link to participant identifying information (other than subject code) without access to a password-protected computer containing the identifying information linking information to a given subject. Access to linked identifiers is limited to research personnel intimately involved with the human subjects. All data and records acquired from subjects is for research purposes only and will be kept confidential and maintained in a secure database identifiable only by subject code. The results of the study may be published for scientific purposes; however, subjects' identities will not be revealed and data will not be traceable to any individuals in any resultant publications. The information gathered during this study will be kept confidential to the extent permitted by law.

3. Potential Benefits of the proposed research to the subjects and others: Subjects with muscle dysfunction who participate in this study may see improvements in their own functional ability and strength, but any benefit cannot be guaranteed. Others may benefit from advancement of scientific knowledge. Given the minimal risks involved and the potential for improved functional capacity, the potential benefits of participation make the potential risks reasonable.

4. <u>Importance of the knowledge to be gained</u>: A better and more specific scientific understanding of muscular changes resulting post-stroke and following rehabilitation intervention may ultimately lead to a better understanding of muscle plasticity and the extent to which training restores muscle function and locomotor ability. *Further, the data obtained during the process evaluation regarding the facilitators and barriers to implementation will be critical to bridge the gap between efficacy and effectiveness and will provide rationale for future studies.* Given the minimal risks involved and the potential to add to the limited base of scientific knowledge describing this population, the potential risks are reasonable.

5. <u>Statement of Inclusion of Women and Minorities in Research</u>: This research will include women and minorities to the extent reflected by composition of the population in Charleston, SC and its surrounding areas. There are no exclusion or inclusion criteria which would exclude or preclude women or minorities from participating in this study.

F. REFERENCES/LITERATURE CITATIONS

1) Grillner S, Control of locomotion in bipeds, tetrapods and fish, In: Handbook of Physiology, J Brookhart, V Mountcastle and V Brooks, Ed. American Physiological Society: Bethesda, MD: 1179-1236, 1981.
2) Rossignol S, Neural control of stereotypic limb movements. In: Handbook of Physiology, Section 12: Exercise: regulation and Integration of multiple systems, L Rowell and J Shepherd, Ed. American Physiological Society: 173-216, 1996.
3) Brouwer B and Ashby P. Corticospinal projections to lower limb motoneurons in man. *Exp Brain Res*, 89: 649-654, 1992.
4) Grillner S. Possible analogies in the control of innate motor acts and the production of sound in speech, In: Speech Motor Control, S Grillner, Ed. Pergamon Press: New York: 217-229, 1982.
5) Yates, J.S., et al., Bicycle ergometry in subacute-stroke survivors: Feasibility, safety, and exercise performance. *Journal of Aging and Physical Activity*, 12(1): p. 64-74, 2004.
6) Bushnell, C.D., D.C. Johnston, and L.B. Goldstein, Retrospective assessment of initial stroke severity: comparison of the NIH Stroke Scale and the Canadian Neurological Scale. *Stroke*, 32(3): p. 656-60, 2001.
7) Studenski, S.A., et al., Predicting stroke recovery: three- and six-month rates of patient- centered functional outcomes based on the Orpington prognostic scale. J Am Geriatr Soc, 49(3): p. 308-12, 2001.
8) Folstein, M.F., S.E. Folstein, and P.R. McHugh, "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, 12(3): p. 189-98, 1975.
9) Folstein, M.F., L.N. Robins, and J.E. Helzer. The Mini-Mental State Examination. *Arch Gen Psychiatry*, 40(7): p. 812, 1983.
10) Duncan P, et al. Randomized clinical trial of therapeutic exercise in subacute stroke. *Stroke.* 34:2173-80, 2003.
11) Mahler DA, Froelicher VF and Miller NH. The American College of Sports Medicine's Guideline for Exercise Testing and Prescription, 5th Edition, Philadelphia, PA: Williams & Wilkins, 1995.
12) Dobkin BH, et al., Methods for a randomized trial of weight-spupported treadmill training versus conventional training for walking during inpatient rehabilitation after incomplete traumatic spinal cord injury. *Neurorehabil Neural Repair*, 17(3): P. 153-67, 2003.
13) AHCPR. Clinical practice guideline: post-stroke rehabilitation. Rockville, MD: AHCPR; 1995.
14) CM Gregory, M Bowden, A Jayaraman, P Shah, A Behrman, S Kautz & K Vandenborne. Resistance training and locomotor recovery after incomplete SCI: A Case Series. *Spinal Cord* 45(7): 522-30, 2007.
15) Rossier P, Wade DT. Validity and reliability comparison of 4 mobility measures in patients presenting with neurologic impairment. *Arch Phys Med Rehabil*; 82(1):9-13, 2001.
16) Bosco C and PV Komi. Influence of aging on the mechanical behavior of leg extensor muscles, *Eur J Appl Physiol Occup Physiol*, 45:209–219, 1980.
17) A Jayaraman, P Shah, CM Gregory, M Bowden, J Stevens, AL Behrman & K Vandenborne. Locomotor training and muscle function after incomplete spinal cord injury: case series. *Journal of Spinal Cord Medicine* 31(2): 185-93, 2008.
18) Moreau NG, Teefey SA, Damiano DL In vivo muscle architecture and size of the rectus femoris and

vastus lateralis in children and adolescents with cerebral palsy. Dev Med Child Neurol 2009; 51: 800-806.
19) Elliott MA, Walter GA, Gulish H, Sadi AS, Lawson DD, Jaffe W, Insko EK, Leigh JS and Vandenborne K. Volumetric measurement of human calf muscle from magnetic resonance imaging. *MAGMA*. 5:93-8, 1997.
20) Vandenborne K, Elliott MA, Walter GA, Abdus S, Okereke E, Shaffer M, Tahernia D and Esterhai JL. Longitudinal study of skeletal muscle adaptations during immobilization and rehabilitation. Muscle Nerve. 21:1006-12, 1998.
21) Cumming RG, Salkeld G, Thomas M, Szonyi G. Prospective study of the impact of fear of falling on activities of daily living, SF-36 scores, and nursing home admission. *J Gerontol A Biol Sci Med Sci*; 55(5):M299-305, 2000.
22) Tinetti ME, Richman D, Powell L. Falls efficacy as a measure of fear of falling. *J Gerontol*; 45(6):P239-43, 1990.
23) Solway S, Brooks D, Lacasse Y, Thomas S. A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory domain. *Chest*; 119(1):256-70, 2001.
24) Rossier P, Wade DT. Validity and reliability comparison of 4 mobility measures in patients presenting with neurologic impairment. *Arch Phys Med Rehabil*; 82(1):9-13, 2001.
25) Wade D. Measurement in neurological rehabilitation. New York; Oxford Univ Press; 1992.
26) Zajac FE, Neptune RR and Kautz SA. Biomechanics and muscle coordination of human walking. Part II: Lessons from dynamical simulations and clinical applications. *Gait and Posture*. 17:1-17, 2003.
27) Bowden MG, Balasubramanian CK, Neptune RR, Kautz SA. Anterior-posterior ground reaction forces as a measure of paretic leg contribution in hemiparetic walking. *Stroke*. 2006, 37(3):872-6, 2006.
28) Patterson SL, Forrester LW, Rodgers MM, Ryan AS, Ivey FM, Sorkin JD, Macko RF. Determinants of walking function after stroke: differences by deficit severity. *Arch Phys Med Rehabil*; 88(1):115-9, 2007.