DO FATIGUING MUSCLE CONTRACTIONS ALTER INHERENT CONTRACTILE PERFORMANCE IN THE SINGLE FIBER?

by

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A THESIS

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The increased fatiguability of muscle in older adults is recognized as a major decrement to quality-of-life accompanying age. The mechanisms by which this increased fatiguability occurs is not well understood. Research has shown that accumulating metabolites play a role in stopping optimal interaction of muscle filaments when contracting. However, older adults exhibit higher resistance to fatigue at low-velocity contractions than younger adults and fatigue more during high velocity contractions, a phenomenon that is not easily explained by accumulating metabolite levels. This study will evaluate the contractile changes in single muscle fibers that accompany fatigue, outside the influence of intracellular metabolites. Therefore, this study will explore the novel hypothesis that inherent changes in muscle cellular function, and not only intracellular metabolic environment per se, contribute to in vivo fatigue.

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Congratulations to all other members of the Clark Honors College graduating class of 2021 and Go Ducks!

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Introduction:

An overview of muscular contraction and fatigue

Muscular contraction occurs through the interaction of two overlapping filamentous proteins called actin and myosin [1,2]. They exist within a lattice of proteins arranged in structures called sarcomeres, the smallest functional unit in the contractile apparatus within muscle cells. The interaction between the molecular motor, myosin, and its binding partner, actin produces the force that allows for muscle shortening and body movement [3]. This force can be used to characterize the contractile function of sarcomere [1–4]. The function of actin, myosin, and other sarcomeric proteins, depends on the intracellular environment that surrounds them. Age is a stressor that can bring about chronic alterations in contractile function. There are also acute stressors, like exercise, in which repeated muscle activations will cause brief and reversable alterations in contractile function. This acute decrease in force generating capacity is known as fatigue [5]. The hope in this study is to develop findings from observation of fatigue's effect on single muscle fiber contractility at an acute level and use it as a precursor to the development of treatment and prevention for health risks associated with increased fatigue that is seen with age [6–8], such as increased risk of falling/fracture or decrease in immune function [9].

In general, at the single fiber level, it is understood that the acute decrease in force generating capacity seen during fatigue is caused by a buildup of metabolites that interfere with the process of myosin interacting with actin, the two proteins involved in causing muscle shortening [10]. What is less understood is that older adults exhibit higher

resistance to fatigue at low-velocity contractions than younger adults and fatigue much more at high velocity contractions. This can't be explained by build-up of metabolites [11,12].

The gap in knowledge resulting from this fatigue resistance suggests alternate mechanisms by which fatigue occurs, which may prove useful clinically in treatment of increased fatigue of older adults at medium to high velocity contractions. In this study, the role of post-translational modifications of sarcomeric proteins will be investigated as to their role in altering the contractile properties of muscle due to fatigue.

Subsequent research question and hypothesis

Research question: If fatigue is caused by elevated levels of inorganic phosphate (Pi) and reduced pH (H+ concentration), will we be able to identify fatigue at optimal levels of Pi and pH by through observation of the contractile properties of the fiber?

Hypothesis: Fatigued fibers will have decreased performance compared to non-fatigued fibers especially in terms of velocity and power because of the increased phosphorylation of proteins associated with fatigue.

Literature Review

Overview of Fatigue

Skeletal muscle contraction involves the shortening of the muscle due to interactions of the proteins actin and myosin [2,13]. This process is called cross bridge cycling [2]. With repeated skeletal muscle contraction, there is an acute reduction in force and power known as fatigue due to the repeated contractile activity of the muscle [2,14]. This phenomenon is a normal physiologic response to repeated contractile activity in healthy individuals [15]. Fatigue has been linked to acute impairments in neuromuscular activation as well as altered contractile function within the muscle cells. In neuromuscular fatigue, there is a contribution from both the peripheral nervous system and the central nervous system known as peripheral fatigue and central fatigue, respectively [16]. In peripheral fatigue, reductions in force and power are due to processes occurring distal to the neuromuscular junction [16–18] while in central fatigue, reductions in force and power occur due to processes proximal to the neuromuscular junction [16,18]. Within the muscle itself, fatigue is caused by mechanisms independent of the nervous system [2,5]. The most understood mechanism by which this happens is metabolite accumulation [5,10,11].

Neuromuscular Activation and Metabolite Accumulation

As previously suggested with age, the ability of adults to generate force is severely negatively affected by increased fatigue when older adults perform moderate to high velocity contractions [3,5,19]. While research has shown that neural activation does play a factor in fatigue along the motor pathway, [11,12] impaired contractile function of

muscle at the sarcomere level has been shown to be the primary mechanism by which fatigue occurs during high-intensity exercise, such as a maximal voluntary contraction [7,8,20]. Additionally, research on the neuromuscular system of older adults shows that the mechanisms of fatigue *with age* are a result of factors within the muscle and not along the motor pathway [7,8,14]. This means a mechanism within the muscle must be causing the increased fatigue seen during high-intensity contractions of older adults, suggesting neural activation does not explain age-related differences in fatiguability [7,8,14]. Instead, research on the neuromuscular system of older adults shows that the mechanisms of fatigue with age are a result of factors *within the muscle* and not along the motor pathway [7,8,11,12,14].Therefore, to more completely understand the mechanisms explaining age-related differences in contractile performance, we focus our efforts on in vitro observation of individual muscle fibers obtained via percutaneous needle muscle biopsy. Thereafter, isolated fibers are activated with exogenous calcium and under ideal biochemical conditions, where the effects of neural activation have been eliminated, allowing for more precise determination of underlying mechanisms explaining age-related alterations in contractile function [12,21]. Research demonstrates that during high intensity-exercise of muscle, the metabolites hydrogen (H+) and inorganic phosphate (Pi) accumulate within the muscle [22]. These metabolites have an inhibitory effect on the contractility of muscle, causing it to fatigue [11,23,24].While this may indicate that the build-up of metabolites is the main mechanism that causes fatigue, it cannot explain why older adults demonstrate fatigue resistance during contractions performed at low velocity, but potentiated fatigue during high velocity contractions when compared to young. This unique phenomenon, when considered in ight of

additional studies demonstrating similar intracellular metabolic perturbations in old and young, suggest that other mechanisms are likely playing a role in these age related fatigue differences.

Fatigue Resistance

Older individuals have lower fatigue resistance than young with dynamic contraction [3] and higher fatigue resistance than young with isometric contraction [25,26]. This means that at low velocity contractions (isometric), older adults will fatigue less and at high velocity contractions (dynamic) that require more velocity and power to complete, older adults will fatigue much faster. The fatigue resistance in isometric contractions with old age can ostensibly be explained by a lesser reliance on non-oxidative glycolytic flux which produces inhibitory contractile metabolites [6,12]. By relying less on glycolytic flux in low velocity contractions, metabolites do not accumulate and therefore fatigue does not occur as quickly. This mechanism could potentially explain the isometric fatigue resistance associated with older adults. In order to test this explanation in a physiologic setting, studies have been conducted in order to show that blood occlusion (ischemia) results in more fatigue in individuals regardless of age than in free-flowing circulatory conditions [11,27,28]. Blood occlusion, which is a viable method to stop oxidative capacity [11], results in older adults increasing glycolytic flux to the same degree as young adults [12]. Metabolite accumulation is less pronounced in ischemic conditions despite glycolytic flux increase [12] and synergistic depression of muscle contractility resulting from accumulated Pi and H+ [10,23,24] does not exhibit a difference between age when saturating in Calcium [10]. This indicates a mechanism by which age-related fatigue resistance can't be explained by accumulating metabolite,

therefore the fatigue differences in old and young can't be fully explained with the metabolite mechanism.

Low Frequency Fatigue

Exploration of the mechanisms that can explain fatigue leads to the phenomenon of low-frequency fatigue. Low-frequency fatigue (LFF) can be characterized by the prolonged recovery and the selective loss of force of muscle when it is activated by low frequency stimulation [29,30]. This phenomenon occurs regardless of the type of protocol used to induce the low frequency stimulation [29–32]. It is relevant to note that the central and peripheral nervous systems are not involved in LFF due to lack of electromyogram alterations in LFF [30]. Furthermore, muscle metabolite levels have been shown to return close to resting levels early in recovery while a significant depression of force remains. This would therefore exclude metabolic mechanisms as a cause of LFF [30]. Since the nervous system and metabolites are suggested as having no involvement in LFF, then fatigue due to LFF must be due to factors within the muscle itself [29–32]. Within the muscle, a failure during the protein interaction involved in E-C coupling has been observed to be the main mechanism by which LFF causes fatigue [29–32]. This observation seems relevant when compared to literature that could potentially indicate relation between age related impairments in calcium release and LFF [29]. The relevant significance of LFF is that its mechanisms indicate a protein modification by which force is eventually restored [30].

Gap in Knowledge

Metabolite accumulation is the main *understood* mechanism by which contractile function is impaired [11,23,24] due to age [7,8,14]. Novel research indicates that accumulating metabolites can't fully explain the phenomenon of low-velocity, isometric fatigue resistance in older adults [10,12]. These findings suggest an alternative mechanism that affects fatigue through altered contractile function with age [7,8,20]. Contractile function due to myofilament protein-protein interaction is regulated by posttranslational modifications of the myofilaments which have the potential to underlie differences in contractile function with age [33,34]. For example, heart failure as a pathology can affect skeletal muscle through posttranslational modification of the ryanodine receptor in muscle fibers, causing fatigue, impaired function and pathological calcium release [35]. In general, evidence supports that contractile function is affected by posttranslational modifications of proteins [33–35], and these changes can be induced by pathology [35]. Posttranslational modifications of proteins in contractile function are not fully understood and therefore have the potential to explain fatiguing differences between old and young. This study is designed to understand the role of PTM in contractile function before and after acute fatigue by separating the muscle fiber from the neuromuscular system and activating it exogenously in optimal metabolite, both methods done to account for the variability of each mechanism. In this way, any changes in contractile function seen in the muscle cell would have to be caused by a mechanism that is not explained by metabolite buildup or neuromuscular activation.

Relevant Applications of Research

With age comes increased fatiguability preventing older adults from completing simple daily tasks [3,36]. This increased fatigue is accompanied by increased risk of falls and decreased mobility, all of which contribute to a lower quality of life for older adults. As of 2015, in the U.S. it was estimated that health care costs for fatal and non-fatal falls alone in older adults 65 and older was about 50 billion dollars [37–39]. The monetary and health related relevance of increased fatigue with age suggests that understanding acute fatigue mechanisms will help improve quality of life for older adults.

Methods

Subjects

The data collection for this project will be conducted out of the Muscle Cellular Biology Lab directed by Dr. Damien Callahan at the University of Oregon. The lab focuses on mechanisms that explain age-related changes in skeletal muscle and function. Written and informed consent gathered from all subjects before their participation in the study. Raw data collected from 2 older males and incomplete data set from 2 females measured from samples that were collected prior to Covid-19 restrictions will be analyzed in order to determine contractile function.

Experimental Protocol and Biopsy Procedure

2 older males and 2 younger females were brought into the lab and baseline measurements were taken before the exercise procedure was conducted. The exercise procedure consisted of continuous leg extensions until activity failure. Activity failure was marked as maximal fatigue. At this point, a percutaneous needle biopsy of the fatigued leg was conducted on the vastus lateralis. A second biopsy was conducted in the contralateral, non-exercised leg to serve as the controlled sample. Extracted muscle samples were stored at -20 deg Celsius in a storage solution containing 50% glycerol to prevent freezing. This effectively maintains conditions of the muscle at the time of fatigue. Once stored, the samples could later be chemically permeabilized with a chemical skinning solution while single muscle fibers could then be dissected from muscle bundles. This single muscle fibers were subsequently attached to the Aurora

Muscle Fiber rig for measurement of relevant data. On the rig, the samples were placed in conditions of optimal pH levels, optimal inorganic phosphate levels, and optimal calcium levels. This was done to ensure that the contractile properties of the muscle fibers due to fatigue were not being affected by the various metabolites as we want to know if fatigue would still have an effect without these metabolites. The contractile properties were measured in the form active contractile tension, velocity, and power of the muscle fiber, making up the force clamp data for this experiment. After measuring contractile performance, fibers were individually stored in lysing buffer and latter assess for myosin heavy chain isoform expression (MHC) using SDS PAGE.

MATLAB and Excel Procedure

The program MATLAB was used to generate a full analysis of the muscle fiber force clamp data. With this analysis generated, all values were stored on Microsoft Excel, sorted by individual subject. The document was further sorted to separate relevant data of Vmax, Pmax, Tmax, Fmax and Topt for each subject, resulting in an Excel sheet made up of data for the desired variables. From this collection of data, statistical analysis could be conducted.

Statistical Analysis

Statistical analysis was conducted via the statistics program SPSS. Univariate analysis was run on the data for each of the five variables, Vmax, Pmax, Tmax, Fmax and Topt. For each variable, fibers were separated by age to account for age related differences in muscle fibers during fatigue. Once separated, a univariate analysis was generated for each of the five variables. This analysis included descriptive statistics that

analyzed three different fiber types individually before and after the fatiguing protocol. Mean differences were found and tested via ANOVA using an alpha level of .05 as the cutoff point for significance.

Post-Experimental Analysis

With collected raw data, the aim of this study could be addressed. With the measurements collected from the Single Muscle Fiber Rig, data analysis was compared numerically with the observations of active contractile tension, velocity, and power of the muscle fiber. Each of these measurements were plotted as an average for the fibers and then compared with other literature that showed the effect of metabolite buildup on contractile function with fatigue. If functional contractile decline was still exhibited due to fatigue even at optimal metabolite levels, then that is indication that some other mechanism of fatigue is at play with regard to contractile function. With the idea of protein modification in mind, a discussion of relevant literature and research can be applied to this study's findings as a way to try and begin to explain the mechanisms of fatigue on contractile function.

Results

For the older male subjects, a total of 74 fibers were analyzed. 42 of these fibers consisted of non-fatigued tissue, leaving 32 to make up the fatigued tissue data. The fiber types consisted of 33 type I's, 21 type II's and 20 type I/IIa's. Overall, there was strong evidence from the data to suggest that fatigue induced contractile deficits were present when looking at older males. The older male subject group was well balanced in fiber type and fatigued vs. non-fatigued samples. For these subjects, there was a trend that showed decreases in max power and decreasing max velocity due to fatigue while the max tension and optimal remained the same before and after fatigue. There was no main effect of fiber type, nor an interaction between fiber type and fatigue, indicating the effects of fatigue were not dependent on fiber type. In the younger subjects, 13 non-fatigued and 14 fatigued fibers were used between three different subjects. Two of the subjects were female and one was male. These fibers consisted of five MHC I, nine MHC IIA, four MHC IIA/X and nine MHC I/IIA fibers. The younger subjects had both a smaller sample and unbalanced MHC abundance compared to the older subjects, making comparison to the older group difficult to interpret. With attention paid to this incomplete data, the young subjects exhibit significant decreases in Pmax, Tmax and Topt due to fatigue while failing to show a significant change in Vmax due to fatigue. While these data support the claim that fatigue will induce contractile deficits of the muscle not caused by intracellular environment, lack of data caused by COVID-19 regulations over the course of this study dictate that the effect of age and fiber type due to fatigue would need more investigation in order for useful evaluation to be made.

OLDER SUBJECTS

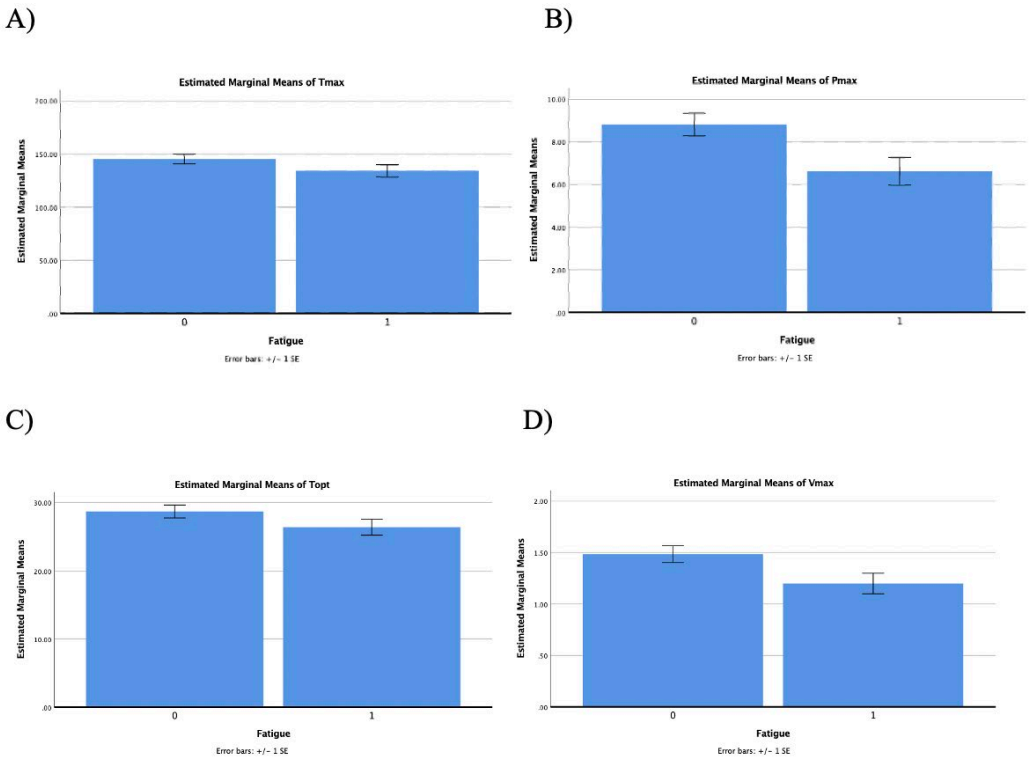

Figure 1: Effects of fatigue alone on the contractile properties of skeletal muscle for older adults

Older age group results for variables of Tmax, Pmax, Topt and Vmax measured before and after fatigue. General trends suggest that Pmax and Vmax have larger decreases due to fatigue than Topt and Tmax which remain closer to their pre-fatigue values post-fatigue. In general, it is clear that there is a loss of contractile function due to fatigue when fiber type is not account for.

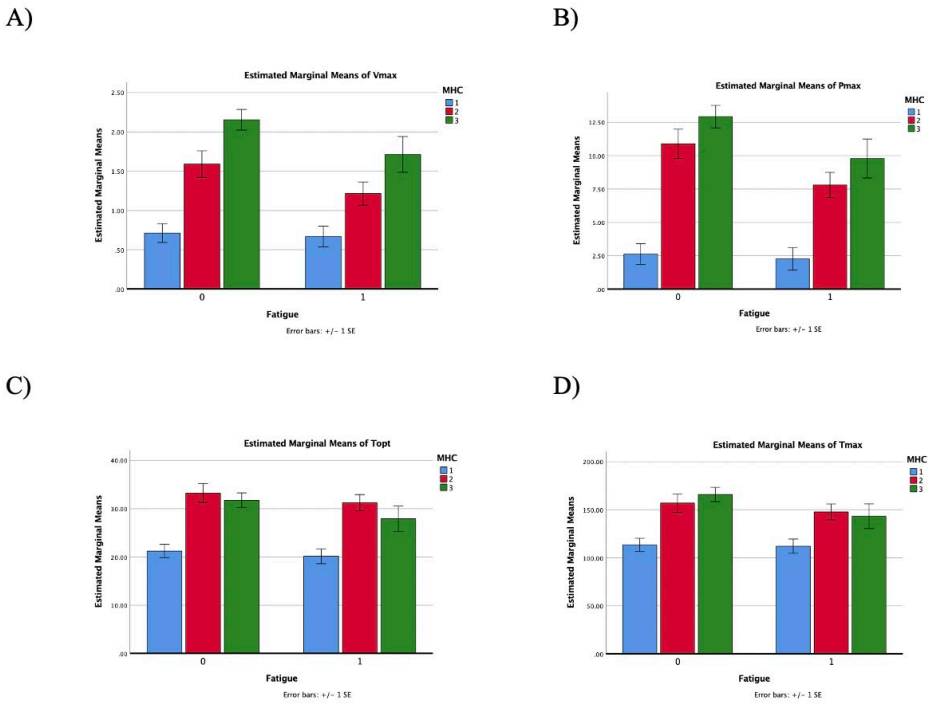

Figure 2: Effects of fatigue on contractile muscle properties when accounting for fiber type in older subjects.

Older age group results for variables of Tmax, Pmax, Topt and Vmax measured before and after fatigue with independent fiber type accounted for. MHC 2 and 3 follow the same general trend that was observed in Figure 1 with decreases in Pmax and Vmax and no change in Topt and Tmax. MHC 1 seems unaffected by the fatiguing protocol. Overall, there is not sufficient evidence to say that the changes due to fatigue are dependent on fiber type.

A)

Tests of Between-Subjects Effects

Dependent Variable: Tmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36936.436[a]	5	7387.287	8.892	.000
Intercept	1208358.80	1	1208358.80	1454.554	.000
Fatigue	1873.530	1	1873.530	2.255	.138
MHC	28026.112	2	14013.056	16.868	.000
Fatigue * MHC	1160.671	2	580.336	.699	.501
Error	56490.439	68	830.742		
Total	1476163.02	74			
Corrected Total	93426.875	73			

a. R Squared = .395 (Adjusted R Squared = .351)

B)

Tests of Between-Subjects Effects

Dependent Variable: Topt

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2203.752[a]	5	440.750	12.650	.000
Intercept	46910.967	1	46910.967	1346.390	.000
Fatigue	81.585	1	81.585	2.342	.131
MHC	1940.408	2	970.204	27.846	.000
Fatigue * MHC	19.609	2	9.805	.281	.756
Error	2369.259	68	34.842		
Total	57177.290	74			
Corrected Total	4573.010	73			

a. R Squared = .482 (Adjusted R Squared = .444)

C)

Tests of Between-Subjects Effects

Dependent Variable: Vmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24.658[a]	5	4.932	19.222	.000
Intercept	111.229	1	111.229	433.542	.000
Fatigue	1.261	1	1.261	4.917	.030
MHC	17.377	2	8.688	33.865	.000
Fatigue * MHC	.564	2	.282	1.099	.339
Error	17.446	68	.257		
Total	158.077	74			
Corrected Total	42.104	73			

a. R Squared = .586 (Adjusted R Squared = .555)

D)

Tests of Between-Subjects Effects

Dependent Variable: Pmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1387.279[a]	5	277.456	26.027	.000
Intercept	3679.706	1	3679.706	345.177	.000
Fatigue	74.045	1	74.045	6.946	.010
MHC	1056.590	2	528.295	49.557	.000
Fatigue * MHC	32.034	2	16.017	1.502	.230
Error	724.904	68	10.660		
Total	5712.540	74			
Corrected Total	2112.183	73			

a. R Squared = .657 (Adjusted R Squared = .632)

Figure 3: ANOVA testing for older subjects.

ANOVA testing for older subject variables. When looking at the fatiguing condition alone, Tmax and Topt were not significant before and after fatigue with a p-value of 0.138 and 0.131 respectively, confirming general trend of no change due to fatigue when alpha level is set at 0.05. Vmax was significantly different due to fatigue with a p-value of 0.030 and Pmax was also significant different due to fatigue with a p-value of 0.010. There was no evidence of fiber type dependent contractile deficits due to fatigue in any of the above cases as seen in the MHC * fatigue condition.

YOUNGER SUBJECTS

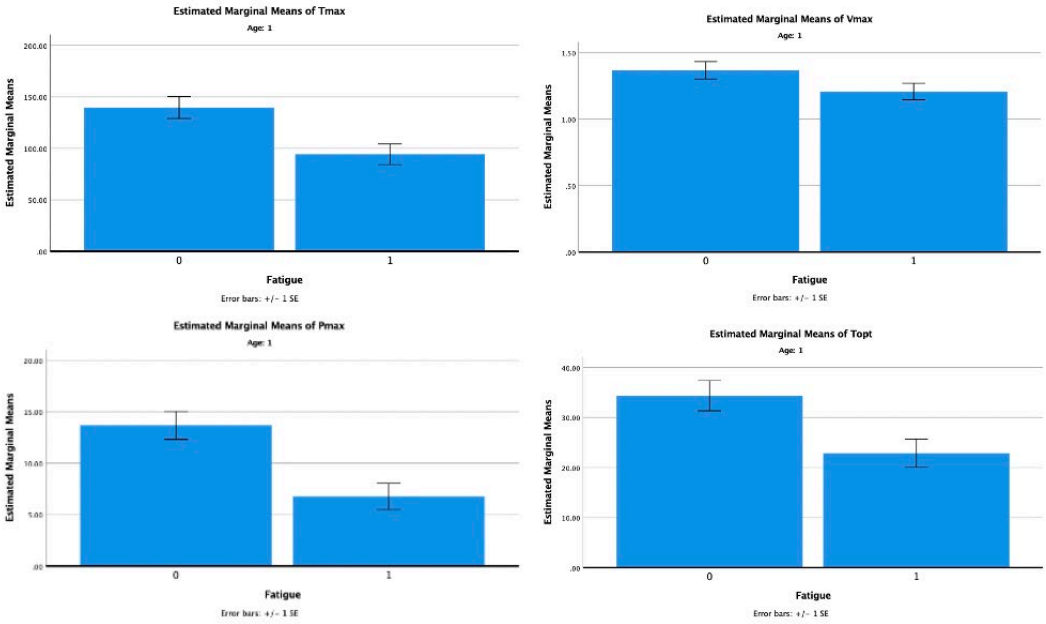

Figure 3: Effects of fatigue alone on the contractile properties of skeletal muscle for younger subjects

Represented are the variables for younger subjects before and after fatigue. General trends show decreases in all variables except for Vmax for younger subjects. It is important to note that this data was severely limited by Covid-19 restrictions and is not sufficient enough to make conclusions on in the case of this study.

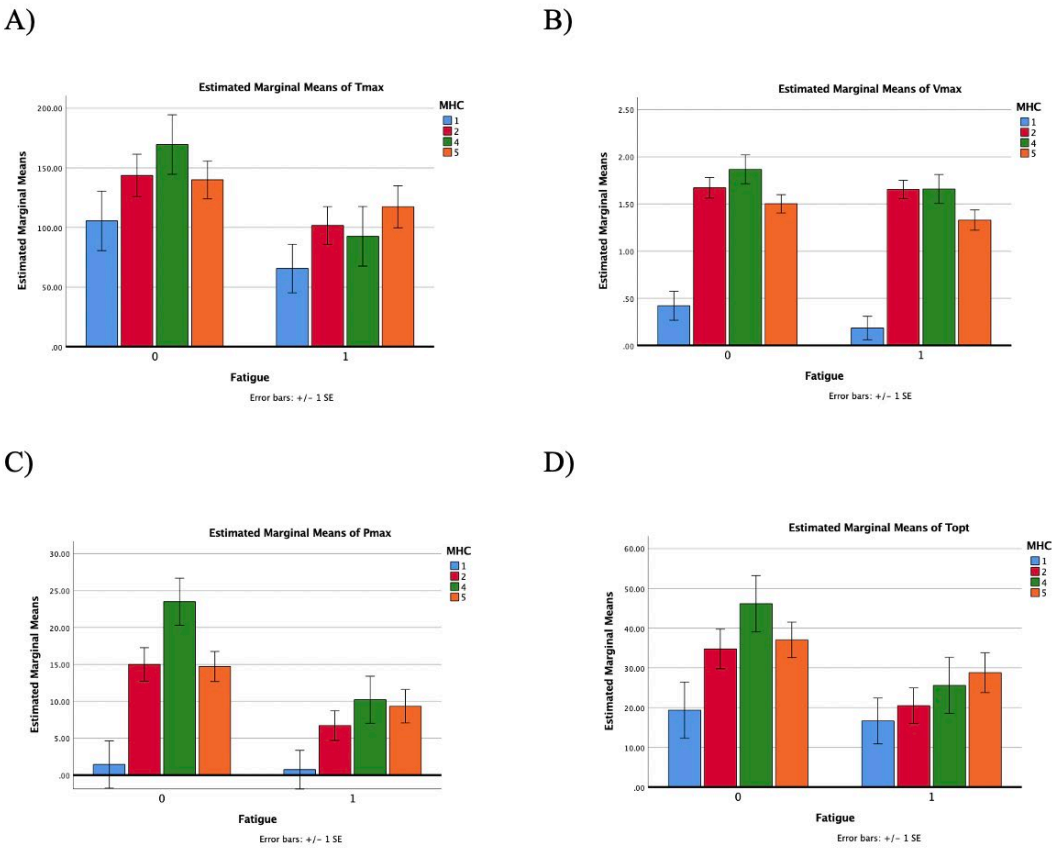

Figure 5: Effects of fatigue on contractile muscle properties when accounting for fiber type.

Younger age group variable changes due to fatiguing conditions. There are clear reductions in contractile function for all of the variables except for Vmax for each fiber type, aside from MHC 1. An important factor to note here is that the younger age group had fiber types not matched by the older age group and types that were not consistent across condition. Sample size was also limited compared to older adults.

A)

Tests of Between-Subjects Effects

Dependent Variable: Tmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21554.222[a]	7	3079.175	2.462	.056
Intercept	320630.265	1	320630.265	256.381	.000
Fatigue	12061.512	1	12061.512	9.645	.006
MHC	6902.015	3	2300.672	1.840	.174
Fatigue * MHC	2044.405	3	681.468	.545	.658
Error	23761.445	19	1250.602		
Total	421067.741	27			
Corrected Total	45315.667	26			

a. R Squared = .476 (Adjusted R Squared = .282)

B)

Tests of Between-Subjects Effects

Dependent Variable: Vmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.514[a]	7	1.073	22.788	.000
Intercept	38.775	1	38.775	823.113	.000
Fatigue	.148	1	.148	3.131	.093
MHC	6.853	3	2.284	48.489	.000
Fatigue * MHC	.051	3	.017	.360	.783
Error	.895	19	.047		
Total	57.091	27			
Corrected Total	8.409	26			

a. R Squared = .894 (Adjusted R Squared = .854)

C)

Tests of Between-Subjects Effects

Dependent Variable: Pmax

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1033.364[a]	7	147.623	7.202	.000
Intercept	2442.766	1	2442.766	119.169	.000
Fatigue	279.934	1	279.934	13.656	.002
MHC	607.664	3	202.555	9.882	.000
Fatigue * MHC	96.105	3	32.035	1.563	.231
Error	389.469	19	20.498		
Total	4269.195	27			
Corrected Total	1422.833	26			

a. R Squared = .726 (Adjusted R Squared = .625)

D)

Tests of Between-Subjects Effects

Dependent Variable: Topt

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2074.606[a]	7	296.372	2.967	.028
Intercept	19173.933	1	19173.933	191.928	.000
Fatigue	770.229	1	770.229	7.710	.012
MHC	922.213	3	307.404	3.077	.052
Fatigue * MHC	215.289	3	71.763	.718	.553
Error	1898.128	19	99.901		
Total	26174.175	27			
Corrected Total	3972.734	26			

a. R Squared = .522 (Adjusted R Squared = .346)

Figure 6: ANOVA testing for younger subjects.

ANOVA testing for younger subjects for each of the four focused variables. Alpha level was set at 0.05. When analyzing for the fatigue condition alone, Tmax and Topt were significant at a p-value of .006 and 0.012 respectively. Pmax was significant at a p-value of 0.002 and Vmax was not significantly different at a p-value of 0.093. Similarly to the older subjects, there was no evidence of a fiber type dependent change in contractile function due to fatigue when looking at the MHC * Fatigue condition.

Discussion

Fatigue alters inherent contractility

This study aimed to determine how the contractile properties of a muscle fiber would be affected by fatigue within an optimal intracellular environment. Overall, there is a clear effect of fatigue on the contractile properties of single muscle fibers when activated in an optimal intracellular environment. Substantial data collected from older male subjects suggests that these changes affect the contractile properties by decreasing both Pmax and Vmax while Tmax and Topt remain relatively the same. What these results suggest is influence of fatigue on the cross-bridge cycling of sarcomeres at the single fiber level. When Vmax decreases and Tmax and Topt remain the same, the decrease in Pmax will result based off of the force-velocity curve for single muscle fibers. In other words, there is a change in the shape of the force-velocity curve from normal.

Potential mechanisms

This shift in the shape of the force-velocity curve would indicate a change in cross-bridge kinetics due to fatigue which can be potentially explained by post-translational modifications of the proteins involved during cross-bridge cycling. In general, analysis of the older male subjects rejects the idea that contractile deficits with fatigue are caused solely by intracellular environment and supports a kinetic deficit that is slowing down cross bridge cycling. While the present data do not support direct evidence for a potential mechanism, pre-clinical studies suggest muscle fatigue may result in post-translational modifications to multiple sarcomeric regulatory proteins such

as myosin binding protein c (MyBP-C) and myosin regulatory light chain (RLC). Indeed, recent data from the Callahan lab suggest enhanced phosphorylation of MyBP-C and reduced phosphorylation of RLC with fatigue in younger adults. These changes would likely result in reduced sensitivity to Ca^{2+} activation and reduced contractile velocity.

Future directions

Further study is necessary to probe the potential mechanisms linking protein modification and contractile performance in human fibers following fatigue. Most immediately, we may simply perform additional studies using our current approach to investigate the potential that reduced sensitivity to Ca^{2+} activation is altered with fatigue. More research would need to be conducted in order to make conclusions on the role age and fiber type has to play on the contractile properties of muscle during the fatiguing process. However, mass spectrometry might allow for the determination of specific post translational modifications in our sarcomeric proteins of interest.

Limitations

This study was limited first and foremost by a lack of balance between samples (young/older) and the limited number of fibers that were analyzed. Ideally, far more fibers for each condition and each fiber type would contribute to a more complete picture of the influence of fatigue on contractile performance. While the data collected for the young subjects suggest deficits in contractile tension with fatigue, we were not able to identify fiber type specific responses, which are necessary to put the physiological consequences of these changes in context. Additionally, young subject

data could not be compared to the old subject data, which was normalized to a greater degree, because the young subjects included a wide range of fiber types that were not even seen in the older subjects.

Conclusions

The overall take home from this study promising. What we observe are parallels at the single fiber level and the whole fiber level that can apply to entire muscle groups together. At the single fiber level, our research demonstrates changes indicating a velocity dependent drop in power in older adults. These same changes can be seen at the whole muscle level suggesting that the single fiber level is relevant for researching fatigue as it affects whole muscle. Finally, the preliminary results for younger adults in this study indicate a tension dependent drop in power despite the low amount of data for this group. In both cases, we are encouraged to research both groups further to understand the contractile deficits seen with fatigue.

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