EMPOWERING OLDER ADULTS: POWER ASSESSMENT BASED ON TYPE IIX MUSCLE FIBER ABUNDANCE IN AGING

by

ARREANNA SAYLOR

A THESIS

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Title:
Empowering Older Adults: Power Assessment Based on Type IIx Muscle Fiber Abundance in Aging

Approved: *Damien Callahan, PhD*
Primary Thesis Advisor

Older adults lose power as they age from the result of multiple aging processes. Older adults loose muscle mass, in particular type IIx muscle fibers, as they age which results in a loss of power. The loss of power is different than just a loss of strength. Power is the product of force and velocity, whereas strength is force alone and at its peak when velocity is zero. Power loss is associated with poor balance and risk of falling. Research has found that specific interventions can increase power output in older adults, but there is not a substantial amount of research that has quantified how much of the change in power from the interventions is the result of the change in MHC IIx abundance. There are also no current models on the relationship between power and type IIx content. Therefore, I compared the ability of two prominent methods of assessing fiber type composition in muscle samples to model fiber type contribution to power production. Finding an accurate model would allow health care providers to better assess the mechanisms contributing to enhanced power output with intervention. I compared the two main methods used for finding muscle fiber abundance, immunohistochemistry (IHC) and sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE), to see which would better predict power. Together, these contributed to an algebraic predictor of muscle power, formulated based on seven healthy subjects. We measured peak power output of each subject using dynamometry. We then took muscle biopsies from the vastus lateralis of each subject and ran SDS-PAGE and IHC on their samples. I used a linear regression analysis to compare each methods prediction ability of power Surprisingly, neither IHC nor SDS PAGE were effective in predicting power output in our cohort. Alternate approaches to assessing the expression of MHC IIx may be necessary to better predict peak power output from voluntary contractions.

Acknowledgements

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Introduction

In my education at the University of Oregon, in my work as an intern for the University of Oregon's Sports and Performance Science Department, and in my work in the Muscle Cellular Biology Laboratory I have been able to learn how the composition of human skeletal muscle contributes to the production of contractile power. Contraction is the shortening of muscle with body movement. It pulls bones closer or further away to allow for motion. Contractile power is the product of contractile force and velocity. Power differs from strength, as strength is only contractile force. Studies have shown that muscle power can be indicative of things like elite sprinting capabilities as well as physical dysfunction of older adults (Trappe et al., 2015; Miszko et al., 2003). After learning how crucial power was, I wanted to apply what I had learned to the patients I get to see in my work as a physical therapy technician.

Muscle atrophy is the loss of muscle – occurring through age, injury, or disease. I work in a physical therapy clinic. Each day I spend time with patients who struggle to walk and move because they have suffered muscle atrophy with age (sarcopenia). Even if a person is a picture of health early in adulthood, nobody is safe from the plague of sarcopenia that occurs during aging. The loss of muscle leads to unfortunate complications like instability, and difficulty performing daily tasks (Miszko et al., 2003). Instability is a killer. According to the CDC "more than one out of four older people falls each year," and "one out of five falls causes a severe injury such as broken bones or a head injury" (CDC, 2015). Seeing the hardship sarcopenia brings for my patients and knowing the dangers it can pose, I knew that this portion of functional capacity is critically important for quality of life. Interventions such as resistance

training and stair climbing have shown to increase muscle power, but it is not clear which interventions are most effective at altering muscle power in ways that directly target the age-related changes to that lead to power deficits. The reason I will focus on the power is that power is not only important for the creation of our movements and stability, but power is a good predictor (better than strength) of mobility performance (Marsh et al., 2006).

My research into this problem begins with the cellular structure of muscle tissue. Muscle is composed primarily of long fibers -- the cells which make up a person's whole muscle and allow the tissue to perform its primary task, to generate force and shorten. Within the muscle fiber is an even smaller component called the muscle fibril, which is made up of proteins actin and myosin. The human body has three types of muscle fibers, defined by their fibril content, each adapted to perform distinct functions. These muscle fibers are classified by the myosin heavy chain (MHC). The pure MHC isoforms are I, IIa, and IIx. Type I is a muscle fiber geared towards endurance in activities, and Type IIx is geared towards speed. Research has shown that both forms of type II, especially type IIx have a higher power output (Fitts et al., 1991; Methenitis et al., 2016). Type IIx is the fastest muscle fiber. However, in a typical population pure type IIx only makes up two percent of muscle fiber composition (Trappe et al., 2015). Along with the three main fiber types there are also three main hybrid types of muscle fiber - the main presentations of these are: I/IIa, IIa/IIx, I/IIa/IIx (Trappe et al., 2015). A majority of most individual's MHC IIx resides in hybrid form. Research has shown that individuals with a higher percentage of type IIx muscle fibers, or larger IIx fibers, in its pure and hybrid form have a faster rate of force development and are faster than

individuals with more type I (Korhonen et al., 2006; Trappe et al., 2015). As we age changes in our body lead to a reduction of power. One reason power declines is that our muscle composition changes favoring loss of powerful type IIx muscle fibers (Lexell et al., 1988).

There are two main methods that allow us to see the abundance of each type of muscle fiber within a muscle: immunohistochemistry (IHC) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). IHC allows us to measure morphology (cross sectional area) and protein expression with muscle fiber isoform specific antibodies. The spatial and protein specific distinction allows researchers characterize the size of muscle fibers by type, including identification of hybridized fibers. By contrast, SDS-PAGE separates a larger, mixed muscle sample by the relative abundance of MHC isoforms expressed within it. However, because the sample is homogenized, fiber morphology and co-expression at the cellular level is lost.

The purpose of my research is to identify which method of assessing MHC IIx content better predicted power output, to allow health providers to assess the mechanisms that might be responsible for enhanced power following successful intervention. My question is: is using IHC or SDS-PAGE better for predicting the power output using MHC IIx abundance? This information will be able to inform future studies that aim to change the relative abundance of contractile proteins in the muscle of older adults with the intent to increase power. Our study will help future efforts by providing an obvious choice regarding the most appropriate means of assessing power with fiber type abundance.

Background

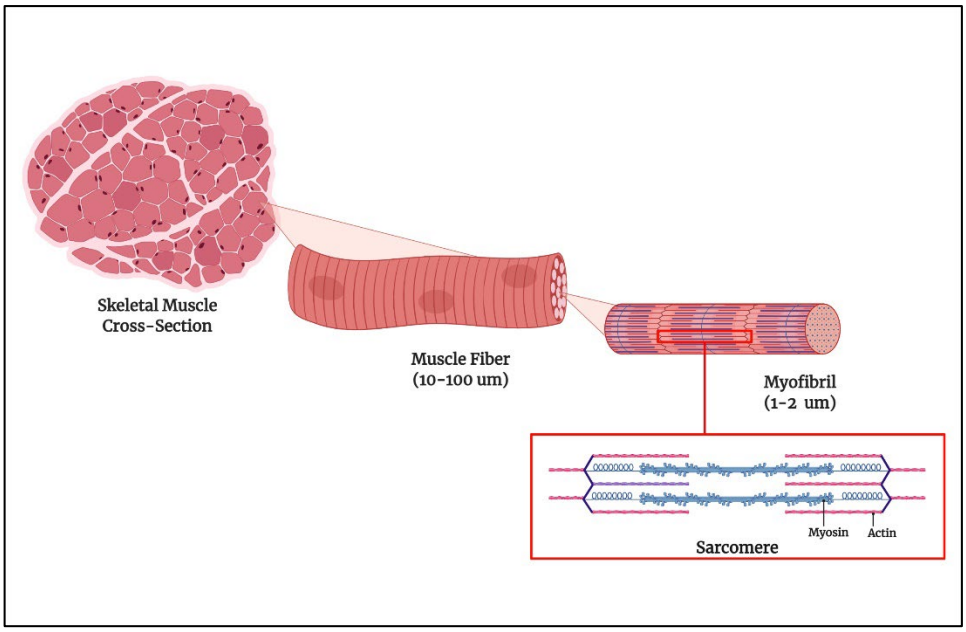

Figure 1. Skeletal muscle composition

This figure illustrates the components of skeletal muscle. It shows how muscle is composed of groups of muscle fibers. The figure also illustrates the placement of myosin within the skeletal muscle. This figure is referenced from "Hierarchical fibrous structures for muscle-inspired soft-actuators" (Gotti et al., 2020). Image created on BioRender.

Skeletal Muscle Fibers

To understand why the type of myosin heavy chain is important, it is necessary to understand how all the components of a muscle fiber interact to create the movement of the human body. The sarcomere is a functional unit that makes up skeletal muscle fiber (Scott et al., 2001). Within the powerhouse that is the sarcomere (Fig 1) there are myosin filaments, actin filaments, and regulatory proteins: troponin, and tropomyosin (Scott et al., 2001). One sarcomere is one length of myosin filament paired with one length of actin filament and regulatory proteins.

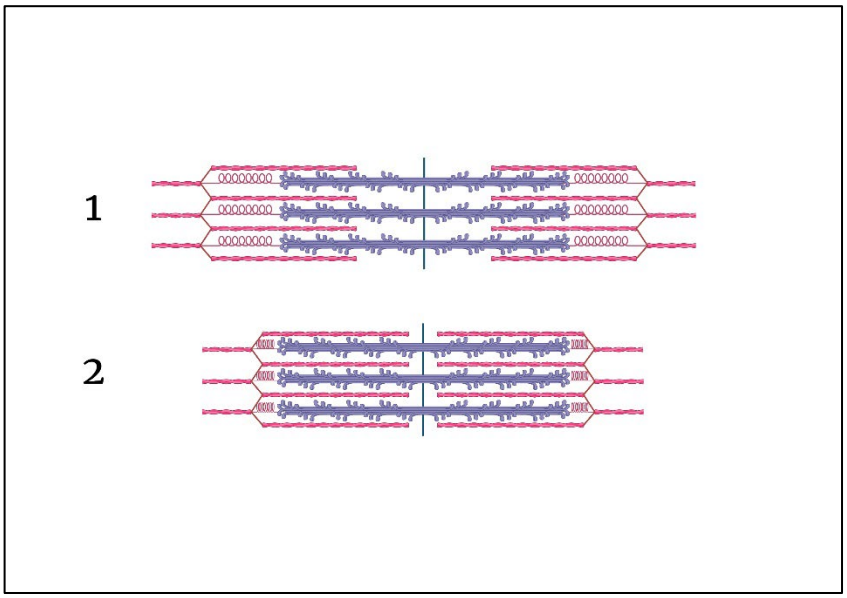

Figure 2. Actin and myosin overlap with sarcomere contraction

This figure pictures a sarcomere which is the grouping of actin and myosin. The myosin is represented by the blue component of the drawing and actin is represented by the red. The number one shows the muscle fiber when it is relaxed. Two shows the muscle fiber when is contracted. This figure is referenced from "Hierarchical fibrous structures for muscle-inspired soft-actuators" (Gotti et al., 2020). Image created on BioRender.

The actin filament spins in spiraled chains and has an activation site found on its face (Scott et al., 2001). Tropomyosin spans the face of actin, and when the system is at rest (Fig 3) it blocks the actin activation site. The troponin sits on the actin and acts as a binding site for calcium, which I will discuss in more detail momentarily. The myosin heavy chain is on the outside of the actin, and throughout the myosin there are globular structures called the myosin heads, which bind to the binding sites on actin (Scott et al., 2001). Myosin is a motor protein and when it binds to actin it creates the movement of the sarcomere (Fig 2).

Motor Units

Understanding the basics of the skeletal muscle is the precursor to understanding muscular contraction. Muscular contraction (shortening) allows people to produce their movements and power. Muscular contraction occurs through motor units. A motor unit is a motor neuron (a neuron connected to skeletal muscle) and the muscle fibers it innervates. Neurons are nerve cells that send signals throughout the body to create movements. There are specific motor units to the type of muscle fiber they are innervating. Slow motor units innervate slow muscle fibers, fast fatigue resistant innervate medium speed fibers while fast fatigable innervate fast fibers.

Contraction begins when motor neurons in the spinal cord receive excitation signals from the cortico-spinal tract. These signals, called action potentials, are then relayed down the stem of the neurons called the axon. The action potentials travel down the axon until they meet the neuromuscular junction, the area where the neuron meets the muscle fiber. Motor units are recruited from slow to fast fatigue as is demanded by the intensity of the movement a person is performing. All movements recruit slow motor units. Simple movements like walking only recruit slow motor units, while the most powerful forceful movements recruit fast fatigable motor units. The process of sending the signal to the contraction of the muscle is referred to as excitation contraction coupling (ECC).

Cross-Bridge Cycling

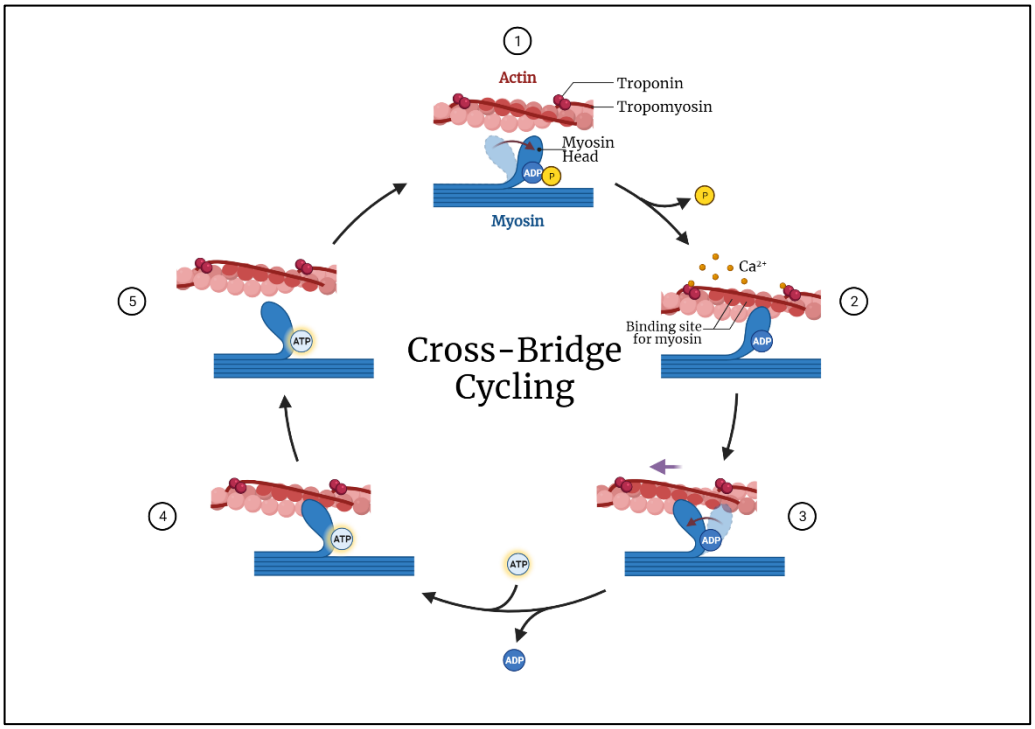

Figure 3. Steps of cross-bridge cycling

This figure provides a visual representation of the actin filament and its interaction with troponin, and tropomyosin at rest – this is a portion of excitation contraction coupling called the cross-bridge cycle. At the top it shows myosin before it is bound to actin. It then shows the binding and contracting of the head in steps two and three to create muscle contraction. It then shows a visual representation of the myosin heavy chain with the myosin head releasing from actin as ADP is traded for ATP. Cycling back to step one the ATP is converted to ADP and the myosin is ready to bind again. Image created on BioRender.

When the action potential reaches the neuromuscular junction, it triggers a process called cross-bridge cycling. Cross-bridge cycling is the interaction between myosin and actin which makes the sarcomere shorten and in doing so shortens the muscle (Fig 2).

There are two substances (calcium and adenosine triphosphate) crucial for excitation contraction coupling. Calcium is not just what we get from milk - calcium is crucial for many systems throughout the body including ECC. Calcium is an intracellular messenger that can bind to different sites, like troponin, in cellular systems and activate them (Schiaffino & Reggiani, 2011). ATP is a source of energy within the body when it is hydrolyzed into phosphate (P) and adenosine diphosphate (ADP) (Matsuoka, 2004).

Each component of the sarcomere works together to allow for the binding of the myosin head and the actin filament during ECC. After the action potential reaches the neuromuscular junction, the signal tells a storage unit in the muscle cell, the sarcoplasmic reticulum (SR), to release calcium it is holding (Schiaffino & Reggiani, 2011). When the SR releases calcium the calcium binds to troponin, which then triggers the movement of tropomyosin away from the actin binding sites. While this process is occurring and making actin ready to bind, myosin functions as an enzyme to break down ATP. Energy is transferred from the high energy phosphate bonds of ATP to the globular head of MHC, which is coupled with the cocking of the myosin head. When the myosin head is cocked, it is then positioned to bind the actin. After these processes have occurred the myosin head binds the actin (Fig 3). This head then pulls back and shortens the sarcomere creating more overlap of actin and myosin (Fig 2; Fig 3).

Myosin Heavy Chain

The main three muscle fiber types: type I, type IIa, and type IIx all have differing properties that make them advantageous for certain activities out of the many movements our bodies can produce. The content of the MHC isoform determines how

the muscle fiber will contract and the properties of that contraction (Andruchov et al., 2004). Each fiber roughly produces the same amount of force. The fibers are distinguished based on their contraction speed. As discussed, fiber type I is slow because it has a slow contraction, while both types of fiber type two are faster because they contract more quickly. The contraction velocity depends on how quickly the globular head of MHC hydrolyses ATP and progresses through steps of the cross-bridge cycle. The rate of hydrolysis is an inherent property of each fiber. Type IIx has the fastest rate of hydrolysis, type IIa has a slower rate, and type I has the slowest rate.

Immunohistochemistry and SDS Page

We can understand MHC isoforms using the two main analytic approaches: IHC and SDS-PAGE. Whereas IHC of human skeletal muscle relies on a single, roughly 8-micron thick slice of tissue, approximately one hundred microns in diameter, SDS-PAGE uses a far larger sample, up to twenty micrograms of tissue. In SDS-PAGE muscle samples are homogenized, proteins are extracted and treated so their amino acids accept a uniform charge. Thereafter, the muscle homogenates are separated by gel electrophoresis, a technique by which proteins within the sample are sorted by molecular weight. The heaviest proteins are pulled the least distance because they are the hardest to move through the gel. Proteins of identical weight will migrate the same distance along the gel. This technique is effective for separating MHC isoforms because they have slightly different molecular weights. At the conclusion of the run, gels are stained with a non-specific dye to reveal all proteins. Bands corresponding to the weight of identical proteins will be large and dark or small and light based on the abundance of protein and thus the intensity of the stain. By comparing the relative intensity of this

stain, one can measure the relative abundance of the 3 MHC isoforms present in human skeletal muscle.

In IHC the muscle fibers section is frozen and cut (like the way you would slice salami, but much thinner) to where it displays a cross-section of the muscle. The muscle slice is then displayed on a glass plate and sent through a series of antibodies and washes to ensure specific antibodies are bound only to fibers expressing their respective protein targets. When imaged the whole muscle slice can be seen with each muscle fiber type illuminated at different wavelengths. This is beneficial because it allows you to see the morphology and co-expression of the muscle – IHC highlights the size and content of a muscle fiber displaying amounts and size of pure and hybrid muscle fibers (Murach et al., 2019). It has been shown that IHC can be advantageous in identifying muscle fiber types including pure fibers and hybrids when compared to SDS-PAGE (Murach et al., 2019).

Aging, Sarcopenia, and Falls Risk

Research by Jan Lexell, Charles C. Taylor, and Michael Sjostrom pioneered the understanding of how sarcopenia affects older adults. In their research they took whole vastus lateralis muscles from cadavers, who were previously physically healthy men ages fifteen to eighty-three (Lexell et al., 1988). They obtained data on muscle area, fiber number, fiber size, and fiber proportion and distribution. They found that the decline of fiber numbers begins at the age of twenty-five and with age type II muscles fibers decreased in size more than type I (Lexell et al., 1988). The research by Lexell set the foundation for understanding sarcopenia in older adults. It is now understood that

type IIx muscle fibers are selectively lost in aging, and that can lead to the power reduction of older adults (McKinnon et al., 2017; Roberts et al., 2018).

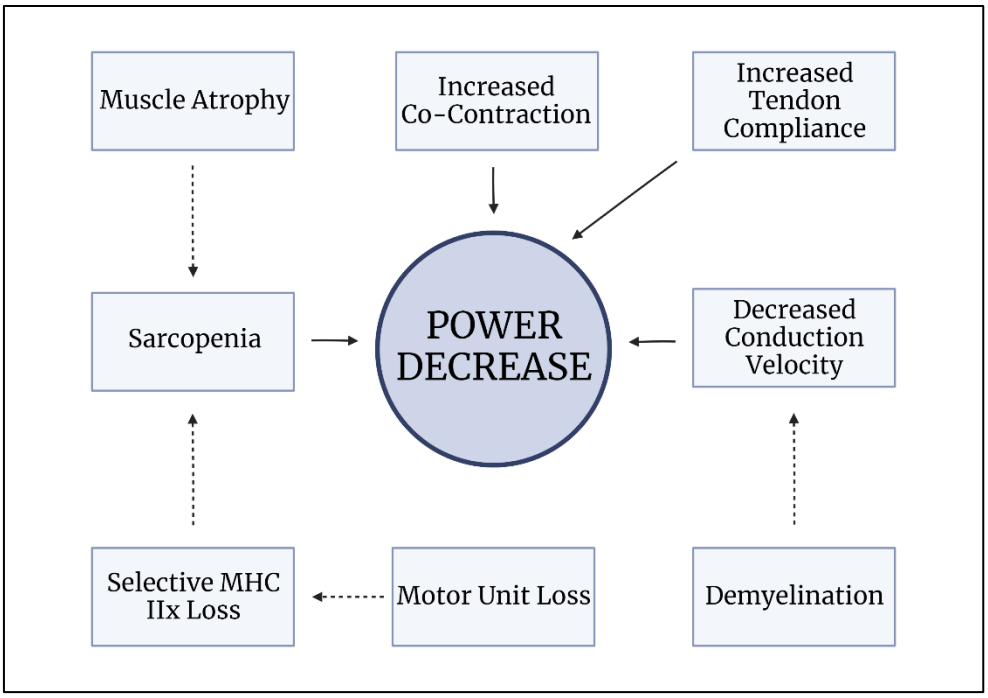

Figure 4. Contributions to power loss with aging

This figure illustrates how muscle fiber changes with age, and the impacts of this. It shows change in myelination, myosin, and motor units. The changes in the body lead to a decrease in power. This figure is referenced from "Neuromuscular contributions to the age-related reduction in muscle power: Mechanisms and potential role of high velocity power training" (McKinnon et al., 2017). Image created on BioRender.

Age-related reduction of power occurs in the motor units in conjunction in changes with neurons (McKinnon et al., 2017). As people age, they lose their motor units (Fig 4) (Clamann, 1993). Their motor neurons also change, losing the integrity of their myelination and demyelinating (Fig 4) (Madden et al., 2004). Both properties complement each other in the reduction of the ability to conduct and send motor commands (McKinnon et al., 2017). Demyelination leads to a decrease in conduction

velocity, reducing the control of motor signaling. The loss of myelin and of motor units reduces the body's ability to quickly communicate what movements it wants to create, reducing velocity and thus reducing power.

Another contributor to reduced power with aging also relies on the signaling through motor units and neurons – this contributor is the change in co-contraction (McKinnon et al., 2017). In a normal movement the antagonist muscle to a movement is inhibited to allow motion to occur. Co-contraction is when normal inhibition does not occur in a movement and the agonist and antagonist muscle are both contracted at the same time. Because aging reduces the net torque about the joint, this inhibits an older individual's ability to create power (McKinnon et al., 2017).

Increased tendon compliance also reduces power with age. It has been shown that stiffer tendons increase power (Kurokawa et al., 2001). With age tendons become less stiff and so it allows muscles to stretch further, and it is more difficult to exert force on bones (McKinnon et al., 2017; Narici et al., 2009). By allowing muscles to stretch further the sarcomere is more stretched, so the sarcomere is at a less optimal length (Narici et al., 2009). The extra stretch of the tendon reduces the intrinsic force of the muscle, reducing power (Narici et al., 2009).

The change most important to note for the main questions of this thesis is the change to the muscle fiber content experienced through sarcopenia. It has been shown that with age MHC IIx size and abundance are decreased more significantly than other fiber types, as motor units are lost (Rowan et al., 2012; Lexell et al., 1988). This can be explained by metabolic needs and the principle of 'use it or lose it.' Due to their high metabolic demands and late recruitment type II motor axons are at risk for oxidative

stress and apoptosis (McKinnon et al., 2017). Apoptosis is the process of maintaining important tissues and cells by allowing for the death of less important cells. As people age, they experience oxidative stress from their environment. This stress causes death of cells, and to protect the most important cells for living the body allows for the death of the least important cells first. Because fast fatigable motor neurons and IIx fibers are recruited last and not used in most movement, the body allows type fast motor neurons and MHC IIx fibers to die first to protect the slow motor units and fiber that are recruited for all movement. Reducing MHC IIx corresponds with a significant reduction of power which leads to an increase in fall risk (Fig 4).

Predicting Muscle Power Based on MHC IIx Assessment

Subjects

Seven healthy individuals from ages (18-35) participated. There were four male subjects and three female subjects. The subjects included both resistance trained and non-resistance trained individuals. The subjects were taking part in a larger study on fatigue in the Muscle Cellular Biology Lab, so the methods of the research for this thesis include some of the larger study's methods.

Dynamometry

Subjects were familiarized with the dynamometer on the day of testing. The BioDex System 3™ Dynamometer (Biodex, USA) was used. The subjects sat in an upright seated position on the dynamometer with their hip and knee flexed at 90 degrees aligned with the dynamometer axis of rotation. Range of motion for the subjects was set to approximately 90 degrees (180 degrees being full leg extension). Subjects then performed three maximum voluntary isometric contractions (MVIC), with a one-minute rest in between each contraction, for the measurement of their peak isometric torque. Subjects left the dynamometer, and under local anesthetic (1% lidocaine) an incision was made on the vastus lateralis muscle and immediately closed with Steri-Strips™ (3M). Subjects then immediately returned to the dynamometer and performed a fatiguing knee extensor exercise to task failure. The fatiguing protocol is a part of the larger study; however, this is when the measurements of peak power were also taken. Subjects were then instructed to kick out as hard and quickly as possible at 30 % of their MVIC in time with a metronome set to 40 bpm. The subjects were given strong

verbal encouragement as well as visual feedback of their force trace. Raw analog data was converted to digital data using an A/D converter (CED,UK) and recorded using Spike2 Software (CED/UK) with a sampling rate of 500Hz. The data was then analyzed on MATAB 2022a (MathWorks, Natick MA). Peak power (P; Watts) was defined as the largest positive product of the torque-time and velocity-time curves in.

Biopsy

A bilateral muscle biopsy of about two hundred milligrams was taken of the vastus lateralis from each subject. The muscles samples were then blotted clean and removed of fat and connective tissue. The samples were then either transferred to a 4 degree Celsius dissecting solution (120mM NaMS, 5mM EGTA, 0.1mM CaCl2, 6 mM MgCl2, 0.25mM KH2PO4, 20mM BES, 1.8mM KOH, 1mM DTT, and 5mM ATP-Mg; pH = 7.0) flash frozen in liquid nitrogen at -80 °C or embedded it in O.C.T gel (Tissue Tek) frozen in liquid isopentane and stored at -80 °C for IHC analysis.

Ultrasound

Ultrasound was used to find vastus lateralis thickness. To begin, the vastus lateralis was marked at the point half-way between the greater trochanter and the popliteal crease. A water-based gel was applied to the marked point on the subject. B-mode ultrasound was used with the Phillips IE33 for imaging. Minimal pressure was applied to avoid compressing the muscle. Three images were taken to ensure operator consistency. The images were processed and analyzed using software from FIJI (Image J). Three measurements of muscle thickness were taken for each image and then averaged to account for varied thickness along the length of the muscle. Muscle

thickness was defined as the vertical distance between the superficial and deep aponeuroses.

IHC

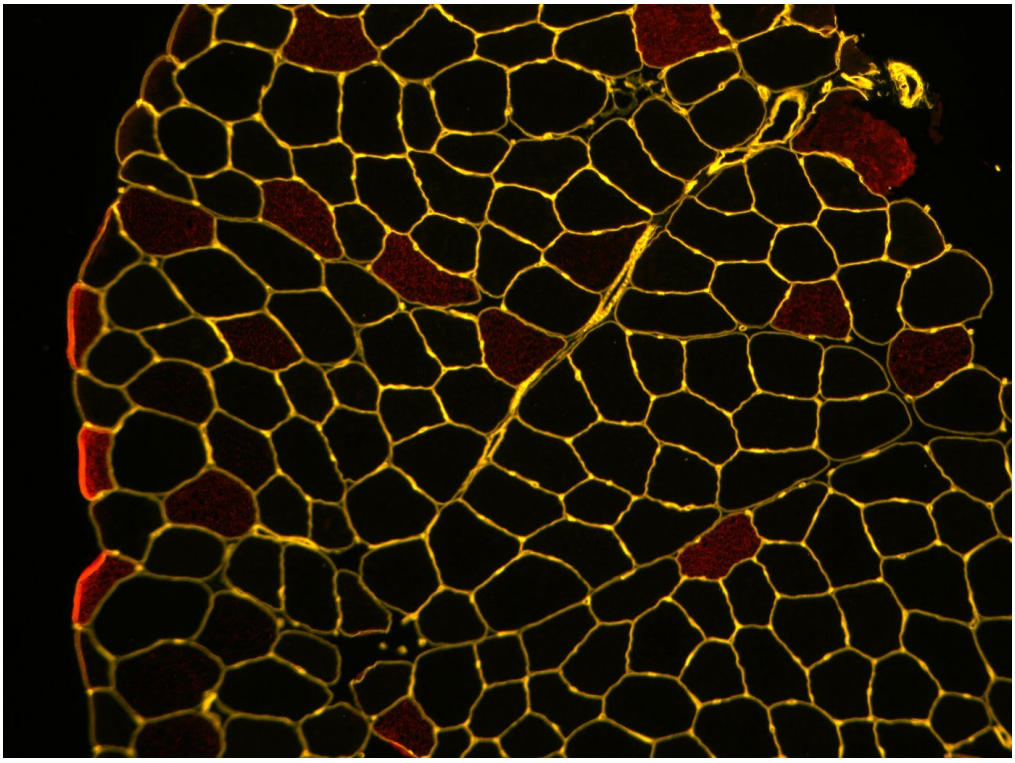

Figure 5. IHC staining for laminin and MHC IIx

This image is a muscle sample image captured while performing IHC. Each circle is a muscle fiber. The red stained circles are type IIx muscle fibers. The yellow stain is laminin. This image was used for statistical analysis.

To begin IHC the muscle fiber sample was mounted on the cryostat chuck and cut into serial cross-sectional slices eight micrometers thick. The tissue slice was then mounted onto a glass slide. A pap-pen was used (Science Device Laboratory) to draw around the slice to provide a hydrophobic barrier and was then allowed to dry overnight. Proceeding, the slice was washed with PBS (phosphate buffer saline) 1%

BSA (bovine serum albumin). The tissue slice was incubated with primary antibodies overnight. The following day the tissue slice was gently washed with PBS to remove any extra antibody that could create noise in our images. Then the tissue slice was incubated with secondary antibodies for one hour. The tissue slice was then put through a series of washes. Drops of mounting media and DAPI (Invitrogen) were applied to the tissue and a cover slip was placed over the tissue. The slides were stored in the dark at 4 ° C until imaging. For imaging, the samples were placed under a microscope (Leica) at 10X and 20X for assessment. The images were captured at a wavelength range of 490-510nm for DAPI excitation, 520-550nm for DAPI emission, 625-655nm for MHC I excitation, 665-715nm for MHC I emission, 532-558nm for MHC IIa and laminin excitation, 572-648nm for MHC IIa and laminin emission, 570-590nm for MHC IIx excitation, and 602-662nm for MHC IIx emission. The images were captured with an exposure of four hundred for muscle fibers, two hundred for laminin, and one hundred for nuclei. The primaries (Developmental Studies Hybridoma Bank) were DSHB BA-D5; IgG2b for MHC I, DSHB SC-71; IgG1 for MHC IIa, and DSHB 6H1; IgM for MHC IIx. The secondaries (Invitrogen) were goat anti-mouse Ig2b AlexaFlour 647 for MHC I, goat ani-mouse IgG1 AlexaFlour 488 for MCH IIa, and goat anti-mouse IgM AlexaFlour 568 for MHC IIx. Laminin was stained with rabbit polyclonal antibody IgG.

SDS-PAGE

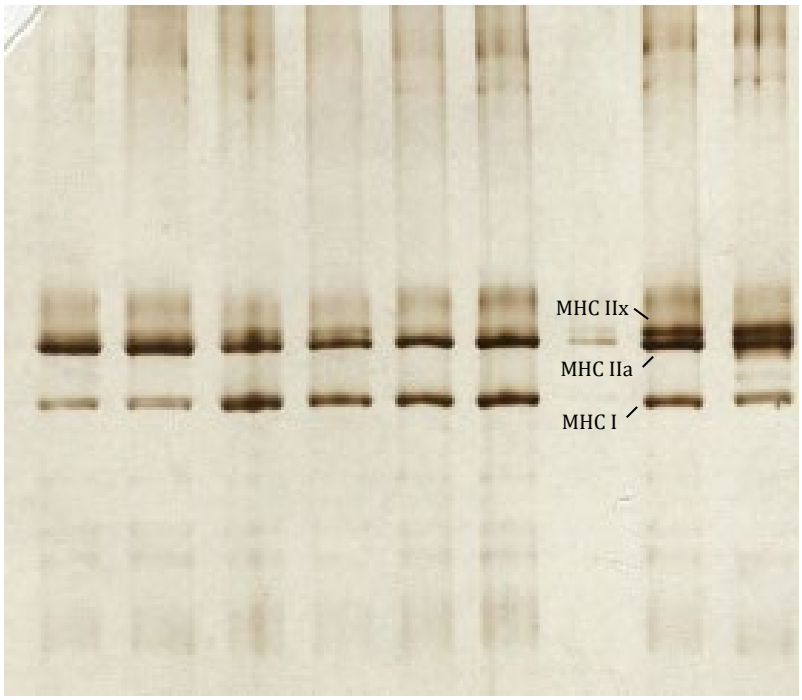

Figure 6. SDS-PAGE protein distribution

This image is a scanned in gel from one of the subjects. The first nine lanes show the composition of the whole muscle fiber sample. MHC IIX, IIa, and I bands are labeled on the figure. This image was used for statistical analysis.

The resolving gel contained 7% acrylamide/Bis (50:1; w/v), 1.5M Tris/HCl (pH 8.8), 1.0 M glycine, 4% SDS, 30% glycerol and the stacking gel contained 4% acrylamide/Bis (50:1; w/v), 0.5M Tris/HCl (pH 6.8), 0.1M EDTA (pH 7.0), 4% SDS, 5% glycerol. For polymerization, the upper and lower chamber running buffers consisting of 1X SDS and 0.5X SDS, respectively, was poured in before the protein was loaded. 2-5 µl protein standard from one subject (1-3mg) was placed into the gel. The gels were run for 3.5 hours at 70V and then 20 hours at 200V. After electrophoresis was complete, the gels were stained with a Pierce Silver Stain Kit (BioRAD) and were either immediately dried or stored in a gel drying buffer (10 glycerol, 20% ethanol) at 4 °C. The MHC isoforms displayed were MHC I, MHC IIa, MHC IIx. MHC IIx is the

heaviest band and so it is the furthest towards the top of the gel, whereas MHC I is the lightest, so its band is the furthest towards the bottom (Fig 6).

Statistical Analysis

Data analysis began with finding the abundance of each muscle fiber type for both IHC and SDS-PAGE. For both methods, the FIJI macros of Image J (NIH, Washington D.C.) were used to quantify relative MHC abundance. For IHC the cross-sectional area (CSA) of each type IIx muscle fiber was found and expressed as a percent of the whole muscle sample CSA. For SDS-PAGE the integrated pixel density corresponding to each MHC isoform was found for each subject's muscle sample. The background integrated density was subtracted from each band and then made a percent of sum for all band's integrated density. To normalize peak power to muscle size for each volunteer, power was divided by VL thickness as assessed via ultrasound. This is important because a larger vastus lateralis could hold more muscle fibers, increasing power without requiring altered fiber type. Each subject's value was compared to their measured power. Lastly, two linear regressions models were based on normalized power and the two means of assessing MHC IIx abundance. The p-value and R^2 values were compared to assess statistical significance and predictive power of the correlation, respectively. The best predictor was then used to create a predictive model.

Model Creation

To create the model, the graph with the highest R^2 value was used. The equation of the graphs best fit line became the model. For this graph percentage was a decimal

point instead of a whole number, so the math would be accurate. The x and y-values were exchanged for the variables from the graph.

Results

Subject #	VL Thickness (cm)	IHC MHC IIx % CSA	SDS-PAGE MHC IIx % CSA	Normalized Power To VL Thickness (W/cm)
1	2.11	12.25	33.87	114.91
2	2.39	5.10	25.73	145.01
3	2.30	9.44	29.57	202.56
4	2.93	2.91	23.47	220.10
5	2.26	9.10	23.71	245.91
6	1.98	4.04	39.07	295.36
7	2.70	5.86	26.01	403.84
Mean	2.38	6.96	28.78	232.53
Standard Deviation	0.33	3.37	5.82	96.63

Table 1. Subject Data

This table shows the values for each subject's vastus lateralis thickness, IHC MHC IIx percentage, SDS-PAGE MHC IIx percentage, and normalized power value. Subjects were sorted from lowest to highest power and numbered one to seven.

IHC

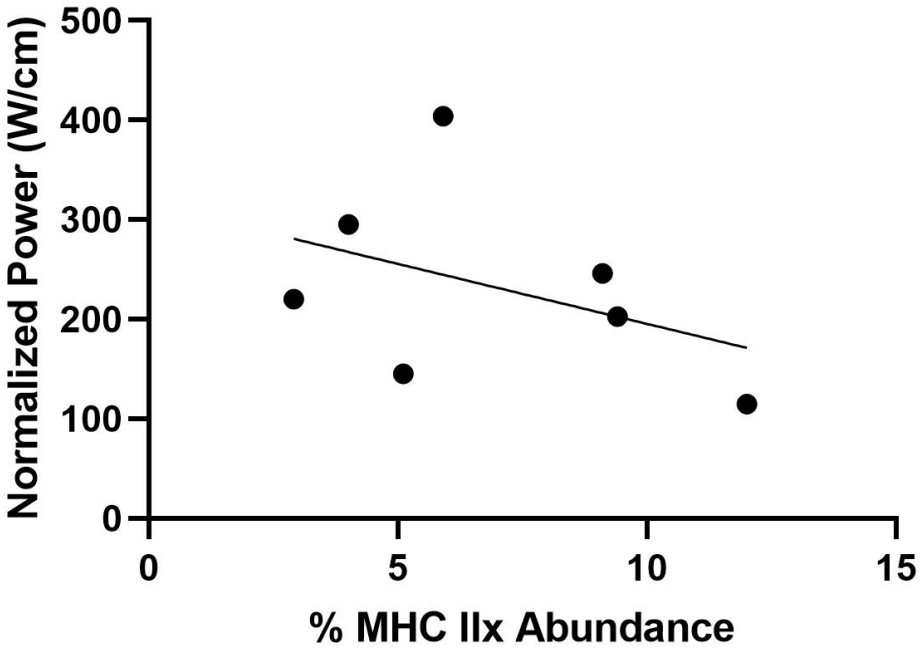

Figure 7. IHC linear regression

This figure shows a comparison of normalized power to IHC Type IIx %. The line represents the linear correlation between the two variables. R^2 is 0.18. The p-value is 0.34.

The results of linear regression between normalized power and the MHC IIx percentage found with IHC had a weak relationship, and no significant correlation. The low R^2 value, as R^2 represents the strength of the correlation between the two variables on a scale from zero to one, demonstrates a weak relationship (Fig 7). The p-value being greater than 0.05 demonstrates that the correlation is not significant.

SDS-PAGE

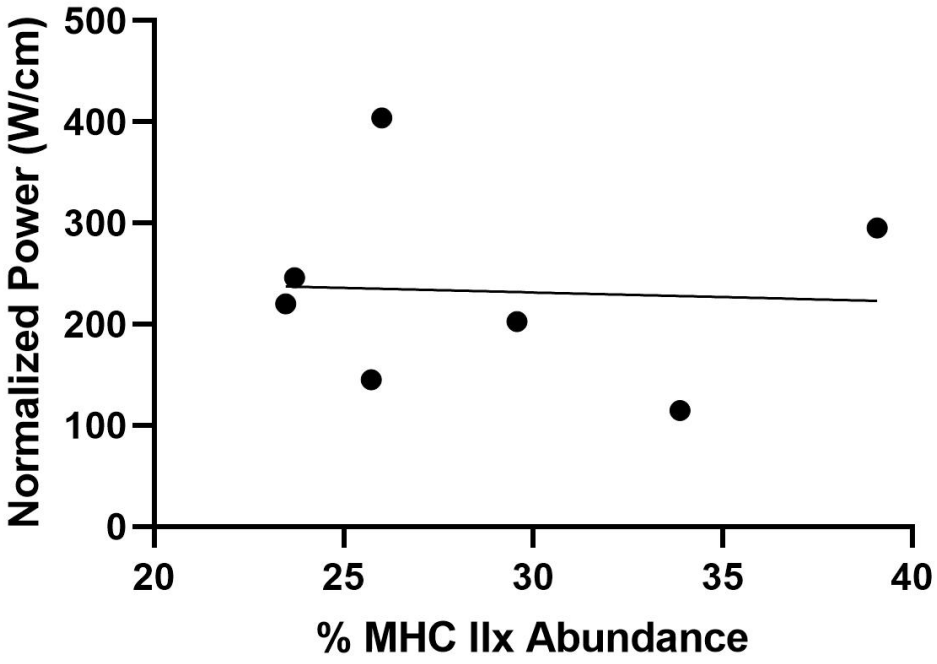

Figure 8. SDS-PAGE linear regression

This figure shows a comparison of normalized power to SDS-PAGE Type IIx %. The line represents the linear correlation between the two variables. The R^2 value is 0.00. The p-value is 0.91.

The results of linear regression between normalized power and the MHC IIx percentage found with SDS-PAGE had almost no predictive power, and no significant correlation. These findings are displayed by the exceptionally low R^2 value (Fig 8). The correlation is not significant because the p-value was greater than 0.05 (Fig 8).

Comparing SDS-PAGE and IHC

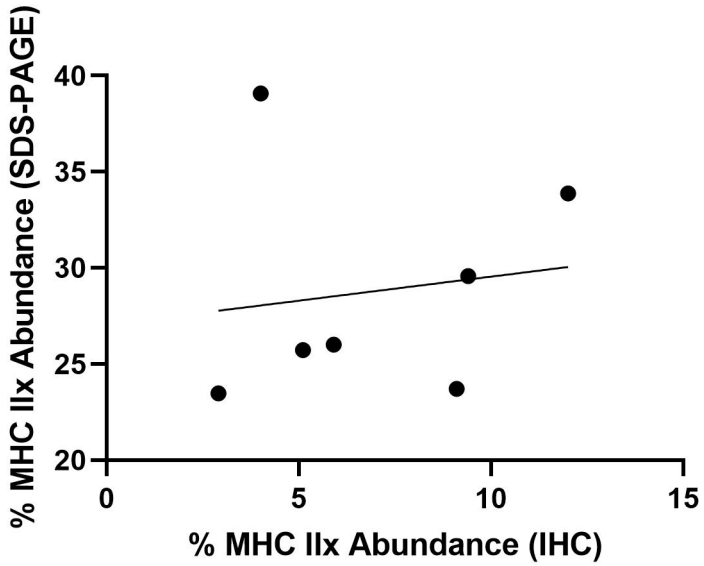

Figure 9. SDS-PAGE V. IHC

This figure shows the comparison of SDS-PAGE to IHC. The R^2 value is 0.02 and the p-value is 0.74.

The results demonstrate that the relationship between the percentage of MHC IIx abundance using IHC and SDS-PAGE has almost no correlation as the R^2 value is almost zero and are not statistically significant (Fig 9).

Model

$$Normalized\ Power = -1210(MHC\ IIx\ \%) + 317$$

Figure 10. Model for normalized power prediction

This model shows that normalized power is equal to MHC percent multiplied by -1210 plus 317.

The method that had the highest R^2 value was IHC. The line of best fit from the IHC graph was to create the model (Fig 10)

Discussion

It makes sense that MHC IIx would have a considerable influence on power. As discussed in the background section, physiologically, type IIx fibers create fast and forceful movements, i.e., powerful movements, because the MHC IIx isoform of myosin completes its cross-bridge cycle and hydrolyzes ATP more rapidly than other isoforms. Also, type IIx has shown to be a good predictor of individuals who run faster and have a faster rate of force development (Korhonen et al., 2006). Rate of force development is related to power because it depends on the speed at which myosin can engage actin. Research has also shown that MHC IIx produce the largest peak power when isolated (Methenitis et al., 2016). It would thus make sense to be a reliable predictor of power, because as the number of MHC IIx fibers increase a person's overall power should increase; however, there are multiple reducers of power that come with aging (Fig 4).

Interventions

Interventions that target power increase in older adults could be influencing any of the properties related to power reduction. However, targeting IIx fibers is the best way to increase power, because MHC IIx creates the power of our muscles. We cannot increase the number of IIx muscle fibers an individual has – changing another muscle fiber to IIx, even with training, is not possible. The way we can change the properties of IIx fibers is by using exercise to increase CSA of the existing fibers (Blocquiaux et al., 2020). There are many interventions that have been successful in increasing power by trying to target IIx fibers.

Resistance training has been a popular muscle-targeted intervention in improving power in older adults. Resistance training is when muscles contract against an external resistance like weights. Individuals over the age of 90 showed increase power when resistance training consisting of leg press and bench press was given (Cadore et al., 2014). In comparing three different methods of resistance training, machine stable, machine unstable, and unstable free-weight training, Eckardt found that each intervention significantly increased power (Eckardt, 2016).

Providers also widely use power training for improving performance in older adults; however, it is unknown whether the power training interventions improve power exclusively through changes in morphology or composition. Comparing a power trained group to a control group, power was significantly increased in the power trained group (Henwood & Taaffe, 2005). Increased velocity specific to task (InVEST) training, where task-specific movement patterns were resisted with a weighted vest and then done as quickly as possible, showed significant increase in power of the elderly (Bean et al., 2009). Power training, with a fast knee extension and leg press, compared to resistance training with the same movements also had a significant increase in the power of an elderly population (Marsh et al., 2009).

In contrast to resistance training power training utilizes quick movements to increase power output. Power output has shown to have a higher increase in power of older adults compared to resistance training in multiple studies (Drey et al., 2012; Marsh et al., 2006; Reid et al., 2013). However, there are some studies that have shown both types of training have no significant difference in effects on power (Henwood et al., 2008).

For both types of training interventions, it is unknown how much MHC IIx CSA change is contributing to the change of power measured. Therefore, a model is necessary. The model would allow medical providers to quantify if their interventions are effectively changing muscle fiber content to increase power. If their interventions were increasing power but not CSA of IIx fibers, then they could try other interventions that could better increase the power of their patients.

Relationship of IHC and SDS-PAGE

As the relationship between IHC and SDS-PAGE had almost no correlation or significance, it demonstrates that there is variance between both methods. What this means that neither method may be an accurate predictor of power. The methods should be very strongly correlated because they are both measuring MHC IIx abundance. The values of IHC are smaller because they are only measuring pure IIx instead of hybridized and pure IIx. Despite this the values still should be correlated, but because they are not it shows either method is not necessarily good predictor of power.

Model

Interestingly, neither of our measures of MHC IIx abundance were associated with variation in normalized power across subjects. Both measures were not statistically significant (Fig 7; Fig 8). This means the model would not be a strong representation of the relationship between MHC IIx abundance and power. For demonstration purposes only the model was still created using the measure that had the higher R^2 value. The model had a negative value being multiplied by percent of MHC IIx, which would indicate that as MHC IIx percent increases normalized power decreases. The decrease

in power with increasing MHC IIx percent physiologically does not make sense. As discussed earlier, IIx is the most powerful muscle fiber so as the number of IIx increase power should increase as well. The small sample size led to the negative relationship seen. There are not enough subjects to account for the variance of individuals. This means the relationship found was weak, and not well representative of the larger population. This also displays how power is not just dictated MHC IIx abundance (Fig 4). Although MHC IIx is a big contributor to power, individuals have other factors that could be impacting their power output like mobility or tendon compliance (Fig 4). For example, subject number two had the highest MHC IIx % for IHC and the second highest for SDS-PAGE but had the lowest power value (Table 1).

Limitations

My research had three major limitations. The first limitation was the small subject pool. There were complications with data storage, and the power data on some subjects were lost. Fortunately, this issue was resolved and seven subjects who had power data that were able to have IHC and SDS-PAGE run on their samples. More subjects diminish the effects of variance between individuals, so the more subjects the better (Martínez-Mesa et al., 2014). Having more of these subjects could also improve the model made, as this model was created using the values from most accurate linear regression. By diminishing the impacts of variance, the model could be refined. The impacts of having a small data pool were reflected in the statistical analysis, because the graphs both had weak correlations and high p-values (Fig 8 & Fig 9). To address this limitation further research on more subjects should be done.

The second limitation was that there were not any older adult subjects. The model was not able to be tested on older adult patients, so the accuracy of the model on this demographic is unknown. Also, older adults typically have lower power values, so adding them to the data set could help create a stronger relationship between power and MHC IIx abundance by introducing a new range of power values. To address this limitation further research on older adult subjects should be done to confirm the model can accurately predicts their power values.

The third limitation was that the power values recorded from the biodex were of the whole quadriceps, not just the vastus lateralis. Some individuals could have muscles that exhibit more power than their vastus lateralis or vice versa. This means the power values obtained are not an exact representation of the vastus lateralis, so the fiber measurements do not exactly relate to the recorded power value.

Conclusion

Interventions have shown to be useful in increasing older adult power, but it is still unknown how useful these interventions are at targeting MHC IIx. More research needs to be done to create an effective model to determine the intervention's impact. My model did not make sense physiologically due to the small sample size and the variance between the subjects. Studying more subjects could help linearize the data by lessening the impact of outliers. Although a model has yet to be created, this research sets the foundation for creating a model. With further research, we can begin to target older adult instability through power increase and improve the lives of our elders.

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