

**POST-STROKE MUSCLE ATROPHY  
AND INTRAMUSCULAR FAT CONTENT  
IN HEMIPARETIC SUBJECTS**

by

John W. Ramsay

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Mechanical Engineering

Summer 2010

© 2010 John W. Ramsay  
All Rights Reserved

**POST-STROKE MUSCLE ATROPHY  
AND INTRAMUSCULAR FAT CONTENT  
IN HEMIPARETIC SUBJECTS**

by  
John W. Ramsay

Approved: \_\_\_\_\_  
Thomas S. Buchanan, Ph.D.  
Professor in charge of thesis on behalf of the Advisory Committee

Approved: \_\_\_\_\_  
Anette M. Karlsson, Ph.D.  
Chair of the Department of Mechanical Engineering

Approved: \_\_\_\_\_  
Michael J. Chajes, Ph.D.  
Dean of the College of Engineering

Approved: \_\_\_\_\_  
Debra Hess Norris, M.S.  
Vice Provost for Graduate and Professional Education

## ACKNOWLEDGMENTS

I would like to acknowledge my advisor Dr. Thomas S. Buchanan for his guidance and insight during the preparation of this thesis. I am also thankful to Dr. Jill S. Higginson for her continuing support and encouragement and for meeting with me regularly to help develop a strong understanding of this subject. I would like to thank Dr. Peter J. Barrance for his willingness to be on my advisory committee and for helping me portray my data in a meaningful manner. Without additional discussions with my collaborators Dr. Kurt Manal, Dr. Darcy S. Reisman, and Dr. Stuart A. Binder-Macleod, much of my understanding and interpretation of this work would not be complete.

I acknowledge the National Institute of Health for the financial support of this work.

I am also grateful to the imaging technicians at the Omega Imaging center located in Newark, Delaware for their help in scanning each subject. Also, this work would not be possible without the individuals who volunteered their time to be subjects in this study.

I would like to thank my family members, especially my fiancé Laura for supporting and encouraging me to work hard and pursue this degree with diligence. Their heartfelt prayers for my success have been appreciated.

Finally, I would like to give thanks and praise to God for giving me wisdom, strength, and perseverance throughout my entire academic career.

## TABLE OF CONTENTS

LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
ABSTRACT .....	viii
INTRODUCTION .....	1
1.1. Focus of the Thesis .....	4
1.1.1. Aim 1: Determine the difference in individual muscle volumes between paretic and non-paretic sides. ....	4
1.1.2. Aim 2: Determine the difference in muscle volume between post-stroke plantar flexors and dorsiflexors. ....	5
1.1.3. Aim 3: Determine the difference in muscle volume between post-stroke knee flexors and knee extensors. ....	6
1.2. Significance of this Research .....	6
1.3. Thesis Outline .....	7
BACKGROUND .....	9
2.1. Stroke .....	9
2.1.1. Post-stroke Strength Changes .....	10
2.1.2. Post-stroke Gait Changes .....	12
2.1.3. Post-stroke Muscle Fiber Atrophy .....	13
2.2. Imaging Methods .....	15
2.2.1. Dual-energy X-ray Absorptiometry .....	15
2.2.2. Computed Tomography .....	16
2.2.3. Magnetic Resonance Imaging .....	17
2.3. Summary .....	18
PARETIC MUSCLE ATROPHY AND INTRAMUSCULAR FAT CONTENT IN INDIVIDUAL MUSCLES OF THE POST-STROKE HEMIPARETIC LOWER EXTREMITY .....	19
3.1. Introduction .....	19
3.2. Methods .....	23
3.2.1. Subjects .....	23
3.2.2. Imaging .....	24
3.2.3. Muscle Volume Reconstruction .....	24
3.3. Results .....	30
3.4. Discussion .....	34
3.5. Conclusions .....	39
MUSCLE GROUP ATROPHY IN THE POST-STROKE LOWER EXTREMITY .....	41

4.1. Introduction .....	41
4.2. Methods .....	45
4.2.1. Subjects .....	45
4.2.2. Imaging .....	46
4.2.3. Muscle Volume Reconstruction .....	47
4.3. Results .....	51
4.4. Discussion.....	55
4.5. Conclusions .....	59
CONCLUSIONS .....	60
5.1. Major findings of this thesis .....	60
5.1.1. Individual muscle atrophy in the hemiparetic lower extremity.....	60
5.1.2. Muscle volume changes in hemiparetic ankle and knee flexors and extensors .....	61
5.2. Contribution of this thesis .....	62
5.3. Limitations.....	63
5.4. Future work .....	64
5.5. Summary.....	66
APPENDIX .....	67
6.1. Validation and Reliability Studies.....	67
6.1.1. Phantom Validation.....	67
6.1.2. Intraobserver reliability .....	68
6.1.3. Cross-Sectional Area Method Validation.....	68
6.1.4. Final Validation Remarks.....	69
6.2. Power Analysis Results .....	70
6.3. Informed Consent Form .....	71

## LIST OF TABLES

Table 3.1.	Subject demographics for 11 post-stroke hemiparetic individuals.....	23
Table 3.2.	Individual muscle volumes, percent differences between paretic and non-paretic sides, and p values for fifteen lower extremity muscles. ....	32
Table 3.3.	Intramuscular fat content for fifteen individual muscles, represented by percentages.....	34
Table 4.1.	Subject demographics and clinical assessment scores for 11 post-stroke hemiparetic individuals.....	46
Table 4.2.	Individual muscles group by their functional roles. ....	50
Table 4.3.	Overall paretic and non-paretic muscle group volumes and volume ratios for the dorsiflexors and plantar flexors.....	52
Table 4.4.	Overall paretic and non-paretic muscle group volumes and volume ratios for the knee flexors and knee extensors.....	53

## LIST OF FIGURES

Figure 3.1.	MR image of both paretic (L) and non-paretic (R) limbs with muscle boundaries located and labeled for three muscles of the shank region..	25
Figure 3.2.	(a) Muscle boundaries for consecutive MR images of one muscle over the entire length of the muscle belly and (b) the triangle-based surface mesh model generated by Nuages software.....	27
Figure 3.3.	MR image with muscle boundaries (top) and the same image (bottom) with intramuscular fat pixels removed .....	29
Figure 3.4.	Mean adjusted muscle volumes (cm <sup>3</sup> ) with standard error bars for fifteen individual muscles .....	31
Figure 3.5.	Intramuscular fat percentages with standard error bars for fifteen individual muscles.....	33
Figure 4.1.	Muscle group volumes, with standard error bars, for both the paretic and non-paretic sides of 11 post-stroke hemiparetic subjects..	51
Figure 4.2.	Intramuscular fat percentages, with standard error bars, for the paretic and non-paretic sides of four muscle groups.....	54

## **ABSTRACT**

Stroke is a leading cause of long term disability in adults, affecting approximately 795,000 adults a year in the United States alone. Following stroke, muscle weakness contralateral to the brain lesion, or hemiparesis, is the most common impairment. Post-stroke hemiparesis is a concern clinically because it restricts many daily living tasks including reaching and grasping, stair-climbing, and most importantly, walking.

Among many factors involved in post-stroke hemiparesis is muscle atrophy – a loss of muscle tissue resulting from immobilization, disuse, inactivation, or a combination thereof. Since muscle force is a function of muscle size, the amount of atrophy a post-stroke muscle undergoes is important in adequately describing any changes to its force-generating capability as a result of stroke. Few studies have measured muscle atrophy in post-stroke individuals, and none have attempted to quantify muscle atrophy for individual paretic and non-paretic muscle.

In this thesis, Magnetic Resonance Imaging (MRI) and digital reconstruction software were used to measure muscle volumes for individual muscles as well as specific muscle groups in the hemiparetic lower extremity. All muscle volumes were

adjusted to exclude non-contractile tissue content, and muscle atrophy was quantified by comparing the volumes between paretic and non-paretic sides.

The results of this study suggest that all individual paretic muscles atrophy in relation to the non-paretic side except the gracilis muscle. Besides the gracilis, an average decrease in muscle volume of 23% was observed for the paretic muscles. The gracilis was larger, with an increase of approximately 11%. The gracilis acts not only as a knee flexor, but also as a hip flexor and hip adductor and may increase in volume as ipsilateral hip flexors have been shown to compensate for plantar flexor weakness. The results also suggest that the gastrocnemius atrophies preferentially in the plantar flexor group. Results observed for the muscle groups suggest that the plantar flexor group atrophies preferentially over the dorsiflexors, and that paretic and non-paretic knee flexors and extensors atrophy approximately the same amount.

This thesis successfully quantified individual muscle and muscle group atrophy between paretic and non-paretic sides of post-stroke lower extremities. The findings can be used in future studies to develop stroke-specific musculoskeletal models that address additional changes to movement strategies following stroke.

## **Chapter 1**

### **INTRODUCTION**

Stroke is a leading cause of long term disability in adults, affecting approximately 795,000 adults a year in the United States alone (NINDS 2004, Heart disease and stroke statistics-2010 update, NSA 2007). A stroke occurs when a blood vessel in the brain becomes blocked (ischemic) or ruptures (hemorrhagic) (NSA 2007). As a result, oxygen and nutrients fail to reach brain cells which cause them to die. The brain lesion that develops is debilitating to the patient, and rehabilitation to restore function is a primary concern of clinicians (Olney and Richards 1996).

Muscle weakness contralateral to the brain lesion, or hemiparesis, is the most common impairment following stroke (Chan 1986, Andrews and Bohannon 2000). Post-stroke hemiparesis is a concern clinically because it restricts many daily living tasks. Impaired functional arm and hand movements have been observed (Wade, et al. 1983), and deficits during tasks such as reaching (Trombly 1992, van Vliet, Sheridan and Kerwin 1995, Roby-Brami, Fuchs, et al. 1997, Roby-Brami, Feydy, et al. 2003, Roby-Brami, Jacobs, et al. 2003, Michaelsen, et al. 2004) and grasping (Michaelsen, et al. 2004) have also been reported. Difficulties in performing lower extremity tasks such as making transfers (Bohannon 1988), stair climbing (Bohannon and Walsh 1991, Bohannon and Walsh 1992), and standing (Bohannon 1989a) have also been

studied. Walking, however, is the primary functional focus for lower extremity rehabilitation (Kim and Eng 2004). Hemiparetic gait deficiencies have been widely studied (Bohannon 1986, Olney, Griffin and McBride 1994, Olney and Richards 1996, Nadeau, et al. 1999, Hsu, Tang and Jan 2003, Chen, Patten, et al. 2005a, Chen, Patten, et al. 2005b) and characterized (Olney and Richards 1996).

Poor mobility can also affect the patient's quality of life following stroke. Assistive devices (e.g. canes) are used when a patient has unstable gait or weak muscles (Joyce and Kirby 1991). However, they reduce overall gait speed (Chen, et al. 2000) and may increase time needed to reach a destination by walking. Decreases in both muscle strength and instability during standing also increase risk of falling (Campbell, Borrie and Spears 1989), and safety around the house must be modified to protect the patient from further injury (ASA 2007).

The strength of a muscle is dependent upon a combination of neurological, mechanical, and structural factors (Patten, Lexell and Brown 2004). Neurologically, muscle weakness can result from a decrease in the ability to activate individual motor units, a loss of functioning motor units (McComas, Sica, et al. 1973), or a reduction in the firing rates for each motor unit (Rosenfalck and Andreassen 1980, Tang and Rymer 1981). Changes in muscle mechanical properties such as muscle stress and force-length or force-velocity relationships will also alter the force-generating capability of a muscle. Structurally, muscle weakness may be due to disuse atrophy (McComas 1994).

Depending on the level of use a muscle endures, skeletal muscles can adapt and change in size (Lieber 2010). Since muscle force is a function of muscle size (Lieber 2010), the amount of atrophy a post-stroke muscle undergoes is important in adequately describing any changes to its force-generating capability as a result of stroke. Many studies have described muscle atrophy in post-stroke hemiparetic limbs using imaging techniques such as dual-energy X-ray absorptiometry (DXA) (Jorgensen and Jacobsen 2001) and computed tomography (CT) (Sunnerhagen, et al. 1999, Ryan, et al. 2002, Metoki, et al. 2003). However, while these imaging techniques have effectively shown a decrease in overall muscle mass and thigh cross-sectional areas, they are limited to their inability to show changes that may occur in individual muscles. Recent studies have used magnetic resonance imaging methods to measure changes in muscle volume for healthy young adults (Holzbaur, et al. 2007) and ACL deficient subjects (Williams, Buchanan, et al. 2005, Williams, Snyder-Mackler, et al. 2005, Tate, et al. 2006). Ploutz-Snyder et al. (2006) used MRI to measure the decrease in cross-sectional area that occurs in the post-stroke upper extremity. However, to our knowledge, the change in muscle volume between paretic and non-paretic sides for individual lower-limb muscles and muscle groups has not been quantified.

Stroke rehabilitation targeting impairments due to muscle weakness is performed by a variety of methods including progressive resistive strength training (Patten, Lexell and Brown 2004, Bohannon 2007), and functional electrical stimulation (Peckham and Knutson 2005, Kesar, et al. 2009). However, it is unclear

whether post-stroke muscle weakness is primarily due to muscle atrophy or impaired activation. Quantifying muscle atrophy between paretic and non-paretic sides is a preliminary step in determining the role of each factor on muscle weakness.

## **1.1. Focus of the Thesis**

The purpose of this study was to quantify muscle atrophy by calculating muscle volume changes in chronic stroke survivors with lower-limb post-stroke hemiparesis. Using Magnetic Resonance Imaging (MRI) and digital reconstruction software, volumes for individual muscles as well as specific muscle groups were found for both the paretic and non-paretic sides of each subject. All muscle volumes were adjusted to exclude non-contractile tissue content, and the results were compared between paretic and non-paretic sides. To accomplish this goal, three specific aims were addressed:

### **1.1.1. Aim 1: Determine the difference in individual muscle volumes between paretic and non-paretic sides.**

Individual muscles were identified on each MR image and the muscle boundary was manually traced using a digitization tablet (Cintiq 18SX, Wacom Technology Corp., Vancouver, WA) and IMOD software (University of Colorado, Boulder, CO) (Kremer, Mastronarde and McIntosh 1996). To ensure that only contractile tissue was investigated, pixels below a threshold that did not represent active tissue were eliminated from the volume calculation. Proximal and distal

tendons were also excluded from the analyses in an attempt to describe only the volume of contractile tissue present. Muscle atrophy was quantified by comparing the difference between the paretic and non-paretic muscle volumes for each muscle. Our hypothesis was that the paretic muscle volumes would be smaller than the non-paretic muscle volumes, and that the differences would be significant.

**1.1.2. Aim 2: Determine the difference in muscle volume between post-stroke plantar flexors and dorsiflexors.**

For each stroke subject, individual muscles were grouped by their functional roles (e.g. plantar flexors) and the volumes from Section 1.1.1 were summed together to represent a cumulative group volume. For this Aim, we manually traced the dorsiflexors as a whole group rather than individual muscles because of the difficulty in distinguishing between each individual dorsiflexor muscle on the MRI scan. Group volumes were compared between sides (paretic vs. non-paretic) as well as within each limb (plantar flexors vs. dorsiflexors). Two separate ratios comparing dorsiflexors and plantar flexors for each limb was determined. Our hypothesis was that the paretic dorsiflexor/plantar flexor ratio would be higher than the non-paretic dorsiflexor/plantar flexor ratio, reflecting greater atrophy of paretic plantar flexors.

### **1.1.3. Aim 3: Determine the difference in muscle volume between post-stroke knee flexors and knee extensors.**

Similar to Aim 2, individual muscles were grouped into either knee flexors or knee extensors and a cumulative group volume was calculated per subject. Group volumes were also compared between sides (paretic vs. non-paretic) and as well as within each limb (knee flexors vs. knee extensors). A ratio of paretic knee flexors/knee extensors and a ratio for non-paretic knee flexors/knee extensors were each determined. Our hypothesis was that the paretic knee flexor/knee extensor ratio would be higher than the non-paretic knee flexor/knee extensor ratio, due to greater knee extensor atrophy on the paretic side.

## **1.2. Significance of this Research**

Musculoskeletal modeling allows researchers to estimate muscle forces during dynamic tasks (Lloyd and Besier 2003). These models typically use muscle force-generating properties from cadavers, which may not properly represent varying subject populations (Holzbaur, et al. 2007). Since muscle weakness is prevalent among stroke survivors, models of stroke-specific individuals and tasks must include updated force-generating properties. Muscle force is directly related to muscle volume (Lieber 2010), and therefore changes observed in hemiparetic stroke volumes should be accounted for in stroke-specific musculoskeletal models. Muscle volume changes in post-stroke populations have been observed using MRI (Ploutz-Snyder, et al. 2006) in the upper extremity, as well as by CT (Metoki, et al. 2003) in the lower extremity.

However, to our knowledge, there have been no studies that have tried to use MRI to quantify individual isolated muscle volumes over multiple joints in the lower extremity. The first study measured volumes of 15 lower extremity muscles and the reported values may be incorporated into stroke-specific models by updating the force-generating properties of each muscle.

A few studies have focused on the size changes between affected and unaffected lower extremities following stroke (Ryan, et al. 2002, Metoki, et al. 2003). Ryan et al. (2002) measured cross-sectional areas of the thigh region using DXA and concluded that mid-thigh area was 20% lower on the paretic side than the non-paretic side. Metoki et al. (2003) measured thigh muscle volumes using CT in subjects with hemiplegia and concluded that overall thigh muscle volume was approximately 20% lower on the affected side than the unaffected side. However, neither groups studied the muscle groups of the lower leg. Our second study measured ankle plantar flexors and dorsiflexors in addition to knee flexors and extensors. We compared each antagonist group using a volume ratio to provide insight into the changes observed within each limb.

### **1.3. Thesis Outline**

The next chapter explains what a stroke is, reviews related research and describes the background and motivation for this study. Chapter 3 presents muscle volumes for individual muscles and describes the amount of atrophy each muscle undergoes between limbs. Chapter 4 focuses on the changes in muscle volume at the

muscle group level, and compares the changes between paretic and non-paretic sides as well as within each limb. Chapter 5 summarizes the key findings of this study, and discusses possible future directions for the use of the muscle volume data.

## **Chapter 2**

### **BACKGROUND**

#### **2.1. Stroke**

Stroke is a leading cause of long term disability in adults and the third leading cause of death in the United States (NINDS 2004, NSA 2007). Approximately 795,000 adults per year are affected in the United States alone (NINDS 2004, Heart disease and stroke statistics-2010 update, NSA 2007). There are two types of stroke: ischemic and hemorrhagic. An ischemic stroke occurs when an artery in the brain is blocked, cutting off blood flow to the surrounding areas. A hemorrhagic stroke occurs when a weakened blood vessel ruptures, pouring blood into or outside of the brain (NSA 2007). Regardless of stroke type, oxygen and nutrients fail to reach brain cells (NSA 2007), damaging motor cells and pathways of the central nervous system (Olney and Richards 1996). The infarct that develops, typically in one hemisphere of the brain, leaves the victim with a variety of impairments including muscle paralysis or weakness (hemiplegia or hemiparesis), impaired speech (aphasia), and additional cognitive problems (NINDS 2009, Heart disease and stroke statistics-2010 update); the most common impairment following stroke is hemiparesis (Chan 1986, Andrews and Bohannon 2000). Goals for stroke rehabilitation often include independent walking and functional performance of daily tasks (Richards and Olney 1996).

### **2.1.1 Post-stroke Strength Changes**

The most common impairment following stroke is hemiparesis (Chan 1986, Andrews and Bohannon 2000). Many studies have tried to quantify muscle weakness by studying strength changes post-stroke (Bohannon 1986, Adams, Gandevia and Skuse 1990, Andrews and Bohannon 2000, Ryan, et al. 2002, Hsu, Tang and Jan 2003, Kim and Eng 2003, Marigold, et al. 2004, Gerrits, et al. 2009). For this thesis, particular focus has been on muscle groups of the lower extremity used in walking, since gait is the primary goal for rehabilitation (Bohannon, Andrews and Smith 1988, Olney and Richards 1996, Kim and Eng 2004).

Isometric strength tests allow researchers to study the maximum net joint torque at a fixed joint angle. Decreases in isometric strength have been observed on the paretic side of the body when compared to the non-paretic side (Adams, Gandevia and Skuse 1990, Andrews and Bohannon 2000, Horstman, et al. 2008, Gerrits, et al. 2009). Gerrits et al. (2009) noticed a significant decrease in the maximum isometric voluntary strength for paretic knee extensors, as well as a significant decrease in both paretic and non-paretic limbs in comparison to healthy controls. Adams et al. (1990) used myometers to measure isometric joint torque for the flexors and extensors of the hip, knee, ankle and hallux joints, and compared the results of paretic and non-paretic sides. The ankle plantar flexor strength was affected the most, knee extensors were affected more than knee flexors, and hip extensors were affected more than hip flexors.

Isokinetic studies are used to observe changes in muscle strength as the joint moves with a constant velocity. Paretic isokinetic torque values are also lower than non-paretic counterparts in the lower extremity (Sharp and Brouwer 1997, Hsu, Tang and Jan 2003, Kim and Eng 2003, Marigold, et al. 2004). Kim and Eng (2003) measured isokinetic lower-extremity torque at the hip, knee and ankle joints for both paretic and non-paretic limbs. They found that the paretic limb torques were lower than those of the non-paretic limb. The ankle joint also exhibited the greatest asymmetry between the affected and unaffected sides. Similarly, Hsu et al. (2003) reported a 24%-50% decrease in paretic isokinetic torque values compared to the non-paretic side, with the more proximal muscle group (hip flexors) being affected the least (24%), and the most distal muscle groups (ankle plantar flexors) being affected the most (50%).

There is a general consensus that for both isokinetic and isometric strength testing methods, the paretic side of the body displays more weakness than the non-paretic side. However, Andrews and Bohannon (2000) report conflicting findings regarding whether distal muscles are affected more than proximal muscles. They found that proximal muscle groups were not impaired less than distal muscle groups on the paretic side, attributing differences in methodologies and muscle groups included for the conflicting results.

### **2.1.2. Post-stroke Gait Changes**

Post-stroke gait is characterized by many spatio-temporal, kinematic, and kinetic deficits compared to normal walking patterns (Olney and Richards 1996). Initially, walking function cannot be performed in two out of three patients (Jorgensen, et al. 1995). Therefore, walking is often the primary goal for post-stroke rehabilitation (Bohannon, Andrews and Smith 1988, Olney and Richards 1996, Kim and Eng 2004). A few gait deficits that have been related to hemiparesis are short stance on the paretic side and longer stance on the non-paretic side (Brandstater, et al. 1983, Wall and Turnbull 1986, Olney, Griffin and McBride 1994, Olney and Richards 1996), a decrease in walking speed (Brandstater, et al. 1983, Burdett, et al. 1988, Olney, Griffin and McBride 1994, Turnbull, Charteris and Wall 1995, von Schroeder, et al. 1995, Witte and Carlsson 1997), and lack of center of mass support (Higginson, et al. 2006).

A person's walking performance after stroke has been closely related to paretic knee extensor strength (Hamrin, et al. 1982, Nakamura, Hosokawa and Tsuji 1985, Nakamura, Watanabe, et al. 1988, Bohannon 1989a, Bohannon 1989b, Bohannon and Andrews 1990, Bohannon and Walsh 1992). Knee extensors function to decelerate the leg at the end of swing phase and prevent the leg from collapsing during stance as body weight is transferred over the leg. Paretic step length is shortened and walking rate is lower because the knee extensors on the paretic side cannot produce the angular velocities needed for rapid knee extension during deceleration of the leg (Nakamura, Hosokawa and Tsuji 1985).

Slow gait speed in stroke patients is also related to the lack of joint moment and power produced at the ankle by the plantar flexors (Olney, Griffin and Monga, et al. 1991, Olney, Griffin and McBride 1994, Nadeau, et al. 1999). In normal gait, plantar flexors generate a large portion of the energy needed to propel the legs forward during push-off (Winter 1983) and are important in gait speed regulation (Nadeau, et al. 1999). Hemiparetic push-off force is reduced on the paretic side as observed by a decrease in vertical ground reaction forces (Claeys 1983, Hesse, Jahnke and Schreiner, et al. 1993, Hesse, Jahnke and Bertelt, et al. 1994). Without substantial push-off force and plantar flexor power, increases in walking speeds may not be obtained (Olney and Richards 1996). Jonkers et al. (2009) found that higher functioning hemiparetic subjects were able to increase walking speed by increasing plantar flexor and hip flexor power; lower functioning hemiparetic individuals failed to adopt this mechanism and were limited in their ability to increase walking speed.

### **2.1.3. Post-stroke Muscle Fiber Atrophy**

Disuse muscle atrophy is common among post-stroke hemiparetic survivors (Adams, Gandevia and Skuse 1990, McComas 1994). Disabilities following stroke typically lead to physical inactivity, which eventually results in reduced muscle mass (Hafer-Macko, et al. 2008). Approximately 2 months after stroke, the number of functional motor units has been observed to decrease by 50% in distal muscles of the leg (McComas, Sica, et al. 1973). Jorgensen and Jacobsen (2001) noticed a decrease in lean muscle mass as early as 2 months following stroke.

Histochemical changes in muscle have been investigated to determine the extent of muscle atrophy of specific muscle fibers following stroke (Fenichel, Daroff and Glaser 1964, Scelsi, et al. 1984, Slager, Hsu and Jordan 1985, Dattola, et al. 1993, Hachisuka, Umezu and Ogata 1997). Muscle biopsies of the tibialis anterior (Scelsi, et al. 1984, Slager, Hsu and Jordan 1985), gastrocnemius (Dattola, et al. 1993), vastus lateralis (Hachisuka, Umezu and Ogata 1997), rectus femoris (Slager, Hsu and Jordan 1985), and gluteus maximus (Slager, Hsu and Jordan 1985) have all been performed. Although both Type I and Type II fibers undergo changes in various muscle types (Slager, Hsu and Jordan 1985), studies consistently show selective Type II fiber atrophy with hypertrophy of Type I muscle fibers (Slager, Hsu and Jordan 1985, Dattola, et al. 1993). Henneman et al. (1965) termed the phrase “size principle” to describe the orderly recruitment of muscle fibers based on the size of the motor unit; smaller units are recruited first and larger units recruited last. In weak muscles mainly Type I muscle fibers are recruited (Dalla Toffola, et al. 2001), which explains the hypertrophy of these muscle fibers. Similarly, atrophy of Type II muscle fibers may be explained by the inability to achieve high levels of activation necessary to excite these large motor units (Dalla Toffola, et al. 2001, Gracies 2005). Exercising post-stroke muscles may not prevent Type II fiber atrophy, but strength training can increase the number of Type I muscle fibers (Dalla Toffola, et al. 2001). However the development of Type II fibers, particularly Type IIa, has been observed with eccentric isokinetic training (Hortobagyi, et al. 1996).

## **2.2. Imaging Methods**

Imaging *in vivo* soft tissue composition in the human body has been performed using a variety of methods including dual-energy X-ray absorptiometry (Fuller, et al. 1999, Jorgensen and Jacobsen 2001, Pang and Eng 2005), computed tomography (Termote, et al. 1980, Borkan, et al. 1983, Ryan, et al. 2002, Metoki, et al. 2003), and magnetic resonance imaging (Williams, Buchanan, et al. 2005, Williams, Snyder-Mackler, et al. 2005, Tate, et al. 2006, Lampe, et al. 2006, Holzbaur, et al. 2007). These imaging techniques allow researchers to not only measure normal weight (mass) and size (area or volume) values of body tissue, but also pathological changes that may occur. *In vivo* imaging methods are non-invasive, and live subject data is more representative of specific subject populations rather than relying on cadaveric data (Holzbaur, et al. 2007).

### **2.2.1. Dual-energy X-ray Absorptiometry**

Dual-energy X-ray Absorptiometry (DXA) uses dual-energy radiation to measure body mass and bone mineral content and density (Mazess, et al. 1990). Skeletal muscle mass can be interpreted from lean muscle mass in DXA (Heymsfield, et al. 1990, Wang, et al. 1999). Few studies have used DXA to measure skeletal muscle mass changes following stroke (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001, Ryan, et al. 2002). Jorgensen and Jacobsen (2001) studied two groups of stroke patients over a one year period: those that regained walking ability within two months of stroke and those that did not regain the ability to

walk. In the paretic limb, lean muscle mass decreased an average of 3% and fat mass increased 9% overall. Walking ability after 2 months played an important role in muscle mass growth. Those with no walking ability at two months had a 5-6% decrease in paretic lean muscle mass, which was sustained over the one year time period. Ryan and colleagues (2002) compared paretic and non-paretic arm and leg lean muscle mass and fat mass in stroke patients. Lean muscle mass was significantly lower on the paretic side for all regions, however fat content was the same between sides (Ryan, et al. 2002) using DXA. Comparable results were found by Iversen et al. (1989) that lean content was significantly smaller on the paretic side, although fat content was reported to be larger on the paretic side. While these studies have effectively used DXA to show that paretic skeletal muscle mass is less than non-paretic mass following stroke, DXA imaging is not without its disadvantages and limitations. DXA exposes patients to radiation, body hydration levels can negatively affect the accuracy of the results (Pietrobelli, et al. 1998), and the resolution limits the technique to provide estimates of total limb mass rather than single muscle mass.

### **2.2.2. Computed Tomography**

Computed tomography (CT) is another method for imaging muscle and fat content (Borkan, et al. 1983, Mitsiopoulos, et al. 1998, Ryan, et al. 2002, Metoki, et al. 2003, Jolivet, et al. 2009). CT uses radiation to construct cross-sectional images over a scanned region of the body (Borkan, et al. 1983), and can also be used to measure muscle volume (Metoki, et al. 2003, Jolivet, et al. 2009). Studies have used CT to

measure changes in muscle cross-sectional, fat content, and muscle volume in stroke subjects (Ryan, et al. 2002, Metoki, et al. 2003). Ryan et al. (2002) reported that overall paretic mid-thigh muscle area was 20% lower than non-paretic area, and subcutaneous fat content was higher on the paretic side. This finding confirmed that muscle atrophy does occur and intramuscular fat content increases post-stroke. Muscle volume can also be measured using CT (Mitsiopoulos, et al. 1998), although the application of the technology to stroke is rare. Metoki et al. (2003) measured thigh muscle volume in stroke patients using CT and found a decrease in muscle volume on the affected side. Muscle volume ratios comparing the paretic to non-paretic sides ranged from 0.75 to 0.83. Although useful in measuring overall cross-sectional areas, exposure to radiation is higher in CT than DXA (Wang, et al. 1999), and may not be favorable to the subject if scanning periods occur for long periods of time (Eng, et al. 2007).

### **2.2.3. Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) is advantageous in measuring skeletal muscle variations because no radiation is required and no negative effects to the patient have been reported (Murphy, Totty and Carroli 1986). For this reason, many studies have used MRI to calculate skeletal muscle volumes in a variety of subject populations and body regions (Murphy, Totty and Carroli 1986, Williams, Buchanan, et al. 2005, Williams, Snyder-Mackler, et al. 2005, Lampe, et al. 2006, Tate, et al. 2006, Holzbaur, et al. 2007, Stackhouse, et al. 2007, Petterson, et al. 2008). MRI also

provides high contrast between different soft tissues (e.g. muscle, fat, connective tissue), which allows muscle borders to be easily distinguished (Eng, et al. 2007). The large field of view allows entire muscles to be viewed (Eng, et al. 2007).

While imaging techniques have been used to study changes in skeletal muscle, there seems to be a lack of information regarding post-stroke changes. To our knowledge, no study has measured post-stroke plantar flexor or dorsiflexor muscle group volumes nor have individual muscle changes been reported for muscles of the entire leg. MRI seems to be the most beneficial method for determining individual skeletal muscle volumes, although it has yet to be used to study post-stroke muscle atrophy.

### **2.3. Summary**

In summary, muscle weakness following stroke limits the functionality of the patient, particularly in walking. Since muscle strength is proportional to muscle size, the amount of atrophy observed post-stroke is an important factor when determining the overall force generating capability of specific muscles. While many studies have confirmed post-stroke muscle atrophy both histochemically and morphologically, there is limited data on the amount of individual muscle atrophy that develops in post-stroke populations as well as any changes within each limb. The motivation of this study was to use MRI to measure individual muscle volumes in post-stroke hemiparetic patients and quantify muscle atrophy and fat content.

## **Chapter 3**

# **PARETIC MUSCLE ATROPHY AND INTRAMUSCULAR FAT CONTENT IN INDIVIDUAL MUSCLES OF THE POST-STROKE HEMIPARETIC LOWER EXTREMITY**

### **3.1. Introduction**

Stroke is the third leading cause of death in the United States and a leading cause of long-term disability (NSA 2009). In the United States, approximately 795,000 people are affected by stroke each year (NINDS 2004, NSA 2009, Heart disease and stroke statistics-2010 update). Following stroke, motor cells and pathways of the central nervous system are damaged (Olney and Richards 1996) leaving the person with a variety of disabilities including impaired speech and cognitive difficulties (NINDS 2009). Most commonly observed is hemiparesis, or muscle weakness contralateral to the brain lesion (Chan 1986, Andrews and Bohannon 2000).

Immediately following stroke, walking is limited in two out of three patients (Jorgensen, et al. 1995). When ambulation ability is regained, post-stroke gait is typically characterized by a number of spatio-temporal, kinematic, and kinetic deficits compared to normal gait (Olney and Richards 1996) such as asymmetric stance periods (Brandstater, et al. 1983, Wall and Turnbull 1986, Olney, Griffin and McBride 1994, Olney and Richards 1996), decreased walking speeds (Brandstater, et al. 1983,

Burdett, et al. 1988, Olney, Griffin and McBride 1994, Turnbull, Charteris and Wall 1995, von Schroeder, et al. 1995, Witte and Carlsson 1997), and lack of center of mass support (Higginson, et al. 2006). A person's walking performance after stroke is related to paretic knee extensor strength (Hamrin, et al. 1982, Nakamura, Hosokawa and Tsuji 1985, Nakamura, Watanabe, et al. 1988, Bohannon 1989a, Bohannon 1989b, Bohannon and Andrews 1990, Bohannon and Walsh 1992) and slow gait speed is associated with reduced joint moment and power produced at the ankle by the plantar flexors (Olney, Griffin and Monga, et al. 1991, Olney, Griffin and McBride 1994, Nadeau, et al. 1999). These predictors of post-stroke gait are directly related to hemiparesis; therefore, determining the underlying factors of muscle weakness may help increase our understanding of post-stroke gait.

Post-stroke hemiparesis is dependent upon a combination of neurological, mechanical, and structural factors (Patten, Lexell and Brown 2004). A decrease in the ability to activate individual motor units, a loss of functioning motor units (McComas, Sica, et al. 1973), and a reduction in the firing rates for each motor unit (Rosenfalck and Andreassen 1980, Tang and Rymer 1981) can contribute to post-stroke muscle weakness. Changes in muscle properties like muscle stress, force-length or force-velocity relationships, and fiber type may also contribute to post-stroke strength deficits. Additionally, post-stroke hemiparesis may be exacerbated by disuse atrophy (McComas 1994).

Skeletal muscles can adapt and change in size depending on the level of use (Lieber 2010). The size (e.g. physiological cross-sectional area) of a muscle is

generally a good indicator of its force generating capability because muscle force is a function of muscle cross-sectional area (Lieber 2010). Therefore, the amount of post-stroke atrophy a muscle or group of muscles undergoes is important in properly describing any changes to the overall ability to generate force. Moreover, intramuscular fat content has also been shown to increase with age and some diseases (Mitsiopoulos, et al. 1998), changing the mechanical properties of the muscle and altering its force generating capacity.

Studies using imaging techniques such as dual-energy X-ray absorptiometry (DXA) (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001) and computed tomography (CT) (Sunnerhagen, et al. 1999, Ryan, et al. 2002, Metoki, et al. 2003) have confirmed muscle atrophy in post-stroke limbs. Jorgensen and Jacobsen (2001) used DXA and reported that lean muscle mass decreased an average of 3% in the paretic leg, with a 5-6% decrease in those who did not regain the ability to walk. Ryan et al. (2002) reported that overall paretic mid-thigh muscle cross-sectional area was 20% lower than non-paretic areas using CT. Metoki et al. (2003) also used CT to measure thigh volume and found a decrease in overall volume on the affected side. Intramuscular fat content also increased on the paretic side (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001, Ryan, et al. 2002). While these studies describe muscle atrophy that occurs post-stroke, they are limited to describing atrophy in entire limbs or muscle groups such as the thigh. The extent of muscle atrophy observed in each individual muscle has not been reported. In

addition, both of these imaging methods expose the patient to radiation and may not be favorable to the subject (Eng, et al. 2007).

Magnetic Resonance Imaging (MRI) is advantageous in measuring skeletal muscle variations because no radiation is required and no negative effects to the patient have been reported (Murphy, Totty and Carroli 1986). For these reasons, MRI may be more useful in calculating skeletal muscle volumes than the previously described techniques. Recent studies have used MR imaging methods to measure changes in muscle volume in people with ACL deficiencies (Williams, Buchanan, et al. 2005, Williams, Snyder-Mackler, et al. 2005, Tate, et al. 2006), young healthy adults (Holzbaur, et al. 2007), and youths with cerebral palsy (Lampe, et al. 2006). Lampe et al. (2006) found that the paretic side of young adults with cerebral palsy had decreased muscle volume. Similarly, Ploutz-Snyder et al. (2006) used MRI to measure the decrease in cross-sectional area changes that occur in the post-stroke upper extremity. These studies were also able to isolate individual muscles and calculate individual muscle volumes, rather than entire muscle groups alone.

To the authors' knowledge, the amount of atrophy an individual muscle undergoes post-stroke has not been quantified for the lower extremity. Therefore, the purpose of this study was to calculate muscle volumes for paretic and non-paretic limbs and compare the results to describe muscle atrophy. We hypothesized that paretic muscle volumes would be significantly smaller than non-paretic muscle volumes, and that intramuscular fat would be higher in paretic muscles.

## 3.2. Methods

### 3.2.1. Subjects

Eleven subjects with post-stroke hemiparesis (mean age  $61.9 \pm 8.0$  years,  $46 \pm 38$  months since stroke) participated in the study. A summary of subject demographics can be found in Table 3.1. Inclusion criteria were (1) chronic stroke occurring at least six months previously; (2) single lesion; (3) age 30-80 years; and (4) ambulatory but with noticeable gait deficits. Subjects were excluded if they had (1) multiple strokes affecting both sides of the body; (2) heart disease or hypertension; (3) dementia; (4) severe aphasia; (5) orthopedic or pain conditions; (6) cancer; (7) any metal implants; or were (8) claustrophobic. The study was approved by the University of Delaware Review Board. Prior to participation, all patients provided their written informed consent (Appendix 6.3).

**Table 3.1. Subject demographics for 11 post-stroke hemiparetic individuals.**

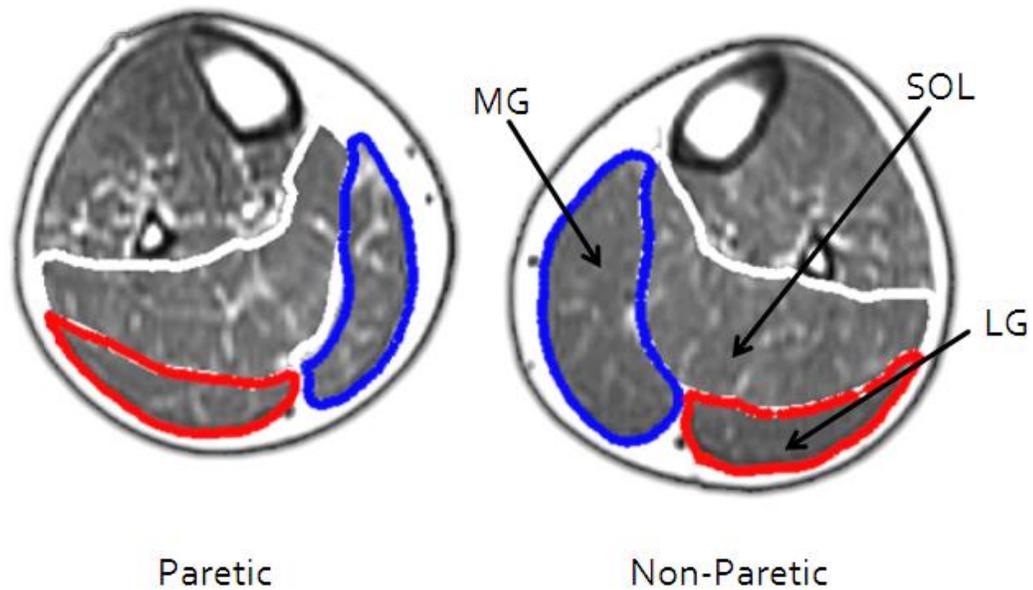
Subject Demographics		Mean	SD	Range
Age (years)		61.9	8.0	47-73
Time since onset of stroke (months)		46	38	9-120
Gender (male/female)	8/3			
Side of hemiparesis (right/left)	6/5			

### 3.2.2. Imaging

Patients lay supine in a 1.5T Signa LX scanner (GE Medical, Milwaukee, WI) with their feet taped at the toes to limit any movement during the scan period and maintain neutral hip rotation. Axial spin-echo T1-weighted MR images were acquired of both legs simultaneously from the ankle mortise to the iliac crest using the scanner's body coil. Images were taken in five overlapping sequences: ankle, lower leg, knee, thigh, and pelvis. A repetition time (TR) of 450 ms, echo time (TE) of 10 ms, slice thickness of 10 mm and space between slices of 11.5 mm was used for all scanned region except for the knee region (slice thickness of 5 mm and space between slices of 6 mm). A matrix size of 256 x 256 and field of view of 400 mm were used for all scans.

### 3.2.3. Muscle Volume Reconstruction

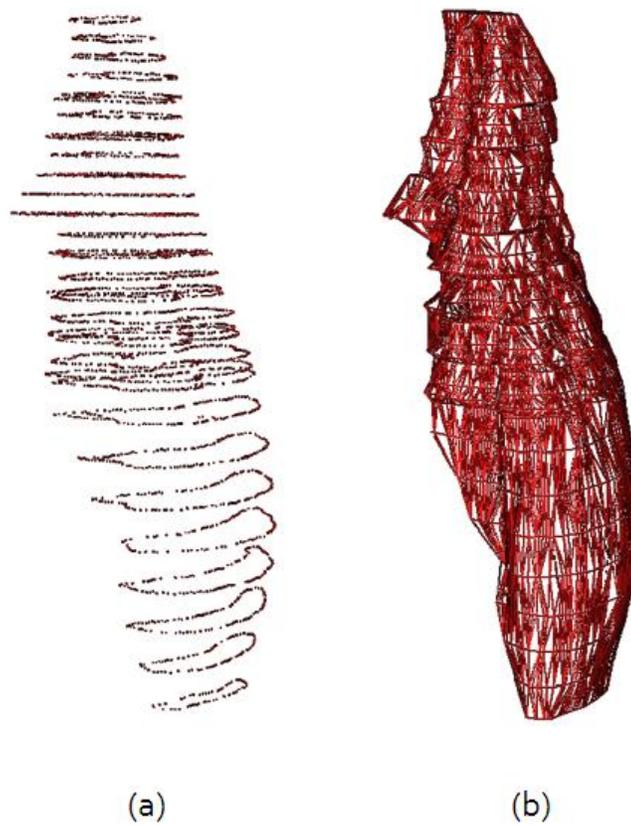
Each muscle was digitally reconstructed by tracing the muscle boundary (Figure 3.1) over the entire length of the muscle belly. Fifteen muscles were traced for each leg: soleus (SOL), medial gastrocnemius (MG), lateral gastrocnemius (LG), tibialis anterior (TA), biceps femoris-short head (BFS), biceps femoris-long head (BFL), semimembranosus (SM), semitendinosus (ST), gracilis (GRA), sartorius (SAR), rectus femoris (RF), vastus medialis (VM), vastus intermedius (VI), vastus lateralis (VL), and tensor fasciae latae (TFL).



**Figure 3.1. MR image of both paretic (L) and non-paretic (R) limbs with muscle boundaries located and labeled for three muscles of the shank region. For both limbs, the medial gastrocnemius (MG) is in blue, lateral gastrocnemius (LG) is in red, and soleus (SOL) is in white.**

Prior to measuring muscle volumes, a validation study comparing a control volume to the measured reconstructed volume was performed; all measured volumes were within 1% of the control volume (See Appendix Section 6.1.1). A single observer manually traced the images of all subjects using a digitization tablet (Cintiq 18SX, Wacom Technology Corp., Vancouver, WA) and IMOD software (University of Colorado,

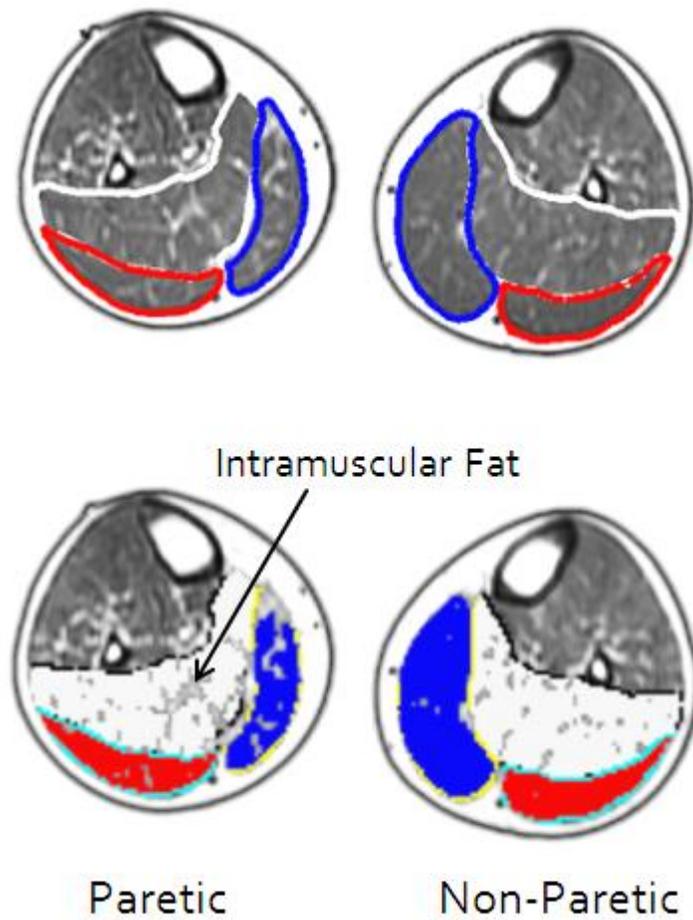
Boulder, CO) (Kremer, Mastronarde and McIntosh 1996). All digitization was performed by a single rater, and intraobserver reliability was established in a test-retest reliability study for three muscles (SOL, MG, SAR) of one subject for both the paretic and non-paretic sides (paretic correlation coefficient = 0.999; non-paretic correlation coefficient = 0.994). A minimum of one month between tracings elapsed to reduce any memory bias from the observer. Subject-specific triangle-based surface mesh models (Figure 3.2) for each muscle were built using Nuages software (Nuages, INRIA, Sophia-Antipolis, France) (Geiger 1993). Unadjusted muscle volumes were calculated from the surface mesh models using subroutines from the Visualization Toolkit (Kitware Inc., Clifton Park, NY) (Schroeder, Martin and Lorensen 1997).



**Figure 3.2.** (a) Muscle boundaries for consecutive MR images of one muscle over the entire length of the muscle belly and (b) the triangle-based surface mesh model generated by Nuages software.

To more precisely describe the actual contractile tissue, proximal and distal tendons were excluded and intramuscular fat content was eliminated. To eliminate intramuscular fat from the net muscle volume calculation, a pixel threshold (out of

600) was determined for each subject by visually inspecting each MRI scan for fatty regions, which are white areas on the image. Other studies have used similar grey-level (pixel-intensity) methods to distinguish between lean skeletal muscle and adipose tissue (Ross, et al. 1996, Kent-Braun, Ng and Young 2000, Goodpaster, et al. 2004). Cross sectional areas were calculated using a trapezoidal integration algorithm, then adjusted for fat content by removing the pixels below the specified threshold that represented fat (Figure 3.3). The adjusted cross sectional areas were then summed over the length of each muscle, and multiplied by the slice thickness to obtain adjusted muscle volumes. Overlapping images from adjacent scan regions (e.g. pelvis region images overlap thigh scan region) and muscles that crossed the knee (e.g. MG and LG) which had different slice thicknesses were accounted for in the adjusted volume calculation. Intramuscular fat content for each individual muscle was determined by comparing the net muscle volume with the adjusted muscle volume and calculating a percent difference between the two values.

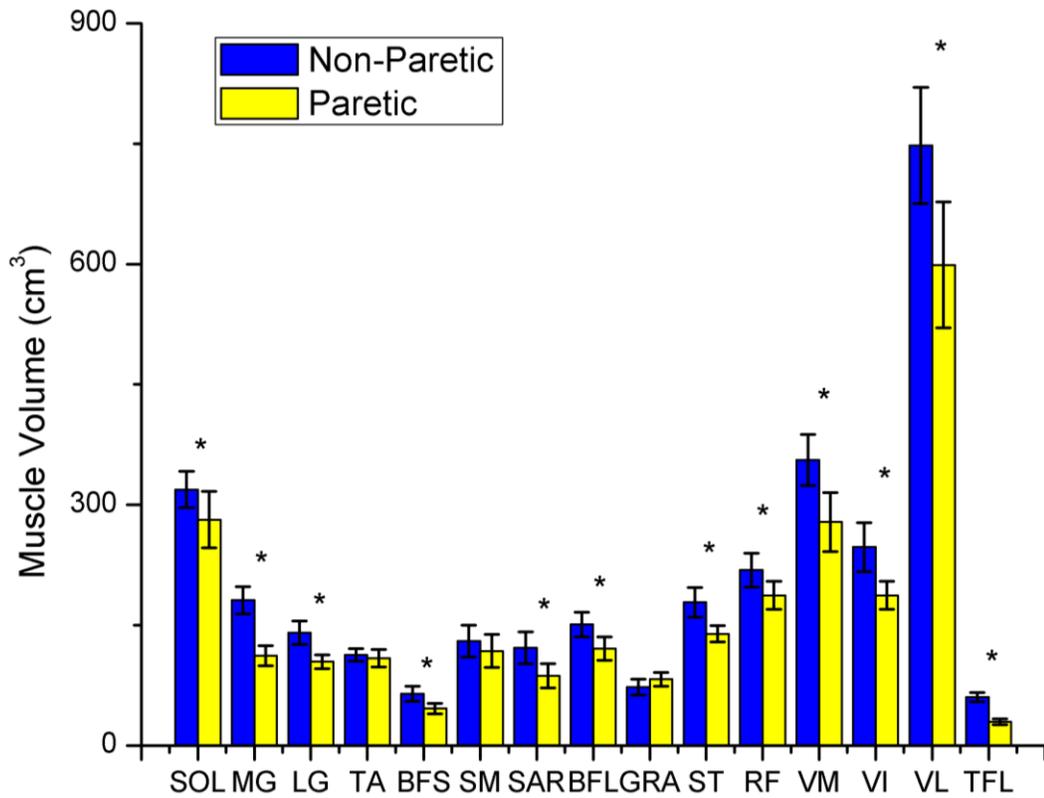


**Figure 3.3. MR image with muscle boundaries (top) and the same image (bottom) with intramuscular fat pixels removed. Pixels representing muscle tissue retain their respective colors and are included in the adjusted volume calculation. For both limbs, the medial gastrocnemius (MG) is in blue, lateral gastrocnemius (LG) is in red, and soleus (SOL) is in white.**

All reported muscle volumes were adjusted for fat content. To quantify atrophy for each muscle, a percent difference was determined between the paretic and non-paretic sides. The sign of the percent difference corresponds to the smaller limb – negative values represent volume reductions on the paretic side, and positive values represent reductions on the non-paretic side. An average percent difference was calculated for all muscles that had lower volumes on the paretic side. One-tailed paired t-tests ( $\alpha=0.05$ ) were used to test for significant differences between paretic and non-paretic muscle volumes for each individual muscle.

### **3.3. Results**

All muscle volumes were lower on the paretic side when compared to the non-paretic side, except the GRA (Figure 3.4). Significant differences between the paretic and non-paretic sides were observed for all muscles except the TA ( $p=0.2647$ ), SM ( $p=0.0974$ ), and GRA ( $p=0.1074$ ) (Table 3.2). The TA was affected the least with a percent difference of 8%, and the largest percent difference occurred in the TFL, with a 57% difference. The mean change in individual muscle volume for the fourteen muscles with smaller paretic volumes was approximately 23%. In the shank region the average decrease in individual muscle volume was 20%. Likewise in the thigh region, the average decrease was 24%. The GRA muscle was the only muscle that showed an increase in muscle volume on the paretic side, with an increase of 11% from the non-paretic side.

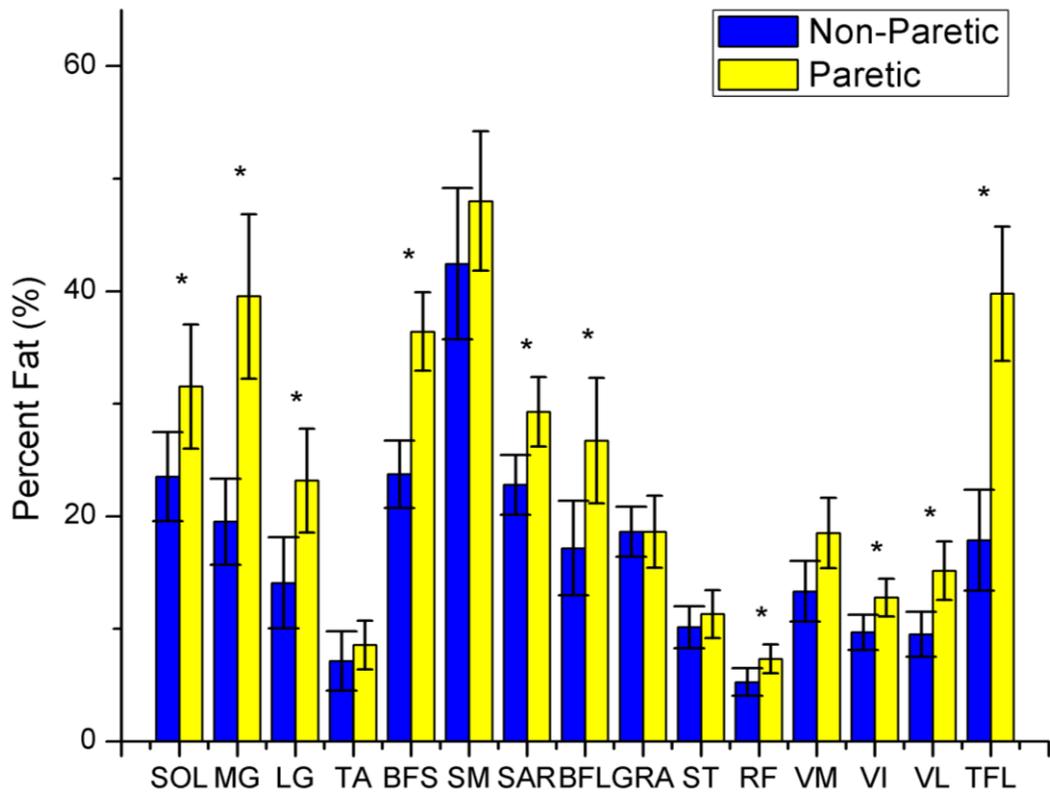


**Figure 3.4.** Mean adjusted muscle volumes (cm<sup>3</sup>) with standard error bars for fifteen individual muscles. Volumes from the non-paretic side are in blue and volumes from the paretic side are in yellow. Significant differences between paretic and non-paretic volumes \*p<0.05 are noted

**Table 3.2. Individual muscle volumes, percent differences between paretic and non-paretic sides, and p values for fifteen lower extremity muscles.**

**Significance was determined for p=0.05.**

<b>Individual Muscle Volumes</b>				
	Paretic (cm <sup>3</sup> )	Non-Paretic (cm <sup>3</sup> )	% Difference	p value
<b>SOL</b>	281.42	319.00	-12	0.0490*
<b>MG</b>	111.82	181.04	-38	0.0050*
<b>LG</b>	104.30	140.46	-26	0.0007*
<b>TA</b>	108.60	112.79	-4	0.2647
<b>BFS</b>	45.99	64.12	-28	0.0036*
<b>SM</b>	117.61	130.10	-10	0.0974
<b>SAR</b>	86.83	122.00	-29	0.0011*
<b>BFL</b>	120.68	150.89	-20	0.0018*
<b>GRA</b>	82.38	72.93	+11	0.1074
<b>ST</b>	139.19	178.41	-22	0.0059*
<b>RF</b>	187.21	218.69	-14	0.0010*
<b>VM</b>	278.39	355.82	-22	0.0026*
<b>VI</b>	187.08	247.23	-24	0.0037*
<b>VL</b>	598.94	748.13	-20	0.0001*
<b>TFL</b>	29.46	60.07	-51	<0.0001*



**Figure 3.5. Intramuscular fat percentages with standard error bars for fifteen individual muscles. Fat percentages from the non-paretic side are in blue and fat percentages from the paretic side are in yellow. Significant differences between paretic and non-paretic fat percentages \* $p < 0.05$  are noted.**

Fat content was higher in all paretic muscles except the gracilis (Figure 3.5). The TFL had the highest percentage of fat (55%) and the ST had the lowest (10%) (Table 3.3). The GRA showed zero difference between paretic and non-paretic fat percentages.

**Table 3.3. Intramuscular fat content for fifteen individual muscles, represented by percentages. The difference between paretic and non-paretic fat content is represented by a percent difference.**

<b>Percent Intramuscular Fat</b>			
	<b>Paretic (%)</b>	<b>Non-Paretic (%)</b>	<b>% Difference</b>
<b>SOL</b>	31.54	23.53	25
<b>MG</b>	39.55	19.52	51
<b>LG</b>	23.18	14.09	39
<b>TA</b>	8.56	7.15	16
<b>BFS</b>	36.42	23.75	35
<b>SM</b>	48.01	42.44	12
<b>SAR</b>	29.29	22.81	22
<b>BFL</b>	26.72	17.18	36
<b>GRA</b>	18.62	18.62	0
<b>ST</b>	11.32	10.15	10
<b>RF</b>	7.33	5.29	28
<b>VM</b>	18.52	13.34	28
<b>VI</b>	12.79	9.69	24
<b>VL</b>	15.16	9.53	37
<b>TFL</b>	39.78	17.88	55

### **3.4. Discussion**

In this study we have presented post-stroke muscle volumes for fifteen muscles from both paretic and non-paretic legs. MR imaging and digital reconstruction techniques allowed us to isolate each individual muscle, adjust for intramuscular fat development, and quantify muscle atrophy by comparing volumes between limbs. Besides the gracilis, all muscles were smaller on the paretic side with an average decrease in muscle volume of 23%. In the shank region a 20% decrease in paretic

muscle volume was observed on average, and in the thigh region an average decrease of 24% was observed. The gracilis was actually larger on the paretic side, with an increase of approximately 11%.

These results are similar to other studies that measured muscle atrophy using imaging techniques (Ryan, et al. 2002, Metoki, et al. 2003, Lampe, et al. 2006) and confirm that muscle atrophy does occur in individual muscles post-stroke. Post stroke cross-sectional areas have been reported to decrease an average of 20% in the paretic thigh (Ryan, et al. 2002), and depending on age, thigh muscle volumes ranged between 17-25% when paretic thighs were compared to non-paretic (Metoki, et al. 2003). Our paretic thigh volumes were 24% smaller than the non-paretic thigh. Metoki et al. (2003) measured thigh volume between a predetermined region of the thigh, and likely excluded portions of individual muscles in their calculation. We calculated muscle volumes over the entire length of the muscle belly, which may explain why our differences were slightly higher. Neither of the previous studies measured shank volume changes in post-stroke individuals, so we were unable to compare our results directly to other post-stroke data. However, Lampe et al. (2006) measured individual muscle volume changes in young hemiparetic patients with cerebral palsy and reported a 28% decrease in paretic shank volumes and a 16% decrease in thigh muscle volumes. Our shank volume was similar in comparison, and although our thigh values were different, both values were within the range reported by Metoki et al. (2003).

Intramuscular fat content increases with age, obesity, and various diseases (Mitsiopoulos, et al. 1998). Although Ramnemark et al. (1999) did not find any significant changes between paretic and non-paretic legs following stroke, some studies have (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001). Post-stroke intramuscular fat mass has been reported to increase in the paretic arm and leg by approximately 15% and 8%, respectively (Iversen, Hassager and Christiansen 1989). Jorgensen and Jacobsen (2001) found that fat content increased significantly in stroke patients at a one-year follow up, but only on the paretic side and among the most impaired subjects. Our results show that all individual paretic muscles besides the gracilis have higher fat content than non-paretic muscles. While we report intramuscular fat content and changes to muscle volumes as a result, our method for determining fat content from MRI has not been validated. Although pixel thresholds set for each subject may not be exact measures of fat content, the same value is used for both limbs and any error would be similar for both sides. By eliminating fat content from our volume calculations, we feel that muscle atrophy and the effect muscle volume changes may have on a muscle's force generating capability is more appropriately represented.

The percent difference between the paretic and non-paretic soleus is 12%; a much smaller difference when compared to the remaining plantar flexors (medial gastrocnemius, 38%, and lateral gastrocnemius, 26%). Such a large difference between muscles of the same group suggests that the gastrocnemius atrophies preferentially in the plantar flexor group. The soleus and gastrocnemius each have

specific roles for forward propulsion and vertical support during normal gait (Neptune, Kautz and Zajac 2001), and plantar flexor weakness has been noted as a limiting factor in post-stroke gait speed (Nadeau, et al. 1999). Since the gastrocnemius is the only muscle of the two that contributes to swing initiation and the gastrocnemius atrophies preferentially over the soleus, our results imply that atrophy of the gastrocnemius plays a key role in limiting swing initiation in post-stroke subjects. This implication has not been thoroughly studied; however it may be possible with the recent development of musculoskeletal modeling software such as OpenSim (Delp, et al. 2007) to account for muscle atrophy during gait simulations. Stroke-specific models that account for individual muscle volume changes may be developed by adjusting the maximum isometric force for each paretic and non-paretic plantar flexor muscle. Stroke-specific models will give further insight into the role of paretic and non-paretic plantar flexors during post-stroke gait.

Another surprising result was the gracilis was actually larger on the paretic side rather than the non-paretic, although the difference was not statistically significant. We hypothesized that all muscles on the paretic side would be significantly lower than their non-paretic counterparts. In the case of the gracilis, this hypothesis was not supported. Since the gracilis is a relatively small knee flexor, an 11% increase in muscle volume observed on the paretic side may not be clinically relevant in regards to the contribution of the gracilis to knee flexion function. However, the gracilis acts not only as a knee flexor, but also as a hip flexor and hip adductor. Our study was limited in that the focus was on muscles crossing the knee

and ankle joints, and therefore we did not investigate muscle volume changes in hip flexors and adductors to the full extent. Nadeau et al. (1999) reported that post-stroke subjects may compensate for paretic plantar flexor weakness with their ipsilateral hip flexors. It is therefore possible that paretic hip flexor muscles such as the gracilis will actually increase in size as a result of increased use as they compensate for plantar flexor weakness during gait.

Our results also show that the tibialis anterior muscle volume is not significantly lower on the paretic side, with only a 4% difference between sides. We also found that the paretic and non-paretic muscle had similar intramuscular fat content, each with less than 10%. Therefore, with similar muscle volumes and intramuscular fat content between sides, it may be suggested that the tibialis anterior atrophies the same amount per side following stroke. During normal gait, the primary function of the tibialis anterior muscle is to lift the foot clear of the ground and control plantar flexion after heel strike (Burrige, et al. 2001). However, post-stroke gait deficits such as foot drop during swing (Burrige, et al. 2007) and limited ankle dorsiflexion at initial contact and during stance are often attributed to dorsiflexor muscle weakness (Olney and Richards 1996) – specifically the tibialis anterior muscle (Burrige, et al. 2007). Since both the paretic and non-paretic tibialis anterior muscles atrophy the same amount, our findings suggest that muscle weakness on the paretic side must be attributed to neurological factors rather than muscle atrophy.

Predictors of post-stroke gait include knee extensor strength (Hamrin, et al. 1982, Nakamura, Hosokawa and Tsuji 1985, Nakamura, Watanabe, et al. 1988,

Bohannon 1989a, Bohannon 1989b, Bohannon and Andrews 1990, Bohannon and Walsh 1992) and ankle joint moment and power (Olney, Griffin and Monga, et al. 1991, Olney, Griffin and McBride 1994, Nadeau, et al. 1999). The overall force a muscle can generate is calculated by the product of muscle stress and physiological cross-sectional area (PCSA), assuming no intramuscular fat has developed. Muscle volume is a determinant of PCSA, and therefore is a critical variable in determining a muscle's force-generating capability.

Musculoskeletal models can be used to evaluate muscle function and motor control, yet most models use force-generating properties derived from cadavers and may not properly reflect the population type being studied (Holzbaur, et al. 2007). In this study, we have provided average muscle volumes for subjects with post-stroke hemiparesis. The presented volumes have been adjusted for intramuscular fat development to better approximate the true force-generating tissue for each muscle. This data can be used in the future to study the effect muscle atrophy has on PCSA and each individual muscle's force-generating capability for both paretic and non-paretic legs.

### **3.5. Conclusions**

To our knowledge no study has investigated individual muscle atrophy following stroke and there have been no reported values for post-stroke individual muscle volumes. We used digital reconstruction software to digitize fifteen leg muscles from MR images. Our study confirmed muscle atrophy in the paretic leg in all

but one muscle, and showed that intramuscular fat is greater on the paretic side.

Paretic muscles that did atrophy were, on average 23% smaller than the non-paretic sides. The gracilis, which was larger on the paretic side, is a hip flexor which may increase in size if it is used to compensate for weak plantar flexors during gait. The tibialis anterior tends to atrophy the same amount on both paretic and non-paretic sides. By eliminating intramuscular fat from our volume calculations, we have presented volume data that represents the true force generating muscle tissue that may be used in future studies to assess the effect muscle atrophy has on post-stroke gait.

**Chapter 4**

**MUSCLE GROUP ATROPHY IN THE POST-STROKE LOWER  
EXTREMITY**

**4.1. Introduction**

Stroke is a leading cause of long term disability in adults, affecting approximately 795,000 adults a year in the United States alone (NINDS 2004, Heart disease and stroke statistics-2010 update, NSA 2007). Stroke damages brain cells and disrupts pathways of the central nervous system. Following stroke, a range of disabilities are often observed including impaired speech, unilateral muscle weakness, and cognitive difficulties (NINDS 2009). Muscle weakness contralateral to the brain lesion, or hemiparesis, is the most common disability following stroke (Chan 1986, Andrews and Bohannon 2000).

Post-stroke hemiparesis is a concern clinically because it restricts many daily living tasks. Difficulties in performing lower extremity tasks such as making transfers (Bohannon 1988), stair climbing (Bohannon and Walsh 1991, Bohannon and Walsh 1992), and standing (Bohannon 1989a) have been observed. Initially, walking is limited in two out of three patients (Jorgensen, et al. 1995) which makes it the primary functional focus for lower extremity rehabilitation (Kim and Eng 2004). Hemiparetic gait deficiencies such as decreased walking speeds (Brandstater, et al. 1983, Burdett,

et al. 1988, Olney, Griffin and McBride 1994, Turnbull, Charteris and Wall 1995, von Schroeder, et al. 1995, Witte and Carlsson 1997), asymmetric stance phases (Brandstater, et al. 1983, Wall and Turnbull 1986, Olney, Griffin and McBride 1994, Olney and Richards 1996), and lack of center of mass support (Higginson, et al. 2006) have been reported. Two predictors of post-stroke hemiparetic gait are knee extensor and plantar flexor muscle strength (Hamrin, et al. 1982, Nakamura, Hosokawa and Tsuji 1985, Nakamura, Watanabe, et al. 1988, Bohannon 1989a, Bohannon 1989b, Bohannon and Andrews 1990, Olney, Griffin and Monga, et al. 1991, Olney, Griffin and McBride 1994, Bohannon and Walsh 1992, Nadeau, et al. 1999). Since these predictors are directly related to hemiparesis, understanding factors associated with muscle weakness may help increase our knowledge of post-stroke gait.

A combination of neurological, mechanical, and structural factors will cause a decrease in muscle strength following stroke (Patten, Lexell and Brown 2004). Neurological changes such as a decrease in the ability to activate individual motor units, a loss of functioning motor units (McComas, Sica, et al. 1973), and reduced firing rates for each motor unit (Rosenfalck and Andreassen 1980, Tang and Rymer 1981) will decrease muscle activation and limit the contractile ability of each muscle. Alterations in the mechanical properties of muscle such as muscle stress or changes in force-length-velocity relationships may also influence the force-generating capability of a muscle. From a structural standpoint, post-stroke hemiparetic muscle weakness may be due to disuse atrophy (McComas 1994).

Depending on the level of use a muscle endures, skeletal muscles can adapt and change in size (Lieber 2010). A good indicator of the force-generating capability of a muscle is its size (e.g. physiological cross-sectional area) (Lieber 2010). Due to this relationship between force generation and muscle size, the amount of atrophy a post-stroke muscle undergoes is important in adequately describing any changes to its force-generating capability. Intramuscular fat increases with age and some diseases (Mitsiopoulos, et al. 1998) as well as stroke, particularly on the paretic side (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001, Ryan, et al. 2002). An increase in fat content following stroke must be accounted for to prevent an underestimation of muscle atrophy.

Many studies have described muscle atrophy in post-stroke hemiparetic limbs using imaging techniques such as dual-energy X-ray absorptiometry (DXA) (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001) and computed tomography (CT) (Sunnerhagen, et al. 1999, Ryan, et al. 2002, Metoki, et al. 2003). Using DXA, Jorgensen and Jacobsen (2001) reported a 3% decrease in lean muscle mass in the paretic leg compared to the non-paretic leg for subjects with acute stroke. For those patients who did not regain the ability to walk within two months of stroke onset, a 5-6% reduction in lean mass was observed. Ryan et al. (2002) used CT to measure overall mid-thigh muscle cross-sectional areas between paretic and non-paretic limbs in subjects with chronic (>6 month) stroke. They found a 20% decrease in paretic cross sectional areas. Paretic thigh muscle volumes calculated using CT by Metoki et al. (2003) were also reported as being smaller on the paretic side for

subjects with duration of stroke between 45 and 90 months. These studies confirm that muscle atrophy does occur more extensively in the post-stroke paretic limb. However, the previous studies were limited in describing muscle atrophy of entire extremities or isolated regions such as the thigh. Muscle atrophy observed in individual muscles or by specific muscle groups, particularly those in the shank region, has not been reported. Both DXA and CT imaging methods also expose the patient to various amounts of radiation and may be unfavorable when performing repeated scans over longer durations of time (Eng, et al. 2007).

An alternative imaging method for measuring changes in muscle size is Magnetic Resonance Imaging (MRI). MRI is advantageous because no radiation is required and no negative effects to the patient have been observed (Murphy, Totty and Carroli 1986). Recent studies have used MRI methods to measure changes in muscle size across a variety of demographics (Williams, Buchanan, et al. 2005, Williams, Snyder-Mackler, et al. 2005, Tate, et al. 2006, Lampe, et al. 2006, Ploutz-Snyder, et al. 2006, Holzbaur, et al. 2007). Lampe et al. (2006) used MRI to show that paretic muscle volumes in young adults with cerebral palsy are lower than the non-paretic side. Cross-sectional areas in the upper extremity were smaller on the paretic side in post-stroke patients (Ploutz-Snyder, et al. 2006).

The difference between paretic and non-paretic muscle volumes for individual lower-extremity muscles and muscle groups has not been quantified. The goal of this study was to measure specific muscle group volumes for the paretic and non-paretic limbs of post-stroke hemiparetic subjects. Since muscle weakness in the knee

extensors and plantar flexors is directly related to deficits in post-stroke gait patterns, we hypothesized that the paretic knee extensors would atrophy more than the knee flexors and the paretic plantar flexors would atrophy more than the dorsiflexors.

## **4.2. Methods**

### **4.2.1. Subjects**

Eleven adults with post-stroke hemiparesis (mean age  $61.9 \pm 8.0$  years,  $46 \pm 38$  months since stroke) participated in the study. Table 4.1 lists the subject demographics and clinical characteristics of those included in this study. Ambulatory subjects aged 30-80 years who had a single lesion, chronic stroke at least 6 months prior to their MRI session and who exhibited noticeable gait deficits were eligible for this study. Those with metal implants or who were claustrophobic were excluded. Other exclusion criteria were: multiple strokes affecting both sides of the body; heart disease; hypertension; dementia; severe aphasia; orthopedic or pain conditions; and cancer. The study was approved by the University of Delaware Review Board and prior to participation all patients provided their written informed consent.

**Table 4.1. Subject demographics and clinical assessment scores for 11 post-stroke hemiparetic individuals.**

<b>Clinical Characteristics of Participants</b>					
Patient	Age (yr)	Gender	Time Since Stroke (yr)	Side of Hemiparesis	Fugl-Meyer Assessment
1	66.50	Female	2.00	R	22
2	58.83	Male	1.00	R	13
3	47.00	Male	1.25	R	15
4	65.58	Female	1.83	R	18
5	52.50	Male	10.00	L	22
6	55.75	Male	7.08	L	26
7	68.75	Male	3.33	L	19
8	60.08	Male	1.50	L	18
9	62.50	Male	6.42	R	25
10	73.00	Male	6.83	R	12
11	70.83	Female	0.75	L	26

#### 4.2.2. Imaging

All imaging took place at Omega Imaging (Diagnostic Imaging Associates, Newark, DE). Each subject laid in a supine position with their feet strapped together at the toes to limit any movement during the scan period and maintain neutral hip rotation. Axial spin-echo T1-weighted MR images from a 1.5T Signa LX scanner (GE Medical, Milwaukee, WI) were acquired of both legs simultaneously from the ankle mortise to the iliac crest using the scanner's body coil. Five overlapping sequences of images were taken: ankle, lower leg, knee, thigh, and pelvis. Images from the ankle, lower leg, thigh and pelvic regions used a repetition time (TR) of 450 ms, echo time (TE) of 10 ms, slice thickness of 10 mm and space between slices of 11.5 mm. For the

knee region, TR and TE were the same; however the slice thickness was reduced to 5 mm and space between slices reduced to 6 mm. A matrix size of 256 x 256 and field of view of 400 mm were used for all scans.

#### 4.2.3. Muscle Volume Reconstruction

Following each scan session, the MR images were digitally reconstructed using a digitization tablet (Cintiq 18SX, Wacom Technology Corp., Vancouver, WA) and IMOD software (University of Colorado, Boulder, CO) (Kremer, Mastronarde and McIntosh 1996). A single observer manually traced the images of all subjects. Thirteen muscles were each digitally reconstructed by tracing the muscle boundary over the entire length of the muscle belly. The muscles were the soleus (SOL), medial gastrocnemius (MG), lateral gastrocnemius (LG), biceps femoris-short head (BFS), biceps femoris-long head (BFL), semimembranosus (SM), semitendinosus (ST), gracilis (GRA), sartorius (SAR), rectus femoris (RF), vastus medialis (VM), vastus intermedius (VI), and vastus lateralis (VL). Also traced was the entire dorsiflexor muscle group (i.e. tibialis anterior, extensor hallucis longus, extensor digitorum longus, and peroneus tertius muscles). An intraobserver reliability of muscle digitization was established by retracing muscle outlines a minimum of one month following the initial tracing. A one month period was used to reduce any memory bias from the observer. The coefficient of determination for the observer was 0.994.

Once all muscles and groups were manually traced, Nuages software (Nuages, INRIA, Sophia-Antipolis, France) (Geiger 1993) was used to generate subject-specific

triangle-based surface mesh models for each muscle. Subroutines from the Visualization Toolkit (Kitware Inc., Clifton Park, NY) (Schroeder, Martin and Lorensen 1997) were used to calculate unadjusted muscle volumes from the surface mesh models. Proximal and distal tendons were excluded and intramuscular fat content was eliminated from volume calculation to properly describe the actual contractile tissue present in each muscle. A subject-specific pixel threshold (out of 600) was determined for each subject by visually inspecting each MRI scan for fatty regions. Intramuscular fat content was eliminated by removing all pixels below a specified threshold that represented fat. A trapezoidal integration algorithm was used to calculate cross sectional areas (Williams, Snyder-Mackler, et al. 2005). The adjusted cross-sectional areas were then summed over the length of each muscle, and multiplied by the slice thickness to obtain fat-adjusted muscle volumes. Images that overlapped adjacent scan regions (e.g. thigh region images overlap knee scan region) were accounted for and excluded from volume calculation.

For each subject, individual muscles were grouped by their functional roles (e.g. plantar flexors) and the volumes were summed together to represent a cumulative group volume (Table 4.2). The values were averaged across all subjects to get a representative mean. Bi-articular muscles of the knee (i.e. MG and LG) were included in both the plantar flexor and knee flexor groups. To determine intramuscular fat content for each muscle group, the net muscle group volume was compared with the adjusted muscle group volume and a percent difference was found between the two values. All reported muscle group volumes have been adjusted for fat content.

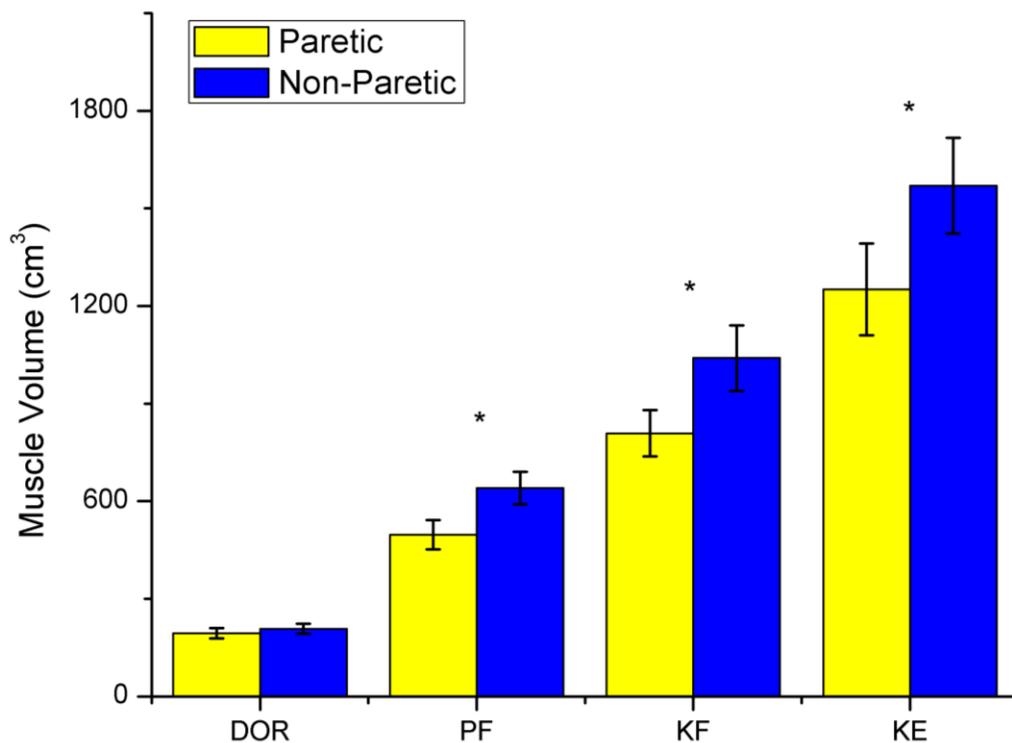
To quantify muscle size differences for each muscle group, each functional muscle group volume was compared between sides (paretic vs. non-paretic) and each opposing functional groups were compared within each limb (e.g. plantar flexors vs. dorsiflexors) using two separate ratios. One-tailed paired t-tests ( $\alpha=0.05$ ) were used to compare the within-limb opposing muscle group ratios between paretic and non-paretic sides.

**Table 4.2. Individual muscles group by their functional roles. The medial (MG) and lateral gastrocnemius (LG) are bi-articular muscles and were included in both the plantar flexor (PF) and knee flexor (KF) groups. The dorsiflexor group was traced as a group during reconstruction.**

<b>Functional Groups</b>	
<b>Dorsiflexors (DOR)</b>	entire group which includes: <i>tibialis anterior</i> <i>extensor hallucis longus</i> <i>extensor digitorum longus</i> <i>peroneus tertius</i>
<b>Plantar Flexors (PF)</b>	<i>soleus (SOL)</i> <i>medial gastrocnemius (MG)</i> <i>lateral gastrocnemius (LG)</i>
<b>Knee Flexors (KF)</b>	<i>medial gastrocnemius (MG)</i> <i>lateral gastrocnemius (LG)</i> <i>biceps femoris-short head (BFS)</i> <i>biceps femoris-long head (BFL)</i> <i>semimembranosus (SM)</i> <i>semitendinosus (ST)</i> <i>gracilis (GRA)</i> <i>sartorius (SAR)</i>
<b>Knee Extensors (KE)</b>	<i>rectus femoris (RF)</i> <i>vastus medialis (VM)</i> <i>vastus intermedius (VI)</i> <i>vastus lateralis (VL)</i>

### 4.3. Results

Overall group volumes can be seen graphically in Figure 4.1. Significant differences were found between the paretic and non-paretic sides for the plantar flexors ( $p < 0.0001$ ), knee flexors ( $p = 0.0004$ ), and knee extensors ( $p < 0.0001$ ), but not for the dorsiflexors ( $p = 0.0572$ ).



**Figure 4.1. Muscle group volumes, with standard error bars, for both the paretic and non-paretic sides of 11 post-stroke hemiparetic subjects. Volumes from the non-paretic side are in blue and volumes from the paretic side are in yellow. Significant differences between paretic and non-paretic volumes  $*p < 0.05$  are noted.**

**Table 4.3. Overall paretic and non-paretic muscle group volumes and volume ratios for the dorsiflexors and plantar flexors**

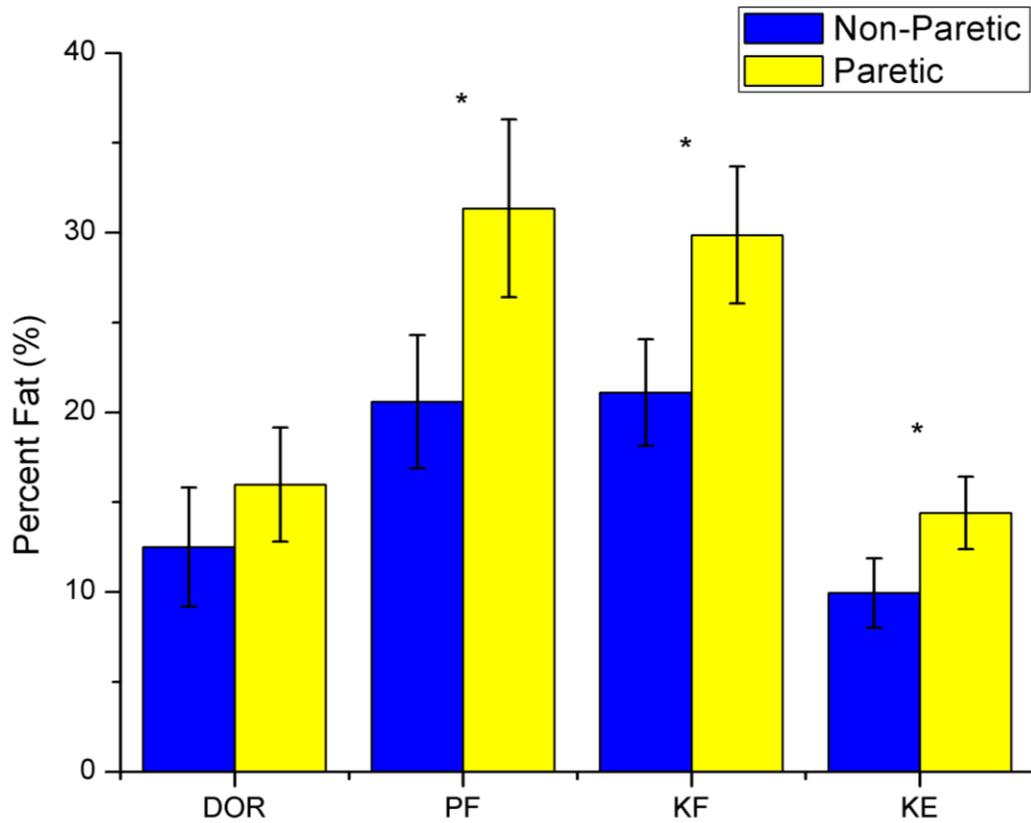
Dorsiflexor and Plantar Flexor Volume Ratios			
n=11	DF (cm <sup>3</sup> )	PF (cm <sup>3</sup> )	DF/PF Ratio
Paretic (cm <sup>3</sup> )	194.027	497.532	<b>0.390*</b>
Non-Paretic (cm <sup>3</sup> )	207.767	640.494	<b>0.324</b>
P/NP Ratio	0.934	0.777	

For all four muscle groups, the paretic to non-paretic (P/NP) ratio is less than 1.0 and therefore the paretic side is smaller than the non-paretic side for all groups. Ratios for the dorsiflexors and plantar flexors can be found in Table 4.3. Comparing the DF/PF ratios between sides, the paretic ratio (0.390) is significantly higher ( $p=0.0285$ ) than the non-paretic ratio (0.324). Knee flexor and knee extensor ratios can be found in Table 4.4. No significant difference was observed between the paretic (0.646) and non-paretic (0.662) KF/KE ratio ( $p=0.3826$ ).

**Table 4.4. Overall paretic and non-paretic muscle group volumes and volume ratios for the knee flexors and knee extensors**

Knee Flexor and Knee Extensor Volume Ratios			
n=11	KF (cm <sup>3</sup> )	KE (cm <sup>3</sup> )	KF/KE Ratio
Paretic (cm <sup>3</sup> )	808.783	1251.607	<b>0.646</b>
Non-Paretic (cm <sup>3</sup> )	1039.959	1569.867	<b>0.662</b>
P/NP Ratio	0.778	0.797	

Intramuscular fat content was higher on the paretic side in all four muscle groups (Figure 4.2), with significant differences observed in the plantar flexors ( $p=0.0002$ ), knee flexors ( $p=0.0008$ ) and knee extensors ( $p<0.0001$ ). No significant difference was found between paretic and non-paretic dorsiflexors ( $p=0.0575$ ). The plantar flexors and knee flexors had similar fat percentages on the paretic side (31% and 30%, respectively); however the plantar flexors had the largest difference between paretic and non-paretic sides, with a percent difference of 52% between sides. The average fat content across all groups for the paretic and non-paretic sides was 23% and 16% respectively. The average difference between sides was 42%.



**Figure 4.2. Intramuscular fat percentages, with standard error bars, for the paretic and non-paretic sides of four muscle groups. Fat percentages from the non-paretic side are in blue and fat percentages from the paretic side are in yellow. Significant differences between paretic and non-paretic fat percentages \*p<0.05 are noted.**

#### **4.4. Discussion**

This study has presented post-stroke hemiparetic muscle volume data of four primary muscle groups in the lower extremity: ankle dorsiflexors, ankle plantar flexors, knee flexors, and knee extensors. To our knowledge, no study has attempted to quantify muscle atrophy in all four of these muscle groups, and few have investigated muscle volume changes – especially over the length of the entire muscle. MR imaging and digital reconstruction methods enabled us to measure individual muscle volumes and group each muscle based on its functional role. Muscle group atrophy was quantified by measuring the difference between paretic and non-paretic group volumes. In addition, antagonist muscle group volume ratios were compared between the paretic and non-paretic limbs.

For all four muscle groups, the paretic/non-paretic volume ratios are less than 1.0. This confirms that the paretic limb does atrophy more than the non-paretic limb in post-stroke hemiparetic subjects. Metoki et al. (2003) used CT to measure thigh muscle volumes of chronic hemiplegic stroke patients. They reported a paretic/non-paretic ratio of 0.75 to 0.83 depending on a subject's age. Our P/NP knee flexor and knee extensor ratios (0.778 and 0.797, respectively) were each within the same range. Similarly, the plantar flexor P/NP ratio (0.777) fell within the range as well. However, although the ratio was less than one, there was no significant difference between paretic and non-paretic dorsiflexors. This suggests that the dorsiflexors do not atrophy any significant amount between sides.

To quantify muscle group atrophy, we calculated a second ratio that compared antagonist muscle groups and tested for significant differences between the paretic and non-paretic sides. This comparison allowed us to first determine any size relationships between antagonist muscle groups (symmetry), and then compare the differences between sides. By comparison, these ratios determine whether one group atrophies preferentially over another. In the shank region, the paretic DF/PF ratio was significantly higher than the non-paretic DF/PF ratio. In the paretic limb, the size of the dorsiflexor group is approximately 40% the size of the plantar flexors. In the non-paretic limb, the dorsiflexors are approximately 32% the size of the plantar flexors. These asymmetric size differences suggest that the dorsiflexors have a higher opposing action on the plantar flexors on the paretic side than the non-paretic side. The higher paretic DF/PF ratio also indicates that the paretic plantar flexors atrophy the most, thus confirming our hypothesis. Paretic plantar flexor muscle atrophy will reduce the overall force-generating capability of this group, which can have debilitating effects during post-stroke gait. Plantar flexors play an integral role during gait by providing vertical support during stance, assisting in push-off, and propelling the leg into swing phase (Neptune, Kautz and Zajac 2001). It has been shown that plantar flexor weakness is a limiting factor to post-stroke walking speed (Nadeau, et al. 1999) and can result in a decrease of COM support (Higginson, et al. 2006). Since the plantar flexors atrophy most on the paretic side, the paretic plantar flexors will not have the overall force-generating capability to function normally during gait, even if they are activated normally.

In the thigh region, the paretic knee flexors are approximately 65% of the size of the paretic knee extensors. Similarly, the non-paretic knee flexors are 66% of the size of the non-paretic knee extensors. It appears that although the paretic muscle volume is smaller (as indicated by a P/NP ratio less than 1.0), the antagonist muscle groups are symmetrical between both paretic and non-paretic sides of the body. There was also no significant difference between the two ratios, indicating that both knee flexors and knee extensors atrophy about the same amount on each side. Our hypothesis that the paretic knee extensors would atrophy more was not supported by these data. However, our results are comparable to one study that measured cross-sectional areas in post-stroke hemiparetic thighs. Ryan et al. (2002) reported a 20% decrease in paretic cross-sectional areas between the paretic and non-paretic thighs. Our P/NP thigh volume ratios show an average decrease of 21% for both knee flexors and knee extensors, similar to Ryan et al. (2002). Although cross-sectional area and volume are not the same measurement, volume depends on cross-sectional area and the relationships are similar.

In regards to post-stroke muscle strength, many studies offer different opinions on the pattern of muscle weakness distribution along a limb (Gowers 1893, Twitchell 1951, Adams, Gandevia and Skuse 1990, Bohannon and Andrews 1995, Andrews and Bohannon 2000, Hsu, Tang and Jan 2003). In the lower limb, the historical consensus was that weakness followed a proximal to distal gradient and that knee extension strength was less impaired than knee flexion strength (Andrews and Bohannon 2000). We would expect that, if muscle volume is representative of the force-generating

capability of each of the lower extremity muscle groups, the proximal-to-distal progression would show in the amount of atrophy each group undergoes. However, this is not the case in our study – in fact, one of the most distal muscle groups (DOR) barely change in volume between paretic and non-paretic sides and the paretic plantar flexors and knee flexors and extensors all atrophy about the same amount when compared to the non-paretic limb. Changes in muscle activation or stress may explain why muscle weakness and not atrophy is pronounced distally.

Some studies have shown that intramuscular fat increases in the paretic limb following stroke (Iversen, Hassager and Christiansen 1989, Jorgensen and Jacobsen 2001, Ryan, et al. 2002). We have reported similar findings in that intramuscular fat increases significantly on the paretic side in all but the dorsiflexor muscle group, with the highest fat content in the paretic plantar flexors and knee flexors. While we report intramuscular fat content in each muscle group, our method for determining fat from MRI has not been validated. A subject-specific pixel threshold was determined to be sufficient for each scan, but may not have resulted in measuring the exact amount of fat content. Although this method may have a subjective component, we feel that by eliminating fat content from our muscle volume calculations, muscle atrophy and the effect changes in muscle volume have on each muscle group's force generating capability are both better represented.

Using a ratio to describe muscle atrophy has its clinical implications as well because it demonstrates whether or not one muscle group atrophies preferentially over another. A subject that has symmetrical volumes (as indicated by similar ratios

between paretic and non-paretic sides) implies that both muscle groups have been affected relatively the same amount. An increase in the paretic ratio indicates greater muscle atrophy of the group in the denominator (i.e. the plantar flexors and knee extensors). Increasing muscle volumes by strength training muscle groups that atrophy preferentially, such as the plantar flexors, has the potential of increasing the group's overall force-generating capability.

#### **4.5. Conclusions**

In this study we grouped individual muscle volumes by their functional roles and calculated group muscle volumes for each of four primary muscle groups in the lower extremity: ankle dorsiflexors, ankle plantar flexors, knee flexors, and knee extensors. By using digital reconstruction software and MR images, we measured muscle volumes for these four muscle groups and calculated intramuscular fat content for each. Our study confirmed muscle atrophy in all four groups, with significant differences on the paretic side in all but the dorsiflexors. Intramuscular fat was higher for all groups on the paretic side, but was highest in the paretic plantar flexors and no significant difference was found in dorsiflexors. We found that the paretic plantar flexors atrophied the most, and that the knee flexors and extensors atrophy relatively the same amount.

## **Chapter 5**

### **CONCLUSIONS**

This thesis used MR imaging and digital reconstruction software to measure both individual muscle volumes and muscle group volumes in post-stroke hemiparetic lower extremities. All muscle volumes excluded intramuscular fat to appropriately represent the force-generating tissue. The results of the individual muscle volume study indicated that all muscles atrophy on the paretic side except the gracilis. The results of the muscle group volume study indicated that paretic muscle groups atrophy more than the non-paretic groups, that the paretic plantar flexors atrophy the most, and that the paretic knee flexors and knee extensors atrophy relatively the same amount.

#### **5.1. Major findings of this thesis**

##### **5.1.1. Individual muscle atrophy in the hemiparetic lower extremity**

Muscle atrophy was quantified in fifteen muscles of the lower extremity by measuring the difference in muscle volume between the paretic and non-paretic sides. Besides the gracilis, all muscles were smaller on the paretic side with an average decrease in muscle volume of 23%. The gracilis was actually larger on the paretic side, with an increase of approximately 11%. These results confirmed that most

individual muscles do atrophy on the paretic side – the exception may be that the hip flexors such as the gracilis may be compensating for weakness in muscles such as the plantar flexors. We found significant differences between paretic and non-paretic sides in all muscles except the soleus and the tibialis anterior. The difference between paretic and non-paretic soleus volumes was much smaller when compared to the other plantar flexors muscles (i.e. medial and lateral gastrocnemius), suggesting that the gastrocnemius atrophies preferentially in the plantar flexor group. Since the gastrocnemius initiates swing during gait (Neptune, Kautz and Zajac 2001), our results imply that the atrophy in the gastrocnemius plays a key role in limiting swing initiation in post-stroke subjects. The tibialis anterior muscle exhibited non-significant muscle atrophy between paretic and non-paretic legs and both paretic and non-paretic sides had similar fat content. This suggests that the tibialis anterior tends to atrophy the same amount per side.

### **5.1.2. Muscle volume changes in hemiparetic ankle and knee flexors and extensors**

In this study, thirteen of the previous fifteen muscles were grouped into four muscle groups based on their functional role: ankle dorsiflexors, ankle plantar flexors, knee flexors, and knee extensors. For all muscle groups, the paretic group volume was lower than the non-paretic volume, although no significant difference was found between paretic and non-paretic dorsiflexors. A ratio was calculated to describe the muscle volume changes between antagonist muscle groups (e.g. plantar flexors and

dorsiflexors). This ratio comparison allowed us to first determine any size relationships between antagonist muscle groups (i.e. symmetry), and then compare the differences between sides to determine whether any group atrophies preferentially. Asymmetry was observed between the paretic and non-paretic sides for both the dorsiflexors and plantar flexors. The paretic dorsiflexor/plantar flexor ratio was larger than the non-paretic ratio, indicating that the plantar flexors atrophied most on the paretic side. At the knee joint, the paretic and non-paretic knee flexor/knee extensor ratios were similar indicating that the muscle volumes were symmetrical between sides and that both knee flexors and extensors atrophy the same amount on the paretic side.

## **5.2. Contribution of this thesis**

This thesis provides a data set representing muscle volumes for fifteen individual muscles that have not been reported in the literature for post-stroke hemiparetic subjects. Muscle atrophy between paretic and non-paretic sides has been observed using imaging techniques besides MRI (Jorgensen and Jacobsen 2001, Ryan, et al. 2002, Metoki, et al. 2003); however, none have quantified atrophy over the length of an entire isolated muscle nor did any investigate changes in the dorsiflexors or plantar flexors. By adjusting for intramuscular fat content and comparing paretic and non-paretic muscle volumes rather than cross-sectional area or mass, we quantified post-stroke muscle atrophy over the entire length of the muscle belly for the first time.

This thesis also provides data for muscle groups rather than overall regions of the leg. Specific muscle groups were studied by grouping each individual muscle by its functional role and summing all muscles within that group. Volume ratios comparing antagonist groups gave insight into the symmetry of muscle group volumes as well as the extent of atrophy each muscle group endured.

### **5.3. Limitations**

One limitation to the methodology of this thesis is that the fat elimination process has not been validated. Pixels were identified visually and no set criterion for determining a proper range of fat thresholds was defined. It is possible that the pixels below the given threshold for each subject represent various types of developing passive tissue rather than intramuscular fat.

An additional limitation to the methodology is in the manner to which adjusted volumes were calculated. Fat-adjusted muscle volumes were calculated as the sum of all areas multiplied by the slice thickness. This numerical integration method may introduce over- or under-estimation errors to the volume calculation. Similarly, because of the slice thickness of 11.5 mm at the proximal and distal ends of most muscles, it is also possible that some portions of the muscle are not included in the volume calculation.

Since this thesis focused on muscle volume changes at the knee and ankle joints, another limitation to this study is that we did not investigate muscle volume changes in the hip flexors. Ipsilateral hip flexors have been shown to compensate for

plantar flexor weakness and may also exhibit some volume changes between paretic and non-paretic sides.

A final limitation is that muscle strength is not determined by muscle size alone, but also muscle activation. In this study we presented the amount of muscle atrophy that each muscle or muscle group endures post-stroke, which gives insight only into the overall force-generating capability of each muscle or muscle group. However, impaired muscle activation following stroke will likely compound the results of muscle atrophy on muscle strength. This study did not account for muscle activation and its role in muscle weakness, leaving that matter for future studies to address.

#### **5.4. Future work**

With individual post-stroke muscle volumes for both paretic and non-paretic limbs now available, muscle atrophy can be included in musculoskeletal models. Stroke specific force-generating parameters such as physiological cross-sectional area and maximum isometric force can be updated in modeling software such as OpenSim (Delp, et al. 2007) and simulations can be used to detect any changes as a direct result of muscle atrophy.

This work has shown that muscle weakness, particularly in the dorsiflexors, cannot be attributed to muscle atrophy alone. Changes in muscle activation and tissue properties are also likely to occur following stroke. To account for the effect post-stroke muscle atrophy has on the overall force-generating capability of each muscle,

the maximum isometric force for each muscle may be updated by estimating stroke-specific physiological cross-sectional areas. Physiological cross-sectional areas can be estimated for post-stroke hemiparetic muscles by dividing the muscle volumes measured in this thesis by fiber lengths and multiplying by the cosine of the pennation angle, which can be found from the literature (Lieber 2010). Maximum isometric force can then be estimated by multiplying PCSA by muscle stress. Once maximum isometric force is calculated, simulation software such as OpenSim can be used to observe any changes in gait as a result of muscle atrophy. Observations can be made on how muscle atrophy affects the role of plantar flexors during stance and push off, as well as the compensatory effect muscles such as the gracilis have during swing phase. Muscle activations may also change to compensate for the decrease in overall force-generating capability in the paretic muscles. By incorporating muscle atrophy into stroke-specific simulation, the relationship between muscle atrophy, impaired activation and muscle weakness can be observed.

Finite element models of paretic and non-paretic muscles may be generated from the surface mesh models created in this study. Finite element models of the musculotendon unit could be used to calculate and compare muscle stress values between the paretic and non-paretic sides and observe any changes that are due primarily to muscle architecture. Quantifying changes in muscle stress will give better insight into additional determinants of muscle force and increase the reliability of muscle force predictions.

## 5.5. Summary

This thesis quantified muscle atrophy in fifteen individual muscles by measuring paretic and non-paretic muscle volumes and comparing the difference between sides. With one exception (i.e. gracilis), individual paretic muscles atrophy more than non-paretic muscles. Overall group muscle volume changes were also observed by combining muscles with similar functional roles into their functional groups and measuring the difference between paretic and non-paretic sides. For the first time, plantar flexors and dorsiflexors were included in the muscle groups studied. All muscle groups exhibited significant decreases in muscle volume on the paretic side except the dorsiflexors. Volume ratios compared antagonist groups and gave insight into the symmetry of muscle group volumes as well as the extent of atrophy each muscle group endured. Paretic plantar flexors atrophied the most in the shank region, and the paretic and non-paretic knee flexors and extensors atrophy relatively the same amount. Although muscles generally atrophy on the paretic side, the direct effect that these changes might have on daily tasks such as gait is a topic left for future studies.

## Chapter 6

### APPENDIX

#### 6.1. Validation and Reliability Studies

##### 6.1.1. Phantom Validation

Prior to measuring individual post-stroke muscle volumes, a validation study was performed to establish that the volume reconstruction method accurately measured volume from a set of MR images. To do this, a rectangular cube calibration phantom with dimensions 15.0 cm x 14.8 cm x 37.2 cm and known volume (8258.4 cm<sup>3</sup>) was scanned using the same procedure outlined in Chapter 3 and Chapter 4. Measured volumes were averaged across five trials and compared to the control volume as a percentage of the known volume. The average measured volume was 8207.9 cm<sup>3</sup>, which is 99.4% of the known phantom volume. All measured volumes were within 1% of the control volume. The difference between measured volumes and the control (0.6%) is small enough to establish that the tracing method and reconstruction programs used to calculate volumes from MR images are valid.

### **6.1.2. Intraobserver reliability**

Intraobserver reliability was needed to assess the variation in muscle volume measurements based on the repeated tracings of a single observer. For this thesis, intraobserver reliability was performed using a test-retest method, which determined whether a single observer could locate and trace specific muscles in a similar manner after a period of time.

One uniarticular (i.e. SOL) and two biarticular muscles (i.e. MG and SAR) were chosen to be retraced for both the paretic and non-paretic sides of one post-stroke subject. The muscles chosen represent a broad range of cross-sectional area and volume shapes. Each muscle overlapped at least two scan regions. To minimize memory-based bias between tests, a minimum of one month elapsed between the initial tracing and the repeated tracing. The correlation coefficient for paretic muscles was 0.999 and for non-paretic muscles the coefficient was 0.994. The results of this reliability study show that the author showed a great deal of reliability between tests.

### **6.1.3. Cross-Sectional Area Method Validation**

All reported muscle volumes in this thesis have been adjusted for intramuscular fat content. However, the method for calculating adjusted muscle volume is different than the surface mesh volume method. Instead of using a surface mesh model to calculate muscle volume, the adjusted volumes have been calculated by summing the cross-sectional areas over the length of the muscle and multiplying by the corresponding slice thicknesses (11.5 mm or 6 mm). Therefore, validation was

needed to determine whether the two measures could be compared when determining fat content. To see whether the two methods were similar, the surface mesh volume was compared to the CSA method volume without removing any fat pixels for all fifteen muscles and one muscle group for two subjects. The two methods were determined to be comparable because the cross-sectional area method calculated muscle volumes that were within  $\pm 5\%$  of the surface mesh model values for each subject.

#### **6.1.4. Final Validation Remarks**

While the two methods were shown to be comparable in how they calculated overall volumes, no validation was performed to determine whether the fat content removed was accurate. Each pixel was defined as a 1.5625 mm x 1.5625 mm area, and all pixels with a value below a specified threshold were removed from the adjusted volume calculation; however pixel values are dependent upon the black and white levels of the MR scan itself which may change between overlapping scans, as well as between subjects.

Also, rather than using the cross-sectional area method for all volume calculations, the surface mesh method demonstrated more reliability in calculating overall muscle volume, and was therefore used as the basis for comparing adjusted and unadjusted volumes for fat content. Any error introduced by the cross-sectional area method was absorbed in the fat content calculation.

## **6.2. Power Analysis Results**

An a priori power analysis was performed using G\*Power 3.1.1 to compute the sample size required to achieve a power of 0.8. The power analysis was based on a one-tailed matched paired t-test using an  $\alpha$  of 0.05. The effect size was determined for each individual muscle and muscle group using the mean and standard deviation of both the paretic and non-paretic sides. The correlation between groups was 0.5. The results for each individual muscle power analysis suggested a sample size range of 4 (TFL) to 75 (TA) subjects. The results for each muscle group suggested a sample size range of 7 (plantar flexors) to 51 (dorsiflexors) subjects. However, the plantar flexors, knee flexors and knee extensors required sample sizes of 7, 10 and 12, respectively, and therefore the current sample size of 11 was deemed sufficient for this study.

A post-hoc power analysis was also performed using the same program described above for all muscles and muscle groups based on the means and standard deviations. Individual muscle results ranged from 0.11 (TA) to 0.99 (TFL, MG) and results for muscle groups ranged from 0.21 (dorsiflexors) to 0.88 (plantar flexors).

## 6.3. Informed Consent Form

### INFORMED CONSENT FORM

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

#### **Morphology, strength and compensatory strategies of leg muscles after stroke**

##### **Summary**

You are invited to participate in a research study conducted by Dr. Jill Higginson which will compare muscle properties, strength and walking performance of stroke survivors and neurologically healthy adults. This is a four-part study involving (1) MRI, (2) muscle twitch test, (3) muscle strength test and (4) walking trials. We hope to identify the underlying changes in muscle function responsible for specific walking impairments following stroke.

##### **Study Description**

*Participants:* Healthy adults, aged 18 to 80 years, will be eligible for this study. Healthy subjects will be recruited by word-of-mouth in the University and local community.

We will also recruit adults between the ages of 18 and 80 years who have been diagnosed with stroke. Potential subjects will be recruited by way of local physical therapy practices, physicians and patient support groups.

If you decide to participate, Dr. Higginson or her research associates will ask you to complete a questionnaire about your health and medical history. You may be excluded based on your responses.

##### **Part 1**

This test will occur at Omega Imaging center, located across the street from Christiana Hospital. Transportation will be provided if needed. You will be asked to wear comfortable clothes and remove all metal accessories. We will obtain Magnetic Resonance Imaging (MRI) scans of your legs. MRI images will be taken while you remain still. You will lie on your back in the MRI machine. Claustrophobia, often experienced by patients while in the imager, is not a problem for this study because your

Participant's initials: \_\_\_\_\_  
Page 1 of 6

**INFORMED CONSENT FORM**

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

head remains outside the confines of the inner bore of the imager. Typical imaging times range from 3 - 7 minutes. Multiple scans may be taken, and the total scan duration will be less than one half hour. The imaging session will take approximately one half hour, and the total time for your participation in Part 1 will be no longer than 1 hour.

**Part 2**

Parts 2, 3 and 4 of the study will occur in the biomechanics lab in Spencer Laboratory and you will be asked to wear shorts, t-shirt and comfortable walking shoes. You will complete several standard clinical tests to assess memory and function. We will determine how completely you can activate your muscles during a set of strength tests. We will ask you to sit in the strength measurement chair with your knee strapped to the movable arm. Two self-adhesive electrodes, 3" x 5", will be placed over your quadriceps muscles. A brief (10 millisecond) burst of electrical stimulation will be delivered by a stimulator to your muscles (you will feel moderate tingling). Next, you will be asked to contract your quadriceps as hard as you can (extend your knee). During the contraction, another very brief burst of electrical stimulation will be delivered. We will measure your strength before and during the electrical stimulation. The electrodes will be adjusted and the test will be repeated at the ankle and on both legs. During this session, you will remain seated and will be permitted to rest as often as needed. The clinical test will take approximately 1 hour, the testing session will take approximately 1 hour, and the total time for your participation in Part 2 will be no longer than 2.5 hours.

**Part 3**

For this session, we will measure your muscle activity during a set of strength tests. Muscle activity will be recorded by the use of surface electrodes and/or fine-wire electrodes. To measure muscle activity, we will attach surface electrodes to your legs with tape. For the fine-wire recordings, small needles will be used to insert wires into three of your muscles. These wires will be used to record the electrical activity of muscle fibers allowing us to make precise measurements of the signals being sent to your

Participant's initials: \_\_\_\_\_  
Page 2 of 6

**INFORMED CONSENT FORM**

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

muscles. While there is some discomfort during passage of the needle, this usually passes quickly, and no local anesthesia is required. At the end of the experiment, all electrodes will be removed. While you are securely seated in the strength measurement chair, we will ask you to perform three trials where you will contract your muscles as hard as you can while straightening and bending the knee. This will be repeated for the ankle. We will also measure the stiffness of your ankle and knee joints. We will also ask you to contract your knee and ankle muscles to push and then pull the strength device while the arm is moving. All trials (4 static, 4 dynamic) will be repeated for the opposite leg. During this session, you will remain seated and will be permitted to rest as often as needed. Setup will take approximately 1 hour, the testing session will take approximately 1 hour, and the total time for your participation in Part 3 will be no longer than 2.5 hours.

**Part 4**

Your weight and height will be measured. We will attach reflective markers to your torso, legs, and feet. To measure muscle activity, we will also attach surface electrodes to your legs with tape. You will first be asked to walk at a comfortable pace along a 10 meter walkway. From this, we will calculate your comfortable walking speed. You will then be asked to walk on a treadmill that is embedded with force plates at two speeds (1) comfortable and (2) fastest possible walking speed for less than 2 minutes, including a 30 second warm-up and a one minute trial for each condition. To calculate your fastest possible speed, we will slowly and steadily increase the treadmill speed until you indicate you have reached your fastest possible speed. For each walking test, the motion analysis cameras will capture the movement of the reflective markers and ground reaction forces will be collected. We may also collect video for comparison with the motion data. The electrical activity of your leg muscles will be recorded during the walking trials. Healthy subjects will also be asked to perform an additional trial for each condition at slower speeds. You will be allowed to sit on a chair to rest as often as needed. Your heart rate will be monitored during walking. We will stop the treadmill and ask you to rest if your

Participant's initials: \_\_\_\_\_  
Page 3 of 6

**INFORMED CONSENT FORM**

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

heart rate exceeds 80% of your maximum target heart rate. Walking will only continue when your heart rate returns to baseline for 2 minutes. In addition, we will ask you to rate how hard you are working during walking and when this level exceeds “light”, we will stop walking for a rest break. You will wear a safety harness attached to an overhead beam for all trials. Plus, an emergency safety switch that shuts off the treadmill will be within reach and an assistant will be accessible at all times when walking on the treadmill. Setup will take approximately 1 hour, the testing session will take approximately 1 hour, and the total time for your participation in Part 4 will be no longer than 2.5 hours.

**Conditions for Participation**

You should not participate in this project if you are currently pregnant or have a condition other than stroke that will affect your walking (e.g. Parkinson’s disease, joint replacement). You will be asked to complete a physical activity readiness questionnaire and may be excluded based on your responses. You should have no bone or joint problems in the legs or spine, nor shortness of breath without exertion in the last 6 months. You are not eligible if you have sustained multiple strokes affecting both sides of the body. In addition, you must be able to understand spoken instructions and be able to communicate with the investigators. You should not participate if you have implanted magnetic metal or electronic devices or other conditions for which MRI poses risks. You must be able to walk without the assistance of another person but may use a leg brace or an assistive device. Your personal information will be stored in a locked cabinet, remain confidential and will not be released (including any publication) without your written consent. Data obtained from this study will be recorded without personal identifiers on a computer and archived indefinitely. If you agree, video acquired during this study may be used as part of educational presentations and we will block out your face so that your identity will not be revealed.

Participant's initials: \_\_\_\_\_  
Page 4 of 6

**INFORMED CONSENT FORM**

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

**Risks and Benefits**

The MRI test uses standard imaging practices. MRI does not involve exposure to radiation. Claustrophobia, often experienced by patients while in the imager, is not a problem for this study because the subject's head remains outside the confines of the inner bore of the imager. The electrical stimulator delivers an electrical current that should not cause any damage to your muscles. Recording the electrical activity of your muscles using surface electrodes poses very little risk. There may be some minor irritation of the skin around the site of the electrode following the experiment. This is most likely due to the mild adhesive. Although the use of fine-wire electrodes is a standard clinical procedure for diagnosis of neural and muscular problems, there are a number of risks involved. There is some discomfort: you will feel a brief prick to your skin as if receiving a shot with a very small needle which should subside immediately. There is also a slight risk of bleeding and of damage to nerves and vessels, and a possibility of delayed infection. These various risks are minimized when the recordings are practiced by an experienced researcher, as will be done here. If you experience redness or swelling where the electrodes were placed, you should contact Dr. Higginson or her research associates. Your muscles may be a little sore after the strength tests, but this should not have lingering effects. As with any physical activity, mild exertion associated with walking at a rapid pace for a short period of time presents risk to the musculoskeletal system (sprains, strains) and the cardiorespiratory system (dizziness, discomfort in breathing, heart attack). While walking on the treadmill, you will wear a protective harness and a handrail will be within reach. You will receive emergency first aid in the event of injury as a direct result of this project. If you require additional medical treatment, you will be responsible for the cost.

**Compensation**

You will receive \$25 for participation in each testing session (for a total of \$100).

Participant's initials: \_\_\_\_\_  
Page 5 of 6

**INFORMED CONSENT FORM**

Project: Morphology, strength and compensatory strategies of leg muscles after stroke  
PI: Jill Higginson, Ph.D.

**Contacts**

Further information regarding this study may be obtained from the project director, Dr. Jill Higginson, at telephone number (302) 831-6622. Other questions about your rights as a research subject can be directed to the Chair of the University of Delaware Human Subjects Review Board at (302) 831-2136.

**Subject Consent**

I agree to participate in the research study described above. I understand that I may withdraw from this study or the principal investigator may terminate the study at any time.

( ) I agree to have video acquired during this study which may be used as part of educational presentations with my face obscured.

( ) I do NOT agree to have video acquired during this study.

Name: \_\_\_\_\_ (please print)

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Participant's initials: \_\_\_\_\_  
Page 6 of 6

## References

- Adams, R.W., S.C. Gandevia, and N.F. Skuse. "The distribution of muscle weakness in upper motoneuron lesions affecting the lower limb." *Brain*, 1990: 1459-1476.
- American Stroke Association. "Let's talk about living at home after stroke." 2007. <http://www.strokeassociation.org/presenter.jhtml?identifier=3018563> (accessed June 2010).
- Andrews, A., and R.W. Bohannon. "Distribution of muscle strength impairments following stroke." *Clinical Rehabilitation* 14 (2000): 79-87.
- Bohannon, R.W. "Strength of lower limb related to gait velocity and cadence in stroke patients." *Physiotherapy Canada* 38 (1986): 204-206.
- Bohannon, R.W. "Determinants of transfer capacity in patients with hemiparesis." *Physiotherapy Canada* 40 (1988a): 236-239.
- Bohannon, R.W., A.W. Andrews, and M.B. Smith. "Rehabilitation goals of patients with hemiplegia." *International Journal of Rehabilitation Research* 11 (1988b): 181-183.
- Bohannon, R.W. "Correlation of lower limb strengths and other variables with standing performance in stroke patients." *Physiotherapy Canada* 41 (1989a): 198-202.

- Bohannon, R.W. "Selected determinants of ambulatory capacity in patients with hemiplegia." *Clinical Rehabilitation* 3 (1989b): 47-53.
- Bohannon, R.W., and A.W. Andrews. "Correlation of knee extensor muscle torque and spasticity with gait speed in patients with stroke." *Archives of Physical Medicine and Rehabilitation* 71 (1990): 330-333.
- Bohannon, R.W., and S. Walsh. "Association of paretic lower extremity muscle strength and standing balance with stair climbing ability in patients with stroke." *Journal of Stroke and Cerebrovascular Diseases* 1 (1991): 129-133.
- Bohannon, R.W., and S. Walsh. "Nature, reliability, and predictive value of muscle performance measures in patients with hemiparesis following stroke." *Archives of Physical Medicine and Rehabilitation* 73 (1992): 721-725.
- Bohannon, R.W., and A.W. Andrews. "Limb muscle strength is impaired bilaterally after stroke." *Journal of Physical Therapy Science* 7 (1995): 1-7.
- Bohannon, R.W. "Muscle strength and muscle training after stroke." *Journal of Rehabilitation Medicine* 39 (2007): 14-20.
- Borkan, G.A., D.E. Hulst, S.G. Gerzof, A.H. Robbins, and C.K. Silbert. "Age changes in body composition revealed by computed tomography." *Journal of Gerontology* 38 (1983): 673-677.
- Brandstater, M.E., H. Bruin, C. Gowland, and B.M. Clark. "Hemiplegic gait: analysis of temporal variables." *Arch Phys Med Rehabil* 64 (1983): 583-587.

- Burdett, R.G., D. Borello-France, C. Blatchly, and C. Poptter. "Gait comparison of subjects with hemiplegia walking unbraced, with ankle-foot orthosis, and with Air-Stirrup brace." *Physical Therapy* 68 (1988): 1197-1203.
- Burrige, J.H., D.E. Wood, P.N. Taylor, and D.L. McLellan. "Indices to describe different muscle activation patterns, identified during treadmill walking, in people with spastic drop-foot." *Medical Engineering & Physics* 23 (2001): 427-434.
- Burrige, J., et al. "Phase II trial to evaluate the ActiGait implanted drop-foot stimulator in established hemiplegia." *Journal of Rehabilitation Medicine* 39 (2007): 212-218.
- Campbell, A.J., M.J. Borrie, and G.F. Spears. "Risk factors for falls in a community-based prospective study of people 70 years and older." *Journal of Gerontology* 44 (1989): M112-M117.
- Chan, C.W.Y. "Motor and sensory deficits following stroke: Relevance to a comprehensive evaluation." *Physiotherapy Canada* 38 (1986): 29-34.
- Chen, C.-L., H.-C. Chen, M.-K. Wong, F.-T. Tang, and R.-S. Chen. "Temporal stride and force analysis of cane-assisted gait in people with hemiplegic stroke." *Archives of Physical Medicine and Rehabilitation* 81 (2000): 43-48.
- Chen, G., C. Patten, D.H. Kothari, and F.E. Zajac. "Gait deviations associated with post-stroke hemiparesis: improvement during treadmill walking using weight support, speed, support stiffness, and handrail hold." *Gait & Posture* 22 (2005a): 57-62.

- Chen, G., C. Patten, D.H. Kothari, and F.E. Zajac. "Gait differences between individuals with post-stroke hemiparesis and non-disabled controls at matched speeds." *Gait & Posture* 22 (2005b): 51-56.
- Claeys, R. "The analysis of ground reaction forces in pathological gait." *International Orthopaedics* 7 (1983): 113-119.
- Dalla Toffola, E., D. Sparpaglione, A. Pistorio, and M. Buonocore. "Myoelectric manifestations of muscle changes in stroke patients." *Archives of Physical Medicine and Rehabilitation* 82 (2001): 661-665.
- Dattola, R., et al. "Muscle rearrangement in patients with hemiparesis after stroke: an electrophysiological and morphological study." *Eur Neurol* 33 (1993): 109-114.
- Delp, S.L., et al. "OpenSim: open-source software to create and analyze dynamic simulations of movement." *IEEE Transactions on Biomedical Engineering* 54 (2007): 1940-1950.
- Eng, C.M., G.D. Abrams, L.R. Smallwood, R.L. Lieber, and S.R. Ward. "Muscle geometry affects accuracy of forearm volume determination by magnetic resonance imaging (MRI)." *Journal of Biomechanics* 40 (2007): 3261-3266.
- Fenichel, G.M., R.B. Daroff, and G.H. Glaser. "Hemiplegic atrophy: histological and etiologic considerations." *Neurology* 14 (1964): 883-890.
- Fuller, N.J., et al. "Assessment of limb muscle and adipose tissue by dual-energy X-ray absorptiometry using magnetic resonance imaging for comparison." *International Journal of Obesity* 23 (1999): 1295-1302.

- Geiger, B. "Three-dimensional modeling of human organs and its application to diagnosis and surgical planning." Technical Report, Institut National de Recherche en Informatique et Automatique, France, 1993.
- Gerrits, K.H., et al. "Isometric muscle function of knee extensors and the relation with functional performance in patients with stroke." *Arch Phys Med Rehabil* 90 (2009): 480-487.
- Goodpaster, B.H., et al. "Skeletal muscle lipid concentration quantified by magnetic resonance imaging." *The American Journal of Clinical Nutrition* 79 (2004): 748-754.
- Gowers, W.R. *A manual of diseases of the nervous system*. 2nd Edition. London: J. and A. Churchill, 1893.
- Gracies, J.-M. "Pathophysiology of spastic paresis. I: Paresis and soft tissue changes." *Muscle & Nerve* 31 (2005): 535-551.
- Hachisuka, K., Y. Umezu, and H. Ogata. "Disuse muscle atrophy of lower limbs in hemiplegic patients." *Arch Phys med Rehabil* 78 (1997): 13-18.
- Hafer-Macko, C.E., A.S. Ryan, F.M. Ivey, and R.F. Macko. "Skeletal muscle changes after hemiparetic stroke and potential beneficial effects of exercise intervention strategies." *Journal of Rehabilitation Research & Development* 45 (2008): 261-272.
- Hamrin, E., G. Eklund, A.-K. Hillgren, O. Borges, J. Hall, and O. Hellstrom. "Muscle strength and balance in post-stroke patients." *Upsala Journal of Medical Sciences* 87 (1982): 11-26.

- "Heart disease and stroke statistics - 2010 update. A report from the American Heart Association." *Circulation* 121 (2010): e54-e69.
- Henneman, E., G. Somjen, and D.O. Carpenter. "Functional significance of cell size in spinal motoneurons." *Journal of Neurophysiology* 28 (1965): 560-580.
- Hesse, S.A., M.T. Jahnke, C. Schreiner, and K.H. Mauritz. "Gait symmetry and functional walking performance in hemiparetic patients prior to and after a 4-week rehabilitation programme." *Gait & Posture* 1 (1993): 166-171.
- Hesse, S.A., M.T. Jahnke, C.M. Bertelt, C. Schreiner, D. Lucke, and K.H. Mauritz. "Gait outcome in ambulatory hemiparetic patients after a 4-week comprehensive rehabilitation program and prognostic factors." *Stroke* 25 (1994): 1999-2004.
- Heymsfield, S.B., et al. "Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry." *The American Journal of Clinical Nutrition* 52 (1990): 214-218.
- Higginson, J.S., F.E. Zajac, R.R. Neptune, S.A. Kautz, and S.L. Delp. "Muscle contributions to support during gait in an individual with post-stroke hemiparesis." *Journal of Biomechanics* 39 (2006): 1769-1777.
- Holzbour, K.R.S., W.M. Murray, G.E. Gold, and S.L. Delp. "Upper limb muscle volumes in adult subjects." *Journal of Biomechanics* 40 (2007): 742-749.
- Horstman, A.M., et al. "Intrinsic muscle strength and voluntary activation of both lower limbs and functional performance after stroke." *Clinical Physiology and Functional Imaging* 28 (2008): 251-261.

Hortobagyi, T., J.P. Hill, J.A. Houmard, D.D. Fraser, N.J. Lambert, and R.G. Israel.

"Adaptive responses to muscle lengthening and shortening in humans."

*Journal of Applied Physiology* 80 (1996): 765-772.

Hsu, A., P. Tang, and M. Jan. "Analysis of impairments influencing gait velocity and

asymmetry of hemiplegic patients after mild to moderate stroke." *Arch Phys*

*Med Rehabil* 84 (2003): 1185-1193.

Iversen, E., C. Hassager, and C. Christiansen. "The effect of hemiplegia on bone mass

and soft tissue body composition." *Acta Neurologica Scandinavica* 79 (1989):

155-159.

Jolivet, E., E. Daguette, V. Bousson, C. Bergot, W. Skalli, and J.D. Laredo. "Variability

of hip muscle volume determined by computer tomography." *IRBM* 30 (2009):

14-19.

Jonkers, I., S. Delp, and C. Patten. "Capacity to increase walking speed is limited by

impaired hip and ankle power generation in lower functioning persons post-

stroke." *Gait & Posture* 29 (2009): 129-137.

Jorgensen, H.S., H. Nakayama, H.O. Raaschou, and T.S. Olsen. "Recovery of walking

function in stroke patients: The Copenhagen stroke study." *Arch Phys Med*

*Rehabil* 76 (1995): 27-32.

Jorgensen, L., and B.K. Jacobsen. "Changes in muscle mass, fat mass, and bone

mineral content in the legs after stroke: A 1 year prospective study." *Bone* 28

(2001): 655-659.

- Joyce, B.M., and R.L. Kirby. "Canes, crutches, and walkers." *American Family Physician* 43 (1991): 535-542.
- Kent-Braun, J.A., A.V. Ng, and K. Young. "Skeletal muscle contractile and noncontractile components in young and older women and men." *Journal of Applied Physiology* 88 (2000): 662-668.
- Kesar, T.M., et al. "Functional electrical stimulation of ankle plantarflexor and dorsiflexor muscles: effects on poststroke gait." *Stroke* 40 (2009): 3821-3827.
- Kim, C.M., and J.J. Eng. "The relationship of lower-extremity muscle torque to locomotor performance in people with stroke." *Physical Therapy* 83 (2003): 49-57.
- Kim, C.M., and J.J. Eng. "Magnitude and pattern of 3D kinematic and kinetic gait profiles in persons with stroke: relationship to walking speed." *Gait & Posture* 20 (2004): 140-146.
- Kremer, J.R., D.N. Mastrorarde, and J.R. McIntosh. "Computer visualization of three-dimensional image data using IMOD." *Journal of Structural Biology* 116 (1996): 71-76.
- Lampe, R., S. Grassi, J. Mitternacht, L. Gerdesmeyer, and R. Gradinger. "MRT-measurements of muscle volumes of the lower extremities of youths with spastic hemiplegia caused by cerebral palsy." *Brain & Development* 28 (2006): 500-506.

Lieber, R.L. *Skeletal muscle structure, function, and plasticity: the physical basis of rehabilitation*. 3rd Edition. Philadelphia: Lippincott Williams & Wilkins, 2010.

Lloyd, D.G., and T.F. Besier. "An EMG-driven musculoskeletal model to estimate muscle forces and knee joint moments in vivo." *Journal of Biomechanics* 36 (2003): 765-776.

Marigold, D.S., J.J. Eng, C.D. Tokuno, and C.A. Donnelly. "Contribution of muscle strength and integration of afferent input to postural instability in persons with stroke." *Neurorehabilitation and Neural Repair* 18 (2004): 222-229.

Mazess, R.B., H.S. Barden, J.P. Bisek, and J. Hanson. "Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition." *The American Journal of Clinical Nutrition* 51 (1990): 1106-1112.

McComas, A.J., R.E.P. Sica, A.R.M. Upton, and N. Aguilera. "Functional changes in motoneurons of hemiparetic patients." *Journal of Neurology, Neurosurgery, and Psychiatry* 36 (1973): 183-193.

McComas, A.J. "Human neuromuscular adaptations that accompany changes in activity." *Medicine & Science in Sports & Exercise* 26 (1994): 1498-1509.

Metoki, N., Y. Sato, K. Satoh, K. Okumura, and J. Iwamoto. "Muscular atrophy in the hemiplegic thigh in patients after stroke." *American Journal of Physical Medicine & Rehabilitation* 82 (2003): 862-865.

- Michaelsen, S.M., S. Jacobs, A. Roby-Brami, and M.F. Levin. "Compensation for distal impairments of grasping in adults with hemiparesis." *Experimental Brain Research* 157 (2004): 162-173.
- Mitsiopoulos, N., R.N. Baumgartner, S.B. Heymsfield, W. Lyons, D. Gallagher, and R. Ross. "Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography." *Journal of Applied Physiology* 85 (1998): 115-122.
- Murphy, W.A., W.G. Totty, and J.E. Carroli. "MRI of normal and pathologic skeletal muscle." *American Journal of Roentgenology* 146 (1986): 565-574.
- Nadeau, S., D. Gravel, A.B. Arsenault, and D. Bourbonnais. "Plantarflexor weakness as a limiting factor of gait speed in stroke subjects and the compensating role of hip flexors." *Clinical Biomechanics* 14 (1999): 125-135.
- Nakamura, R., T. Hosokawa, and I. Tsuji. "Relationship of muscle strength for knee extension to walking capacity in patients with spastic hemiparesis." *Tohoku Journal of Experimental Medicine* 145 (1985): 335-340.
- Nakamura, R., S. Watanabe, T. Handa, and I. Morohashi. "The relationship between walking speed and muscle strength for knee extension in hemiparetic stroke patients: a follow-up study." *Tohoku J. exp. Med.* 154 (1988): 111-113.
- National Institute of Neurological Disorders and Stroke. "What you need to know about stroke." 2004. <http://stroke.nih.gov/materials/needtoknow.htm> (accessed January 2010).

National Institute of Neurological Disorders and Stroke. "Stroke: challenges, progress, and promise." 2009. <http://stroke.nih.gov/materials/strokechallenges.htm> (accessed January 2010).

National Stroke Association. "Explaining Stroke Brochure." 2007. <http://www.stroke.org/site/PageServer?pagename=STROKE> (accessed April 2010).

National Stroke Association. "Stroke 101 Fact Sheet." 2009.

Neptune, R.R., S.A. Kautz, and F.E. Zajac. "Contributions of the individual ankle plantar flexors to support, forward progression and swing initiation during walking." *Journal of Biomechanics* 34 (2001): 1387-1398.

Olney, S.J., M.P. Griffin, T.N. Monga, and I.D. McBride. "Work and power in gait of stroke patients." *Archives of Physical Medicine and Rehabilitation* 72 (1991): 309-314.

Olney, S.J., M.P. Griffin, and I.D. McBride. "Temporal, Kinematic, and Kinetic Variables Related to Gait Speed in Subjects With Hemiplegia: A Regression Approach." *Physical Therapy* 74 (1994): 81-94.

Olney, S.J., and C. Richards. "Hemiparetic gait following stroke. Part I: Characteristics." *Gait & Posture* 4 (1996): 136-148.

Pang, M.Y.C., and J.J. Eng. "Muscle strength is a determinant of bone mineral content in the hemiparetic upper extremity: implications for stroke rehabilitation." *Bone* 37 (2005): 103-111.

- Patten, C., J. Lexell, and H.E. Brown. "Weakness and strength training in persons with poststroke hemiplegia: Rationale, method and efficacy." *Journal of Rehabilitation Research & Development* 41 (2004): 293-312.
- Peckham, P.H., and J.S. Knutson. "Functional electrical stimulation for neuromuscular applications." *Annual Reviews of Biomedical Engineering* 7 (2005): 4.1-4.34.
- Petterson, S.C., P. Barrance, T. Buchanan, S. Binder-Macleod, and L. Snyder-Mackler. "Mechanisms underlying quadriceps weakness in knee osteoarthritis." *Medicine & Science in Sports & Exercise* 40 (2008): 422-427.
- Pietrobelli, A., Z. Wang, C. Formica, and S.B. Heymsfield. "Dual-energy X-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration." *American Journal of Physiology Endocrinology and Metabolism* 274 (1998): E808-E816.
- Ploutz-Snyder, L.L., B.C. Clark, L. Logan, and M. Turk. "Evaluation of spastic muscle in stroke survivors using magnetic resonance imaging and resistance to passive motion." *Arch Phys Med Rehabil* 87 (2006): 1636-1642.
- Ramnemark, A., L. Nyberg, R. Lorentzon, U. Englund, and Y. Gustafson. "Progressive hemiosteoporosis on the paretic side and increased bone mineral density in the nonparetic arm the first year after severe stroke." *Osteoporosis International* 9 (1999): 269-275.
- Richards, C.L., and S.J. Olney. "Hemiparetic gait following stroke. Part II: Recovery and physical therapy." *Gait & Posture* 4 (1996): 149-162.

- Roby-Brami, A., S. Fuchs, M. Mokhtari, and B. Bussel. "Reaching and grasping strategies in hemiparetic patients." *Motor Control* 1 (1997): 72-91.
- Roby-Brami, A., S. Jacobs, N. Bennis, and M.F. Levin. "Hand orientation for grasping and arm joint rotation patterns in healthy subjects and hemiparetic stroke patients." *Brain Research* 969 (2003a): 217-229.
- Roby-Brami, A., A. Feydy, M. Combeaud, E.V. Biryukova, B. Bussel, and M.F. Levin. "Motor compensation and recovery for reaching in stroke patients." *Acta Neurologica Scandinavica* 107 (2003b): 369-381.
- Rosenfalck, A., and S. Andreassen. "Impaired regulation of force and firing pattern of single motor units in patients with spasticity." *Journal of Neurology, Neurosurgery, and Psychiatry* 43 (1980): 907-916.
- Ross, R., J. Rissanen, H. Pedwell, J. Clifford, and P. Shragge. "Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men." *Journal of Applied Physiology* 81 (1996): 2445-2455.
- Ryan, A.S., C.L. Dobrovoly, G.V. Smith, K.H. Silver, and R.F. Macko. "Hemiparetic muscle atrophy and increased intramuscular fat in stroke patients." *Arch Phys Med Rehabil* 83 (2002): 1703-1707.
- Scelsi, R., S. Lotta, G. Lommi, P. Poggi, and C. Marchetti. "Hemiplegic atrophy: morphological findings in the anterior tibial muscle of patients with cerebral vascular accidents." *Acta Neuropathologica* 62 (1984): 324-331.
- Schroeder, W., K. Martin, and B. Lorenzen. *The Visualization Toolkit*. 2nd Edition. Upper Saddle River, NJ: Prentice-Hall, Inc, 1997.

Sharp, S.A., and B.J. Brouwer. "Isokinetic strength training of the hemiparetic knee: effects on function and spasticity." *Arch Phys Med Rehabil* 78 (1997): 1231-1236.

Slager, U.T., J.D. Hsu, and C. Jordan. "Histochemical and morphometric changes in muscles of stroke patients." *Clinical Orthopaedics and Related Research* 199 (1985): 159-168.

Stackhouse, S.K., S.A. Binder-Macleod, C.A. Stackhouse, J.J. McCarthy, L.A. Prosser, and S.C.K. Lee. "Neuromuscular Electrical stimulation versus volitional isometric strength training in children with spastic diplegic cerebral palsy: a preliminary study." *Neurorehabilitation and Neural Repair* 21 (2007): 475-485.

Sunnerhagen, K.S., U. Svantesson, L. Lonn, M. Krotkiewski, and G. Grimby. "Upper motor neuron lesions: their effect on muscle performance and appearance in stroke patients with minor motor impairment." *Archives of Physical Medicine and Rehabilitation* 80 (1999): 155-161.

Tang, A., and W.Z. Rymer. "Abnormal force-EMG relations in paretic limbs of hemiparetic human subjects." *Journal of Neurology, Neurosurgery, and Psychiatry* 44 (1981): 690-698.

Tate, C.M., G.N. Williams, P.J. Barrance, and T.S. Buchanan. "Lower extremity muscle morphology in young athletes: An MRI-based analysis." *Medicine & Science in Sports & Exercise* 38 (2006): 122-128.

- Termote, J.L., A. Baert, D. Crolla, Y. Palmers, and J.A. Bulcke. "Computed tomography of the normal and pathologic muscular system." *Radiology* 137 (1980): 439-444.
- Trombly, C.A. "Deficits of reaching in individuals with left hemiparesis: a pilot study." *American Journal of Occupational Therapy* 46 (1992): 887-897.
- Turnbull, G.I., J. Charteris, and J.C. Wall. "A comparison of the range of walking speeds between normal and hemiplegic subjects." *Scandinavian Journal of Rehabilitation Medicine* 27 (1995): 175-182.
- Twitchell, T.E. "The restoration of motor function following hemiplegia in man." *Brain* 74 (1951): 443-480.
- van Vliet, P., M. Sheridan, and D.G. Kerwin. "The influence of functional goals on the kinematics of reaching following stroke." *NeuroReport* 19 (1995): 11-19.
- von Schroeder, H.P., R.D. Coutts, P.D. Lyden, E. Billings Jr., and V.L. Nickel. "Gait parameters following stroke: a practical assessment." *Journal of Rehabilitation Research and Development* 32 (1995): 25-31.
- Wade, D.T., R. Langton-Hewer, V.A. Wood, C.E. Skilbeck, and H.M. Ismail. "The hemiplegic arm after stroke: measurement and recovery." *Journal of Neurology, Neurosurgery, and Psychiatry* 46 (1983): 521-524.
- Wall, J.C., and G.I. Turnbull. "Gait asymmetries in residual hemiplegia." *Arch Phys Med Rehabil* 67 (1986): 550-553.

- Wang, W., Z. Wang, M.S. Faith, D. Kotler, R. Shih, and S.B. Heymsfield. "Regional skeletal muscle measurement: evaluation of new dual-energy X-ray absorptiometry model." *Journal of Applied Physiology* 87 (1999): 1163-1171.
- Williams, G.N., L. Snyder-Mackler, P.J. Barrance, and T.S. Buchanan. "Quadriceps femoris muscle morphology and function after ACL injury: a differential response in copers versus non-copers." *Journal of Biomechanics* 38 (2005a): 685-693.
- Williams, G.N., T.S. Buchanan, P.J. Barrance, M.J. Axe, and L. Snyder-Mackler. "Quadriceps weakness, atrophy, and activation failure in predicted noncopers after anterior cruciate ligament failure." *The American Journal of Sports Medicine* 33 (2005b): 402-407.
- Winter, D.A. "Energy generation and absorption at the ankle and knee during fast, natural, and slow cadences." *Clinical Orthopaedics and Related Research* 175 (1983): 147-154.
- Witte, U.S., and J.Y. Carlsson. "Self-selected walking speed in patients with hemiparesis after stroke." *Scandinavian Journal of Rehabilitation Medicine* 29 (1997): 161-165.