THE EFFECT OF NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) IN INDUCING MUSCLE HYPERTROPHY AND IMPROVEMENT IN MUSCLE TORQUE WITHIN THE QUADRICEPS MUSCLE OF ELDERLY PEOPLE

by

Ibrahim Mustafa Altubasi

B.S. PT, University of Jordan, 2003

M.S. University of Pittsburgh, 2006

Submitted to the Graduate Faculty of

School of Health and Rehabilitation Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

[2012]

UNIVERSITY OF PITTSBURGH

SCHOOL OF HEALTH AND REHABILITATION SCIENCES

This dissertation was presented

by

Ibrahim Mustafa Altubasi

It was defended on

July, 17, 2012

and approved by

Anthony Delitto, PhD, Professor and Associate Dean of Research

Sara Piva, PhD, Assistant Professor

Bret H. Goodpaster, PhD, Associate Professor

Dissertation Advisor: G. Kelley Fitzgerald, PhD, Associate Professor

THE EFFECT OF NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) IN INDUCING MUSCLE HYPERTROPHY AND IMPROVEMENT IN MUSCLE TORQUE WITHIN THE QUADRICEPS MUSCLE OF ELDERLY PEOPLE

Ibrahim Mustafa Altubasi, PhD

University of Pittsburgh, 2012

Aging is associated with structural changes in skeletal muscles. One such change is loss of muscle mass. Muscle fiber atrophy is another structural transformation. Muscle fiber atrophy is selective to type II muscle fibers. The reduced mass of aging muscles has functional consequences like reduced performance of daily activities. Although exercise can be used to hypertrophy type II muscle fibers, it requires high intensity training that may not be feasible for elderly people. Due to the ability of neuromuscular electrical stimulation (NMES) to activate type II muscle fiber at relatively low intensity compared to voluntary exercises, NMES might be an alternative method to train type II muscle fibers in the elderly population.

The purpose is to test the effectiveness of NMES compared to exercise that is performed at the same intensity in inducing structural changes in quadriceps muscle of elderly subjects. The aims are to 1) compare changes in muscle hypertrophy, 2) compare changes in quadriceps muscle power output, and 3) Compare changes in the performance-based functional power tests in subjects who receive NMES and those who receive isometric strengthening exercise at the same intensity level.

Twenty subjects (71.2 +/- 4.41) were randomized to receive NMES or exercise. Computed tomography (CT), and muscle biopsy were performed to assess changes in cross sectional area (CSA) and fiber types of the quadriceps. Isokinetic quadriceps muscle power and

performance based functional power were measured to assess changes in quadriceps strength and performance based physical function.

The patterns of change from pre to post training on total quadriceps CSA, lean quadriceps CSA, and type IIA CSA were significantly higher for the NMES group. There was no difference between the groups in isokinetic quadriceps muscle power. The change in ramp power test from pre to post training was significantly higher for the NMES group.

NMES might be an alternative to exercises in inducing type II fiber hypertrophy in older adults. NMES training induced improvements in performance based functional power tests compared to voluntary isometric exercise. There were no differences between the groups in the change in isokinetic quadriceps muscle power test scores.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to acknowledge the contribution of my committee member, especially Dr. Kelley Fitzgerald for supporting and helping me completing this dissertation. Also I would like to acknowledge the contribution of the Pittsburgh Claude D. Pepper Older American Independent Center for funding this project

I would like to thank my wife and my family members for supporting me. Finally I would like to dedicate this work to my late parents who were waiting for this moment to arrive.

1.0   CHAPTER ONE: INTRODUCTION

1.1   SPECIFIC AIMS AND HYPOTHESES

1.1.1   Specific Aim 1

This study will compare changes in muscle hypertrophy in subjects who receive NMES and those who receive isometric strengthening exercise at comparable torque output levels.

1.1.1.1 Hypothesis 1.1

Subjects who receive NMES will demonstrate larger increases in the cross sectional area of lean quadriceps muscle mass compared to subjects who receive isometric strengthening exercise, as measured by CT-scanning.

1.1.1.2 Hypothesis 1.2

Subjects who received NMES will demonstrate larger increases in cross sectional area of type II muscle fibers compared to subjects who received isometric strengthening exercise, based on muscle biopsies.

1.1.2   Specific aim 2

This study will compare changes in quadriceps muscle power output in subjects who received NMES and those who received isometric strengthening exercise.

1.1.2.1 Hypothesis 2.1

Subjects who received NMES will show greater increases in quadriceps muscle power output compared to the subjects who received isometric strengthening exercise, based on measures of power output at different contraction velocities using an isokinetic dynamometer.

1.1.3   Specific Aim 3

This study will compare changes in the performance-based functional power tests in subjects who receive NMES and those who receive isometric strengthening exercise.

1.1.3.1 Hypothesis 3.1

Subjects who received NMES will demonstrate greater increases in power output scores during the timed stair climb power test and the ramp power test, compared to subjects that receive isometric strengthening exercise.

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 Skeletal Muscle Structure and Function

Declines in whole skeletal muscle strength and size are features commonly associated with aging (29). Aging reduces the number of fibers in whole muscle, which primarily explains the loss of muscle mass (1). Frontera et al (3) studied muscle strength, cross sectional area, and contractile properties of skinned parts of single fibers of the quadriceps muscle. They found that the strength and the whole muscle cross sectional area of knee extensors were significantly greater in younger men compared to older men, but there were no differences between old and young subjects when whole muscle strength was adjusted to the whole muscle cross sectional area (specific tension). They also found that muscle fibers from young men were stronger than muscle fibers from old men expressing the same myosin heavy chain isoform even after adjusting for size. Therefore Frontera et al (3) concluded that abnormality in the quantity and/or function of the contractile or regulatory proteins could explain the reduction in the ability of muscle fibers to generate force.

Moreover Morse et al (51) studied 19 elderly (mean age = 73.83) and 12 young men (mean age = 25.30) to determine whether a decrease in the fascicle force (Ff) could be accounted for by muscle atrophy. The lateral head of the gastrocnemius (GL) muscle was assessed in this study. The volume of the GL muscle and the Achilles tendon moment arm length were evaluated using magnetic resonance imaging (MRI). The fiber fascicle length (Lf) and the pennation angle were measured using B-mode ultrasonography during isometric voluntary contraction of planter flexors. The physiological cross-sectional area (PCSA) was measured in vivo as GL volume/Lf. GL Ff was computed by dividing Achilles tendon force by the cosine of the pennation angle. The Achilles tendon force was measured by dividing the planter flexors torque by the Achilles tendon

3

moment arm. The results of this study showed that planter flexors isometric torque was 47% lower in older compared to younger men. The moment arm of the Achilles tendon was the same in both groups; accordingly the difference in the Achilles tendon torque is due to difference in Achilles tendon force. After accounting for both the agonist and antagonist muscle activity, the Achilles tendon force was 37% lower in the elderly group compared to young group. The pennation angle was 12% lower in the older men compared to younger men. Decreased pennation angle means greater value of cosine the pennation angle; consequently GL Ff (Achilles tendon force/cosine the pennation angle) will be smaller. After accounting for both the agonist and antagonist muscle activity, GL Ff was 38% lower in the older compared to younger men. GL volume and PCSA were lower in older compared to younger men by 28% and 16% respectively. Differences in the GL Ff between older and younger men could be explained by the differences in PCSA of the GL between both groups. The next question is, if the groups have equal PCSA do they have equal GL Ff? In order to answer this question, specific force was computed which is equal to the force generated divided by the cross sectional area. The specific force will be explained as the amount of force generated per one unit area. Morse et al found that GL specific force (Ff/PCSA) was 30% smaller in older compared to younger men. The results of this study suggest that additional factors, other than reduced PCSA, contribute to the reduction in fascicle force in older adults.

In addition to the loss of muscle fibers, atrophy of the fibers also has been attributed to the aging process. This atrophy has been found to be selective to type II muscle fibers (5, 8). Lexell et al (5) found in a study done on the cross sections of the whole vastus lateralis muscle from 20 men (19-84 years of age) that the mean cross sectional area (CSA) of type II fibers from muscles of older individuals was on average 35 % smaller than those of younger individuals. In

contrast the mean CSA of type I muscle fibers in older subjects was only 6 % smaller than that of young individuals. Larsson et al (52) studied muscle fibers distribution and cross sectional area in 51 male subjects aged 20-65. The results indicate that with aging there is lower percentage of type II muscle fibers and a corresponding increase in the percentage of type I muscle fibers. Average cross sectional area of type II muscle fibers was decreased with aging while there was no significant change in type I muscle fiber cross sectional areas.

Loss of skeletal muscle mass, strength and quality has functional consequences. Weakness of the lower extremities has been associated with difficulties in rising from a chair and ascending and descending stairs (53). Schiller et al (53) studied 82 healthy community dwelling women aged 20-78 years (37 Caucasians, 45 Hispanic). The hypothesis underlining this study was that Hispanic women may undergo greater age-related reduction in physical functional capacity compared with Caucasian women, and a greater rate of reduction in muscle strength with aging could contribute to this reduction in physical function. Quadriceps muscle strength was measured using a 1 Repetition Maximum test (1RM). Functional performance was determined by three timed tests; 10 m walking, repetitive chair stands and stair ascending and descending. Both groups had similar rates of age-related decline in quadriceps muscle strength, and there was no difference between the groups in the mean absolute quadriceps muscle strength. There were also age-related increases in the time to perform functional performance tasks with no differences between the groups. Moreover there was an inverse relationship between quadriceps muscle strength and each measure of functional performance in both groups except for 10 m walking for Caucasians. A stepwise multiple regression model was performed to predict the age-related reduction in functional performance. Quadriceps muscle strength, normalized for fat free mass, was the primary predictor in both groups; accounting for 40% of variance in age-

related reduction in functional performance. Age was the secondary predictor for both groups, accounting for an additional 12% and 9% in the variance for Caucasians and Hispanic, respectively.

Alexander et al (54) measured the maximum knee extension torques from 22 healthy young (mean age 22) and 23 healthy older adults (mean age 71) while rising from a chair under eight different conditions: 1) from raised seat that allowed 45 degrees knee flexion, 2) 100% of floor to knee height (KH), 3) 80% KH, 4) 65% KH, 5) seat kept at 100% KH with fast rising, 6) seat at 100% KH with slow rising, 7) at comfortable rate and seat at 100% KH while keeping the feet on 12 cm wide beam (wide beam condition) and 8) at a comfortable rate with seat at 100% of KH while keeping the feet on a beam ¼ of their foot length (narrow beam condition). The joint torques used during these tasks did not differ substantially between young and old adults. Greater knee torques were needed during lower seat height and during fast rises. However the maximum voluntary isokinetic knee extensor strength at 120 degrees/sec was greater for the younger compared to older adults ($p<0.001$). Accordingly the percentage of maximum available knee strength used for each task was greater in older compared to younger adults ($p<0.001$). Alexander et al concluded that older adults have more difficulty rising from a chair than younger adults. Therefore when rising from a low chair, the strength required may exceed the capacity available in older adults making them unable to rise successfully.

Hurley et al (55) compared quadriceps muscle strength, activation pattern, proprioceptive activity, postural stability, and functional performance in 20 young (mean age 23), 10 middle aged (mean age 56) and 15 old (mean age 72) adults. All subjects were healthy and living independently. Isometric quadriceps muscle strength was measured at 90 degrees of knee flexion. Quadriceps activation was measured by superimposing percutaneous electrical

stimulation on isometric quadriceps MVC. Proprioceptive activity was measured by the joint position sense; the subjects were blindfolded and asked to slowly extend their knees to random angles measured by electro-goniometer. Subjects were then allowed to see their knee positions, and were instructed to reproduce the test angles from when they were blindfolded. The mean error between the test and reproduced angles was calculated. Postural stability was also measured using a sway meter under three different tasks; bipedal stance with eyes open, bipedal stance with eyes closed and one leg stance with eyes open. Measures of functional performance included 50 feet walking speed, Get Up and Go Test, and stair ascent and descent. The results indicated that the older subjects were weaker compared to younger and middle aged subjects and quadriceps muscle strength was negatively correlated with age (r = -.511), which means that it decreased with aging. There was no difference between the groups in quadriceps activation and it was not associated with age. Joint position sense was worse in older adults group compared to the other groups, and it was associated negatively with age (r = -.603), which means it became worse with aging. The three groups were similar in postural stability during bipedal stance with eyes open. But with eyes closed, the middle aged and older subjects were less stable than younger subjects. During one leg stance, the older and middle-aged subjects were not able to maintain stance for 15 seconds, therefore data for those groups were collected for 7 seconds. Older subjects were less stable than the young and middle aged subjects. Older subjects also were slower in all measures of functional performance than the young and middle aged subjects. The aggregate functional performance time was calculated for all individual functional performance tests and it was used to determine its association with age, quadriceps muscle strength, and joint position sense. As subjects became older, they needed longer aggregate time

to perform all functional tests. Moreover, longer aggregate functional performance time was associated with weak quadriceps muscle strength and poor joint position sense.

According to the above evidence, there are age-related structural changes in the quadriceps muscle and decline in quadriceps muscle strength. These impairments are associated with slow gait speed (33, 55), and also associated with slow performance of physical functional tasks such as chair rise (53, 54), stair ascending and descending (53, 55). These functional impairments can lead to loss of physical functional independence (35).

1.2.2   The Effects of Strength Training on Skeletal Muscles in Elderly Subjects

Maintaining muscle strength and size with aging has become a significant issue for elderly people who are trying to maintain independent living and good quality of life (30). Several studies on elderly men and women have reported on the effect of resistance exercises in reversing sarcopenia related problems (30, 56).

Trappe et al (30) studied the effect of high intensity resistance exercises performed once per week for maintaining skeletal muscle strength and size in a group of older men who completed a resistance-training regimen. Ten elderly men participated in the study. Quadriceps muscle strength and cross sectional area were measured at the beginning of the study (T1) by using one repetition maximum (1RM) and CT scans respectively. All subjects performed a 12 week training program to strengthen the quadriceps muscle. Bilateral isotonic leg extension at 80% of 1RM was used as the strengthening exercise. There were 3 training sessions per week; each session was consisted of 2 sets of 10 repetitions and a third set until failure. Following the 12 week program (T2), subjects were divided into 2 groups; a maintenance program group which performed resistance exercises (3 sets of 10 repetitions at 80 % of 1 RM) once per week for 6

months (T3), and the other group returned to their normal free-living life style. From T1 to T2 there were increases in the quadriceps muscle strength by 45% for the maintenance group and 53% for the other group. From T2 to T3 the maintenance group maintained their quadriceps muscle strength over 6 months of strength training once per week; however quadriceps muscle strength decreased by 11% in the other group over the 6 months period following the training program. Moreover after the 12 week training program, the maintenance group and the other group had 7.4% and 6.5% increases in the quadriceps muscle cross sectional area, respectively. From T2 to T3 the maintenance group had no change in the muscle area; however the other group had a 5% decreases in muscle size.

Sipila et al (56) also investigated the effects of exercises on muscle mass and strength in 42 elderly women. Subjects were randomized to strength (n=16), endurance (n=15), and control (n=11) groups. The duration of the training program for both experimental groups was 18 weeks. The strength group was trained by using the leg press, leg extension curl, leg flexion curl in standing and heel raise exercises. The intensity of exercises was gradually increased from 60-75% of 1 RM during the 18-week period. The endurance group was trained by progressive track walking twice a week and step aerobics once a week. The controls were told to continue their normal life. The results of this study showed no significant interaction of groups by time on cross sectional area of type I and type IIA muscle fibers. When comparing baseline to 18 months for the separate groups, the strength group showed a significant increase in the cross sectional area of type I muscle fibers.

Frontera and colleges (4) studied the effect of strength conditioning at 80 % of 1 RM of knee flexors and extensors in 12 healthy untrained volunteers aged 60-72 years. The training program consisted of 3 sets of eight repetitions, 3 times / week for 12 weeks. The results of this

study showed weekly increments of 1 RM strength for both knee extensors and flexors. Isokinetic peak torque of knee extensors and flexors was significantly increased at 60 and 240 degrees/second. He found that the area of type I fibers had significantly increased by 33.5% and the area of type II fibers by 27.6%. Under this high intensity exercise one could expect type II fibers to be most affected because they are responsible for producing high amounts of tension. However, in Frontera's study, the cross sectional areas of both fiber types increased and type I showed greater hypertrophy. Based on these findings, if elderly subjects are capable of exercising at intensities equivalent to 80% of their maximum effort, then voluntary exercises could be helpful for inducing hypertrophy of either type I and type II muscle fibers.

Larson et al (16) studied 18 male subjects 22-65 years of age to determine the effect of physical training on muscle morphology and strength in different age groups. The training program was of low intensity (body weight and light weight), and large numbers of repetitions (20-30 repetitions), but it was considered a high volume-training program; it lasted for 60-80 minutes. The program consisted of a 10 minutes warm up, followed by a circuit strength training program. The circuit training consisted of 10 different exercise stations, with exercises including knee extensors every second station. Subjects ran 20 m twice at maximal speed. A 1 min rest was given between each station. Larson and colleges found that for the old age group (56-65 years); the cross sectional areas of type I and type II muscle fibers of the vastus lateralis muscle had significantly increased.

Taaffe et al (57) investigated the effect of exercise intensity on the musculoskeletal system of elderly women aged 65-79 years. Women were randomly assigned to 3 groups; control, high and low intensity resistance training groups. The resistance exercises included leg press, knee extension and knee flexion. The exercise groups performed 3 sets of each exercise, 3

days per week. The low intensity group trained at 40% of 1RM for 14 repetitions in each set. The high intensity group trained at 40% of 1RM for 14 repetitions in the first set, and then they performed 2 sets of 7 repetitions at 80% of 1RM. Leg press, knee extensors and knee flexors strength increased in both exercise groups following 52 weeks of training. There was neither interaction effect nor group effect across time points (0, 3, 6, 9, and 12 months) for each exercise, which means no difference between the training regimens. However there was time effect: knee extensors strength gain from baseline to 3 month was significantly greater for the high intensity group compared to low intensity group. Muscle biopsies were performed pre and post the 52 week training program to measure cross sectional areas of muscle fibers. Type I muscle fiber areas increased by 27.5% (significantly) in the high intensity group and by 9.7% (not significantly) in the low intensity group compared to baseline. However, compared to baseline, type II muscle fiber areas increased, but not significantly, by 22.1% in the high intensity group and by 18.3 % in the low intensity group.

Furthermore Trappe et al (58) investigated the effect of resistance training on single muscle fiber contractile function in elderly women. Seven women (mean age = 74 years) performed a 12-week progressive resistance-training program to strengthen the quadriceps muscle. The program consisted of bilateral isotonic leg extensions at 80% of 1RM. The subjects performed the program 3 times per week. During each session, subjects performed 2 sets of 10 repetitions and a third set until failure. The 1RM was reevaluated every 2 week to ensure that the intensity of the training was maintained at 80% of 1RM. The number of single muscle fibers that were analyzed was 160 pre-training and 153 post-training. The diameter of the type I muscle fibers increased by 24% form baseline, however there was no significant change in the diameter of type IIA muscle fibers. At baseline, type I muscle fibers were 8% larger compared to type IIa

muscle fibers. After the training program, type I muscle fibers were 27% larger compared to type IIA muscle fibers. Single muscle fiber peak tension increased significantly by 33% and 14% in type I and type IIA muscle fibers, respectively, compared to baseline. Before training, there was no difference in peak tension between type I and type IIA muscle fibers. However after the training, type I muscle fibers peak tension was 22% greater compared to type IIA muscle fibers. The single muscle fiber specific tension (peak tension/CSA) was not different form pre to post training. Type I muscle fibers specific tension decreased 18% after the training but this change was not statistically significant. There was no difference in specific tension at baseline between muscle fibers type I and type IIA. However, type IIA muscle fibers specific tension was 25% greater compared to type I muscle fibers after the training program. Single muscle fiber unloaded shortening velocity did not change in both muscle fiber types from pre to post training, however, type IIA muscle fibers unloaded shortening velocity was three times faster compared to type I muscle fibers at both pre and post training. Absolute peak power developed by type I muscle fiber increased significantly by 50%, compared to 25% for type IIA muscle fibers after the training program. On average, type IIA muscle fibers had 5 times greater peak power than type I muscle fibers. When peak power was normalized for cell size, there was no difference from pre to post training in power output for both types of muscle fibers.

Trappe et al (59) also studied the effect of the same resistance training protocol on single muscle fibers in older men. For type I muscle fibers, elderly men and women had similar improvements in the percent change of fiber size, peak tension, and specific tension (peak tension/ CSA). However, type I muscle fibers in elderly men had greater improvements in shortening velocity, absolute power, and normalized power output compared to elderly women. For type IIA muscle fibers, elderly men and women had similar improvements in fiber size, peak

tension, specific tension, and absolute power. However elderly men had greater improvements in type IIA muscle fiber shortening velocity and normalized power output. There was significant difference between elderly men and women at baseline in single muscle fiber contractile properties. Type I muscle fibers from elderly women were larger, stronger, faster and more powerful compared to type I muscle fibers from elderly men. Type IIA muscle fibers were similar in elderly men and women in size and peak tension. However, type IIA muscle fibers in elderly women were faster and produced more peak power than type IIA muscle fibers in elderly men.

In summary according to these studies, quadriceps muscle strengthening exercises at 80% of 1RM, performed 3 times per week for 3 months, are able to increase the strength and the size of the muscle as a whole in elderly people. Frontera et al (4) found that at this level of intensity, type I and type II muscle fibers could be increased (4). However, Trappe et al (58, 59) showed that type I single muscle fibers' cross sectional area increased after training at 80% of 1RM but not type II single muscle fiber. Traaffe et al (57) also found that training at high intensity (80% of 1RM) for 1 year increased the cross sectional area of type I but not type II muscle fibers. Training at 60-75% of 1RM for 18 weeks produced a significant increase in type I muscle fiber cross sectional area (56). In contrast, with low resistance exercises, there was no change in type II muscle fibers' area (57), unless the exercise was very intense and done with a large number of repetitions (16).

1.2.3 The Role of Neuromuscular Electrical Stimulation in Improving Skeletal Muscle Strength

The use of neuromuscular electrical stimulation is well documented for improving quadriceps femoris muscle torque output. Delitto et al (60) compared the effectiveness of electrical stimulation vs. voluntary exercise training protocols in strengthening quadriceps and hamstring muscles in patients with anterior cruciate ligament (ACL) injury. Twenty subjects whom undergone ACL reconstruction surgery participated in the study. Subjects in the electrical stimulation group were treated 5 times a week for 3 weeks. The treatment consisted of 15 electrically induced quadriceps and hamstring muscles co-contractions. Subjects in the voluntary exercise group were instructed to perform 15 maximal co-contractions of their thigh muscles 5 times a week for 3 weeks. Gravity corrected quadriceps and hamstring muscles strength percentages of the involved relative to the uninvolved knees were calculated. After training, the electrical stimulation group had higher quadriceps and hamstring muscles strength percentages (78.8%, 94.1% respectively) compared to the voluntary exercise group (51.7%, 70% respectively). Snyder Mackler et al (61) also studied the effectiveness of NMES for strengthening the quadriceps muscle as an adjunct to ongoing intensive rehabilitation in the early postoperative phase after ACL reconstruction. Subjects were randomly assigned to 4 treatment groups; high intensity NMES, high-level voluntary exercise, low intensity NMES, and combined low and high intensity NMES groups. All subjects received the intensive rehabilitation program 3 times per week from the first to the sixth postoperative week. Subjects in the high intensity NMES group were treated 3 times a week throughout the entire treatment period. The treatment consisted of 15 electrically elicited contractions of the quadriceps muscle at 65 degrees of knee flexion. The amplitude of the current was set to the maximal tolerable level of each subject.

Subjects in the high-level voluntary exercise group were asked to perform 3 sets of 15 maximal quadriceps muscle contractions 3 times a week for the entire treatment period. The low intensity NMES group self-administered the NMES using a portable device designed for home use. These subjects were asked to apply the stimulation on their quadriceps muscle for 15 minutes 4 times a day, 5 days a week. Isometric quadriceps muscle strength of the involved knee was measured and expressed as percentage of the isometric quadriceps muscle strength of the uninvolved side. Quadriceps muscle strength was at least 70% of the strength of the uninvolved knee in the groups that were treated with high intensity NMES, 57% of the uninvolved side in the group treated with high-level voluntary exercise, and 51% in the low intensity NMES group. Moreover, Fitzgerald et al (62) studied the effectiveness of a modified NMES training program as additional treatment to improve quadriceps muscle strength following ACL reconstruction. Forty-three subjects with ACL reconstruction were randomly assigned to either the NMES + rehabilitation group or the rehabilitation only group. The results of this study showed greater quadriceps muscle strength (effect size= .48) at 12 weeks of rehabilitation for the NMES group compared to the rehabilitation only group. Subjects in the NMES group reported better levels on the knee function measures at both 12 (effect size= .72) and 16 (effect size= .65) weeks of rehabilitation compared to the rehabilitation only group.

During voluntary muscle contraction, small motor units, type I muscle fibers, are recruited first to meet the required force to accomplish the task, and as the required force of contraction increases, larger motor units (type II muscle fibers) are recruited to meet the demand of increasing force. However the Henneman principle of motor units' recruitment does not apply during electrically induced muscle contraction. Stephens et al (63) found that cutaneous

stimulation of the first dorsal interosseous muscle would reverse the pattern of recruitment of muscle fibers; type II first then type I.

The theory of reversal of recruitment pattern has since been adopted in electrical stimulation research. For example, Kubiak et al (64) compared isometric and isokinetic strength in 3 groups; isometric exercise, electrical stimulation and control groups. The outcome measurements were: 1) isometric quadriceps muscle strength at 60 degrees of knee flexion, 2) isokinetic quadriceps and 3) isokinetic hamstring muscles strength at 60 degrees/sec using a CYBEX II device. The exercise group performed 10 maximum voluntary isometric contractions (MVIC) while seated on the CYBEX II device with their knees maintained at 60 degrees of flexion. Each contraction lasted for 10 sec, and rest between contractions was 50 sec. Subjects in the electrical stimulation group were positioned in the same way as the subjects in the exercise group. The quadriceps muscle was stimulated by using the Electrostem-180. Each contraction lasted for 10 sec again; 50 sec of rest was given between contractions. This protocol sought to evoke muscle contraction of at least 45% of the MVIC. There were 15 training sessions for the exercise and the electrical stimulation groups. The duration of the study was 5 weeks. Subjects in the electrical stimulation group took 9 sessions to attain strength of contractions equal to 45% of their MVIC. By the end of the study, subjects in the electrical stimulation group tolerated intensity that produced a contraction of at least 75% of their MVIC.

There was no difference between the groups on isokinetic quadriceps and hamstring muscles strength after the training program. Similarly there was no difference between the electrical stimulation and the isometric exercise groups on isometric quadriceps muscle strength after the training program, although the isometric exercise group showed a trend of a greater percentage of change in strength. However, both groups had significantly greater strength

compared to the control group. Kubiak et al (64) explained the trend of more gain in isometric strength in the exercise group as the reversal of the recruitment pattern with electrical stimulation. Kubiak et al (64) also discussed that stressing muscle fibers within the motor units are required to increase their strength. They further explained that electrical stimulation would first recruit type II muscle fibers because their axons are large in diameter. However type I muscle fibers might not be recruited unless the intensity of electrical stimulation is very high that could produce a contraction at least equal to MVIC. Subsequently, not all type I muscle fibers would be recruited; and therefore not stressed. Furthermore, voluntary isometric strengthening exercise and the pre and post training measurement of isometric quadriceps muscle strength would follow the Henneman principle of motor unit recruitment. In this case type I muscle fibers would be recruited first and used most. According to Kubaik's point of view, stressing type I muscle fibers in isometric exercise but not in electrical stimulation might explain the trend in isometric strength difference between the training groups.

Sinacore et al (20) also found that electrical stimulation selectively activated type II muscle fibers in a single case study to determine the pattern of activation of muscle fibers. The subject's quadriceps muscle was stimulated at 80 % of his maximum voluntary contraction. A modified glycogen depletion method was used to determine the activation of muscle fibers; pre stimulation muscle biopsy showed uniform staining with Periodic Acid Schiff (PAS) positive in all muscle fibers. Immediately after stimulation, muscle biopsy showed glycogen depletion from type II muscle fibers.

Recent research on the effect of constant and variable frequency train of electrical stimulation in augmenting torque output in fatigued skeletal muscle has changed the belief that electrical stimulation preferentially activates type II muscle fibers (65, 66). Variable frequency

17

train (VFT) is a method to counteract force loss during electrical stimulation due to fatigue (65, 66). VFT is a variable pattern of electrical stimulation, in which few high frequency pulses are followed by several lower constant frequency pulses within a train of stimulation administered to counter fatigue (66).

Slade et al (66) studied the effect of VFT compared to constant frequency train (CFT) in augmenting torque output in fatigued quadriceps muscles at 2 different stimulation amplitudes. Six subjects participated in the study. Maximum voluntary contractions were performed and the peak MVC was recorded. Then the current intensity necessary to produce 25% or 50% of MVC was determined for a moderate or high amplitude protocol. Then the quadriceps muscle was potentiated with six pulse CFTs (200-μs square wave pluses, 70 ms interpulse interval) that were delivered every 5 sec until force leveled. When the muscle was potentiated, a CFT and a VFT were delivered. Then the muscle was fatigued by using 180 six pulse CFTs delivered at a 50% duty cycle. After fatigue, a CFT and a VFT were delivered randomly. Moderate and high amplitude protocols were performed more than 3 weeks apart. The mean MVC was 204 N.m. Peak torque produced by moderate and high amplitude electrical stimulation protocols, before fatigue, were 27 and 51 N.m, respectively.

During the fatigue protocol, peak torque decreased 60% and 65% in the moderate and high amplitude protocols, respectively. If reversal of muscle fibers recruitment occurred during electrical stimulation, type I muscle fibers would be recruited as the intensity of electrical stimulation increases from moderate to high. Because type I muscle fibers are fatigue resistant, peak torque produced by high amplitude CFT would be decreased to a lesser degree compared to moderate amplitude CFT during fatigue protocol. However peak torques associated with both moderate and high amplitude protocols decreased similarly during fatigue protocol. Slade et al

(66) compared the difference between VFT and CFT in the time from 20% to 80% (T20-80) of peak torque induced by moderate and high amplitude electrical stimulation. There was significant difference between VFT and CFT in T20-80; VFT had a faster rise in torque (form 20% - 80% of peak torque) compared to CFT. However there was no difference between moderate and high amplitude in the T20-80. If reversal of muscle fibers recruitment occurred during electrical stimulation, the T20-80 of peak torque produced by high amplitude electrical stimulation would be greater compared to T20-80 of peak torque produced by moderate amplitude electrical stimulation. Because type I muscle fibers are slow twitch fibers; the time to reach their peak force is longer compared to type II muscle fibers. Therefore as type I muscle fibers are recruited after type II in high amplitude electrical stimulation, the time needed to reach peak torque induced by high amplitude electrical stimulation would be longer compared to the time needed to reach peak torque induced by moderate amplitude electrical stimulation. However, both times were the same both before and after fatigue, which also suggests that there is no preferential recruitment of type II muscle fibers during electrical stimulation.

Bickel et al (65) also studied the effect of VFT compared to CFT in augmenting torque output from tibialis anterior and quadriceps femoris muscles. The experimental procedure was similar to the one used in Slade et al (66). However just the current necessary to produce 25% of MVIC was used in the study. Tibialis anterior muscle has 50% more type I muscle fibers compared to quadriceps muscle. Therefore, it was hypothesized that VFT and CFT would have different effects on muscles that have different muscle fibers composition. The stimulation before fatigue resulted in similar peak torques relative to the MVIC; 27% of MVIC for quadriceps muscle and 28% of MVIC for the tibialis anterior muscle. Torque output declined during the fatigue protocol similarly in both muscles; peak torque declined 57% and 55% for the

quadriceps and tibialis anterior muscles, respectively. The time from 20% - 80% (T20-80) of MVIC was shorter for the VFT compared to CFT for both muscles. The T20-80 for the tibialis anterior was longer compared to quadriceps muscle. Bickel et al explained that it is unlikely that current intensity that produces 25% of MVIC could activate more than 50% of the mass on both muscles. Therefore, if reversal of recruitment occurred, type II muscle fibers would be recruited in both muscles and accordingly the T20-80 would be expected to be the same. However the T20-80 was longer for the tibialis anterior muscle compared to the T20-80 for the quadriceps muscle, which suggested that a random pattern of recruitment had occurred. Furthermore, the similarity in peak torque decline during the fatigue protocol in both muscles also suggests that random pattern of recruitment had occurred.

According to the above evidence, NMES is an effective method of strengthening skeletal muscles. NMES is able to activate both types of muscle fibers randomly, and it may be more likely to activate type II muscle fibers at relatively low force levels compared to voluntary exercise (6, 21). If this is found to be true, then we may have an alternative treatment to improve muscle size and strength for elderly individuals who cannot perform high intensity exercises to achieve these same benefits. This alternative approach might result in improved overall physical function for these individuals because there is an association between strengthening exercises and improvements in functional abilities (33).

In chapter two, the effect of NMES compared to an isometric strengthening exercise in inducing structural changes within the quadriceps muscle of elderly subjects will be investigated. Chapter two represents a sub study that is devoted to specific aim 1 in this project. In this sub study we will investigate the ability of NMES to create hypertrophy of the quadriceps muscle of elderly subjects at both the macro and microscopic levels.

1.2.4   Assessment of Muscle Hypertrophy

1.2.4.1 Whole Muscle

CT Images have been used to quantify areas of skeletal muscle and adipose tissues (41 42 44). Mitsiopoulos et al (45) used MRI and CT acquisition and image analysis methods to compare arm and leg adipose tissue free skeletal muscles (ATFSM) cross-sectional area estimates with cadaver estimates. They found that the cross-sectional areas of ATFSM measured by MRI, CT scans, and cadaver analyses were not different from each other. Furthermore, they found that both MRI and CT estimates of ATFSM were highly correlated with the corresponding cadaver values. The findings of this study strongly support that MRI and CT scans are accurate methods for quantifying areas of skeletal muscles in vivo.

Computed tomography has the ability to objectively quantify muscle composition in vivo (40 - 45), and can differentiate tissues in vivo based on their attenuation characteristics (40). The CT image is composed of a 512 X 512 matrix of pixels, and each pixel has a specific number that indicates a specific location within the patient (40). The numeric value of each pixel in the CT image matches to a specific level of gray within the image (40). These values are called Hounsfield Units (HU), which correspond to the linear attenuation coefficient that is associated with the physical properties of the tissues (40). Different tissues have different attenuation value ranges: fat tissues have negative attenuation values (-190 to -30 HU), and muscles have positive attenuation values (0 to 100 HU) (40 47).

CT scans can supply spatial maps of attenuation coefficients within tissues, which can be used to compute tissue areas within a specific range of attenuation values which can be used to calculate mean tissue attenuation values (40). Several studies have taken the advantage of this quantitative ability of the CT scans to calculate muscle sections of the limb without the adipose

tissue intermingled in and around muscles (41-47). Overend et al (46) used CT scans to quantify components of the thigh muscles in young and old men. Cross sectional areas of total thigh, total muscle plus bone, quadriceps compartment, hamstring compartment, bone, and non-muscle tissue within muscle compartments were measured. The results indicated that there was no difference between young and old men on total thigh cross sectional area. However old men had smaller total muscle plus bone, quadriceps, and hamstring muscle areas. By using the attenuation coefficient characteristics in the CT images, old men had greater cross sectional areas of non-muscle tissue within the muscle compartments. This study demonstrated the ability of CT scans to quantify different tissues within the thigh and show differences between young and old men regarding thigh composition.

Goodpaster et al (47) studied the effect of weight loss on regional adiposity and its relation to insulin sensitivity. Thirty-eight obese men and women participated in the study along with 15 men and women who served as controls. One of the regions studied was the thigh. Computer tomography was used to measure the cross sectional area of the thigh muscles, bone, and adipose tissue, and to determine the muscles attenuation characteristics. Muscle area was divided into two parts; normal density muscle which has HU range from 31-100, and low-density muscle which has HU range from 0-30. These measurements were taken before and after caloric restriction induced weight loss. Their results indicate that after the weight loss program, the total adipose tissue was decreased in the obese people. At baseline, obese subjects had greater thigh skeletal muscle area compared to controls. They also had different muscle composition in terms of lower muscle mean attenuation values, and more low density muscle area than controls. However they had similar normal density muscle area as controls. After the weight loss program, there was no change in the normal density muscle area, but there was reduction in low density

muscle area. This study demonstrates the ability of CT images to detect changes in muscle composition as a result of clinical intervention (40).

In summary, CT scans provide a quantitative method of measuring cross sectional areas of skeletal muscles, and its composition. It can detect changes in the area of skeletal muscles and its composition as results of clinical intervention.

1.2.4.2 Muscle Fibers

Histochemical analysis of muscle biopsies has been widely used to identify muscle fibers. It can differentiate muscle fiber types based on ATPase activity. This procedure allows scientists to measure cross sectional areas of muscle fibers. D'Antona et al (48) studied the structure and function of skeletal muscle fibers in male body builders and active untrained male subjects. Muscle biopsies were taken from the vastus lateralis muscle, and fibers have been classified based on the Myosin Heavy Chain (MHC) isoform composition. Their results regarding cross sectional area of muscle fibers indicated that untrained subjects had larger cross sectional areas (CSA) of type I muscle fibers than type IIAX and type IIX fibers. However body builders had smaller CSA of type I than type IIA IIAX and IIX muscle fibers. Type II muscle fibers were larger in body builders than untrained subjects, but type I muscle fibers were not different between the groups. This study demonstrated how muscle biopsy techniques could be used to identify muscle fibers and study their structure; it also can detect individual differences on CSA of different muscle fiber types.

Chilibeck et al (49) studied the effect of strengthening exercises on mitochondrial distribution within muscle fibers in young individuals. Muscle biopsies were taken from the quadriceps muscles pre and post 12 week strength training. Along with mitochondrial distribution, CSA of muscle fibers were also measured in the exercise and control groups. The

23

results indicated that fiber areas in the strengthening exercise group increased by 26% and 28% in type I and type II muscle fibers respectively, but muscle fiber areas in the control group did not change. This study also demonstrated how muscle biopsy techniques could detect changes in CSA of muscle fibers due to clinical intervention.

1.2.5 Power Measurement to Quantify Changes in Type II Muscle Fibers

Type II muscle fibers are fast-twitch and are responsible for generating strength at higher contraction speeds (50). Power varies as a function of both the force exerted and the velocity at which it is produced (50). As a measure of the rate of doing work, power may best represent type II fibers activity because these fibers are the ones that produce greater tension per unit time (50).

Rothstein et al (50) compared isokinetic measures of peak torque and power in patients with rheumatic diseases, who were on long-term corticosteroid therapy, to healthy control subjects to examine if isokinetic measures could reflect the deficits that accompany the atrophy of type II muscle fibers in people with rheumatic diseases. Because long term corticosteroid use is associated with atrophy of type II muscle fibers, and type II muscle fibers are responsible for higher contraction speeds and higher tension production, it was hypothesized that there would be consistently lower power output as the speed of contraction increased during isokinetic testing for the rheumatoid subjects compared to the controls (50). An isokinetic dynamometer was used to measure peak torque and power output from the left quadriceps muscle at 4 different velocities of contraction; 30, 60, 90, and 120 degrees per second. Peak torque and power values for each velocity, and the slopes of the peak torque-velocity and power-velocity regression lines were compared between the diseased group and the control group.

The results of Rothstein's study indicated that the mean peak torque was higher for the control group at all velocities of contractions, but they were statistically significant only at 30 and 120 degrees per second. The slopes for the peak torque-velocity regression lines for both groups were identical, which means that the lines were parallel. The power outputs for the control group were significantly higher at all velocities of contractions, and the slopes for the power velocity regression line for both groups were significantly different from each other.

According to Rothstein's results (50), the parallel regression lines for the peak torque-velocity relationship could not be used for describing the functional deficits in the diseased group. However, the divergent regression lines for power-velocity relationship for both groups seemed to be a differentiating measure for both groups. The diseased group had a clear deficit in power output that became more prominent at higher velocities of contraction. This deficit in power output may be due to the atrophy of type II muscle fibers that is associated with long term corticosteroid use, because those fibers are responsible for generating higher tension at higher velocities of contraction. The power output is a function of both muscle torque output and velocity of contraction. Therefore atrophy of type II muscle fibers will be manifested in less force generated at higher contraction speeds and accordingly less power output.

Based on Rothstein's study (50), it seems reasonable that we should examine differences in power output between subjects who receive NMES and those that perform voluntary exercise in this study. If the NMES intervention is likely to activate a greater proportion of type II muscle fibers at lower contraction intensities compared to voluntary exercise, then the changes in power from pre to post treatment should be higher at faster speeds of contractions for subjects receiving NMES compared to those who received voluntary exercise at comparable torque output levels.

The effect of NMES compared to a voluntary isometric strengthening exercise in improving the isokinetic quadriceps muscle power and the performance based functional power will be examined in chapter 3. Chapter 3 represents the second sub study for this project and it covers specific aims 2 and 3. In this sub study, we will investigate whether the probable NMES induced structural changes within the quadriceps muscles of the elderly could translate in improvements in quadriceps muscle power and the performance based functional power.

2.0 CHAPTER TWO: NMES INDUCED STRUCTURAL CHANGES IN QUADRICEPS MUSCLE OF ELDERLY SUBJECTS: A RANDOMIZED TRIAL

2.1 BACKGROUND AND SIGNIFICANCE

Aging is associated with structural changes within the skeletal muscles. One of these structural changes is the loss of muscle mass, which could be attributed to the reduction in the number of muscle fibers within the aging muscle (1). Therefore the abnormality in the quantity of the contractile tissue could explain the reduced ability of the elderly to generate muscle forces (3). Atrophy of muscle fibers is another structural transformation within the aging muscle. Atrophy of muscle fibers has been shown to be selective to type II muscle fibers (5, 8). The reduced mass and size of aging muscles has functional consequences; weakness of the lower extremity muscles that happen with aging is associated with reduced ability to perform activities of daily living (33, 53, 54, 55).

The need to maintain muscle strength and size with aging has become an important issue for elderly people who are trying to keep independent living and good quality of life (30). Several studies have reported the effects of resistance exercises in reversing sarcopenia related problems (30, 56). Trappe et al (30) studied the effect of quadriceps muscle strengthening program that is performed at 80% of 1 repetition maximum (1 RM), 3 times per week for 12 weeks, then training 1 time per week at 80 % of 1 RM for 6 months. Thigh muscle cross

sectional area (CSA) was increased by 7.4% for the first 12 weeks of training, and this effect did not change for the next 6 months of training. Sipila et al (56) found that performing strengthening exercises, at gradually increasing intensities from 60-75% of 1 RM for 18 weeks, increased the CSA of type I muscle fibers significantly. Frontera et al (4), found that a knee extension and flexion strengthening program at 80% of 1 RM, 3 times per week for 12 weeks, was able to improve the CSA of type I and type II muscle fibers. Taaffe et al (57) studied the effect of high and low intensities of resistance training for 52 weeks on elderly women. This study showed that the high intensity exercise program improved type I muscle fibers' area significantly, but the area of type II muscle fibers did not improve significantly. The low intensity exercise program did not increase the area of either fiber types. Larson et al (16) studied the effect of low intensity, but high volume training program on muscle morphology at different age groups. The older subjects had significant increase in both types of muscle fibers. Accordingly in order to induce structural changes within the aging muscles, it appears that the elderly will need to either tolerate performing strengthening exercises at high levels of intensity or execute low intensity but high volume strengthening.

Electrical stimulation has also been used to strengthen muscles (17, 18). The use of neuromuscular electrical stimulation (NMES) is well documented for improving quadriceps femoris muscle torque output (60, 61, 62). During voluntary muscle contraction, small motor units (type I muscle fibers) are recruited first to meet the required force to accomplish the task, and as the required force of contraction increases, larger motor units (type II muscle fibers) are recruited to meet the demand of increasing force. However the pattern of recruitment of muscle fibers during NMES induced muscle contraction has been found to be different than that during the voluntary muscle contraction. Recent research on the effect of constant and variable

frequency train of electrical stimulation have found that both types of muscle fibers have equal chance of being activated (6, 19, 20). Furthermore, compared to voluntary exercise, NMES is more likely to activate type II muscle fibers at relatively low force levels (6, 21). Therefore, NMES could be an alternative method to train type II muscle fibers in elderly people without requiring them to exert 80% of their effort or participate in a low intensity but high volume-strengthening regime.

The purpose of this project is to test the effectiveness of NMES that produces 40 % of maximum voluntary contraction (MVC) compared to an exercise that is performed at the same level of intensity in inducing hypertrophy at the macroscopic and the microscopic levels of the quadriceps muscle of elderly population. We hypothesized that subjects who receive NMES will demonstrate larger increase in the cross sectional area (CSA) of the quadriceps muscle and the CSA of type II muscle fibers compared to subjects who receive isometric strengthening exercise.

2.2   METHODOLOGY

2.2.1   Study Sample

Subjects were enrolled in this study if they met the inclusion and exclusion criteria, which included an age between 65-80 years and walking independently without the use of assistive devices. The study exclusion criteria included uncontrolled hypertension, history of cardiovascular disease, history of neurological disease, history of chronic and significant respiratory disease, inflammatory arthritis, muscle disease, the use of anticoagulant and platelet inhibitors, history of quadriceps tendon rupture or patellar fracture, corticosteroid injection to the

quadriceps muscle or patellar tendon within one year or long-term use of corticosteroid medication, and current participation in a regular strengthening exercise program. The Institutional Review Board (IRB) of the University of Pittsburgh approved all study procedures and all subjects signed an informed consent form before participating in the study.

2.2.2   Examination Procedures

2.2.2.1 CT Scan

Axial CT image of the mid-thigh was obtained to quantify changes in the total CSA of the quadriceps femoris muscle and the CSA of fat free portion of the quadriceps muscle mass, which is called normal density muscle (NDM) (40). Subjects were imaged in the supine position with the legs extended flat on the table. An anterior posterior scout image of the entire femur was used to determine the femoral midpoint. The femoral length was measured from the most lateral part of the greater trochanter to the most lateral part of the lateral femoral condyle. A single, 10 mm thick axial image slice was obtained at the femoral midpoint. Scanning parameters of the image are 120kVp and 200-250mA. Total quadriceps muscle CSA was calculated from the CT images using commercially available software (Slice-O-Matic, Tomovision, Montreal, Canada). Areas of skeletal muscle are measured electronically by selecting regions of interest defined by attenuation values: (Hounsfield Units) 0 to 100 HU for the total muscle, and 35 to 100 HU for NDM (40, 73). Quadriceps muscles were distinguished from the Hamstrings by manual tracing. The coefficient of variation for test retest reliability of the mid-thigh attenuation was 0.51% (43).

2.2.2.2 Muscle Biopsy

A physician performed the muscle biopsy procedure on the vastus lateralis muscle of the dominant leg. Using 2% lidocaine as an anesthetic, a small ¼ inch incision was made in the skin and fascia to place the needle, obtaining approximately 100-150mg of tissue. The incision was closed with sterile adhesive strips, and a pressure bandage was applied for a period of 24 hours. Specimens were mounted in OCT mounting-medium (Miles, Inc., Elkhart, IN), and frozen in Isopentane cooled at -160ºC by liquid nitrogen and stored at -80ºC for histochemical analysis. Histochemical analysis was performed on light-microscopic micrographs of 10 μm thick transverse cryostat sections at -29ºC. Initial sections from each frozen muscle block were inspected without stain to ensure that proper cross-sectional cuts were being obtained. Pre and post intervention muscle cryosections for each participant were placed on the same microscope slide so that histochemical staining and image analysis be performed under identical conditions for each participant, eliminating potentially confounding inter- assay variations in the treatment effect. Images were obtained for analysis using a Leica DM 4000b light microscope (Leica Microsystems GmbH, Wetzlar, Germany). The cross sectional area of type I and type IIA muscle fibers were computed using manual planimetry to outline of each muscle fiber using Northern Exposure imaging software (MVIA Inc, Monaca, PA). Intra-assay variability for this method was < 5% (73).

2.2.3 General Training Procedures

2.2.3.1 Training Volume

The training program for both the NMES group and the isometric strengthening group consisted of quadriceps muscle isometric contractions. Subjects in both groups were asked to come for training 3 times per week for 12 weeks.

2.2.3.2 Training Intensity

Maximum voluntary contraction (MVC) was used to set the intensity of training for both the NMES group and isometric strengthening exercise group. The MVC was measured at the beginning of the first session and after every 2 weeks of training using a maximum isometric quadriceps torque test. This test was performed on an isokinetic dynamometer (Biodex System 3 Pro, Shirley NY). Subjects were seated on the isokinetic dynamometer with their hips flexed to 90 degrees. Straps were secured on the trunk to prevent confounding the quadriceps torque output. Then subjects were positioned so that the lateral femoral epicondyle of the knee joint is aligned with the rotational axis of the dynamometer. The force-sensing arm secured to the ankle. The subjects' limb was weighted for gravity correction. The knee being tested was positioned in 70° of flexion. Subjects were asked to exert as much force as possible while trying to extend the knee against the force-sensing arm of the dynamometer. The subjects performed 2 warm up submaximal voluntary isometric contractions and then 2 maximum voluntary isometric contractions on each leg and the greatest peak torque from the 2 contractions was used to set the intensity of training. Each contraction was held for 5 seconds and there was a 30 second rest between repeated contractions.

2.2.4   NMES Training Procedure

Subjects in this group were prepared on the isokinetic dynamometer in the same manner as described before for the MVC testing. Portable stimulator (infinity plus, embi company, St. Paul, Minnesota) was used to stimulate the quadriceps muscle. Two electrodes were placed on the quadriceps muscle; one proximally on the vastus lateralis muscle and the other was placed distally on the vastus medialis muscle (figure 2-1). Markers of 40% +/- 5% of MVC were displayed on a monitor as a target intensity of training (figure 2-2). Then NMES was turned on and its amplitude was gradually increased to produce a torque curve that was maintained at the target intensity. The NMES parameters include a pulse rate of 75 pulses per second and a pulse duration 250 microseconds (26). Subjects had 10 electrically induced quadriceps contractions on each limb. Each contraction lasted for 14 seconds; 4 seconds ramp up to reach the required intensity (40 % of MVC), 6-second stimulation at the required intensity, and 4 seconds of ramp down to zero intensity. There was 50 seconds rest between contractions.

2.2.5   Isometric Exercise Training Procedures

Subjects in this group were prepared in the same manner as those in the NMES group. Subjects were asked to voluntarily perform isometric quadriceps muscle contractions until the torque output, displayed on the monitor screen of the dynamometer, reaches the window of 40% +/- 5% of the torque produced during subject's maximum voluntary contraction. They were asked to hold their muscle contraction for 6 seconds, and then relax slowly to zero torque level. Rest between contractions was 50 seconds. They repeated the same process 10 times for each side.

2.2.6 Data Analysis

Statistical analyses were performed using SPSS version 19 for Mac (IBM, SPSS statistics). Average quadriceps CSA, average quadriceps NDM CSA, type I and type IIA muscle fibers' CSA were analyzed using mixed ANOVA (group X time) model. The probability of error was set at 5% for all the analyses.

2.3   RESULTS

Baseline characteristics of study sample are shown in Table 2-1. Our study sample was comparable to the participants who were included in a sub study of the Health ABC study cohort (40) in terms of age, height, weight and quadriceps muscle CSA. However, our sample was healthier that the study sample that participated in the ancillary project to the LIFE-P Study (70) based on the short physical performance battery (SPPB). Twenty-three subjects were enrolled in this study. Three subjects withdrew from participation after signing the informed consent form due to inability to dedicate time for the training sessions. Twenty subjects (mean age +/- SD = 71.2 +/- 4.41, 13 women) completed the baseline visits then were randomized using sealed envelopes to either the exercise group or NMES group. Nine subjects were randomized to the exercise group, and 11 subjects were randomized to the NMES group. One subject from the exercise group was lost to follow up due to unwillingness to continue with the study. One subject from the NMES group was not able to perform follow up muscle biopsy due to medical reasons. One subject's muscle sample from the NMES group has freezing effect and we were not able to extract data from it. Follow up muscle biopsy procedure was not successful in 2 subjects, one in

each group, due to the inability to get muscle tissue from the samples. Therefore, complete CT scan data was extracted from 19 subjects, and complete muscle biopsy data was extracted from 15 subjects (Figure 2-3). There were 3 subjects in the NMES group who were not complaint with the training procedures: 1 subject was not able to tolerate NMES to produce 40% of MVC, 2 subjects were not able to attend more than 7 training sessions.

Mean Changes and confidence intervals of the outcome measures are presented in Table 2-2. Statistical significance and effect sizes for the main and interaction effects are presented in table 2-3. There was no difference between the groups on the quadriceps muscle CSA averaged across time. Also there was no difference from pre to post training on quadriceps muscle CSA averaged across group. However the NMES group had a significant increase in the average quadriceps muscle CSA from pre to post training (partial η^2 = .665, p= .001) while the exercise group had no change in the average quadriceps muscle CSA during this time frame (partial η^2 = .133, p= .375). Therefore there was an interaction effect between groups over time (Figure 4). The change in the average quadriceps muscle CSA from pre to post training was significantly larger among the NMES group than that for the exercise group (mean change= -1.36, SD= 3.76).

There was no difference between the groups on the quadriceps NDM CSA averaged across time. Also there was no difference from pre to post training on the quadriceps NDM CSA averaged across groups. However the NMES group had a significant increase in the average quadriceps NDM CSA from pre to post training (partial η^2 = .475, p= .013) while the exercise group had no change in average quadriceps NDM CSA during this time frame (partial η^2 = .461, p= .064). Therefore there was an interaction effect on the average quadriceps NDM CSA between the groups from pre to post training (Figure 5). The change in the average quadriceps

NDM CSA from pre to post training was significantly larger for the NMES group (mean change= 2.41, SD= 2.66) than that for the exercise group (mean change= -2.15, SD= 2.51).

At the microscopic level, the minimum and the maximum number of type I muscle fibers that were analyzed was 35 to 352 fibers pre training and 20 to 332 fibers post training. The range of type IIA muscle fibers that were analyzed pre training was 25 to 288 and for post training the range was 32 to 345 fibers. There was no difference between the groups on type I muscle fibers CSA averaged across time. Muscle fibers type I CSA was significantly different from pre to post training averaged across group. Follow up analysis was performed to test how the CSA of type I muscle fibers changed from pre to post training for each group. Muscle fibers type I CSA was significantly smaller post training than pre training among subjects in the exercise group (partial η^2 = .694, p= .010). However there was no change in the CSA of type I muscle fibers among subjects in the NMES group (partial η^2 = .069, p= .493). The interaction effect on the type I muscle fibers CSA between groups from pre to post training was not statistically significant (Figure 6)

There was no difference between the groups on type IIA muscle fiber CSA averaged across time. There was no difference on type IIA muscle fibers CSA from pre to post training averaged across groups. There was an interaction effect on the CSA of type IIA muscle fibers between the groups from pre to post training (Figure 7). The change in muscle fibers type IIA CSA from pre to post training was significantly larger for the NMES group (mean change= 567.37, SD= 767.19) than that for the exercise group (mean change= -690.13, SD= -692.13).

2.4   DISCUSSION

This study showed that NMES is able to induce structural improvements within the quadriceps muscles of elderly subjects using a dosage of 40 % MVC. Quadriceps muscle isometric contractions induced by using NMES significantly increased the muscle's CSA and the NDM CSA by 2.61 cm² (5.8%) and 2.41 cm² (6.27%) respectively. However performing voluntary quadriceps muscle isometric contractions at the same torque level was not able to change the CSA of the quadriceps muscle (-1.36 cm²) or the quadriceps muscle NDM (-2.15 cm²). The change in quadriceps muscle CSA that occurred with NMES training at 40% of MVC was comparable with the reported changes by other investigators during strength training with voluntary muscle contractions at higher percentages of MVC. Sipila et al (71), found that elderly women who performed strength training for 18 weeks at 60-75% of the 1 RM was able to increase the quadriceps muscle CSA and lean tissue CSA by 2 cm² (4.9%) and 2.3 cm² (6.1%) respectively. Frontera et al (4) reported 9.3% increase in the quadriceps muscle CSA after 12 weeks of training at 80% of 1 RM. Grimby et al (72) reported 3% increase in quadriceps muscle CSA in elderly men who performed 8 weeks of strength training.

To further examine whether the improvements in the quadriceps muscle CSA could lead to improvements in the quality of the quadriceps muscle of the elderly subjects, quadriceps muscle specific torque was calculated. Specific torque provides information about the amount of torque generated per unit area of the contracted muscle. Quadriceps muscle torque was calculated at 3 different isokinetic quadriceps muscle contractions (60, 150 and 240 degrees/second) using isokinetic dynamometer. Quadriceps muscle specific torques was significantly smaller post training than pre training within the NMES group at all speeds of isokinetic contractions (Table 2-4). However, quadriceps muscle specific torque did not change

from pre to post training within the isometric exercise group (Table 2-4). Since the specific torque is inversely related with the CSA of the muscle, the decrease in the quadriceps muscle specific torque within the NMES group could be explained by the significant increase in the CSA of the quadriceps muscle that happened with the NMES training. On the contrary, there was no change in the CSA of the quadriceps muscle within the isometric exercise group, which may explain the unchanged specific torque among subjects in this group.

At the cell level, by looking at figure 6, it seems that the decrease in type I muscle fibers CSA was larger in one group than the other. The exercise group had significant decrease in the CSA of type I muscle fibers by -1221.8 µm² (-28%). But in the NMES group, type I muscle fibers' CSA decreased by -253.7 µm² (-.87%), which is not significant. However, this interaction between the groups from pre to post training on type I muscle fibers CSA failed to reach statistical significance (p= .068), likely due to lack of statistical power. In contrast, the interaction effect on muscle fibers type IIA CSA between the groups from pre to post training was significant (figure 7). The change in type IIA CSA was higher in the NMES group compared to the exercise group. The CSA of type IIA muscle fibers post training appeared to be larger than pre training (change= 567.38 µm², 21.46%) among the NMES groups. In the exercise group the CSA of type IIA appeared to be smaller than pre training (change = -692.13 µm², -18.66%) yet neither the increase in the CSA of type IIA among the NMES group nor the decrease in type IIA CSA among the exercise group reached statistical significance, p values = .075, .088 respectively. Possibly again, the lack of statistical power was the reason for the statistical insignificance.

Sipila et al (56) reported a significant increase in type I muscle fibers CSA (1146 µm² (34%) after 18 weeks of strength training in elderly women. However there was an insignificant

increase in type IIA muscle fibers CSA (change =142 µm², 7%). Frontera et al (4) found that the CSA of type I and type II muscle fibers increased by 33.5% and 27.6% respectively after training at 80% of 1RM for 12 weeks. Trappe et al (58) found that training at 80 % of 1 RM for 12 weeks was able to increase the diameter of type I muscle fibers significantly by 24%, but did not significantly change the diameter of type IIA fibers (6%) in elderly women. Taaffe et al (57) found that the CSA of type I muscle fibers significantly increased by 27.5% after high intensity exercises for 52 weeks, however the change in CSA of type II muscle fibers (22.1%) was not significant. Furthermore, the changes in type I and type II muscle fibers CSA (9.7% and 18.3% respectively) was not significant after low intensity exercise. Accordingly, in this study NMES performed at an intensity of percent MVC that was considerably less than what has been used in previous studies on strength training with voluntary exercise was able to achieve comparable changes in CSA of the quadriceps muscle as a whole and the CSA of the muscle fiber.

High intensity exercises would be able to increase type I muscle fibers CSA, however there is a debate on its effectiveness on type IIA muscle fibers. On the other hand, low intensity exercises might not be able to induce changes in both fiber types. In this study, the NMES group had significant change in type IIA muscle fibers CSA compared to the exercise group. Even though the percent increase in type IIA muscle fibers CSA compared to baseline was not statistically significant, it was equivalent to the increase that is reported with high intensity exercises. Furthermore NMES was able to maintain type I muscle fibers CSA from pre to post training. In contrast, there was a significant decrease in type I muscle fibers CSA in the voluntary exercise group. This result underscores the finding that NMES may be able to minimize atrophy of type I muscle fibers at considerably lower dosages of training than what would be required using voluntary muscle contraction.

These different results could be explained by the pattern of activation of muscle fibers during both voluntary and NMES induced muscle contractions. According to the Henneman principle of motor units' recruitment; during voluntary muscle contraction, small motor units, type I muscle fibers, are recruited first and as the required force of contraction increases, larger motor units, type II muscle fibers, are recruited to meet the demand of increasing force (6). Therefore during low intensity isometric contractions, the need to activate type II muscle fibers becomes less relevant. Furthermore, the level of resistance might not be enough to challenge the muscle and activate structural changes in type I muscle fibers and consequently might not able to maintain its cross sectional area. Some investigators have demonstrated maintenance of type I and IIA CSA using moderate resistance voluntary muscle contractions, however the exercise programs incorporated a variety of exercises (leg press, knee extensions, etc.) and relatively large volumes of exercise (more repetitions and sets). These programs were more intensive than the one used in the current study, which only included 10 isometric contractions at 40% of MVC per session. Yet we were able to demonstrate comparable improvements in CSA of type IIA fibers and maintenance of type I muscle fibers using NMES at considerably less intensity and volume of exercise. Therefore, we believe the results of our study supports our original premise that NMES is able to induce structural changes in the quadriceps muscle at lower levels of MVC that normally would not happen with exercise.

The pattern of activation of muscle fibers is quite different with NMES. Kubiak et al (64) and Sinacore et al (20) proposed the reversal of recruitment pattern with NMES in which type II muscle fibers are recruited first and followed by the recruitment of type I muscle fibers as the intensity of NMES increases. However, the research that has been done on the constant and variable frequency train of electrical stimulation (65, 66) supported the theory of nonselective

40

pattern of muscle fibers recruitment. Therefore type II muscle fibers could be activated even with small levels of torque development. In this study, the NMES group had larger change in the CSA of type IIA muscle fibers than that for the exercise group. Furthermore, NMES was able to maintain the CSA of type I muscle fibers from pre to post training contrary to the exercise group who had significant decrease in the CSA of type I muscle fibers. Our findings seem to be consistent with a non-selective pattern of muscle fibers recruitment for NMES.

Table 2-5 represents a comparison between the subjects in the NMES group who were compliant with the NMES training procedures and those who were not. Subjects are considered compliant if they attended 80% of the proposed training sessions and tolerated NMES that produced 40% of quadriceps muscle MVC. There were larger increases in the quadriceps muscle CSA and the quadriceps NDS CSA among the compliant subjects than the non-compliant ones. The increases in type I and type IIA muscle fibers CSA were larger among the subject who was not able to tolerate NMES to produce 40 % of MVC than the compliant ones. Therefore, in order to induce improvements in the quadriceps muscle CSA, elderly subjects will need to perform larger number of NMES training session that produce 40% of MVC. However improvements in the muscle fibers CSA could be induced with less than 40% of MVC using larger number of training sessions.

2.5   CONCLUSION

The results of the present study indicate that NMES plays an important role in inducing structural changes at the macroscopic and microscopic levels of the quadriceps muscle of elderly subjects. NMES might serve as an alternative training tool to exercises in inducing hypertrophy

of type II muscle fibers for older adults who might not be able to tolerate high intensity exercises. Further research should be done to explore the effect of NMES in comparison to high intensity strength training programs, or investigate the effect of combining NMES with exercises compared to exercises only in inducing structural changes of the muscle fibers in elderly subjects. Further research should be done to explore whether the NMES induced structural changes in skeletal muscles translates into improved functional performance of daily activities for the elderly.

Figure 2-1 Electrodes placement on the quadriceps muscle
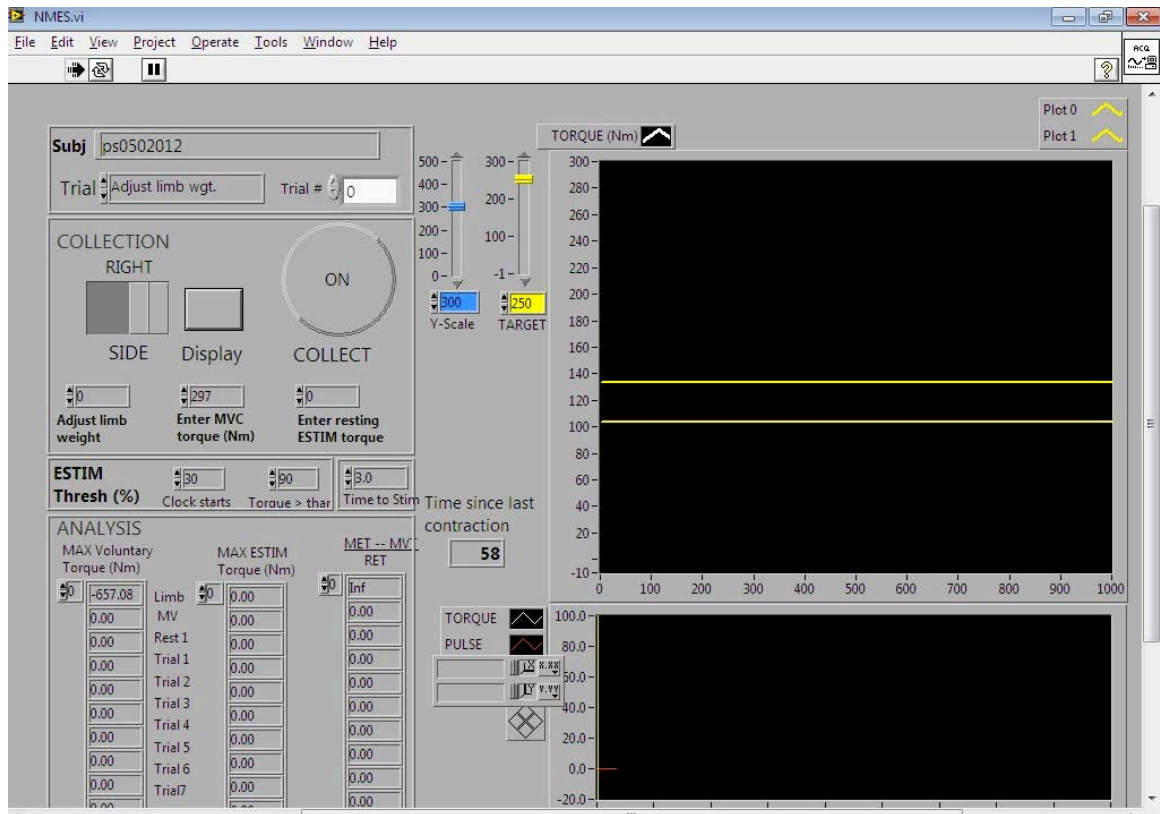
Figure 2-2 A screenshot of the view on the monitor that the subjects see during administration of the training procedures. The 2 yellow lines represent the target intensity window and they are set at 35% and 45% of the MVC
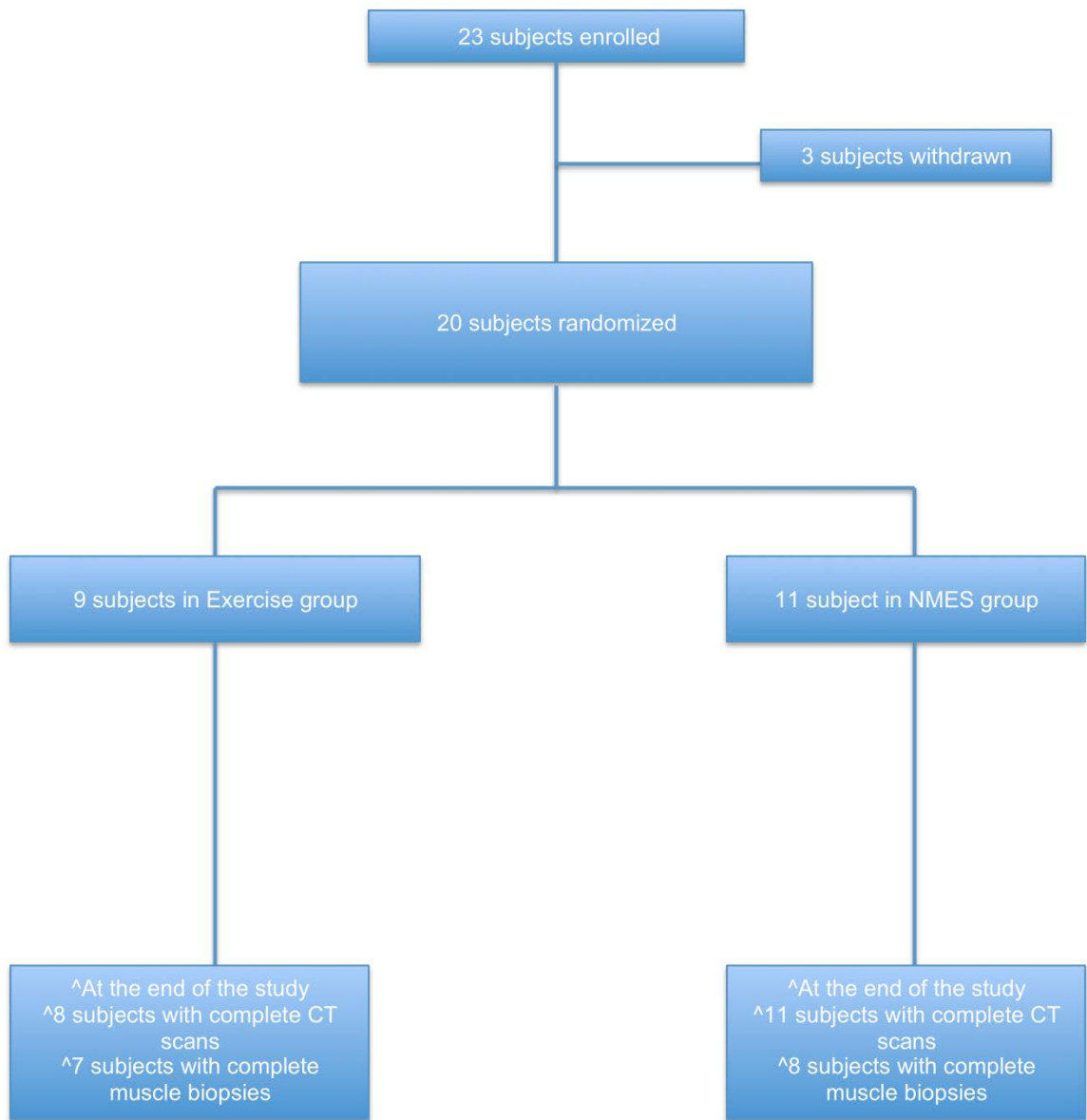
Figure 2-3 Study Flow Diagram

Table 2-1 Baseline characteristics of study sample

	NMES	Exercise
Number	11	9
Age, years, mean (SD)	71.18 (4.38)	71.22 (4.71)
Gender, female / male	7 / 4	6 / 3
Weight, kg, mean (SD)	75.69 (10.04)	81.81 (20.96)
Height, m, mean (SD)	1.68 (.07)	1.66 (.12)
Timed up and go test, min, (SD)	7.87 (2.41)	7.53 (.91)
SPPB (SD)	10.64 (1.43)	11 (1.32)
Quadriceps Muscle CSA cm² (SD)	48.27 (9.60)	48.79 (16.5)
Type I CSA µm² (SD)	3755.22 (1690.23)	4831.52 (1523.53)
Type IIA CSA µm² (SD)	3088.65 (905.28)	3709.31 (1166.44)

Table 2-2 Mean change, confidence intervals and % change of the outcome measures

	NMES			Exercise		
	Mean Change	Confidence interval	% Change	Mean change	Confidence interval	% Change
Quad CSA cm²	2.61	1.30 – 3.92	5.80	-1.36	-4.84 – 2.12	-1.52
Quad NDM CSA cm²	2.41	.62 – 4.20	6.27	-2.15	-4.47 - .17	-4.42
Type I CSA µm²	-253.66	-1083.85 – 576.53	-.87	-1221.80	-2031.74 - -411.86	-28
Type II CSA µm²	567.38	-74.00 – 1208.77	21.46	-692.16	-1524.83 – 140.58	-18.66

Table 2-3 Statistical significance and effect size on the dependent variables

	Group Main Effect			Time Main Effect			Interaction Effect		
	η^2	p value	Observed power	η^2	p value	Observed power	η^2	p value	Observed power
Quadriceps muscle CSA cm²	.004	.798	.057	.052	.365	.142	.355	.009*	.795
Quadriceps NDM CSA cm²	.015	.626	.075	.003	.837	.054	.450	.002*	.924
Type I CSA µm²	.004	.834	.055	.414	.010*	.800	.233	.068	.453
Type IIA CSA µm²	.003	.853	.054	.006	.776	.058	.397	.012*	.772

- Significant at alpha level = .05
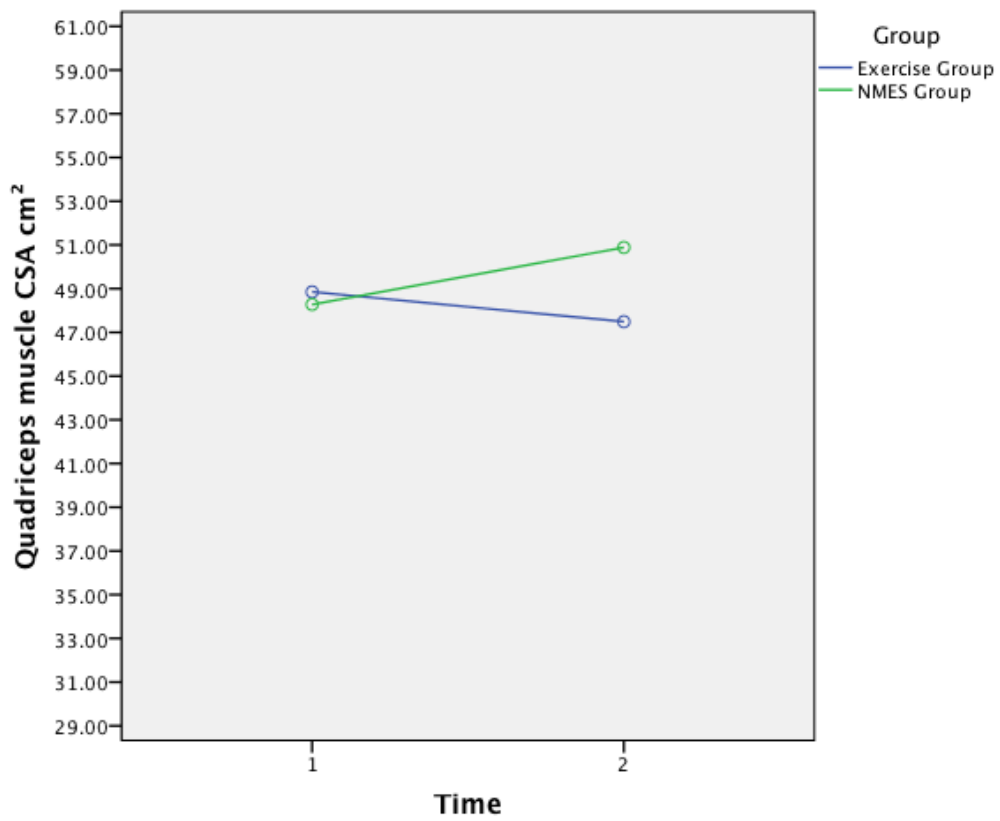
Figure 2-4 Quadriceps muscle CSA by group and time

The interaction effect was significant, F (1, 16) = 8.804, p= .009, partial η² = .355.
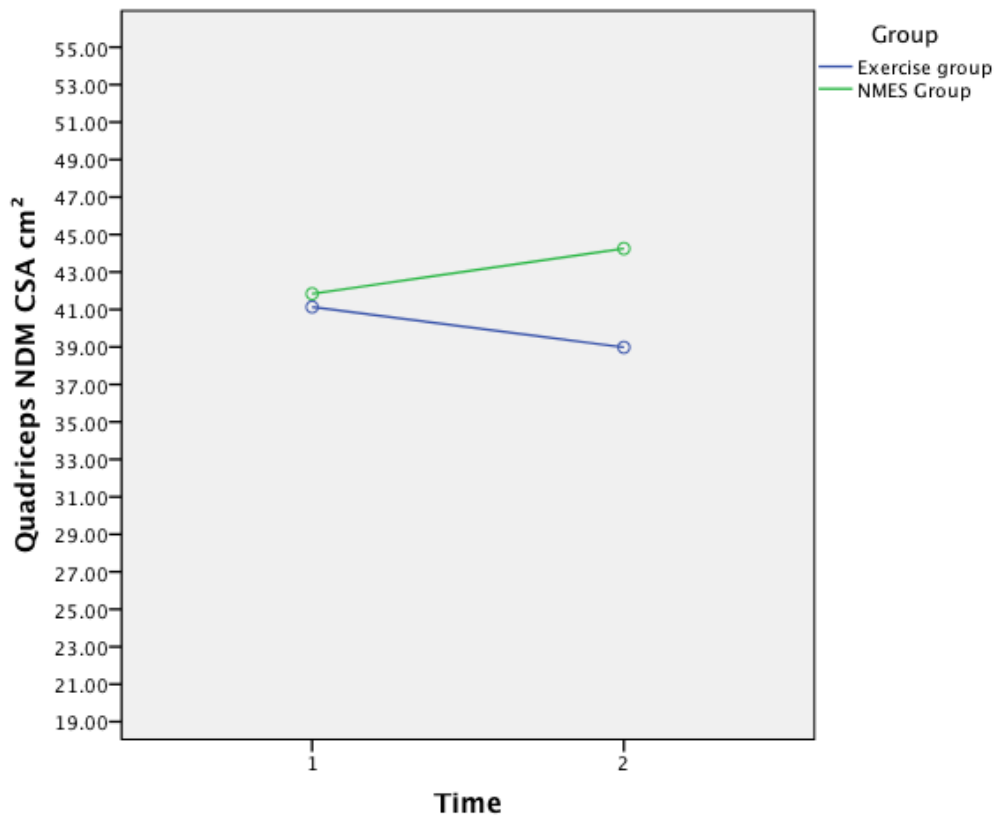
Figure 2-5 Quadriceps Muscle NDM CSA by group and time

The interaction effect was significant, F (1, 16) = 13.11, p= .002, partial η² = .450
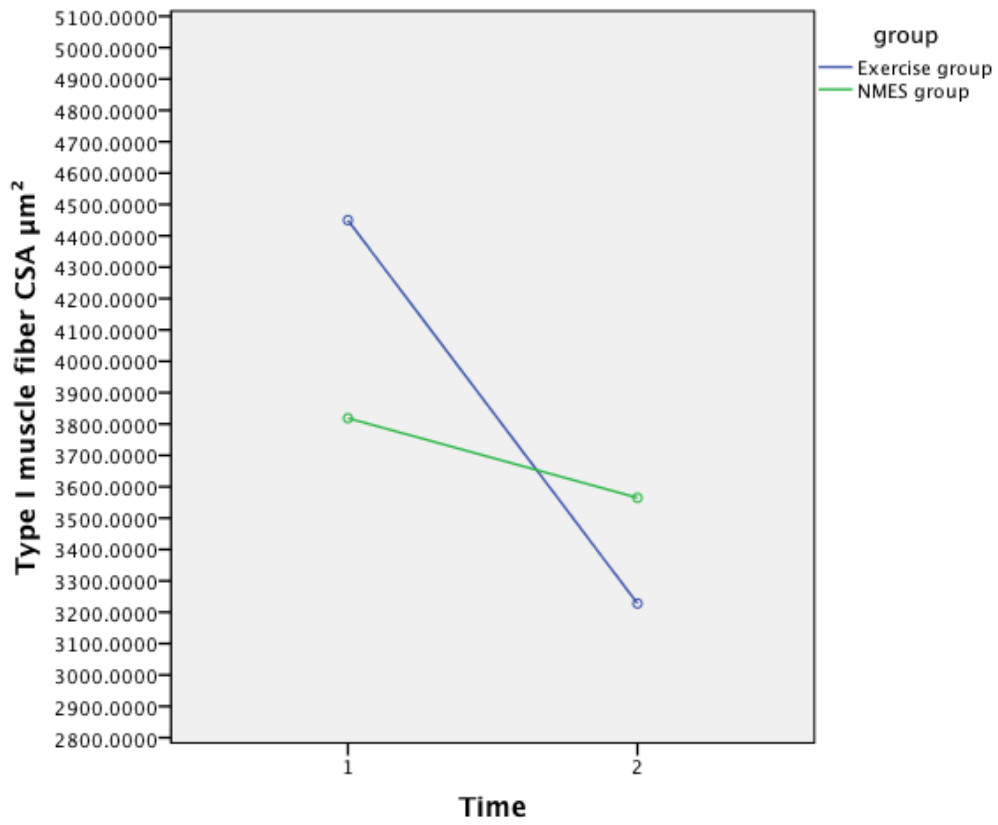
Figure 2-6 Muscle fibers type I CSA by group and time

Interaction effect was not significant, $F_{(1, 13)} = 3.954$, $p = .068$, partial $\eta^2 = .233$.
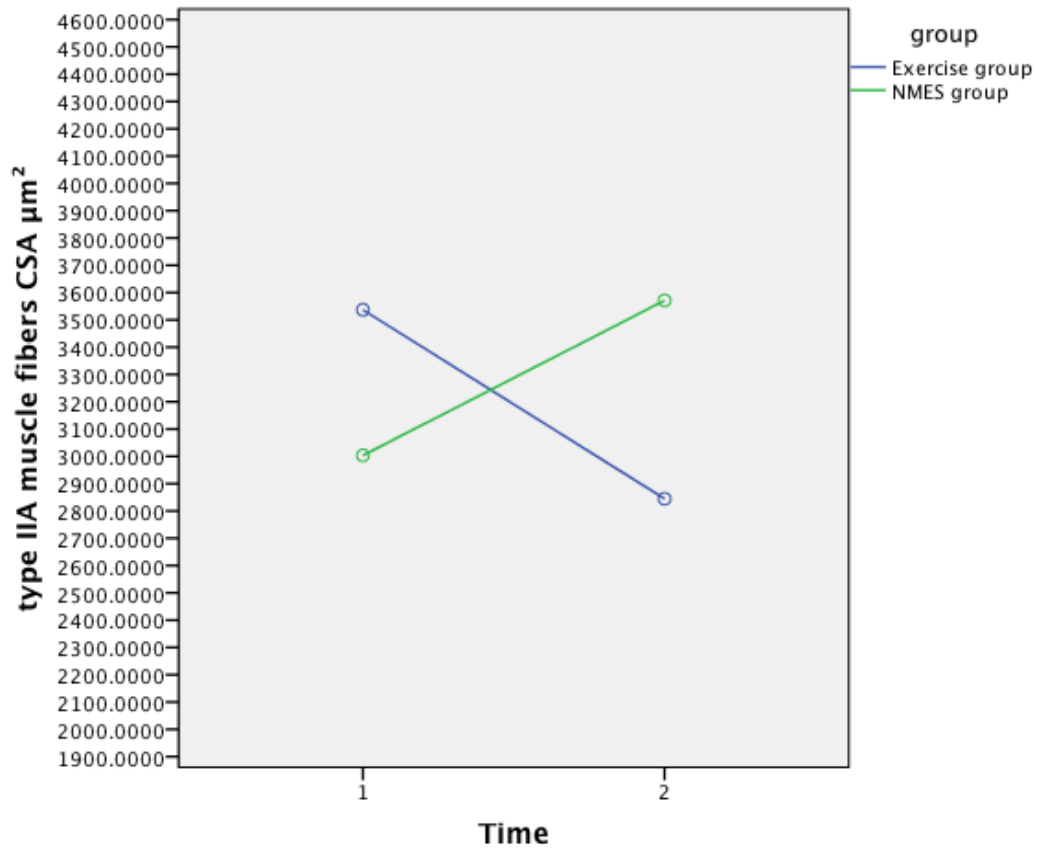
Figure 2-7 Muscle fibers type IIA CSA by group and time

The interaction effect was significant, $F_{(1, 13)} = 8.57$, p= .012, partial $\eta^2 = .397$.

Table 2-4 Specific torque at 60, 150 and 240 degree/ second of isokinetic contraction

		Mean Change	Confidence interval	Percent Change	Effect Size η²	p value	Observed power
Specific Torque	NMES	-.30	-.43 - -.18	-12.01	.737	.000	.123
60	Exercise	.24	-.39 - .88	15.86	.105	.396	.997
Specific Torque	NMES	-.12	-.22 - -.02	-2.29	.432	.020	.125
150	Exercise	.20	-.32 - .71	-1.94	.107	.391	.701
Specific Torque	NMES	-.19	-.29 - -.09	-8.86	.651	.002	.972
240	Exercise	.19	-.12 - .49	1.46	.230	.191	.241

Table 2-5 Comparison between compliant and non-compliant subjects within the NMES group on quadriceps muscle structural changes

	N	Change in Quad CSA cm²	Change in Quad NDM CSA cm²	Change in Type I CSA µm²	Change in Type IIA CSA µm²
Complain with NMES	8	3.31	3.70	-429.75	508.95
Non-Compliant with NMES Number of session	2	1.92	-.65	-	-
Non-Compliant with NMES intensity < 40%	1	-1.60	-1.76	978.99	976.37

3.0   CHAPTER THREE: THE EFFECT OF NMES IN IMPROVING PERFORMANCE BASED PHYSICAL FUNCTION AND QUADRICEPS MUSCLE STRENGTH IN ELDERLY SUBJECTS

3.1   BACKGROUND AND SIGNIFICANCE

Aging is one of the major causes of reduced muscle strength and size, a condition known as sarcopenia (1). Aging is associated with the reduction in the number of muscle fibers in the muscle (3), which might explain the loss of muscle mass. In addition to reducing the number muscle fibers, aging is also associated with reduced size of muscle fibers (5). This atrophy of muscle fibers has been found to be selective to type II fibers (5, 8). Furthermore these age-related structural changes within muscles of the elderly are associated with less muscle force generation compared to young adults. Loss of skeletal muscle mass, strength and quality has functional consequences. Weakness of the lower extremities has been associated with difficulties in rising from a chair, getting out of bed (31, 32), it is related to slow gait speed (33), as well as balance problems and increased risk of falls (34). These functional impairments can lead to loss of physical functional independence (35).

Type II muscle fibers are considered fast twitch fibers, they are responsible of generating force at higher contraction speeds (50). Muscle power is defined as the rate of doing work, and it varies as a function of the force exerted and the velocity at which it is generated (50). Therefore

muscle power might be an important measure to reflect on the performance of type II muscle fibers (50). Rothstein et al (50) compared a group of subjects with rheumatoid arthritis disease to healthy subjects on the quadriceps muscle power outputs at different velocities of isokinetic contractions. Rothstein et al (50) studied the rheumatoid arthritis disease because of its association with atrophy of type II muscle fibers. Rothstein et al (50) found that at all speeds of isokinetic contractions, quadriceps muscle power was significantly lower among the rheumatoid arthritis groups than the healthy group. Furthermore, the slope for power velocity relationship among the rheumatoid arthritis group was lower than that for the healthy group. Therefore isokinetic muscle power was able to discriminate a group that may have deficits in type II muscle fibers from a healthy group. Based on Rothstein's study, if we can target type II muscle fibers in strengthening exercises, improvements in type II muscle fibers could be detected by improvements in isokinetic muscle power output at higher contraction speeds.

Several studies have reported the effects of resistance exercises on muscle fibers. Frontera et al (4) found that quadriceps muscle strengthening exercises at 80% of 1RM, performed 3 times per week for 3 months is able to increase type I and type II muscle fibers' cross sectional area (4). However, Trappe et al (58, 59) demonstrated increases in cross sectional area of single type I muscle fibers but not in single type II muscle fibers after training at 80% of 1RM. Traaffe et al (57) also found that training at high intensity (80% of 1RM) for 1 year increased the cross sectional area of type I but not type II muscle fibers. Training at 60-75% of 1RM for 18 weeks produced a significant increase in type I muscle fiber cross sectional area (56). In contrast, with low resistance exercises, there was no change in the area of both types of muscle fibers (57). Larson et al (16) studied the effect of low intensity, but high volume training program on muscle morphology at different age groups. The older subjects had significant

56

increases in the area of both types of muscle fibers. Accordingly, in order to induce structural changes in type II muscle fibers within the aging muscles, the alternatives would be to use high intensity exercises, or low intensity exercise with high volume, provided that the elderly subjects were able to tolerate these exercise programs.

Electrical stimulation is an effective method of strengthening skeletal muscles (17, 18). Neuromuscular electrical stimulation (NMES) is well documented for improving quadriceps femoris muscle torque output (60, 61, 62). The pattern of recruitment of muscle fibers during NMES induced muscle contraction has been found to be different than that during the voluntary muscle contraction. Kubiak et al (64) and Sinacore et al (20) adopted the theory of reversal of recruitment of muscle fibers during electrically induced muscle contraction. However, recent research on the effect of constant and variable frequency train of electrical stimulation have found that both types of muscle fibers have equal chance of being activated (6, 19, 20). Moreover, compared to voluntary exercise, NMES is more likely to activate type II muscle fibers at relatively low force levels (6, 21). Furthermore, in the experiment described in chapter 2 above we found that subjects in the NMES group had higher change (567.38 µm²) in the CSA of type IIA muscle fibers than that for the subjects in the exercise group (-692.13 µm²). Therefore, NMES could be an alternative method to train type II muscle fibers in elderly people without requiring them to exert 80% of their effort or participate in a low intensity but high volume-strengthening regime. We would also expect that if NMES increased the CSA of type IIA fibers, then this would translate into improvements in muscle power output and functional power.

The purpose of this study is to test the effectiveness of NMES that produces 40 % of maximum voluntary contraction (MVC) compared to an exercise that is performed at the same level of intensity in improving isokinetic quadriceps muscle power and performance based

functional power. We hypothesized that subjects who receive NMES will show greater increase in isokinetic quadriceps muscle power output at higher isokinetic contraction speeds and also will demonstrate larger increase in the performance based functional power compared to subjects who receive isometric strengthening exercise.

3.2   METHODS

3.2.1   Study Sample

Subjects were enrolled in this study if they were between 65-80 years of age and walking independently without the use of assistive devices. Subjects were excluded if they had uncontrolled hypertension, history of cardiovascular disease, history of neurological disease, history of chronic and significant respiratory disease, inflammatory arthritis, muscle disease, the use of anticoagulant and platelet inhibitors, history of quadriceps tendon rupture or patellar fracture, corticosteroid injection to the quadriceps muscle or patellar tendon within one year or long-term use of corticosteroid medication, and current participation in a regular strengthening exercise program. The Institutional Review Board (IRB) of the University of Pittsburgh approved all study procedures and all subjects signed an informed consent form before participating in the study.

3.2.2 Examination Procedures

3.2.2.1 Isokinetic Quadriceps Muscle Power

Isokinetic quadriceps muscle power from the dominant limb was measured using Isokinetic dynamometer (Biodex System 3 Pro, Shirley NY), at 3 contraction velocities; 60, 150, 240 degrees per second. Subjects were seated on the isokinetic dynamometer with the force-sensing arm secured to the ankle. Subjects were asked to exert as much force as possible while extending the knee against the force-sensing arm of the dynamometer through the range from 90 to 0 degrees of flexion. Estimates of intra-tester reliability for measuring isokinetic quadriceps muscle power at 60, 150 and 240 degrees per second has been done in our laboratory and they were high (ICC = .987, .978, .979 for 60, 150 and 240 degrees per second respectively). The subjects performed 4 maximum voluntary contractions at each speed (60, 150, 240 degrees per second) and the mean value of power output for each velocity condition was used in the analysis. During the eccentric phase data were not collected and the subjects were asked to relax their muscles to allow the dynamometer to return the subjects' knees back to 90 degrees of flexion. The value of the isokinetic power at each speed of contraction was extracted from the isokinetic dynamometer during the extension range from 90 to 0 degrees of flexion.

3.2.2.2 Timed Stair Climb Power Test

The timed stair climb test was used as a measure of functional power (67, 68). Subjects were instructed to climb a flight of stairs consisting of 11 steps; each step is 17 cm in height, as fast as they can. The time it took the subject to climb the flight of stairs was recorded. Stair climbing power output was calculated using the formula described by Henwood, et al (68) which is as follows:

$$\text{Power} = \frac{\text{Weight (kg) x Acceleration of Gravity (9.8 m/s}^2\text{) x step height (.17m) x number of steps (11)}}{\text{Time (sec)}}$$

Subjects were given 1 practice trial, followed by 2 test trials. Subjects were given a 2-minute rest between each trial. Power was calculated for each trial as described above and the average of the two trials was used as the power score. Test retest reliability for this measure was high (R= 0.99) (67).

3.2.2.3 The Ramp Power Test

The ramp power test was used as a measure of functional power (69). Subjects were instructed to walk up, as fast as possible, a ramp with a 3.66 m run and a .32 m rise. The time it took to walk up the length of the ramp was recorded. The reliability of ramp power test was high (ICC= 0.966) (69). Ramp climbing power was calculated as described by Signorile, et al (69) according to the following formula:

$$\text{Power} = \frac{\text{Weight (kg) x Acceleration of Gravity (9.8 m/s}^2\text{) x Vertical Distance (.32 m)}}{\text{Time (sec)}}$$

3.2.3   General Training Procedures

3.2.3.1 Training Volume

The training program for both the NMES group and the isometric strengthening group consisted of quadriceps muscle isometric contractions. Subjects in both groups were asked to come for training 3 times per week for 12 weeks.

3.2.3.2 Training Intensity

Maximum voluntary contraction (MVC) was used to set the intensity of training for both the NMES group and isometric strengthening exercise group. The MVC was measured at the beginning of the first session and after every 2 weeks of training using a maximum isometric quadriceps torque test. This test was performed on an isokinetic dynamometer (Biodex System 3 Pro, Shirley NY). Subjects were seated on the isokinetic dynamometer with their hips flexed to 90 degrees. Straps were secured on the trunk to prevent confounding the quadriceps torque output. Then subjects were positioned so that the lateral femoral epicondyle of the knee joint is aligned with the rotational axis of the dynamometer. The force-sensing arm secured to the ankle. The subjects' limb was weighted for gravity correction. The knee being tested was positioned in 70° of flexion. Subjects were asked to exert as much force as possible while trying to extend the knee against the force-sensing arm of the dynamometer. The subjects performed 2 warm up submaximal voluntary isometric contractions and then 2 maximum voluntary isometric contractions on each leg and the greatest peak torque from the 2 contractions was used to set the intensity of training. Each contraction was held for 5 seconds and there was a 30 second rest between repeated contractions.

3.2.3.3 NMES Training Procedure

Subjects in this group were prepared on the isokinetic dynamometer in the same manner as described before for the MVC testing. Portable stimulator (Infinity Plus, Empi company, St. Paul, Minnesota) was used to stimulate the quadriceps muscle. Two electrodes were placed on the quadriceps muscle; one proximally on the vastus lateralis muscle and the other was placed distally on the vastus medialis muscle (figure 2-1). Markers of 40% +/- 5% of MVC were displayed on a monitor as a target intensity of training (figure 2-2). Then NMES was turned on

and its amplitude was gradually increased to produce a torque curve that was maintained at the target intensity. The NMES parameters include a pulse rate of 75 pulses per second and pulse duration 250 microseconds (26). Subjects had 10 electrically induced quadriceps contractions on each limb. Each contraction lasted for 14 seconds; 4 seconds ramp up to reach the required intensity (40 % of MVC), 6-second stimulation at the required intensity, and 4 seconds of ramp down to zero intensity. There was 50 seconds rest between contractions.

3.2.3.4 Isometric Exercise Training Procedures

Subjects in this group were prepared in the same manner as those in the NMES group. Subjects were asked to voluntarily perform isometric quadriceps muscle contractions until the torque output, displayed on the monitor screen of the dynamometer, reaches the window of 40% +/- 5% of the torque produced during subject's maximum voluntary contraction. They were asked to hold their muscle contraction for 6 seconds, and then relax slowly to zero torque level. Rest between contractions was 50 seconds. They repeated the same process 10 times for each side.

3.2.4   Data analysis

Statistical analyses were performed using SPSS version 19 for Mac (IBM, SPSS statistics). Isokinetic quadriceps muscle power output was analyzed using mixed ANCOVA (group X time) model and baseline subject's weight was used as a covariate. Timed stair climb power test and ramp power test were analyzed using mix ANOVA (group X time) model. The probability of error was set at 5% for all the analyses.

3.3   RESULTS

Baseline characteristics of the study sample are shown in Table 2-1. Twenty-three subjects were enrolled in this study. Three subjects withdrew from participation after signing the informed consent form due to inability to dedicate time for the training sessions. Twenty subjects (mean age +/- SD = 71.2 +/- 4.41, 13 women) completed the baseline visits then were randomized using sealed envelopes to either the exercise group or NMES group. Nine subjects were randomized to the exercise group, and 11 subjects were randomized to the NMES group. All 20 subjects finished the follow up testing sessions (figure 3-1). There were 3 subjects in the NMES group who were not complaint with the training procedures: 1 subject was not able to tolerate NMES to produce 40% of MVC, 2 subjects were not able to attend more than 7 training sessions.

Mean changes and confidence intervals of the outcome measures are presented in table 3-1. Statistical significance and effect sizes for the main and interaction effects are presented in table 3-2. There was no significant difference on isokinetic quadriceps muscle power at each speed of contraction between the groups averaged across time after adjusting for weight as a covariate (table 3-2). Also there was no significant difference on isokinetic quadriceps muscle power at each speed of contraction from pre to post training averaged across groups after adjusting for weight as a covariate (table 3-2). There was no interaction between groups from pre to post training on isokinetic quadriceps muscle power at each speed of contraction after adjusting for weight as a covariate (figure 3-2, 3-3, 3-4).

There was no significant difference on the timed stair climb power test between the groups averaged across time (table 3-2). Also there was no significant difference on the timed stair climb power test from pre to post training averaged across groups (table 3-2). The pattern of

change on timed stair climb power test from pre to post training was not statistically different between the groups (figure 3-5).

For the ramp power test, there was no significant difference between the groups averaged across time (table 3-2). Also there was no significant difference on ramp power test from pre to post training averaged across groups (table 3-2). However, the pattern of change on ramp power test from pre to post training was significantly higher for the NMES group than that for the exercise group (figure 3-6).

3.4   DISCUSSION

The purpose of this study was to examine the effectiveness of the NMES in improving isokinetic quadriceps muscle power and performance based functional power of elderly subjects. We hypothesized that subjects in the NMES group will demonstrate higher scores on the isokinetic quadriceps muscle power and performance based functional power. The basis for our hypothesis is the ability of the NMES to activate type II muscle fibers at relatively low MVC levels (6, 21). Type II muscle fibers are responsible of generating high force at short period of time (50) which encompass the 2 components of the power measure. Therefore power measures may represent the status of type II muscle fibers. The results of this study showed that isokinetic quadriceps muscle power did not change from pre to post training for both groups at any speed of isokinetic contraction. Muscle power is a function of the torque generated during the muscle contraction multiplied by the velocity of that contraction. Since the isokinetic contractions are set at pre-defined velocities i.e. 60, 150 and 240 degrees per second, the only variable that could determine the muscle power at each velocity is the torque generated. One explanation for the unchanged

64

isokinetic quadriceps muscle power output in both groups is the specificity of training. Isometric quadriceps muscle training by either voluntary or electrically induced contractions at 40% of MVC might not be enough to transform the isometric strength into improvements in isokinetic torque at different speeds. Although, Folland et al (75) was able to demonstrate the ability of isometric training using voluntary muscle contractions to improve isokinetic torques, the intensity of training was 75% of the maximum force measured before initiation of training, which was considerably higher than the intensity of training in our study. The reason for using low MVC level (40%) as an intensity of training in our study was so that we could test whether NMES could induce training effects in type II muscle fibers at lower intensity of exercise that would not be expected to induce training effects through voluntary exercise.

On visual inspection of the graph of the timed stair climb power test (figure 3-6), it seems that the change form pre to post training for the NMES group (change = 25.57 watt, SD = 42.11) was higher than that for the exercise group (change = -12.16 watt, SD = 53,31). However, this interaction effect between the groups on the timed stair climb power test from pre to post training failed to reach statistical significance (p= .094), likely due to lack of statistical power. In contrast, the interaction effect between the groups on the ramp power test from pre to post training (figure 3-7) was statistically significant (p= .016). The NMES groups had statistically higher change from pre to post training on the ramp power test (change = 9.79 watt, SD= 13.37) (partial η^2 = .371, p= .036). However the exercise group had no change from pre to post training on the ramp power test (change= -4.41, SD= 9.84) (partial η^2 = .184, p= .216).

Based on the results of this study, it seems that the performance based functional power was able to differentiate between the NMES and the exercise groups. However the isokinetic quadriceps muscle power was not able to show differences between the groups. Based on the

equations to measure power from the timed stair climb power and ramp power tests, time to complete the test is the main variable to determine the functional power. Therefore shorter time to complete the test will result in higher scores on the functional power. Performance based functional power tests may allow the muscle torque and the velocity of muscle contraction to interact to produce greater power output than the isokinetic muscle power test that maintains the velocity constant. Therefore functional power tests might be more valid as they permit the interaction of muscle torque and velocity of muscle contraction to occur more naturally.

The inability of the isokinetic quadriceps muscle power test to differentiate between subjects who received NMES and those who received voluntary exercise raises doubts about the usefulness of the isokinetic muscle power tests to reflect on the status of type II muscle fibers in elderly subjects. Even though Rothstein et al (50) was able to demonstrate that isokinetic muscle power was able to discriminate a group with presumed type II muscle fibers atrophy from a healthy group, the maximum velocity that was used in Rothstein's study was 120 degrees per second. Rothstein et al (50) assumed that the subjects during isokinetic testing made maximum effort, which means all available motor units were involved during the isokinetic testing at all chosen speeds of contraction. However, Barnes et al (76) found that motor-unit electrical activity decreases as the velocity of the elbow flexors isokinetic contraction increases from 60,120, 180, 240 to 300 degrees per second. Therefore not all motor units would be activated as the velocity of isokinetic contraction increases. Rothstein's assumption that all motor units were involved might have been correct in the context of his study as velocities were never higher than 120 degrees per second. However, in the present study 2 of the 3 chosen velocities of contraction (60, 150 and 240 degrees per second) are higher than what Rothstein et al (50) used. Since the electrical activity of motor units decrease as the velocity of contractions increases (76), the

66

number of type II muscle fibers that were activated may have decreased as the velocity of isokinetic contraction increases. Consequently isokinetic muscle power at higher contraction speeds might not be reflective of the status of type II muscle fibers.

To further examine whether isokinetic quadriceps muscle power and the performance based functional power tests may be used to reflect the integrity of the CSA of type II muscle fibers, we performed post-hoc Pearson product correlation analysis to examine the relationship between the change in the power test scores and the changes in the CSA of type II muscle fibers. Scatter plot graphs and Correlation coefficients were developed between changes in the CSA of type IIA muscle fibers; isokinetic quadriceps muscle power at each speed of contraction and performance based functional power tests. Pearson product correlations coefficients are presented in table (3-3). Figures (3-7, 3-8, 3-9) represent the scatter plots for the change in the CSA of type IIA muscle fibers and the change in the isokinetic quadriceps muscle power at 60, 150 and 240 degrees per second respectively. There was no correlation between the change in the CSA of type IIA muscle fibers and changes in the isokinetic quadriceps muscle power at any speed of contraction (table 3-3). This finding further supports our premise that isokinetic quadriceps muscle power might not reflect the status of type II muscle fibers.

On the contrary, there was strong significant positive correlation between the change in the CSA of type IIA muscle fibers and the change in the stair climb power test (r= .634, p= .011) and the change in the ramp power test (r= .668, p= .006). Figures (3-10, 3-11) show how higher changes in the CSA of type IIA muscle fibers were associated with greater improvement in scores on stair climb power test and the ramp power test respectively. Therefore performance based functional power tests might be more valid in assessing the integrity of type II muscle

fibers function as they appear to be associated with changes in the CSA of type II muscle fibers compared to isokinetic muscle power.

Table 3-4 represents a comparison between the subjects in the NMES group who were compliant with the NMES training procedures and those who were not on power measure. Subjects are considered compliant if they attended 80% of the proposed training sessions and tolerated NMES that produced 40% of quadriceps muscle MVC. There were minimal changes in the isokinetic quadriceps muscle power and larger increases in the performance based functional power among the compliant subjects than the non-compliant ones.

3.5   CONCLUSION

The results of this study indicate that subjects who received NMES training had improvements in the functional power tests while those in the voluntary exercise group had no change or declined in the performance on the functional power tests. There were no differences between the groups in the change in isokinetic quadriceps muscle power test scores. Post hoc analyses indicated there was a significant positive association between changes in the CSA of type IIA muscle fibers and changes in the performance based functional power test but not with changes in the performance on the isokinetic power test. Therefore the ease of administering the functional performance power tests along with their association with changes in the CSA of type II muscle fibers of elderly subjects make them more valid for assessing muscle power output than the isokinetic muscle power test, which requires special and expensive equipment. However, further research is needed to validate the use of the performance based functional power test to predict improvements in cross sectional area of type II muscle fibers in the elderly.
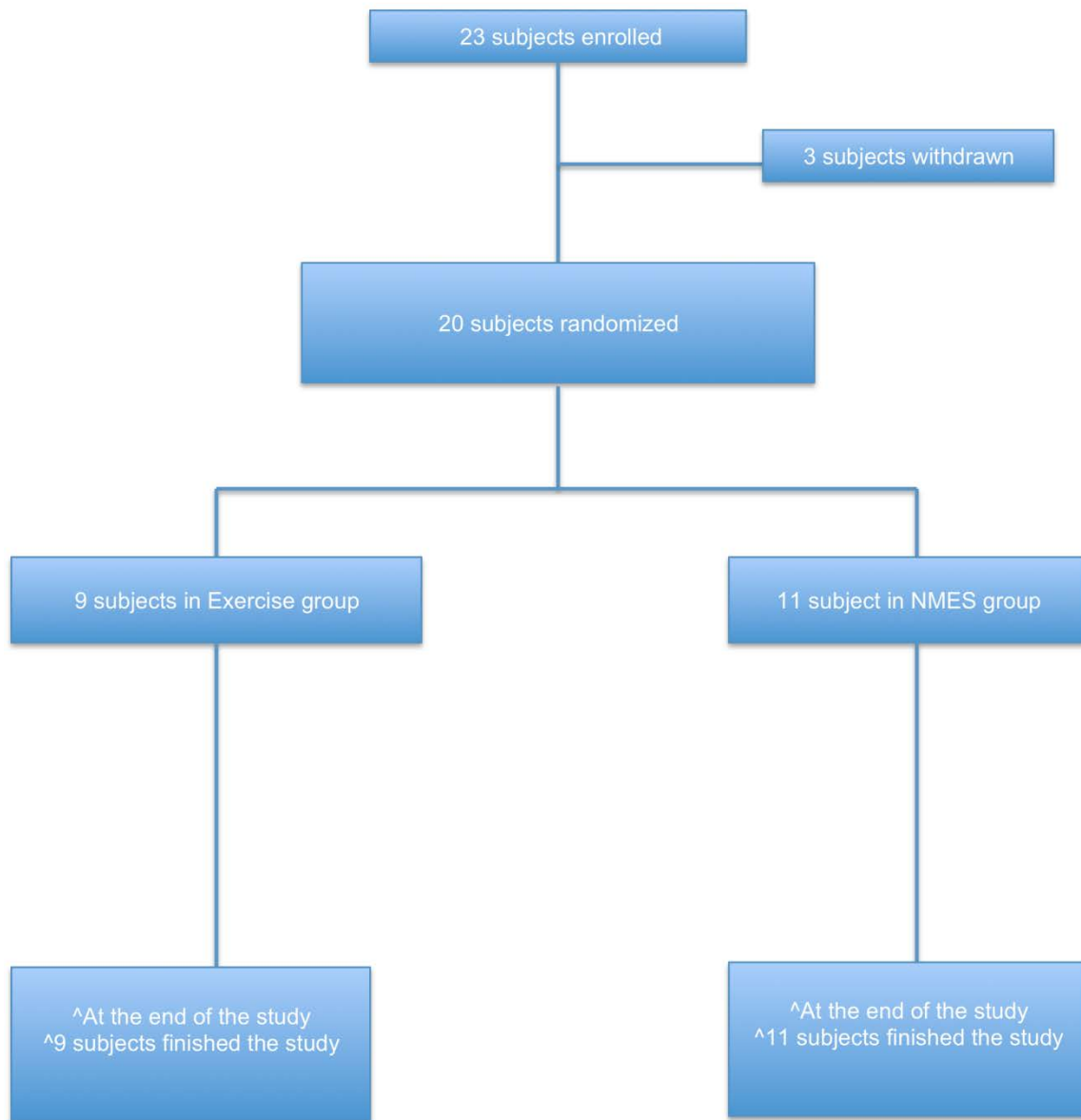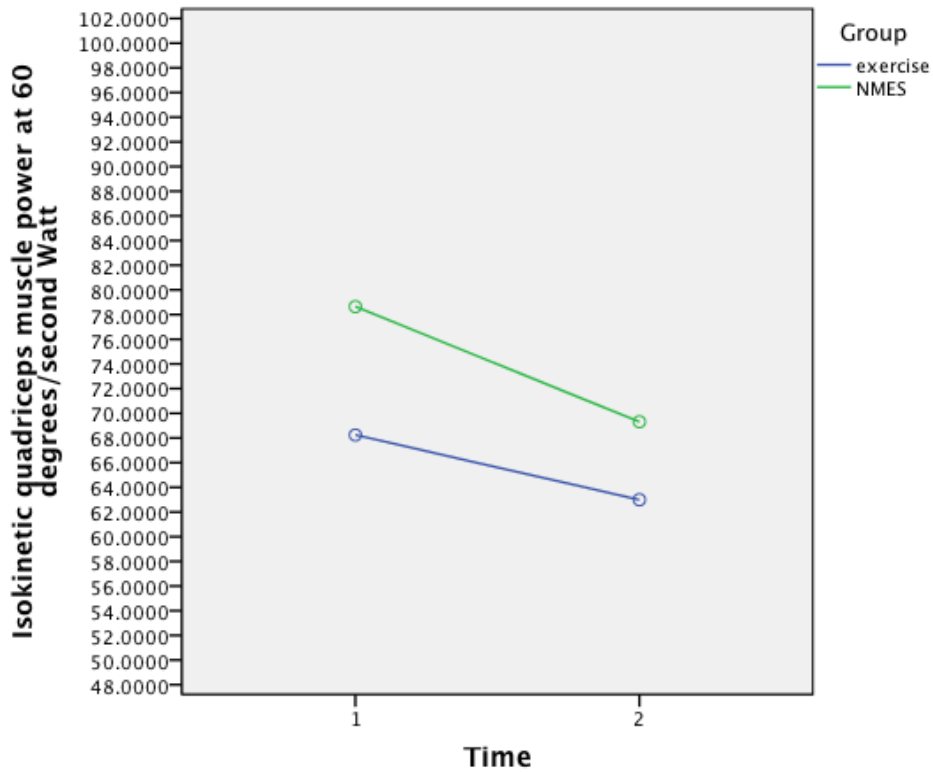
Figure 3-1 Study Flow Diagram

Table 3-1 Mean change, confidence interval and percent change of power outcome measures

	NMES			Exercise		
	Mean Change	Confidence Interval	% Change	Mean Change	Confidence Interval	% Change
Power 60 Watt	-8.95	-20.00 – 2.11	-11.64	-5.73	-13.64 – 2.19	-7.05
Power 150 Watt	-4.93	-13.64 – 3.78	-7.11	-2.56	-25.57 – 20.45	-1.33
Power 240 Watt	-3.06	-19.14 – 13.02	-1.18	9.34	-22.41 – 41.10	17.08
Timed Stair climb power Watt	25.57	-2.72 – 53.86	12.34	-12.16	-53.14 – 28.82	-2.42
Ramp power Watt	9.79	.81 – 18.78	8.01	-4.41	-11.98 – 3.16	-3.20

Table 3-2 Statistical significance and effect size on the dependent variables

|	Group Main Effect			Time Main Effect			Interaction Effect		
	η^2	p value	Observed power	η^2	p value	Observed power	η^2	p value	Observed power
Power 60 Watt	.034	.449	.113	.003	.830	.048	.022	.541	.091
Power 150 Watt	.046	.380	.136	.001	.905	.051	.005	.784	.058
Power 240 Watt	.074	.259	.197	.001	.880	.052	.034	.426	.120
Timed Stair climb power test Watt	.005	.778	.058	.022	.537	.092	.148	.094	.388
Ramp power test Watt	.015	.611	.078	.053	.329	.158	.280	.016*	.707

* Significant at alpha level = .05

Covariates appearing in the model are evaluated at the following values: Weightba_Kg = 78.445

Figure 3-2 Isokinetic quadriceps muscle power at 60 degrees per second

The interaction effect was not significant, $F_{(1, 17)} = .389$, $p = .541$, partial $\eta^2 = .022$.
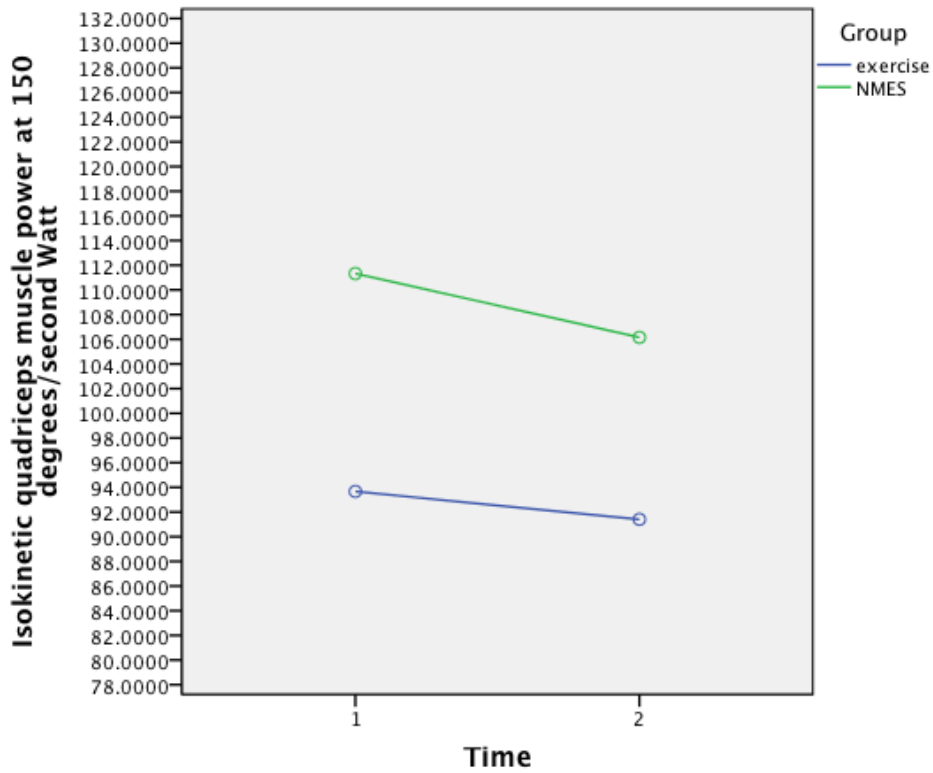
Covariates appearing in the model are evaluated at the following values: Weightba_Kg = 78.445

Figure 3-3 Isokinetic quadriceps muscle power at 150 degrees per second

The interaction effect was not significant, $F (1, 17) = .078$, p= .784, partial $\eta^2 = .005$.

Covariates appearing in the model are evaluated at the following values: Weightba_Kg = 78.445

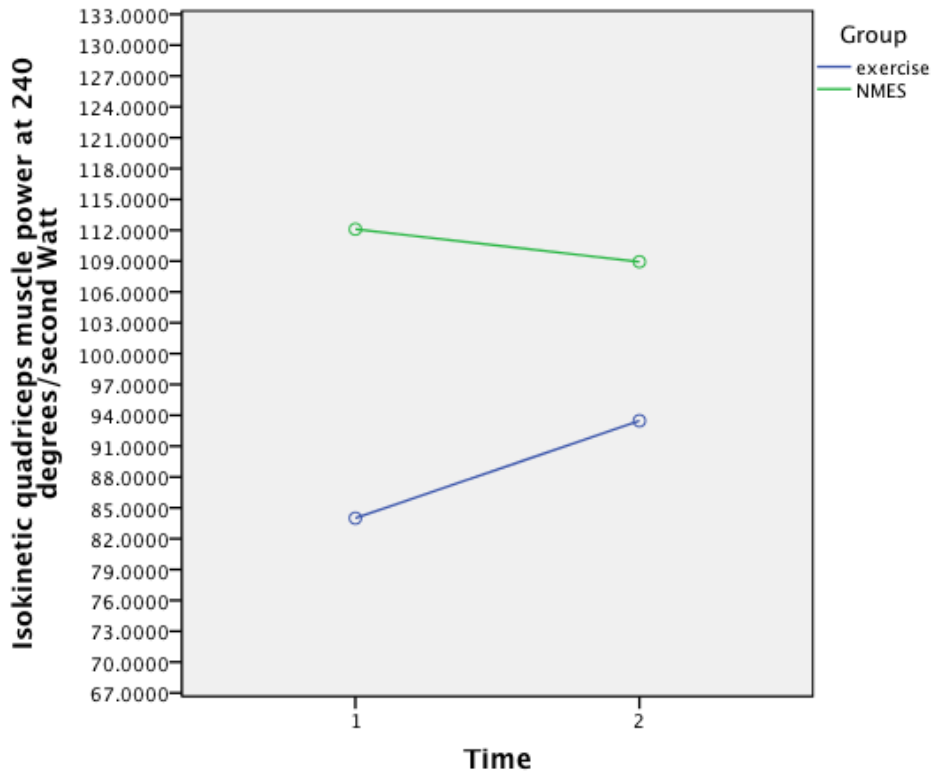**Figure 3-4** Isokinetic quadriceps muscle power at 240 degrees per second

The interaction effect was not significant, $F_{(1, 17)} = .666$, p= .426, partial $\eta^2 = .038$.
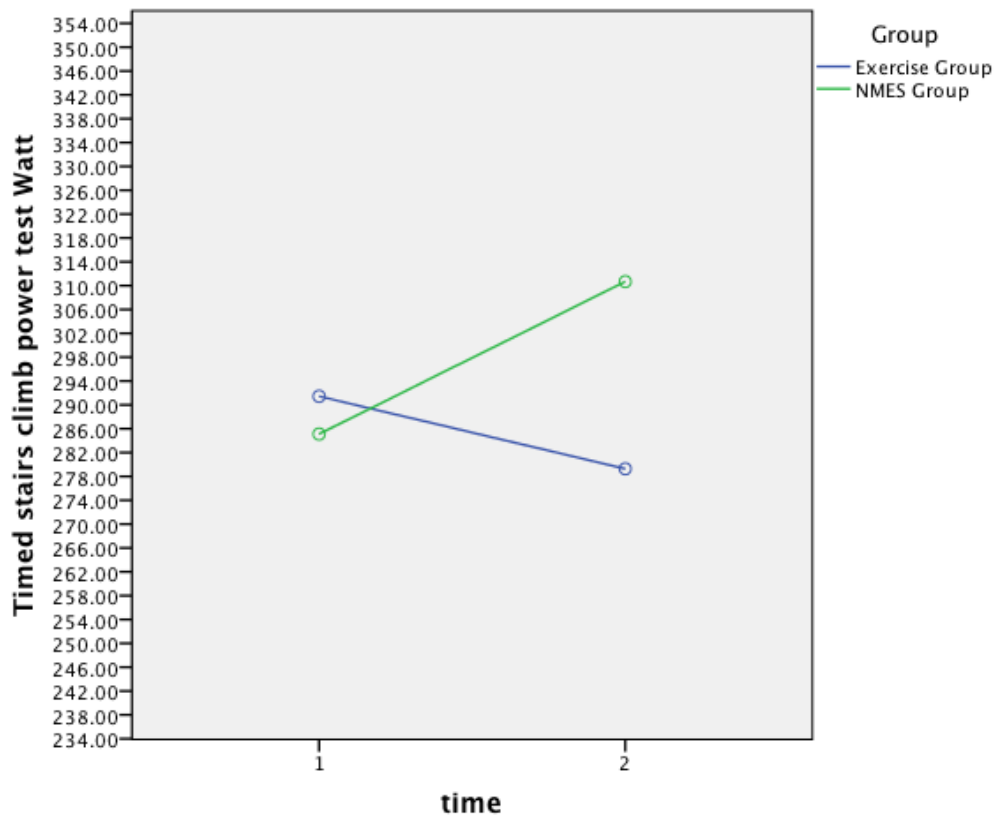
Figure 3-5 Timed stairs climb power test

The interaction effect was not significant, $F_{(1, 18)} = 3.134$, p= .094, partial $\eta^2 = .148$.
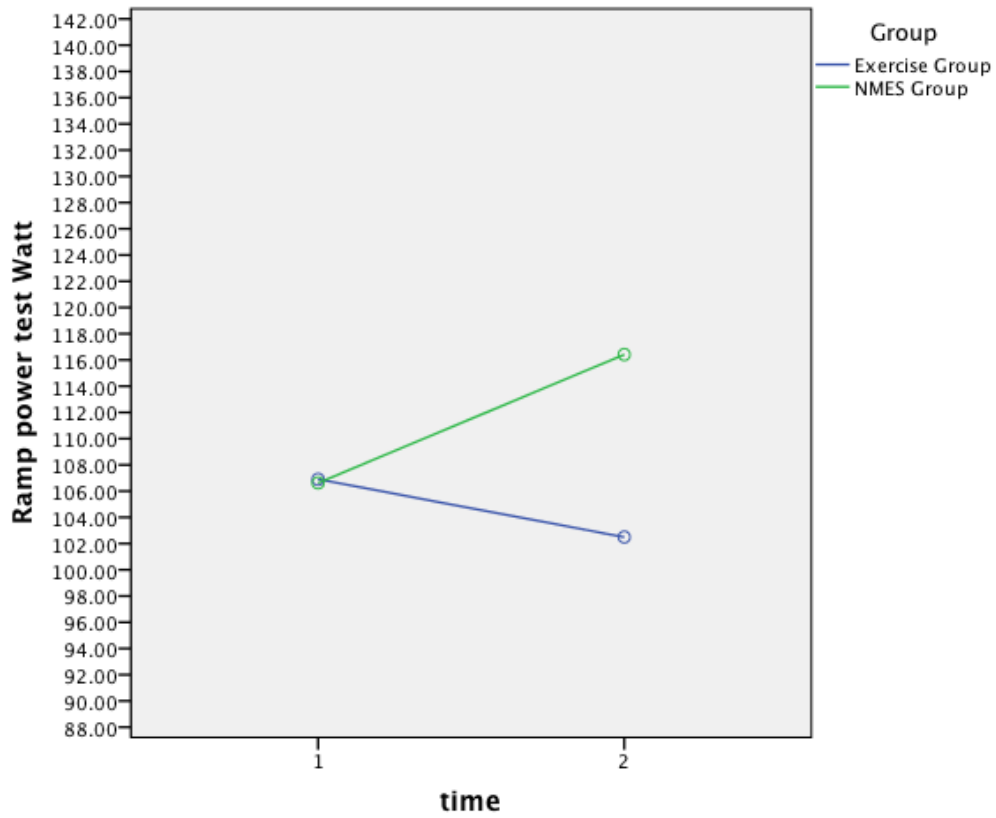
Figure 3-6 Ramp power test

The interaction effect was significant, F (1, 18) = 7.014, p= .016, partial η² = .280.

Table 3-3 Pearson product correlation matrix for the change of type IIA muscle fibers, change in isokinetic quadriceps muscle power and performance based functional power

		Type IIA change	Power 60 change	Power 150 change	Power 240 change	Timed stair climb power test change	Ramp power test change
Type IIA change	r	1	-.259	-.063	-.409	.634[*]	.668[**]
	p		.351	.825	.130	.011	.006
	n		15	15	15	15	15
Power 60 change	r		1	.731[**]	.662[**]	.010	.038
	p			.000	.001	.965	.874
	n			20	20	20	20
Power 150 change	r			1	.743[**]	.298	.202
	p				.000	.203	.393
	n				20	20	20
Power 240 change	r				1	.201	.126
	p					.396	.597
	n					20	20
Timed stair climb power test change	r					1	.408
	p						.074
	n						20
Ramp power test change	r						1
	p						
	n						

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 3-7 Scatter plot of the change in the CSA of type IIA muscle fibers and the change in the isokinetic quadriceps muscle power at 60 degrees per second

Figure 3-8 Scatter plot of the change in the CSA of type IIA muscle fibers and the change in the isokinetic quadriceps muscle power at 150 degrees per second

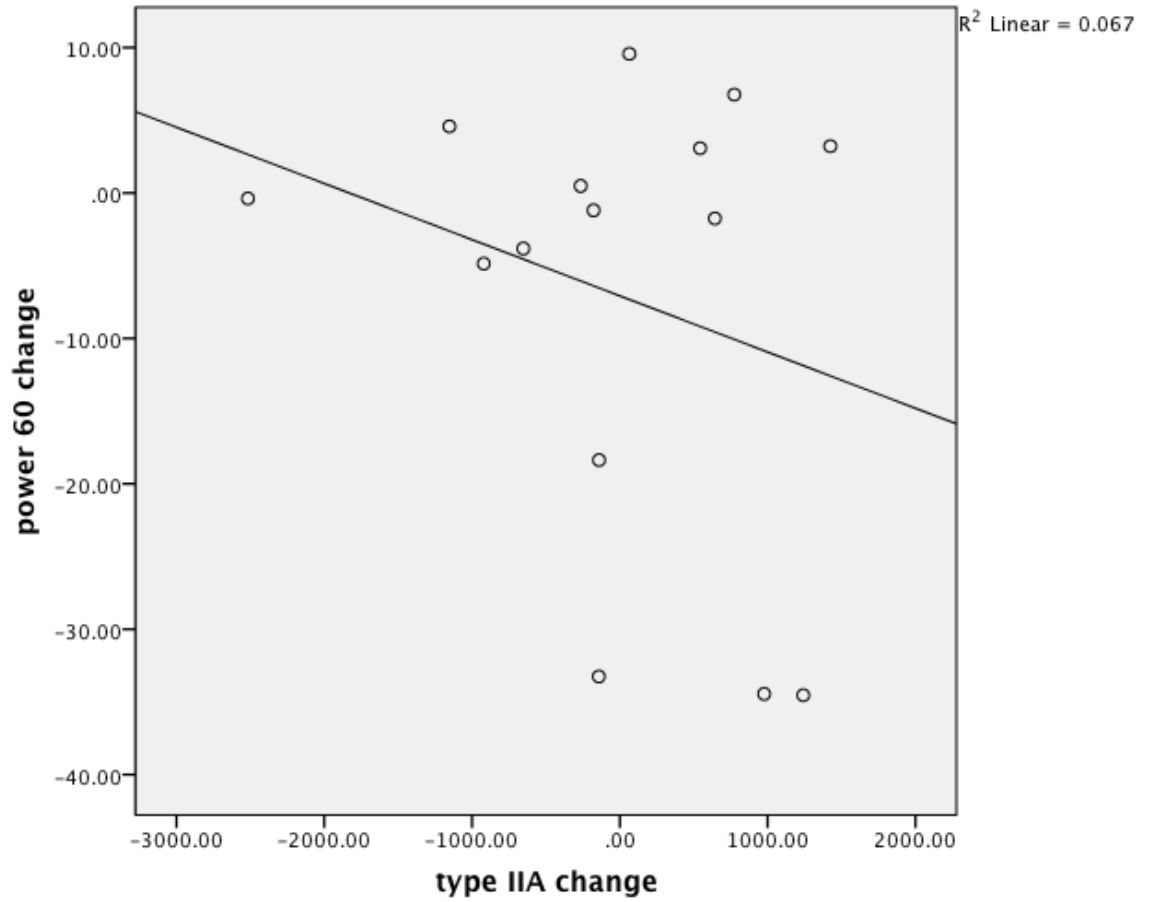**Figure 3-9** Scatter plot of the change in the CSA of type IIA muscle fibers and the change in the isokinetic quadriceps muscle power at 240 degrees per second

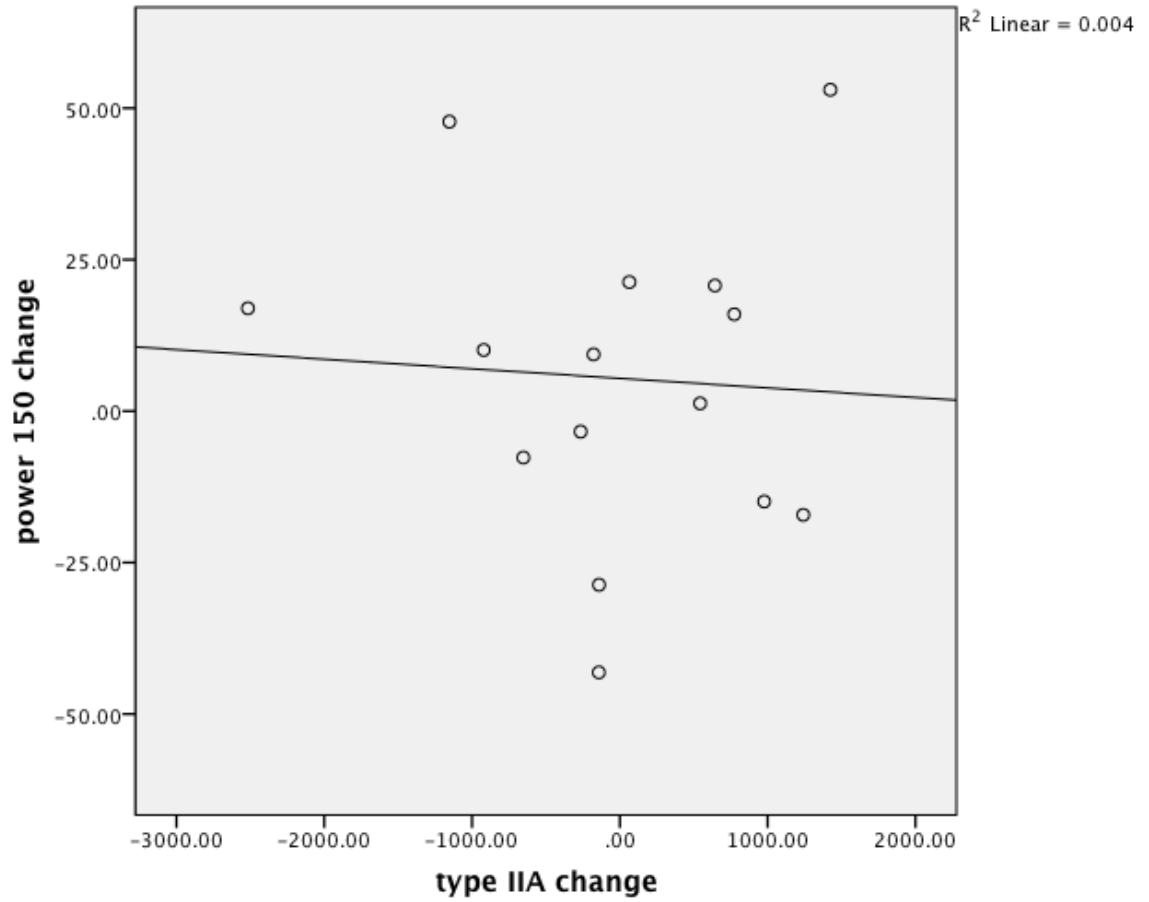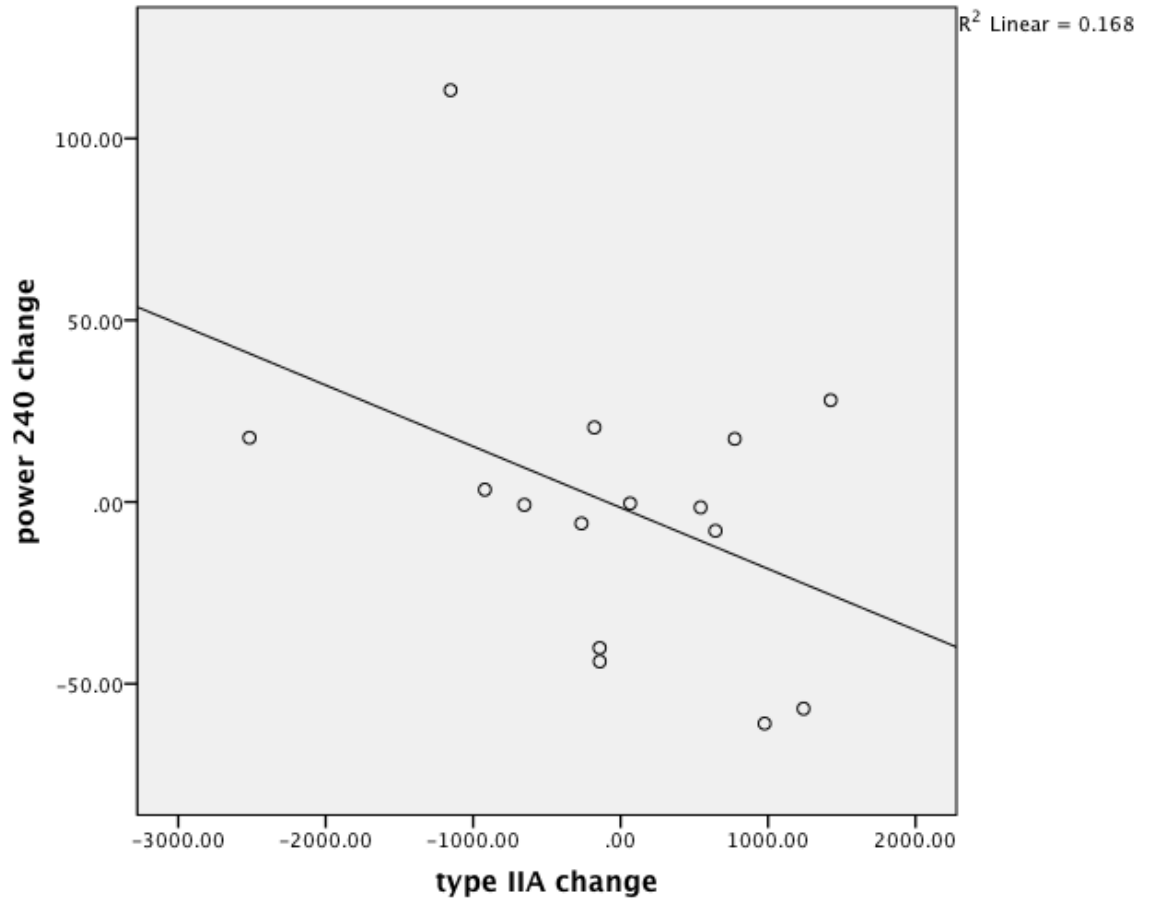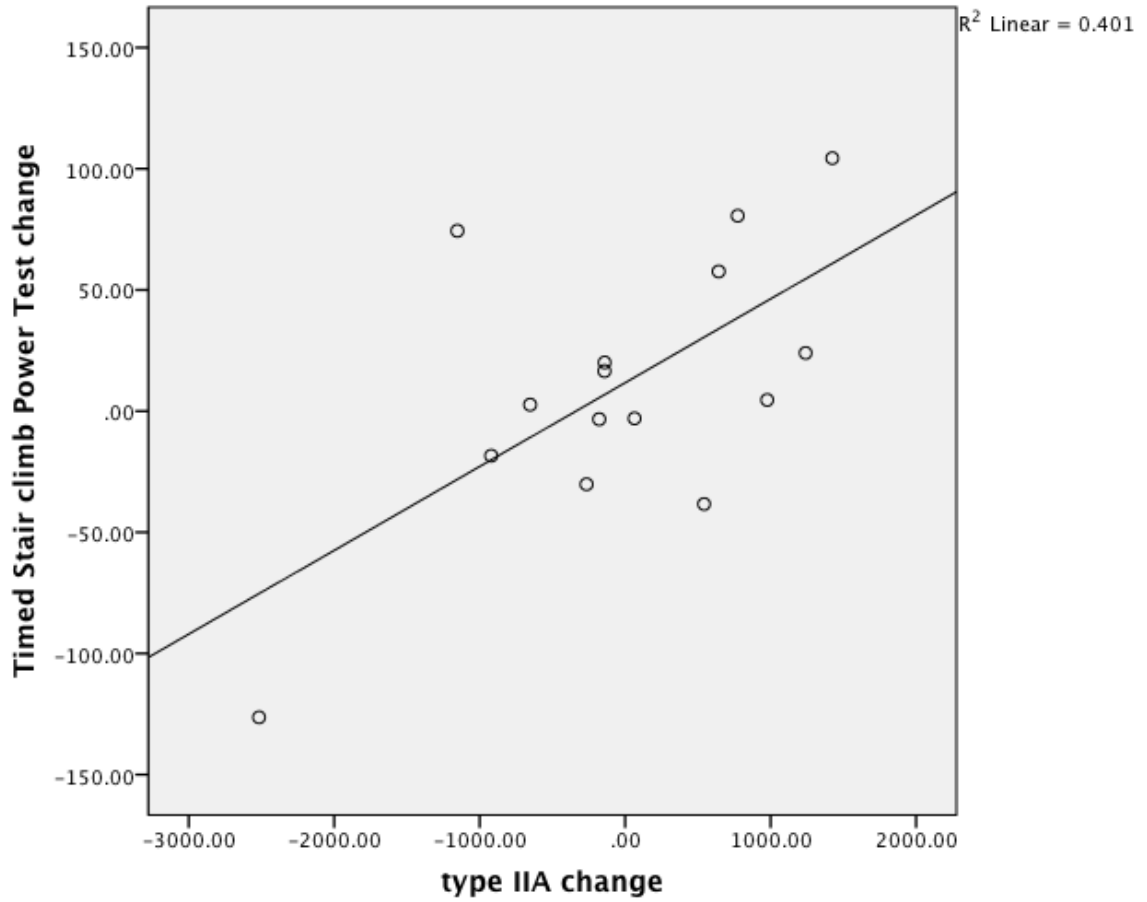**Figure 3-10** Scatter plot of the change in the CSA of type IIA muscle fibers and the change in the stair climb power test
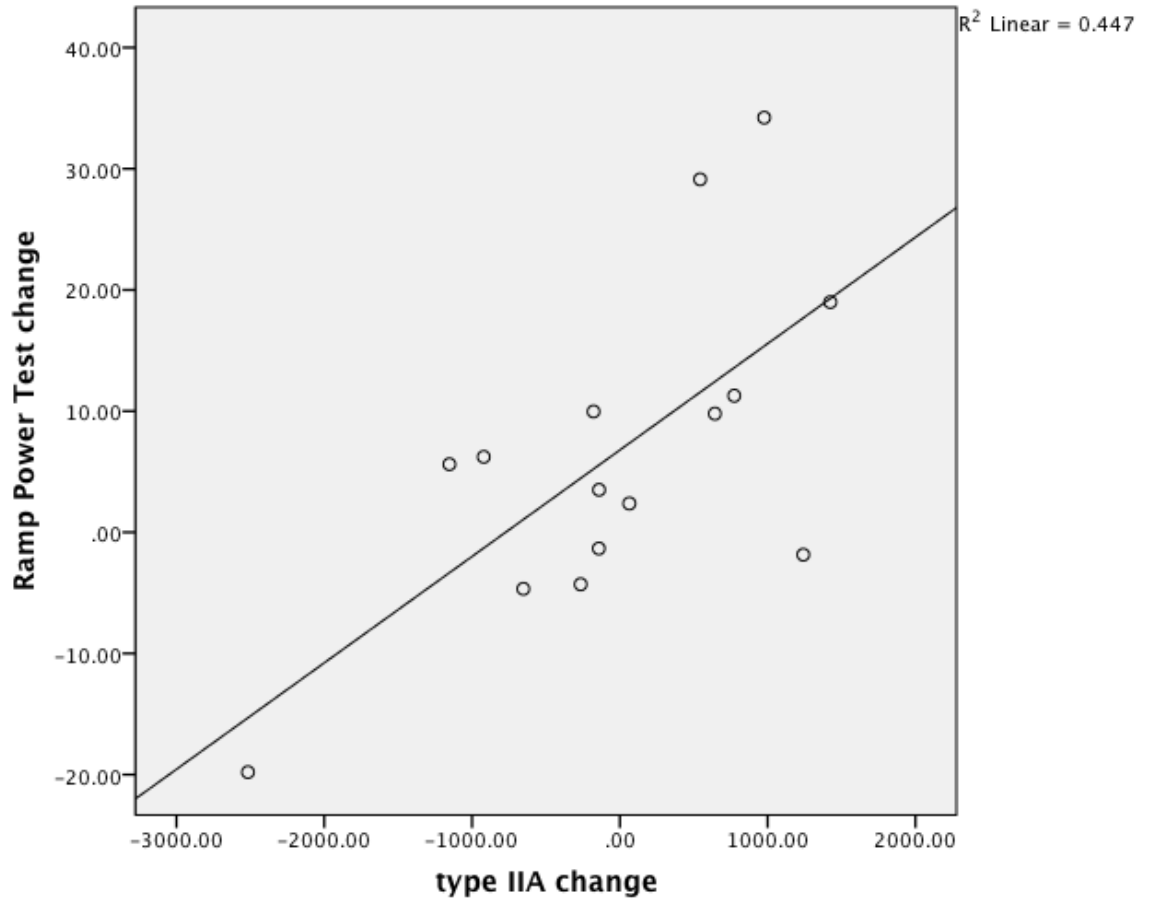
Figure 3-11 Scatter plot of the change in the CSA of type IIA muscle fibers and the change in the

ramp power test

Table 3-4 Comparisons between compliant and non-compliant subjects on isokinetic power and performance based functional power

	N	Change in Power at 60 Watt	Change in power at 150 watt	Change in Power at 240 Watt	Change in timed stair climb power Watt	Change in ramp power Watt
Complain with NMES	8	-7.08	4.80	-12.95	33.39	9.54
Non Complaint with NMES number of session	2	-15.43	-10.35	-6.19	4.92	-1.39
Non Complaint with NMES intensity < 40%	1	-34.45	-14.92	-60.95	4.61	34.22

4.0 CHAPTER FOUR: SUMMARY AND FUTURE RESEARCH

The selective atrophy of type II muscle fibers with aging (5,8) was the main topic of this project. The purpose of this study was to test the hypothesis that NMES training at relatively low percentage of MVC could induce structural changes in type II muscle fibers within the quadriceps muscles of elderly subjects that would not usually occur with strength training at the same level of MVC. Since muscle fiber activation during voluntary muscle contraction follows the Henneman principle of motor units recruitment (6), type II muscle fibers might not be activated during low intensity exercises. However, the pattern of activation of muscle fibers during electrically induced muscle contraction was found to be nonselective (65, 66). Furthermore, NMES is more likely to activate type II muscle fibers at relatively low MVC levels (6, 21). Therefore, the difference in muscle fibers activation between voluntary or electrically induced muscle contractions was the reasoning behind our hypothesis.

In order to explore the effectiveness of NMES in inducing structural changes within the quadriceps muscle, CT images and muscle biopsies were obtained from the quadriceps muscles of elderly subjects. Furthermore, quadriceps muscle power and performance based functional power were measured to determine if the structural changes within the quadriceps muscle could translate into functional improvements of the elderly subjects.

The overall findings of this project were:

1. There was an interaction effect between the groups over time on the quadriceps muscle CSA and the quadriceps NDM CSA.

2. Quadriceps muscle CSA and quadriceps NDM CSA had increased significantly among the subjects in the NMES group, but they did not change among the subjects in the exercise group.

3. Muscle fibers type I CSA was significantly smaller post training than pre training among subjects in the exercise group. However there was no change in the CSA of type I muscle fibers among subjects in the NMES group.

4. The interaction effect on muscle fibers type IIA CSA between the groups from pre to post training was significant. The change in the CSA of type IIA muscle fibers was higher in the NMES group compared to the exercise group. The CSA of type IIA muscle fibers increased from pre to post training among the NMES groups. However, in the exercise group the CSA of type IIA muscle fibers decreased compared to pre training yet neither the positive change among the NMES group nor the negative change among the exercise group on type IIA muscle fibers reached statistical significance, p values = .075, .088 respectively.

5. Isokinetic quadriceps muscle power was not different either between the groups or from pre to post training at each speed of isokinetic contraction after adjusting for the subjects' weight as a covariate.

6. There was an interaction effect on the ramp power test between the groups from pre to post training. The change in the ramp power test from pre to post training was significantly higher for the NMES group than that for the exercise group.

7. The timed stairs climb power test had a similar pattern of change as the ramp power test however it did not reach statistical significance (p= .094).

The results of this study provide evidence for the effectiveness of the NMES to induce structural changes within the quadriceps muscle of the elderly subjects at the macro and the microscopic levels. This study supports what has been shown in the literature regarding the ability of NMES to activate muscle fibers without a specific pattern of activation. Additionally, the NMES induced structural changes within the quadriceps muscle of the elderly subjects were translated into improvements in functional performance. Performance based functional power might be more responsive to changes in type II muscle fibers than isokinetic muscle power output. Functional power tests may allow muscle torque and muscle contraction speed to interact with each other to produce higher power compared to isokinetic muscle power tests that hold the velocity constant. Therefore performance based functional power tests might be good outcome measures in clinics and they might be a better alternative to the expensive isokinetic dynamometer.

In this study, there were significant strong positive associations between the changes in the performance based functional power tests and the change in the CSA of type IIA muscle fibers. These associations are helpful in determining how changes in the CSA of type IIA muscle fiber in response to training could be detected by changes in performance based functional power. However they would not inform us as to whether performance based functional power tests could identify patients with deficits in the CSA of type II muscle fibers prior to treatment. Therefore we further examined the correlation between the baseline functional power tests and the baseline CSA of type IIA muscle fibers using Pearson product correlation analysis. There was no association between the baseline CSA of type IIA muscle fibers and the baseline timed

stair climb power test (r= .049, p= .853) and the baseline ramp power test (r= .146, p= .575).

Therefore although the performance based functional power tests might be good for assessing changes in type IIA muscle fibers in response to treatment, they may not be useful in identifying patients who have deficits in type IIA muscle fibers prior to intervention.

Since type II muscle fibers are able to generate high force in short periods of time (50), the rate of torque development might reflect the performance of type IIA muscle fibers and could possibly be a better alternative for identifying patients with type IIA muscle fibers pathology prior to treatment. Further research should be done to explore the ability of the rate of torque development to examine the integrity of type IIA muscle fiber in elderly subjects.

There were some limitations to our study. The small sample size did not allow us to examine if the effect of the NMES training would be similar between males and females. Another important limitation to this study is the participation of subjects who were health. Those subjects might not have a large margin for improvement, which may result in under estimation of the training effect. In spite of having those limitations, we believe that this study served as a good preliminary report for undergoing a larger randomized clinical trail to examine the effect of NMES in inducing structural changes in the quadriceps muscle of the elderly subjects.

The results of this study have raised other questions that might be worth exploring in future studies. It would be interesting to examine the effect of the NMES on the composition of quadriceps muscle in the elderly subjects. NMES was able to increase the CSA of the quadriceps muscle and the fat free portion of the quadriceps muscle mass. Therefore NMES training may results in reduction of the fat infiltrate within the quadriceps muscle. Goodpaster et al (70) found that moderate physical activity was able to prevent gain of inter-muscular adipose tissue within

the quadriceps muscle of elderly subjects. Therefore it would be imperative to examine the effect of NMES on the composition of the quadriceps muscle.

Further study in needed to determine the predictive validity of the performance based functional power test in detecting changes in the CSA of the type II muscle fibers in the elderly subjects. In this study we found an association between the change in the performance based functional power tests and the change in CSA of type IIA muscle fibers. However, due to the small sample size we were not able to quantify the amount of change in the CSA of type IIA muscle fibers that is associated with 1-point change in the performance based functional power tests. Therefore a larger scale study should be done to allow performing regression analysis to quantify this relationship

In our study, NMES was compared to a voluntary isometric exercise that both produced a torque equivalent to 40% of MVC. This MVC level will likely not result in actual training effect for those involved in a voluntary exercise program. Therefore it would be important to compare NMES to a voluntary exercise program at higher dosages of MVC that might result in actual training effect or examining whether the combination of NMES with voluntary exercises would result in a bigger effect compared to exercise only

Finally it would be useful to determine the effect of NMES in reversing atrophy of type II muscle fibers that is associated with some diseases. Aging is associated with atrophy of type II muscle fibers (5, 8). The results of our study have shown that NMES, compared to voluntary isometric training, is able to induce hypertrophy of type II muscle fibers within the quadriceps muscle of elderly subjects. Perhaps this intervention approach might also be beneficial in targeting type II muscle fiber atrophy in conditions such as rheumatoid arthritis, prolonged

steroid use, or muscle atrophy observed in association with some cancers. Therefore further research should be done to examine the potential use of NMES in patients with these diseases.

BIBLIOGRAPHY

1. Deschenes MR. Effects of aging on muscle fibre type and size. [Review] [188 refs][Journal Article. Review] Sports Medicine. 34(12):809-24, 2004.

2. Greenlund LJ. Nair KS. Sarcopenia--consequences, mechanisms, and potential therapies. [Review] [128 refs] [Journal Article. Review] Mechanisms of Ageing & Development. 124(3):287-99, 2003 Mar.

3. Frontera WR. Suh D. Krivickas LS. Hughes VA. Goldstein R. Roubenoff R. Skeletal muscle fiber quality in older men and women. [Journal Article] American Journal of Physiology - Cell Physiology. 279(3):C611-8, 2000 Sep.

4. Frontera WR. Meredith CN. O'Reilly KP. Knuttgen HG. Evans WJ. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. [Journal Article] Journal of Applied Physiology. 64(3):1038-44, 1988 Mar.

5. Lexell J. Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: effects of increasing age. [Journal Article] Journal of Anatomy. 174:239-49, 1991 Feb.

6. Gregory CM. Bickel CS. Recruitment patterns in human skeletal muscle during electrical stimulation. [Review] [31 refs] [Journal Article. Review] Physical Therapy. 85(4):358-64, 2005 Apr.

7. Thrane G. Joakimsen RM. Thornquist E. The association between timed up and go test and history of falls: the Tromso study. [Journal Article. Research Support, Non-U.S.Gov't]BMCGeriatrics.7:1,2007.

8. Lars Larsson, Gunnar Grimby, AJanKarlsson. Muscle strength and speed of movement in relation to age and muscle morphology. J. Appl. Physio.: Respirat. Environ. Exercise Physiol. 46(3): 451-456, 1979.

9. Short KR. Vittone JL. Bigelow ML. Proctor DN. Coenen-Schimke JM. Rys P. Nair KS. Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, N.I.H., Extramural. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 99(1):95-102, 2005 Jul.

10. Allen DL. Roy RR. Edgerton VR. Myonuclear domains in muscle adaptation and disease. [Review] [82 refs] [Journal Article. Review] Muscle & Nerve. 22(10):1350-60, 1999 Oct.

11. Balagopal P. Schimke JC. Ades P. Adey D. Nair KS. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, U.S. Gov't, P.H.S.] American Journal of Physiology - Endocrinology & Metabolism. 280(2):E203-8, 2001 Feb.

12. Bamman MM. Ragan RC. Kim JS. Cross JM. Hill VJ. Tuggle SC. Allman RM. Myogenic protein expression before and after resistance loading in 26- and 64-yr-old men and women. [Clinical Trial. Controlled Clinical Trial. Journal Article. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 97(4):1329-37, 2004 Oct.

13. Lindle RS. Metter EJ. Lynch NA. Fleg JL. Fozard JL. Tobin J. Roy TA. Hurley BF. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. [Clinical Trial. Journal Article. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 83(5):1581-7, 1997 Nov.

14. Chekanov VS. Karakozov P. Rieder M. Zander G. Age related skeletal muscle response to electrical stimulation. [Journal Article] ASAIO Journal. 46(4):474-81, 2000 Jul-Aug.

15. Aniansson, A., and E. Gustafsson. Physical training in elderly men with special reference to quadriceps muscle strength and morphology. Clin. Physio. 1:87-98, 1981.

16. Larsson, Lars. Physical training effects on muscle morphology in sedentary males at different ages. Med. Sci. Sport exercise, Vol. 14, No. 3. pp. 203-206, 1982.

17. Snyder-Mackler L. Delitto A. Stralka SW. Bailey SL. Use of electrical stimulation to enhance recovery of quadriceps femoris muscle force production in patients following anterior cruciate ligament reconstruction.[see comment]. [Clinical Trial. Journal Article. Multicenter Study. Randomized Controlled Trial. Research Support, Non-U.S. Gov't] Physical Therapy. 74(10):901-7, 1994 Oct.

18. Lyons CL. Robb JB. Irrgang JJ. Fitzgerald GK. Differences in quadriceps femoris muscle torque when using a clinical electrical stimulator versus a portable electrical stimulator. [Comparative Study. Journal Article] Physical Therapy. 85(1):44-51, 2005 Jan.

19. Delitto A. Snyder-Mackler L. Two theories of muscle strength augmentation using percutaneous electrical stimulation. [Review] [36 refs] [Journal Article. Review] Physical Therapy. 70(3):158-64, 1990 Mar.

20. Sinacore DR. Delitto A. King DS. Rose SJ. Type II fiber activation with electrical stimulation: a preliminary report. [Case Reports. Journal Article. Research Support, Non-U.S. Gov't] Physical Therapy. 70(7):416-22, 1990 Jul.

21. Talbot LA. Gaines JM. Ling SM. Metter EJ. A home-based protocol of electrical muscle stimulation for quadriceps muscle strength in older adults with osteoarthritis of the knee. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of Rheumatology. 30(7):1571-8, 2003 Jul.

22. Malisoux L. Francaux M. Nielens H. Theisen D. Stretch-shortening cycle exercises: an effective training paradigm to enhance power output of human single muscle fibers. [Clinical Trial. Journal Article. Research Support, Non-U.S. Gov't] Journal of Applied Physiology. 100(3):771-9, 2006 Mar.

23. Pincivero DM. Lephart SM. Karunakara RA. Reliability and precision of isokinetic strength and muscular endurance for the quadriceps and hamstrings. [Journal Article] International Journal of Sports Medicine. 18(2):113-7, 1997 Feb.

24. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E, Newman AB. Attenuation of skeletal muscle and strength in the elderly:The health ABC study. J ApplPhysiol 90 (2001): 2157-65.

25. Caggiano E. Emrey T. Shirley S. Craik RL. Effects of electrical stimulation or voluntary contraction for strengthening the quadriceps femoris muscles in an aged male population. [Clinical Trial. Journal Article. Randomized Controlled Trial] Journal of Orthopaedic& Sports Physical Therapy. 20(1):22-8, 1994 Jul.

26. Piva SR. Goodnite EA. Azuma K. Woollard JD. Goodpaster BH. Wasko MC. Fitzgerald GK. Neuromuscular electrical stimulation and volitional exercise for individuals with rheumatoid arthritis: a multiple-patient case report. [Case Reports. Journal Article] Physical Therapy. 87(8):1064-77, 2007 Aug.

27. Studenski S. Perera S. Wallace D. Chandler JM. Duncan PW. Rooney E. Fox M. Guralnik JM. Physical performance measures in the clinical setting. [Comparative Study. Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of the American Geriatrics Society. 51(3):314-22, 2003 Mar.

28. Landi F, Russo A, Cesari M, Pahor M, Bernabei R, Onder G. HDL-cholesterol and physical performance: results from the ageing and longevity study in the sirente geographic area (ilSIRENTE Study). Age Ageing. 2007 Sep;36(5):514-20.

29. Trappe S. Williamson D. Godard M. Porter D. Rowden G. Costill D. Effect of resistance training on single muscle fiber contractile function in older men. [Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 89(1):143-52, 2000 Jul.

30. Trappe S. Williamson D. Godard M. Maintenance of whole muscle strength and size following resistance training in older men. [Journal Article. Research Support, U.S. Gov't,

P.H.S.] Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 57(4):B138-43, 2002 Apr.

31. Alexander NB. Schultz AB. Warwick DN. Rising from a chair: effects of age and functional ability on performance biomechanics. [Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of Gerontology. 46(3):M91-8, 1991 May.

32. Alexander NB. Fry-Welch DK. Marshall LM. Chung CC. Kowalski AM. Healthy young and old women differ in their trunk elevation and hip pivot motions when rising from supine to sitting. [Comparative Study. Journal Article. Research Support, U.S. Gov't, P.H.S.] Journal of the American Geriatrics Society. 43(4):338-43, 1995 Apr.

33. Judge JO. Underwood M. Gennosa T. Exercise to improve gait velocity in older persons. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't] Archives of Physical Medicine & Rehabilitation. 74(4):400-6, 1993 Apr.

34. Wolfson L. Judge J. Whipple R. King M. Strength is a major factor in balance, gait, and the occurrence of falls. [Journal Article. Research Support, U.S. Gov't, P.H.S.] Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 50 Spec No:64-7, 1995 Nov.

35. Dutta C. Significance of sarcopenia in the elderly. [Review] [23 refs] [Journal Article. Review] Journal of Nutrition. 127(5 Suppl):992S-993S, 1997 May.

36. Moxley Scarborough D. Krebs DE. Harris BA. Quadriceps muscle strength and dynamic stability in elderly persons. [Journal Article. Research Support, U.S. Gov't, P.H.S.] Gait & Posture. 10(1):10-20, 1999 Sep.

37. Trappe S. Gallagher P. Harber M. Carrithers J. Fluckey J. Trappe T. Single muscle fibre contractile properties in young and old men and women.[Comparative Study. Journal Article. Research Support, U.S. Gov't, P.H.S.]Journal of Physiology. 552(Pt 1):47-58, 2003 Oct 1.

38. Brown M. Sinacore DR. Host HH. The relationship of strength to function in the older adult.[Comparative Study. Journal Article. Research Support, U.S. Gov't, P.H.S.] *Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 50 Spec No:55-9, 1995 Nov.*

39. Skelton DA. Kennedy J. Rutherford OM. Explosive power and asymmetry in leg muscle function in frequent fallers and non-fallers aged over 65.[Journal Article. Research Support, Non-U.S. Gov't] *Age & Ageing. 31(2):119-25, 2002 Mar.*

40. Goodpaster BH. Thaete FL. Kelley DE. Composition of skeletal muscle evaluated with computed tomography. [Review] [19 refs] [Journal Article. Review] Annals of the New York Academy of Sciences. 904:18-24, 2000 May.

41. Visser M. Kritchevsky SB. Goodpaster BH. Newman AB. Nevitt M. Stamm E. Harris TB. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. [Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of the American Geriatrics Society. 50(5):897-904, 2002 May.

42. Goodpaster BH. Carlson CL. Visser M. Kelley DE. Scherzinger A. Harris TB. Stamm E. Newman AB. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. [Clinical Trial. Journal Article. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 90(6):2157-65, 2001 Jun.

43. Goodpaster BH. Kelley DE. Thaete FL. He J. Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. [Journal Article. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 89(1):104-10, 2000 Jul.

44. Goodpaster BH. Thaete FL. Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. [Journal Article. Research Support, U.S. Gov't, P.H.S.] American Journal of Clinical Nutrition. 71(4):885-92, 2000 Apr.

45. Mitsiopoulos N. Baumgartner RN. Heymsfield SB. Lyons W. Gallagher D. Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography.[see comment]. [Journal Article. Research Support, Non-U.S. Gov't] Journal of Applied Physiology. 85(1):115-22, 1998 Jul.

46. Overend TJ. Cunningham DA. Paterson DH. Lefcoe MS. Thigh composition in young and elderly men determined by computed tomography.[Journal Article. Research Support, Non-U.S. Gov't] Clinical Physiology. 12(6):629-40, 1992 Nov.

47. Goodpaster BH. Kelley DE. Wing RR. Meier A. Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity.[Journal Article. Research Support, U.S. Gov't, P.H.S.] Diabetes. 48(4):839-47, 1999 Apr.

48. D'Antona G. Lanfranconi F. Pellegrino MA. Brocca L. Adami R. Rossi R. Moro G. Miotti D. Canepari M. Bottinelli R. Skeletal muscle hypertrophy and structure and function of skeletal muscle fibres in male body builders. [Journal Article. Research Support, Non-U.S. Gov't] Journal of Physiology. 570(Pt 3):611-27, 2006 Feb *1*.

49. Chilibeck PD. Syrotuik DG. Bell GJ. The effect of strength training on estimates of mitochondrial density and distribution throughout muscle fibres. [Clinical Trial. Journal Article. Randomized Controlled Trial] European Journal of Applied Physiology & Occupational Physiology. 80(6):604-9, 1999 Nov-Dec.

50. Rothstein JM. Delitto A. Sinacore DR. Rose SJ. Muscle function in rheumatic disease patients treated with corticosteroids. [Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Muscle & Nerve. 6(2):128-35, 1983 Feb.

51. Morse CI. Thom JM. Reeves ND. Birch KM. Narici MV. In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. [Clinical Trial. Journal Article. Research Support, Non-U.S. Gov't] Journal of Applied Physiology. 99(3):1050-5, 2005 Sep.

52. Larsson L. Grimby G. Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. [Journal Article] Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology. 46(3):451-6, 1979 Mar.

53. Schiller BC. Casas YG. Tracy BL. DeSouza CA. Seals DR. Age-related declines in knee extensor strength and physical performance in healthy Hispanic and Caucasian women. [Comparative Study. Journal Article. Research Support, U.S. Gov't, P.H.S.] Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 55(12):B563-9, 2000 Dec.

54. Alexander NB. Schultz AB. Ashton-Miller JA. Gross MM. Giordani B. Muscle strength and rising from a chair in older adults. [Review] [19 refs] [Journal Article. Research Support, U.S. Gov't, Non-P.H.S.. Research Support, U.S. Gov't, P.H.S.. Review] Muscle & Nerve Supplement. 5:S56-9, 1997.

55. Hurley MV. Rees J. Newham DJ. Quadriceps function, proprioceptive acuity and functional performance in healthy young, middle-aged and elderly subjects. [Journal Article. Research Support, Non-U.S. Gov't] Age & Ageing. 27(1):55-62, 1998 Jan.

56. Sipila S. Elorinne M. Alen M. Suominen H. Kovanen V. Effects of strength and endurance training on muscle fibre characteristics in elderly women. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't] Clinical Physiology. 17(5):459-74, 1997 Sep.

57. Taaffe DR. Pruitt L. Pyka G. Guido D. Marcus R. Comparative effects of high- and low-intensity resistance training on thigh muscle strength, fiber area, and tissue composition in elderly women. [Clinical Trial. Comparative Study. Journal Article. Randomized Controlled Trial. Research Support, U.S. Gov't, Non-P.H.S.] Clinical Physiology. 16(4):381-92, 1996 Jul.

58. Trappe S. Godard M. Gallagher P. Carroll C. Rowden G. Porter D. Resistance training improves single muscle fiber contractile function in older women. [Journal Article. Research Support, U.S. Gov't, P.H.S.] American Journal of Physiology - Cell Physiology. 281(2):C398-406, 2001 Aug.

59. Trappe S. Williamson D. Godard M. Porter D. Rowden G. Costill D. Effect of resistance training on single muscle fiber contractile function in older men. [Journal Article. Research

Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Journal of Applied Physiology. 89(1):143-52, 2000 Jul.

60.   Delitto A. Rose SJ. McKowen JM. Lehman RC. Thomas JA. Shively RA. Electrical stimulation versus voluntary exercise in strengthening thigh musculature after anterior cruciate ligament surgery.[erratum appears in Phys Ther 1988 Jul;68(7):1145]. [Clinical Trial. Comparative Study. Journal Article. Randomized Controlled Trial] Physical Therapy. 68(5):660-3, 1988 May.

61.   Snyder-Mackler L. Delitto A. Bailey SL. Stralka SW. Strength of the quadriceps femoris muscle and functional recovery after reconstruction of the anterior cruciate ligament. A prospective, randomized clinical trial of electrical stimulation. [Clinical Trial. Comparative Study. Journal Article. Multicenter Study. Randomized Controlled Trial. Research Support, Non-U.S. Gov't] Journal of Bone & Joint Surgery - American Volume. 77(8):1166-73, 1995 Aug.

62.   Fitzgerald GK. Piva SR. Irrgang JJ. A modified neuromuscular electrical stimulation protocol for quadriceps strength training following anterior cruciate ligament reconstruction. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't] Journal of Orthopaedic & Sports Physical Therapy. 33(9):492-501, 2003 Sep.

63.   Stephens JA. Garnett R. Buller NP. Reversal of recruitment order of single motor units produced by cutaneous stimulation during voluntary muscle contraction in man. [Journal Article] Nature. 272(5651):362-4, 1978 Mar 23.

64.   Kubiak RJ, Whitman KM, Johnston RM. Changes in quadriceps femoris muscle strength using isometric exercise versus electrical stimulation. J Orthop Sports Phys Ther. 1987;8(11):537-41.

65.   Bickel CS. Slade JM. Warren GL. Dudley GA. Fatigability and variable-frequency train stimulation of human skeletal muscles. [Clinical Trial. Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Physical Therapy. 83(4):366-73, 2003 Apr

66.   Slade JM. Bickel CS. Warren GL. Dudley GA. Variable frequency trains enhance torque independent of stimulation amplitude. [Clinical Trial. Journal Article. Research Support, Non-U.S. Gov't. Research Support, U.S. Gov't, P.H.S.] Acta Physiologica Scandinavica. 177(1):87-92, 2003 Jan.

67.   Bean JF. Kiely DK. LaRose S. Alian J. Frontera WR. Is stair climb power a clinically relevant measure of leg power impairments in at-risk older adults?. [Journal Article] Archives of Physical Medicine & Rehabilitation. 88(5):604-9, 2007 May.

68.   Henwood TR. Taaffe DR. Short-term resistance training and the older adult: the effect of varied programmes for the enhancement of muscle strength and functional performance.

[Journal Article. Randomized Controlled Trial] Clinical Physiology & Functional Imaging. 26(5):305-13, 2006 Sep.

69. Signorile JF. Sandler D. Kempner L. Stanziano D. Ma F. Roos BA. The ramp power test: a power assessment during a functional task for older individuals. [Journal Article. Research Support, Non-U.S. Gov't] Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 62(11):1266-73, 2007 Nov.

70. Goodpaster BH. Chomentowski P. Ward BK. Rossi A. Glynn NW. Delmonico MJ. Kritchevsky SB. Pahor M. Newman AB. Effects of physical activity on strength and skeletal muscle fat infiltration in older adults: a randomized controlled trial. Journal of Clinical Endocrinology & Metabolism. 96(2):494-503, 2011 Feb.

71. Sipila S. Elorinne M. Alen M. Suominen H. Kovanen V. Effects of strength and endurance training on muscle fibre characteristics in elderly women. Clinical Physiology. 17(5):459-74, 1997 Sep.

72. Grimby, G., A. Aniansson, M. Hedberg, G.-B.Henning, U.Grangard, and H. Kvist. Training can improve muscle strength and endurance in 78- to 84-yr-old men. J. Appl. Physiol. 73: 2517-2523, 1992.

73. Dubé JJ, Amati F, Stefanovic-Racic M, Toledo FG, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. Am J Physiol Endocrinol Metab. 2008 May;294(5):E882-8. Epub 2008 Mar 4.

74. David J Clark, Carolynn Patten, Kieran F Reid, Robert J Carabello, Edward M. Phillips, Roger A. Fielding. Impaired voluntary neuromuscular Activation limits muscle power in mobility-limited older adults. J Gerontol A Biol Sci Med Sci. 2010 May; 65(5):495-502

75. Folland JP. Hawker K. Leach B. Little T. Jones DA. Strength training: isometric training at a range of joint angles versus dynamic training. Journal of Sports Sciences. 23(8):817-24, 2005 Aug.

76. Barnes WS. The relationship of motor-unit activation to isokinetic muscular contraction at different contractile velocities. Phys Ther 60:1152-1158. 1980.