SKELETAL MUSCLE MITOCHONDRIAL CAPACITY AND MUSCLE-SPECIFIC ENDURANCE IN PERSONS WITH FRIEDREICH'S ATAXIA

by

HANNAH MICHELLE BOSSIE

(Under the Direction of Kevin McCully)

ABSTRACT

PURPOSE: Evaluate skeletal muscle mitochondrial capacity and muscle-specific endurance using noninvasive methods in persons with Friedreich's Ataxia (FRDA) and able-bodied controls (AB). **METHODS:** Participants with FRDA (n=16) and AB controls (n=10) were tested (ages 8-55 yrs). Forearm mitochondrial capacity was measured using the rate of recovery of oxygen consumption after electrical stimulation with near-infrared spectroscopy. Mechanomyography was used to assess muscle-specific endurance after electrical stimulation for 3 minutes at 2Hz, 4Hz, and 6Hz sequentially. **RESULTS:** There was no difference in mitochondrial capacity (FRDA and AB: 1.8 ± 0.3 1/min). Muscle-specific endurance was significantly impaired by 14% in the FRDA participants in comparison to AB controls ($p < 0.001$). Correlations show a positive, moderate relationship between these two measurements and a negative, moderate relationship with disease severity in the FRDA group. **CONCLUSIONS:** Impairments in endurance and relationships between disease severity, endurance and mitochondrial capacity suggest that these measurements can be useful to monitor people with FRDA.

The ease of testing and noninvasive nature of these methods also make them appropriate for futures studies.

INDEX WORDS: NIRS, Friedreich's Ataxia, mitochondrial capacity, mechanomyography

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by

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DEDICATION

I would like to dedicate my Master's thesis to my parents, Tom and Angela Bossie. I cannot express the depth of my appreciation for your unconditional love and support. My admiration of who you are as parents, professionals, and people grows with each passing day. I could not ask for any greater role models, not just because of who you are today, but because of the journey you both have taken to get here. Thank you for your encouragement and belief in me.

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I would like to thank my committee members, Dr. Pat O'Connor and Dr. Ellen Evans for their support throughout my degree process. Lastly, I would like to thank the man, the myth, the legend: Dr. Kevin McCully. Dr. McCully's passion for research is absolutely contagious and is driven from a genuine desire to help others. He has been the greatest teacher and mentor that I have ever had the pleasure to learn from. I am eternally grateful for the opportunities he has provided me and for the profound impact he has had on my career path and professional development.

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CHAPTER 1

INTRODUCTION

Friedreich's ataxia (FRDA) is a progression neurodegenerative disorder affecting 1 in every 50,000 people (1, 2). FRDA is the most common hereditary ataxia and it is estimated the 1 in every 120 people of European descent are carriers (3). FRDA is associated with the deterioration of large sensory neurons in the dorsal root ganglia, dorsal columns, spinocerebellar and pyramidal tracts of the spinal cord (2, 3). Clinical symptoms may include progressive ataxia of limbs and gait, loss of proprioception, diabetes mellitus, cardiomyopathy, dysarthria, tendon areflexia, lower limb weakness, sensory loss, and scoliosis (1, 2, 4). Symptom onset typically occurs before the age 25 and most people affected by FRDA are confined to a wheelchair by their late 20s (2, 3, 5).

The primary genetic mutation accounting for 95-98% of FRDA cases is an expanded GAA trinucleotide repeat in the first intron of both alleles of the FRDA gene located on chromosome nine which encodes a protein called "frataxin" (1, 2, 5). Normally this gene has up to 33 GAA repeats, but in FRDA patients 67 to 1700 GAA repeats may be present (4). This mutation results in a severely reduced production of functional frataxin (2). The reductions of frataxin mRNA and protein levels are inversely related to the size of the GAA repeat of the smaller alleles (3, 4).

Frataxin mRNA is expressed abundantly in tissues with high metabolic rates, including heart, liver, pancreas, skeletal muscle, and the spinal cord (5). Frataxin is associated with the mitochondrial inner membrane and is thought to play a role in mitochondrial iron homeostasis and iron-sulfur biogenesis (2, 6). Frataxin deficiency is associated with mitochondrial iron accumulation, however mechanisms of this accumulation are still being explored(6). Due to challenges with models of FRDA and the variation in clinical phenotype, noninvasive assessments of skeletal muscle are greatly needed in this population.

Traditionally, *in vitro* and *in vivo* methods have been used to assess mitochondrial function (7). *In vitro* methods of evaluating mitochondrial function are able to assess specific aspects of, and changes in, regulatory pathways including the production of reactive oxygen species (8). Despite the benefits of the specificity of data from *in vitro* methods, they require painfully invasive muscle biopsies, which hinder the rationality of its application to clinical trials in humans. The *in vivo* method of assessing mitochondrial function that can be regarded as the "gold standard" is phosphorous magnetic resonance spectroscopy (^{31}P-MRS), which measures the rate of recovery of phosphocreatine (PCr) after a brief exercise (9, 10). This technique requires a phosphorus MRI unit, which are very costly and in limited availability across the country. Another *in vivo* method is near infrared spectroscopy (NIRS) which also measures skeletal muscle mitochondrial capacity, but by measure the rate of recovery of oxidative metabolism after exercise (11). While this test method is not able to measure the functionality of the mitochondria, it is able to measure the capacity of the mitochondria within skeletal muscle to utilize oxygen while accounting for the influence of oxygen delivery. NIRS is an advantageous

alternative to the MRS due to its accessibility, non-invasive nature and lower cost of administration.

The McCully lab has extensive experience with the NIRS mitochondrial capacity protocol. Briefly, a rapid inflating cuff inflation system that effectively cuts off oxygen delivery temporarily during recovery from exercise, allowing the measurement of the rate of recovery of skeletal muscle oxygen consumption ($musVO_2$) following a brief bout of electrical stimulation (12). This recovery after exercise is indicative of mitochondrial ATP production and has been shown to be easily replicable (13), comparable to MRS (14), and sensitive to changes and differences among test groups (11, 15). Our NIRS techniques have been applied to several clinical populations, including measuring mitochondrial capacity in people affected by spinal cord injuries and calf oxygenation in people affected by peripheral arterial disease (16, 17). I anticipated that patients affected by Friedreich's ataxia will be tolerant of our test and that the data yielded will provide measurable benefits to this population.

Statement of Problem

There is a need to noninvasively characterize skeletal muscle dysfunction in patients affected by Friedreich's Ataxia (FRDA).

Specific Aims

Specific Aim 1: Measure skeletal muscle mitochondrial capacity in participants affected by FRDA and AB participants using near-infrared spectroscopy.

Specific Aim 2: Measure muscle-specific endurance in participants affected by FRDA and AB participants using surface mechanomyography.

Specific Aim 3: Measure disease severity in participants affected by FRDA using questionnaires on symptoms of energy and fatigue, functional daily activity level, physical activity level, and GAA repeat length.

Hypotheses

Hypothesis 1: Skeletal muscle mitochondrial capacity will be reduced in participants affected by FRDA, compared to AB participants, as measured by near-infrared spectroscopy.

Hypothesis 2: Muscle-specific endurance will be reduced in participants affected by FRDA, compared to AB participants, as measured by surface mechanomyography.

Hypothesis 3: Mitochondrial capacity and muscle-specific endurance will be moderately and positively related to each other and moderately negatively related to disease severity in participants affected by FRDA.

Significance of Study

The findings of this study will evaluate the feasibility of the near-infrared spectroscopy and surface mechanomyography measurements in the FRDA population.

There is a need for clinical outcome measures that are easy to administer and sensitive to change over time in the FRDA population (2, 18). Many people affected by FRDA are ineligible for clinical trials due to the advanced stage of their disease and their inability to complete trial measurements that involve physical activity. If these methods are effective, then NIRS and surface mechanomyography protocols may provide novel, noninvasive assessments for clinical trials while also allowing a larger portion of the FRDA population to be eligible for participation. With these measurements, we will expand the knowledge of skeletal muscle mitochondrial capacity as well as muscle-specific endurance in the FRDA population. The information learned is expected to help guide future research by targeting modifiable factors to enhance muscle function and by providing tools to assess changes in muscle over time.

CHAPTER 2

REVIEW OF LITERATURE

Friedreich's Ataxia

Friedreich's ataxia (FRDA) is a progression neurodegenerative disorder affecting 1 in every 50,000 people (1, 2). FRDA is the most common hereditary ataxia and it is estimated the 1 in every 120 people of European descent are carriers (3). Pathologically, FRDA is associated with the deterioration of large sensory neurons in the dorsal root ganglia, dorsal columns, spinocerebellar and pyramidal tracts of the spinal cord (2, 3). FRDA does not have the homogeneity in clinical phenotype as many other recessive traits display (4). Clinical symptoms may include progressive ataxia of limbs and gait, loss of proprioception, diabetes mellitus, cardiomyopathy, dysarthria, tendon areflexia, lower limb weakness, sensory loss, and scoliosis (1, 2, 4). Symptom onset typically occurs before the age 25 and most people affected by FRDA are confined to a wheelchair by their late 20s (2, 3, 5). Currently, there are no effective clinical treatments for FRDA (2).

FRDA and Fatigue

Chronic fatigue is a common symptom amongst neurological disorders (19). Fatigue, and particularly the physical component, is a significant symptom of Friedreich's ataxia (FRDA) (20). Fatigue in FRDA patients has been shown to correlated

with disease severity and duration (20). It is thought that fatigue is a frequent yet under recognized symptom of FRDA (20). Symptoms of weakness and fatigue in iron overload disorders have been attributed to reduced cardiac function, but the role and impact of skeletal muscle of these symptoms warrants further investigation (21).

Frataxin

The primary genetic mutation accounting for 95-98% of FRDA cases is an expanded GAA trinucleotide repeat in the first intron of both alleles of the FRDA gene located on chromosome nine which encodes a protein called "frataxin" (1, 2, 5). Normally this gene has up to 33 GAA repeats, but in FRDA patients 67 to 1700 GAA repeats may be present (4). This mutation results in a severely reduced production of functional frataxin (2). The reductions of frataxin mRNA and protein levels are inversely related to the size of the GAA repeat of the smaller alleles (3, 4).

Frataxin mRNA is expressed abundantly in tissues with high metabolic rates, including heart, liver, pancreas, skeletal muscle, and the spinal cord (5). Frataxin is associated with the mitochondrial inner membrane and is thought to play a role in mitochondrial iron homeostasis and iron-sulfur biogenesis (2, 6). Frataxin deficiency is associated with cellular iron metabolism dysregulation and mitochondrial iron accumulation due to decreased iron-sulfur cluster enzyme activity, however mechanisms of this accumulation are still being explored (2, 6). Additionally, the resulting mitochondrial respiratory chain defects are associated with increased free radical generation and oxidative damage (4, 22).

Effect of Iron on Muscle Fatigue

Free iron in skeletal muscle has been shown to increase the production of reactive oxygen species (ROS), which is proposed to accelerate skeletal muscle fatigue (21, 23). Iron has been shown to accumulate in the heart, liver, and spleen in FRDA patients (1, 24). A study by *Reardon et al* used a mouse model of skeletal muscle iron overload to determine the effect of free iron on skeletal muscle (21). The iron overload mice had reduced muscle endurance, strength, and muscle weight as well as increased markers of oxidative stress, when compared to controls (21). This study suggests that iron accumulation may play a role in reduced exercise capacity seen in iron overload disorders (21).

Mitochondria and Frataxin

Evidence of mitochondrial involvement in the pathophysiology of FRDA is increasing as research accumulates. In a study by *Schöls et al*, mitochondrial defects were assessed through skeletal muscle biopsies in a variety of degenerative ataxias (25). In this study, 31% of Friedreich's ataxia participants displayed mitochondrial enzyme defects (25). Additionally, mitochondrial dysfunction has been shown *in vivo* in the cardiac muscle of FRDA patients as well (26). *Bradley et al* showed that respiratory chain and aconitase activity were reduced in skeletal muscle, but not significantly (1). This study also showed that mitochondrial DNA levels were significantly reduced in the heart and skeletal muscle (1). However, the mtDNA levels were matched with a decline in citrate synthase activity (1). In addition to biopsy data, in vivo assessments of skeletal muscle mitochondrial function have also been made (5, 27-29).

In Vivo Assessments of Muscle Metabolism

Traditionally, *in vitro* and *in vivo* methods have been used to assess mitochondrial function (7). *In vitro* methods of evaluating mitochondrial function are able to assess specific aspects of, and changes in, regulatory pathways (8). Despite the benefits of the specificity of data from *in vitro* methods, they require painfully invasive muscle biopsies, which hinder the rationality of its application to clinical trials in humans. The *in vivo* method of assessing mitochondrial function that can be regarded as the "gold standard" is phosphorous magnetic resonance spectroscopy (^{31}P-MRS), which measures the rate of recovery of phosphocreatine (PCr) after a brief exercise (9, 10). This methodology has been applied to the FRDA population. *Lodi et al* reported that participants affected by FRDA had significantly reduced initial rates of PCr postexercise resynthesis and maximum rates of mitochondrial ATP production (27). *Vorgerd et al also* reported that participants affected by FRDA had significantly delayed PCr recovery time when compared to controls (5). This technique requires a phosphorus spectroscopy MRI unit, which are very costly and in limited availability across the country.

Another *in vivo* method is near infrared spectroscopy (NIRS) which also measures skeletal muscle mitochondrial capacity, but by measure the rate of recovery of oxidative metabolism after exercise (11). While this test method is not able to measure the functionality of the mitochondria, it is able to measure the capacity of the mitochondria within skeletal muscle to utilize oxygen while accounting for the influence of oxygen delivery. A study by *Lynch et al* examined tissue oxygenation after exercise with near-infrared spectroscopy in FRDA (28). FRDA patients performed incremental treadmill walking exercise and tissue oxygenation was measured before, during, and after the

exercise (28). This study showed that FRDA participants had a significantly delayed half-time of recovery, assessed by changes in oxygenation levels during and following exercise (28). NIRS is an advantageous alternative to the MRS due to its accessibility, non-invasive nature and lower cost of administration.

Need for Clinical Measurements

Due to challenges with models of FRDA and the variation in clinical phenotype, noninvasive assessments of skeletal muscle are greatly needed in this population. The role of skeletal muscle and mitochondrial capacity is incompletely characterized due to poor methodologies, limited sample sizes and clinical phenotypes tested, and the limited number of studies conducted. Additionally, very limited studies have examined the role of fatigue in FRDA (20). The purpose of this study is to evaluate our two noninvasive skeletal muscle measurements for clinical utility in the FRDA population.

CHAPTER 3

SKELETAL MUSCLE MITOCHONDRIAL CAPACITY AND MUSCLE-SPECIFIC ENDURANCE IN PERSONS WITH FRIEDREICH'S ATAXIA

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Abstract

PURPOSE: Evaluate skeletal muscle mitochondrial capacity and muscle-specific endurance using noninvasive methods in persons with Friedreich's Ataxia (FRDA) and able-bodied controls (AB). **METHODS:** Participants with FRDA (n=16) and AB controls (n=10) were tested (ages 8-55 yrs). Forearm mitochondrial capacity was measured using the rate of recovery of oxygen consumption after electrical stimulation with near-infrared spectroscopy. Mechanomyography was used to assess muscle-specific endurance after electrical stimulation for 3 minutes at 2Hz, 4Hz, and 6Hz sequentially. **RESULTS:** There was no difference in mitochondrial capacity (FRDA and AB: 1.8 ± 0.3 1/min). Muscle-specific endurance was significantly impaired by 14% in the FRDA participants in comparison to AB controls ($p < 0.001$). Correlations show a positive, moderate relationship between these two measurements and a negative, moderate relationship with disease severity in the FRDA group. **CONCLUSIONS:** Impairments in endurance and relationships between disease severity, endurance and mitochondrial capacity suggest that these measurements can be useful to monitor people with FRDA. The ease of testing and noninvasive nature of these methods also make them appropriate for futures studies.

Keywords: Friedreich's Ataxia, mitochondrial capacity, mechanomyography, muscle-specific endurance

Introduction

Friedreich's ataxia (FRDA) is a progression neurodegenerative disorder affecting 1 in every 50,000 people (1, 2). FRDA is the most common hereditary ataxia and it is estimated the 1 in ever 120 people of European descent are carriers (3). The primary genetic mutation accounting for 95-98% of FRDA cases is an expanded GAA trinucleotide repeat in the first intron of both alleles of the FRDA gene located on chromosome nine which encodes the protein "frataxin" (1, 2, 5). This mutation results in a severely reduced production of functional frataxin, which results in mitochondrial iron accumulation (2, 6). Pathologically, FRDA is associated with the deterioration of large sensory neurons in the dorsal root ganglia, dorsal columns, spinocerebellar and pyramidal tracts of the spinal cord (2, 3).

Clinical symptoms of FRDA may include progressive ataxia of limbs and gait, loss of proprioception, diabetes mellitus, cardiomyopathy, dysarthria, tendon areflexia, lower limb weakness, sensory loss, and scoliosis (1, 2, 4). Fatigue, and particularly the physical component, is a significant symptom of Friedreich's ataxia (20). Fatigue in FRDA patients has been shown to correlated with disease severity and duration (20).

Currently, there are no effective clinical treatments for FRDA (2). There is a great need for clinical outcome measures for use in clinical trials as FRDA treatment research progresses(2). The purpose of this study was to examine near-infrared spectroscopy and surface mechanomyography methods of evaluating skeletal muscle dysfunction in a sample of patients affected by FRDA. It was hypothesized that skeletal muscle mitochondrial capacity and muscle-specific endurance would be directly correlated with

each other and inversely correlated with measures of disease severity in the FRDA population.

Methods

Study Participants

Participants with genetically confirmed Friedreich's Ataxia (FRDA) were recruited for participation in this study through listserv and email recruitment. Participant diagnostic and demographic information is provided in Table 1. Some participants were unable to retrieve their genetic results and some were unwilling to provide the information due to their preference to not know their GAA repeat number.

To be eligible for the study, participants affected by FRDA had to have a diagnosis of Friedreich's Ataxia, be between the ages of 7 and 65, volunteer to be involved in the study, and provide informed consent. Able-bodied (AB) participants were recruited as controls from the local Athens, GA and University of Georgia community through listserv and email recruitment. To be eligible for the study, AB participants had to volunteer to be involved in the study, provide informed consent, and be between the ages of 7 and 65. The exclusion criteria for all study participants were as follows: mental impairment such that informed consent cannot be obtained or that subject would not be safe with the protocol, evidence of drug dependency or heavy alcohol use that would interfere with testing, evidence of any unstable medical condition that would make participation unsafe, presence of significant vascular disease, and any recent prior injury to the arm that would make testing unsafe such as an orthopedic injury to bone muscle or connective tissue.

The study was approved by the Institutional Review Board at the University of Georgia. We certify that all applicable instructional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research. All participants provided written informed consent prior to data collect. Data was collected from August 2015 to March 2016.

Study Design and Procedures

This study was a cross-sectional study looking at skeletal muscle mitochondrial capacity, muscle-specific endurance, and disease severity in participants affected by FRDA and comparing them to AB controls. Testing consisted of one session during which mitochondrial capacity, muscle-specific endurance, and perceptions of energy and fatigue were measured. Mitochondrial capacity and muscle-specific endurance were measured in the forearm flexor muscles of the nondominant arm.

Measurements

Near-infrared Spectroscopy (NIRS)

The testing protocol included resting metabolism measurements, physiological calibration, and three mitochondrial capacity tests (16, 30). The participants were positioned supine on a padded testing table for the duration of the test session. The arm tested was positioned with the elbow joint extended, approximately 45 degrees away from the body. The NIRS probe (Oxymon MK III, Artinis Medical Systems, the Netherlands) was placed over the surface of the forearm flexor muscles and secured on the forearm with biadhesive tape and two Velcro straps. The probe contained one light

source and two detectors. The optode separation distances varied from 2.5 and 3.5 cm, to 4.5 and 5.5 cm depending on the amount of subcutaneous adipose tissue thickness (ATT). ATT was measured at the beginning of each testing session utilizing B-Mode imaging (LOGIQ e; GE Healthcare, USA). A Hokanson vascular cuff (SC 5 or SC 7 depending on arm circumference) was wrapped around the distal end of the upper arm, proximal to the elbow and the NIRS probe. The cuff was fit to the circumference of the limb. The vascular cuff was attached to a Hokanson AG101 Rapid Cuff Inflation system (Hokanson E20 control box and AG101 compressor). Neurostimulation electrodes (2' by 4' rectangle, Pro Advantage by NDC) were positioned on the proximal and distal side of the NIRS optode. Participants were asked to remain as still as possible in order to avoid motion artifact during testing. Electrical stimulation (Theratouch 4.7; Rich-Mar, USA) was used to increase metabolic rate for the recovery kinetics measurements. Electrical stimulation frequency was set 6Hz and the intensity was set to the same current used for the surface mechanomyography testing.

Resting Muscle Oxygen Consumption Rate

Three resting arterial occlusions (250 mmHg for 30 seconds on-30 seconds off) were performed. Resting oxygen consumption was calculated from the average slope of the three occlusions.

Skeletal Muscle Oxidative Capacity

Mitochondrial capacity was measured as the rate of recovery of muscle metabolism after exercise (30, 31). Electrical stimulation was used to exercise the muscle

and NIRS was used to measure metabolic rate. Electrical stimulation (Theratouch 4.7; Rich-Mar, USA) frequency was set 6Hz and the current intensity was selected based on the visual inspection of a strong contraction that was tolerable to the participant. Electrical stimulation was performed for 10-15 seconds, immediately followed by a series of short duration cuffs as follows: cuffs 1 to 5 (5-seconds on/6-seconds off), cuffs 6 to 10 (7-seconds on/7-seconds off), cuffs 11 to 15 (10-seconds on/10-seconds off) and cuffs 16-20 (10-seconds on/20-seconds off) over 5 minutes. The rate of change in relative oxygenated hemoglobin and deoxygenated hemoglobin levels from each cuff was used to measure the return of metabolic rate from exercised to resting conditions. The slope values were fit to an exponential curve and a rate constant determined.

$$y = End - Delta \times e^{-kt} \qquad \text{(Equation 1)}$$

For this equation, y represents relative metabolic rate during the arterial occlusion, *End* is the metabolic rate immediately after the cessation of exercise, *Delta* is the change in metabolic rate from rest to end exercise, and k is the fitting rate constant. The rate constant, which was considered a measurement of muscle mitochondrial oxidative capacity (32). This recovery kinetics test was performed three times.

Physiological Calibration

A physiological calibration was performed to establish a range of tissue oxygenation as previously described (33, 34). Electrical stimulation at 6 Hz was performed for 10-15 seconds, followed by a 3-5 minute arterial cuff occlusion. The electrical stimulation acts to increase metabolic rate within the muscle, and the cuff is applied until the oxygenated hemoglobin signal reaches a steady state value (for at least 1

minute), which represents the relative 0% oxygenation. Upon the release of the cuff, the peak value from the resultant hyperoxygenation is used to represent the relative 100% oxygenation capacity of the tissue. This calibration allows analysis of resting metabolic rate relative to the total oxygenated signal from the physiological calibration.

Surface Mechanomyography (MMG)

The participants were positioned supine with arm placed in the same position as for the NIRS measurements. A tri-axial accelerometer (WAX-9; Axivity, UK) was placed over the surface of the forearm flexor muscles and secured on the forearm with biadhesive tape. Neurostimulation electrodes (Pro Advantage 2' by 4' rectangle; NDC, USA) were positioned 2cm from the accelerometer on the proximal and distal sides. A clinical electrical stimulator (Theratouch 4.7; Rich-Mar, USA) was used to stimulate the forearm flexor muscles. A Velcro strap was placed across the wrist to restrict forearm movement during stimulation. Electrical stimulation intensity was selected based on the visual inspection of a strong contraction that was tolerable to the participant. The protocol consisted of 30 seconds of baseline, 3 minutes of electrical stimulation at 2Hz, 4Hz, and 6Hz sequentially with 5 seconds of rest in between each stage, and 30 seconds of baseline at the end. The surface accelerometer measured the contractile acceleration resulting from the muscle twitches being produced throughout the protocol via wireless Bluetooth transmission, collecting at a sampling frequency of 100 data points per second. The endurance index is calculated as the difference between the previous peak acceleration with the acceleration at the end of each stage.

Questionnaires

Perceptions of Energy and Fatigue

Mental and Physical State and Trait Energy and Fatigue Scales were used to assess the influence of Friedreich's Ataxia on feelings of energy and fatigue. Previous studies have supported the validity of these scales, including negative correlations between fatigue scores and both physical activity and body mass index, structural validity based on confirmatory factor analysis and sensitivity to change with interventions known to influence fatigue or energy (35-41). This instrument was chosen partly due to its ability to measure energy and fatigue separately, from both acute and chronic perspectives, which are important factors of quality of life for people affected by Friedreich's Ataxia. The distinction between physical and mental aspects of energy and fatigue are of particular interest for this population since cognition was not traditionally thought to be involved in the pathophysiology of FRDA, however the role of cerebellar degeneration and its impact on cognition is being explored (42). Previously, mental and physical energy and fatigue have not been examined separately in a sample of people affected by Friedreich's Ataxia.

The first part of the questionnaire, which includes background information used to interpret the state and trait scales, was excluded due to the overlap with other data collected through screening. This questionnaire was administered in two parts: the state and trait scales. The same items are used for both the state and trait scales. First, four mood states were measured by 3 items each: Physical Energy State, Physical Fatigue State, Mental Energy State, and Mental Fatigue State (41). These items were presented as a visual analogue scale in order to measure the intensity of current feelings (41). For

participants who were unable to physically complete the visual analogue scale, this part was administered orally and the participant was instructed to assign a value, 0-100 to indicate their feelings. Next the mood traits were measured by three items each: Trait Physical Energy, Trait Physical Fatigue, Trait Mental Energy, and Trait Mental Fatigue (41). These questions were aimed at measuring the frequency of usual feelings, with the possible responses of "never", "a little bit of the time", "sometimes", "most of the time", and "always" (41). Each of these responses is assigned a value from 5-1 and each 3-item subscale is calculated from totally the score for each included response (41). For participants who were unable to physically complete the trait scales, this part was also administered orally.

Physical Activity

Prior week physical activity were obtained with the Godin Leisure-Time Exercise Questionnaire (43). For participants who were unable to physically or visually complete the questionnaire, it was administered orally. This instrument was selected partly due to its simplicity in design and administration, as well as its distinction between light, moderate, and high intensity activities. This questionnaire is scored by multiplying the number of times a participant engages in each intensity of physical activity by a different number: high intensity score is multiplied by 9, moderate intensity score is multiplied by 5, and light intensity score is multiplied by 3. These three values are then totaled for their Godin score. Comparative data taken from 411 undergraduate students showed an average score of 67.47 ± 43.2 (44).

Functional Independence in Activities of Daily Living

Activities of daily living were assessed with the Barthel's Index of Daily Living Activities in the participants affected by FRDA. This assessment was designed for people affected by neurological or musculoskeletal disorders undergoing rehabilitation to evaluate changes in functional independence and is one of the most widely used assessments of functional status (45, 46). The Barthel Index has been shown to have reliability, validity, and reproducibility in disabled populations (46-48). The ten activities of daily living that are assessed include: feeding, bathing, grooming, dressing, bowel control, bladder control, toileting, chair transfer, ambulation, and stair climbing (45, 48). Caretakers or the participants themselves assign a 0, 1, or 2 for each activity, indicating how much assistance they need in executing that task (45, 48). Further details on the differences between scores are provided as well in case clarification is needed (45, 48). This tool is advantageous due to the ability of participants to self-report their functional independence with this measure. This instrument was used to more thoroughly characterize disease severity for the participants affected by FRDA and to account for wheelchair usage.

Statistical Analysis

Data are presented as means ± standard deviations. Unpaired t-tests were performed to compare mitochondrial capacity, questionnaire data, and demographic variables between FRDA and AB groups. A two-way ANOVA was performed to evaluate the effect of group and electrical stimulation frequency on muscle-specific endurance scores. Cohen's d effect sizes were calculated for each of the three endurance

indices separately and combined. Two-tailed Pearson correlations were used to examine relationships between mitochondrial capacity, muscle-specific endurance, Barthel Index of Daily Living Activities, Godin Leisure-Time Activity Questionnaire, and the number of GAA repeats for the FRDA group. Partial correlations were also explored controlling for the Godin Leisure-Time Activity Questionnaire when examining the relationships between mitochondrial capacity, muscle-specific endurance, Barthel Index of Daily Living Activities. Significance was accepted at $p < 0.05$.

Results

Sixteen participants affected by FRDA and ten AB controls were tested. Summary characteristics of both groups are presented in Table 1. Characteristics of the participants affected by FRDA are presented in Table 2. No adverse events occurred during testing. No significant differences on age ($p = 0.9$), BMI ($p = 1.0$), or subcutaneous adipose tissue thickness (ATT) ($p = 0.5$) occurred between groups. Self-report data from the 8-year-old participant affected by FRDA was not included due to his age. The Godin Leisure-Time Physical Activity scores ($p < 0.001$) significantly differed between groups. The average Godin score for the participants affected by FRDA was 15.1 ± 16.7 and in the AB group it was 62.2 ± 28.8. Of the participants affected by FRDA who used a wheelchair, five had self-propelling wheelchairs and five had power chairs.

Due to the fact that our mitochondrial capacity and muscle-specific endurance measurements are not anticipated to change from day to day, we focused on interpreting the trait results from the Mental and Physical State and Trait Energy and Fatigue Scales. Significant differences occurred between groups for Trait Physical Energy ($p = 0.02$)

with an effect size of d = -1.2. The average score for Trait Physical Energy for the participants affected by FRDA was 4.9 ± 2.7 and for the AB group it was 7.4 ± 2.3. Average Trait Physical Fatigue for the participants affected by FRDA was 6.0 ± 2.1 and in the AB group it was 4.6 ± 1.8 (p = 0.1) with an effect size of d = 0.5. Average Trait Mental Energy for the participants affected by FRDA was 7.0 ± 2.9 and in the AB group it was 7.2 ± 2.4 (p = 0.9). Average Trait Mental Fatigue for the participants affected by FRDA was 4.6 ± 2.9 and in the AB group it was 5.1 ± 2.6 (p = 0.7). The results of the AB group are in agreement with normative data from 202 adults (41).

Forearm mitochondrial capacity, as measured by NIRS, was not significantly different between groups (p = 0.5). Average rate constant in the participants affected by FRDA and AB participants were both 1.8 ± 0.3 1/min. Results of the mitochondrial capacity test are presented in Figure 1. Time to half magnitude of the recovery of oxygenated heme signal following the release of the arterial cuff from the physiological calibration was not significantly different between groups (p = 0.6). Average time to half magnitude for the participants affected by FRDA was 22.2 ± 9.3 seconds and for the AB group was 24.2 ± 7.7 seconds.

A two-way ANOVA was performed to evaluate the effect of group and electrical stimulation frequency on muscle-specific endurance scores. Due to the fact that there was no significant interaction of group and frequency on endurance scores F (2,72) = 0.6 (p = 0.5), we were able to interpret our main effects. There was a significant main effect of group on endurance score F (1,72) = 15.0 ($p < 0.001$). Estimated marginal mean in the AB group was 84.8 ± SE 2.8 (CI: 79.1-90.4) and in the participants affected by FRDA was 70.7 ± SE 2.3 (CI: 66.2-75.2). There was also a significant main effect of frequency

on endurance score F (2,72) = 24.8 ($p < 0.001$). Results of the endurance index are presented in Figure 2. Cohen's d effect sizes were calculated for the three frequencies separately (2Hz, 4Hz, 6Hz): d = 1.2, 0.8, 1.2, respectively. Effect size was also calculated for group means across all frequencies, which yielded the largest effect size (d = 1.6).

Barthel scores from the FRDA group are presented in Figure 4. Average mitochondrial capacity was moderately correlated with the Barthel's Index of Daily Living Activities in the participants affected by FRDA (r = 0.55, p = 0.04) and is presented in Figure 4. The significance of this correlation was lost when controlling for Godin leisure-time physical activity score (r = 0.53, p = 0.08), indicating that this relationship is confounded by the influence of physical activity level on both mitochondrial capacity and functional independence. Average endurance was moderately correlated with the Barthel Index of Daily Living Activities in the participants affected by FRDA (r = 0.60, p = 0.01) and is presented in Figure 5. The significance of this correlation remained even when controlling for Godin leisure-time physical activity score (r = 0.77, p = 0.003), indicating that there is a significant relationship between mitochondrial capacity and functional independence that is not confounded by physical activity level. Average muscle-specific endurance and mitochondrial capacity were also moderately correlated in the participants affected by FRDA (r = 0.59, p = 0.03) and are presented in Figure 6.

Discussion

We successfully measured skeletal muscle mitochondrial capacity and muscle-specific endurance in 16 FRDA participants and 10 AB participants. We did not find evidence of impaired skeletal muscle mitochondrial capacity, as measured by rate constant of recovery of muscle metabolism measured with NIRS. These results are not consistent with previous studies that examined mitochondrial capacity by phosphorous nuclear magnetic resonance (^{31}P-MRS) (5, 27). A study by *Lodi et al* reported that participants affected by FRDA had significantly reduced initial rates of PCr postexercise resynthesis and maximum rates of mitochondrial ATP production (27). A study by *Vorgerd et al also* reported that participants affected by FRDA had significantly delayed PCr recovery time when compared to controls (5). Both of these studies were conducted in the calf muscles of FRDA participants. It is possible that the difference between our results and previous studies of mitochondrial capacity in people affected by FRDA is due to the difference in muscle tested. It is plausible that greater impairments would be seen in the calf muscles is a result of the reduced activity levels and wheelchair usage observed in people affected by FRDA. In our study, we chose to test the non-dominant forearm muscles and believe that the difference in activity levels for the forearm muscles between our participants affected by FRDA and control subjects was much less than if we were comparing the calves.

A possible explanation for the lack of mitochondrial capacity deficit observed in the FRDA group is the potential for mitochondrial compensation. Wredenberg *et al* created a mouse model of mitochondrial myopathy, which showed increased mitochondrial mass within the skeletal muscle and normal ATP production (49).

Although these results are surprising, the author's rationale was that the impaired respiratory chain function was compensated for by this increase in mitochondrial mass. This study concluded that the pathophysiology of mitochondrial myopathies may not be largely driven by reduced mitochondrial ATP production as previously thought (49). We have unpublished data on mitochondrial myopathies (MITO) showing normal mitochondrial capacity values in the non-dominant forearm as well. We believe that similar protective mechanisms may be at play in our sample of people affected by FRDA, particularly since skeletal muscle in people affected by FRDA is incompletely characterized and the exact function of the frataxin protein is still being understood. A recent study by *Martelli et al* suggests that iron regulatory protein 1 (IRP1) activation upon frataxin deficiency may help to sustain mitochondrial functioning (50). The expression and ratio of IRP1 and IRP2 vary between different tissues and may play a role in modulating impact of this IRP1 activation on iron metabolism (51). These studies exemplify the fact that protective mechanisms, which are still being understood, may be at play in maintaining mitochondrial capacity in the forearm.

The results of the muscle-specific endurance index showed that FRDA participants had an approximately 14% reduction in muscle-specific endurance, indicating greater fatigue in comparison to AB participants. This evidence is supported by the proposed role of iron accumulation in reduced exercise capacity observed in iron overload disorders (21). Previously, reported symptoms of weakness and fatigue in patients affected by iron overload diseases were attributed to cardiac function (21), however our results provide support for the contribution of skeletal muscle to these

physiological consequences. However, we would have anticipated a larger difference between groups but we believe this could be due to potential compensatory mechanisms.

We found relationships between muscle mitochondrial capacity, muscle-specific endurance, and disease severity as indicated by the Barthel's Index of Daily Living Activities. These findings are consistent with mitochondria assessments made with muscle biopsies and near infrared spectroscopy, in that there is heterogeneity in mitochondrial function. Schöls *et al* took skeletal muscle biopsies of the vastus lateralis muscle from 16 people affected by FRDA and found the 31% had significant mitochondrial defects (25). One previous study examined recovery of tissue oxygenation after exercise in FRDA participants with near-infrared spectroscopy (28). The study by *Lynch et al* showed that on average, participants affected by FRDA had delayed recovery time of oxygenation ($t_{1/2}$) following incremental exercise treadmill walking, (28). However, 14 of the 22 participants had normal recovery time of oxygenation (28), demonstrating the heterogeneity of participants affected by FRDA even when only people who were able to walk were included. This measurement reflects both recovery of oxidative metabolism and reperfusion of muscle, and was performed while the participants stood using the muscles that were being tested. Therefore, this outcome measure is not as directly related to mitochondrial capacity as the skeletal muscle biopsies or ^{31}P-MRS methodologies.

One of the limitations of our study was the heterogeneity of clinical phenotype observed in people affected by FRDA. It is possible that only some people affect by FRDA have skeletal muscle abnormalities, and it is not known if the forearm muscles are as affected as other muscles in the body (5). We used the Barthel index as a measurement

of functional independent and disease severity, while many studies use clinical ataxia rating scales, such as the Friedreich's Ataxia Rating Scale (FARS), which requires a neurological examination (52, 53). The advantage of the Barthel index is that it is a self-report scale that does not require trained professionals to perform the assessment. Our study also tested a diverse group of patients, from 8 to 55 years of age and with a wide range of functional impairments. This heterogeneity can influence the measurement variability in our study. If there are some people affected by FRDA who have greater skeletal muscle involvement, then a larger sample size would have helped in identifying and characterizing this subpopulation.

There is a need for assessment tools that can be used in clinical trials for FRDA. Currently clinical trials use measurements such as the 9-hole peg test, PATA test, and low-contract letter acuity, timed 25-foot walk, ataxia rating scales, and treadmill walking speed as functional assessments (28, 53, 54). Both of our skeletal muscle measurements were well tolerated and allowed participants who many not have complete motor control to be included. These tools allow for a much broader range of disease severity to be included in FRDA research. Since both measurements were moderately correlated with disease severity, we believe they are potentially useful tools for characterizing and tracking disease progression and should be further exploded.

Conclusion

There was no different in mitochondrial capacity between people affected by FRDA and AB controls. Muscle-specific endurance was significantly reduced in participants affected by FRDA in comparison to AB controls. Both mitochondrial

capacity and muscle-specific endurance were correlated to disease severity. Additionally, mitochondrial capacity and muscle-specific endurance were correlated with each other in the FRDA group. Our results are inconsistent with previous studies that examined mitochondrial capacity by phosphorous nuclear magnetic resonance (^{31}P-MRS), perhaps due to our testing a muscle not involved in locomotion (5, 27). However our results are consistent with previous skeletal muscle biopsy and NIRS-measurements in that there was heterogeneity in mitochondrial defects in people affected by FRDA (25, 28). The correlations between mitochondrial capacity, muscle-specific endurance, and disease severity suggest that this heterogeneity is important and that these measurement tools are potentially useful. NIRS and MMG measurements of mitochondrial capacity and muscle-specific endurance should be further investigated as potential clinical tools for assessing disease severity, progression, and tracking changes in muscle function over time in people affected by FRDA.

Figure Legends

Figure 3.1: Comparison of mitochondrial capacity between FRDA and AB groups. Circles represent individual FRDA participants and squares represent individual AB participants. Solid black shapes represent group averages. Bars represent standard deviations. There were no differences between groups ($p = 0.5$).

Figure 3.2: Comparison of muscle-specific endurance indices between FRDA and AB groups. Blue striped columns represent AB group averages. Pink columns represent FRDA group averages. Bars represent standard deviations.

Figure 3.3: Comparison of average muscle-specific endurance between FRDA and AB groups. Blue striped columns represent AB group average. Pink columns represent FRDA group average. Bars represent standard deviations. Significant differences occurred between groups ($p < 0.001$).

Figure 3.4: Comparison of perceptions of trait energy and fatigue between FRDA and AB groups. Blue striped columns represent AB group average. Pink columns represent FRDA group average. Bars represent standard deviations. Significant differences occurred between groups for physical energy ($p = 0.02$).

Figure 3.5a: Pearson correlation between mitochondrial capacity and the Barthel Index of Daily Living Activities in FRDA participants ($R^2 = 0.55$, $p = 0.04$).

Figure 3.5b: Pearson correlation between muscle-specific endurance and the Barthel Index of Daily Living Activities in in FRDA participants. ($R^2 = 0.61$, $p = 0.02$).

Figure 3.5c: Pearson correlation between mitochondrial capacity and muscle-specific endurance in in FRDA participants ($R^2 = 0.59$, $p = 0.03$)

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Table 3.1

	Gender (M/F)	**Age** (yrs)	**BMI** (kg/m2)	**ATT** (cm)	**Godin** (score)
FRDA	(3/13)	31.8 ± 12.8	22.2 ± 5.3	0.7 ± 0.2	15.1 ± 16.7*
Control	(2/8)	31.4 ± 12.2	22.1 ± 2.6	0.6 ± 0.2	62.2 ± 28.8

Values are presented as means ± SD. *$p < 0.05$

Table 3.2

Barthel Index	Gender (M/F)	Age (yrs)	Short Arm GAA Repeat	Wheelchair (Y/N)	Godin (score)
6	F	30	967	Y	21
7	F	36	-	Y	3
7	F	11	733	Y	3
9	F	44	850	Y	0
9	F	55	380	Y	3
9	F	43	-	Y	6
12	F	32	254	Y	3
14	M	36	244	Y	25
14	F	29	-	Y	27
15	F	26	-	Y	19.5
14	F	41	850	Y	54
16	F	44	550	Y	0
17	F	30	350	Y	10.9
18	F	30	400	N	6
20	M	14	766	N	45
20	M	8	600	N	-
13 ± 5	(3M/13F)	31 ± 13	579 ± 252	(13Y/3N)	15.1±16.7

Figure 3.1

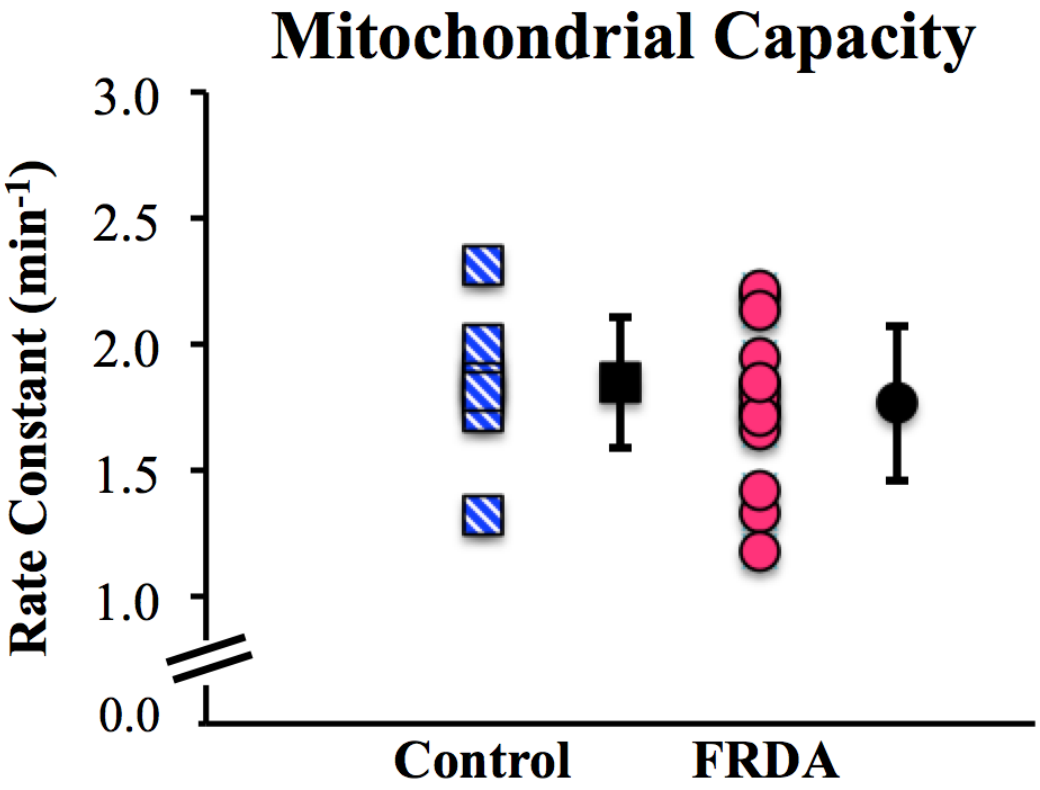

Mitochondrial Capacity
Rate Constant (min-1)
3.0
2.5
2.0
1.5
1.0
0.0
Control
FRDA

Figure 3.2

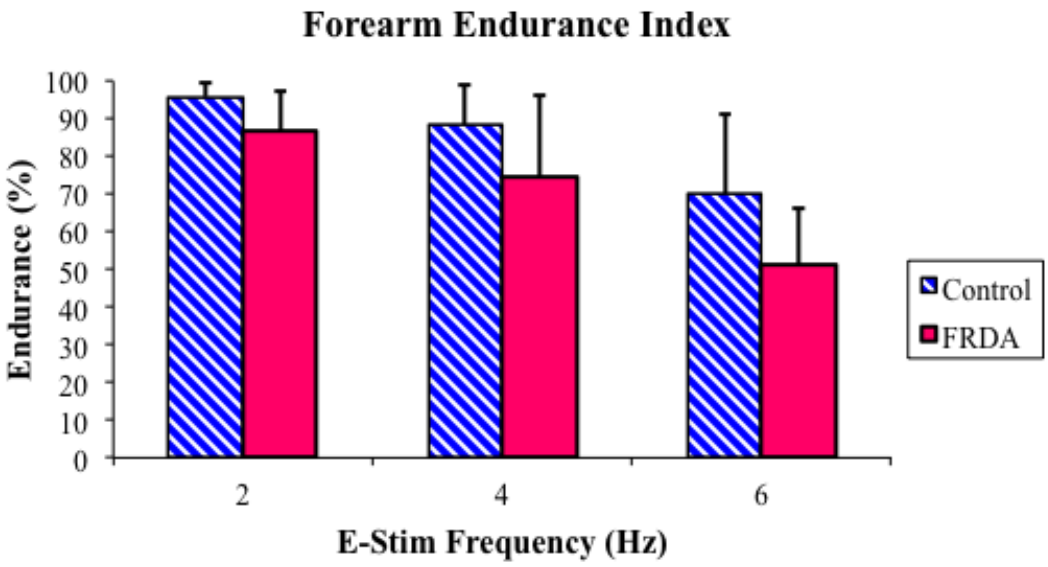

Forearm Endurance Index
Endurance (%)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
E-Stim Frequency (Hz)
Control
FRDA

Figure 3.3

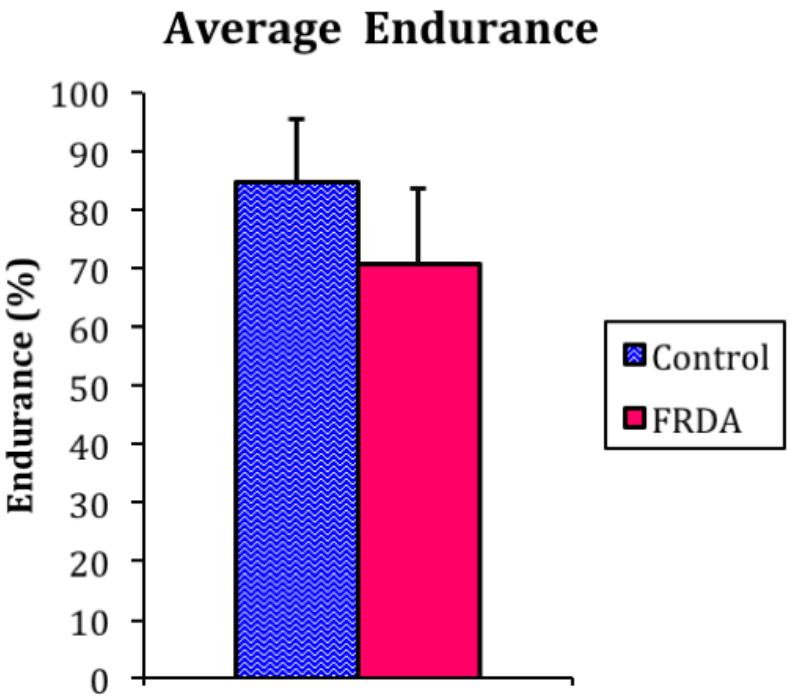

Average Endurance
Endurance (%)
100
90
80
70
60
50
40
30
20
10
0
Control
FRDA

Figure 3.4

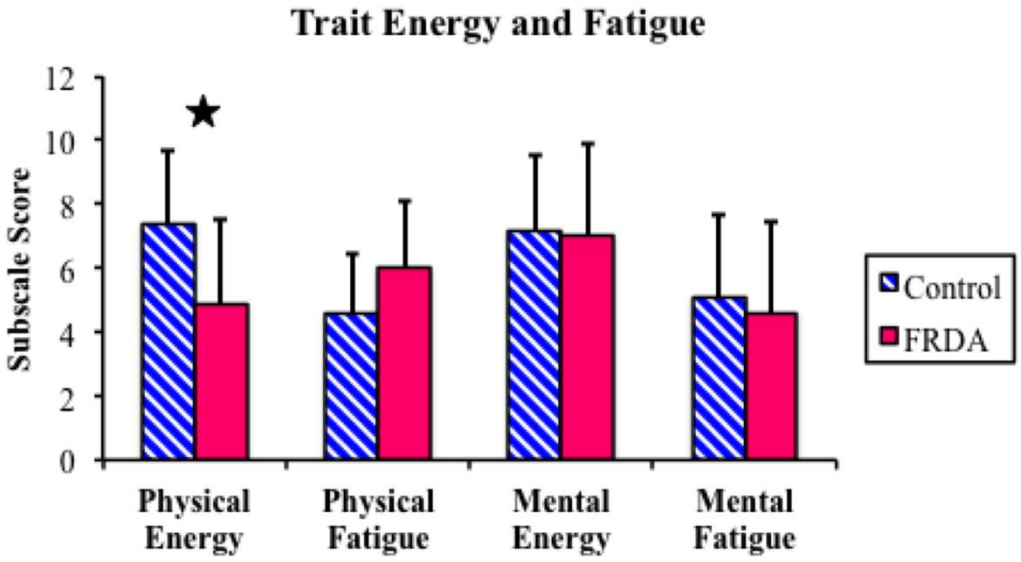

Trait Energy and Fatigue
Subscale Score
12
10
8
6
4
2
0
Physical Energy
Physical Fatigue
Mental Energy
Mental Fatigue
Control
FRDA

Figure 3.5a

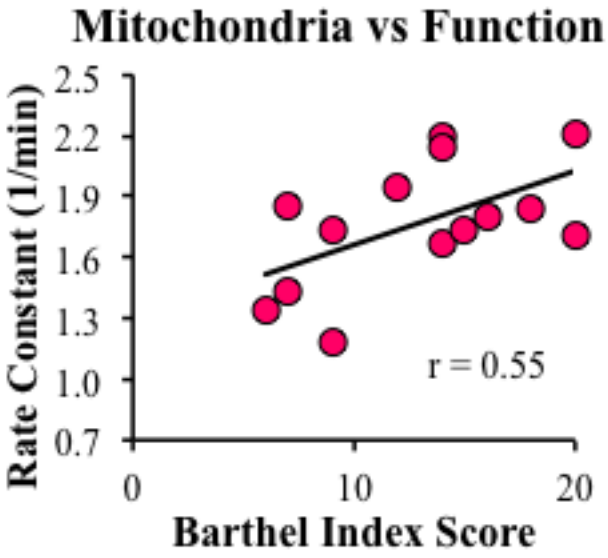

Mitochondria vs Function
Rate Constant (1/min)
2.5
2.2
1.9
1.6
1.3
1.0
0.7
0
10
20
Barthel Index Score
r = 0.55

Figure 3.5b

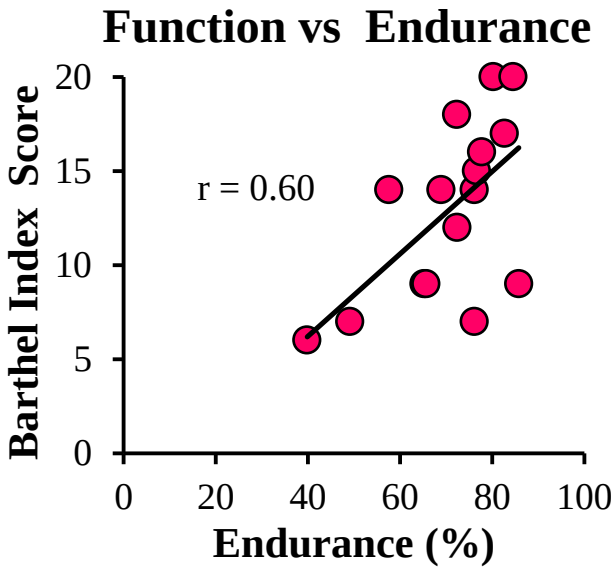
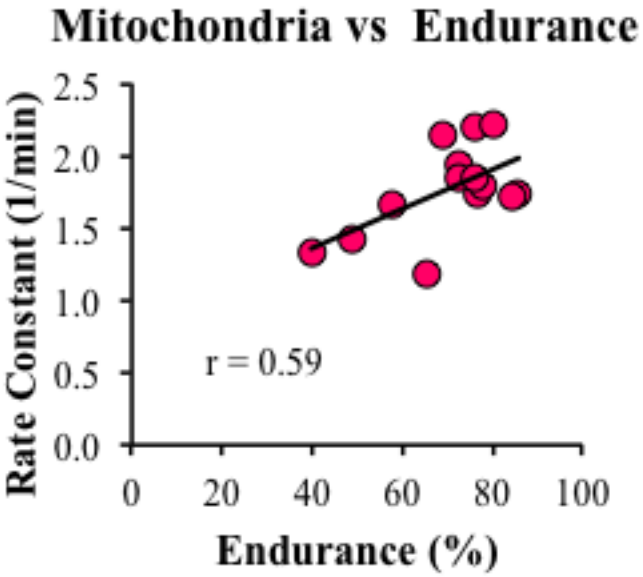

Function vs Endurance
Barthel Index Score
20
15
10
5
0
r = 0.60
0
20
40
60
80
100
Endurance (%)

Figure 3.5c


Mitochondria vs Endurance
Rate Constant (1/min)
2.5
2.0
1.5
1.0
0.5
0.0
r = 0.59
0
20
40
60
80
100
Endurance (%)

CHAPTER 4

SUMMARY AND CONCLUSION

Purpose and Feasibility

Current FRDA research is limited by the lack of clinical measures of disease progression (18). Clinical trials to test therapeutic interventions ultimately rely on the ability to characterize patients and track functional changes over time (54). These measurements need to be easy to administer and sensitive to change over time (2). There are many challenges in monitoring disease progression in people affected by FRDA. FRDA does not have the homogeneity in clinical phenotype as many other recessive traits display (4). Subsequently, the variability in age of onset and speed of progression contribute to barriers to characterizing the population (55). Additionally, with the incomplete understanding of the function of frataxin it is hard to develop a tool that will be sensitive to FRDA-specific outcomes. Currently clinical trials use measurements such as the 9-hole peg test, PATA test, and low-contract letter acuity, timed 25-foot walk, ataxia rating scales, and treadmill walking speed as functional assessments (28, 53, 54). The purpose of this study was to test the clinical utility of our two noninvasive measurements of skeletal muscle in people affected by FRDA.

Study Strengths

We successfully recruited a large sample of participants (n=16) affected by FRDA who represented a fully range of disease severity, from asymptomatic to advanced (ages 8-55 yrs). We demonstrated that these measurements could be successfully made on this population regardless of age or disease severity. Although mitochondrial capacity was not different between groups, it was correlated with disease severity, as measured by functional independence. This correlation indicates that our NIRS test can distinguish differences within a range of physical functioning for this population. Our results indicate that the FRDA group had significantly impaired muscle-specific endurance in comparison to AB controls. This method provides a functional assessment of muscle that is also correlated to disease severity, as measured by functionality in daily living activities. Additionally, our two noninvasive assessment of skeletal muscle were correlated in the FRDA group, indicating that there is a relationship between our measurements. These two tools should be further investigated as clinical tools of characterizing and assessing changes over time in skeletal muscle function.

Study Limitations

Limitations of our study may include our choice of the forearm muscle, which yielded insignificantly results and are in disagreement with previous studies of mitochondrial capacity on the calf muscles (5, 27, 28). With FRDA characterized by gait ataxia, studying a muscle group within the leg may have yielded significant differences in mitochondrial capacity. However, the leg was not chosen due to the confounding factor of disuse in those who are wheelchair bound. In a previous study by *Lynch et al*, the calf

was studied in only participants who were not wheelchair bound (28). However, only 3 of the 16 FRDA participants tested in our study were able to walk, so this would not have been feasible with our recruitment. In order to study the leg muscles, we would need to have a wheelchair control group in order to account for the effect of disuse. Additionally, a greater exercise stimulus in the forearm may have elicited a difference in mitochondrial capacity. Despite the insignificant results, the correlation with muscle-specific endurance and disease serenity indicate that our NIRS measurement may still be a clinical tool for this population, perhaps with alterations in muscle tested and exercise stimulus. Although we did find a significant impairment in muscle-specific endurance, it was a smaller difference that we would have anticipated. This may be due to compensatory mechanisms or also the choice of muscle.

NIRS Test Failures

Three NIRS measurements, two in the FRDA group and one in the AB control group, could not be completed or accurately analyzed for multiple reasons. Two tests was unable to be analyzed due to incomplete cuff occlusion, resulting from excessive adipose tissue thickness and/or loose skin from recent weight loss on the proximal arm. One participant did not tolerate the sensation of the electrical stimulation above a very low current, which resulted in insufficient activation of the muscle and a failed test.

Surface Mechanomyography

The muscle-specific endurance test demonstrated a significant impairment in the FRDA group. This protocol is only nine minutes long with three 3-minute stages at 2, 4,

and 6Hz sequentially. The muscle-specific endurance protocol was well tolerated by all participants with zero test failures. The protocol was designed to be able to be applied to any patient population, by having multiple stages at increasing frequencies we are able to determine not only how much a participant fatigues, but at what stage in the protocol does this occur. We have also tested protocols in AB populations that just use 5 minutes at 6Hz frequency, and we considered just interpreting the endurance index at the 6Hz frequency of our protocol. However, by interpreting all three stages we are able to identify different patterns of progression, including a gradual loss of contractile acceleration, as well as more sudden determents that seemed to reach a minimum acceleration that was maintained for the duration of the test. An additional advantage of our progressive protocol is that it provided familiarization of the electrical stimulation at lower frequencies before reaching the maximum 6Hz. This proved to be advantageous when testing participants who had no prior electrical stimulation experience.

Unlike other muscular fatigue tests, which require participants to complete voluntary exercise, this protocol and technique allows even those who do not have complete motor control to be successfully tested. With the rapid progression of FRDA, providing tools such as this that would allow clinical trials to include patients of all disease severities could drastically increase the number of eligible participants in FRDA research. Additionally, we are able to apply this protocol to many different muscles, including the calf, quadriceps, back, and trapezius muscles, giving this methodology an even greater potential as far as application goes.

Future Directions

My master's thesis is the first application of these surface mechanomyography and near-infrared spectroscopy methods to the FRDA population. For future research, I think we need to further explore these measurements and see if studying skeletal muscle as a main outcome in the FRDA population is an appropriate focus in developing clinical tools. I believe we should follow up with a test of the calf or thigh muscles and use a wheelchair-bound control group to see if the lower limbs are more affected than the forearm in the FRDA population. I think that we should conduct an electrical stimulation training study to examine whether or not these measurements would be enhanced after electrical stimulation training. I also think we should conduct a trial looking at antioxidant supplementation and see if any changes in these measurements occur over time. These trials would provide strong evidence for the inclusion of these measurements in clinical trials for FRDA treatments.

Insights

I anticipated that I would have found impairments in mitochondrial capacity and even greater impairments in muscle-specific endurance. I initially thought that these measurements would've shown stark differences between people affected by Friedreich's Ataxia and controls. However, the fact that both of our noninvasive methods correlate with disease severity, as measured by functional independence in daily living activities, indicates that these tests may be useful indicators of overall function in individuals affected by FRDA. The fact that we saw a subgroup of individuals affected by FRDA who showed impairments in both measures leads me to think that these measurements

may help to identify a subgroup of people affected my FRDA with more severe muscular impairments, as previous studies have found. Both of these tests are entirely noninvasive, quick, easy to administer, and able to be applied to any level of disease severity. Many established tests of muscle endurance require motor control, automatically excluding many people affected by FRDA. By utilizing these noninvasive tools in clinical FRDA research, more participants would be able to be included.

Overall Conclusion

Our results are consistent with previous biopsy and NIRS measurements in that there is heterogeneity in mitochondrial dysfunction in people affected by FRDA. The correltations that we observed between mitochondrial capacity, muscle-specific endurance, and disease severity indicate that this heterogeneity is important and that these methodologies are potentially useful. These noninvasive, quick, and easy methods should be further evaluated as clinical research tools to track disease progression and test treatment efficacy. The application of these measurements has the potential to broaden recruitment and participation in FRDA research, ultimately accelerating efforts towards finding efficacious treatments for FRDA.

CHAPTER 5

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