Myosin Heavy Chain Characteristics and Their Relationship to Exercise Performance

By

Michael T. Lane

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Chairperson Andrew C. Fry Ph.D.

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Trent J. Herda Ph.D.

_____

Ashley A. Herda Ph.D.

_____

Joseph P. Weir Ph.D.

_____

Vicki Peyton Ph.D.

Date Defended: 7/22/2014

The Dissertation Committee for Michael T. Lane

certifies that this is the approved version of the following dissertation:

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Chairperson Andrew Fry Ph.D.

Date approved:

Abstract

There are a large variety of factors that affect performance in physical tasks. One factor is the muscle myosin heavy chain (MHC) composition of the muscle involved in the task. Differences in MHC can affect not just exercise performance, but can be related to general health. **Purpose:** The purpose of this study was to examine the relationship between exercise performance and MHC composition. **Methods:** Forty two (n=42) males of a variety of training backgrounds (aerobically trained, resistance trained, recreationally trained, and sedentary) took part in this study (Age = 22.4±3.5 years, Height 1.78±.07m, Weight 78.7±13.3 kg). Subjects were familiarized with vertical jumps, as well as maximal and fatiguing leg extensions during their first visit. A DEXA scan was performed on each subject along with thigh girth and skinfold caliper measurements. The following visit, subjects performed 2 reps of the following jump variations: counter movement jump, with and without arm swing, and depth jumps. Each jump was recorded utilizing a 2 dimensional force plate. Subjects then were tested for leg extension one repetition maximum (1RM). During the final testing session, subjects performed maximal velocity leg extensions at 30, 40, 50, 60, 70, 80, and 90% of their 1RM. Subjects then performed 15 maximal velocity repetitions at 70% of their 1RM on a cadence. Each rep of leg extension performance was recorded for velocity and torque. Immediately afterwards, a muscle biopsy was taken from the vastus lateralis that would be analyzed for MHC content utilizing SDS-PAGE separation (IIX = 13.8±12.9%, IIA =49.5±10.3 %, I = 36.8±11.3%). Data analysis of vertical jump and leg extension performance was analyzed for a number of variables. **Results:** Significant relationships were found between jump peak and mean power relative to cross sectional area of the thigh with type IIX fiber (r = .429-.459*)(* = significance p <.05). Negative relationships were noted with vertical jump height and type I fiber (r=-.355*), velocity load slope

and intercept to type IIA fiber (r=.377-.480*), predicted peak and mean power maximum to type IIA (r=-.328-.352*) and type I fiber (r=.314*), and mean velocity fatigue index to IIA fiber (r=-.319*). **Conclusion:** This research further establishes a number of links joining exercise performance to muscle MHC, and promotes further research linking muscle MHC to performance at varying resistance levels along the potential load spectrum for skeletal muscle.

Key Words: Vertical Jump, Myosin Heavy Chain, Muscle Fiber type, Leg extension

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Figures, Tables, and Formulas

Chapter 1 - Problem

Introduction

Of the contractile tissue in the body, skeletal muscle is the only under volitional control. Without muscle mass daily tasks as basic as locomotion are impossible. Having a large amount of muscle mass has been shown to increase survivability and independence in an elderly population. However, not all muscle is the same and there are a variety of fiber types in humans. Although they all share the same basic properties of contractility, there are a number of differences in fatigability, force, and power production. The three primary fiber types are type I, IIA, and IIX (sometimes called IIB). Type I fibers are "slow twitch" fibers, which are not only the most fatigue resistant, but also have lowest power production. Type IIA and IIX are fast twitch fibers which not only have higher fatigue rates, but a greater power production, more specifically type IIX fibers, which have the highest power production. It is also important to note that humans have hybrid fibers. This is where a single muscle fiber has multiple myosin heavy chain (MHC) types expressed. These fibers express multiple MHC isoforms along their length. With training, it is possible to cause these hybrid fibers to selectively express the MHC most compatible to the form of training (Fitts & Widrick, 1996; Pette, 2001; Pette & Staron, 2001).

With these different fiber types, there have been a variety of effects seen on performance. Having higher amounts of type I fibers predisposes someone to be a greater aerobic athlete whereas having higher amounts of Type II fibers predisposes someone to be a better power athlete. There has been little research that has shown the positive effects of having higher amounts of IIX fibers on performance. It is theorized that having a higher amount of type IIX fibers will lead to greater power or speed performance, yet this is very hard to analyze. Previous studies have tried to do so with mixed results. Some studies have shown that there is a positive

effect of having type IIX fibers, as they are expressed in high level sprinters (Baumann, Jaggi, Soland, Howald, & Schaub, 1987), but still more research must be performed in this area. However, high numbers of IIX fibers are also found in sedentary controls and are rarely expressed in well trained subjects (Fry, Schilling, et al., 2003; Fry, Webber, et al., 2003). Some have seen a decrease in the expression of the type IIX fibers in the skeletal muscle with training (Andersen, Klitgaard, & Saltin, 1994; Trappe et al., 2000). This is due to a conversion of fiber type in hybrid fibers to IIA or I fiber types (Staron et al., 1994). There has yet to be a study that has shown a high amount of type IIX fibers in well trained subjects of a large sample size, more specifically not in hybrids utilizing myosin ATPase techniques (Pette, 2001). There is limited research linking IIX concentration to vertical jump performance, specifically in the depth jump (Eckerson, 2005; Fry, 2002).

In order to measure muscle fiber type, it is necessary to take a muscle biopsy, which is an invasive procedure (Staron et al., 1990). There are a few studies that have developed models or shown relationships between muscle fiber type and performance on different muscle tasks. However, these tasks typically require expensive equipment or measurement tools. Furthermore, these methods typically end at attempting to quantify the difference between heavy chain fast and slow fibers, without differentiation between the two fast isoforms of IIA and IIX (Herda et al., 2010; MacIntosh, Herzog, Suter, Wiley, & Sokolosky, 1993; Sadoyama, Masuda, Miyata, & Katsuta, 1988; Schilling, Fry, Chiu, & Weiss, 2005; Schilling, Fry, Weiss, & Chiu, 2005; Thorstensson, Grimby, & Karlsson, 1976). Being able to mechanically test subjects for fiber type conversion is of importance and should be evaluated.

Purpose

The purpose of this research study is to explore exercise performance relationships with muscle fiber type, specifically IIX during isotonic and plyometric exercises.

Rationale and experimental approach to the problem

By creating a predictive model for muscle MHC a glimpse into potential reasons for certain athletic performances, and allow researchers to create MHC composition estimates without the need for muscle biopsies. Furthermore, a predictive model will hopefully allow the test to be performed in a setting that utilizes less cost prohibitive equipment and to be performed and analyzed more easily (Baguet et al., 2011; Herda et al., 2010; Thorstensson et al., 1976).

Independent and dependent variables

To create a predictive equation to estimate muscle MHC, a number of variables were tracked in order to try to create a model which remained accurate for a number of subjects with a variety of genetic predispositions and training backgrounds. Individuals with different training backgrounds were divided into groups based upon self-reported exercise health histories. The four groups subjects were allocated into were:

Resistance trained – These subjects were capable of back squatting at least 1.5x their own body weight in the barbell back squat. A squat was defined as to top of the thighs, (quadriceps) parallel to the ground. They utilized the stance of their own preference (which was be measured and documented for consistency throughout the study), with a belt and no knee wraps.

Endurance trained – These subjects were capable of running a mile in less than 6 minutes and had a total weekly running volume of 30 miles or more. Subjects that are cyclists or other type of aerobic athlete were evaluated on a case by case basis.

Recreationally trained – These subjects were active most days of the week and not following an organized, periodized, resistance training program or aerobic program. Subjects were not able to run a mile in less than 6 minutes and squatted less than 1.5x bodyweight. Final judgment on inclusion in this group was reserved for the research team.

Sedentary – These subjects did not take part in any exercise program or sports for at least 2 years prior to the study. They also did not participate in more than 2 hours per week of exercise.

Other independent variables include subject MHC characteristics (determined through muscle biopsies and analyzed utilizing SDS-PAGE gel electrophoresis methodology), muscle cross sectional area, and lean body mass. Subject performances on isotonic and isometric leg extension tests and performance (power, impulse, and rate of force development) in the vertical jump tests were the dependent variables in the study. These tests are readily available in most laboratories and moderately easy to perform. Specifically, the velocity and power performance metrics, if effectively linked to MHC, will allow for field testing of MHC that will be both cost-effective and easy to perform. Through these parameters we showed a significant relationship with the relative expression of types I, IIA, and IIX MHC and performance in this research.

Hypotheses

Vertical jump performance

Hypothesis 1: Vertical jump impulse, rate of force development, jump height, and power will have a positive relationship to vastus lateralis muscle fast MHC composition, specifically when variables are calculated relative to body mass and cross sectional area of the muscle.

Leg extension performance

Hypothesis 2: Isotonic leg extension velocity-load linear relationship will be related to muscle MHC with fast fiber positively correlated to the intercept of the line and negatively correlated to the slope of the line.

Hypothesis 3: Isotonic leg extension power-load curves will be related to IIA, specifically the point of which the peak occurs at; earlier peaks relative to percentage of the total load will be negatively related to fast fiber composition.

Hypothesis 4: Isotonic leg extension test fatigue index will be positively related to total concentration of the combination of both fast isoforms.

Limitations and delimitations

Delimitations

In order to make this research study be as applicable and general as possible, there were a number of delimitations that were made:

In order to control for any potentially complicating effects of having both males and female participants, only males were recruited to take part in this study. Subjects were healthy, with no major orthopedic, metabolic, or neurological diseases or disorders as a means to remove any possible confounding effects of utilizing subjects who might not be able to optimally express force or muscle recruitment. Subjects were all aged 18-35 years, to adjust for any possible effects of development or age-related sarcopenia that could affect muscle mass, more specifically MHC conversion. We recruited subjects who had a variety of training backgrounds: sedentary, aerobically trained, resistance trained, and recreationally trained (criteria listed earlier). By utilizing subjects with a wide variety of backgrounds we hoped to obtain varied muscle fiber

expression. This wide –range of fiber expression and physical capacity allowed for more variable exercise performance. This allowed the research team a greater opportunity to find significance and decreased the amount of subjects required for participation in the study.

There is an extensive amount of muscle groups in the body, but we only related the tests to the quadriceps and biopsied the vastus lateralis. This allowed examination and performance measures to be focused on this muscle. The vastus lateralis was chosen, as it is a very large muscle, and is easy to test and biopsy, with only a minimal risk for complications. By utilizing a large muscle group, we had the advantage of working with greater force and power production. The higher variability of the data collected provided greater versatility and relation to performance. Follow up studies could potentially examine the effect in other muscle groups, but first a relationship must be found in this study to justify another.

We quantified the proportion of MHC through homogenized samples run through SDS-PAGE page. There are a number of different ways to perform this type of examination, but we were solely interested in linking performance to present MHC expression it was not necessary to complete myosin ATPase staining. ATPase analysis would have shown the same type of results that we obtain through only the MHC SDS-PAGE, and also provided the proportion of hybrid muscle fibers, fiber number, and fiber size, which is not of added interest in this study.

With the Biodex, we performed isotonic testing, and no isokinetic testing. By utilizing isotonic testing, we were able to generate results that allowed for greater application of the protocol. The reasoning for this is to lower needs for specialized equipment and invasive tissue collection so that this can be developed into a field test for MHC. This will be more useful to the practitioner as a means to quantify MHC in subjects, athletes, or clients.

Assumptions

We assumed that all of our subjects would give their best effort on each test. We anticipated that subjects would not prematurely fatigue during the power load test, which would affect final results. Subjects informed researchers if they were ill or had any other type of problem that would decrease exercise performance on any given testing day (trained the day before).

We assumed that the distribution of MHC throughout the muscle did not vary too greatly along the length of the muscle, so that our muscle biopsy would construe a representative sample of the subject's true MHC. We assume that by performing the biopsy on the same biological landmark for each subject will further help control for variability on the biopsy. We also assumed that there would not be enough training days to cause significant MHC shifting in the subjects. Furthermore, that there would not be significant neurological adaptations to the exercise that would change the effectiveness of the tests with the subjects.

All of our equipment was calibrated accordingly to give us accurate and reliable data. Our method of heavy chain analysis was consistent and the means of quantifying our results was accurate and reliable.

Definitions

Isoforms – This is slight variations on the same base structure. In reference to human muscle, these slight variations cause differences in speed of contraction and shortening rate. This can also effect power production.

Velocity-load curve – This is a typically linear relationship between maximal velocity attained with a given load. As load increases, peak velocity decreases. This relationship varies by individual.

Power-load curve – This is the 2nd order polynomial relationship between maximal power attained at a given load. As load increases, power will peak and then decline as a subject gets close to their max. This relationship is different from individual to individual.

One Repetition Maximum (1RM) – This is the greatest amount of resistance that can be lifted for only one complete repetition in a given movement.

Myosin Heavy Chain (MHC) – The component of the contractile fiber that comes in various forms and each form as a different contraction speed and fatigability, activation of this protein causes muscles to contract.

Fatigue Index – The decline in performance of an event from the greatest performance to the lowest performance in trials that induce a decrease in performance. Mathematically defined as: fatigue index = (greatest performance – lowest performance) / greatest performance X 100.

Impulse – The sum of the Average force multiplied by the time of period the force was applied.

Maximal Voluntary Contraction (MVC) – The greatest amount of force that can be volitionally produced by subjects participating in the study against an isometric task.

Chapter 2 - Review of Literature

Basic muscle architecture and contractile units

In humans, contractile tissue comes in 3 different forms; skeletal, smooth, and cardiac. Skeletal muscle is the only type of muscle that is under volitional control of the nervous system. Autonomic (sympathetic and parasympathetic) innervation can affect functioning of the smooth and cardiac muscle; therefore it is not under conscious control and can be fully contracted and relaxed by other regulators. The organization of each of these muscle types differs, but only skeletal muscle architecture will be analyzed in this review.

Skeletal muscle is organized into groups of overlapping myosin and actin. Myosin and actin form the basic contractile unit where myosin attaches to actin and "pulls" itself along the actin. Individually, one head of myosin produces very little force (approximately $0.4pN^.nm^{-1}$) (Finer, Simmons, & Spudich, 1994). However, a single myosin protein with multiple heads is surrounded by actin to form a sarcomere, and hundreds of these sarcomeres make up one muscle fiber. Not only are they grouped together laterally, but they are oriented end to end longitudinally along the entire muscle cell from the origin to the end point of a given muscle. When increased laterally (cross-sectionally), this orientation has been shown to increase force production. Simply put, a larger muscle is a stronger muscle.

Description of Fiber Types

Definitions of Fiber Types (abbreviations)

There is a number of MHCs (ten forms) identified in humans, and only three different isoforms that are found in adult skeletal muscle under normal conditions: I, IIA, and IIX. This review and proposal will only focus on those three in detail. Other isoforms might be mentioned,

but are rarely found in skeletal muscle. Those rarely found fibers are typically found only due to disease state, early in development, or transiently after large amounts of stress. Their abundance in healthy muscle is negligible for any effect on performance in healthy adults (Weiss, Schiaffino, & Leinwand, 1999).

Fiber Types in Humans

In humans there are a number of different types of contractile tissue. The three different types of contractile tissue in humans are skeletal, cardiac, and smooth. Each of these fiber types has different kinds of arrangements, both structurally and within interactions between actin and myosin. These fibers also vary based upon the types of electrical action potentials and contraction mechanisms. This review will focus on the different isoforms of skeletal muscle tissue MHC.

Throughout all of the skeletal muscles there are varying distributions of each of the fiber types. Some muscles are more predominantly type I (soleus), while other muscles typically contain higher amounts of fast twitch (both IIA and IIX) (Triceps surae) (Elder, Bradbury, & Roberts, 1982). Within certain muscles there is a shifting of expression in different locations throughout the muscle, such as the trapezius (Lindman, Eriksson, & Thornell, 1990). Muscle MHC composition varies from person to person, but the manner in which an individual stresses the body (resistance training, aerobic training, sedentary behavior, etc.) greatly effects their muscle MHC composition (Simoneau & Bouchard, 1995). There is also a 20-50% genetic component to muscle fiber type that will be briefly visited later in this review (Bouchard et al., 1986).

Each of these muscle fiber types has characteristics that differentiate it from another. Enzymatic capacities of individual fibers are found in higher concentrations according to the

metabolic conditions of preference to the fiber (type I aerobic, type IIA/X anaerobic). These enzymatic concentrations, due to energy production, are to an extent trainable, and will increase or decrease in concentration according to training stimulus (Bouchard et al., 1986; Galpin et al., 2012). Power production from individual fiber types is shown to be highest in IIX fibers, followed by IIA, and then type I fibers. Shortening velocity is shown to have the same relationship where IIX is the fastest and I is the slowest (Bottinelli, Schiaffino, & Reggiani, 1991). However, total force produced, controlled for cross sectional area, is essentially the same for each of the fiber types.

Beyond the MHC effects there are additional effects of the myosin light chain characteristics. These light chains have a variety of isomers that differ on the speed and amount of power production that they can create. It is also thought that these isomers might shift due to training, but these effects have not been thoroughly investigated and do not have as large of an effect as MHC characteristics can have on performance (Fitts & Widrick, 1996).

In human skeletal muscle samples from cadavers, the biceps brachii has an expression of a multitude of isoforms. Across a number of samples, slow twitch, type I fibers were found to also express alpha cardiac fibers, embryonic, and fetal isoforms. In fast (type IIA and IIX) fibers embryonic and fetal isoforms were also detected. This shows the variable expression of human skeletal muscle fiber, which lends to the plasticity of muscle according to the demands placed upon it, as well as the amount of reformation that is occurring within it (Liu, Eriksson, Thornell, & Pedrosa-Domellöf, 2002).

Some of the other varieties of skeletal muscle fibers in the body are found in specific regions of the body such as the muscles that control the motion of the eyes. In extraocular muscles, a variety of MHC expression has been seen beyond that typically seen in the other

skeletal muscle; through immunoblots and immunocytochemistry, Kjellgren et al. showed the

presence of heavy chain isoforms I, IIA, IIX, embryonic, and extraocular isoform (Kjellgren,

Thornell, Andersen, & Pedrosa-Domellof, 2003).
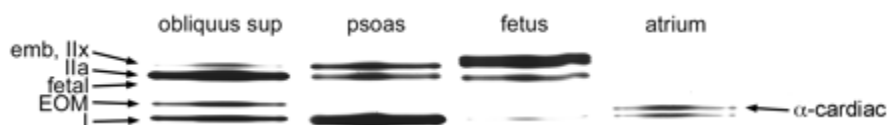
Figure 1 (Kjellgren et al., 2003) display of multitude of MHC separation utilizing SDS-
PAGE methods

When older men (over 70) were immobilized for 3.5 months, neonatal MHC was found

to be expressed in samples from their vastus lateralis. This isoform was found in none of the

other subjects (healthy, non-immobilized old, or young), and is linked to disuse of the muscle.

The same sample from the immobilized group also had much higher amounts of hybrid fibers

than the other groups sampled in the study (D'Antona et al., 2003).

Note on Fiber Type Names

There is some debate in the nomenclature of the IIX fibers that are sometimes labelled

IIB/D (Staron, 1997). In the animal model, there are more than 3 heavy chain isoforms that are

found expressed in skeletal muscle (Bottinelli et al., 1991; Termin, Staron, & Pette, 1989),

however, in mature human skeletal muscle only I, IIA, and IIX are observed. The debate over the

labelling arises from genomic similarities and translation that occurs since the sequence does

closely resemble animal IIB in the mRNA sequence (Smerdu, Karsch-Mizrachi, Campione,

Leinwand, & Schiaffino, 1994).

Muscle Fiber Type Variability

In human skeletal muscle, aside from having variable expression of MHC between different muscles in the human body (Johnson, Polgar, Weightman, & Appleton, 1973), there is also variability of expression throughout the length of a muscle. Research in this area, which compared biopsies to full muscle excision from cadavers resulted in a correction equation listed in Figure 2 (Elder et al., 1982). This equation indicating that the mean percentage of fast twitch fibers (both IIX and IIA) equals the mean portion of the number of sites (n) with number of areas counted on each site (m) would have a standard error where (σ^2w) within site variance and (σ^2b) between site variance. Essentially, the greater number of biopsy sites taken and fields viewed utilizing ATPase techniques the lower the potential standard error. Overall, this study found that a single biopsy of the vastus lateralis could have a nine percent standard deviation of the total fast twitch fiber percentage with only one field observed which decreases to just over seven percent when up to 10 fields are observed (Elder et al., 1982).

$$\text{SE (mean FT proportion)} = \sqrt{\frac{\sigma_{\text{B}}^2}{n} + \frac{\sigma_{\text{W}}^2}{nm}}$$

Figure 2 Elder et al., 1982 - generated standard error equation based upon number of fields viewed per biopsy and number of muscle biopsies performed

There is some variability in muscle fiber expression based upon the depth that samples are taken from. Superficial fibers tend to have a greater expression of IIX fibers compared to deeper fibers, which tend to have a greater expression of type I (Dahmane, Djordjevič, Šimunič, & Valenčič, 2005). As individuals age there seems to be an increase of this variability, potentially due to selective atrophy of type II fibers (Lexell, Henriksson-Larsén, Winblad, & Sjöström, 1983)

Fiber Type Transitioning

Skeletal muscle fiber has a high degree of plasticity. It adapts to greater working demands by increasing its energy producing enzyme concentration (Borges & Essen-Gustavsson, 1989), and increasing the number of cell nuclei (MacDougall, Elder, Sale, Moroz, & Sutton, 1980), and mitochondria (Hoppeler et al., 1985). Skeletal muscle fiber can also alter the MHC composition through gene expression (Vechetti-Junior 2013); this adaptation has been observed in humans and animals.

There is a variety of research conducted with rats showing adaptations in MHC to different stimuli. This model has also shown intracellular mechanisms behind both genetic and intramuscular signals for fiber type transitions. In Wistar rats, just 8 weeks of swim training increased type IIA fiber in soleus (decreased type I) and plantaris (decreased type IIX) muscles. This fiber transition activated the NFATc1-3 gene expression, which is specific to fiber type expression, yet did not activate the NFATc4 level or calcineurin expression (CaN) (Vechetti-Junior 2013). Other animal research has utilized nerve blocks and chronic electrical stimulation of musculature. This research has shown a significant conversion of fast fiber muscle to include greater amounts of type I fiber. However, in slow twitch, dominant muscles there was not a significant increase in fast fiber (specifically B) (Termin, Staron, & Pette, 1989).

In humans, shifting of muscle fiber type can occur after a moderate amount of time with consistent application of stress. 10 weeks of circuit weight training decreased the proportion of type IIX fibers in sedentary individuals (Harber, Fry, Rubin, Smith, & Weiss, 2004). Loads in this training protocol were typically between 40 and 60% of the subjects 1RM, which showed that low intensity circuit training was capable of decreasing IIX concentration. There have also been studies which have shown a decrease in type I fibers and an increase in type IIA muscle

fibers during 6 week stretch shortening, cycle power training in addition to resistance training. (Liu, Schlumberger, Wirth, Schmidtbleicher, & Steinacker, 2003).

Longer training studies, utilizing untrained individuals, have shown an increase in type IIA fiber and a decrease in IIX proportion, specifically a decrease in hybrid fibers, which likely shifted to present more IIA throughout the fiber, as opposed to multiple isoforms. This adaptation occurs in both sexes (Staron et al., 1990; Williamson, Gallagher, Carroll, Raue, & Trappe, 2001). Few studies have shown how fiber type adapts to training and then regresses with detraining. Anderson and Aagaard had subjects first participate in 3 months of resistance training, which showed a decrease in the proportion of IIX muscle fibers and an increase in the amount of IIA fibers. Following this phase, subjects then completed 3 months of detraining. Following detraining, subjects actually experienced an increase in IIX fibers to a higher proportion than they had experienced during their baseline (Andersen & Aagaard, 2000). In older men, 12 weeks of moderate resistance training decreased the amount of hybrid fiber expression and increased the concentration of type I muscle fiber. However, no significant change in the total concentration of pure IIA or IIX fibers was observed (Williamson, Godard, Porter, Costill, & Trappe, 2000). This training response has been seen in athletic populations that are well trained. For example, female soccer players taking part in a strength training program experienced a decrease in the amount of their type IIA fiber area compared to soccer players who did not take part in the resistance training protocol (Andersen, Klitgaard, Bangsbo, & Saltin, 1994).

Sprint trained, older individuals experience a decrease in type IIX expression, but no decrease in amount of IIA as they age. This is potentially a training adaptation; the use of the fibers caused them to maintain function. Skinned fibers in the study maintained the same

velocity in both older and younger individuals (Korhonen et al., 2006). Women, specifically, experienced a decrease in power production in type IIA fibers with aging. Yet the same effect was not seen in older men (matched for adequate activity levels) when compared to young, active, men and women (Miller et al., 2013). This is an area that warrants further exploration, due to the fact that women during this generation had significantly less access to athletics at a younger age. This lack of athletic opportunity could be a contributor to the decrease in the muscle fiber functionality.

In sprinters who underwent a hard training protocol, researchers have seen a decrease in both type I and IIX concentration, with an equivalent increase in the IIA isoform. The greater decrease was seen in the type I, however, hybrid fibers were ATPase stained with still moderate amounts of IIX with IIA expression, opposed to purely IIX fibers which were quite rare (1 in a 1000 fibers analyzed) (Andersen, Klitgaard, & Saltin, 1994). When older sprinters took part in an organized resistance training program on top of their sprint training, they experienced an increase in type IIA fiber size, but no significant change in fiber composition or function when compared to age matched sprinters not in the intervention (Cristea et al., 2008). Transitions also occurred in athletes who were performing sprints on a bicycle, not on land. High intensity sprint, stationary, cycling for 15 weeks showed a decrease in IIX concentration, an increase in type I concentration, as well as an increase in the fiber size in each of the isoforms (Simoneau et al., 1985).

Some studies do not show any significant changes in fiber type with a training intervention; often this is related to too brief of a training stimulus or too small of a training stress. One week of acute, resistance overtraining showed no shift in MHC characteristics, or any other significant changes in the isoform concentrations (Fry, Schilling, Weiss, & Chiu, 2006).

Swimmers who went through an intense, 10 day training showed no change in muscle MHC composition in their deltoid muscle, yet experienced a change in the shortening velocity; Type I muscle fibers increased shortening speed, while the type II fibers decreased their shortening speed. The authors theorized this could be due to opposite heavy chain expression in the muscle fibers related to the intensity of the training (Fitts, Costill, & Gardetto, 1989). When subjects performed a bike sprint protocol for 6 weeks, there was no change in the MHC composition in either the vastus lateralis or gastrocnemius. The training volume, however, was only composed of 12, 3 second sprints per week, broken up over 4 training sessions. This volume was understandably inadequate to cause any shift in fiber type in recreationally trained individuals (Harridge et al., 1998).

After 8 weeks of jump squat training at two different intensities, (30 and 80 % of 1RM load) researchers saw no significant change in muscle fiber type expression in active and athletic males (McGuigan et al., 2003). 54 weeks of progressive resistance training program provided no significant shift in MHC IIA or IIX fibers. This could have been due to lack of adherence, or possibly lack of sufficient training volume to induce a large enough change, since the authors did publish their training program it is not possible to compare the training program to other successful studies. An interesting note is they saw a significant, negative correlation between amount of time subjects spent playing recreational activities and type IIX fiber concentration (Puhke et al., 2006).

Few studies have used a mixed model of resistance training with aerobic training to look at MHC transition. In a training study examining fiber type change with a strength training program, strength and endurance program, or endurance only program, fiber shifting was seen in every group. Most interestingly, IIX fibers were no longer found in the strength and endurance

group only. The resistance training group had an increase in the amount of hybrid I/IIA fibers, while the endurance group had an increase in type I fibers (Putman, Xu, Gillies, MacLean, & Bell, 2004).

Not only does MHC adapt in human skeletal muscle, but this adaptation can also been seen in the other contractile tissues. In the heart, fiber type transition from the alpha (which is more powerful isoform) to the beta isoform of MHC occurred in end stage heart failure (Miyata, Minobe, Bristow, & Leinwand, 2000). In individuals with cardiac failure, there was not only an increase in IIX concentration, but a decrease in type I concentration The degree to which this occurs is outside the realm of a mere training effect and may be a sign of a greater skeletal muscle dysfunction while in heart failure (Sullivan et al., 1997).

Finally, changes of MHC composition, though modifiable with adequate training stress, can regress back to baseline with the removal of the training stress. With both training and detraining, fiber type shifting has been seen in healthy individuals in an intervention of 3 months (Andersen et al., 2005). This showed a decrease in the amount of IIX fibers with training. However, when the training stimulus was removed for three months, levels of IIX increased slightly over baseline values.

Individual Muscle Fibers and Performance

Type II fiber types shorten at a faster rate than type I fibers in rat tissue (R. Bottinelli et al., 1991), specifically type IIX shorten faster than the type IIA. In humans, the same is true with the slowest being type I (Pette & Staron, 2000). Power production is similar with IIX being the highest, followed by IIA and type I. All fiber types produce nearly identical amounts of isometric force when standardized for cross sectional area. When it comes to fatigue resistance and metabolic enzyme concentrations, type I fibers have the greatest fatigue resistance and aerobic

capacity, followed by IIA, which are well adapted for anaerobic glycolysis, and then by IIX, which have limited metabolic capacities and fatigue the most quickly (Trappe et al., 2004; Trappe et al., 2000).

Myosin light chain isoforms also effect muscle shortening velocity and help to explain some of the variation in shortening velocity within MHC fiber types. This can assist in explaining the overlap, specifically in the shortening velocity of Type IIA and IIX (Bottinelli, Betto, Schiaffino, & Reggiani, 1994). The speed that muscle fiber contracts is independent of the muscle it comes from, however the type of heavy chain and light chain that is expressed causes a difference in contraction velocity and power production (Sweeney, Kushmerick, Mabuchi, Sreter, & Gergely, 1988). To date, five different isoforms have been identified and their distribution varies in each heavy chain fiber type (Staron & Pette, 1987a, 1987b).

Metabolically, type I fibers have the greatest concentration of oxidative enzymes compared to both of the fast isoforms (Type IIA and IIX), which have greater amounts of glycolytic enzymes. Type II fibers have a greater amount of myokinase concentration than type I fibers as well (Borges & Essen-Gustavsson, 1989). The greater amount of aerobic enzymes in type I fiber lends individuals with high percentages of type I to have an advantage with endurance activities and tests. Along with knee flexor endurance, and knee extensor strength, the percentage of type I fiber explained 40% of variance in VO2 performance during a cycling test when analyzed utilizing a stepwise regression (Segerstrom et al., 2011).

When it comes to varied expression of MHC characteristics, studies with well-trained subjects have shown very little to no IIX expression when compared to sedentary subjects (Beck et al., 2008). Strength athletes, such as weight lifters and power lifters, have greater amounts of hypertrophy of their type II fibers (both A and X) compared with body builders, who have equal

amounts of hypertrophy of their type I and II (both isoforms) fibers. This is likely a training effect since weight lifters and power lifters consistently train at a higher percentage of their 1RM (Fry, 2004).

Space Flight

After 11 days of space flight, the concentration of type I fibers significantly decreases, while there is an increase in both type IIA and IIX fibers in the muscles of the calf (Trappe et al., 2009). There was no significant shift to one of the fast isoforms or the other (Zhou et al., 1995).

Hyperplasia

Another possible cause for muscle hypertrophy and an increase in muscle fiber concentration in any one of the isoforms is hyperplasia. This is when satellite cells divide and the daughter cells become contractile muscle fibers that are then innervated, and then used by the muscle or continue to divide. This type of occurrence has not been directly documented in humans and is unlikely to occur to any significant level (Abernethy, Jürimäe, Logan, Taylor, & Thayer, 1994). This however, does not mean it does not occur. In the animal model, it has been shown to transpire in both mammalian and avian species (Antonio & Gonyea, 1993). Specifically, more in birds than mammals, and can be caused by a large, stretching stress or intense training (Kelley, 1996). Stretching has been shown to cause a greater degree of hyperplasia than training (Kelley, 1996).

Only a few studies have had results that suggest the occurrence of hyperplasia in humans. In male subjects who participated in 12 weeks of hard resistance training, muscle biopsies were performed before and after training. Utilizing myosin ATPase techniques, their increases in cross sectional area for the muscle could not be explained by only the increases in fiber size for the

type I and II (both isoforms). This increase in the cross sectional area was attributed to the possibility of hyperplasia having occurred (increase in fiber number); however this theory was unable to be confirmed. What is also of interest in this study is the capillary density of both the muscle fiber types increased with the training protocol (McCall, Byrnes, Dickinson, Pattany, & Fleck, 1996).

Finally, the possible mechanism for how this occurs in humans has been identified to a limited extent in humans. By utilizing electron microscopy, subjects who participated in 6 weeks of aerobic bicycle training had small myofibers with central nuclei, which upon further analysis turned out to be myotubes. This type of formation showed satellite cells had started to fuse with muscle fibers, which denotes that hyperplasia was occurring to some degree in the muscle (Appell, Forsberg, & Hollmann, 1988).

Effects of Training on Fiber Types

Human muscle fibers contain a high degree of plasticity. Through training, muscle fiber type can start to shift towards those that it is mostly utilized for. If an individual trains for endurance events, there is an increase in type I fiber concentration, and if they train for strength/power events, there is a shift towards a greater amount of type II fibers, specifically type IIA concentration (Wilson et al., 2012). There are a number of studies that have shown this transition in humans.

In resistance training, specifically, adaptations in muscle fiber types can occur rapidly. In as few as two weeks, females participating in a twice a week resistance training program saw a decrease in percentage of type IIX fibers, while males with the same program, saw this change occur in 4 weeks (Staron et al., 1994). Fiber type shifting seems to occur no matter the repetition range trained, as long as there is adequate training frequency and volume (Campos et al., 2002).

With resistance training, an adequate amount of training stress is also required to alter fiber type expression. When sedentary individuals trained one leg with high intensity resistance training (70%) and the other leg with low intensity (17%), matched for total training volume load, there was no change in the expression of type IIX fibers in the low intensity group, but there was a significant decrease in the high intensity group (Holm et al., 2008). In subjects older than 90 years of age, a response to resistance training is still seen, with an increase in size and strength of the skeletal muscle, specifically hypertrophy of the IIA fibers, and a slight decrease in type I fibers (Kryger & Andersen, 2007).

Power training has shown mixed results on fiber type change with exercise. Sprint training can also cause a fiber type shift. With just 6 weeks of sprint cycle training, a significant decrease was seen in type IIX fibers, whereas no significant changes in type I or IIA concentration was reported (Allemeier et al., 1994). However, after 15 weeks of power training with moderate loads no significant changes in muscle fiber type in the gastrocnemius occurred in subjects (Kyröläinen et al., 2005).

Endurance training can cause a shift in muscle fiber type as well. Gastrocnemius muscle biopsies from young runners showed very few hybrids (1%) and a high percentage of type I fibers (66%). Composition of the MHCs did not significantly change over the 12 weeks that the athletes participated in the study, but there was a significant increase in type I fiber size over baseline at 8 and 12 weeks (Harber, Gallagher, Creer, Minchev, & Trappe, 2004). High intensity endurance training was shown to decrease type IIX concentration after only 8 weeks of training, and when comparing professional cyclists' muscle fibers to sedentary controls they have, on average, 20 times fewer IIX fibers. This population had 80% type I fibers in the vastus lateralis (Baumann et al., 1987).

A combination of resistance training and endurance training can change muscle fiber composition and type. This change is specific to the emphasis of the type of activity and previous training status of the subjects. After 16 weeks of strength and endurance training, elite level cyclists saw a decrease in type IIX muscle fiber concentration and an increase in type IIA proportion (Aagaard et al., 2011).

Some research has shown that just the act of playing a sport competitively throughout a season can reduce IIX fibers. Men's hockey players experienced a decrease in IIX fiber concentration over the course of one season and as well as an increase in IIA fiber concentration (Green, Thomson, Daub, Houston, & Ranney, 1979).

Age and Fiber Type

As people age, there is a transition in fiber type to a greater amount of type I fiber, and a loss of type II (A and X) fibers (Venturelli & Richardson, 2013). These effects start to occur as young as age 25 and continue as we age. Specifically, there is a decrease in the number of fibers and type II fiber size (Larsson, Grimby, & Karlsson, 1979; Lexell, Taylor, & Sjöström, 1988). Additionally, the quality of older muscle fiber particularly type IIX has been found to decline (Narici & Maffulli, 2010). This is possibly due to the lack of utilization of this particular fiber type with older age individuals (Larsson & Karlsson, 1978), yet the exact mechanism for the change of muscle fiber type with age has not been fully explored or understood. It is also interesting that not all of the loss in muscle force can be attributed simply to a decrease in cross sectional area. When examining a group of individuals across a very wide age range (23-80), only 39% of the force decline could be attributed to a loss in cross sectional area and there was no significant difference in muscle fiber type between the individuals or a shift with age. This suggests that there are underlying mechanisms for the decrease in force with aging that are not

limited to changes in cross sectional area and MHC shifting (Jubrias, Odderson, Esselman, & Conley, 1997).

Aside from changes in MHC distribution, there is an alteration in contractile properties of the fibers. Older individuals have been shown to have lower force production with individual fibers compared to younger counterparts (Yu, Hedström, Cristea, Dalén, & Larsson, 2007), possibly attributed to an increase in fiber force production in single fiber studies with training in older individuals. These studies show us that fibers underperform what they are capable of with sufficient training stress (Trappe et al., 2000). Aside from exercise performance, muscle MHC, and muscle fiber quality, it is important with older subjects who are experiencing a decline to not just review type II fiber content, but also the quality of the muscle fibers themselves (Slivka, Raue, Hollon, Minchev, & Trappe, 2008). We also see changes in individuals limited to bed rest (Andersen, Gruschy-Knudsen, Sandri, Larsson, & Schiaffino, 1999; Trappe et al., 2004) and astronauts (Caiozzo, Baker, Herrick, Tao, & Baldwin, 1994; Trappe et al., 2009), who have an increase in the expression of type IIX fibers. This increase in expression of type IIX is found to be related to detraining.

There are a number of changes that occur with aging and performance of the musculature. Some of the causes for the change in muscle function with age are neuronal. There is a loss of motor nerve numbers as we age, which leads to a decrease in muscle performance despite branching, which to a degree causes re-innervation. However, this mechanism eventually slows and re-innervation is less likely (Stålberg et al., 1989). Some research has shown a greater concentration of hybrid muscle fiber in older individuals compared to younger, healthy adults (Andersen et al., 1999). This type of adaptation could be beneficial as a means of maintaining aerobic capacity and also the muscle power production potential that the type II fibers provide.

Older individuals typically experience a decline in speed of recovery and adaptation from stress. When younger and older men were immobilized for a short period of time, the older population took longer to regain original muscle size and strength compared with the younger population, specifically the type IIA muscle fibers (Hvid, Ortenblad, Aagaard, Kjaer, & Suetta, 2011). However, older individuals do show an increase in efficiency with age. They have a greater concentration of type I fibers, which produce less power than type IIA and IIX isoforms, but are more efficient with energy substrates, which allows for less metabolic stress on their body (Venturelli & Richardson, 2013). A decrease in hybrid fibers in the biceps, specifically fibers that express both IIA and IIX isoforms, (Klitgaard, Zhou, et al., 1990) has also been noted. Aside from a decrease in total amount of fibers, size, and MHC, researchers have seen a decrease in certain intracellular enzymes related to metabolism. Lower amount of citrate synthetase (CS) are present in older individuals when compared to younger individuals, which effects maximal oxygen consumption performance, as CS concentration is positively correlated with this measure of cardiovascular fitness (Houmard et al., 1998).

As humans age the risk of developing sarcopenia increases, as does the risk for further compounding effects of sarcopenic obesity. Sarcopenia is a loss in skeletal muscle mass, and underline causes continue to be studied. Disuse is one cause of sarcopenia, along with mitochondrial oxidation and exhaustion of muscle satellite cell reserves. Sarcopenic obesity is the associated increase in fat mass and reduction of muscle mass. This can affect metabolic function, and lead to metabolic diseases, such as type II diabetes mellitus (Narici & Maffulli, 2010).

Older individuals do still adapt to exercise, but a variety of training studies have shown that these changes are often different than the adaptation that occurs in younger individuals. With

three to four sessions per week of aerobic exercise for 12 weeks, older women (approximately 70 years old) increased their muscle function, specifically their type I fiber size and content. There was also a decrease in the expression of type IIA and IIX fibers in the mRNA expression and protein levels (Konopka, Trappe, Jemiolo, Trappe, & Harber, 2011). Three groups of subjects: young, middle-aged, and older, who took part in a resistance training program for 3 months, had their mRNA analyzed for each of the specific MHC isoforms, and found that older individuals do not transcript as high of levels of type II fibers (either isoform). The older individuals did show an increased expression of type I fiber, indicating that there is still adaptability to training stimulus later in life, however without the same expression of the fast isoforms as occurs during youth and middle age (Balagopal, Schimke, Ades, Adey, & Nair, 2001). Other research has also shown that skeletal muscle fibers still maintain adaptability in older individuals. With the use of a progressive resistance training program, older men were able to increase the shortening velocity and power of their type I and IIA fibers. There was a larger change in the type I fibers than the type IIA (Trappe et al., 2000). With progressive resistance training in older men and women (mean age of 68) there was significant increases in type I muscle fiber size after 15 weeks of training as well as a significant increase in type IIA fibers after 30 weeks. The earlier significance of type I fiber size, and later increase in IIA fiber size further solidifies that type I fibers are more adaptable in older individuals (Pyka, Lindenberger, Charette, & Marcus, 1994). Older men (average age of 82 years) were put through a resistance training program for three sessions maximum per week over a period of 12 weeks. Researchers found no significant changes in muscle MHC composition or contractile abilities. This could potentially be due to the low training volume, or perhaps due the greater age of this population, as multiple studies with a

slightly younger population and greater training volume have shown a change (Slivka et al., 2008).

Not all age-related muscle declines are necessarily going to occur. Those individuals, who trained consistently over their entire life, are able to better maintain their muscle fiber and performance compared to sedentary, older individuals. Older men who had been active for over a decade in running, swimming, or resistance training had higher amounts of type I fiber than younger sedentary individuals (Klitgaard et al., 1990). Older male sprinters, when compared to younger male sprinters, had lower amounts of type II fibers specifically type IIX, however, their functional strength and power of the type IIA fibers were still at the same level as the fibers found in the younger individuals. A longitudinal, consistent, training modality seems to lead to better preservation of function in specific fibers, suggesting a use of sprint training for maintaining type IIA fibers (Korhonen et al., 2006).

Performance and Fiber Types

With large differences in energetic and power performance between the different fiber types in humans, it is important to look at how these effects compound in vivo. A number of studies with varying levels of control have looked at the effects of different proportions of fiber type on performance in a variety of tasks. These tasks will be broken down into muscle action performed and the increasing number of joints involved.

In Vitro

When muscle fibers are isolated from the organism, direct measures of force, time to start of force production, and shortening velocity are measurable. In general, there is little difference in peak force production between any of the MHCs (when controlled for cross sectional area),

however the rate of which contraction occurs and calcium sensitivity differs between each of the isoforms (Fitts & Widrick, 1996). Studies have shown that twitch time to peak torque, maximal rate of torque development, frequency response, and fatigability are related to muscle MHC in the type I, IIA, and IIX (Harridge et al., 1996). The same effect was found when looking at rat skeletal muscle where both the IIB and IIX fibers had a greater contraction power production (Bottinelli et al., 1991). The same study also found that force production per unit of cross sectional area was higher in the type II fibers than in the type I, and it was not differentiated to the different isoforms of the type II.(Bottinelli et al., 1991)

With disuse, there is a decrease in the force produced by individual muscle fibers across both MHCs with immobilization. There is also an age-dependent decrease in calcium sensitivity, specifically a decrease in type I fibers in the young and type IIA fibers in the old (Hvid et al., 2011). In older women, there was no reported difference in force production in either type I or type IIA fibers compared to younger women (Raue, Slivka, Minchev, & Trappe, 2009). There was a significant difference in adaptation to progressive resistance training. Older women were not able to adapt to the exercise protocol\, whereas the young women were.

Single Joint

Single joint movements allow all of the muscle shortening to cause rotation only across one joint. This joint can either stay stationary or move at a variety of velocities. Each of these actions can illustrate different attributes of muscle performance and will be analyzed for their effectiveness at estimating muscle MHC.

Isometric

When attempting to predict muscle MHC it is important to minimize the number of joint

actions and muscles involved in order to remove as many confounding factors as possible. One

way to do this is to utilize single joint movements so that all of the action of the joint is caused

by a limited number of muscles. Specifically, when looking at basic hinge joints, such as knee

extensors, it is relatively easy to gauge muscle action of the large muscle group, the quadriceps.

This muscle group, in healthy individuals, is a large muscle that is relatively easy to not only test,

but biopsy (vastus lateralis portion). Current research has analyzed the quadriceps from a variety

of approaches. One such approach is to test muscle isometric force production analyzing

different features of this muscle action (Clarkson, Kroll, & Melchionda, 1981; Herda et al., 2010;

Schilling, Fry, Chiu, et al., 2005; Viitasalo & Komi, 1978).

This type of research has a variety of results that are conflicting. A research study strictly

looking at maximal force produced found no relationship between muscle strength and fiber

composition. Additionally, the muscle strength to cross sectional area ratio did not have a

relationship to MHC either (Maughan & Nimmo, 1984). This study, though good for its time,

would now be able to generate more information due to increased access and technological

advancements. This study did help researchers to see that in isometric tests, total amount of force

produced is not different due to variations in MHC composition. When looking at the differences

between MVCs and fast MVCs the difference between the forces created were linked to the

proportion of fast fiber and this link was stronger in older individuals (Clarkson et al., 1981).

Schilling et al. found that IIX fibers, when added to a correlation model, explained a significant

amount of the relative peak force (19.9%). This research also showed that there was trend

towards significance on the effects of the type IIX fibers in the first half of the rise phase by

force produced during the MVC (Schilling, Fry, Chiu, et al., 2005). One study found a positive relationship between isometric MVC force and slow twitch fiber concentration, which is contrary to most of the research that is found in this area (Kroll, Clarkson, Kamen, & Lambert, 1980).

Other research has examined the relationship between peak force development and MHC composition. The rate of which peak force was attained was related to the proportion of slow twitch MHC. Specifically, individuals with longer times to peak force typically had a greater amount of slow twitch (type I) muscle fiber in the vastus lateralis (Viitasalo & Komi, 1978). Further research has agreed with the positive correlation between time to fatigue in isometric leg extension tests and concentration of type I muscle fiber (Hulten, Thorstensson, Sjödin, & Karlsson, 1975). Tesch and Karlsson reported that there is a positive correlation between MHC characteristics (percent fast twitch fiber) and maximal isometric strength (Tesch & Karlsson, 1978).

Other research which compares different types of well-trained individuals has shown MHC specific results. When comparing sprinters to marathon runners in performance on an isokinetic leg extension test it was found that there was a positive relationship after correcting for cross sectional area for type IIA fiber area and peak torque, instantaneous power, integrated EMG, and contractional work at specifically a contraction speed of 180 degrees per second (Johansson, Lorentzon, Sjöström, Fagerlund, & Fugl-Meyer, 1987).

Mechanomyography (MMG) and Electromyography (EMG)

MMG and EMG are methods of looking at muscle function. MMG can measure the rate at which a muscle is vibrating due to active contraction. EMG can show the amount of electrical activity that is occurring in the muscle due to muscle recruitment, specifically depolarization of the tissue. Complex systems now are able to differentiate between motor firing rates of

individual motor units and interrelate this to muscular function with the use of MMG signals. This method requires the use of isometric testing currently to assure the degree of accuracy, representation, and muscle recruitment.

MMG has been used to find differences in muscular performance between the different MHCs. The speed of a fiber's electrical conduction measured during an MVC has been shown to be related to muscle MHC. Fast isoform (IIA and IIX) skeletal muscle has a higher speed of conduction than slow twitch, dominate muscle fiber (Sadoyama et al., 1988). When subjects performed ramped contractions, EMG mean power, frequency, and MHC were positively correlated. There was also a negative correlation between type I fiber concentration and the torque – mean power frequency intercept (Gerdle, Henriksson-Larsen, Lorentzon, & Wretling, 1991).

Different types of fatiguing protocols and muscular tracings have been used as a means to compare muscular function for MHC characteristics. Beck et al. found that when performing 45 second isometric contractions at 50% of MVC there was no significant difference in the MMG-EMG signals from a resistance trained or aerobic trained group to predict muscle MHC (Beck et al., 2009b). There was trending toward differences in mean power frequency performance, but due to the small sample size the difference was not significant (Beck et al., 2009a). Previous research showed no relation to MMG amplitude and torque production utilizing a ramping protocol with three different sample groups of strength trained, aerobic trained, and sedentary individuals (Beck et al., 2008). Herda et al., utilizing a ramp contraction from 5% to 90% of maximal voluntary contraction, found there were significant differences in the slope derived from linear regression lines created by log transforming the EMG data. This data was related to the muscle MHC differences in the three groups tested (resistance trained, aerobically trained,

sedentary). The intercept seemed to be more related to the level of subcutaneous fat (Herda et al., 2010).

Isotonic

Isotonic muscular tasks cause joints to move through a range of motion against a constant load. When greater forces are applied, the joint is able to move at greater velocities once the required force to initiate movement is overcome. Some research studies have looked at isotonic exercise performance as an indicator of muscle MHC characteristics. When subjects gave their greatest velocity/force effort against a fixed load relative to their 1RM on the leg extension machine at either 40% or 70%, there was no relationship to muscle MHC, possibly due to the small sample size (Schilling, Fry, Weiss, et al., 2005). Other research looking at isotonic leg extension performance found that repetitions to failure at different percentages of a subjects 1RM were not predictive of muscle MHC (Rodriguez, 2005).

Further research has examined relationships between velocity, power production, and MHC. A positive relationship between peak acceleration and MHC has been seen for fast twitch (both A and X) percentage (r=.40, p=.04) with an unloaded leg extension test. There was no difference in the peak velocity between the subjects of differing muscle MHC compositions (Houston, Norman, & Froese, 1988). When subjects were divided by MHC, an isotonic leg extension showed that large power outputs at matched relative loads were different between the two groups. Individuals with higher amounts of fast twitch fiber (no differentiation between IIA and IIX) had greater power productions than slow twitch dominant athletes (Tihanyi, Apor, & Fekete, 1982). Tihanyi, found that higher powers, created along with higher velocities, were achieved at higher loads in individuals with a higher amount of fast twitch fiber proportion than their slow twitch counterparts (Tihanyi et al., 1982).

Multi joint

    Vertical Jump

When subjects performed vertical jump testing there was a greater amount of stored kinetic energy in subjects with larger amount of fast twitch muscle fibers (type IIA and IIX) compared to those with more slow twitch (type I). When a longer pause occurred the difference in stored force dissipated, then shifted to giving a greater efficiency of force return in the slow twitch dominate subjects (Bosco, Tihanyi, Komi, Fekete, & Apor, 1982). Other vertical jump tests have shown that jump height, average force, net impulse, and average power all were significantly related to fast twitch fibers in a static start jump; additionally jump height and net impulse significantly related to fast twitch fibers in a counter movement jump. This study however, did not disseminate between type IIA and IIX fibers (Bosco & Komi, 1979). The only research found in this area looking at type IIX has been presented as an abstract (Eckerson, 2005; Fry, 2002)

In athletic populations, relationships between MHC and jump performance have also been seen. When track and field athletes performed a vertical jump power test for 60 seconds the first 15 seconds of the test's power output correlated strongly with fast twitch fiber concentration ($r = 0.86$, $p < .005$). After the first 15 seconds, the following 15 seconds still had some relationship to fast twitch concentration, but were no longer significantly related after 30 seconds (Bosco, Komi, Tihanyi, Fekete, & Apor, 1983). With Olympic weight lifters, there was a positive correlation with proportion of IIA fiber and weight lifting and vertical jump performance. There was not a significant relationship to performance with the type IIX fibers (Fry, Schilling, et al., 2003).

When subjects performed a fatiguing protocol, the store and return of elastic energy was greater in slow twitch dominant subjects before having to undergo 60 seconds of continuous jumping. This relationship then changed and a greater return of elastic energy was seen after the fast twitch fibers. (Bosco et al., 1986). Finally muscle damage from plyometric exercise has been shown to induce a greater amount of tissue damage to the fast isoforms compared to slow twitch fiber (Macaluso, Isaacs, & Myburgh, 2012), which could show both more activation and greater predisposition for fiber damage in the fast isoforms.

Wingate

Other multi-joint, but controlled tests, have been used to predict muscle MHC; specifically anaerobic power tests. Utilizing an anaerobic, 30 second Wingate test a positive relationship was seen between peak power, total work, and power decrease with fast twitch fiber percentage (Bar-Or et al., 1980). This effect was found only in the male subjects, not the females; this was attributed to the high resistance level for females (Froese & Houston, 1987). Further research with a Wingate has shown relationships between the % fast twitch and peak power, max peak torque, and power decrease. The same study found that sedentary individuals did not show a significant relationship and that a 40m sprint speed was positively related to fast twitch percentage while a 2000m speed was negatively related to fast twitch percent (Inbar, Kaiser, & Tesch, 1981).

Isokinetic

Specific machines have been designed to limit the speed at which a movement can occur. This type of exercise machine is referred to as an isokinetic device. If a subject produces greater force, an internal brake is applied so that the speed of displacement or rotation cannot be increased over a set velocity. This testing allows for movement to be limited so subjects cannot

become too ballistic and possibly risk injury. Additionally, isokinetic devices allow for unique testing modalities with a multitude of applications. Literature has not been able to find significance in reference to type IIX fibers, but there are some published studies in this area. One research study performed by Gregor et al in 1979, found that as the isokinetic speed was increased, athletes with a higher proportion of type II to type I muscle fibers were capable of generating higher amounts of torque. This study, however, did not disseminate the contributing proportion of type IIX to type IIA fibers (Gregor, Edgerton, Perrine, Campion, & DeBus, 1979). Other articles have also come to the same conclusions regarding the correlation of fast isoforms (IIA and IIX) when the strength in the peak torque was produced at higher speeds of rotation on isokinetic leg extension tests (Coyle, Costill, & Lesmes, 1979). Further research has seen specific relationships with MHC and speed of movement, where no relationship between MHC and force production at lower speeds was seen, but one was at higher speeds (Aagaard & Andersen, 1998). This study did not disseminate between IIA and IIX fibers.

The torque ratio of isokinetic leg extensions performed at 240 degrees to 30 degrees/second was positively related to type II (A and X) fiber concentration in both males and females (Gür, Gransberg, Knutsson, & Larsson, 2003). The same types of relationships were not seen in a population of physical education students and bodybuilders. In this study, as the velocity of movement increased, no relationship was determined between the concentration of type I fibers and torque produced in the leg or elbow extension movements (Schantz, Randall-Fox, Hutchison, Tyden, & Åstrand, 1983).

Fatiguing leg extension tests can also be utilized for testing on isokinetic machines. One of the first studies to predict MHC content from isokinetic leg extension performance was Thorstensson. He found that a muscle with a higher amount of fast twitch fibers contracted at a

faster velocity. This allowed for the creation of a predictive model to look at fatigue index and leg extension performance (Thorstensson et al., 1976; Thorstensson & Karlsson, 1976). Other research has been used in high level athletes with isokinetic testing. When sprinters were compared with marathon runners on their performance in a fatiguing isokinetic leg extension test, it was found that sprinters had a much greater decline in performance, which was negatively correlated with their proportion of fast twitch fiber (IIA and IIX) (Lorentzon, Johansson, SjÖStrÖM, Fagerlund, & Fugl-Meyer, 1988).

Sports and Fiber Type

Muscle fiber type is linked to performance in a number of different sports. Specifically, sports that require one end of the power-endurance continuum tend to favor, at the highest level, specific MHC distributions in the athletes. Overall strength power sports tend to have elite level athletes with greater proportions of fast twitch fiber (e.g. sprinters) and endurance sports tend to have athletes with greater amounts of slow twitch fiber (e.g. marathon runners). The research in this area has also examined sports that require both great endurance and short bursts of power (e.g. soccer).

Some research has linked MHC in athletes to performance in certain exercise tasks. Gregor et Al. found that the MHCs of athletes affected their performance in maximal isokinetic leg extension testing and that there was a positive relationship between the amount of type II fibers and the ability to create a greater amount of torque at a higher velocity. Furthermore, long distance athletes tend to have greater amounts of type I muscle fibers and sprinters tend to have greater amounts of type II muscle fibers, showing a specificity of MHC characteristics and performance. There was also a difference in the fiber size in athletes, with the fiber of most frequent use being larger than the other, less utilized fibers.(Gregor et al., 1979)

Higher amounts of type II fibers, and specifically type IIX fibers, are correlated with being a better sprinter, whereas Caracals had higher amounts of IIX fibers than the lions which still have much greater levels compared to humans (Kohn et al., 2011). In humans, sprinters tend to have a high amount of IIA fiber content (Korhonen et al., 2006). In weightlifting, competitors with higher amounts of type IIA fibers had greater performances in the lifts compared to competitors that had lower proportions of IIA fibers (Fry, Schilling, et al., 2003).

Long distance runners had a higher proportion of type I fibers compared to middle distance runners or controls in a study by Harber et al. (Harber, Gallagher, Trautmann, & Trappe, 2002). What is also interesting to note with this study was that the middle distance runners had the highest amounts of type IIA while the sedentary subjects had the highest amount of type IIX and hybrid fibers. This type of expression seems to be consistent with training status, and shows that untrained individuals have a larger proportion of both hybrid fibers and IIX expression (Harber et al., 2002). Long distance athletes had high percentages of type I fibers (60-70%) compared to fast fibers. Type IIX fibers were found in small concentrations in the vastus lateralis and gastrocnemius (<6%) of the runners. This type of adaption is advantageous to the athlete whose sport requires continuous exercise output for over 15 minutes at a time (Saltin et al., 1995). In a study analyzing cycling efficiency and performance, subjects with a higher proportion of type I muscle fiber were more efficient and performed better on exercise tests (Coyle, Sidossis, Horowitz, & Beltz, 1992). Cycling performance has been linked to a higher power output in individuals with a higher percentage of type I fibers (Horowitz, Sidossis, & Coyle, 1994) when matched with individuals who had comparable VO2 maxes.

When compared to sedentary females, female track and field athletes had a greater amount of type IIA fibers and a lower amount of type I fibers. Overall, there was not a

significant difference in the total amount of type IIX fibers in either group. This is interesting since the biopsy was taken 14 weeks into the training program for the track athletes and typically IIX fibers are less prevalent in well trained individuals than sedentary subjects. Furthermore, all of these athletes were national or international caliber athletes whose furthest event was the 400m or heptathlon (Parcell, Sawyer, & Craig Poole, 2003). Female soccer players, regardless of taking part in a strength training program or otherwise, minimally expressed IIX fibers, and only expressed in hybrid fibers that also contained IIA (Andersen, Klitgaard, Bangsbo, et al., 1994).

So far, research has shown a much greater degree of IIX fiber presence in untrained individuals. Not only do they present with more IIX, but it is common to have hybrid fibers which express both IIA and IIX isoforms. Well trained aerobic athletes tend to show a higher amount of hybrid fibers by expressing the type I and IIA isoforms in their muscle (Klitgaard, Bergman, et al., 1990).

Genetics

Heredity plays a factor in a person's muscle fiber type. The MHC gene is found on the short arm of chromosome 17 in humans (Leinwand, Fournier, Nadal-Ginard, & Shows, 1983). Studies looking at twins have found genetically inherited factors to comprise about 45% of an individual's fiber type (Simoneau & Bouchard, 1995). Certain genetic traits, such as being born with a special angiotensin-converting enzyme gene, have been seen to have a positive effect on having higher amounts of type I muscle fiber (Zhang et al., 2003). In older women, having different genetic traits has been shown to have an effect on training response. The most recent study specifically looked at the ACE I/D trait and $ACTN_3$ R/X trait (Pereira et al., 2013). The $ACTN_3$ R/X is related to increased amounts of fast fiber in humans, and there is still a great deal of research into the heritability of muscle fiber type that needs to be performed.

Fiber Type Analysis Methods

There are many different methodologies for finding MHC composition with muscle tissue. Each of these methodologies has their own advantages and disadvantages that must be appropriately analyzed to determine which fits best for the type of testing that will occur in a study.

Large format gels have been utilized in a number of studies to find the MHC composition. This allows for a glimpse of type fiber expression throughout the entire sample. Typically mounted in traganth gum to allow for an even cross section of tissue (Staron et al., 1994). Samples are frozen in isopentane to keep from lysing the cells, which occurs with flash freezing in liquid nitrogen typically used in other extractions for muscle protein activation. Homogenate does not allow for the quantification of hybrid muscle fibers, but it shows the concentration of fiber isoforms relative to one another, which provides a percentage of each isoform present in the muscle overall (Fry, Webber, et al., 2003). SDS-page separation can also be done with small format gels, and has specifically been shown to work well with rat tissue (Talmadge & Roy, 1993). Furthermore this method has been shown to be positively correlated with myosin ATPase analysis (Staron, 1991).

Single fiber (Harber et al., 2004) analysis method allows for the quantification of hybrid muscle MHC. This can be a useful marker due to its ability to show if fiber types have shifted, what fiber type is now expressed, or as a possible marker of training status (taper, in season, etc.). This method requires special chemical preparation to individually extract the fibers, and then allows for testing the fibers for force production and other variables. Fibers can then be lysed in an extraction buffer and ran individually, utilizing MHC large format SDS-page methods discussed above.

Myosin ATPase staining (Baumann et al., 1987) allows for the analysis of hybrid muscle fiber types and for the analysis of mean fiber size, which can be indicative of training effects (Tesch & Larsson, 1982). This method has been available since the 1960s. By staining the cross sections of cut muscle fibers it allows for counting of the individuals fibers. Each fiber type stains to a different darkness levels depending on the type of ATPase that is expressed in that fiber (Bárány, 1967). Changes in fiber size overall and per fiber can be seen with this method. It is useful for tracking changes in expression of hybrid muscle fibers, and easily measures increases or decreases in their concentration. It is, however, very time consuming since a large number of individual fibers must be counted in order to achieve a significant sample that will be representative of the rest of the muscle.(Brooke & Kaiser, 1970)

By analyzing the presence of the different types of MHC mRNA expression it is possible to start quantifying muscle fiber type transitioning at a much earlier point (Flück & Hoppeler, 2003). This type of cellular signaling to build more protein, specifically heavy chain, can show if a training regime or intervention is causing fiber type to shift or maintain its current expression characteristics (Balagopal et al., 2001; Plomgaard et al., 2006). What is also interesting is there is a correlation between the mRNA expression for fiber type and for mRNA specific to different metabolic enzymes. This link is seen in a more aerobic enzyme mRNA expression in type I MHC mRNA upregulated cells, and more anaerobic enzyme mRNA expression in type II (A and X) MHC (both isoforms) mRNA upregulated cells (Plomgaard et al., 2006). This is sensible as the metabolic demands placed on the fiber would be fiber type specific.

Western immunoblots have been utilized as a means to quantify MHC composition (Kjellgren et al., 2003). This method utilizes homogenized muscle samples that have been extracted in the same methods as the SDS-PAGE separation, but samples are transferred to a

receptive membrane (typically PVDF). This protein is transferred by electric current. Once proteins are transferred, the membrane is then washed in a blocking protein so any other possible binding sites are covered. At this point, the blot is then treated with an antibody that is specific to the protein of interest (Lucas, Kang, & Hoh, 2000). The blot is then washed and a secondary antibody is applied that is specific to the primary antibody. The blot is then imaged via different methods of chemiluminescence or scanned for infrared radiation. Imaging proteins are then quantified based upon image saturation and normalizing to housekeeping proteins.

Predicting Myosin Heavy Chain Concentrations

There are currently a limited amount of tests that are used for predicting MHC expression based on performance of different tasks. All of these tests have moderate degrees of ability to predict MHC; however, all require expensive equipment or training. The most popular isokinetic test currently used is the Thorstensson. This test was originally developed in the 1970's and is still used today. (Thorstensson & Karlsson, 1976) When comparing fatigue indexes found from the testing methodology by Thorstensson et al. there was no significant differences between sexes, but males on average had a higher amount of peak torque (Stock, Beck, DeFreitas, & Ye, 2013). There are also relationships between EMG and MMG signals that relate to MHC as mentioned before (Herda et al., 2010). Each of these methods has varying levels of ability to predict MHC.

Other tests based upon resistance training are somewhat invasive, but have a limited amount of predictability. Lactate levels produced from heavy resistance training on a leg press machine were positively correlated with proportion of fast twitch fiber (both IIA and X) (Linnamo et al., 2000).

There is also more advanced technology which been used as a means to predict MHC. One study attempted to predict MHC characteristics of human muscle samples by utilizing proton magnetic resonance spectroscopy to analyze muscle carnosine levels (Baguet et al., 2011). This predictive model was only based on looking at the difference between type I and both type II isoforms.
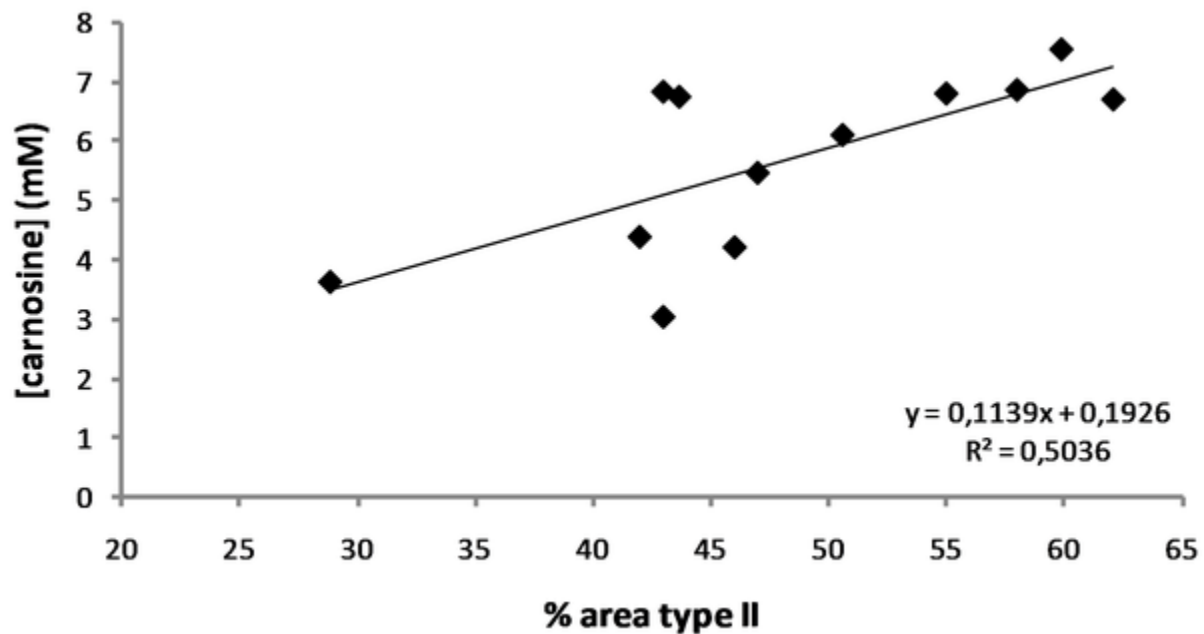
Figure 3 (Baguet et al., 2011) Correlation of intramuscular carnosine levels to MHC content type II area

Power Load Curves
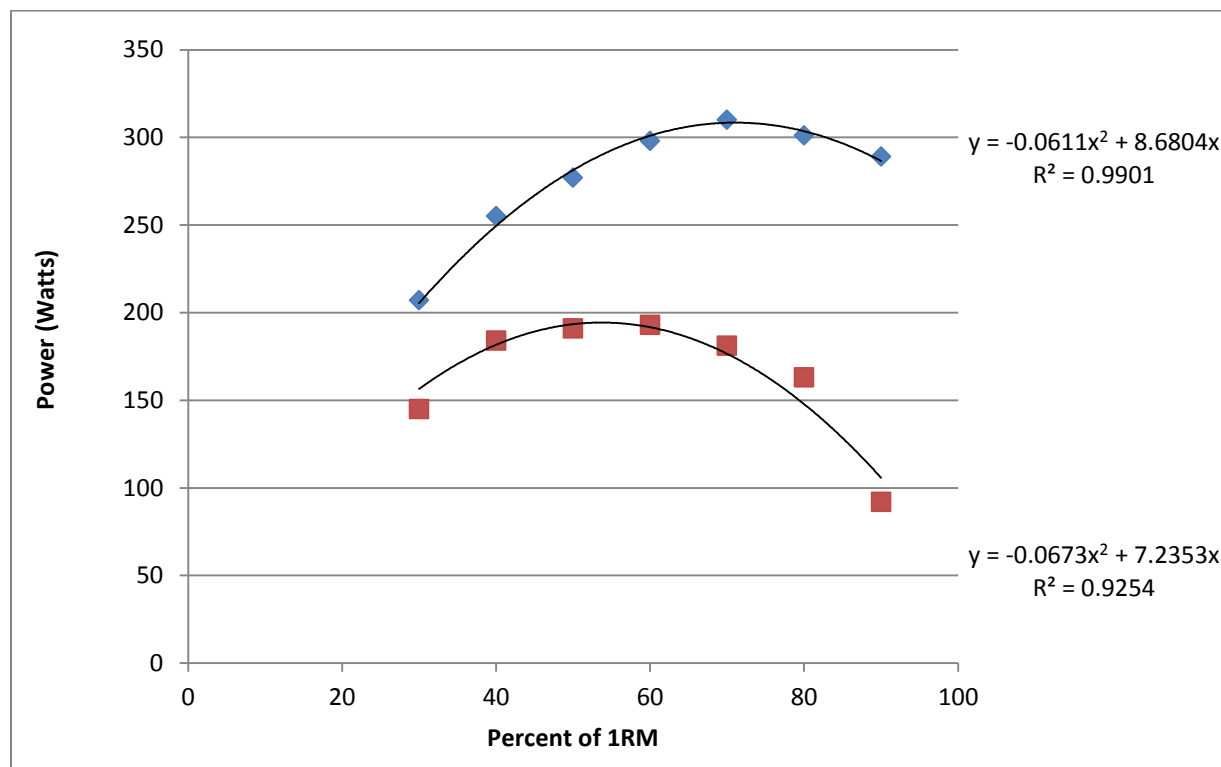
Figure 4 Power-load curve pilot data – displays two different subject performance on a leg extension performance.

Pictured above is pilot data from the lab graphing mean power performance to percentage of 1RM in two separate subjects (Figure 4). After graphing a best fit line, based on a second order polynomial agreement, there are very different performances relative to the 1RM. Best fit lines are given an intercept of zero for all participants. Both of these subjects were recreationally trained, one subject was strength trained, while the other aerobically trained. Their training history and performance suggests MHC differences. Deriving estimate peak performance along the percentage of 1RM line requires the solving of the polynomial best fit line for the greatest "y" value.

Velocity Load linear agreement
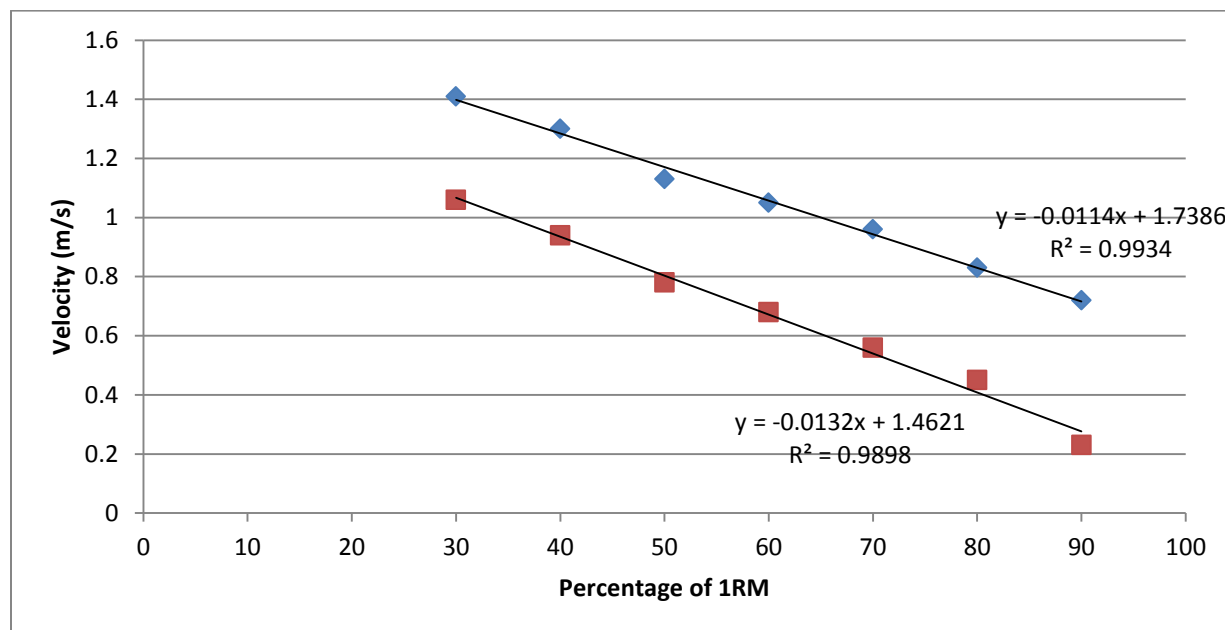
Figure 5 Velocity load linear agreement pilot data - displays two different subject performance on a leg extension performance.

Pictured above is more pilot data from the lab showing the linear agreement to velocity performance as subjects get closer to their 1RM (Figure 5). These are the same subjects from the previous graph. It is possible that the different performance can be attributed partially to MHC differences in the subjects. This pilot data has fallen in line with the same great deal of variability that we have found when testing well trained athletes in the barbell back squat.

Chapter 3 Methodology

Subjects

Forty two males between the ages of 18-36 were recruited for this study. Subjects had no history of major medical problems or surgeries based upon self-reported questionnaires. Subjects were then placed in sub-groups dependent on current activity level based upon health/training history responses. Criteria for the groups were as follows:

Resistance trained (n = 13) – These subjects were capable of back squatting at least 1.5x their own body weight in the barbell back squat. A squat was defined as to top of the thighs, (quadriceps) parallel to the ground.

Endurance trained (n = 12) – These subjects were be capable of running a mile in less than 6 minutes and had a total weekly running volume 30 miles or more.

Recreationally trained (n = 11) – These subjects were active most days of the week and not following an organized, periodized, resistance training program or aerobic program. Subjects were not able to run a mile in less than 6 minutes and squatted less than 1.5x bodyweight.

Sedentary (n = 6) – These subjects had not taken part in any exercise program or sports for at least two years prior to the study. They were not active for more than 2 hours per week of exercise.

Testing Preparation

Subjects participated in several exercise tests to predict MHC characteristics. Subjects were instructed not to train the day directly prior to each testing session. If a subject did train the day before testing, their testing was to be rescheduled so there would be no fatigue pre-training

to negatively affect performance. If subjects had an acute illness on the day of testing subjects were rescheduled at the next earliest opportunity for their session once they had recovered.

Testing Schedule

Subjects signed consent forms, a health history, and a training history questionnaire before taking part in anthropometric testing and familiarization. The subjects then scheduled their next testing day. During the next testing session, subjects performed the vertical jump test and dynamic constant external resistance (DCER) isotonic leg extension 1RM test. Subjects then returned 3-9 days later and performed a leg extension power load test, and modified Kansas squat power test on the leg extension. After completion of the tests, a muscle biopsy was performed.

Subjects were instructed to come in at the same time each testing day. The time of day has been shown previously to have an effect on muscle activation and performance, as one study noted an effect on the elbow flexors (Gauthier, Davenne, Martin, Cometti, & Hoecke, 1996). The same effect was seen using a leg extension MVC test (Guette, Gondin, & Martin, 2005) and a Wingate power test (Chtourou et al., 2011). There is also a component of trainability of which this peak performance occurs during the time of day (Souissi, Gauthier, Sesboüé, Larue, & Davenne, 2002). This adaptation seems to be mostly through peripheral mechanisms, not neurological (Sedliak, Finni, Peltonen, & Häkkinen, 2008). To control for this, subjects started each session of testing within a one hour window of the time they tested during their first session.
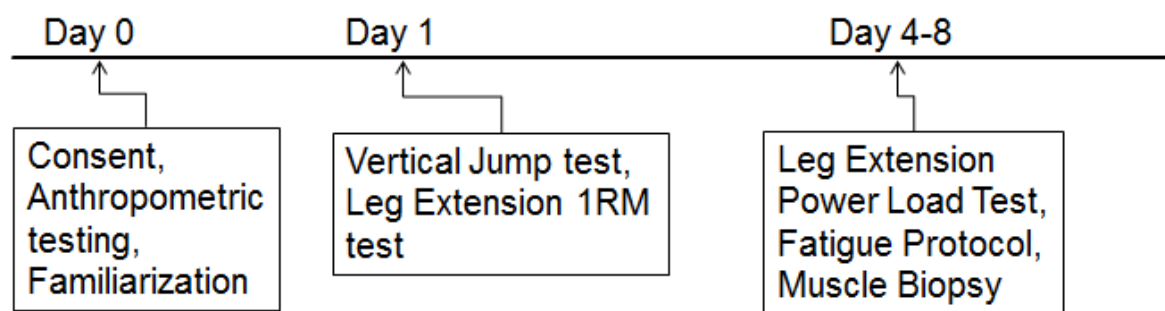
Figure 6 Timeline of subject testing for the visits in the study

Body Composition and Anthropometrics

A Dual-energy X-ray absorptiometry (DEXA) scanner was utilized to determine body fat percentage, as well as local and systemic lean body mass (Fuller, Laskey, & Elia, 1992). Additionally, thigh circumference measurements and skinfold thicknesses were taken to estimate thigh cross sectional area using the methods of Housh et al. (Housh et al., 1995). Height was taken with subjects standing without shoes on against a tape measure that is mounted to the wall. Weight was taken utilizing a floor scale (Toledo industrial floor scale 2136 8140 Digital, Mettler Toledo Columbus, OH).

Familiarization

Subjects completed informed consent forms and health history questionnaires. Subjects were instructed to detail their training from the previous week as a means to help classify the appropriate category to place the subject based upon their training history. Any questions that researchers had with the subjects exercise program were addressed at this point. After this, subjects underwent a DEXA scan for body composition and then performed jump familiarization.

Subjects performed two practice jumps where they attempted to touch as high on the Vertec as possible. They then performed 2 jumps without the use of their arms and 2 depth jumps off of a 12" box. Each jump was given a minimum of a one minute rest period.

Subjects were then moved to the DCER leg extension machine (Biodex) (Biodex Leg extension Humac System 3, Biodex Medical Systems, Shirley, NY). The seat position along with thigh pad was outfitted appropriately for each subject's size and recorded for uniformity across each of the subjects testing sessions. Subjects were seated appropriately on the Biodex chair allowing for approximately one inch of clearance between the posterior portion of their lower leg and the bottom of the Biodex seat. Subjects were restrained by a waist strap, thigh strap, and shoulder straps to minimize any body movements. Subjects then grasped the handles at the sides of the chair for each trial throughout all of the tests in the study.

Subjects performed 3 sets of slightly increasing resistance. Loads for these sets were 5 repetitions at 10lbs. of resistance, 20lbs. of resistance, and 40lbs. of resistance. At this point subjects were then built up to an attempted 1RM based upon performance of previous repetitions, with no larger than a 20lb. increase. They performed a familiarization of the fatiguing protocol of 5 repetitions at 70% of the highest load attained. Rest periods between each of these sets were at least one minute. At this point subjects were finished with their familiarization and were scheduled for their following visit.

Vertical jump performance

Subjects warmed up for 5 minute ride on a stationary bicycle at a self-selected cadence and resistance. Afterwards, they performed 2 maximal, vertical jumps utilizing an arm countermovement swing on a force plate. Following this, subjects performed 2 maximal, vertical jumps with their hands on their waist. Finally, subjects performed 2 maximal depth jumps from a

12" box. For the depth jump the subjects were instructed to step off the box so that they would drop from the height for the rebound. This was to avoid any variability in jumps off the box or stepping down and losing the effect on the vertical jump. Between each jump, subjects were given a one minute rest period before the following jump. Jumps were performed on a force plate (Rough Deck, Rice Lake) to allow for force production and flight time to be recorded. Force plate data was interfaced with a Biopac MP150 voltage interface (Biopac Systems Inc, Goleta, CA) and visual displayed and signals recorded with AcqKnowledge software (version 3 Biopac Systems Inc.) at a sampling frequency of 2000 Hz. Signals were filtered utilizing a Butterworth low-pass filter with cut off frequencies of 10Hz. Jump height, power, rate of force development, and impulse was later derived utilizing Biopac interfaced AcqKnowledge software. A Vertec (Power Systems Inc. Knoxville, TN) was utilized to give the subjects a visual objective to jump and touch on the countermovement jump. Standing reach was recorded and utilized to measure the highest jump and touch achieved. Standing reach was measured by both hands overlapping at the middle finger when reached as far above the head as possible on flat feet, measured to the nearest half inch. Peak and mean power performance was estimated utilizing the Harman equations (Harman 1991). Force plate was calibrated daily utilizing a load of 300kg.

Vertical jumps were analyzed for rate of force development, which was defined as the amount of time which passed between the point when force was equivalent to the body weight of the subject until the peak force was attained. Impulse was calculated as the sum of the average force during concentric portion of the jumping movement multiplied by the ground contact time. Lastly, peak and mean power for vertical jump performance were calculated utilizing the Harman equation for each of the different jumps (Harman, Rosenstein, Frykman, Rosenstein, & Kraemer, 1991).

Maximal isotonic leg extensions

Subjects were tested on the Biodex machine for a right leg extension for their 1RM. Each repetition was measured utilizing national instruments CDAQ-9174 with NI 9215 ports (National Instruments Corporation, Austin, TX) receiving signals directly from the Biodex Humac System 3 unit (Biodex inc.). Each sets was performed on the isotonic testing mode in with the Humac Software (2009v 10.000.0047, Biodex Inc.). Signals were recorded with researcher created custom programs with Labview software (NI Labview 2013, National Instruments Corporation), which recorded torque, velocity, direction, and position sampled at a frequency of 1000Hz. Data was conditioned with Butterworth 10Hz cut off frequencies. All data was analyzed utilizing Microsoft Excel software for performance. The start of each repetition was defined as the point of which rotational velocity exceeded one degree per second, end of repetition was defined as the point of which the direction signal voltage changed to negative direction. Subjects warmed up with a set of 5 repetitions at 10lbs, 3 repetitions at 20lbs. and 2 repetitions at 40lbs. Following the warm-up, the subjects started perform single attempts at 50lbs-60lbs. Each set, the load was increased by 5-20lbs. depending on the subject's familiarization 1RM and the rated exertion on the previous set. There was a 60-90 second rest period between sets. Once the subject attained their 1RM they were done with their participation for the day and the final testing session was scheduled. Range of motion calibration data was saved from each subject and calibration of torque and velocity signals were performed weekly to show consistence of values which correlated at $r = 1.0$ ($P < .001$).

Leg extension power testing

For the final testing session, subjects performed 5 minutes of riding on the stationary bike at a self-selected cadence and resistance. After the warm up, subjects performed maximal velocity repetitions at 30%, 40%, 50%, 60%, 70%, 80%, and 90% of their 1 RM. Loads were set to the nearest tenth of a pound of resistance. Each set consisted of two maximal velocity repetitions. Each repetition was separated by a three second pause. Torque, direction, position, and velocity were recorded for each repetition (Schilling, Fry, Weiss, et al., 2005). Subjects were given two minute rest periods between each set. Greatest rep by peak velocity was utilized for later analysis from each different percentage load.

Following this test the fatiguing protocol was performed, subjects performed 15 repetitions at 70% of their 1RM with 3 second rest intervals for each rep. Cadence was maintained by researcher providing a verbal and visual signal. Subjects were instructed give their best effort on each repetition (Fry et al., 2013). Adjustments for limb weight and size were not made (Winter, Wells, & Orr, 1981). At the end of this test, the muscle biopsy on the subject's vastus lateralis was performed.

Muscle Biopsy

Muscle biopsies were taken from the vastus lateralis on the midpoint of the thigh, midway between the inguinal ligament and the patella on the right leg after the fatiguing protocol. Subjects were instructed to lie on the biopsy table face up. The area where the muscle biopsy was performed was cleaned with betadine and shaved to decrease infection risk and possible scaring. Subjects then had lidocaine injected to numb the area. After 5-10 minutes had elapsed, subjects were then tested to ensure that the lidocaine had taken effect. If it had not, subjects were either given more time, more lidocaine, or both. Once a subject was numb, a sterile

scalpel was used to make an approximately 1 cm incision that was roughly 2.5 cm deep. A Bergstrom biopsy needle was then inserted, utilizing the double chop method and suction utilized by Staron et al. (Staron et al., 1990) and a muscle sample was taken. Samples were then mounted on traganth gum and frozen with isopentane for later analysis of MHC characteristics.

The subject's leg area was then cleaned with isopropyl alcohol around the area of the biopsy, but not directly in the incision site. Skin adhesive was applied directly above and below the incision and steri strips were used to close the wound. A sterile bandage was then placed over the incision site and cotton gauze and a pressure wrap bandage were placed over the bandage, to help keep the area clean of any possible debris. The leg was then bound with flex wrap. Subjects were instructed to keep the incision dry and clean and to return in 24 hours so that the wound area could be examined and re-bandaged. They were given contact information of the staff in case a situation occurred with the incision and they needed medical guidance. No such situations occurred with any of the subjects.

Myosin Heavy Chain Analysis

Sample Extraction

Muscle samples were extracted utilizing a cryostat (MC1800 by Leica Inc.) taking 8-12 40um serial cross sections into one microcentrifuge tube. Tubes were then put in solution with 500ul of a lab recipe extraction buffer (A. C. Fry, 2004) (100ml total of 62.5mm Tris HCl (Fisher Scientific), 5 ml beta-mercaptoethanol (Pierce Scientific), 2.3g SDS (Fisher), glycerol (National Diagnostics) and were heated for 10 minutes at 60˚C. Samples were then vortexed and three drops of glycerol were added (National Diagnostics). Afterwards, samples were stored in a -80˚C freezer for later analysis.

Myosin Heavy Chain Separating

Samples were run through a large format 4-8% gradient gel (using a Gradient Maker by Hoefer Inc.) utilizing a lab recipe for gels made of (protogel (National Diagnostics), ultra-pure DI water, glycerol, 1.5M Tris (Aldrich Scientific)) stacking gel of 4% (.5M Tris). Fresh running buffer was made daily with a lab recipe (glycine (Fisher), Tris, SDS) and approximately 5 liters will be utilized each time. Running buffer was pH controlled at 8.8 for each run at room temperature. After samples were loaded, the gels were run at a constant voltage of 120V for 18-24 hours (Thermo Scientific EC250-90 power source), with a constant water cooling system maintaining case temperature at 19˚C (Fisher Isotemp 3016). After the leading edge of the gel had run off the bottom of the gel, the run was stopped and the gel was extracted to be washed in a commassie brilliant blue staining solution (methanol (Sigma Aldrich), glacial acetic acid (Fisher), ultra-pure Di water, commassie brilliant blue R (ICN Biomedical)) for half an hour to forty five minutes. Then it was washed in a lab recipe stripping buffer (water, methanol, glacial acetic acid) for at least one hour. Gels were imaged utilizing a protein simple laboratory camera system (model U4000, firmware 27 by Alta Inc.). The bands on each gel were analyzed utilizing Fluorchem 2 software (Protein Simple alpha view Fluorchem 2 version 3.4.0.0). All bands were analyzed at least three times for reliability between the analyses.

Data analysis

Demographic data for the subjects was analyzed utilizing a simple ANOVA, if there was a significant difference amongst the groups; post hoc analyses would utilize Bonferroni corrections. One-way ANOVAs were utilized to compare MHC composition among groups. Subject data was analyzed to compare muscle MHC characteristics to performance measures that were obtained.

Task	Variable	Variable	Variable	Variable
Leg Extension	Power Curve	Linear velocity slope	Linear velocity intercept	Fatigue Index
Vertical Jump	Force	Rate of force development	Jump height	Impulse
Anthropometrics	Body weight	Body fat	Thigh circumference	Thigh mass
MHC	Type I	Type IIA	Type IIX	

Figure 7 Variables recorded from the study to be utilized for correlation to one another

  A correlation matrix of the above variables was performed for each variable to find where high covariance naturally occurs as a means to remove any unnecessary, extra variables. Vertical jump data was also analyzed relative to cross sectional area and to lean body mass for power and force measurements. After significant variables (alpha set at .05) were identified utilizing a correlation matrix with the heavy chain characteristics, significant correlation variables were then analyzed utilizing a regression or if there were multiple significant relationships data was analyzed utilizing a forward stepwise multiple regression.

Chapter 4 - Results

This dissertation analyzed plyometric and isotonic performance and its relationship to muscle MHC composition. Each of the hypotheses proposed were answered utilizing the methodology outlined in chapter 3 and the results of each portion of the experiment were statistically analyzed from the quantified data and are reviewed below.

Subject Attrition

Of 46 subjects who enrolled initially in the study, 42 completed the study. The subjects who failed to complete the study were unable to do so due to personal reasons or inability to match schedules, not contraindications from testing.

Demographics

Averages below are representative of subject participants, divided by sample group (Table 1, 2):

Table 1 – Demographics - All units are mean ± standard deviation

Group	N	Age (years)	Height (m)	Weight (kg)	Body Fat (%)
Aerobic	12	22.5±2.9	1.79±.08	70.1±5.7	10.9±4.1
Resistance	13	22.0±1.8	1.75±.04	80.8±11.5	14.7±5.9
Recreational	11	22.0±2.6	1.81±.08	84.2±17	17.3±7.0
Sedentary	6	24.0±7.6	1.81±.09	81.2±14.7	23.2±6.2*
Total	42	22.4±3.5	1.78±.07	78.7±13.3	15.5±6.9

* denotes significance of $p < .05$

Table 2 - Leg Size Information - All units are mean ± standard deviation

Group	Lean Mass (kg)	Lean Mass Right Leg (kg)	Quad CSA (cm^2)	Thigh CSA (cm^2)
Aerobic	58.7±5.0	9.7±.8	73.4±8.2	141.3±14.9
Resistance	65.1±7.0	10.9±1.4	86.2±8.6*	165.9±16.4*
Recreational	64.8±9.3	10.7±1.4	81.5±10.1	157.7±19.0
Sedentary	57.9±7.2	10.0±1.7	67.0±12.2	131.6±21.4
Total	62.2±7.7	10.4±1.4	78.6±11.5	151.8±21.4

* denotes significance of $p < .05$

In general, there were only significant differences between groups with body fat percentage ($p = .001$), and quad and thigh cross sectional area (CSA) ($p = .001$, and $p = .001$ respectively). The sedentary group had statistically significantly greater amounts of body fat than the other groups, whereas the resistance trained group had significantly larger quad and thigh size than the other groups (utilizing Bonferroni corrections for post hoc tests).

Leg Extension Maximum –

Table 3 - Maximum Leg Extension - All units are mean ± standard dev.

Group	Maximum Leg Extension (kg)	Max Leg Extension Relative to CSA
Aerobic	31.5±6.5	.94±.15
Resistance	36.5±7.3	.93±.15
Recreational	37.4±10.2	1.00±.21
Sedentary	29.9±4.2	.99±.13
Total	34.4±8.0	.96±.16

There was no significant difference seen in leg extension strength between each of the groups or when controlled for cross sectional area (Table 3).

Muscle Myosin Heavy Chain –

Table 4 - Myosin Heavy Chain data for all of the subjects across groups and total - All units are mean ± standard dev.

Group	IIX (%)	IIA (%)	I (%)	Total Fast Twitch (%)
Aerobic	10.3±7.1	47.3±8.8	42.2±9.9	59.4±10.8
Resistance	9.9±9.8	54.9±9.1	35.4±9.1	64.8±9.0
Recreational	14.0±13.3	49.9±11.2	36.4±13.1	62.0±13.5
Sedentary	31.1±17.1*	39.5±11.6	29.4±12.2	70.6±10.9
Total	13.8±12.9	49.5±10.3	36.8±11.3	63.3±11.3

* denotes significance of $p < .05$
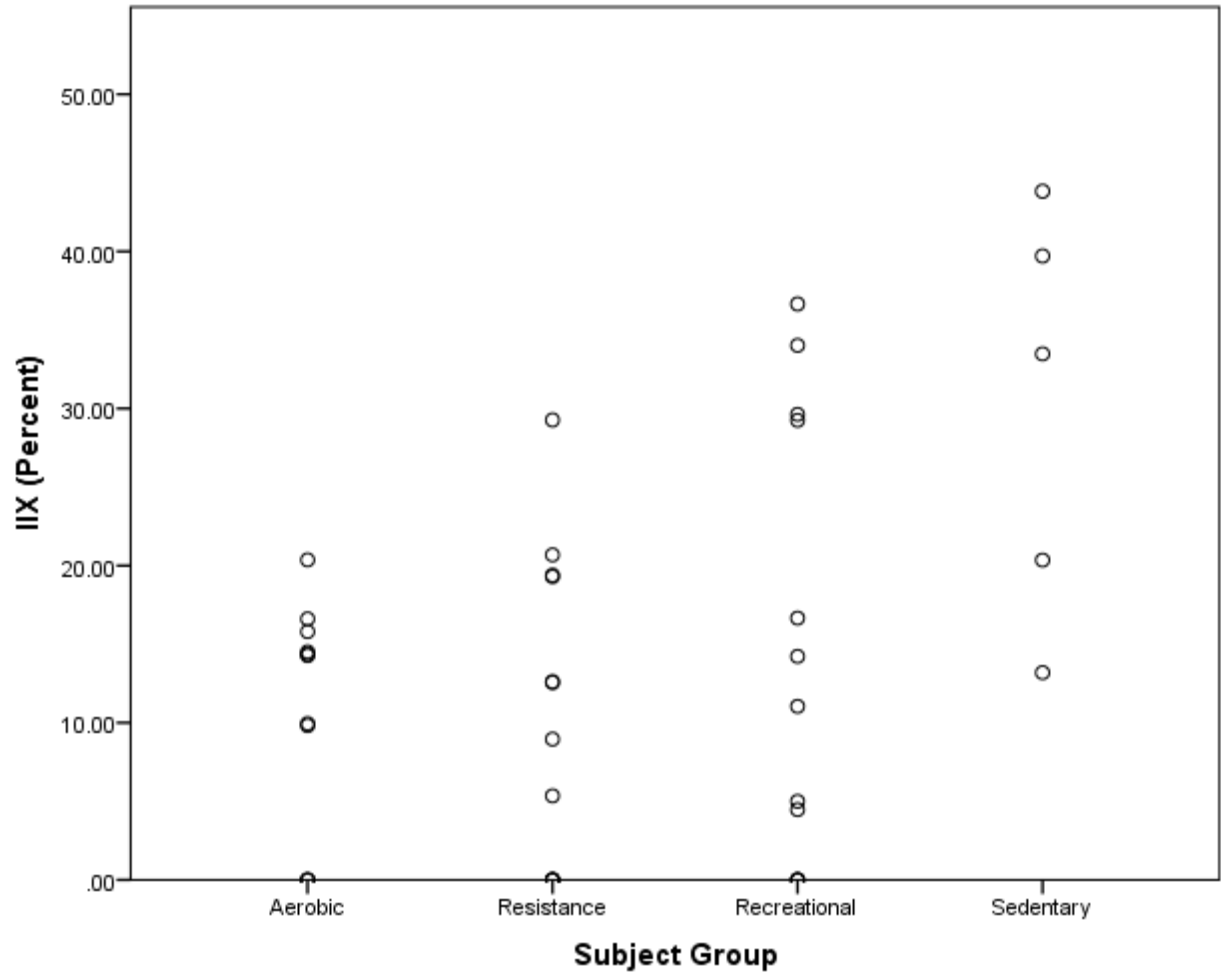
Figure 8 IIX MHC content across subject groups
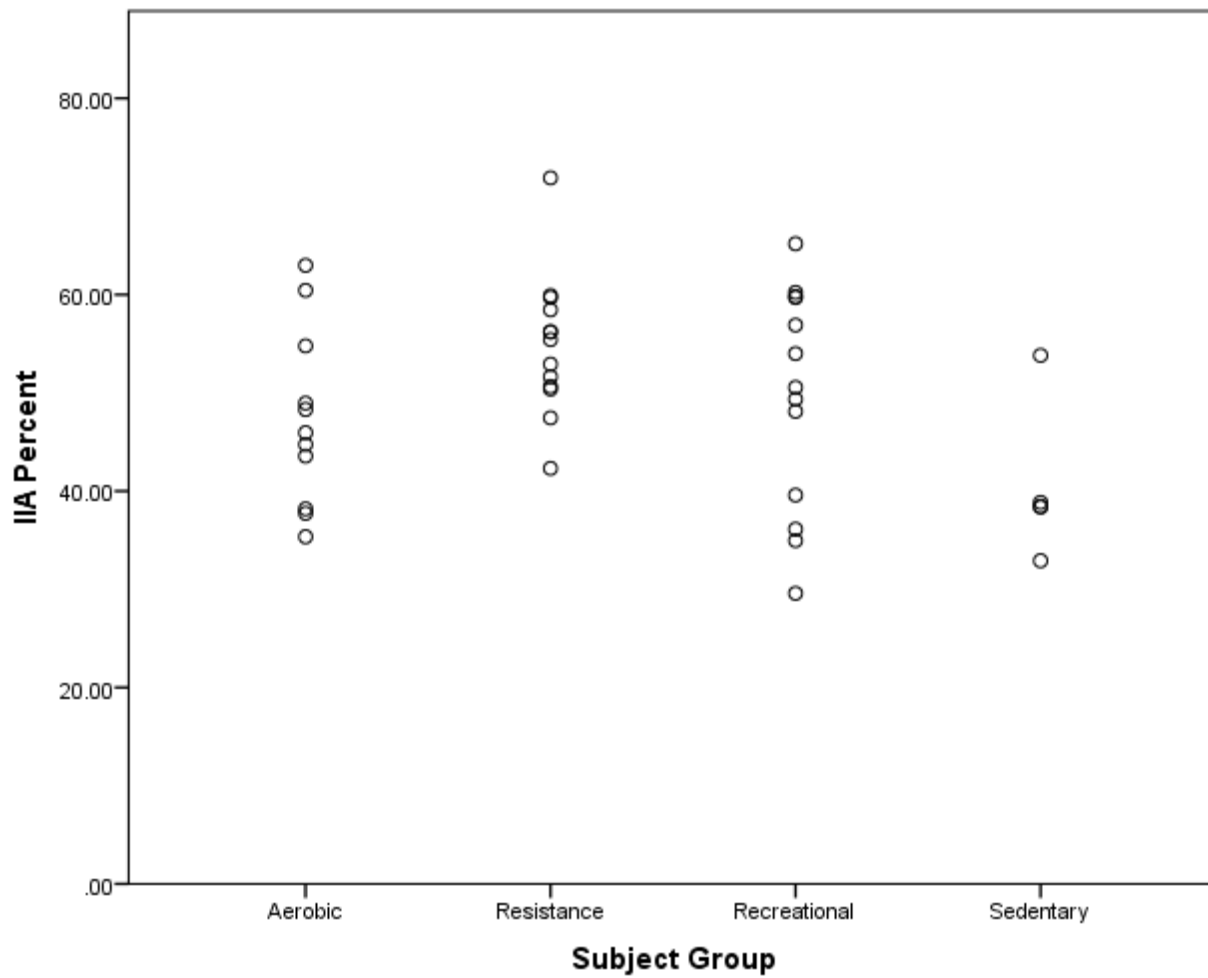
Figure 9 IIA MHC content across subject groups
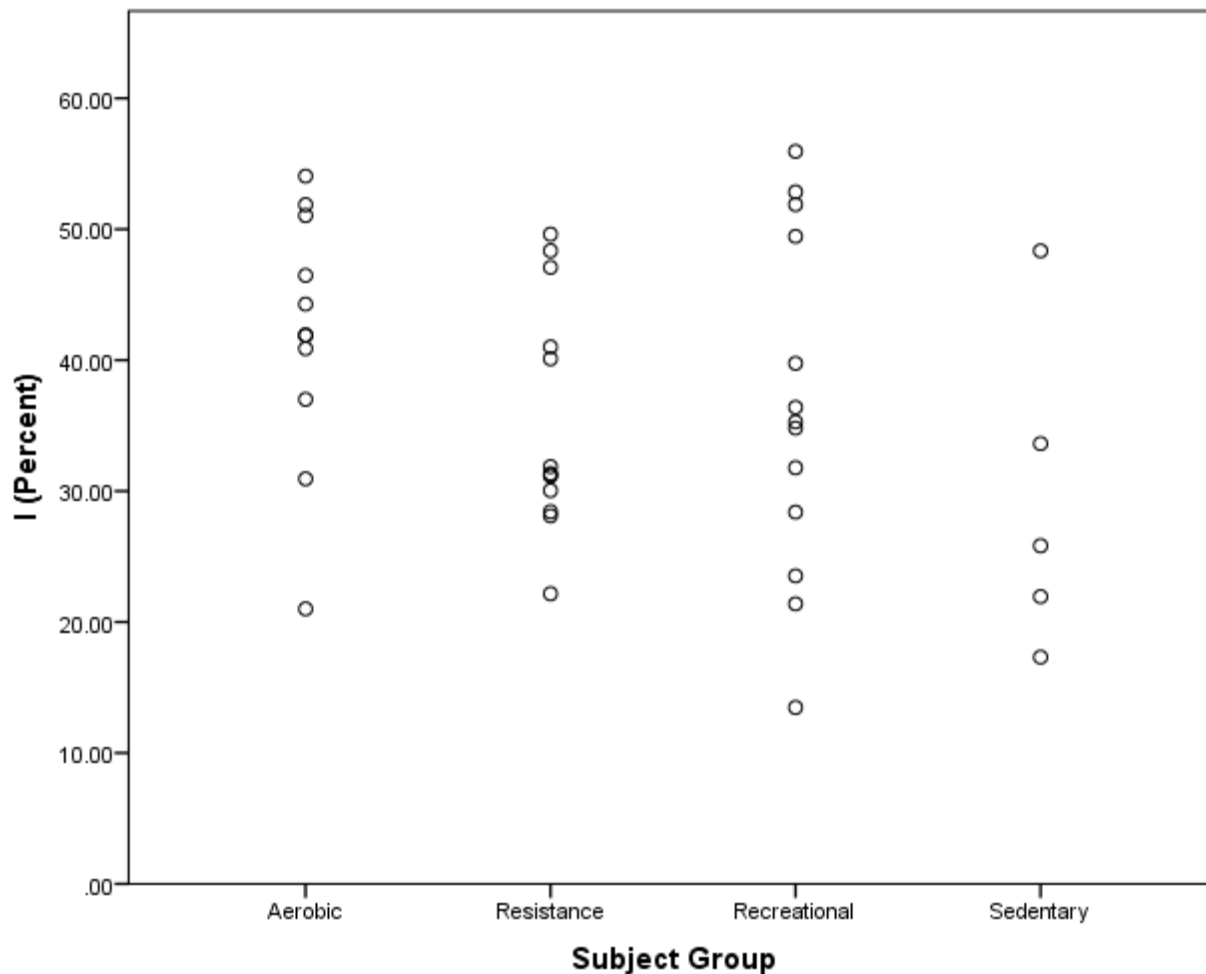
Figure 10 I MHC content across subject groups

No significant differences were found in the type IIA or I muscle MHC between any of

the groups. There were significant differences in IIX MHC between groups (Table 4) (Figure 8,

9, 10). A post hoc analysis utilizing Bonferroni corrections, found that the sedentary group had a

significantly greater amount of IIX fiber than any other group (p> .05).

Correlations of muscle MHC composition to one another results were as follows:

Table 5 - Correlations between each MHC Isoform

MHC	IIX	IIA	I
IIX	1	-0.545*	-0.646*
IIA		1	-0.285
I			1

* denotes significance of p < .05

Hypothesis 1

Vertical jump impulse, rate of force development, jump height, and power will have a positive relationship to vastus lateralis muscle fast MHC composition (IIA and IIX separately as well), specifically when variables are calculated relative to body mass and cross sectional area of the muscle.

Jump Height

Subjects performed each variation of the jump in the study. Most subjects performed two maximal repetitions of each jump. For statistical purposes, maximum height jump data was used for statistical analysis. Vertical jump performance data is listed in table 6.

Table 6 - Jump Height Performance - All units are mean ± standard dev.

Group	CM Jump (m)	No arm Jump (m)	Depth Jump (m)
Aerobic	0.699±.036*	0.659±.037*	0.703±.031
Resistance	0.746±.046^{+}	0.705±.042^{+}	0.748±.045
Recreational	0.713±.040	0.677±.038	0.717±.044
Sedentary	0.681±.050	0.633±.027	0.678±.053
Total	0.715±.047	0.674±.044	0.717±.047

* denotes significant below group average
+ Denotes significant above group average (p < .05)

Table 7- MHC Correlation to Jump Height Performance

	Vertical Jump Height					
	Counter movement		No Arms		Depth Jump	
MHC	r	p	r	p	r	p
IIX	0.238	0.129	0.111	0.486	0.164	0.299
IIA	0.089	0.574	0.129	0.415	0.021	0.895
I	-0.355*	0.021	-0.249	0.111	-0.209	0.184

* denotes significance of $p < .05$
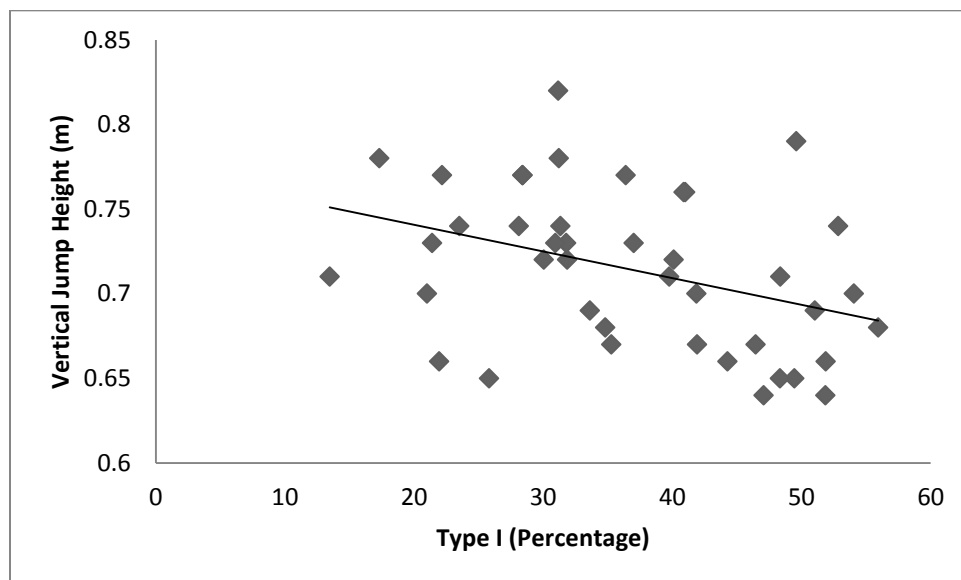
Figure 11 - Scatterplot for Type I MHC correlation to Countermovement Jump Height

A one way ANOVA showed significant differences in vertical jump height between each of the testing groups. With Bonferroni post hoc analysis, the aerobically trained group performed significantly below average in the counter movement jump and no arm jump, whereas the resistance trained group performed above average ($p<.05$). There was a significant negative

relationship between vertical jump height (only counter movement jump trial) and type I fiber

concentration (r = -.355, p = .021) (Table 7)(Figure 11).

Force

Force produced during the jumping phase was calculated through the point of which

force on the concentric portion of the jump was equal to body weight. Peak and mean force on

only the counter movement jump was significantly related to type IIX MHC composition (r=

.354, p = .021 and r = .356, p = .021 respectively). There were no other significant relationships

to force and MHC (Table 8, 9) (Figure 12, 13).

Table 8 - Peak force relative to Body Weight (N/kg)

MHC	Counter movement		No Arms		Depth Jump	
	r	p	r	p	r	p
IIX	0.354*	0.021	0.228	0.146	0.238	0.129
IIA	-0.258	0.099	-0.096	0.545	-0.153	0.333
I	-0.173	0.272	-0.167	0.289	-0.123	0.437

* denotes significance of $p < .05$
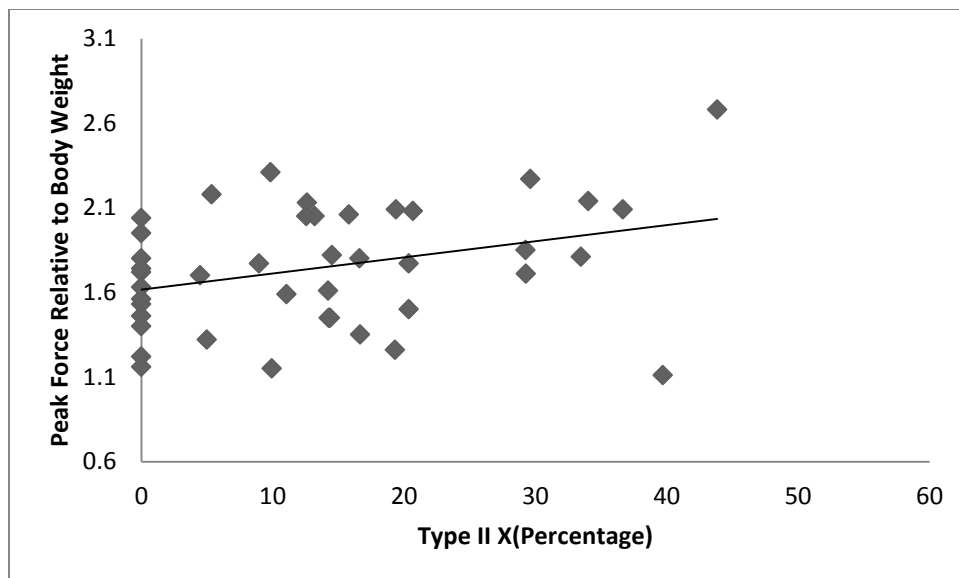
Figure 12 - Scatterplot of MHC IIX correlation to Peak Force Relative to Body Weight

Table 9 - Mean force relative to Body Weight (N/kg)

MHC	Counter movement		No Arms		Depth Jump	
	r	p	r	p	r	p
IIX	0.356*	0.021	0.144	0.361	0.129	0.414
IIA	-0.177	0.261	0.072	0.651	0.021	0.897
I	-0.241	0.124	-0.209	0.183	-0.154	0.332

* denotes significance of $p < .05$
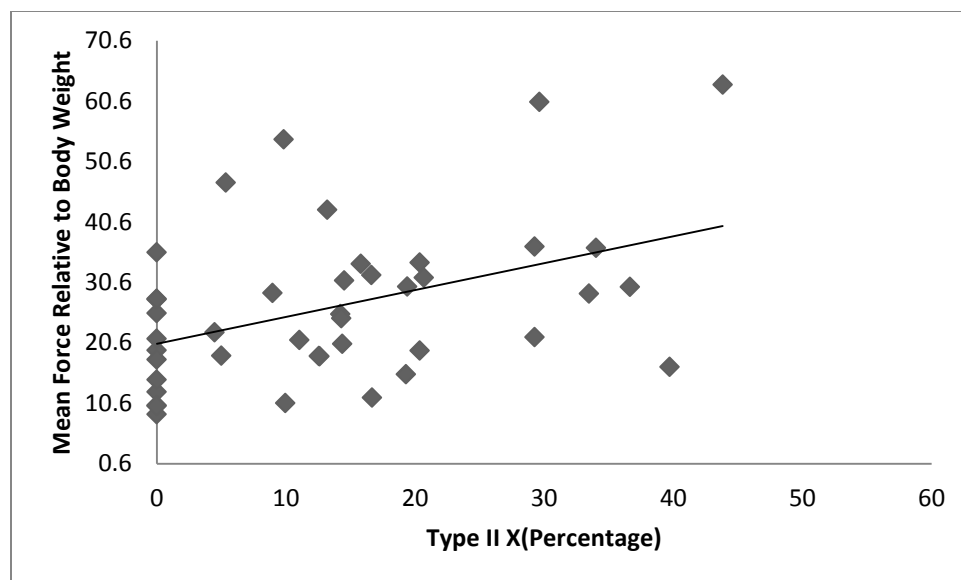
Figure 13-Scatterplot of IIX MHC correlation to Mean Force Relative to Bodyweight

Jump Power

Results showed a significant, positive relationship for vertical jump power between IIX MHC across all jump types. Peak power in each of the jumps relative to cross sectional area of the thigh had significant relationships (counter movement jump r = .449 p=.003, no arm jump r = .429 p=.005, depth jump r = .437 p=.004). The same results were seen in mean power in each of the jump trials relative to cross sectional area of the thigh (counter movement jump r = .483 p = .001, no arm jump r = .434 p = .004, depth jump r = .459 p = .002) (Table 10, 11).

There were significant negative relationships between IIA MHC composition and power performance. Peak power in each of the jumps to cross sectional area of the thigh had significant relationships (counter movement jump r = -.411 p=007, no arm jump r = -.407 p=008, depth jump r = -.422 p=005). The same results were seen with mean power in each of the jumps relative to cross sectional area of the thigh (counter movement jump r = -.341 p = .027, no arm jump r = -.318 p = .040, depth jump r = -.373 p = .015) (Table 10, 11) (Figure 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25).

No significant relationship was seen between type I fiber and vertical jump performance across any of the force or power metrics in any of the jumps (Table 8, 9, 10, 11).

Table 10 – Correlation of peak power relative to cross sectional area of the thigh to MHC content

	Peak Power					
	Counter Movement		No Arms		Depth Jump	
MHC	**r**	**p**	**r**	**p**	**r**	**p**
IIX	0.446*	0.003	0.429*	0.005	0.437*	0.004
IIA	-0.411*	0.007	-0.407*	0.008	-0.422*	0.005
I	-0.143	0.368	-0.128	0.421	-0.122	0.441

* denotes significance of p < .05
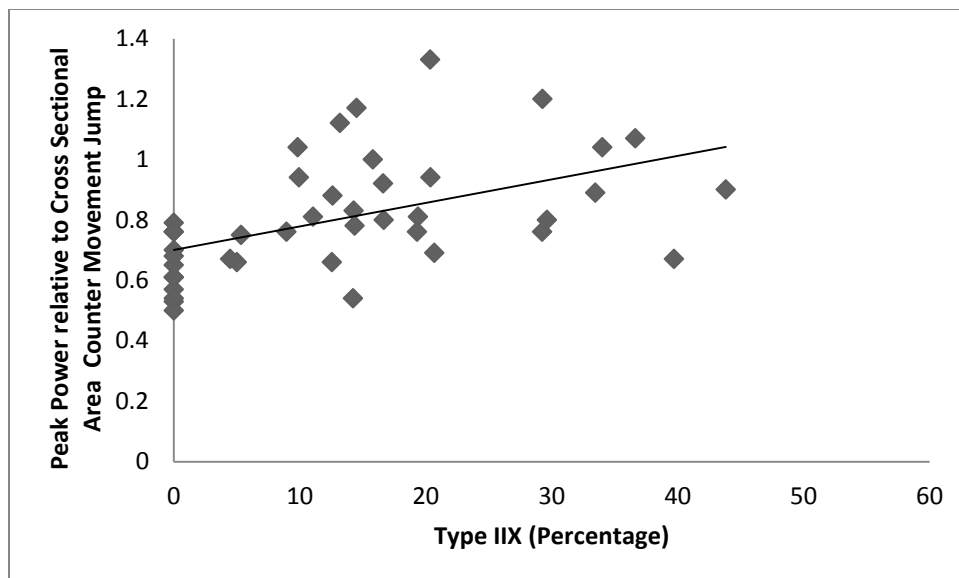
Figure 14 Scatterplot of IIX MHC content to Peak Power relative to cross sectional are in the countermovement jump
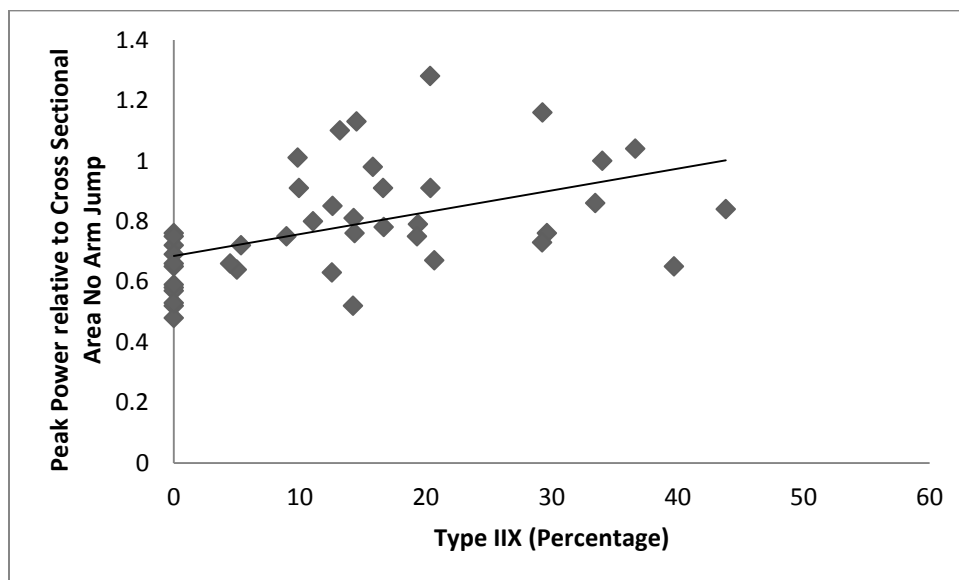
Figure 15 Scatterplot of IIX MHC correlation to Peak Power relative to Cross Sectional Area No Arm Jump
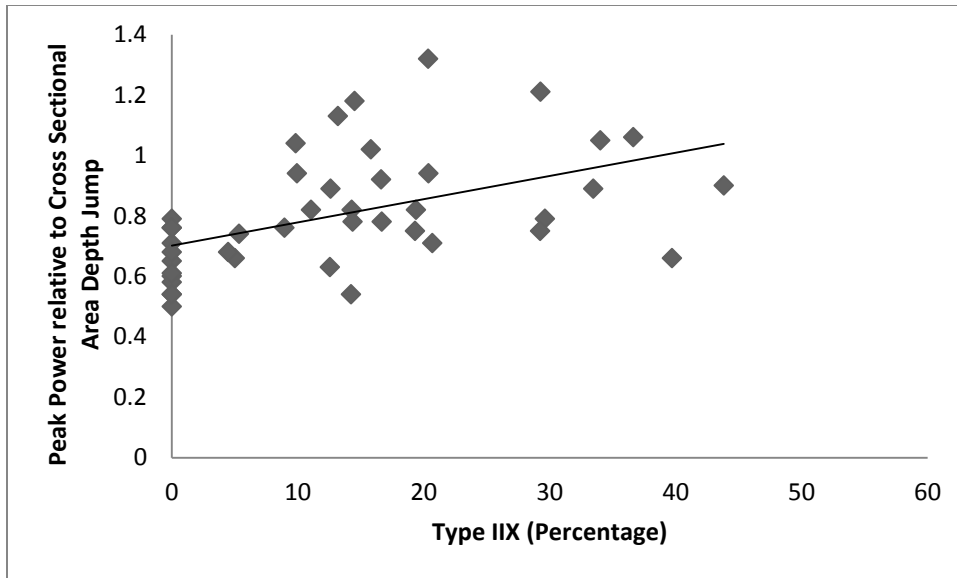
Figure 16 Scatterplot IIX MHC correlation to Peak Power relative to Cross Sectional Area Depth Jump
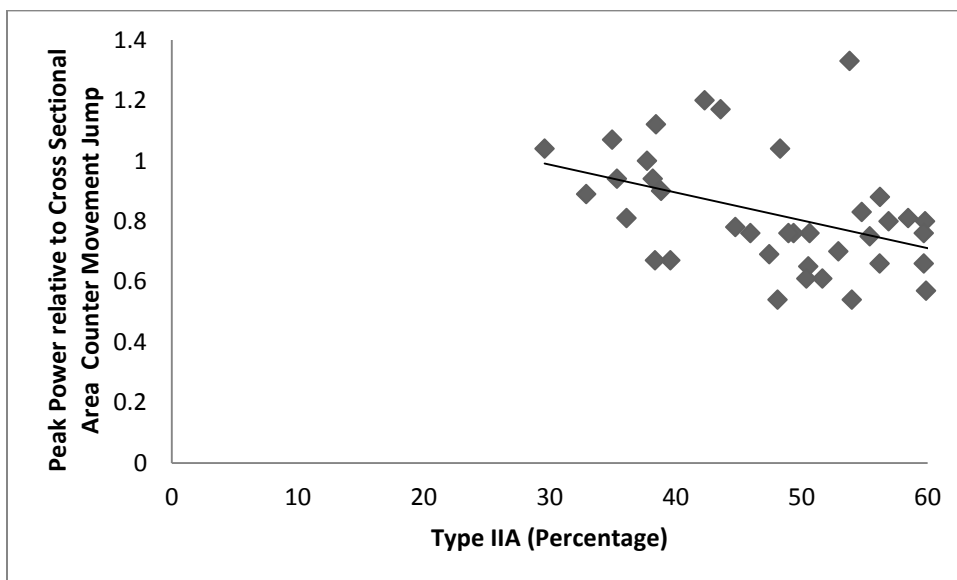
Figure 17- Scatterplot of IIA MHC correlation to Peak Power relative to Cross Sectional Area Counter Movement Jump
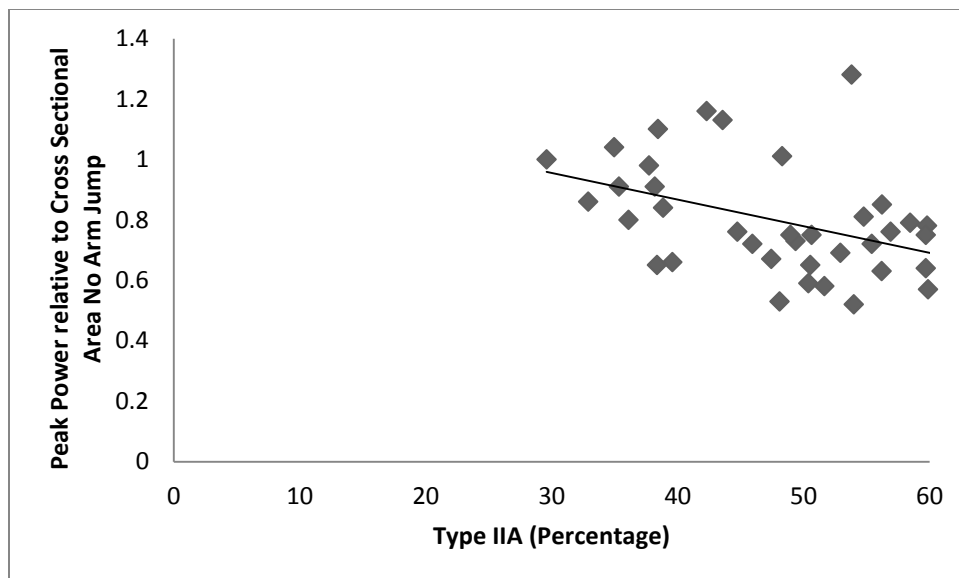
Figure 18 Scatterplot of IIA MHC correlation to Peak Power relative to Cross Sectional Area No Arm Jump
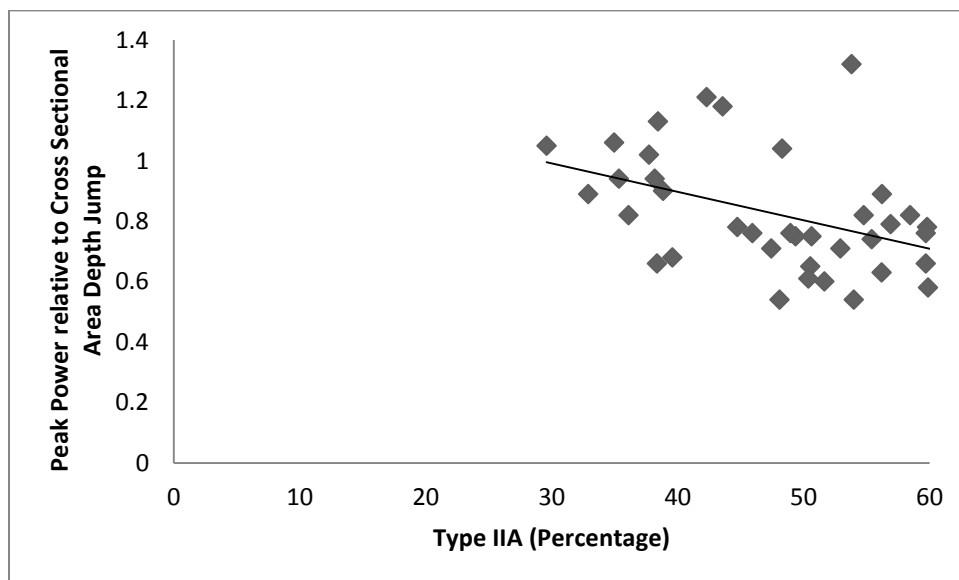
Figure 19 Scatterplot of IIA MHC correlation to Peak Power relative to Cross Sectional Area Depth Jump

Table 11 - Correlation of mean power relative to cross sectional area of the thigh to MHC content

Mean Power

MHC	Counter Movement		No Arms		Depth Jump	
	r	p	r	p	r	P
IIX	0.483*	0.001	0.434*	0.004	0.459*	0.002
IIA	-0.341*	0.027	-0.308*	0.04	-0.373*	0.015
I	-0.247	0.115	-0.213	0.176	-0.191	0.227

* denotes significance of p < .05
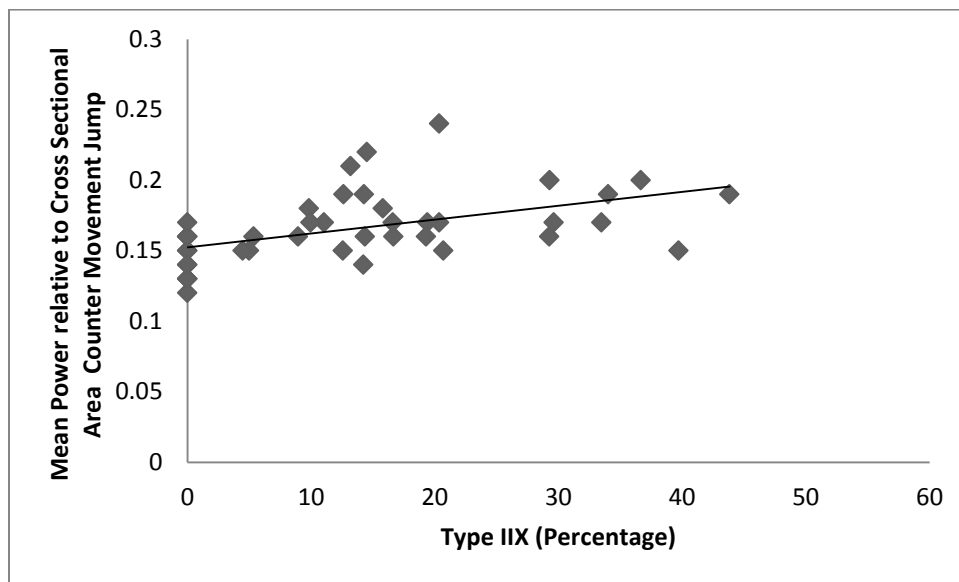
Figure 20 Scatterplot of IIX MHC correlation to Mean Power relative to Cross Sectional Area Counter Movement Jump
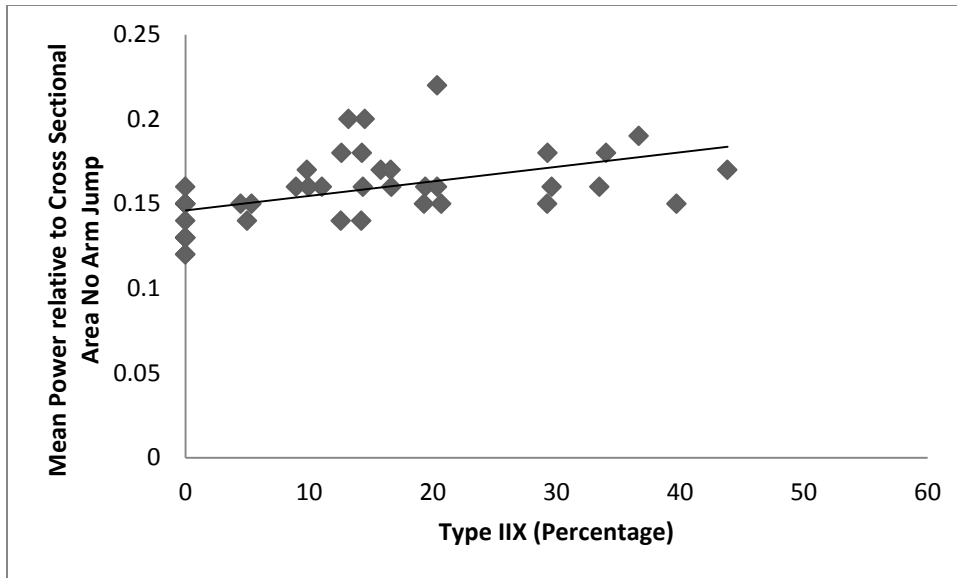
Figure 21 Scatterplot of IIX MHC correlation to Mean Power relative to Cross Sectional Area No Arm Jump
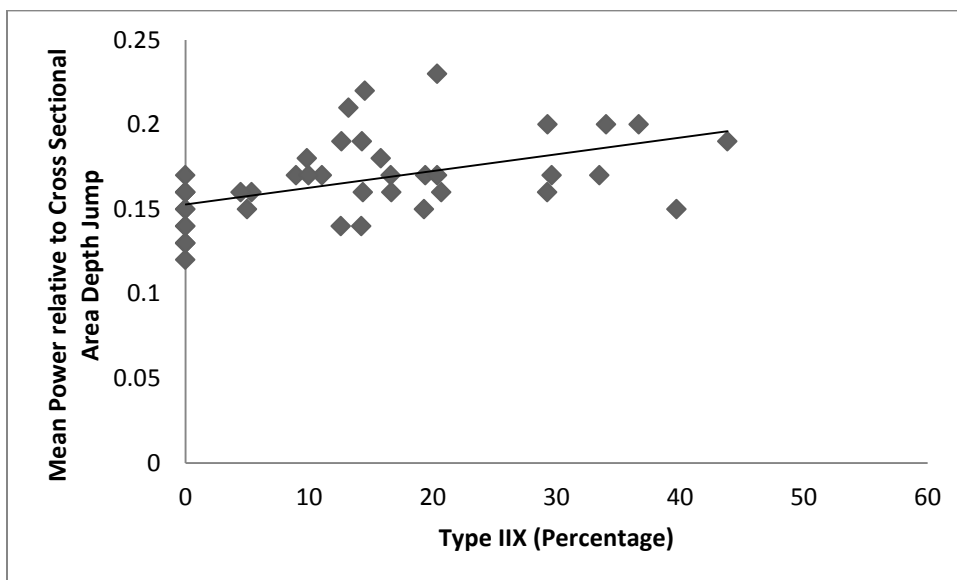
Figure 22 Scatterplot of IIX MHC correlation to Mean Power relative to Cross Sectional Area Depth Jump
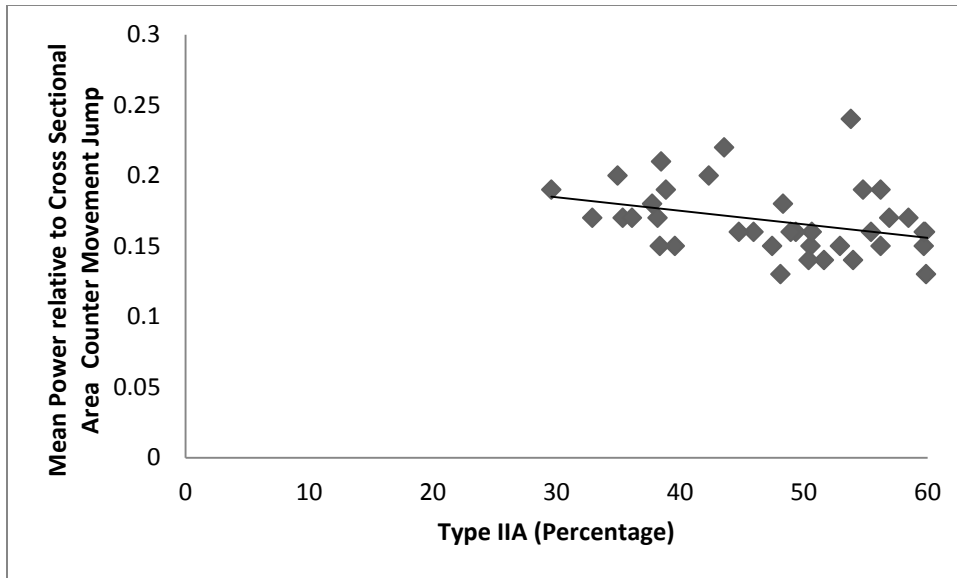
Figure 23 Scatterplot of IIA MHC correlation to Mean Power relative to Cross Sectional Area Counter Movement Jump
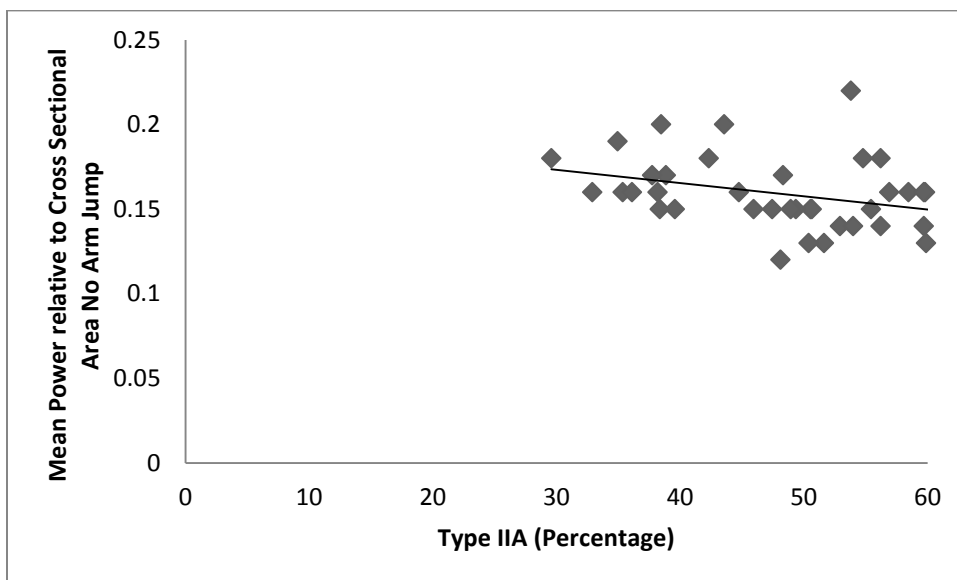
Figure 24 Scatterplot of IIA MHC correlation to Mean Power relative to Cross Sectional Area No Arm Jump
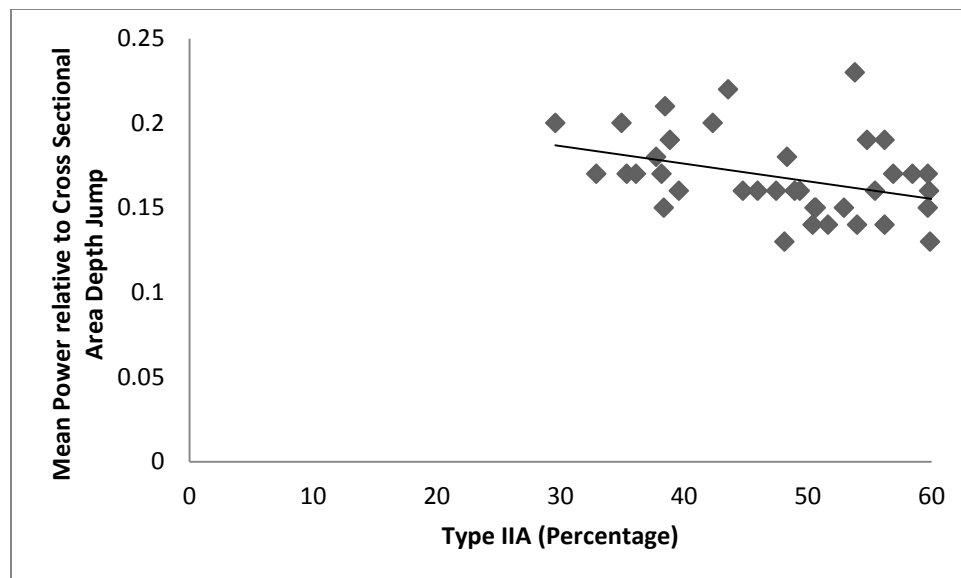
Figure 25 Scatterplot of IIA MHC correlation to Mean Power relative to Cross Sectional Area Depth Jump

Rate of Force of Development –

No significant correlations were seen between rate of force development across any of the jump conditions and MHC, with the exception of a positive relationship between IIX fiber concentration and rate of force development relative to cross sectional area in the counter movement jump trial ($r = .329$, $p = .033$) (Table 12) (Figure 26).

Table 12 - Rate of force development Relative to Cross Sectional Area correlation to MHC

MHC	Counter movement		No Arms		Depth Jump	
	r	p	r	p	r	p
IIX	0.329*	0.033	0.119	0.454	0.1	0.53
IIA	-0.205	0.192	0.087	0.584	-0.242	0.123
I	-0.192	0.224	-0.199	0.206	0.104	0.512

* denotes significance of $p < .05$

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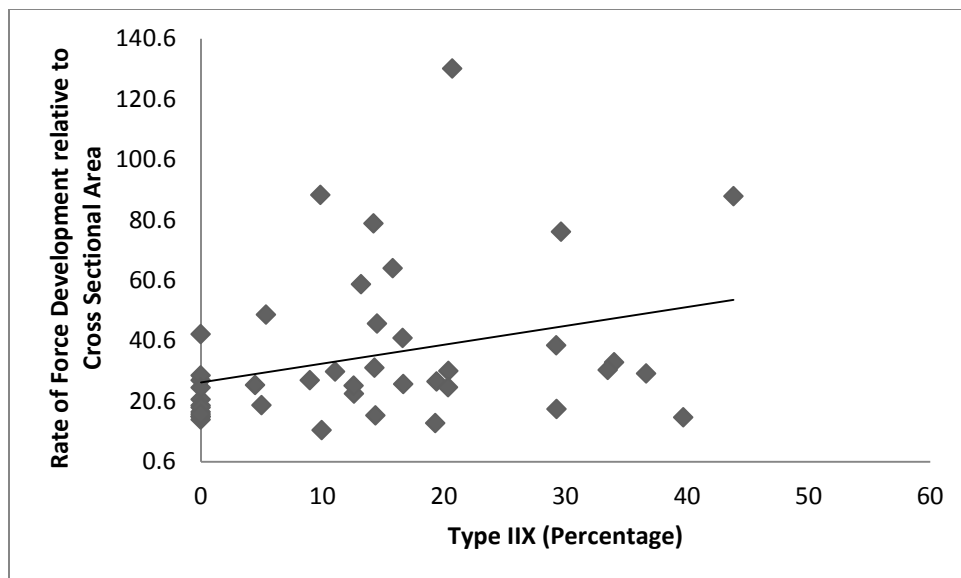
Figure 26 Scatterplot of IIX MHC correlation to Rate of Force Development relative to Cross Sectional Area

Impulse

No significant correlations were seen between impulse and MHC across any of the jump conditions. Impulse (N*sec.) for the counter movement jump was 1829.4 ± 917.4, no arm jump was 1599.7 ± 911.5, and the depth jump was 3073.5 ± 2386.6.

Hypothesis 2

Isotonic leg extension velocity-load linear relationship will be related to muscle MHC with fast fiber (specifically IIA) positively and negatively correlated, respectively, to the intercept of the line and the slope of the line.

Velocity Load

After leg extension data was broken down by individual repetitions, the best repetition of each percentage set was utilized to create a linear equation for leg extension velocity performance at each percentage of their individual 1RM. Figure 27 displays one of the subjects graphs (Figure 27). The average intercept for peak velocity was 415.1 deg/sec $\pm$ 47.7 with a

slope of -2.13 ± .50 deg/sec by increase in percentage of max. Mean intercept for average

velocity 217.7 ± 16.2 deg/sec with a slope of -1.03 ± .26 deg/sec by increase in percentage of

max (Table 13). There were no significant differences between of the groups.

Table 13 - Velocity load best fit lines - All units are mean ± standard dev.

Velocity Load Best Fit Lines	Slope ((deg/sec)/percentage of 1RM)		Intercept ((deg/sec)/percentage of 1RM)	
Group	Max Velocity	Mean Velocity	Max Velocity	Mean Velocity
Aerobic	-2.022±.348	-1.037±.270	397.49±32.97	214.01±15.53
Resistance	-2.251±.628	-1.042±.321	436.06±50.16	222.32±13.89
Recreational	-2.154±.587	-1.017±.195	411.29±58.82	214.64±16.97
Sedentary	-2.026±.250	-0.9934±.291	411.67±37.10	220.36±21.81
Total	-2.128±.499	-1.027±.264	415.07±47.70	217.65±16.24
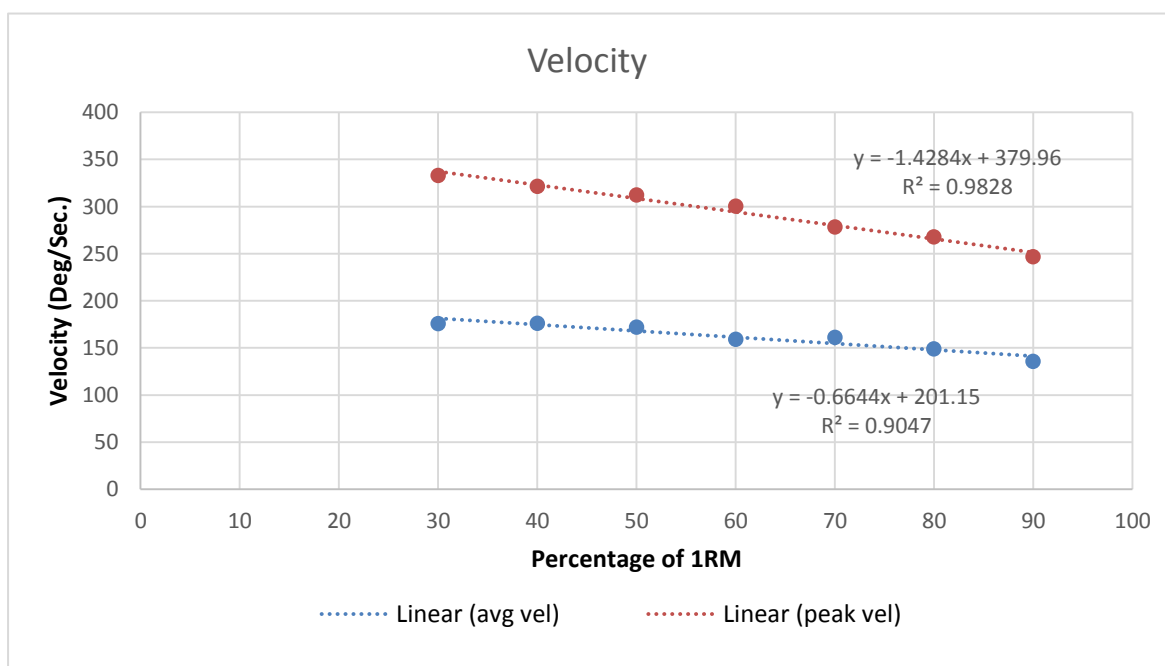
Figure 27 - Velocity Load relationship graph for one of the subjects in the study

Intercept

The estimated velocity attainable in an unloaded condition for the muscle was estimated utilizing linear regression for both peak and mean velocity data. Intercepts were correlated with MHC (Table 14) (Figure 28, 29); peak velocity intercept related to IIA concentration r = .380 (f = 6.752, p = .013, df = 1, 40) creating an intercept equation of:

Intercept = 1.759 (percent IIA) + 327.950 (deg./sec)

Equation 1 - Peak Velocity Intercept to MHC

Mean velocity intercept related to IIA concentration r = .410 (f = 8.106, p = .007, df = 1, 40) creating an intercept equation of:

Intercept = .647 (percent IIA) + 185.611 (deg./sec)

Equation 2 - Mean Velocity Intercept to MHC

Table 14 Velocity Load Intercept correlation to MHC

	Peak Intercept		Mean Intercept	
MHC	**r**	**p**	**r**	**p**
IIX	-0.228	0.147	-0.209	0.185
IIA	0.380*	0.013	0.410*	0.007
I	-0.085	0.592	-0.141	0.373

* denotes significance of p < .05
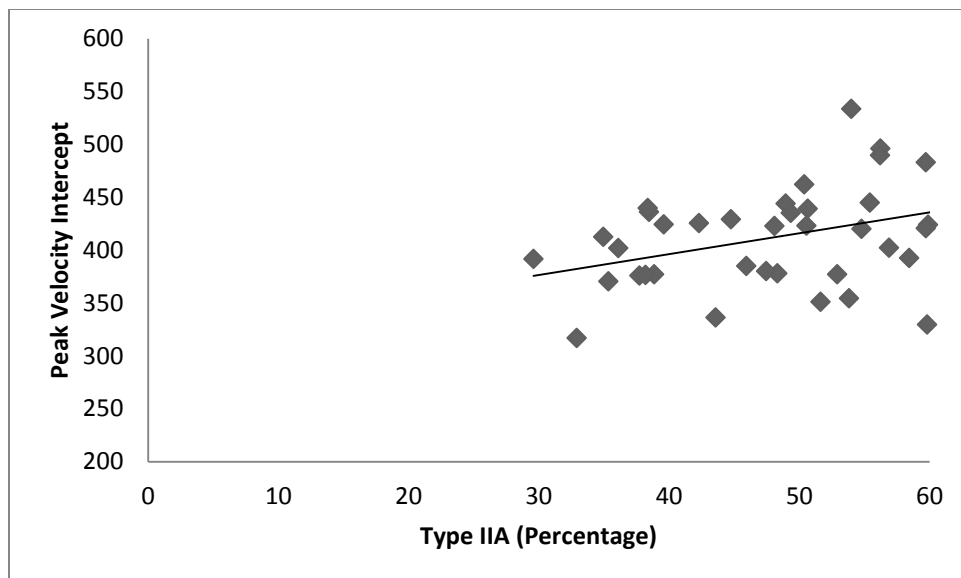
Figure 28 Scatterplot of IIA MHC correlation to Peak Velocity Intercept
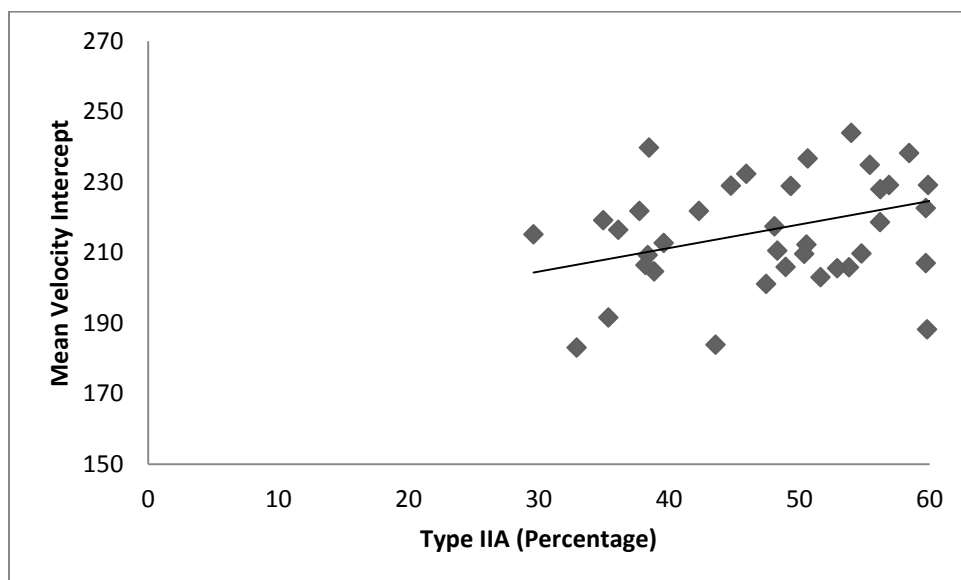
Figure 29 Scatterplot of IIA MHC correlation to Mean Velocity Intercept

Slope

The slope of best fit regression line for velocity attained along the differing percentage of the subject's 1 RM for the leg extension was compared to determine a relationship with type IIA and IIX fibers. A relationship was seen between the peak and mean velocity slopes and IIA fiber

concentration (r = -.480 p = .001, and r=-.377 p=.014, respectively). No other significant relationships were seen between other MHC and slope (Table 15) (Figure 30, 31).

Table 15 - Velocity load slope correlation to MHC

	Peak Slope		Mean slope	
MHC	**r**	**p**	**r**	**p**
IIX	0.221	0.159	0.183	0.247
IIA	-0.480*	0.001	-0.377*	0.014
I	0.185	0.242	0.143	0.367

* denotes significance of $p < .05$
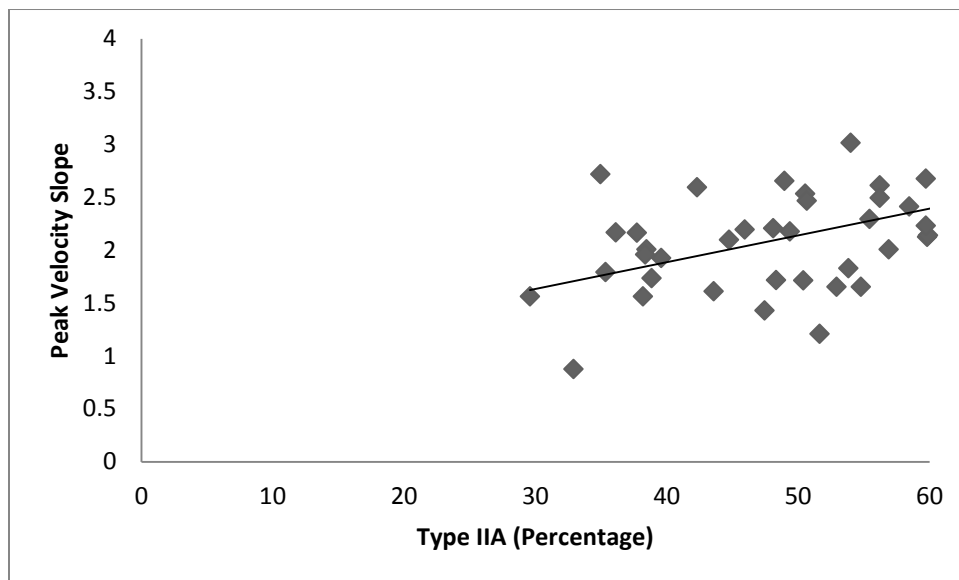
Figure 30 Scatterplot for IIA MHC correlation to Peak Velocity Slope
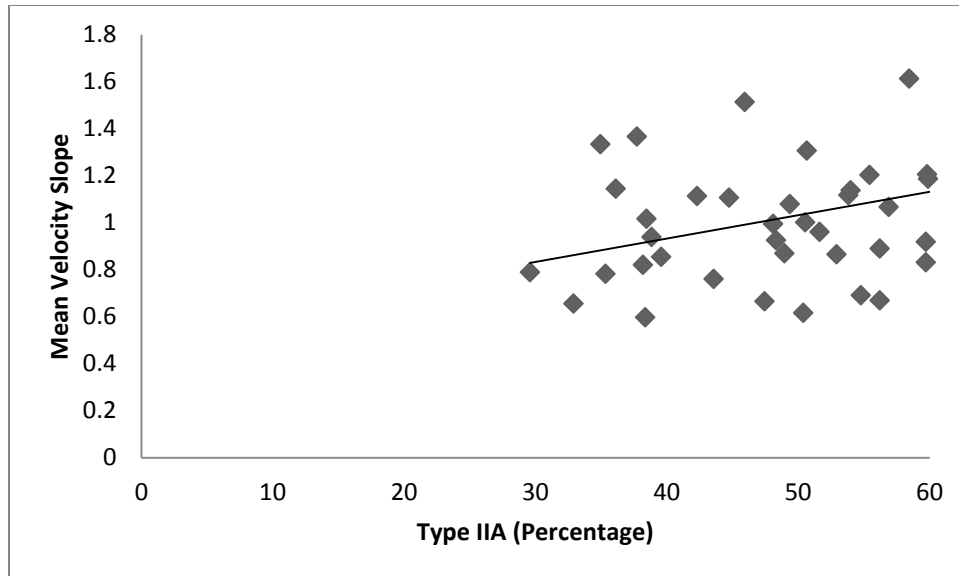
Figure 31 Scatterplot for IIA MHC correlation to Mean Velocity Slope

Significant relationships were seen with slope and peak velocity (f=11.951, p=.001, df =

1, 40) with an r = -.480:

Slope equation = -.023 (percent IIA) -.977

Equation 3 - Peak Velocity Slope to MHC equation

The relationship of the slope to mean velocity was significant r=.377 (f=6.607, p=.014 df

1, 40) with an equation of:

Predicted slope = -.01 (percent IIA) -.550).

Equation 4 - Mean Velocity Slope to MHC equation

Hypothesis 3

Isotonic leg extension power-load curves will be related to % MHC IIA, specifically the

point of which the peak occurs at; earlier peaks relative to percentage of the total load will be

negatively related to fast fiber composition.

After participation in speed trials, peak and mean power performance for each repetition was calculated, and a best fit, 2nd order polynomial line was created with an intercept of 0. Slope equations for these lines were then solved for each subject based upon where the anticipated peak performance for both power measures would occur as a percentage of 1 RM (Figure 32). Results for each group and subjects are listed in (Table 16) (Figure 33, 34, 35). Values were then compared to the MHC composition for each subject. There were no differences between groups with predicted power values.

Table 16 - Predicted max power percentage - All units are mean ± standard dev.

	Predicted Peak	
Group	**Max Power (%)**	**Mean Power (%)**
Aerobic	62.0±3.6	61.7±5.6
Resistance	62.6±6.0	62.0±4.1
Recreational	62.8±4.7	61.4±3.1
Sedentary	61.7±2.0	60.2±2.8
Total	62.5±4.5	61.5±4.1

Peak Power

A weak relationship (p= .034, f = 4.830, df = 1, 40) between peak power and IIA fiber was seen, but no significant relationship to type IIX or I fiber (r = -.328) (Table 17). The relationship between predicted maximal power percentage and IIA MHC composition is:

Percent max power occurs = -.142 (percent IIA) + 69.410.

Equation 5 - Percent Max Power Occurs (Peak Power)

Mean Power

Mean power was also weakly related to IIA and I fiber, but not to type IIX (r = -.352 p= .022 and r = .314, p = .043, respectively) (Table 17). The relationship between predicted mean power

maximum percentage and IIA and I MHC composition is (r = .417, f = 4.103, p = .024, df = 2, 39):

Percent max power occurs = -.114 (percent IIA) + .085 (percent I) + 64.025

Equation 6 - Percent Max Power Occurs (Mean Power)

Table 17 Correlation of predicted power maximum to MHC

	Predicted Peak Power		**Predicted Mean Power**	
MHC	**r**	**p**	**r**	**p**
IIX	0.034	0.83	0.011	0.946
IIA	-0.328*	0.034	-0.352*	0.022
I	0.26	0.097	0.314*	0.043

* denotes significance of p < .05
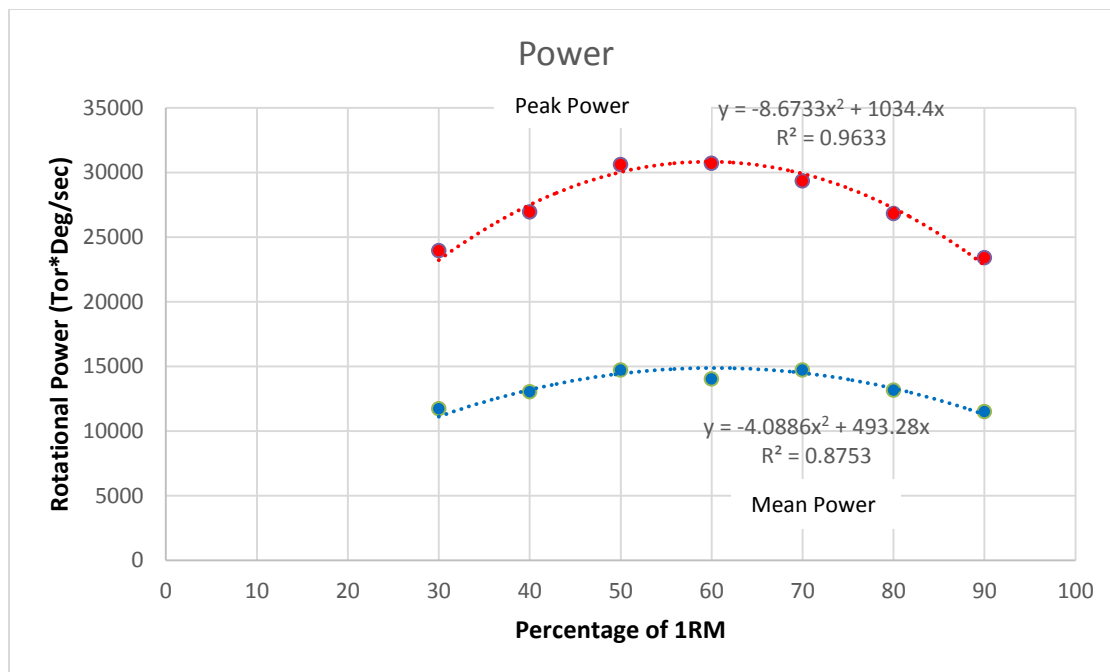
Figure 32 - Power load curve generated for one of the subjects in the study
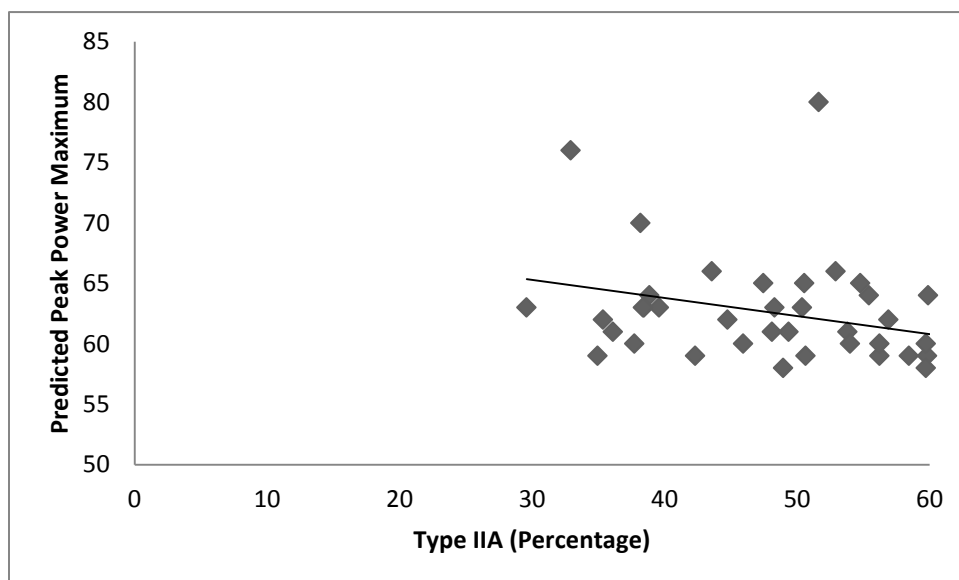
Figure 33 Scatterplot for IIA MHC correlation to Predicted Peak Power Maximum
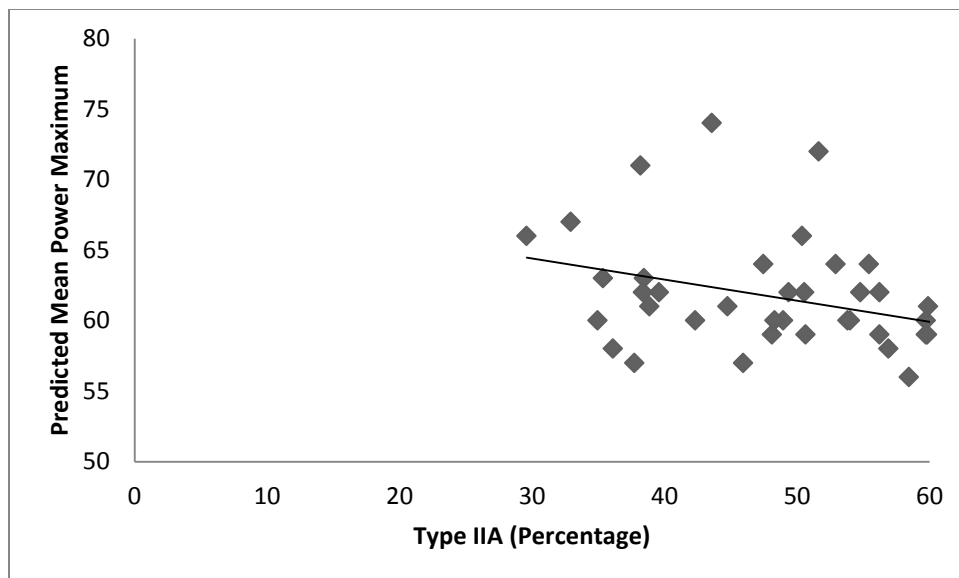
Figure 34 Scatterplot for IIA MHC correlation to Predicted Mean Power Maximum
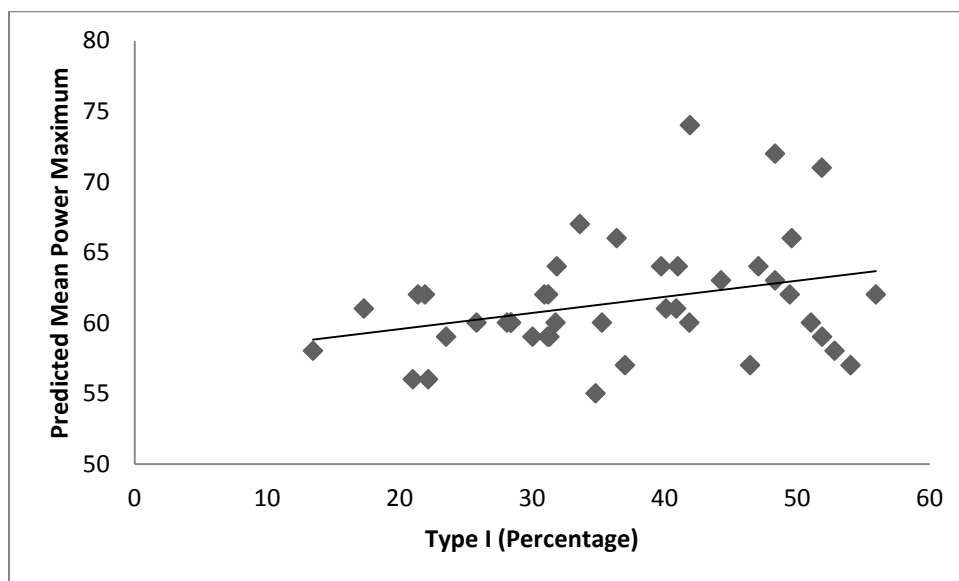
Figure 35 Scatterplot for I MHC correlation to Predicted Mean Power Maximum

Hypothesis 4

Isotonic leg extension power test fatigue index will be positively related to total concentration of the combination of both fast isoforms.

Each subject completed the fatiguing protocol for 15 repetitions with a full range of motion (Figure 36). Data was analyzed for the highest performance of each variable and a fatigue index was derived by comparing highest and lowest performance in the repetitions after the peak performance.

Fatigue index formula= ((highest rep – lowest rep)/highest rep) x 100.
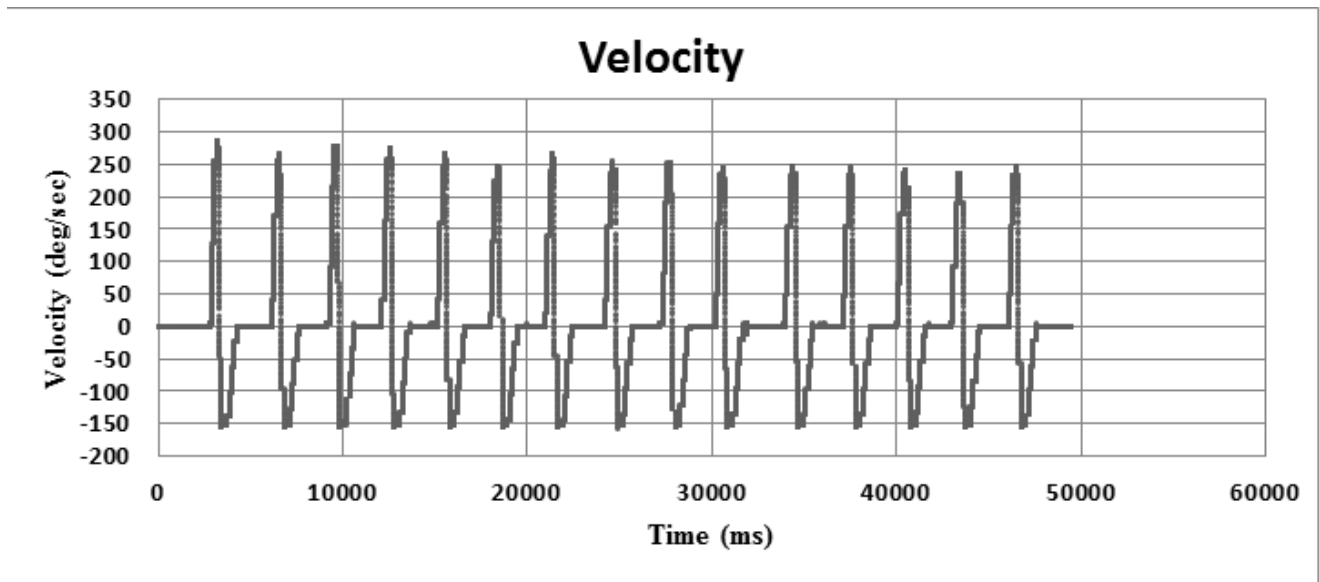
Figure 36 Velocity fatigue graph performance for one of the subjects in the study

Fatigue indexes were computed for both mean and maximal velocity, torque, and power.

Table 18 - Fatigue index values - All units are mean ± standard deviation (all measurements are in percent decline)

Fatigue Index

Group	Mean Velocity	Mean Torque	Mean Power	Maximal Velocity	Maximal Torque	Maximal Power
Aerobic	23.0±10.0	16.2±6.9	30.1±10.4	21.1±7.6	11.0±4.0	28.4±10.4
Resistance	23.7±10.0	13.7±5.5	30.3±9.5	20.7±9.4	12.4±4.5	27.8±9.4
Recreational	24.6±5.3	13.1±7.4	30.1±4.4	25.1±7.6	13.5±3.3	32.3±7.7
Sedentary	24.3±9.4	16.9±4.9	33.1±7.6	23.5±6.7	15.2±4.8	31.7±6.3
Total	23.8±8.6	14.7±6.3	30.6±8.3	22.4±8.0	12.7±4.2	29.7±8.9

Results are as follows (Table 18):

One way analysis of the fatigue protocol showed no significant differences in fatigue between groups.

Table 19 - Fatigue index correlation to MHC

	Mean Velocity		Mean Torque		Mean Power		Peak Velocity		Peak Torque		Peak Power	
MHC	r	p	r	p	r	p	r	p	r	p	r	p
IIX	-0.006	0.968	0.283	0.069	0.074	0.642	-0.013	0.932	0.043	0.786	0.02	0.899
IIA	0.319*	0.039	-0.269	0.085	0.237	0.13	0.298	0.055	0.464*	0.002	0.292	0.061
I	-0.277	0.075	-0.072	0.651	-0.292	0.061	-0.255	0.103	-0.473*	0.002	-0.29	0.062

Figure 37 Scatterplot for IIA MHC correlation to Fatigue Index Mean Velocity
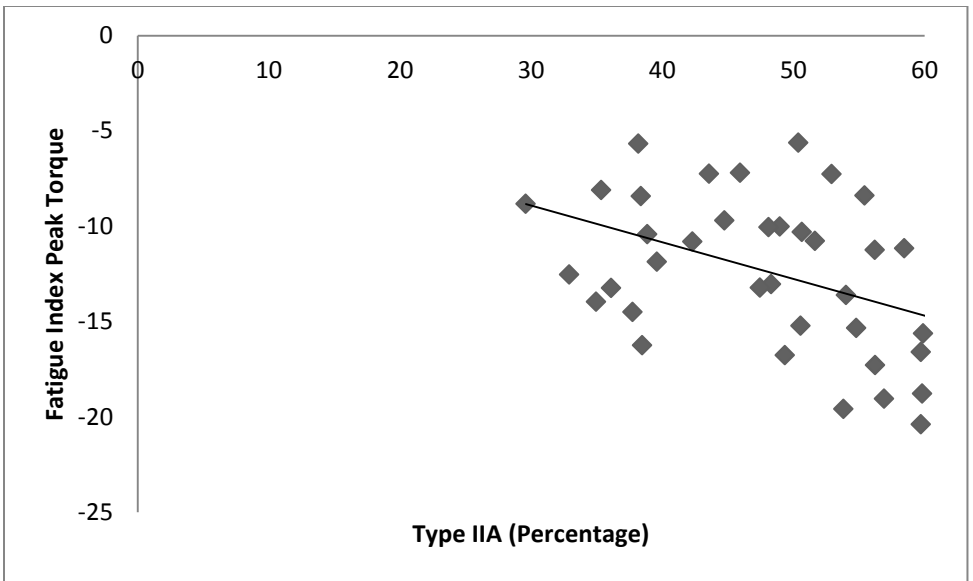
Figure 38 Scatterplot for IIA MHC correlation to Fatigue Index Peak Torque
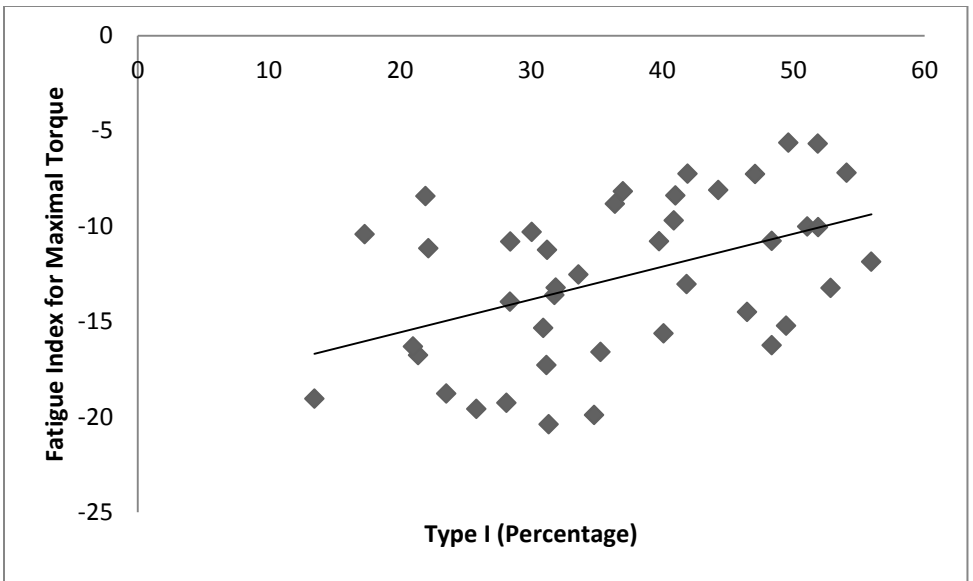
Figure 39 Scatterplot for I MHC correlation to Fatigue Index Peak Torque

Fatigue index scores were correlated with total fast fiber content and the only significant relationship was found to be with maximal torque. This relationship had a relationship of r = -.474 (p = .002). Fatigue index scores were then analyzed based upon each MHC and relationships were found as follows (Table 19) (Figure 37, 38, 39).

Velocity

Velocity fatigue index showed a relationship to IIA MHC concentration and mean velocity fatigue index values of r = -.319 p=.039 (f = 4.54, df 1, 40) (Table 19). There was no relationship of any other MHC to velocity fatigue index for the other mean maximal values. The predicted regression equation for velocity fatigue drop off to IIA MHC was:

Mean Velocity Fatigue Index = -.268 (percent IIA) – 10.549.

Equation 7 - Mean Velocity Fatigue Index MHC relationship
Power

No significant relationship between peak power or mean power fatigue index was observed with any MHC isoform (Table 19).

Torque

There was a significant relationship between maximum torque fatigue index values and both IIA (r = -.464, p=.002) and type I fiber (r = .473 p =.002) content (Table 19). When entered into a step-wise linear regression with IIA fibers, followed by type I fibers, resultant relationships were as follows: r = .586 (f=10.118, p < .001, df = 2, 39) with a prediction equation of:

Maximum Torque Fatigue Index = .137 (percent I) - .146 (percent IIA) – 10.508

Equation 8 – Maximum Torque Fatigue Index MHC relationship

Stepwise Linear Regressions for Myosin Heavy Chain

Stepwise linear regressions were performed to find the strongest statistical prediction possible for each of the MHCs based upon the strongest correlating variable from each of the 4 hypotheses. The variables chosen and the results seen for each are as follows.

Type IIX Fiber - The stepwise linear regression for IIX fiber best fit followed the order of rate of force development relative to cross sectional area in the vertical jump, mean power relative to cross sectional area on the vertical jump, and peak velocity intercept. This model ended with an r = .544, f = 5.318 p = .004 with df = 3, 38. The final regression equation was:

IIX Percent = 225.527 (mean power relative to cross sectional area of the thigh in the vertical jump) + .126 (rate of force development relative to cross sectional area in the vertical jump) - .010 (peak velocity intercept) – 23.846.

Type IIA Fiber– The stepwise linear regression for IIA fiber best fit followed the order of peak velocity slope and intercept, percent mean power maximum predicted, fatigue index for maximum torque, and peak power relative to cross sectional area on the depth jump. This model ended with an r = .679, f = 6.153 p < .001 with df = 5, 36. The final regression equation was:

IIA percent = 7.655 (peak velocity slope) - .035 (peak velocity intercept) + .079 (percent mean power maximum predicted) - .967 (fatigue index for maximum torque) – 21.734 (peak power relative to cross sectional area on the depth jump) + 48.292.

Type I Fiber – The stepwise linear regression for type I fiber best fit followed the stepwise regression entry order of: maximum torque fatigue index, percent mean power maximum predicted, vertical jump height, and peak velocity slope. This model had an r = .597, f = 5.118, p = .002, df 4, 37. The final regression equation produced was:

I percent = 1.231 (maximum torque fatigue index) + .334 (percent mean power maximum predicted), -87.317 (vertical jump height), 3.183 (peak velocity slope).

Chapter 5 - Discussion

Through the multiple tests implemented in this dissertation, a variety of relationships were observed between MHC and performance. These relationships will be explored individually based upon the hypotheses covered.

Subject Sample

There was a large variability in MHC composition between the subjects in the study. The greater diversity in MHC expression in between the subject sample allowed for more relationships to be seen. Future research could find even higher level aerobic and resistance trained athletes for greater concentrations of type I and IIA respectively to help increase the fiber variability between subjects even further (Schilling, Fry, Weiss, & Chiu, 2005). The variability in IIX was very good, one indication being the significance in the concentration of the MHC in the sedentary group compared to the other groups.

Our research is limited in that we only took one muscle biopsy for indication of muscle MHC which does vary along the length and depth of the muscle (Dahmane, Djordjevič, Šimunič, & Valenčič, 2005; Elder, Bradbury, & Roberts, 1982; Lexell, Henriksson-Larsén, Winblad, & Sjöström, 1983). We did sample from the same biological landmark on each of the subjects, and the vast majority of MHC studies have been based off of a single muscle biopsy (Fry, 2002; Fry, Schilling, et al., 2003; Fry, Webber, et al., 2003).

There was a significant relationship between IIX MHC and both IIA and I fiber in the form of a negative correlation. There was however, no significant relationship between IIA and type I fiber. This is a logical result since IIX is the most readily decreased MHC with training

and so would be found in high amounts in the sedentary, and be decreased in trained individuals no matter the training stimulus (Pette & Staron, 2001; Schilling et al., 2005).

Vertical Jumps

This study collaborated the finding of a relationship previously reported by Eckerson and Fry (Eckerson, 2005; Fry, 2002) regarding the relationship between jump performance and IIX fiber composition. Previous research by Fry et al. utilizing a sample of weightlifters, observed a correlation between jump power and type II fiber characteristics. However, this study did not find the same relationship that resulted from our data, possibly due to a difference in training methods and techniques between power lifters and weight lifters, potentially affecting neurological recruitment properties of the muscle (Fry, Schilling, et al., 2003). We however, did not find a relationship with rate of force development and impulse, except for rate of force development in the counter movement jump trial when controlling for cross sectional area with IIX fiber. This is unlike the results from Eckerson and Fry, however, the sample size for this study was much greater (Eckerson, 2005; Fry, 2002). Further research in this area could have a greater amount of familiarization time with all of the jump varieties to allow for greater jumping efficiency with the subjects.

By incorporating cross sectional area of the muscle mass significance was found in a number of variables to type IIX fiber proportion and jump performance. Since IIX fibers have ten times the power production of type I fibers and two times the power production of IIA fibers in single fiber studies (Bottinelli, Canepari, Pellegrino, & Reggiani, 1996; Bottinelli, Pellegrino, Canepari, Rossi, & Reggiani, 1999; Bottinelli, Schiaffino, & Reggiani, 1991; Widrick, Trappe, Costill, & Fitts, 1996) it makes sense that a muscle with a greater proportion would be able to create a greater amount of power relative in size. The negative relationship to type IIA MHC and

jump performance is of interest, since the resistance trained subjects and recreationally trained subjects had larger thigh girths when this extra size was accounted for this translated to a lower power production. This result has not been seen in any other research and should be investigated further to find if this result is consistent. In general utilizing cross sectional area testing should be incorporated in future field tests for MHC as a means to making performances relative to muscle size.

These results lead to possible future research which should examine the same type of sample groups, but utilizing a greater variety of jumps to better explore the relationships to MHC and performance. Furthermore research should test with performing series of jumps, this has previously been seen in research (Bosco, Tihanyi, Komi, Fekete, & Apor, 1982) to be related to muscle MHC when examining fatigue index and holding static positions in the bottom of the squat for longer periods of time. These relationships were only analyzed with fast to slow MHC differentiation not the individual analysis of both fast isoforms. Further research examining rate of force development and vertical jump performance could also utilize smaller time intervals at the initial production of force along the lines of the research done by Andersen, which showed a greater, short time frame production of force (Andersen & Aagaard, 2006). Perhaps an additional analysis of this data set with a different definition of rate of force development could be used to examine this relationship.

Velocity Load

Velocity load performance results confirmed the hypothesis that both the intercept and slope of the best fit line would be related to muscle MHC, specifically IIA fiber. This builds on the research performed by Schilling et al. where little to no relationship to type IIX (or type I) was seen, but by increasing the number of sets at different velocities this current study was able

to observe significant relationships, however, not to IIX MHC (Schilling et al., 2005). Though there is a significant positive relationship between IIA fiber and the height of the intercept and the steepness of the slope, it was not a strong enough relationship to create a predictive model for MHC based upon exercise performance (r=.346-.410). Further research in this area could expand upon the model by recruiting a wider variety of subjects (different training histories) to participate and utilize a greater number of attempts at a wider variety of percentages of the 1RM. The reasoning for this is some research with an unweighted apparatus have shown a relationship to fast fiber (greater power and acceleration), but did not distinguish the fast fiber into both isoforms for statistical analysis (Houston, Norman, & Froese, 1988). So, perhaps additional sets at 0%, 10%, and 20% could add information to the model.

Ancillary analyses could also look at the relative differences in performance at individual percentages and their relationships to MHC when controlling for cross sectional area which has been shown to occur in previous research (Tihanyi, Apor, & Fekete, 1982).

These results also fall in line with the research done using isokinetic devices. They have observed greater amounts of torque being produced from individuals with greater amounts of fast fiber. Our research further compartmentalized this data into the two fast isoforms, which shows a greater contribution of the IIA fiber and no significant relationship with the IIX fiber composition (Aagaard & Andersen, 1998; Coyle, Costill, & Lesmes, 1979; Gregor, Edgerton, Perrine, Campion, & DeBus, 1979), where some research has shown a negative relationship between performance and IIX fiber (Jürimäe, Abernethy, Quigley, Blake, & McEniery, 1997). This corresponds with the torque ratio that was observed by Gür et al., who saw a relationship between the decrease in peak torque as isokinetic speeds increased relating to fast fiber (Gür, Gransberg, Knutsson, & Larsson, 2003). There are however other studies which detected no

significant results when examining the same variables, but were looking at slow fiber

relationships and utilized slower isokinetic speeds (Schantz, Randall-Fox, Hutchison, Tyden, &

Åstrand, 1983). Though our testing was isotonic, there seems to be some relationship with other

studies and different performances across a variety of performed speeds due to MHC.

Power Load

Mathematically predicted max power for both mean and peak power measures was

related to IIA fiber. This relationship showed that individuals with greater amounts of IIA fiber

hit peak power at a lower percentage of their 1RM compared with individuals with a lower

amount of IIA fiber. This could be attributable to a number of facts. First, previous research has

generally related greater power outputs in general (Suter, Herzog, Sokolosky, Wiley, &

Macintosh, 1993; Tihanyi et al., 1982), and at lower percentages (Froese & Houston, 1985), or

even unweighted lever arm performance to greater amounts of fast twitch fiber (Houston et al.,

1988). Secondly, this could be related to differences in motor unit activation and control through

the subjects, as well trained subjects with greater motor control and activation could simply

produce greater amounts of force, regardless of the load on the apparatus compared with subjects

who have greater amounts of type IIX and I fiber. However, post hoc analysis of predicted

maximum power values by group showed no significant differences between each group.

Our study found that the point of which peak power occurs is much higher (in deg/sec)

than found with isokinetic testing in previous research (Coyle et al., 1979; Lorentzon, Johansson,

SjÖstrÖm, Fagerlund, & Fugl-Meyer, 1988; Thorstensson, Grimby, & Karlsson, 1976), but this

is also comparing greatly different testing modalities in resistance application. This would be

attributable to the differences in how force is applied between isokinetic and isotonic testing. The

performance of force application during isokinetic testing allows for greater force to be produced without the velocity of the arm being able to increase unlike isotonic testing.

This relationship was not strong enough to be predictive of MHC (r=.328-352), but it is significant, indicating that further research in this area should build on this testing model utilizing a greater number of testing trials and a wider variety of percentages of the 1RM to be tested. With larger testing data, it could be possible that a predictive relationship can be found and utilized for future scientific investigations.

Fatigue

The fatiguing protocol showed a significant relationship to type IIA muscle fiber and mean velocity fatigue index and max torque (r = .319, .464 respectively) a relationship between type I muscle fiber and max torque fatigue index (r = -.473). These relationships, though significant, were not strong enough to be strong predictors of muscle MHC based upon exercise performance.

Fatiguing protocols such as Wingates have successfully linked fast twitch fiber and exercise performance, but have not been able to disseminate between the two different fast fiber isoforms (Bar-Or et al., 1980). Additionally, sprinting fatigue trials at longer distances had a negative relationship to fast MHC, whereas the antithesis was seen in short distance trials (Inbar, Kaiser, & Tesch, 1981). This research agrees with previous research that was done with isokinetic testing (Lorentzon et al., 1988; Suter et al., 1993; Thorstensson & Karlsson, 1976) in that the fatigue index is related to heavy chain fiber content. However, the relationship was not as strong as that seen in previous research. The successful previous protocols did utilize a greater number of repetitions and due to their isokinetic nature did not run the risk of having prematurely

induced concentric failure. However, not all high rep fatiguing protocols have found success with linking MHC and fatigue rates with higher rep isokinetic performances (Clarkson, Kroll, & Melchionda, 1982; Clarkson et al., 1982).

The results of this study also show a relationship with isotonic fatiguing protocols whereas previous studies have shown such a result (Rodriguez, 2005). This type of testing protocol looked instead at reps to failure, which could have incorporated a greater amount of subject motivation to perform to their full capacities, whereas a fatiguing protocol with a fixed number of reps may have an advantage psychologically to maintaining maximal performance.

Successful fatiguing protocols that utilize leg extension testing all have a greater number of repetitions that are performed in the task compared to the tests that are not as successful, however these higher repetition sets are isokinetic so subject concentric failure is unlikely compared to lower repetition test which have been isotonic (Rodriguez, 2005; Thorstensson & Karlsson, 1976). Another approach to strengthening this model would be to utilize a sample population with little to no type IIX fiber, such as highly trained aerobic athletes (mile times less than 5 minutes) and resistance trained athletes (over two times bodyweight squatters) (Fry, Schilling, et al., 2003; Fry, Webber, et al., 2003). Since there was no statistically significant link between this MHC and performance, it would stand to reason that utilizing a population of only well trained individuals would make this a stronger test for prediction. Further research could also possibly slightly increase the repetitions but would still want to maintain a protocol that would end with subject full performance of each repetition.

Stepwise Regression

An exploratory analysis of taking the strongest relationships from each of the different tasks to the individual MHC isoforms was performed utilizing a stepwise multiple regression. This data analysis found the strongest relationship to the tests predicting IIA fiber explaining approximately one third of the variance. This is not meant to be an end all, but given the great deal of variables that can affect exercise performance (neurological, diurnal, biochemical, etc.) this does create a moderate amount of prediction. However, the ability to predict muscle MHC with a high degree of certainty from isotonic tasks performed in this dissertation was not discovered.

Conclusions

A number of relationships between muscle MHC and performance were seen in both the vertical jump and leg extension tasks. While these relationships were significant, they were not strong enough to fully be indicative of muscle MHC. However, relationships between jumping power, jump height, velocity load, power max estimate, and fatigue index were seen with MHC. This indicates a broad array of indicators of muscle MHC to exercise performance humans of varying degrees of velocity and power production. Future research in this area should aim to control for more possible confounding variables such as motivation, subject abstinence from training, and motor efficiency with tasks should improve the relationships observed to MHC.

Each MHC had different relationships to exercise performance, and no test was able to find significant relationships to all of the MHCs simultaneously. Though all muscle fibers are utilized in different manners, typically maximal efforts should illicit the use of all fibers. This research shows that individuals not just differentiating between fast and slow MHC is important,

but differentiating between the two fast MHCs is important when looking for significant differences between MHC and performance.

There were a number of limitations to this study, in that, like all research with human subjects, there is a great deal of variability from subject to subject in motor control efficiency when performing the movements and subject motivation. Due to the wide variety of training backgrounds from the subjects, motor disparities on a number of tasks was notable by visual observation of jumping mechanics. Subject motivation to give their best performance on each repetition for the tasks could also be a confounding variable where a subject could underperform compared to their potential for each of the tasks which would introduce more error in to the model (Enoka et al., 2003; Kidgell & Pearce, 2011). Muscle pennation angles, attachment, general anthropometrics, and diet were not controlled for and could be other sources for greater variability in performance.

Practical Applications

MHC content is related to exercise performance. The strength and types of relationships are dependent on the speed of muscle action and the amount of power produced. This study did not create a predictive model for MHC based up on task performance, but did show various relationships to performance. In general high power movements and lower loads performance are related to both fast fibers for greater performance and lower performance with higher amounts of type I fiber. Resistance exercises variability of velocity performance as loads reach higher percentages of 1RM is also related to fast fiber IIA concentration.

Implications for further research

Directions for further research have been outline above. The large gain in this study is to compare performance on relative metrics may that be relative to cross sectional area, percentage of 1RM, or fatigue index. By removing any confounding effects on performance that can occur from simply being a large or small subject has a positive effect on resultant observation for MHC differences. Vertical jump research should incorporate a greater variety of jumps to establish other links. Research linking the velocity load and percent that maximal power output is estimated to occur would be more effective with an even larger and more diverse sample size than this study offered. The link between isotonic fatiguing protocols and muscle MHC must be further examined, but incorporation of means to measure drop in performance in velocity, power, and torque are important for establishing a MHC relationship to performance. A greater number of biopsies feasible not only from the vastus lateralis but other muscles of the quadriceps (and perhaps the other major muscle groups of the lower body for jump performance) could further increase the strength of such a predictive model, but would introduce a greater amount of subject discomfort and risks.

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Appendices

Appendix A: Informed Consent

Myosin Heavy Chain Characteristics and Exercise Performance.

INTRODUCTION

The Department of Health Sport and Exercise Sciences at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or the University of Kansas.

PURPOSE OF THE STUDY

The primary purpose of this study is to examine the possible relationships between exercise performance and muscle fiber type composition (myosin heavy chain expression [MHC]). The secondary purpose of this study is to examine the differences in exercise performance based upon training history.

PROCEDURES

A time-line of the testing procedures and an overview of the testing sequence for the testing days are presented below. All procedures will be conducted in the Neuromechanics Laboratory and the Applied Physiology Laboratory at the University of Kansas and will be supervised by trained personnel. During the initial visit (visit 0) we will collect your baseline information (height, weight, etc.) and determine if you are eligible for this study. Visit 1 will last approximately 1 hour with visit 2 lasting approximately 1.5 hours. If you are eligible, visit 3 and 4 will take approximately 1 hour each. You will only be eligible for sessions 3 and 4 if have been resistance training for over one year and can estimate your barbell back squat.

Visit 0: Consent Form, pre-exercise testing health and exercise status questionnaire, determination of eligibility, demographics (height, weight, etc.), and familiarization with testing measurements.

Visit 1 (day 0): DEXA test

     Vertical jump test

     Leg extension maximal force test

     Leg extension isometric tracings

Visit 2 (day 2-7): Kansas leg extension power test

     Adapted Kansas squat power test

     Vertical Jump

     Muscle Biopsy

Visit 3 (day 8-13) (only if eligible): Squat one repetition maximum test

Visit 4 (day 14-20) (only if eligible): Kansas squat power curve test

     Kansas squat power test

DXA scan – This scan will require you to lie on a DXA machine while your body is scanned with a low level of x-rays (e.g. similar to flying in a commercial airplane) in order to determine your muscle mass, body composition, and bone mineral density. This scan will take approximately 7 minutes.

Vertical jumps – You will be given a standardized warm up and then directly there after you will perform 3 maximal vertical jumps on a force plate reaching as high as you can on a vertec (vertical jump tester that you reach up and touch). You will be given a one minute rest period

between each jump. Then you will perform 3 maximal vertical jumps with your hands on your hips with the same rest periods. Finally you will perform 3 maximal jumps from a 21cm (approximately 9 inches) box.

Leg Extension maximal force test - You will be positioned in the isokinetic dynamometer for leg extensor (thigh muscles) strength testing. After familiarization you will perform a standardized warm up on the biodex. Then the load will be increased in 5-10lbs. after each successful repetition until you are unable to complete a rep through a full range of motion.

Isometric strength testing – You will be positioned in the isokinetic dynamometer for leg extensor (thigh muscles) strength testing. After the positioning and prior to the strength tests, electromyographic (EMG) and mechanomyographic (MMG) electrodes will be placed on the skins surface of your right thigh. You will then be instructed to contract your leg maximally for 5 seconds in to the pad. You will then be given a minute rest period and repeat the process.

Isometric tracing test – You will then perform submaximal ramped contractions on the isometric leg extension. Each test will be graphically displayed the amount of force you are producing when compared to the force production that. After this test you are done with testing for the day.

Leg extension power test – Based upon your performance on the leg extension maximal force test you will then perform sets of 3 repetitions at 20, 30, 40, 50, 60, 70, 80, and 90% of the maximal load that you successfully performed a full repetition with. Each rep on each set performed will be done at the maximal force production that you can accomplish. You will be given 2 minute rest periods between each set.

Leg extension adapted Kansas squat test – After performing each of the power sets the load will be set at 70% of your one repetition maximum and you will perform 15 repetitions with a 3 second rest between each rep. Each rep will be performed maximally.

Muscle Biopsy –You have been informed that one of the purposes of this study is to measure the muscle fiber type from the muscle samples that we collect. By obtaining a small sample of your muscle tissue (size of a pea), the different types of proteins in your muscle may be determined which will be helpful in the evaluation of health and exercise performance. All muscle tissue samples (biopsies) will be taken from the outer portion of the front of the thigh using a needle

biopsy technique by either Philip Gallagher or Andy Fry. Philip Gallagher, PhD., Assistant Professor of HSES, and Andrew Fry, PhD., Professor of HSES, have performed over 400 muscle biopsies over the past seven years and have assisted on over 1000 biopsies over the past ten years on various populations (athletes, sedentary people, elderly etc.) with no significant complications and nothing more than minimal adverse reactions. The procedures are supervised by Jeff Burns, M.D., who is a medical doctor in Neurology at the KU Medical Center in Kansas City, Kansas. The total size of the muscle biopsy will be approximately the size of a pea. You will be placed on an examination table lying down on your back (supine) so that the muscles of the leg are relaxed. The skin will be thoroughly cleaned with antiseptic solution (Betadine) using sterile cotton swabs after which a surgical cover will be placed around the sampling site. If you are allergic to Betadine, an alternative antiseptic solution will be used to clean the skin. A small amount (3ml or 3cc) of a local aesthetic (2% Lidocaine) will be injected into the tissue under the skin around the site to be sampled. During this injection you may feel a slight burning sensation. If you are allergic to the local anesthetic, or have had allergic reactions to other anesthetics (i.e.: Novocain) then you will be disqualified from the study. Following the injection of the numbing agent into your thigh, a minimum of five (5) minutes will be allowed to pass to ensure adequate time for the agent to take effect in the area where the incision will be made. A small incision (1 cm) will then be made in the skin overlying the muscle and the biopsy needle inserted into the middle of the muscle (muscle belly) at a depth of 3 cm (about 1 inch). During the time that the sample is being taken (about 5 seconds) you may feel some deep pressure and cramping that will be moderately painful. You have been informed that if you have been previously diagnosed as having a bleeding disorder, a blood clotting problem, take blood thinning medication or have sensitivity to scarring, you will tell the researcher and not participate in the biopsy procedure.

Following the biopsy procedure, firm and constant pressure will be placed on the wound to stop any bleeding. The incision site will be closed with a Steri-Strip and covered with a large Band-Aid and the site compressed using a 10 cm strip of elastic tape for a period of 24-hrs. You have been informed that the procedure will take about 20 min.

Sterile disposable instruments and sterile gloves will be used for the preparation of the site and the reusable biopsy needle will be thoroughly sterilized (via steam and heat autoclave) after each biopsy. Approximately 10-12 mg (size of the exposed lead on a pencil) of skeletal muscle tissue will be removed. With the invasive skeletal muscle biopsy technique there is the possibility of a blood related infection (HIV, Hepatitis B), but the reusable needles will be cleaned and sterilized using an autoclave, a generally accepted method of cleaning surgical instruments.

The following three tests will only be performed if you are eligible:

Squat maximum test – Squatting depth in this study will be defined as the upper portion of the thighs parallel to the ground. You will be allowed to perform the squat with your preferred technique or style as long as it is a back squat. You will not be allowed to use a weight belt or knee wraps, but unsupportive knee sleeves will be allowed. After a 5 minute walking warm up you will then be given an empty bar to squat for a set of 5 to 10 repetitions. From this point on

based upon a predicted squat maximum that you have supplied us you will perform 5 repetitions at 30% or your predicted max, 5 repetitions at 50%, 5 repetitions at 60%, 3 repetitions at 70%, 2 repetitions at 80%, 1 repetition at 90%, 1 repetition at 95%, and 1 repetition at 100%. You will be given a 2 minute rest period between each set minimum up to 3 minutes rest. If you feel capable of making another attempt after your predicted maximum has been attained you will be allowed to do so with the same rest periods applied. After you have attained your maximum for that day you are done with testing.

Day 4 testing

Kansas basketball squat power test – You will take part in a standardized warm up and then perform maximal velocity repetitions at 30, 40, 50, 60, 70, 80, and 90% of your one repetition maximum. The first 5 sets you will perform 3 repetitions. The final two sets you will perform 2 or less repetitions depending on your own voluntary decision for fatigue. You will be given 2 minutes of rest between each set.

Kansas squat test – 70% of your system mass (body weight plus squat 1 repetition maximum) will be loaded on the bar and you will perform 15 maximal velocity repetitions with a 6 second rest between each rep. After you have finished this test you are done with testing for the day.

RISKS

As a participant there is the potential to experience some physical stress during and muscle soreness following the maximal voluntary contractions and the submaximal step contractions. In addition, you may have skin abrasions due to shaving and cleansing the skin with alcohol prior to electrode placement.

Muscle Biopsy - The use of local anesthetics will result in a slight burning sensation, lasting approximately 5 seconds. There is a risk of allergic reactions to the local injection (1 in 1 million). There is a small chance of bleeding from the biopsy site. The principle concern would be prolonged bleeding which would produce a bruise in the area. This would extend the muscle soreness, but is adequately treated with rest, ice, a compression bandage, and keeping the leg elevated as much as possible. Nausea, dizziness, and fainting can occur (1 in 100) during the biopsy process. As a result, the subjects will be in a supine position during the biopsy procedure. There is a risk of infection (1 in 1000) and irritation associated with the biopsy procedure. The

use of aseptic techniques, careful cleaning of the skin and keeping the area dry will minimize the risk of infection. There is a risk of bruising (1 in 100) from the biopsy procedure. This will be minimized by placing ice over the site following the procedure and by applying a compression bandage on the site for the 24 hours following the biopsy. In some instances (1 in 200), some motor nerves may be damaged which may cause local muscle atrophy (decrease in the size of muscle fibers, with a small dimple on the skin). There is likely to be a small scar where the biopsy is performed. This scar usually dissipates over a period of 6-12 months at which time the scaring is very modest.

DXA Scan - You are volunteering to participate in a study in which your body composition will be assessed using Dual-Energy X-Ray Absorptiometry, commonly referred to as DXA. This research study involves a procedure that uses x-rays. The evaluation is being done not because you are sick and the procedure will help you feel better or because it will help your doctor diagnose the problem. There may be no direct benefit to you. The information that will be gained from the x-ray procedure may, however, provide important research data.

The University of Kansas has adopted the philosophy of safety that all exposures to radiation, other hazardous materials, and risks from physical hazards shall be kept 'as low as reasonable achievable.' There are certain limitations placed upon this procedure to achieve that aim.

If you have received nuclear medicine imagining studies (radionuclide and radiopaque agents) within the past two months you may not participate in the study.   These agents will interfere with the DXA whole body scan. If you have metal devices or implants you may not participate in the study. These devices will make whole body measurements difficult to interpret when the devices or implants are located within the scan field. If you have recently undergone CT (Computed Tomography), PET, or fluoroscopic, studies, you cannot participate in this study.

The parts of your body that will receive the most radiation are the skin, although your whole body will be exposed to the radiation. During a measurement, the shutter opens to let the x-ray beam of radiation pass through the scanner table and patient. The radiation field at the table top is 0.75 inches by 0.12 inches. You will see the arm of the DXA pass systematically back and forth over you. Lead oxide shielding surrounds the x-ray tube insert inside the tube housing assembly and reduces radiation levels around the scanner table.

The amount of radiation that you will receive from a DXA whole body scan is twenty time less than a chest x-ray, and comparable to a Trans-Pacific flight. The Radiation Safety Officer at the University of Kansas can provide you with more information about radiation exposure if you are interested.

The DXA measurements are being made by trained personnel who have received formal GE training following installation of the DXA for total body scans. A physician, M.D., Board Certified advisor and medical consultant to the program, will provide medical assistance and supervision for those who voluntarily participate in the research program.

The system is equipped with a Class II Laser device. A Class II rating indicates a low power visible laser that is not normally hazardous to eyesight but has the potential to be hazardous if viewed directly for an extended period of time. Because of the potential hazard, DO NOT stare directly into the beam while the laser is in operation.

FOLLOW UP CARE

Following the procedure you will be provided with a biopsy care sheet, extra bandages and contact information for Philip Gallagher, Ph.D, Andrew Fry, Ph.D, and Mike Lane. After 24-hrs you must report back to the testing coordinator to check the wound. At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound. You will again be asked to report to the test coordinator after 3-days and be contacted via phone one week after the biopsy or whenever necessary to ensure normal recovery. The biopsy procedure often results in a small fine scar at the site of the incision, however, all care post-treatment will aid in reducing the potential for scarring. All care will be taken to aid in the healing of the wound. The entire biopsy procedure will be performed under sterile conditions. All testing staff and associated personnel will be trained in first aid and will be familiar with emergency procedures.

There have been no other major complications reported in the scientific literature as a result of taking small tissue samples from the skeletal muscle using the percutaneous needle biopsy technique described above. This procedure has been performed on numerous subjects by qualified personnel in many institutions worldwide with only slight discomfort being reported.

During the muscle biopsy it is common to feel a strong cramping sensation in the muscle while the biopsy is being performed. However, muscle function is not impaired. In fact, subjects have been reported to continue participation in sporting events immediately following a muscle biopsy. It is common for subjects to experience mild soreness, moderate pain and bruising near the biopsy site, similar to a "Charlie-horse" the day after the procedure. In order to allow the incisions to heal properly and minimize the risk of infection, you should not get the biopsy site wet for 24-hours and avoid prolonged exposure to water for 4-days. Daily showers are acceptable (after the first 24-hours), but baths, swimming, sauna's etc. should be avoided for 4-days following the biopsy procedure.

BENEFITS

You will not directly benefit from participating in this study. However, you will gain an increased understanding of your skeletal muscle function. Specifically, you will learn about your level of muscular strength, muscle fiber type, and body composition. A copy of all personal data from the tests will be provided to you and your data will be completely explained to you by a member of the investigation team.

PARTICIPANT CONFIDENTIALITY

Your name will not be associated in any publication or presentation with the information collected about you or with the research findings from this study. Instead, the researcher(s) will use a study number or a pseudonym rather than your name. Your identifiable information will not be shared unless (a) it is required by law or university policy, or (b) you give written permission.

Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for purposes of this study at any time in the future.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose further information collected about you, in writing, at any time, by sending your written request to: Mike Lane, 1301 Sunnyside Avenue 101DC, Robinson Center, Lawrence KS 66045.

If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

QUESTIONS ABOUT PARTICIPATION

Questions about procedures should be directed to the researcher(s) listed at the end of this consent form.

PARTICIPANT CERTIFICATION:

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or (785) 864-7385, write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7568, or email irb@ku.edu.

I agree to take part in this study as a research participant. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

_____   _____

Type/Print Participant's Name   Date

_____

Participant's Signature

Researcher Contact Information

Mike Lane   Andrew Fry

Principle Investigator   Applied Physiology Lab

Applied Physiology Lab   160 Robinson Hall

101DC Robinson Hall   University of Kansas

University of Kansas   Lawrence, KS 66045

Lawrence, KS 66045   785-864-0784

785-864-0773

Appendix B: Health History Questionnaire

PRE-EXERCISE TESTING

HEALTH & EXERCISE STATUS

~~QUESTIONNAIRE~~

THE UNIVERSITY OF
KU KANSAS

Name _____   Date_____

Home Address_____

Work Phone _____   Home Phone _____

Person to contact in case of emergency   _____

Emergency Contact Phone   _____   Birthday
(mm/dd/yy)_____/_____/_____

Personal Physician   _____   Physician's Phone_____

Gender_____   Age _____(yrs)   Height _____(ft)_____(in)   Weight_____(lbs)

Does the above weight indicate: a gain_____   a loss_____   no change_____   in the past year?

If a change, how many pounds?_____(lbs)

A.   JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

<u>Joint Areas</u>   <u>Muscle Areas</u>

(   ) Wrists   (   ) Arms

(   ) Elbows   (   ) Shoulders

(   ) Shoulders   (   ) Chest

(   ) Upper Spine & Neck   (   ) Upper Back & Neck

(   ) Lower Spine   (   ) Abdominal Regions

(   ) Hips   (   ) Lower Back

(   ) Knees   (   ) Buttocks

(   ) Ankles   (   ) Thighs

(   ) Feet   (   ) Lower Leg

(   ) Other_____   (   ) Feet

                                                             (   ) Other_____

B.   HEALTH STATUS (✓Check if you currently have any of the following conditions)

(   ) High Blood Pressure   (   ) Acute Infection

(   ) Heart Disease or Dysfunction   (   ) Diabetes or Blood Sugar Level Abnormality

(   ) Peripheral Circulatory Disorder   (   ) Anemia

(   ) Lung Disease or Dysfunction   (   ) Hernias

(   ) Arthritis or Gout   (   ) Thyroid Dysfunction

(   ) Edema   (   ) Pancreas Dysfunction

(   ) Epilepsy   (   ) Liver Dysfunction

(   ) Multiply Sclerosis   (   ) Kidney Dysfunction

(   ) High Blood Cholesterol or   (   ) Phenylketonuria (PKU)

     Triglyceride Levels   (   ) Loss of Consciousness

( ) Allergic reactions to rubbing alcohol

** NOTE: If any of these conditions are checked, then a physician's health clearance will
    required.*

C.   PHYSICAL EXAMINATION HISTORY

Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of
physical exertion?   _____YES   _____NO

If YES, what limitations were recommended?_____

_____

D.   **FEMALE REPRODUCTIVE HISTORY**

If you are male, skip to Section E.

Did you begin menses within the past year?   _____YES   _____NO

Have you had consistent menstrual periods for the last 3 months?
YES_____   NO_____

Date of onset of last menstrual period_____

Have you used a hormonal contraceptive within the last 3 months?

YES_____ NO_____

E.   **CURRENT MEDICATION USAGE** (List the drug name, the condition being managed, and the length of time used)

MEDICATION   CONDITION   LENGTH OF USAGE

_____   _____   _____

_____   _____   _____

_____   _____   _____

F.   **PHYSICAL PERCEPTIONS** (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA   SED   PA   SED

( )   ( ) Chest Pain   ( )   ( ) Nausea

( )   ( ) Heart Palpitations   ( )   ( ) Light Headedness

( )   ( ) Unusually Rapid Breathing   ( )   ( ) Loss of Consciousness

( )   ( ) Overheating   ( )   ( ) Loss of Balance

( )   ( ) Muscle Cramping   ( )   ( ) Loss of Coordination

( )   ( ) Muscle Pain   ( )   ( ) Extreme Weakness

( )   ( ) Joint Pain   ( )   ( ) Numbness

( )   ( ) Other_____   ( )   ( ) Mental Confusion

G.   **FAMILY HISTORY** (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)

( ) Heart Disease

(   ) Heart Attacks or Strokes (prior to age 50)

(   ) Elevated Blood Cholesterol or Triglyceride Levels

(   ) High Blood Pressure

(   ) Diabetes

(   ) Sudden Death (other than accidental)

H.   EXERCISE STATUS

Do you regularly engage in aerobic forms of exercise (i.e., jogging, cycling, walking, etc.)?   YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly lift weights?   YES   NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly play recreational sports (i.e., basketball, racquetball, volleyball, etc.)?   YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

I.   EXERCISE PERFORMANCE (answer only what is applicable to your training)

What is your mile time?_____

What is your 5k time?_____

What is your 10k time?_____

What is your half marathon/marathon time?_____

What is your maximum back squat?_____

What is your maximum clean?_____

What is your maximum snatch?_____

Appendix C: Biopsy Care Sheet

CARE AND CLEANING OF YOUR BIOPSY SITE INCISION

Applied Physiology Laboratory
University of Kansas
Department of Health, Sport and Exercise Sciences

Following the percutaneous needle biopsy procedure, the incision is closed using steri-strip bandages and covered with a large sterile adhesive and pressure bandage. For the proper care of the incision site please use the following steps as a guideline. You will receive a 'care package' that will include additional steri-strips and large and small Band-Aids. Please return to the Applied Physiology Lab if you need assistance replacing or require additional bandages.

If you have any questions at anytime while the incision site is healing, please contact:
Andrew Fry, Ph.D. at **(785) 864-0784** or **Phil Gallagher**, Ph.D. at **(785) 864-0772** or **(785) 550-6300** for further instructions.

1. Keep the pressure bandage on for 24 hrs; and avoid getting the area wet during this time.

2. **The following day, report back to the testing coordinator to check the wound.** At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound.

3. If the steri-strip bandage does come off, immediately apply a new steri-strip taking care to position the edges of the incision as close together as possible. Please be sure to wash your hand before doing this to reduce the likelihood of infection.

4. Leave the area covered for at least 24-48 hrs. Keep the large adhesive Band-Aid on for 3 days & report back to the test coordinator.

5. At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound.

6. Leave the regular size Band-Aid on for an additional day. After day 4 you can remove the Band-Aid and the steri-strip. If you wish you can then just put a regular size Band-Aid over the incision site.

7. When washing, wash around the incision site taking care not to scrub directly over the incision or scab that may have formed.

8. Once you have washed the surrounding area, scrub a few inches above the incision site and allow the soap and water to trickle down over the site.

9. When drying off after washing, take care to lightly dab over the incision site – DO NOT rub over the incision site.

10. After about 3-4 days you may see a scab begin to form. Please allow the scab to heal and fall off without assistance.

11. You need to contact to project coordinator via phone one week after the biopsy or whenever necessary to ensure normal recovery.

The biopsy procedure often results in a small fine scar at the site of the incision, however, all care post-treatment will aid in reducing the potential for scarring. All care will be taken to aid in the healing of the wound. The entire biopsy procedure will be performed under sterile conditions. All testing staff and associated personnel will be trained in first aid and will be familiar with emergency procedures.

This procedure has been performed on numerous subjects by qualified personnel in many institutions worldwide with only slight discomfort being reported. Following the muscle biopsy, muscle function is not impaired. In fact, subjects have been reported to continue participation in sporting events immediately following a muscle biopsy.

It is common for subjects to experience mild soreness, moderate pain and bruising near the biopsy site, similar to a "Charlie-horse" the day after the procedure. It is beneficial to periodically apply an ice back to the site the following day to reduce any swelling or residual soreness. **Do not take any pain medication or over the counter pain relievers until after the final 72 hour post exercise biopsy.**

In order to allow the incisions to heal properly and minimize the risk of infection, you should avoid prolonged exposure to water for 4 days. Daily showers are acceptable, but baths, swimming, sauna's etc. should be avoided for 4 days following the biopsy procedure.