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Effects of high and low velocity muscle contraction on myosin heavy chain mRNA and protein expression in conjunction with muscle performance in the elderly

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Effects of high and low velocity muscle contraction on myosin heavy chain mRNA and protein expression in conjunction with muscle performance in the elderly

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DEDICATION

This thesis is dedicated to my father, Dave Englund, PhD, a lifelong teacher and seeker of wisdom.

TABLE OF CONTENTS

ABSTRACT

Title: Effects of high and low velocity muscle contraction on myosin heavy chain mRNA and protein expression in conjunction with muscle performance in the elderly

Introduction: Aging decreases skeletal muscle mass and contractile ability. This decreases the capacity for an individual to successfully carry out activities of daily living, eventually leading to physical disability. Recent research demonstrates that when compared to conventional resistance training, high velocity resistance training (HVRT) may be more effective in slowing or reversing age related declines in skeletal muscle. However, there is currently a paucity of research which has aimed to investigate the transcriptional and translational events taking place within senescent skeletal muscle in response to HVRT.

Purpose: To examine the velocity specificity of resistance training by directly comparing changes in muscle transcription, translation, performance, and function in older adults.

Methods: Twenty-six older adults were randomized to partake in 6 weeks of either low velocity resistance training (LVRT) or HVRT. Subjects underwent pre- and post-training strength and functional testing. A subsample of subjects also underwent subcutaneous needle biopsies of the vastus lateralis pre- and post-training.

Results: From baseline to post-training, there were several significant ($P < 0.05$) differences in muscle performance and functional characteristics in LVRT (n = 13) and HVRT (n = 13) groups. Our results demonstrate HVRT provides a greater number of muscular enhancements when compared to LVRT, particularly under conditions of high velocity muscle contraction. MyHc-a mRNA showed a significant ($P < 0.01$) decrease

(.93-fold ± 0.12) in LVRT and a significant ($P < 0.01$) increase (2.0-fold ± .62) in HVRT. MyHc-IIa mRNA showed a significant ($P < 0.05$) increase (1.2-fold ± 0.01) in HVRT. MyHc-IIx mRNA showed a significant ($P < 0.01$) decrease (0.99-fold ± 0.004) in LVRT). MyHc-b/slow had a significant ($P< 0.05$) decrease in HVRT (1.0 ± 0.12 vs 0.84 ± 0.13, pre v post). MyHc-IIx decreased ($P < 0.01$) in LVRT (1.0 ± 0.06 vs 0.87 ± 0.06, pre vs post).

Conclusion: HVRT is emerging as the optimal training stimulus for the older adult. The present study demonstrates, in addition to increased muscular performance and functional outcomes, HVRT may also evoke a favorable (i.e., slow-to-fast) transcriptional and translational response in MyHC.

CHAPTER I

INTRODUCTION

Inactivity, certain diseases, and aging decrease skeletal muscle mass and contractile ability. This decreases the ability for an individual to successfully carry out activities of daily living (ADLs), eventually leading to physical disability (Guralnik, Ferrucci, Pieper, Leveille, Markides & Ostir, 2000; Janssen, Heymsfield & Ross, 2002; Kortebein, Ferrando, Lombeida, Wolfe & Evans, 2008). Age-related declines in skeletal muscle mass was given the name sarcopenia ("sarcos" meaning flesh and "penia" meaning a lack of) and presents itself in a number of mammalian species (Brooks & Faulkner, 1988; Lexell, 1995; Rosenberg, 1989). While no universal definition for sarcopenia exists, diagnostic criteria introduced by Baumgartner and colleagues (Koehler, Gallagher, Romero, Heymsfield, Ross, Garry & Lindeman, 1998) and Janssen et al. (2002) are commonly implemented. Both methods utilize comparing relative amounts of muscle mass to total body mass, classifying individuals as sarcopenic if they fall 1 to 2 (depending on the chosen diagnostic criteria) standard deviations below the mean of large sex specific reference populations.

Several mechanisms have been proposed to play a role in the decline of muscle mass and function with age: endocrine changes, increased nuclear apoptosis, reduced satellite cell content /regeneration, muscle fiber denervation, oxidative stress, altered protein synthesis/proteolysis, changing contractile characteristics, decreased neuromuscular function, and increased content of muscular fat (Buford, Anton, Judge,

Marzetti, Wohlgemuth & Carter, 2010; Cruz-Jentoft, Vandewoude, Bauer, Baeyens, Zamboni & Topinkova 2010; Johnston, Lisio & Parise, 2008; Larsson & Ansved, 1995).

Findings from Janssen (2004) suggest 45% of the older U.S. population to be sarcopenic, while Manton (2001) report 20% of older adults in the U.S. are functionally disabled. The impact of sarcopenia on the older adult population is far reaching. Individual and societal burdens include: increased morbidity, disability, mortality and health care costs (Gale, Martyn, Cooper & Sayer, 2007; Sayer, Dennison, Syddall, Gilbody, Phillips & Cooper, 2005; Janssen et al., 2002; Janssen et al., 2004). Janssen et al. (2004) report that healthcare costs directly attributable to sarcopenia in the U.S. were $18.5 billion in 2000. This figure will continue to rise as the percentage of older adults in the U.S is expected to increase from 11% to 20% by 2030 (National Institute on Aging and Statistics. 2007). A 10% decrease in the prevalence of sarcopenia would result in a $1.1 billion saving per year in health care costs (Janssen et al., 2004). Thus, reducing the prevalence and negative outcomes of sarcopenia is a critical challenge worthy of further investigation.

Declines in muscle mass in older adults seem to be primarily due to the loss and atrophy of Type-2 (fast) fibers (Nilwik, Snijders, Leenders, Groen, van Kranenburg, Verdijk & van Loon, 2013; Klitgaard, Mantoni, Schiaffino, Ausoni, Gorza, Laurent-Winter, Schnohr & Saltin, 1990; Lexell et al. 1995), with the losses being most robust in the leg musculature (Janssen et al., 2000). Supporting this notion, Verdijek, Koopman, Schaart, Meijer, Savelberg & van Loon (2007) reported 10% to 40% smaller Type-2 fibers in older adults when compared to Type-2 fibers of young controls, with Type-1 (slow) fiber size remaining relatively unchanged with age.

While muscle mass is an important factor influencing muscle strength (Visser, Goodpaster, Kritchevsky, Newman, Nevitt, Rubin & Harris, 2005), a growing body of literature demonstrates measures of muscular performance (e.g., muscular strength and power), which provide an indication of muscle quality (quality referring to the physiological functional capacity of muscle tissue), to be more effective in assessing mobility, functional status, and risk for morbidity and mortality in aging populations (Bassey, Fiatarone, O'Neill, Kelly, Evans & Lipsitz, 1992; Newman, Kupelian, Visser, Simonsick, Goodpaster, Kritchevsky & Harris, 2006). Research indicates that when compared to skeletal muscle strength, measures of power have a greater influence on the capacity to perform ADLs, the risk of hip fracture, and mobility impairment (Bassey et al., 1992; Bean, Kiely, Herman, Leveille, Mizer & Frontera 2003; Phillips, Woledge & Bruce, 1998). Progressively, this has led some to advocate for including declines in muscle quality and function in the diagnostic criteria for sarcopenia (Cruz-Jentoft et al., 2010).

Dynamic muscle strength is typically quantified by assessing either the maximal amount of force generated by a muscle or muscle group during a one repetition maximum (1RM) testing protocol, or the peak torque that can be developed against a lever that moves through an arc at a fixed angular velocity (isokinetic strength). Muscular power is the product of the velocity and force of muscle contraction. In older adults, peak power is generally achieved between 60% to 70% 1RM and at an angular velocity of 180 deg/sec under isokinetic conditions (Cuoco, Callahan, Sayers, Frontera, Bean & Fielding 2004; Fielding, LeBrasseur, Cuoco, Bean, Mizer & Singh, 2002).

Assessing muscle quality by testing muscular performance can serve as an important prognostic tool, as declines in muscle mass, strength, and power take place in a dissimilar fashion. The median rate of muscle loss is reported to be 0.47% and 0.37% per year in older men and women, respectively (Mitchell, Williams, Atherton, Larvin, Lund & Narici, 2012). Declines in muscle strength occur 2 to 5 times more rapidly than muscle mass, with the rate of decline in muscle power exceeding that of strength (Martin, Farrar, Wagner & Spirduso, 2000; Metter, Conwit, Tobin & Fozard 1997; Mitchell et al., 2012). Additionally, declines in power become more evident as movement speeds increase (Clark, Patten, Reid, Carabello, Phillips & Fielding, 2010) and the velocity at which peak power is generated also declines (Davies, White & Young, 1983; Martin et al., 2000; Pearson, Cobbold, Orrell & Harridge, 2006). Several studies have demonstrated the independent influence of the velocity component of muscle power on functional performance, highlighting its importance (Bean, Kiely, LaRose, O'Neill, Goldstein & Frontera, 2009; Cuoco et al., 2004; Mayson, Kiely, LaRose & Bean 2008; Sayers, Guralnik, Thombs & Fielding, 2005). It is likely that declines in the velocity and power generating capacity of skeletal muscle is influencing the increased risk of falls seen in older adults (Whipple, Wolfson & Amerman, 1987), since powerful counter movements are frequently required for the prevention of falls (Pijnappels, Bobbert & Dieën, 2005).

As the ability to produce quick and powerful movements degenerates more rapidly (Martin et al., 2000; Metter et al., 1997; Mitchell et al., 2012) and serve as better indicators for the capacity to successfully carry out ADL's and future risk for impairment than strength (Bassey et al., 1992; Bean et al., 2003; Phillips et al., 1998), many

resistance training interventions have employed a training stimulus with an aim to enhance these abilities within the muscle groups being trained. Early studies evaluating whether resistance training (RT) could improve skeletal muscle function utilized low velocity resistance training (LVRT), involving slow contraction of skeletal muscles against "high" (relative to the maximal force generating capacity of the muscles performing work) loads. LVRT commonly leads to hypertrophy, enabling a more forceful contraction to take place (Campos, Kraemer, Ratamess, Ragg, Murray & Hagerman 2002; Fiatarone et al., 1990; Kosek, Jeong-sur & Petrella, 2006; Taaffe, Pruitt, Pyka, Guido & Marcus, 1996; Trappe, Williamson & Godard, 2000; Trappe, Godard & Gallagher, 2001) and can reverse or slow some age-related declines within skeletal muscle (Campos et al., 2002; Mangione, Miller & Naughton, 2010; Kosek et al., 2006; Trappe et al., 2000, Trappe et al., 2001). However, LVRT has been shown to be inadequate in improving the ability of older individuals to perform ADLs and provides only modest increases in muscular power (Bottaro, Machado, Nogueira, Scales & Veloso, 2007; Fielding et al., 2002; Latham, Bennett, Stretton & Anderson, 2004), partly due to the low movement speeds employed (Henwood, Riek & Taaffe, 2008).

The recognition of LVRT's limitations led to the investigation of more powerful, higher velocity, movements during resistance training in older adults (Bottaro et al., 2007; Earles et al., 2001; Fielding et al., 2002; Marsh et al., 2009; Puhke et al., 2006). This style of power, or high velocity resistance training (HVRT), is characterized by performing the concentric phase of each contraction as quickly as possible. HVRT has been shown to enhance peak power and functional outcomes more effectively than LVRT (Earles et al., 2001; Fielding et al., 2002; Marsh et al., 2009; Puhke et al., 2006; Sayers &

Gibson, 2012; Sayers & Gibson, 2014). Results from Earles et al. (2001) demonstrate increases of up to 141% in peak power after 12 weeks of HVRT in older adults, while Fielding et al. (2002) report an 84% greater increase in peak power in the HVRT group compared to LVRT.

In particular, trials investigating HVRT which have utilized movement speeds exceeding velocities of peak power production yield promising results, improving muscle quality and providing a real world carry over (Sayers et al., 2012; Sayers et al., 2014). This carry over seems to be related to increasing the velocity at which peak power is produced (Sayers et al., 2012; Sayers et al., 2014). This noted enhancement in limb contraction velocity can benefit the older adult in many ways. For an older individual carrying out ADLs, an emphasis is placed on force generating capabilities at the velocities required for carrying out a particular task (Pijnappels et al., 2005; Sayers et al., 2012; Whipple et al., 1987). Force development at these higher velocities will be the critical factor in determining if an older individual can move a lower limb rapidly enough to maintain safety in situations such as crossing the street, stabilizing the body to prevent a fall, or shifting a limb from a car accelerator to the break (Pijnappels et al., 2005; Sayers et al. 2012; Whipple et al., 1987). From a practical outlook, most activities of daily living do not require maximal force or power development (Buchner et al., 1996). Moreover, it has been demonstrated that if one possesses the strength to complete a functional task, further strength gain does not inherently enhance functionality (Buchner et al., 1996).

The neural benefits of HVRT have been demonstrated (Van Cutsem, Duchateau & Hainaut, 1998). However, there is currently a paucity of literature which has aimed to

investigate the transcriptional and translational events taking place within senescent skeletal muscle in response to HVRT. Such work is of great importance since it can provide insights into the downstream mechanisms influencing older adults to respond favorably to a HVRT stimulus.

The myofilament myosin heavy chain (MHC) serves as the molecular motor of a muscle fiber, completing the mechanical work necessary to elicit a muscle contraction (Bottinelli, Canepari, Pellegrino & Reggiani, 1996; Bottinelli, Pellegrino, Canepari, Rossi & Reggiani, 1999). Classically, human muscle fibers have been known to express three MHC isoforms: MHC-1, MHC-2A, and MHC-2X (Biral, Betto, Danielli-Betto & Salviati, 1998; Klitgaard et al., 1990). Muscle fibers can express an exclusive isoform (pure fiber) or multiple isoforms (hybrid fiber), resulting in six different fibers types: 1, 1/2A, 2A, 2A/2X, 2X, 1/2A/2X (Biral et al., 1998; Klitgaard et al., 1990). Each myosin isoform has a unique set of contractile characteristics, with speed of contraction, tension, and power development increasing from: MHC-1 $\rightarrow$ MHC-2A $\rightarrow$ MHC-2X (Bottinelli et al., 1996; Bottinelli et al., 1999). Of interest, is the isoform MyHC-α, which was identified in an animal model and is thought to serve as an intermediate between MyHC-β/slow and MyHC-IIA in skeletal muscle (Peuker, Conjard & Pette, 1998). It has been shown that a shift in the type of MHC isoform present within a muscle fiber can affect the contractile ability of that fiber and can also alter whole muscle performance (D'Antona, Lanfranconi, Pellegrino, Brocca, Adami & Rossi, 2006; Harridge et al., 1996; Ryushi & Fukunaga, 1986).

While a preponderance of work demonstrates RT and endurance exercise results in a fast-to-slow shift in MHC protein and mRNA (MHC-2X $\rightarrow$MHC-2A) (Hortobagyi,

Hill, Houmard & Fraser, 2000; Short, Vittone & Bigelow, 2005; Staron et al., 1990), research which has applied a sprint or high velocity training stimulus found a slow-to-fast (MHC2A $\rightarrow$ MHC2X) or bidirectional (MHC-1 $\rightarrow$MHC-2A $\leftarrow$MHC2X) shift in histochemical and biochemical properties and mRNA (Andersen et al., 1994; Esbjornsson, Hellsten-Westing, Balsom, Sjödin, & Jansson 1993; Jansson et al., 1978; Jansson et al., 1990; Liu, Schlumberger, Schmidtbleicher & Steinacker, 2003; Malisoux et al., 2006; Paddon-Jones, Lonergan, Abernethy & Leveritt 2001). Paralleled with this slow-to-fast shift in MHC are training adaptations which have proven to be favorable in older adults, such as increases in movement speed, peak power, and the velocity at which peak power takes place. Consequently, the purpose of this study was to investigate the transcriptional and translational events which occur in older adults in response to HVRT, since the information gained has the potential to elucidate a mechanism underlying the noted favorable training adaptations.

The hypotheses for this study were that HVRT would induce a slow –to-fast shift in MHC composition and lead to a greater number of favorable muscle performance outcomes, when compared to LVRT

CHAPTER II

REVIEW OF LITERATURE

Sarcopenia

The progressive decline in muscle mass, strength and functionality associated with aging is known as sarcopenia (Cruz-Jentoft et al., 2010). This noted decline in muscle mass, strength and size with age is well documented in a range of mammalian species (Brooks et al., 1988; Lexell et al., 1995).

Depending on the definition used for sarcopenia, the prevalence in people 60 to 70 years old is 5 to 13%, while the prevalence ranges from 11 to 50% in people >80 years in the United States (Morley et al., 2008). However, considerable disparity exists when quantifying the prevalence of sarcopenia in older populations as a result of various definitions and criteria for classification. While no universal definition for sarcopenia exists, diagnostic criteria introduced by Baumgartner et al. (1998) and Janssen et al. (2002) are commonly implemented. Both utilize comparing relative amounts of muscle mass to total body mass. The method proposed by Baumgartner et al. (1998) utilizes a measure of relative amounts of muscle mass by dividing absolute muscle mass, (quantified by dual-energy X-ray absorptiometry (DXA)) by height squared, with sarcopenia being defined as relative muscle mass being more than two standard deviations below the mean of a large sex specific reference population. The method proposed by Janssen et al. (2002) utilizes a skeletal muscle index (SMI), dividing total muscle mass (attained via bioelectrical impedance analysis (BIA)) by total body mass, classifying sarcopenia as being between one and two standard deviations below young adult values.

Findings from Janssen and colleagues (2004) suggest 45% of the older U.S. population to be sarcopenic, while Manton and colleagues (2001) report 20% of older adults in the U.S. are functionally disabled. The impact of sarcopenia on the older adult population is far reaching. Individual and societal burdens include: increased morbidity, disability, mortality and health care costs (Gale et al., 2007; Sayer et al., 2005; Janssen et al., 2002; Janssen et al., 2004). Janssen and colleagues (2004) report that healthcare costs directly attributable to sarcopenia in the U.S. was $18.5 billion in 2000. This figure will continue to rise as the percent of older adults in the U.S is expected to increase from 11% to 20% by 2030 (National Institute on Aging and Statistics). A 10% decrease in prevalence of sarcopenia would result in a $1.1 billion saving per year in health care costs (Janssen et al., 2004). Thus, reducing the prevalence and negative outcomes of sarcopenia is a critical challenge, worthy of further investigation.

The etiology and progression of sarcopenia is multi-faceted. Several mechanisms have been proposed to play a role: increased nuclear apoptosis, reduced satellite cell content / regeneration, muscle fiber denervation, oxidative stress, altered protein synthesis/proteolysis, decreased neuromuscular function, and increased content of muscular fat (Buford et al., 2010; Cruz-Jentoft et al., 2010; Johnston et al., 2008; Larsson et al., 1995).

Fiber type delineation (histochemical, immunohistochemical, and electrophoretic techniques)

Dating back to 1678, attempts have been made to classify skeletal muscle fiber types (Nowak et al., 2008). Initially, fiber types were grossly classified as red and white based on their hue due to varying magnitudes of myoglobin (Nowak et al., 2008).

Improvements in nomenclature came about with the classification of fibers based on contractile characteristics (slow and fast twitch) and metabolic pathway utilization (glycolytic and oxidative) (Nowak et al., 2008).

More sophisticated methods were developed in order to analyze fiber types based on enzymatic histochemical reactions, such as myofibrillar ATPase (mATPase) staining (Brooke et al., 1970). During the cross-bridge cycle, the myosin molecule binds and hydrolyzes ATP during force generation, and because myosin ATPase activity is positively correlated with muscle contraction velocity, measures of ATPase activity can be interpreted in terms of contraction speed (Nelson et al., 2008). Staining for ATPase results in differentiation of muscle types by color based on the pH of the preincubation solution. This process reveals a spectrum of fibers: Type-1 $\rightarrow$ Type-2A $\rightarrow$ Type-2C $\rightarrow$ Type-2X (Nowak et al., 2008). This hierarchy reflects increases in contractile and glycolytic capacity as levels of ATPase increase from Type-1 $\rightarrow$ Type-2X. While mATPase histochemistry provided further insight into varying fiber type characteristics (Staron et al., 1991), biochemical truths cannot be ascertained from histochemistry (Brooke et al., 1974).

Electrophoretic techniques are utilized to separate protein molecules by size and can detect Myosin Heavy Chain (MHC) levels as low as 2–3% of the protein content (Giulian et al., 1983). Three electrophoretically distinct MHC isoforms, termed MHC-1, MHC-2A and MHC-2X, have been identified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (Biral et al., 1998).

Present day immunohistochemical techniques, such as the western blot, utilize antigen-antibody techniques to identify specific proteins, even in very small amounts,

within a tissue sample (Brooke et al., 1974). Immunohistochemistry has been used to identify many proteins which play pivotal roles in the structure and function of muscle fibers (Nowak et al., 2008). This technique was implemented in the identification of the motor protein MHC and its isoforms (Nowak et al., 2008). Following electrophoretic separation, MHC identification via antibody probing is currently the most valid method for fiber typing (Schiaffino et al., 2011). Unlike several other mammalian species, in human muscles, MHC-2B is not detectable, although the corresponding MYH4 gene is present in the genome (Smerdu et al., 1994). In addition to pure fiber types, which express a singular MHC isoform, human skeletal muscles contain a significant proportion of fibers with hybrid myosin composition. Most commonly, these hybrid fibers co-express MHC-1 with MHC-2A or MHC-2A with MHC-2X (Biral et al., 1998; Klitgaard et al., 1990).

Functional diversity among fiber types

There is considerable functional diversity between fiber types and accompanying sub-types (Bottinelli et al., 1996; Bottinelli et al., 1999). The extent to which a given MHC isoform is expressed within a muscle fiber influences contractile capacity and rate of ATP utilization (Bottinelli et al., 1996; Bottinelli et al., 1999; Schiaffino et al., 2011).

The functional capacity of an activated muscle fiber is quantified through measures of force, velocity, and power. The force generating capacity of a half-sarcomere during an isometric contraction can be represented as the number of strongly bound cross bridges (XB) between actin and myosin multiplied by the force generated per XB (F_{uni}) (Brenner, 1988). The number of strongly bound XBs at a given point in time is determined by the total number of functional myosin heads (N) multiplied by the

fraction of time a strongly bound XB is formed (t_{on}) as a function of total myosin cycle time ($t_{on} + t_{off}$), where t_{on} is the amount of time myosin is strongly bound to actin and t_{off} is the amount of time myosin is detached from actin. The amount of force that can be produced in the half sarcomere under isometric conditions can be expressed as: N $(t_{on}/(t_{on}+t_{off}))F_{uni}$. Force generated per XB interaction is also dependent upon the elastic stiffness of the XB (k_{stiff}) multiplied by the unitary displacement of the power stroke (d_{uni}).

The maximum isometric force per unit cross-sectional area, or specific tension (*P*o/CSA), is approximately 2 times lower in Type-1 fibers than Type-2A and Type-2X, with no significant difference being reported between the fast fiber types (Bottinelli et al., 1996; Bottinelli et al., 1999). Two factors contribute to the higher force development in fibers expressing fast MHC isoforms compared with those expressing slow isoforms: a greater number of strongly attached myosin heads and higher force development (F_{uni}) per myosin head (Brenner, 1988; Linari et al., 2004). Contractile velocity is determined by d_{uni} divided by t_{on}, with a shorter t_{on} representing a higher myosin detachment rate (Wallcot et al., 2012) and is exemplified in the differing contraction speeds of human myosin isoforms. In humans, fibers comprised of MHC-2X (Type-2X fast fiber) display up to 9 times greater unloaded shortening velocity (V_0), and maximal power (W_{max}) output than those with a MHC-1 (Type-1 slow fiber) composition (Bottinelli et al., 1996; Bottinelli et al., 1999). Fibers comprised of MHC-2A (Type-2A fast fiber) display intermediate V_0 and W_{max} outputs (Bottinelli et al., 1996; Bottinelli et al., 1999). Trappe et al., (2003) report Type-2 fibers generate four to six times more power than Type-1 fibers.

Quantifying maximal rates of *V*o and *P*o are of consequence since they set the ceiling for other contractile parameters (Bottinelli et al., 1999). However, *in vivo*, muscles rarely contract isometrically, and never contract against zero load. This being the case, other performance variables need to be considered to get a clearer idea of a muscle fiber's *in vivo* capacity. Power output, a measure of mechanical energy released per unit of time, is a more meaningful performance variable in relation to *in vivo* muscle performance (Bottinelli et al., 1999; Schiaffino et al., 2011). A muscle fiber's peak power ($W_{max)}$ is determined by the maximum velocity of unloaded shortening (*V* max when determined from the force velocity curve or *V*o when determined with the slack test), the isometric force (*P*o), and the curvature of the relationship. The less the curvature, the greater the force generated at a particular velocity, resulting in a greater power output (Bottinelli et al., 1999; Schiaffino et al., 2011). This places an emphasis on the shortening velocity and the amount of force developed when peak power is reached within a muscle fiber. These values are termed optimal velocity and optimal force. Optimal velocity (V_{opt}) is reached at ~20% *V*o in fast fibers and ~15% *Vo* in slow fibers, resulting in *V*opt being about four times higher in fast fibers than in slow fibers (Bottinelli et al., 1999). *In vivo*, *V*opt has been shown to be the velocity preferentially adopted during movement, which suggests fibers are recruited not only on the need to increase force development but also in relation to the speed at which a movement must be performed (Lutz et al., 1994; Rome et al., 1988).

These fiber type characteristics are further expressed at the motor unit level. Fast motor units produce 10 to 20 times greater isometric tension than slow motor units (Henneman et al., 1981). Being that a fast motor unit contains a larger amount of fibers,

and fast fibers produce up to 9 times greater power outputs than slow fibers, a fast motor unit will produce a power output hugely surmounting that of a slow motor unit (Bottinelli et al., 1999). Outside of peak power values, a motor unit which is comprised of fast fibers can effectively maintain force development at velocities which surpass that of peak power development (Bottinelli et al., 1999).

In essence, if one is to produce large amounts of force rapidly, it will come from recruiting fast motor units (Henneman et al., 1981; Bottinelli et al., 1996; Bottinelli et al., 1999). The contractile profile of these fast motor units (i.e., to what degree they are composed of MHC-2A or MHC-2X) will influence the amount of force that can be generated at a given velocity (Bottinelli et al., 1996; Bottinelli et al., 1999; Pearson et al., 2006).

Of note, while the fast fiber types do not differ significantly in (*P*o/CSA), they vary significantly in every other quantifiable performance variable. In fact, greater heterogeneity can be displayed between Type-2A and Type-2X than between Type-1 and Type-2A fibers (Bottinelli et al., 1999). This highlights how distinct each of the myosin heavy chain isoforms are from one another, as well as the importance of classifying fiber types based on the particular fast MHC isoforms which are present, and not defaulting to a Type-1 or Type-2 classification.

Type-2 fiber atrophy and functional consequences

In aging human populations, declines in muscle mass seem to be primarily due to the atrophy of Type-2 fibers, with the losses being most robust in the leg musculature (Janssen et al., 2000; Nilwik et al., 2013; Klitgaard et al., 1990; Lexell et al. 1995). Supporting this notion, Verdijek and colleagues (2007) reported 10 to 40% smaller Type-

2 fibers when compared to young controls, with Type-1 fiber size remaining relatively unchanged. Kosek and colleagues (2006) demonstrate a robust atrophy of Type-2X fibers with age. Decrease in Type-2 fiber CSA decreases force generating capacities within the muscle (Trappe et al., 2003).

Transcriptional and translational events have been investigated in search for a mechanism underlying Type-2 fiber atrophy with age. The effects of aging on the transcription and translation of MHC isoforms are functionally important, since MHC serves as the molecular motor of a muscle fiber, completing the mechanical work necessary to elicit a muscular contraction (Schiaffino et al., 2011). Translation is affected by a decrease in the rate of MHC-2A and MHC-2X synthesis (Blazejowski et al., 1984, Hasten et al., 2000; Welle et al., 1993). Declines in MHC contraction characteristics have also been reported with age (Hook et al., 1999; Larsson et al., 1997). However, conflicting results have been reported (Trappe et al., 2003).

MHC-1 transcript levels remain relatively static with age (Balagopal et al., 2001; Welle et al., 1996). However, aging's effects on MHC-2A and MHC-2X transcription are not as well understood. Welle and colleagues (1996) reported no differences in MHC-2A and MHC-2X mRNA levels between young and old men and women. However, a decrease in MHC-2A mRNA, but not in MHC-2X mRNA, has been observed in old men (Welle et al., 2000). Balagopal and colleagues (2001) further reported significant decreases in both MHC-2A and MHC-2X mRNA with aging in men and women. The decreased MHC-2A and MHC-2X mRNA levels reported by Welle and colleagues (2000) and Balagopal and colleagues (2001) provide a logical mechanism underlying the decreased synthesis of fast MHC isoforms within senescent muscle tissue.

The equivocal results reported when investigating MHC mRNA could be partly due to the absence of quantifying MHC isoform expression at the protein level. While the protein isoform and corresponding mRNA share an association, normalizing MHC mRNA to MHC protein is paramount (Hortobagyi et al. 2000; Periasamy et al., 1989). This is because there is significant variability in skeletal muscle MHC isoform expression between individuals as a result of genetic and environmental influence (Simoneau et al., 1995).

When considering the fundamental characteristics of the MHC isoforms, it comes as no surprise that this preferential loss of MHC-2A and MHC-2X (Type-2 fibers) is directly related to decreases in power producing capacities which greatly exceed muscle loss in older populations (Davies et al., 1983; Martin et al., 2000; Metter et al., 1997; Trappe et al., 2003). Declines in power production become more evident as movement speeds increase (Clark et al., 2010). The angular velocity at which peak power is generated (Vopt) is also reduced (Davies et al., 1983; Martin et al., 2000; Pearson et al., 2006). It is likely that this decline in velocity and power generating capacity within the muscle is influencing the increased risk of falls seen in older adults (Whipple et al., 1987), as it has been demonstrated that powerful counter movements are frequently required for the prevention of falls (Pijnappels et al., 2005). Further risks of muscle weakness include immobility, dependency, and disability (Fiatarone et al., 1990).

MHC isoforms and mRNA in transition

Skeletal muscle is a heterogeneous tissue with an incredible propensity to adapt to the stimulus it is placed under (Canepari et al., 2005; Schiaffino et al., 2011). Alterations of myosin heavy chain isoform composition within the muscle can, in part, explain the

muscular adaptations achieved through exercise (Canepari et al., 2005; Schiaffino et al., 2011).

Shifts in the expression of MHC at the protein and mRNA levels can vary greatly in response to patterns of mechanical loading or deloading (Larsson et al., 1985; Short et al., 2005). Longitudinal studies have documented a slow to fast transformation (MHC-I → MHC-2A → MHC-2X) under conditions of immobilization or detraining (Haggmark et al., 1986; Larsson et al., 1985) and a fast to slow transformation (MHC-2X →MHC-2A → MHC-1) as a result of endurance exercise (Howald et al., 1985; Short et al., 2005).

The MHC-I → MHC-2A → MHC-2X shift in response to immobilization or detraining has led to characterizing Type-2X fibers as "default fibers" which will respond to any form of mechanical loading in a MHC-2X →MHC-2A (fast to slow) fashion. A preponderance of works demonstrates resistance and endurance exercise leading to the noted response: MHC-2X →MHC-2A in MHC protein and mRNA (Hortobagyi et al., 2000; Short et al., 2005; Staron et al., 1990). However, the direction of shifting seen in MHC at the protein and mRNA level seem to be highly dependent upon the training stimulus (Andersen et al., 1994; Esbjornsson et al., 1993; Jansson et al., 1978 Jansson et al., 1990; Liu et al., 2003; Malisoux et al., 2006; Paddon-Jones et al., 2001). Research employing a high velocity training stimulus has induced a slow to fast shift in histochemical properties (Esbjornsson et al., 1993; Jansson et al., 1978 Jansson et al., 1990; Paddon-Jones et al., 2001) and MHC isoforms (Liu et al., 2003). Others have demonstrated an increase in MHC-2A, with no decrease in MHC-IIX or a bidirectional shift towards MHC-2A (Andersen et al., 1994; Malisoux et al., 2006).

During states of training or detraining, the presence of hybrid fibers (fibers co-expressing MHC-1 with MHC-2A or MHC-2A with MHC-2X) are a common finding (Andersen et al., 1997; Marx et al., 2002). Andersen and colleagues (1997) suggest hybrid fibers are generally present when fiber type transition occurs. Under these conditions, hybrid fibers will display a mismatch between the distribution of MHC mRNA and protein isoforms (Andersen et al., 1997; Marx et al., 2002). In response to training and detraining, Andersen and colleagues (1997) demonstrate mismatched fibers representing transitional fibers at various stages of the transformation process. This mismatch can be utilized in clarifying the direction in which the fiber is shifting. The up-or-down regulation of mRNA will occur first in a transitional process (MHC mRNA first in, first out) with protein changes following (MHC protein last in, last out) (Andersen et al., 1997; Marx et al., 2002). In other words, when transformation is initiated there will be upregulation of mRNA which expresses the direction of the current shift, but the corresponding protein will not be expressed, while the mRNA and protein of the original fiber type will still be expressed (Andersen et al., 1997). During the intermediate stages of the transitional process, a better match between the distribution of MHC mRNA and protein is expected (Andersen et al., 1997). During the final phase of transformation, both the newly induced mRNA and protein should be detected, as well as the protein but not the mRNA of the original fiber type (Andersen et al., 1997). This is in response to a more rapid turnover of MHC mRNAs, compared with the slow turnover of the corresponding proteins (Gupta et al., 1992; Russell et al., 1992).

It has been reported that the delayed appearance of new MHC isoforms is not due to delayed synthesis, but delayed incorporation of the isoforms into the sarcomere (Pette

et al., 1992). This delay is thought to be caused by the disassembling of older filaments before newly synthesized isoforms are introduced into the sarcomere (Brown et al., 1989; Eisenberg et al., 1984). This inherent delay of MHC incorporation would be amplified in the older adult due to decreased rates of MHC-2A and MHC-2X synthesis (Blazejowski et al., 1984, Hasten et al., 2000; Welle et al. 1993). This suggests, if a study were not long enough in duration, expression of new MHC isoforms may not be detected, even if there were increased expression at the mRNA level. This highlights the importance of investigating mRNA expression in situations where MHC shifting would be expected. Furthermore, normalizing MHC mRNA to MHC protein expression allows control of the variability seen from person to person in MHC isoform expression at the protein level (Simoneau et al., 1995). Increases in MHC mRNA occur rapidly at the onset of training and have a half-life up to 3 days post training (Nadal-Ginard et al., 1982), with corresponding proteins having a half-life of 2-3 weeks (Bates et al., 1983).

Low velocity resistance training (LVRT)

Early studies evaluating whether resistance training (RT) could improve skeletal muscle function utilized low velocity resistance training (LVRT) (i.e., slow contraction) of skeletal muscles against "high" (relative to the maximal force generating capacity of the muscles performing work) loads. This low velocity style of training commonly leads to hypertrophy, enabling a more forceful contraction to take place (Campos et al., 2002; Fiatarone et al., 1990; Kosek et al., 2006, Taaffe et al., 1996 Trappe et al., 2000; Trappe et al., 2001) and can reverse or slow some age-related declines within muscle tissue (Campos et al., 2002; Kosek et al., 2006; Mangione et al., 2010; Widrick et al., 2001). However, LVRT has been shown to be inadequate in improving the ability of older

individuals to perform activities of daily living and provides only modest increases in muscular power (Earles et al., 2001; Latham et al., 2004). The lack of carry over into real world situations is in part due to the low movement speeds employed (Henwood et al., 2008).

High velocity resistance training (HVRT)

The recognition of LVRT's limitations led to the investigation of more powerful, higher velocity, movements during resistance training in older adults (Bottaro et al., 2007; Earles, 2001; Fielding et al., 2002; Marsh et al., 2009; Puhke et al., 2006). This style of power, or high velocity, resistance training (HVRT), is characterized by performing the concentric phase of each contraction as quickly as possible and has been shown to enhance peak power and functional outcomes more effectively than low velocity training (Earles, 2001; Fielding et al., 2002; Marsh et al., 2009; Puhke et al., 2006 Sayers et al., 2012 Sayers et al., 2014). In particular, utilizing movement speeds which exceed velocities of peak power generation demonstrate promising results for improved function and real world carry over (Sayers et al., 2012). This carry over seems to be, in part, explained by increasing the velocity at which peak power is generated (Sayers et al., 2012; Sayers et al., 2014). This noted enhancement in limb contraction velocity can benefit the older adult in many ways: for an older individual carrying out ADL's, an emphasis will be placed on the level of force generation one is capable of at the velocities required for carrying out a particular task (Pijnappels et al., 2005; Sayers et al., 2012; Whipple et al., 1987). Force development at these higher velocities will be the critical factor in determining if an older individual can move a lower limb rapidly enough to maintain safety in situations such as crossing the street, stabilizing the body to prevent

a fall, or shifting a limb from the accelerator to the break (Pijnappels et al., 2005; Sayers et al. 2012; Whipple et al., 1987). From a practical outlook, most activities of daily living do not require maximal force or power development (Buchner et al., 1996). Furthermore, it has been demonstrated that if one possesses the strength to complete a functional task, further strength gain does not inherently enhance functionality (Buchner et al., 1996).

Conclusion

The neural benefits of HVRT have been demonstrated (Van Cutsem et al., 1998). However, there is currently a paucity of literature which has aimed to investigate the transcriptional and translational events taking place within senescent skeletal muscle in response to HVRT. Such work is of great importance since it can provide insights into the mechanisms influencing older adults to respond favorably to a HVRT stimulus.

The myofilament myosin heavy chain (MHC) serves as the molecular motor of a muscle fiber, completing the mechanical work necessary to elicit a muscle contraction (Schiaffino et al., 2011). Human muscle fibers express three MHC isoforms: MHC-1, MHC-2A, and MHC-2X (Biral et al., 1998; Klitgaard et al., 1990). Muscle fibers can express an exclusive isoform (pure fiber) or multiple isoforms (hybrid fiber), resulting in six different fibers types: 1, 1/2A, 2A, 2A/2X, 2X, 1/2A/2X (Biral et al., 1998; Klitgaard et al., 1990). Each myosin isoform has a unique set of contractile characteristics, with speed of contraction, tension and power development increasing from: MHC-1 $\rightarrow$ MHC-2A $\rightarrow$ MHC-2X (Bottinelli et al., 1996; Bottinelli et al., 1999). A shift in the type of MHC isoform present within a muscle fiber will impact the contractile ability of that fiber

and can also alter whole muscle performance (D'Antona et al., 2006; Harridge et al., 1996; Ryushi et al., 1986).

While a preponderance of work demonstrates RT and endurance exercise leads to a fast- to-slow shift in in MHC protein and mRNA (MHC-2X →MHC-2A) (Hortobagyi et al., 2000; Short et al., 2005; Staron et al., 1990), studies which have applied a sprint or high velocity training stimulus have led to a slow-to-fast (MHC2A → MHC2X) or bi directional (MHC-1 →MHC-2A ←MHC2X) shift in MHC protein and mRNA (Andersen et al., 1994; Esbjornsson et al., 1993; Jansson et al., 1978 Jansson et al., 1990; Liu et al., 2003; Malisoux et al., 2006; Paddon-Jones et al., 2001). Paralleled with this slow to fast shift in MHC are training adaptations which have proven to be favorable in aging populations: increases in movement speed, peak power, and the velocity at which peak power takes place. If a slow to fast shift in MHC protein and mRNA also presents itself in older adults in response to HVRT is worthy of investigation, as it aids in elucidating a mechanism underlying the noted favorable training adaptations.

CHAPTER III

METHODS AND MATERIALS

Subjects

Subjects were recruited from the Ames, IA. area through advertisements and email. Potential subjects were initially screened by telephone or in person. To be eligible for the training portion of the study, subjects needed to be aged 60 and older, ostensibly healthy, and not have participated in structured progressive resistance training within the prior 6 months. Exclusion criteria included any preexisting condition that would inhibit successful participation in the exercise program, such as cardiovascular disease, musculoskeletal disorders, and neurological, or cognitive impairment. Exclusion criteria for the biopsy portion of the studyincluded cardiovascular, neurological or musculoskeletal disease; a history of bleeding disorders; taking any anticoagulant (blood-thinning) medications or other medications that affect blood clotting. Written, informed consent was obtained from each participant after all procedures were approved by the Institutional Review Boards of Iowa State University. Gender, age, height, body weight and BMI were recorded (Table 1). The racial distribution of the participants was 100% Caucasian.

Table 1. Anthropometric data

Group	n	n (f)	n (m)	Age (yr)	Height (cm)	Body Weight (kg)	BMI
LVRT	13	8	5	65.1 ± 1.8	171.1 ± 2.2	80.0 ± 2.8	27.3 ± 1.1
HVRT	13	8	5	64.5 ± 0.7	170.4 ± 2.2	79.1 ± 4.8	27.1 ± 1.3

Values are means ± SE; LVRT, low velocity resistance training; HVRT, high velocity resistance training; n, number of subjects; f, female; m, male; BMI, body mass index

Study design

Subjects were randomized to partake in 6 weeks of low velocity resistance training (LVRT) or 6 weeks of high velocity resistance training (HVRT) (details below). Subjects underwent pre-and-post training strength and functional testing (details below). A subsample of subjects also underwent subcutaneous needle biopsies of the vastus lateralis pre-and-post training (details below). The first visit that subjects made to the lab was a familiarization session. The following visit was to undergo the initial muscle biopsy procedure. Three days later, subjects came in for baseline strength and functional testing. Two days after this, subjects began 6 weeks of training. Two days after the final training session, subjects came to the lab for the final biopsy procedure. Two to three days later subjects came in for post-training strength and functional testing. No subjects missed more than 2 training sessions throughout the 6 week training period. Subjects were asked to maintain all prior physical activity and dietary habits and not to partake in any form of lower body resistance training outside of the study.

Progressive Resistance Training: Resistance training was performed 3 times per week for 6 weeks. Each resistance training session was separated by at least 48 hours. Resistance training sessions consisted of performing unilateral knee extensions for both lower limbs. The target muscles were the knee extensors (vastus intermedius, vastus medialis, vastus lateralis and rectus femoris). Subjects performed 3 sets of 8 repetitions, consecutively, for each leg on a calibrated Biodex Multi-Joint System Pro dynamometer (Biodex Medical Systems, Shirley, NY). Subjects assigned to LVRT performed each repetition at an angular velocity of 75 degrees/second. Subjects assigned to HVRT

performed each repetition at an angular velocity of 240 degrees/second. The interval between repetitions and sets was 3 to 4 s and 3 min, respectively.

Muscle performance measurements

Isokinetic Strength Testing Procedure: Concentric, isokinetic knee extensions were performed on the dominant leg at ordered velocities of 75, 180, and 240 deg/sec. Outputs for peak torque, max rep work, total work, torque at .2 sec, the rate of force development, average power and peak power were measured at each velocity using a calibrated dynamometer (Biodex Medical Systems, Shirley, NY). Subjects were seated with a restraining strap over the pelvis and trunk in accordance with the Biodex User's Guide. The input axis of the dynamometer was aligned with the axis of the knee, and the non-working leg was braced against the contralateral- limb stabilization bar. Three submaximal warm-up trials preceded 3 maximal muscle actions at each velocity, with the highest value selected as the representative score. A 4-minute rest was allowed between testing at each velocity.

Functional measurements

Balance: *Tandem, semi-tandem and side by side stands*: For each stand, the test administrator first demonstrated the task, then supported one arm while participants positioned their feet, asked if they were ready, then released the support and began timing. The timing was stopped when the participants moved their feet or grasped the interviewer for support, or when 10 seconds had elapsed. Each participant began with the semi-tandem stand, in which the heel of one foot was placed to the side of the first toe of the other foot, with the participant choosing which foot to place forward. Those unable to hold the semi-tandem position for 10 seconds were evaluated with the feet in a side by

side position. Those able to maintain the semi-tandem position for 10 seconds were further evaluated with the feet in full tandem position, with the heel of one foot directly in front of the other (Guralnik et al., 2000).

Usual walking speed: Subjects were instructed to perform three bouts of 4-m walking at a usual ("As if you were walking down the street to go to the store") speed. Time to completion was recorded using a stop watch. Trials were separated by 2 minutes. The fastest time to completion was used for analysis (Guralnik et al., 2000).

Maximal walking speed: Subjects were instructed to perform three maximum effort bouts of 4-m walking, beginning 2-m before the start line in order to achieve optimal speed. Time to completion was recorded using a stop watch. Trials were separated by 3 minutes. The fastest time to completion was used for analysis (Guralnik et al., 2000).

Sit-to-stand test: A straight-backed chair was placed next to a wall. Subjects were asked to fold their arms across the chest and to stand up from a seated position on the chair. From the sitting position, the subject stood up to full height, then completely back down. After this was completed, the subject was instructed to stand up and sit down five times as quickly as possible Time to completion was recorded using a stop watch. Trials were separated by 3 minutes. The fastest time to completion was used for analysis (Guralnik et al., 2000).

Get-up and go: Subjects were instructed to sit on a chair with their back straight, feet flat on the floor, and hands on their thighs. The timer was started on the signal "go," whether or not the participant actually started to move, and terminated when the subject covered 8 ft. and sat back down. The procedure was visually demonstrated in advance,

and each participant was allowed to practice it once. Following the practice, three test trials were administered. Time to completion was recorded using a stop watch. Trials were separated by 3 minutes. The fastest time to completion was used for analysis (Rikli and Jones, 1999).

Biochemical measurements

Muscle biopsy: A percutaneous biopsy was obtained using a 5mm Bergström needle, under local anesthesia from the vastus lateralis muscle of the dominant leg. The muscle samples (~40 mg of tissue) were removed, immediately frozen in liquid nitrogen and then stored at -80°C. Biopsies were obtained at rest and on non-training days, 3 to 4 days before baseline testing and 2 days after the final training session. The post training biopsy was obtained from the same leg, 3–4 cm distal to the site of the pre training biopsy.

RNA isolation and qRT-PCR: Muscle samples were powdered with a dry-ice-chilled mortar and pestle. Total RNA was extracted from ~30 mg of powdered muscle using TRIizol (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Powdered muscle was homogenized with a handheld homogenizer in TRIzol reagent, centrifuged and extracted with chloroform, and precipitated with ethanol. Total RNA was DNase treated (RNaseFree DNase set, Qiagen Inc., Valencia, CA; cat. no 79254) and purified using a column (RNeasy kit, Qiagen Inc.; cat. no 74106) to remove potential genomic DNA contamination. RNA concentration and purity was determined using an ND-1000 Spectrophotometer (λ = 260/280 nm; NanoDrop technologies, Inc., Wilmington, DE). Total RNA (60ng/µl) was reverse transcribed to cDNA utilizing the Superscript III One-Step qPCR (Qiagen). cDNA was diluted (1:20) in RNase-free water.

Diluted cDNA (2 µl) was amplified on an Eppendorf PCR Master cycler using Quantitect SYBR Green PCR kit (Qiagen). Primers for *Myh7*, *Myh6*, *Myh2*, *Myh1* and *Actb* were designed by Primer 3 Input Version (0.4.0) and are listed in Table 2**.** The regular cycling program consisted of a 15-min hold at 95 °C and 45 cycles of denaturing at 95 °C for 15 s, annealing at 58 °C for 15 s, and extension at 72 °C for 20 s at which point data were acquired. Gene expression was determined using the 2−ΔΔCt method (Livak et al., 2000). Delta (Δ) CT values were calculated by subtracting the CT value from the experimental gene from the CT value of the control. These values were used for statistical comparisons. ΔΔCT was calculated by subtraction of the ΔCT of a treated muscle from the ΔCT of the corresponding control. Each sample was normalized to *Actb* before quantification.

Protein extraction: Protein was isolated from the interphase and phenol-chloroform layer saved from RNA isolation. Briefly 300 µl of 100% ethanol was added to phenol-chloroform layer and centrifuged at 5000 rpm for 10 mts at 4 °C. Phenol-ethanol supernatant was added to 1 ml of isopropanol and incubated at room temperature for 10 mts. After incubation, samples were centrifuged at 5000 rpm for 10 mts at 4 °C to pellet proteins. Protein pellets were washed with 0.3 M guanidine hydrochloride in 95% ethanol followed by 20 mts incubation at room temperature and centrifugation at 5000 rpm for 10 mts at 4 °C. This process was repeated 3 times. Finally, pellets were resuspended in 200 µl of lysis buffer containing protease and phosphatase inhibitors. Protein concentration was measured using a BCA protein assay kit. All samples were stored at -80 °C until further use.

Western blot analysis: All samples were diluted to a protein concentration of 4 μg/μl in loading buffer (62.5 mM Tris, pH 6.8; 2% SDS; 10% glycerol; 2.5% β mercaptoethanol; and 0.002 bromophenole blue) and heated at 95 °C for 5 min. Protein (60 μg; 15 μl) was separated at 80V (through the stacking gel) and then run at 120V for 3 h in a 4-20% gradient polyacrylamide gel (Lonza, Rockland, ME, USA). Following separation, the protein was transferred to a nitrocellulose membrane (GE Water and Process Technologies, Feasterfille-Trevose, PA, USA) overnight at 20V at 4°C. All membranes were stained with Ponceau S to verify equal loading and transfer. Membranes were blocked for 1 h in 5% milk in Tris-buffered saline with 0.1% Tween 20 (TTBS) and incubated with primary antibody diluted in 5% milk in TTBS a 4°C overnight as follows: MyHC-β/slow (1:500 rabbit polyclonal), MyHC-α (1:500 rabbit polyclonal), MyHC-IIa (1:500 mouse polyclonal) MyHCIIx (1:500 rabbit polyclonal) (Proteintech, Chicago, IL, USA). The next day, membranes were washed 3 times for 10 min in TTBS and incubated with species specific (anti-rabbit IgG horseradish peroxidase or anti-mouse IgG horseradish peroxidase) secondary antibodies (1:2000) for 1 h at room temperature. Membranes were then washed 3 times for10 min with TTBS. Following the final wash, membranes were incubated in the chemiluminescence detection substrate ECL (Millipore) for 5 minutes and emitted light was captured with film. To analyze the protein abundance, band density was measured of using ImageJ software (NCBI). Equal protein loading was confirmed by Ponceau S densitometry values.

Table 2. Primer sequences for qPCR

Gene	Forward Primer	Reverse Primer
MYH1	tctttggtcacctttcagcagt	ctggtggacaaactgcaagc
MYH2	cttcacccgcagtttgttca	catgagaggcgagtgaagga
MYH6	attacaggttggcaagagtgag	cggctacaggacctggtggaca
MYH7	tcaagatgtggcaaagctac	acaagctgcagctaaaggtc
ACTB	cgtacaggtctttgcggatg	ccctggagaagagctacgag

Statistical analysis

Muscle performance and functional data were analyzed by two way repeated measures ANOVA. Differences were located using the post hoc Holm-Sidak test using SigmaPlot (Version 11.0). Gene and protein expression data were analyzed by paired t-tests comparing within group pre-and post-training values. If normalized gene expression data for an individual sample was greater than 2 standard deviations from the mean, the sample was excluded as an outlier. Significance was set at $P < 0.05$. All data are expressed means ± SE.

CHAPTER IV

RESULTS

Subjects

At baseline, there were no differences between HVRT (n = 13) and LVRT (n=13) groups in age, gender, height, weight or body mass index ($P < 0.05$; Table1). There was no significant weight gain or loss within groups ($P < 0.05$). From baseline to post training, there were several significant differences in muscle performance and functional characteristics within groups. These are described below.

Muscle Performance

Muscle performance results are presented in Table 3 and also described below.

Peak Torque. Peak torque values are shown in Figure 1. Peak torque values in LVRT improved significantly at 75 deg/s ($P < 0.001$) and 180 deg/s ($P < 0.05$) with no significant difference at 240 deg/s ($P > 0.05$). Peak torque values in HVRT displayed a trend towards significance at 75 deg/s ($P = 0.052$) and showed significant improvements at 180 deg/s ($P < 0.01$) and 240 deg/s ($P < 0.001$).

Maximal repetition total work. Maximal repetition total work values are shown in Figure 2. Maximal repetition total work values in LVRT improved significantly at 75 deg/s ($P < 0.05$) with no significant difference at 180 deg/s and 240 deg/s ($P > 0.05$). Max rep total work values in HVRT did not improve significantly at 75 deg/s or 180 deg/s ($P > 0.05$) and displayed a trend towards significance at 240 deg/s ($P = 0.054$).

Total Work. Total work values are shown in Figure 3. Total work values in LVRT improved significantly at 75 deg/s ($P < 0.001$) with no significant difference at 180 deg/s and 240 deg/s ($P > 0.05$). Total work values in HVRT did not improve

significantly at 75 deg/s or 180 deg/s ($P > 0.05$). Improvements were significant at 240 deg/s ($P < 0.05$).

Torque at 0.2 seconds. Torque at 0.2 seconds values are shown in Figure 4. Torque at .2 seconds values in LVRT improved significantly at 75 deg/s ($P < 0.001$) and at 180 deg/s ($P < 0.01$) with no significant difference at 240 deg/s ($P > 0.05$). Torque at .2 seconds values in HVRT improved significantly at 75 deg/s ($P < 0.05$), 180 deg/s ($P < 0.05$), and 240 deg/s ($P < 0.01$).

Average power. Average power values are shown in Figure 5. Average power values in LVRT improved significantly at 75 deg/s ($P < 0.01$) with no significant differences at 180 deg/sec and 240 deg/s ($P > 0.05$). Average power values in HVRT improved significantly at 75 deg/s ($P < 0.05$), 180 deg/s ($P < 0.01$), and 240 deg/s ($P < 0.01$).

Peak Power. Peak power values are shown in Figure 6. Peak power values in LVRT improved significantly at 75 deg/s ($P < 0.001$) and at 180 deg/sec ($P < 0.05$) with no significant differences at 240 deg/s ($P > 0.05$). Peak power values in HVRT improved significantly at 75 deg/s ($P < 0.05$), 180 deg/s ($P < 0.01$), and 240 deg/s ($P < 0.001$).

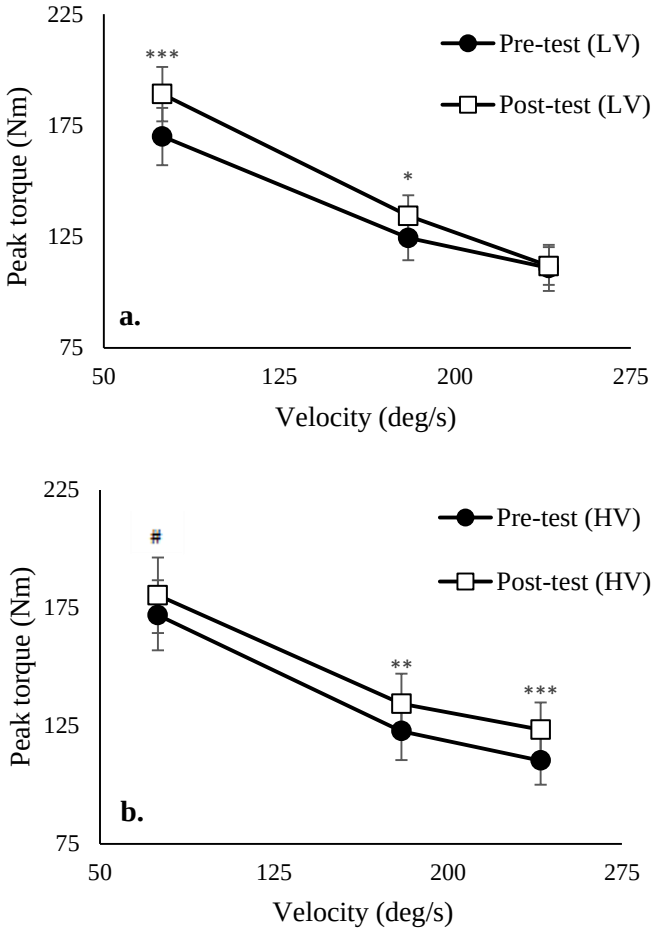


Fig 1. Change in peak torque at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, #P =0.052 vs pre-test.

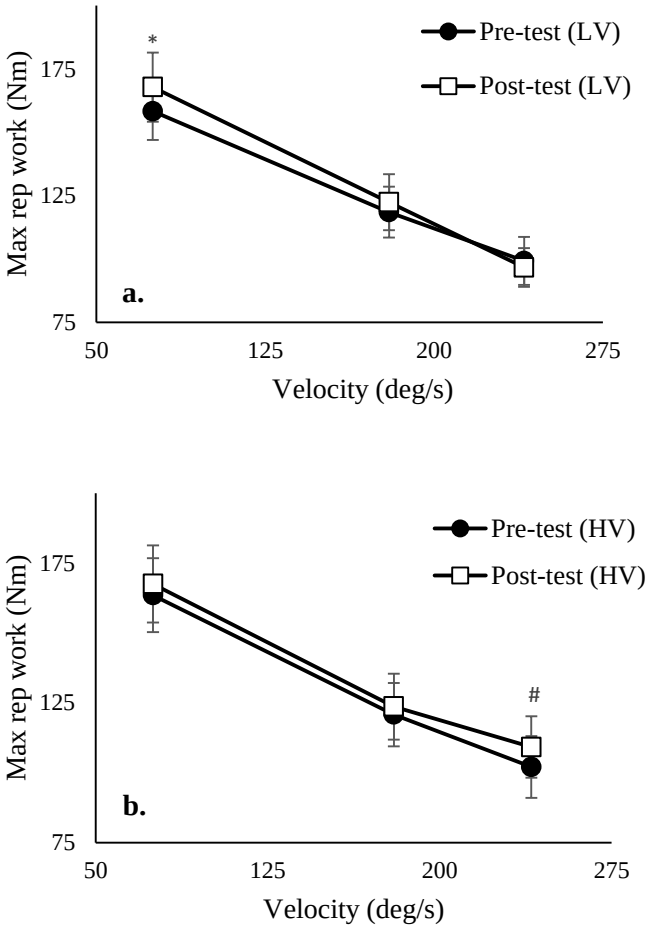


Fig 2. Change in max rep work at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, #P = 0.054 vs pre-test.

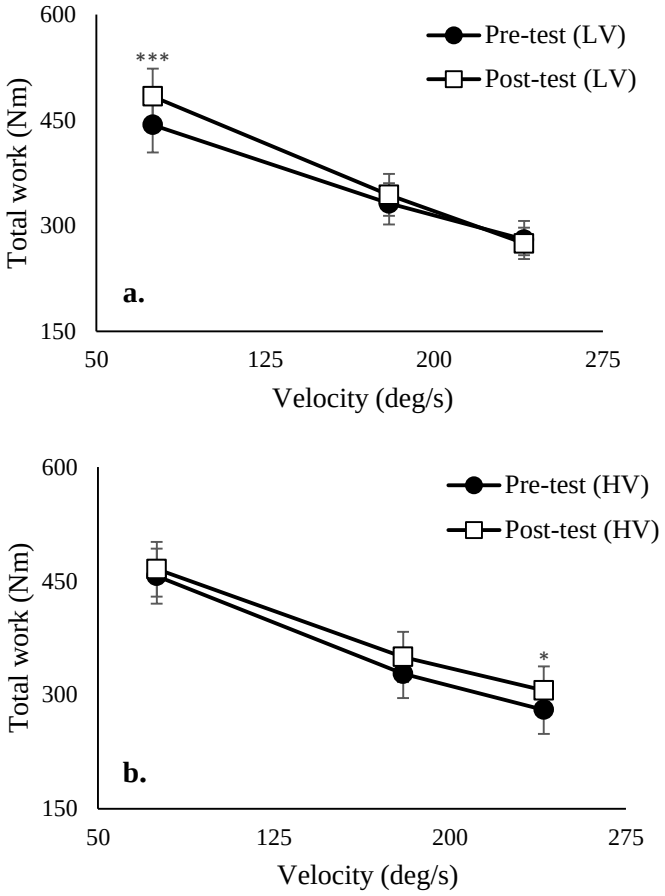


Fig 3. Change in total work at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, ****P* < 0.001 vs pre-test.

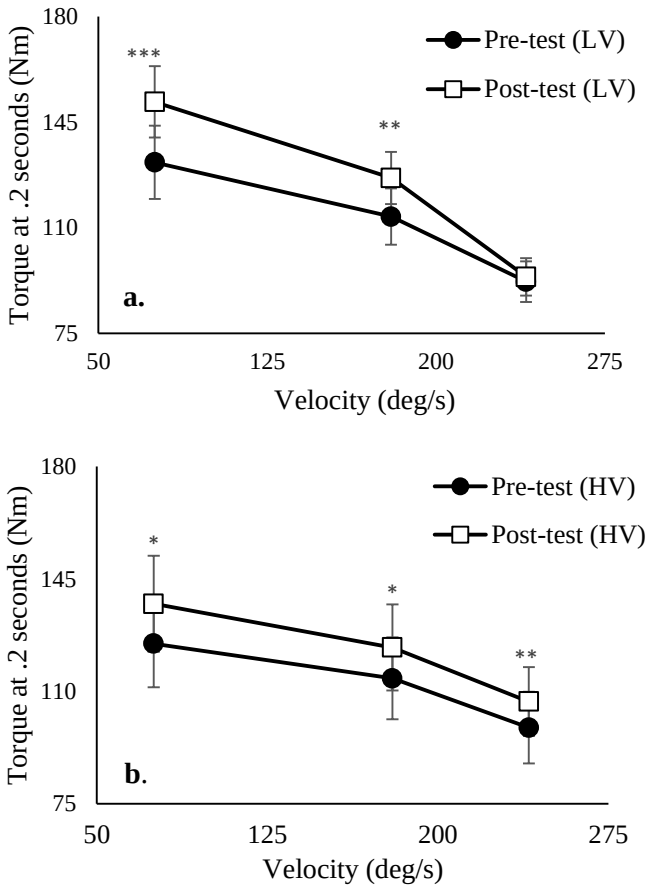


Fig 4. Change in torque at .2 seconds at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs pre-test.

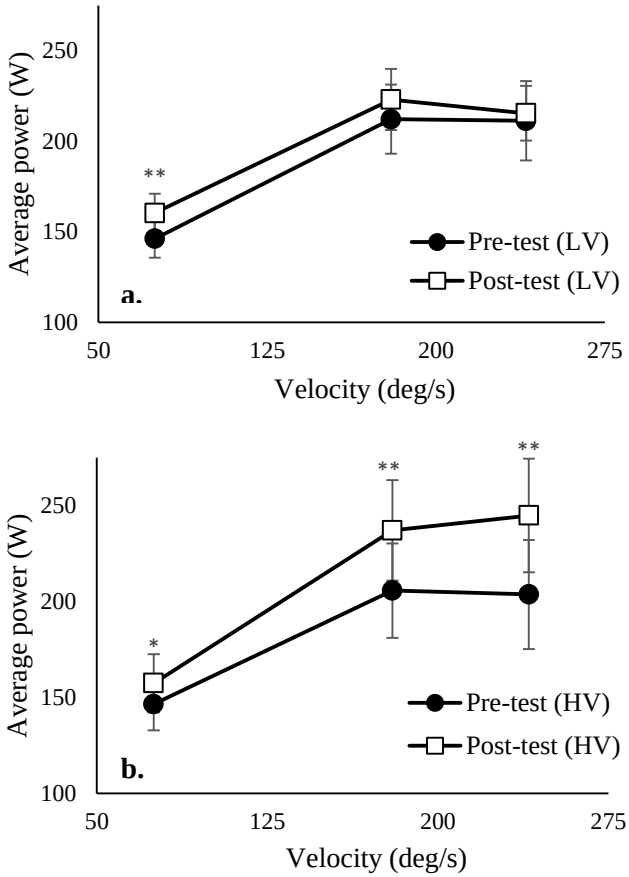


Fig 5. Change in average power at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, ***P* < 0.01, vs pre-test.

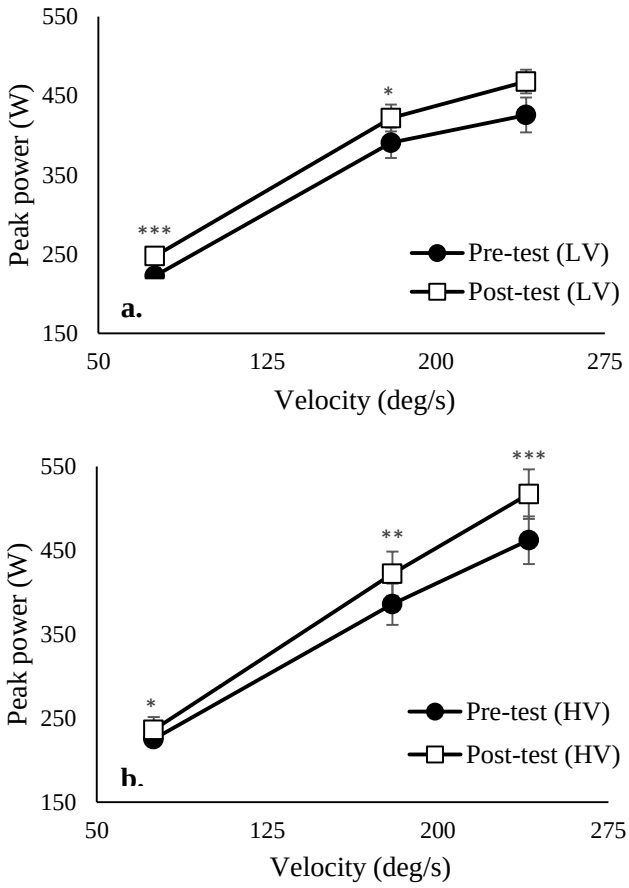


Fig 6. Change in peak power at 75 deg/s, 180 deg/s and 240 deg/s for **a** *LVRT* and **b** *HVRT* groups pre and post 6 weeks of training. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs pre-test.

Table 3. Absolute changes in muscle performance variables

Velocity	Group	Trial	Peak torque (Nm)	Max rep work (Nm)	Total Work (Nm)	Torque at .2 sec (Nm)	Average power (w)	Peak power (w)
75 (deg/s)	LV	Pre	170 ± 13	158 ± 11	444 ± 32	132 ± 12	146 ± 11	223 ± 17
		Post	189 ± 12***	167 ± 14*	483 ± 39***	151 ± 12**	160 ± 11**	247 ± 16***
	HV	Pre	172 ± 15	164 ± 13	456 ± 36	125 ± 14	146 ± 14	225 ± 19
		Post	180 ± 15	168 ± 14	466 ± 36	137 ± 15*	157 ± 15*	236 ± 21*
180 (deg/s)	LV	Pre	124 ± 10	118 ± 10	332 ± 28	114 ± 9	212 ± 19	391 ± 31
		Post	134 ± 9*	122 ± 11	344 ± 30	126 ± 9**	223 ± 17	422 ± 29*
	HV	Pre	123 ± 12	121 ± 11	328 ±32	114 ± 13	206 ± 25	386 ± 39
		Post	134 ± 13**	124 ± 12	350 ± 33	123 ± 13*	237 ± 26**	422 ± 40**
240 (deg/s)	LV	Pre	111 ± 10	99 ± 9	280 ± 26	92 ± 7	211 ± 22	426 ± 55
		Post	112 ± 8	97 ± 8	275 ± 22	94 ± 6	216 ± 15	468 ± 36
	HV	Pre	110 ± 10	102 ± 11	280 ± 32	99 ± 11	204 ± 28	462 ± 43
	HV	Post	123 ± 12**	109 ± 11	305 ± 32*	106 ± 11**	244 ± 30**	517 ± 48***

*P < 0.05, **P < 0.01, ***P < 0.001 vs pre-test

Function

Semi-tandem stand test. Pre and post intervention results for the semi-tandem stand test results are shown in Figure 7. There were no significant differences between or within groups ($P > 0.05$).

Tandem stand test. Pre and post intervention results for the tandem stand test results are shown in Figure 8. There were no significant differences between or within groups ($P > 0.05$).

Usual walking speed. Pre and post intervention results for usual walking speed are shown in Figure 9. There were no significant differences between or within groups ($P > 0.05$).

Maximum walking speed. Pre and post intervention results for maximum walking speed are shown in Figure 10. Prior to training, maximum walking speed was significantly lower in HVRT than LVRT ($P < 0.05$). Max walking speed improved significantly in both LVRT ($P < 0.001$) and HVRT ($P < 0.05$).

Sit to stand. Pre and post intervention results for the sit to stand test are shown in Figure 11. Sit to stand time improved significantly in LVRT ($P < 0.001$) and HVRT ($P < 0.001$) with a trend towards greater improvement in HVRT (*P=0.083).*

Get up and go. Pre and post intervention results for the get up and go test are shown in Figure 12. Get up and go time improved significantly in LVRT ($P < 0.01$) and HVRT ($P < 0.001$) with a trend towards greater improvement in HVRT (*P=0.056).*

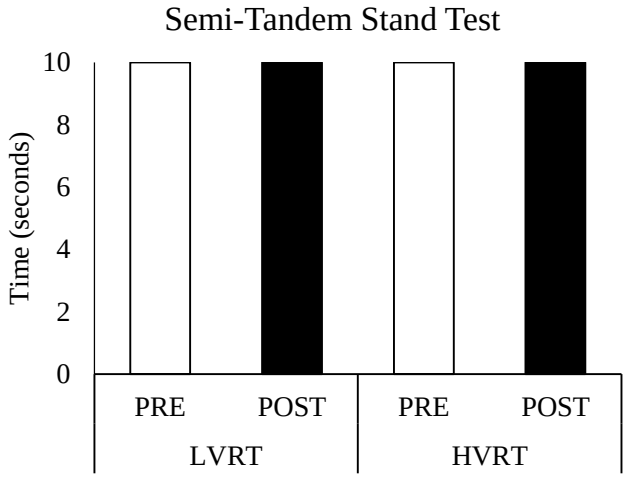


Fig 7. Changes in semi-tandem stand test.

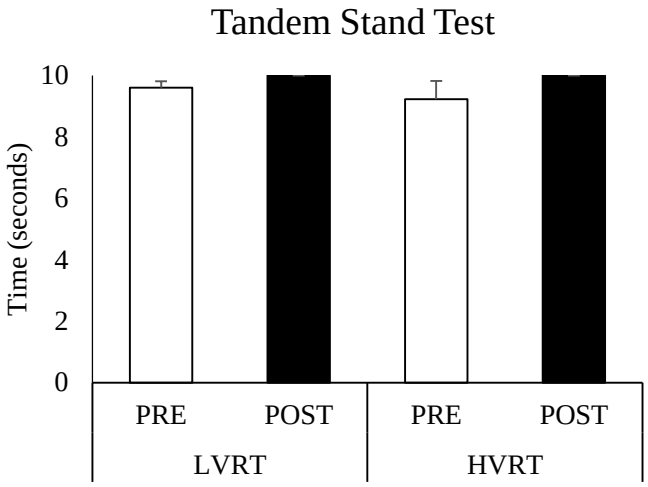


Fig 8. Changes in tandem stand test.

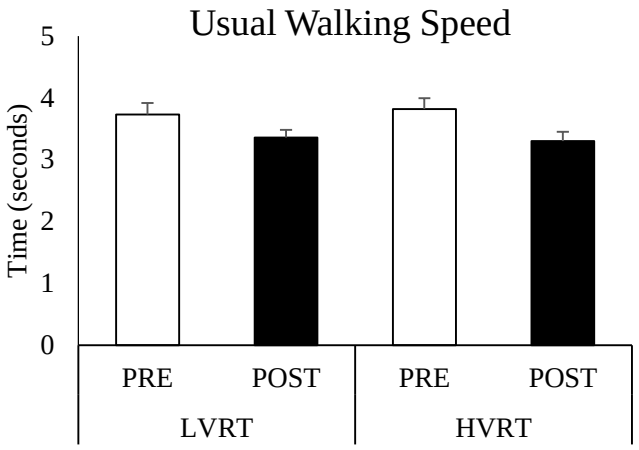


Fig 9. Changes in usual walking speed.

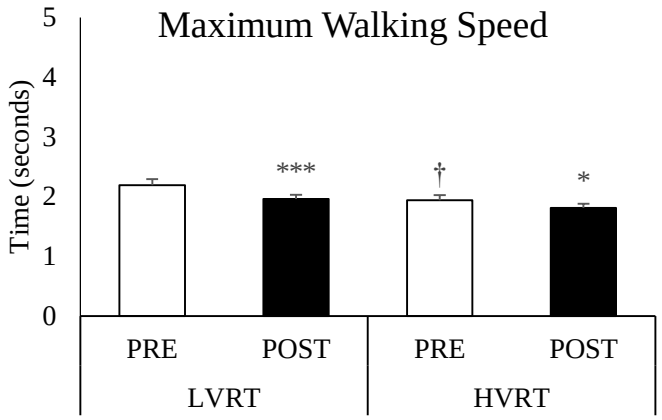


Fig 10. Changes in maximum walking speed. *$P < 0.05$, ***$P < 0.001$ vs pre-test.

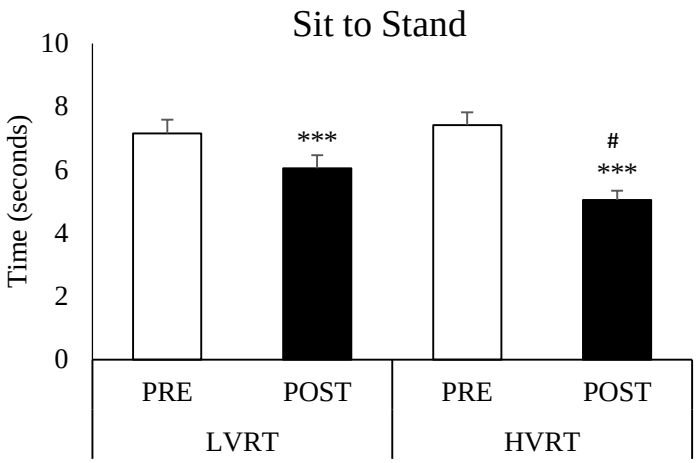


Fig 11. Changes in sit to stand time. ***$P < 0.001$ vs pre-test. #P = 0.083 between groups

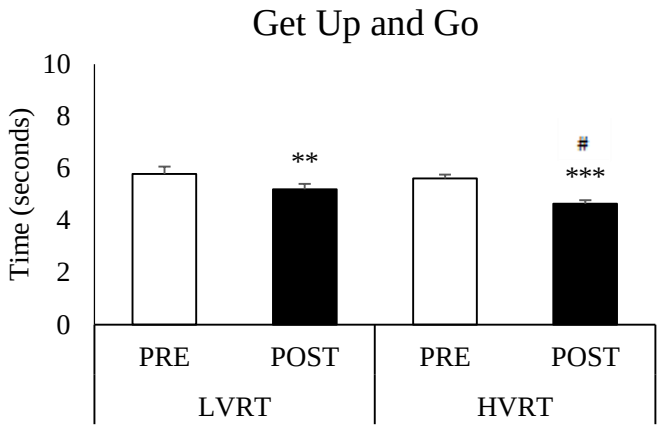


Fig 12. Changes in get up and go time. $^{**}P < 0.01$, $^{***}P < 0.001$ vs pre-test. #P = 0.056 between groups

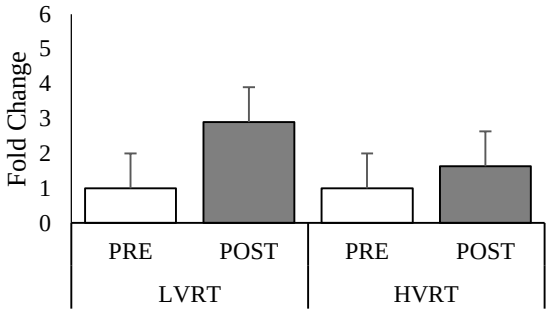
Gene expression

Pre and post intervention results for MyHc-β/slow mRNA, MyHc-α mRNA, MyHc-IIa mRNA, and MyHc-IIx mRNA expression are shown in Figure 13. MyHc-β/slow mRNA showed no significant change ($P > 0.05$) within LVRT (n = 5) or HVRT (n = 6). MyHc-α mRNA showed a significant ($P < 0.01$) decrease (.93-fold ± 0.12) in LVRT (n = 5) and a significant ($P < 0.01$) increase (2.0-fold ± .62) in HVRT (n=7). MyHc-IIa mRNA showed no significance ($P > 0.05$) in LVRT (n = 6) and a significant ($P < 0.05$) increase (1.2-fold ± 0.01) in HVRT (n=7). MyHc-IIx mRNA showed a significant ($P < 0.01$) decrease (0.99-fold ± 0.004) in LVRT (n = 6) with no significant change in HVRT (n = 7) ($P > 0.05$).

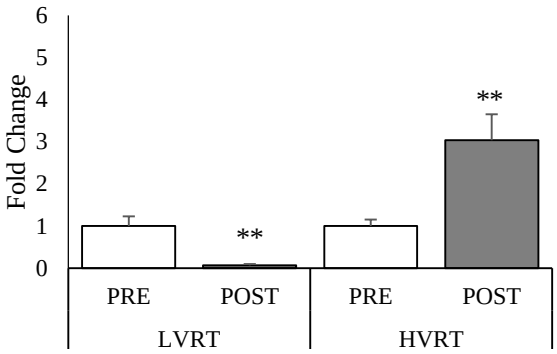
Protein expression

Pre and post intervention results for MyHc-β/slow, MyHc-IIa, and MyHc-IIx expression are shown in Figure 14. MyHc-β/slow had no significant ($P > 0.05$) change in LVRT (n = 7, 1.0 ± 0.06 vs 1.15 ± 0.09, pre vs post) with a significant ($P < 0.05$) decrease in HVRT (n = 7, 1.0 ± 0.12 vs 0.84 ± 0.13, pre v post). MyHc-IIa did not change in LVRT (n = 3, 1.0 ± 0.36 vs 2.45 ± 0.52, pre vs post) or in HVRT (n = 4, 1.0 ± 0.63 vs 0.86 ± 0.31, pre vs post). MyHc-IIx decreased ($P < 0.01$) in LVRT (n = 7, 1.0 ± 0.06 vs 0.87 ± 0.06, pre vs post) but not in HVRT (n = 7, 1.0 ± 0.08 vs 1.06 ± 0.13).

A. MyHC-β slow mRNA



B. MyHC-α mRNA



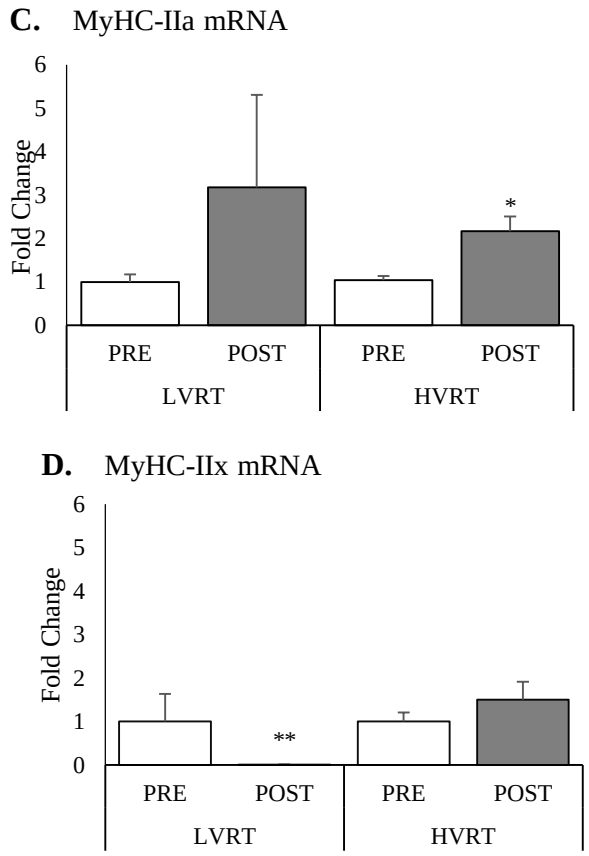


Fig 13. Relative changes in MyHC mRNA expression. **P* < 0.05, ***P* < 0.01, significant within group difference.

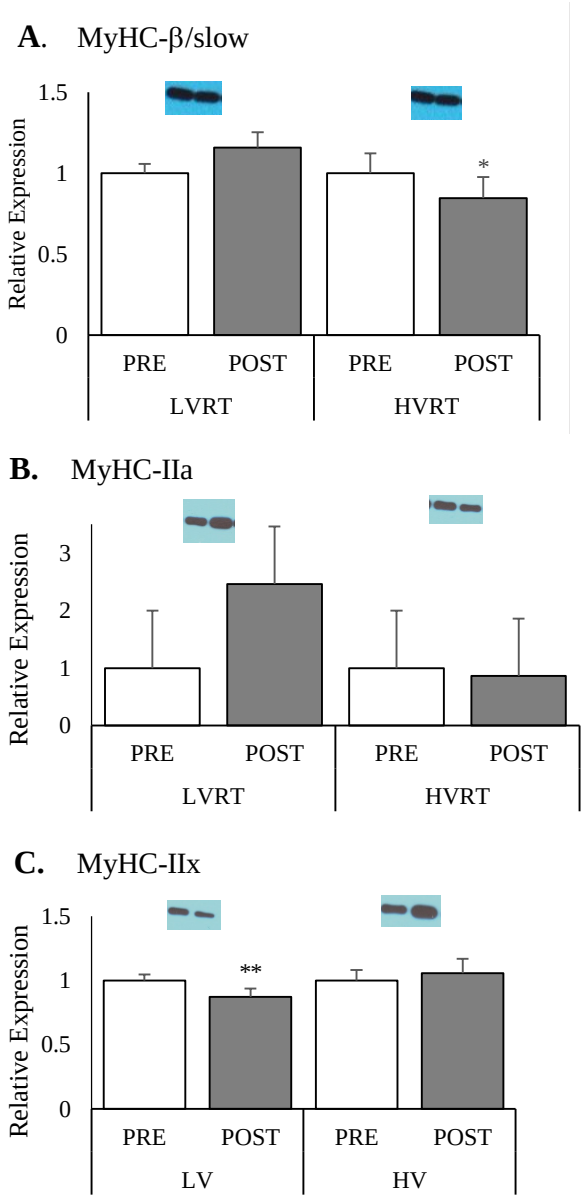


Fig 14. Relative changes in MyHC expression and representative Ponceau S stain *P < 0.05, **P < 0.01, significant within group difference.

CHAPTER V

CONCLUSION

In the case of older adults, the ability to generate adequate levels of force at high movement speeds deteriorates rapidly (Martin et al., 2000; Metter et al., 1997; Mitchell et al., 2012). This decline in muscle function is associated with several unfavorable outcomes (Bassey et al., 1992; Bean et al., 2003; Phillips et al., 1998). This being the case, determining which mode of exercise can most effectively slow or reverse losses of muscle function with age is essential. Of equal importance is the ability to identify mechanisms which influence how older adults adapt to varying training stimuli. To our knowledge, this is the first study to examine velocity specificity of resistance training by directly comparing changes in muscle transcription, translation, performance, and function in older adults.

Both LVRT and HVRT increased several muscular performance and functional measurements. Supporting recent research, our results demonstrate HVRT provides a greater number of muscular enhancements when compared to LVRT, particularly under conditions of high velocity muscle contraction (Earles, 2001; Fielding et al., 2002; Marsh et al., 2009; Puhke et al., 2006 Sayers et al., 2012 Sayers et al., 2014). The LVRT group showed an overall improvement of 9 strength characteristics at testing velocities of 75 deg/sec and 180 deg/sec. The HVRT group showed an overall improvement of 12 strength characteristics at testing velocities of 180 deg/sec and 240 deg/sec. While statistical significance was not achieved, HVRT displayed a trend ($P<0.10$) towards improvement in 2 additional strength characteristics. These results demonstrate that while

velocity-specific training improvements were present in both groups, the HVRT group displayed a greater carry over into testing velocities which were not utilized during training. Both groups showed improvements in maximum walking speed, the get up and go test, and the sit to stand test. It is worthy of note, in the get up and go test and the sit to stand test there was a trend towards greater improvements in the HVRT group. This trend would be expected as the HVRT group enhanced a greater number of strength characteristics which are known to positively influence functional outcomes.

The present study further demonstrates that the transcriptional and translational events which take place in response to mechanical loading are influenced by the velocity of muscle contraction. In the LVRT group, there was a downregulation of MyHC-IIx mRNA and MyHC-α mRNA and a decrease in MyHC-IIx at the protein level. Taken together, this suggests that the low velocity training stimulus was inducing a fast-to-slow MyHC shift within the muscle. This finding is in agreement with a number of studies investigating MyHC response to exercise (Hortobagyi et al., 2000; Short et al., 2005; Staron et al., 1990). In the HVRT group, there was an upregulation of MyHC-α mRNA and MyHc-IIa mRNA and a decrease in MyHC-β/slow, indicating a slow-to-fast MyHC shift. While some studies have shown a similar phenomenon to take place in younger adults (Andersen et al., 1994; Esbjornsson et al., 1993, Jansson et al., 1978; Jansson et al., 1990; Liu et al., 2003; Malisoux et al., 2006; Paddon-Jones, et al., 2001), this finding is novel in older adults.

We are not certain why the different movement velocities produced different alterations in MyHC mRNA and protein. However, it is likely motor unit recruitment took place in a dissimilar fashion between groups in order to effectively meet the unique

mechanical demands being placed on the musculature (Hather et al., 1999; Hortobagyi, et al., 1996; Rome et al., 1988). It is also difficult to discern what exactly lead to the different muscle performance and functional outcomes. It is likely that on the onset of training neural adaptations, such as improved intra-and intermuscular coordination, took place in both groups (Hortobagyi et al., 1996; Sale et al., 1996). The HVRT group may have experienced additional neurological training effects. It has been demonstrated through electromyography (EMG) that higher velocity movements may lead to earlier motor unit activation and higher maximal firing rate (Van Cutsem et al., 1998). It is commonly accepted that after initial strength gains in RT, both neural factors and myofibrillar alterations contribute to increases in muscular performance, with myofibrillar alterations becoming the dominant factor after 3-5 weeks (Moritani et al., 1979). The dissimilar responses seen between groups at the protein level also provide insights into the divergent muscle performance outcomes, as changes in MyHC composition have been shown to alter whole muscle performance (D'Antona et al., 2006; Harridge et al., 1996; Ryushi et al., 1986).

With age, skeletal muscle takes on a slow, type-1 phenotype which is associated with declines in muscle function (Martin et al., 2000; Pearson et al., 2006). This phenotypic shift is partly due to a decrease in the rate of MyHC-2A and MyHC-2X synthesis with age (Blazejowski et al., 1984, Hasten et al., 2000; Welle et al., 1993). While it is not a uniform finding, decreases in MyHC-2A mRNA and MyHC-2X mRNA are reported with age and provide a logical mechanism underlying the decreased synthesis of fast MyHC isoforms (Balagopal et al., 2001; Welle et al., 2000). In the present study, HVRT led to the upregulation of MyHC-IIa mRNA while preserving

MyHC-IIx mRNA and protein. This resulted in HVRT targeting age related declines in translation and proposed declines in transcription with greater specificity than LVRT. This could partly explain the favorable muscle performance and functional outcomes in the HVRT group.

As with all research, the present study had several limitations. If the present study had been longer in duration there would have a better understanding of how muscular vs neurological enhancements influenced training adaptations. Also, being able to test for single fiber function could have effectively ruled out the nervous system influence and informed us if there were improvements in cross bridge contraction kinetics and/or mechanical properties. Additionally, if the study had been longer in duration, we may have observed greater changes at the protein level. The up-or-down regulation of MyHC mRNA has been shown to occur rapidly at the onset of training (Jaschinski et al., 1998; Nadal-Ginard et al., 1982). There is a more rapid turnover of MyHC mRNAs, compared with the slow turnover of the corresponding proteins (Gupta et al., 1992; Russell et al., 1992). This delay is thought to be caused by the disassembling of older filaments before newly synthesized isoforms are introduced into the sarcomere (Brown et al., 1989; Eisenberg et al., 1984). While the MyHC mRNA response to training has been shown to be indicative of, and at times mirror, subsequent alterations in MyHC protein expression (Andersen et al., 1997; Jaschinski et al., 1998; Marx et al., 2002), a longer training period would have provided more time for MyHC isoforms to respond to the training stimulus and to be present in the sarcomere. Also, having a greater amount of subjects to take biopsies from would have helped in the statistical analysis of these results. There was a large amount of variability surrounding gene expression and MyHC-IIa protein could not

be detected in all the samples. This variability could be due to a host of genetic and environmental influences (Simoneau et al., 1995). Future studies should employ a longer training stimulus with a larger sample size in order to gain a better understanding of neurological vs. muscular enhancements, and to better determine how MyHC responds to HVRT at the protein level.

In conclusion, HVRT is emerging as the optimal training stimulus for the older adult. The present study demonstrates, in addition to increased muscular performance and functional outcomes, HVRT may also evoke a favorable (i.e., slow-to-fast) transcriptional and translational response in MyHC.

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