ASSESSING RESPIRATORY FUNCTION USING THE 5-MINUTE ENDURANCE TEST PROTOCOL

by

HALLIE R WACHSMUTH

(Under the Direction of Kevin McCully)

ABSTRACT

There is a need for objective, diaphragm-specific respiratory function measurements applicable to clinical settings. **PURPOSE:** To establish an improved measurement of respiratory function via assessment of diaphragm endurance. **METHODS:** 20 healthy subjects, highly active (HA) (n=10) or inactive (IN) (n=10), were tested using electrical stimulation (5 Hz, 5-minutes) on one occasion. Stimulation electrodes were placed on one phrenic nerve, and a current producing a vigorous contraction was used. An accelerometer collecting at 400Hz was placed on the abdomen. An endurance index (EI) was calculated from the acceleration values at 2 (EI2) and 5 minutes (EI5). **RESULTS:** EI2 of IN and HA were 68.8±16.3% and 92.8±8.7% respectively (p=0.001). EI5 of IN and HA were 62.6±12.4% and 85.7±13.6% respectively (p=0.001). The correlation of EI2 and EI5 was 0.588. **CONCLUSIONS:** Highly active subjects had higher diaphragm endurance index values than inactive subjects, supporting the use of the test to evaluate respiratory function.

INDEX WORDS: accelerometry, diaphragm, electrical twitch mechanomyography, muscle fatigue, muscle endurance, respiration, respiratory function,

ASSESSING RESPIRATORY FUNCTION USING THE 5-MINUTE ENDURANCE TEST PROTOCOL

by

HALLIE R WACHSMUTH

BSEd, University of Georgia, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2020

ASSESSING RESPIRATORY FUNCTION USING THE 5-MINUTE ENDURANCE TEST PROTOCOL

by

HALLIE R WACHSMUTH

Major Professor: Kevin McCully

Committee: Jarrod Call
Nathan Jenkins

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
May 2020

ACKNOWLEDGEMENTS

I would like to thank Dr. McCully for his guidance throughout this thesis project and my time here at the University of Georgia. I greatly appreciate all that you have taught me as both a mentor and an instructor.

To my committee members, your guidance and insightfulness to allow me to think holistically about my project was invaluable. Learning from you about the subjects you are passionate about throughout my bachelor's degree and my master's degree has been an absolute pleasure.

To my lab mates, Megan, Uma, and Adeola, you all were inexplicably helpful throughout this time as both my friends and my lab mates. Thank you for making these two years some of my best.

To my parents, I am so grateful for you. You have always urged me to be the best that I can be, personally and professionally. I am forever grateful for your limitless love, patience, and encouragement.

To my brother, Luke, thank you for consistently raising the bar. First one to Dr. wins! Ready, set, go!

To my friends, I appreciate all of the laughs we've shared and all of the support you've given throughout the years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Title

Assessing Respiratory Function Using the 5-minute Endurance Test Protocol

Purpose

To establish an improved method for measurement of respiratory function via assessment of diaphragm endurance.

Background

Respiratory dysfunction and respiratory failure are severe medical issues that profoundly impact the cost of health care and both long-term and short-term patient survival. While respiratory dysfunction is a major health issue, for the purpose of this thesis, respiratory failure will be identified as the major health problem that is the focus of this project. Acute respiratory failure (ARF) is commonly seen in hospitalized patients and can lead to further complications and, if severe, death. In a recent epidemiological study, researchers determined a 56% increase in occurrence of ARF and a 37% increase in in-hospital deaths associated with ARF from 2001 to 2009.[1] There are many different causes of respiratory dysfunction and failure such as mechanical ventilation, lung disease, nerve damage, and neuromuscular diseases.[2-5] The current methods of clinically assessing respiratory function are either subjective or secondary to the respiratory muscles.[6] There is a need for an inexpensive, non-invasive, direct way to assess respiratory function in the clinical setting.

The diaphragm is the primary inspiratory muscle and is comprised of two parts: costal and crural. The costal portion of the diaphragm is split into right and left halves, which are innervated by the right and left phrenic nerve respectively. The phrenic nerves originate in the spinal cord at the C3-C5 vertebral level. These nerves typically run behind the sternocleidomastoid muscle in the neck and can be stimulated both internally and externally. During inspiration, the diaphragm contracts and reduces the thoracic pressure by increasing the volume of the thoracic cavity. This reduction in thoracic pressure allows for air outside of the body to flow down a pressure gradient into the lungs. The diaphragm is the main inspiratory muscle used during breathing at rest and plays a significant role in inspiration during exercise despite other respiratory muscles being recruited.[7] Since the diaphragm plays a critical role in voluntary inspiration, it is necessary to measure the diaphragm to determine respiratory function; respiratory failure could be associated with diaphragm fatigability or dysfunction. The diaphragm should be assessed during respiratory function assessments to determine inspiration function.

Fatigability of other muscles such as skeletal muscles has been widely studied. There are two different components of fatigue: central and peripheral. Central fatigue is a reduction in muscle contraction due to neuronal components such as reduced motivation or drive in voluntary contractions, while peripheral fatigue is a reduction in muscle contraction force due to muscular conditions.[8, 9] In this thesis, peripheral fatigue will be the focus of the study because the method of electrical stimulation bypasses voluntary contraction and thus eliminates the central component of fatigue. Peripheral fatigue is defined as the temporary decrease in force production of skeletal muscle resulting from activation; this is likely due to metabolite buildup in the muscles.[9, 10]

One method of determining muscle fatigue and fatigability is through an endurance test.[11-13] The endurance test we are basing this study off of is a 5-minute electrical stimulation protocol which has been previously studied on various muscles.[11, 14] This endurance test consists of electrical stimulation and assessment of muscle twitch acceleration via tri-axial accelerometry. The endurance of a muscle is evaluated via analysis of the change in acceleration of the muscle twitch determined via the accelerometry data. The endurance of a muscle is the inverse of muscle fatigue; for example, if the endurance index levels are high, the muscle is not highly fatigable.[11-13, 15, 16] This method could also be employed to assess the endurance of the diaphragm; one half of the diaphragm can be stimulated non-invasively via electrode stimulation of one of the phrenic nerves for 5 minutes. For this study of the diaphragm, this 5-minute endurance protocol is also being evaluated against a 2 minute endurance protocol to determine the validity of a 2-minute test.

The current methods of measuring respiratory function in clinical and research settings are varied. There are many tests that rely on patient/subject motivation or are subjective to the healthcare provider/researcher performing the test.[5, 6] Some of the common measures of respiratory function include: maximum voluntary ventilation, vital capacity, maximal inspiratory pressure, maximal expiratory pressure, and measurement of blood gasses. Assessment of the fatigability of the diaphragm has included supramaximal tetanic contraction stimulation via the phrenic nerve which can be uncomfortable or painful to many subjects.[6] These tests can be improved upon by using the technique that we have developed: a non-invasive, 5-minute endurance protocol to determine diaphragm endurance. This is a relatively inexpensive technique that requires an accelerometer and equipment that most clinical sites have access to.

Statement of the Problem

Assessment of diaphragm muscle endurance using phrenic nerve stimulation needs validation but has the potential to transform how respiratory muscle function is assessed.

Aims and Hypotheses

Aim 1: To further validate a measurement of respiratory function, as assessed by endurance index of the 5-minute endurance test protocol, in 2 different groups: (1) young, highly active adults, (2) young, inactive adults.

Hypothesis 1A: Highly trained subjects will have higher endurance index values in the diaphragm than the inactive subjects.

Hypothesis 1B: Highly trained subjects will have higher endurance index values in their vastus lateralis muscles than inactive subjects.

Hypothesis 1C: Across groups, the diaphragm will have lower muscle endurance than the vastus lateralis muscle.

Aim 2: Evaluate the use of acceleration after two minutes of stimulation (2-minute endurance test) in the diaphragm.

Hypothesis 2A: There will be a statistically significant correlation between the endurance index values at the 2-minute and 5-minute time points for the diaphragm muscle.

Hypothesis 2B: There will be a statistically significant correlation between the endurance index values at the 2-minute and 5-minute time points for the vastus lateralis muscle.

General Approach

In this study 20 participants will be tested with the following group breakdown: young, inactive adults (10) and young, highly active adults (10). There will be 1 day of testing in this study design. The data collected include collection of demographic information and 2, 5-minute endurance tests on the diaphragm and the vastus lateralis muscle. The measurement of the endurance index of the vastus lateralis muscle will be taken as a control to determine if there are any muscular issues with the participants. In the endurance test of the vastus lateralis, results are expected to be similar between the two groups.

The phrenic nerve, and thus the diaphragm, will be stimulated by pencil electrodes placed on either side of the right or left sternocleidomastoid muscle. The accelerometer will be placed 1 inch below the xyphoid process and ½ inch from the midline towards the side that will be stimulated. The vastus lateralis will be stimulated by two adhesive electrodes placed at both the distal and proximal ends of the muscle. The accelerometer will be placed in between the electrodes on the belly of the muscle. Both of these tests will follow the 5-minute endurance test protocol; this consists of stimulation at

5 hertz for 5 minutes at a current that creates a clear, strong contraction. For the diaphragm, the values for the 2-minute endurance test will be extrapolated from the data collected for the 5-minute test.

CHAPTER 2

REVIEW OF LITERATURE

Respiratory Weakness and Disuse

Respiratory dysfunction and respiratory failure are severe medical issues, caused by a multitude of different conditions, which profoundly impact the cost of health care and both long-term and short-term patient survival. There are many different causes of respiratory dysfunction and failure that can be categorized into respiratory weakness and respiratory disuse. Some of these causes are mechanical ventilation, lung disease, nerve damage, and neuromuscular diseases. Respiratory dysfunction is a variable condition ranging in severity, whereas respiratory failure is the inability to maintain normal gas exchange. Respiratory failure is determined as when partial pressure of oxygen is below 60mmHg or partial pressure of carbon dioxide levels are higher than 50mmHg.[17] While respiratory dysfunction is a major health issue, for the purpose of this paper, respiratory failure will be used as the focus of this project.

Acute respiratory failure (ARF) is commonly seen in hospitalized patients; it can lead to further complications and, if severe, death. ARF accounted for 137.1 out of 100,000 hospitalizations of patients 5 or more years old according to the 1994 Nationwide Inpatient Sample.[18] An epidemiological study conducted in 2001 determined that 32% of patients admitted into a participating ICU were admitted for ARF. Throughout the study it was determined that 56% of the ICU patients had ARF at some point during their stay, either during admission or during the treatment period. Patients with ARF had a mortality rate that was twice as large as the mortality rate seen in other patients.[19] In agreement with the previous study, a recent epidemiological study reported an increase of 37% for in-hospital deaths associated with ARF. This study also discovered an increased population occurrence of 56% in ARF.[1] Concurrent with the increase in ARF morbidity and mortality associated with ARF, there is an increased medical cost; the total impatient cost of ARF patients increasing from $30.1 billion in 2001 to $54.3 billion in 2009.[1] These increases in ARF prevalence can be linked to the increased prevalence causes that will be further explained.

Mechanical ventilation is a common practice in intensive care units (ICU) of hospitals for patients with and without ARF;[20-22] approximately 40% of patients in the ICU received mechanical ventilation.[23] Mechanical ventilation is the use of a medical device to fill and empty the lungs with gas that will allow for the patient to have normalized blood gas values. This process is done without work from patient respiratory muscles; either through positive pressure or negative pressure. The mechanical ventilator is an important invention that allows for individuals unable to breathe to maintain necessary blood gas levels, but can be detrimental to respiratory muscles.[2, 3, 21, 24-27] A study conducted in North Carolina found that the use of mechanical ventilation increased by 11% from the years 1996 to 2002 with patients over 64 years old making up most of the mechanically ventilated population. This study also found that the percentage of patients being discharged home after receiving mechanical ventilation as treatment decreased from 1996 to 2002 by about 10%.[18] While mechanical ventilation is proven to improve acute patient mortality rates it is also a source of future respiratory failure through muscle disuse.[2, 21] Mechanical ventilation can cause many negative effects to the respiratory muscles (specifically the diaphragm) such as: decreased force-generation and muscle injury or atrophy.[3, 21, 25, 26] There are no current criteria for deciding to continue with ventilation of the patient; it is based on physician judgement. Although there is one recent paper that evaluated the efficacy of a non-invasive mechanical ventilation advisory system with results that had a high agreement with recommendations from a respiratory specialist.[28] One of the ways that physicians determine if the patient is healthy enough to stop mechanical ventilation is via spontaneous breathing trial. This consists of removing the ventilator and measuring oxygenation of the blood, determining if there is increased breathing effort, diaphoresis, and tachycardia.[29] Most of these mentioned are subjective measures and can vary based on the physician; finding an objective measure of respiratory function could help physicians make a more informed decision when to remove the patient from the ventilator.

Another cause of ARF is pulmonary disease; there are many different kinds of pulmonary diseases that can cause ARF. Chronic obstructive pulmonary disease is a lung disease with high morbidity and mortality rates.[30] This disease severely impacts quality of life in those affected by making activities of daily living difficult or impossible to perform.[31] Over 15 million people in the United States have been diagnosed with chronic obstructive pulmonary disease, but it is projected that as many as twice that amount may have the disease but are undiagnosed.[32] In the United States, chronic obstructive pulmonary disease is the 3rd most common cause of death and has a total health care cost of ~$54 billion.[30, 33-35]

Acute spinal cord injury incidents in developed countries occur at a relatively low rate of 11.5 to 53.4 per million persons, but in the United States the prevalence of spinal cord injury is approximately 14 times higher with a prevalence of 721 per million persons.[36, 37] Since the nerves innervating the diaphragm originate at cervical vertebrae numbers 3-5 (C-3-C-5), spinal cord injury at or above C-5 results in partial or complete respiratory muscle paralysis respectively.[4] For those patients with spinal cord injuries that do not completely paralyze the respiratory muscles, a method of objectively measuring respiratory function would be invaluable in ensuring proper care without over-ventilation. As discussed previously over-ventilation can exacerbate the disuse and ultimate weakness of the respiratory muscles and thus perpetuate respiratory failure.

Some more common neuromuscular diseases that can cause acute respiratory failure are Guillian-Barrè syndrome and myasthenia gravis.[38-40] These neuromuscular diseases can cause respiratory failure by a few different ways with the most likely causes being weakness in the muscles of the throat and mouth or weakness in the primary and secondary respiratory muscles in the chest.[5, 38-40] The clinical assessment for determining future respiratory failure in these populations consists of 4 objective measurements and largely subjective measurements including the palpation of the trapezius and neck muscles to correlate to and assess diaphragmatic strength.[5]

The current assessments of respiratory function in these previously mentioned populations are lacking; a better method of assessment is necessary. There should be a low-cost, objective assessment of respiratory function that can be used to both stratify at-risk populations and to determine whether continued use of a ventilator is beneficial. The method we are evaluating is an objective, non-invasive, and diaphragm-specific measurement.

The Respiratory System: The Role of the Diaphragm

The diaphragm is one of the primary inspiratory muscles at rest and during exercise, and it is essential for respiration.[41, 42] The diaphragm includes 2 parts: costal and crural. The costal portion of the diaphragm is the contractile portion and is split into 2 halves, or hemidiaphragms.[43-45] Each hemidiaphragm is innervated by the phrenic nerve that originates at the C3-C5 vertebral level of the spinal cord; both run down the neck behind the sternocleidomastoid muscle on their respective sides. The left phrenic nerve innervating the left hemidiaphragm and the right nerve innervating the right hemidiaphragm.[44, 46, 47] During inspiration in healthy humans, the costal portion of the diaphragm contracts (both hemidiaphragms contracting simultaneously) and reduces thoracic pressure by increasing the volume of the thoracic cavity. This reduction in thoracic pressure allows for air to flow down a pressure gradient from outside of the body into the lungs.[44] The diaphragm is a unipennate muscle made up of about 50% slow and 50% fast fibers. These fast fibers are further broken into fast oxidative and fast glycolytic fibers, which are in equal proportion of 25% and 25% of the total muscle fiber type distribution.[48, 49] This indicates that it can contract quickly but not with great force; this is beneficial for its role in exercise. The diaphragm is also the main inspiratory muscle during exercise, although there are also other accessory muscles that help decrease the thoracic pressure by further increasing the thoracic cavity volume such as the sternocleidomastoid, scalene, and external intercostal muscles.[7, 41, 42, 50] Because of the role that the diaphragm plays as the main inspiratory force in both rest and exercise, it will be the main focus of this thesis.

Muscle Fatigue

Skeletal muscle fatigue is a common issue in certain populations, and increased fatigability of the diaphragm would be problematic considering its role in inspiration. Skeletal muscle fatigue has been widely studied in many different muscles and muscle groups and studied in a multitude of different ways. Muscle fatigue is considered a temporary decrease in muscle contraction force due to muscle activation.[9, 51, 52] There are two components to muscle fatigue: central and peripheral. Central fatigue is a reduction in neuronal activation of the muscle causing a transient decrease in muscle contraction force.[8] Peripheral fatigue is defined as a transient decrease in muscle contraction force due to a buildup of metabolites arising from repeated contraction.[51] The main metabolites that buildup and cause peripheral fatigue are adenosine diphosphate (ADP), hydrogen ions, and inorganic phosphate.[9, 53-60] ADP buildup causes a change in cross bridge cycle turnover and decreasing the shortening velocity of the muscle fiber due to its role as a competitive inhibitor of ATP binding.[57, 59] Inorganic phosphate and hydrogen ions have been determined to cause fatigue via a different mechanism. Inorganic phosphate and hydrogen ion buildup appear to cause a decrease in tension of contraction via either decreased number of attachments of actin and myosin (small and large contractile filaments) or a decrease in the strength of attachment of these filaments.[57-60] These metabolites are theorized to buildup and cause fatigue as a protection mechanism for the muscle; fatigue prevents the muscle from damaging itself.[61] This becomes an issue when the muscle contracts in modalities that would normally be considered within healthy ranges yet still causes fatigue. Assessment of the rate of peripheral fatigue will be the focus of the study; it will be considered the fatigability of the muscle. This was chosen as the scope of the study because assessing peripheral fatigue via bypass of the central fatigue component allows for an evaluation of muscle quality. The less fatigable the muscle, the greater the work capacity of the muscle. This indicates that the muscle can contract for longer under a given contraction rate.

The fatigability of a muscle is determined by two factors: fiber type distribution and training status. There are 2 main fiber types in humans[62, 63], type I and type II fibers, which are further broken down into 3 types: type I, type IIa, and type IIx.[64] There is a difference in energy producing pathways in the type II muscle fiber subgroups; type IIa fibers are considered fast oxidative fibers while type IIx fibers are fast glycolytic fibers.[9, 65] Type I fibers are considered slow muscle fibers, meaning that they mostly use slower oxidative pathways to generate energy for muscle contraction; these are most resistant to fatigue but produce the least force. Type II muscle fibers are considered fast muscle fibers, meaning that they can contract quickly and produce more force than the type I fibers, but they rely more on faster glycolytic pathways to generate the energy necessary to contract which cause a greater increase in the previously mentioned metabolites.[54-56, 66-68] The fatigability of the muscle fibers is, in increasing fatigability: type I, type IIa, and type IIx. Type IIa fibers, while relying more on the glycolytic energy pathway than type I fibers, use the oxidative energy pathway more than type IIx fibers.[9, 65] An increase in the ability to use the oxidative pathway decreases the fatigability of these fibers, and the efficiency of the type IIa fibers to use oxidative pathways is dependent on training.[69] These muscle fibers are activated during higher work rate activities that require more forceful contractions due to the motor unit recruitment principle of size.[69, 70] This principle states that the first motor units recruited to perform a certain exercise are the smaller motor units which are comprised of mostly type I fibers. As the intensity of the exercise increases and more power is necessary to complete contraction, more and larger motor units are activated to increase the muscle force production to match the force necessary to complete the exercise; these larger motor units contain more of the type II fibers.[70] The more often that the type II fibers are activated, the greater the molecular signals are to increase the oxidative capacity of the muscle. This can cause a shift in phenotype and metabolic characteristics of type IIx fibers to type IIa fibers.[65, 68, 69, 71] This metabolic adaptation of muscle fibers has also been reported to occur in the diaphragm.[72, 73] An increase in oxidative capacity of the type II fibers is important because, if highly fatigue resistant, they allow the muscle to be able to produce higher forces over a longer period of time. This will allow for an increase the total work capacity of the muscle. Muscle fatigability is a good indicator of muscle quality and could be assessed in the diaphragm to determine respiratory health and function. There are many different ways to study fatigue, but none are more clinically relevant and physiologically more encompassing than our endurance test.[9, 11]

The Endurance Test

One way to assess muscle fatigability is an endurance test. This test is non-invasive and completely bypasses the central nervous system and thus, the central aspect of fatigue.[11] There have been studies testing the usability and clinical relevance of this method of assessing muscle endurance in both healthy and clinical populations with success.[11-16] This approach to assessing muscle endurance uses submaximal muscle twitches generated via low-frequency (5Hz) electrical stimulation for 5 minutes. This endurance protocol has been previously used in a 9-minute format of 2, 4, and 6 Hertz for 3 minutes each and since shortened to 5 Hertz for 5 minutes.[11-14, 74] As the muscle contracts in twitches, the twitch contraction accelerations produced from the electrical stimulation are measured via a triaxial accelerometer placed on the muscle being assessed. A decline in the acceleration values collected indicates the onset of muscle fatigue. The accelerometry data are analyzed and used to calculate an endurance index; the more fatigable the muscle, the lower the endurance index. This means that if there is a large decline in the acceleration of the muscle twitches throughout the duration of the 5-minute test, the calculated endurance index will be low and the fatigability of the muscle high. This endurance test has been established on multiple different skeletal muscles[12-14] and once on the diaphragm with moderate success (manuscript under review);[74] if this method can be successfully used on the diaphragm there would be an objective, noninvasive, and inexpensive method of directly assessing respiratory function.

Stimulation of the Phrenic Nerve

Previous assessment of the fatigability of the diaphragm has been studied via phrenic nerve stimulation in healthy populations and clinical populations with varying success.[6, 75-77] This is done by stimulating either one phrenic nerve or both nerves simultaneously to contract the hemidiaphragm or entire diaphragm, respectively.[77] This stimulation can be done via needle electrodes (invasive) or externally (noninvasive) by placing two electrodes on either side of one of the sternocleidomastoid muscles.[75, 76, 78, 79] These phrenic nerve stimulation techniques require a current and frequency that induces a supramaximal contraction; this could be considered uncomfortable or painful and is disadvantageous for clinical populations.[75, 76] These techniques, while generating useful data, have not been received well in clinical settings due to the discomfort and technical concerns surrounding them.[6] These downfalls can be improved upon with our method of phrenic nerve stimulation to generate an assessment of diaphragm endurance. In our testing method we will stimulate one phrenic nerve via external, non-invasive stimulation using pencil electrodes; tin foil electrodes wrapped around pencils to create a circular, 2mm-diameter stimulation area. Testing just one phrenic nerve (and hemi diaphragm), our test uses electrical stimulation in a submaximal, twitch contraction setting that will be comfortable to most people and will not cause task failure post-test in any population.

Current Methods of Measuring Respiratory Function

The current methods of clinically assessing respiratory function are either subjective or secondary to the diaphragm.[6, 80, 81] The measurements can be categorized as strength measurements or endurance measurements. The most common strength measurement is maximal inspiratory pressure, but there are many different ways to measure this.[82] A non-exhaustive list of other strength measures are: sniff nasal inspiratory pressure, inspiratory mouth pressure, transdiaphragmatic pressure, electrical supramaximal stimulation of the phrenic nerve. There are disadvantages with each of these tests performed.[6, 76, 79-82] Vital capacity another common measure of respiratory strength; it is a volitional test requiring a best effort by the patient measuring the total exhalation volume from a patient who has maximally inspired. This can be problematic because there may be decreased motivation to perform the test at maximal effort and thus the data would be skewed.[80] Another reason that this test is not reliable is because it does not give specific information about the respiratory muscles.[6, 80] The data collected by this test could be a result of other issues besides respiratory muscle impairment present in the patient because systemic diseases can also negatively affect the measurements.[6] Testing mouth pressures is also a common practice performed to determine respiratory function; this test includes the patient giving a maximal inhalation and a maximal exhalation to determine the strength of the muscles.[81] This is a volitional test requiring the patient to have adequate motivation to perform at their maximum and solely measures strength, which may not be the best way to assess respiratory function.[6] Lastly, a method that is not volitional and does measure a major respiratory muscle is phrenic nerve stimulation.[6, 75-80] This method is similar to the method we are proposing in this thesis, but there remains a fundamental difference in the testing protocol and what the outcome measure is. The method currently used requires muscle stimulation noninvasively through the phrenic nerves located in the neck to produce a supramaximal contraction of the diaphragm. This contraction may be uncomfortable to patients due to the stimulation frequency used to achieve the desired force.[75-77] This test determines diaphragmatic muscle strength. Respiratory muscle endurance may be a more applicable measurement to obtain.[6]

Respiratory muscle endurance may be a more reliable measure of respiratory function. These measurements are taken less often because they are not as simple as the respiratory strength measurements. There have been a few attempts to create a clinically relevant measurement technique assessing respiratory endurance, but it has proven difficult.[83] Some of the methods tried are negative-pressure inspiratory-threshold loading, maximal voluntary ventilation, or maximal loading test.[84, 85] A threshold loading test is a volitional test requiring the participant to inspire against increasing resistance loads until exhaustion.[86] A maximal loading test requires the participant to inspire against 80% of their determined maximal inspiratory resistance load and then measures the amount of time until failure.[84] All of these measures are volitional, which as explained previously, has distinct disadvantages. Based off of current techniques and the downfalls of both measurement collection and what is measured, there is a need for an inexpensive, non-invasive, direct way to assess respiratory function in the clinical setting.

CHAPTER 3

ASSESSING RESPIRATORY FUNCTION USING THE 5-MINUTE ENDURANCE TEST PROTOCOL[1]

[1] Wachsmuth, H.R. and K.K. McCully. To be submitted to MMSE

Abstract

There is a need for objective, diaphragm-specific respiratory function measurements applicable to clinical settings. **PURPOSE:** To establish an improved measurement of respiratory function via assessment of diaphragm endurance. **METHODS:** 20 healthy subjects, highly active (HA) (n=10) or inactive (IN) (n=10), were tested using electrical stimulation (5 Hz, 5-minutes) on both the phrenic nerve and vastus lateralis on one testing occasion. Stimulation electrodes were placed on one phrenic nerve, and a current producing a vigorous contraction was used. An accelerometer collecting at 400Hz was placed on the abdomen. The same protocol was used on the vastus lateralis muscle. An endurance index (EI) was calculated from the acceleration values at 2 (EI2), 5 minutes (EI5), and 5 minutes for the vastus lateralis (EIVL). **RESULTS:** EI2 of IN and HA were 68.8±16.3% and 92.8±8.7% respectively (p=0.001). EI5 of IN and HA were 62.6±12.4% and 85.7±13.6% respectively (p=0.001). The correlation of EI2 and EI5 was .588. EIVL of IN and HA were 94.4±7.0% and 98.6±4.3% respectively (p>0.1). **CONCLUSIONS:** The higher trained subjects had higher diaphragm muscle endurance with all analysis approaches, supporting the use of the endurance test to evaluate respiratory muscle endurance.

KEY WORDS: accelerometry, diaphragm, electrical twitch mechanomyography, muscle fatigue, muscle endurance, respiration, respiratory function, respiratory health

Introduction

Respiratory dysfunction and respiratory failure are severe medical issues, caused by a multitude of different conditions, which profoundly impact the cost of health care and both long-term and short-term patient survival. Some of these causes are mechanical ventilation, lung disease, nerve damage, and neuromuscular diseases. Acute respiratory failure (ARF) is commonly seen in hospitalized patients; it can lead to further complications and, if severe, death. ARF accounted for 137.1 out of 100,000 hospitalizations of patients 5 or more years old according to the 1994 Nationwide Inpatient Sample.[1] Concurrent with the increase in ARF morbidity and mortality associated with ARF, there is an increased medical cost; the total impatient cost of ARF patients increasing from $30.1 billion in 2001 to $54.3 billion in 2009.[2]

The diaphragm is one of the primary inspiratory muscles, and it is essential for respiration.[3, 4] The diaphragm includes 2 parts: costal and crural; the costal portion of the diaphragm is the contractile portion and is split into 2 halves, or hemidiaphragms.[5-7] Each hemidiaphragm is innervated by a phrenic nerve that originates at the C3-C5 vertebral level of the spinal cord and running down the neck behind the sternocleidomastoid muscle on their respective sides. The left phrenic nerve innervating the left hemidiaphragm and the right nerve innervating the right hemidiaphragm.[6, 8, 9] During inspiration, the costal portion of the diaphragm contracts and reduces thoracic pressure by increasing the volume of the thoracic cavity. This reduction in thoracic pressure allows for air to flow down a pressure gradient from outside of the body into the lungs.[6] The diaphragm is made up of about 50% slow and 50% fast fibers.[10, 11] This indicates that it can contract quickly but not with great force; this is beneficial for its role in exercise.

Because of the role that the diaphragm plays as the main inspiratory force in both rest and exercise, it should be assessed when determining respiratory health.

The current methods of clinically assessing respiratory function are either subjective or secondary to the respiratory muscles.[12] Based off of these techniques used commonly and the downfalls of both measurement collection and what is measured, there is a need for an inexpensive, non-invasive, direct way to assess respiratory function in the clinical setting.

Muscle fatigability is a good indicator of muscle quality and could be assessed in the diaphragm to determine respiratory health and function. One way to assess muscle fatigability is an endurance test. There have been studies testing the efficacy and clinical relevance of this method of assessing muscle endurance in both healthy and clinical populations with success.[13-18] This endurance test has been established on multiple different skeletal muscles[14, 15, 18] and once on the diaphragm with moderate success (manuscript under review);[19] if this method can be successfully used on the diaphragm there would be an objective, noninvasive, and inexpensive method of directly assessing respiratory function.

The aims of this study are to validate a novel measurement of respiratory function via measuring the endurance of the diaphragm in young inactive and highly active populations and to evaluate the use of a shortened, 2-minute endurance test. The vastus lateralis will also be tested via endurance test to have another marker of training status and to see if there is a correlation between the endurance indexes of the two muscles. We believe that the highly active participants will have higher endurance in the diaphragm at both the 2-minute and 5-minute parts of the endurance test. We believe that the highly active group will also have greater endurance of the vastus lateralis and that the vastus lateralis endurance values will be higher across all subjects than their diaphragm endurance values. We believe that there will be a statistically significant correlation between the 2-minute and 5-minute endurance values for the diaphragm muscle. We believe that the vastus lateralis 5-minute endurance index will correlate with both the 2-minute and 5-minute endurance index of the diaphragm.

Methods

Study Participants:

Twenty healthy, able-bodied, young adults (18-40 years old) were recruited to participate in this study (Table 1). There were two groups recruited during this study: healthy, inactive young adults and healthy (IN) (n=10), highly active young adults (HA) (n=10). The activity categorization was determined by a blinded researcher with expertise in the area. This study was approved by the Institutional Review Board at the University of Georgia. All participants provided an informed consent prior to testing procedures beginning. Individuals were excluded based on the following criteria: previous or current musculoskeletal injury or illness that would make electrical stimulation of the neck painful or unsafe, previous or current musculoskeletal injury or disease that would make electrical stimulation of the diaphragm unsafe, or evidence of any medical condition that would make participation in the study unsafe. Participation was voluntary and the participants were able to stop participating at any time.

Experimental Design:

The study consisted of one test occasion lasting 15-30 minutes and three parts. Part 1: Validation of a measurement of respiratory function, as assessed by endurance index of the 5-minute endurance test protocol, in 2 different groups: young, highly active adults and young, inactive adults. Part 2: Evaluate the use of acceleration after two minutes of stimulation (2-minute endurance test) in the diaphragm. Part 3: Evaluate the endurance of the vastus lateralis muscle of the quadriceps and determine the relationship of the vastus lateralis endurance and the diaphragm endurance indexes.

Measurements:

The Endurance Index (EI) of both the diaphragm and the vastus lateralis was determined in this study. The protocol for getting the EI of the diaphragm is to stimulate one hemidiaphragm via stimulation of the phrenic nerve. This is achieved with two pencil electrodes made by the researchers and ultrasound gel as a conducting agent; a tin foil electrode is placed on the end of a pencil so as to create a 2mm diameter electrode that can be placed and held on the participant's neck throughout the duration of the test. The left hemidiaphragm was the first attempt to stimulate, if locating that was unsuccessful or generated vigorous arm movement via stimulation of the brachial plexus, the right hemidiaphragm was stimulated. Once the phrenic nerve was located and the stimulation current was adequate to generate a vigorous contraction (36.5±10.9mA), the assistant researcher unplugged the stimulation cord so as to not keep stimulating the muscle before the accelerometer was placed. The assistant then placed a wireless triaxial accelerometer (WAX3, Axivity, UK) on the abdomen of the participant (1 inch below the xyphoid process and ½ inch medial to the rib cage of the side of stimulation) and plugged the stimulation cord in to begin the test. This test protocol was electrical stimulation at 5Hz for 5 minutes. Participants were in a supine position throughout the test. This same protocol was followed for the vastus lateralis with one main deviation: adhesive electrode pads were used instead of pencil electrodes. The pads were placed at the distal and proximal ends of the muscle (1 inch from the distal end and 3 inches from the proximal end. The accelerometer was placed in between the electrode pads on the belly of the muscle.

Data Analysis

The accelerometer was collecting data at a frequency of 400Hz and the parameters were set at 2gs to ensure a higher quality resolution of smaller magnitude forces. The data from the accelerometer was then exported as a raw CSV file and analyzed in MATLAB (Mathworks, Natick, MA). A custom analysis programs was written to analyze the endurance test. The diaphragm specific program was written to collect the maximum value generated every 10 seconds by the diaphragm twitch contraction. This was written to negate the inspiratory breathing effect of overriding the stimulation current and making acceleration values decrease. The EI5 values calculated for the diaphragm are an average measure of the final 3 (30 seconds) maximum acceleration values compared to the average acceleration values in the first 3 values (30 seconds); the EI2 values calculated for the diaphragm were an average measure of 2 acceleration values at the 110 and 120 second points compared to the average acceleration in the first 2 acceleration values of the first 2 points (20 seconds). These values were determined by a blinded researcher. The vastus lateralis endurance test was analyzed by a custom analysis program in MATLAB. This analysis program takes continuous data points throughout the entire test to create an endurance index from an average of the maximum 3 acceleration values in the first 1 minute and the average of the acceleration values in the last 20 seconds of the test. These were analyzed using SPSS software (IBM Corp., Armonk, NY).

Results

Participant characteristics are listed in Table 1. The test was successfully completed 28/42 times. Twenty out of the 28 successful tests were included in this study; they were chosen based on the quality of the tests. The right phrenic nerve was used in the case of 4 participants, in which the left phrenic nerve could not be isolated. No participants reported any major adverse effects from testing.

Two representative samples of the diaphragm endurance test demonstrating the two distinct breathing patterns of twitch (A) and inspiration override (B) are shown in Figure 1. There were some participants that demonstrated a 'mix' breathing pattern exhibiting qualities of both the twitch and the inspiration override patterns. The breathing patterns were significantly different between groups with the IN group exhibiting n=7 twitch, n=3 mix and HA exhibiting n=2 twitch, n=4 mix, and n=4 inspiration override (p=0.006).

The average 5-minute endurance index (EI5) was significantly different between the two groups (IN=62.6±12.4%, HA=85.7±13.6%, p=0.001) (Figure 2). The average 2-minute endurance index (EI2) was also significantly different between groups (IN=68.8±16.3%, HA=92.8±8.7%, p=0.001) (Figure 3). A Pearson Correlation was run on the EI2 and EI5 values to test their relationship; it was significant at the p=0.01 level with R=0.588 and an R^2 = 0.345 (Figure 4A). A Bland-Altman plot was generated to show outliers; there were 11 points outside of the 95% confidence interval (-0.815, 14.081). There was no significant slope of the plot (figure 4B). There was a significant difference in EI2 and EI5 between groups based on groups of activity hours/week; activity hours were grouped as follows: 0=0hr/wk, 1=1-3hr/wk, 2=4-6hr/wk, 3=7-9hr/wk, 4=>9hr/wk. EI2: Groups 3 and 4 were significantly different than groups 0 and 1; 0*3 (p=0.1), 0*4 (p=0.1), 1*3(p=0.05), and 1*4(p=0.05) (Figure 5). EI5: Groups 3 and 4 were significantly different than groups 0 and 1 and group 4 was also significantly different than group 2; 0*3 (p=0.05), 0*4 (p=0.01), 1*3(p=0.05), 1*4(p=0.01), and 2*4(p=0.1) (Figure 6).

The average 5-minute endurance index of the vastus lateralis (EIVL) was not significantly different between groups (IN=94.4±7.0%, HA=98.6±4.3%). A Pearson Correlation was run on the EI2 and EIVL values to test their relationship; it was significant at the p=0.05 level with R=0.568 (Figure 7). There was no significant relationship between the EI5 and EIVL values. 4 participants (IN=3, HA=1) did not have the endurance test conducted on their vastus lateralis muscle and therefore did not have EIVL data.

The time to fatigue of each group was: IN=30.0±33.3s and HA=206.0±106.4s and was significantly variable between groups, so significance could not be measured. This data was further exemplified in a dot plot with means and standard deviations to illustrate individual data points (Figure 8).

The average current used, age, weight, height, time to find the phrenic nerve, and side used were not significantly variable between the two groups. Sex was significantly variable between the two groups, but there were no significant differences in the EI2 values (p=0.47). There was a significant difference in the EI5 values based on sex (p=.05).

Discussion

This study showed that the diaphragm-specific endurance test was sensitive to training status as expected based on previous studies[20] and can be used to evaluate respiratory function in healthy populations. Diaphragm fatigue previously reported in another phrenic nerve stimulation study of healthy subjects was 28.1% which is consistent with the values from this study and the previous study conducted in our lab[19]; it should be noted that the protocol to induce fatigue was different than this protocol.[21] The sensitivity of this test to respiratory function differences in other populations should be tested to determine the clinical significance of this testing method. One of the most important potential future uses for this test is to determine populations 'at risk' for developing respiratory failure due to mechanical ventilation methods. Due to the diaphragmatic atrophy and weakness caused by prolonged mechanical ventilation,[22-24] this protocol could be used to determine whether a physician needs to consider other options or use preventative methods prior to or during any procedure requiring mechanical ventilation.

This study demonstrates the difficulty surrounding this testing method and the usefulness of a 2-minute endurance index for the diaphragm, which may be better tolerated by clinical populations and have a higher test success rate. The success rate was 64% which is lower than the success rate seen in previous studies, but that could be due to the decreased number of practice tests prior to data collection. Our lab has reported a success rate of 88% with this test before with the tester having approximately 15 practice tests prior to data collection.[19] The success rate for the EI2 was not analyzed in this study but should be to determine whether or not to create a modified protocol. Since the 2-

minute endurance index is not highly correlated with the 5-minute endurance index values this relationship and the validity of the EI2 should be further explored. This 5-minute endurance protocol has been used in a 9-minute format previously and since been adapted based on the high correlation of values of the two tests.[14-16, 18]

There were two distinct breathing patterns seen in this study; a 'twitch breathing pattern' and an 'inspiration override breathing pattern' with a mixture also being present in some participants (Figure 1). The difference of frequency in the breathing patterns in the two groups could be another indicator of respiratory function, but further testing should be done to determine this. Whether these breathing patterns have an effect on the subsequent endurance index values should also be determined via future tests.

Conclusions

The endurance of the diaphragm as assessed by this test increased with training status, indicative of the sensitivity of this test to respiratory function. This test is difficult to perform without many practice trials, but the 2-minute protocol could increase success rates if the values are reliable. There are more tests that should be conducted on this protocol to determine its reliability and readiness for the clinical setting. This may be a useful tool to be used in the clinical setting to determine populations requiring preventative measures during mechanical ventilation.

Figure Legends

Figure 3.1: A representative diaphragm endurance test showing the resultant vector of acceleration for A) a twitch breathing pattern and B) an inspiration override breathing pattern.

Figure 3.2: 5-minute endurance index test values of the inactive and highly active groups. There was a statistically significant difference between groups (p=0.001). Data shown are individual data points with mean and standard deviation.

Figure 3.3: 2-minute endurance index test values of the inactive and highly active groups. There was a statistically significant difference between groups (p=0.001). Data shown are individual data points with mean and standard deviation.

Figure 3.4: Relationship between EI5 and EI2. A Pearson Correlation was run and there was a statistically significant correlation of 0.588 (p=0.01). B) A Bland-Altman plot of the differences and the means of individuals. The red line indicates the mean of the differences and the green lines indicate the upper and lower limits of the 95% confidence interval.

Figure 3.5: 2-minute endurance test values based on number of activity hours per week. As indicated by a single star, group 0 was significantly different from groups 3 and 4 (p=0.1). As indicated by a double star, group 1 was significantly different from groups 3 and 4 (p=0.05). Data shown are mean and standard deviation.

Figure 3.6: 5-minute endurance test values based on number of activity hours per week. As indicated by a single star, group 2 was significantly different from group 4 (p=0.1). As indicated by a double star, group 3 was significantly different from groups 0 and 1 (p=0.05). As indicated by a triple star, group 4 was significantly different from groups 0 and 1 (p=0.01). Data shown are mean and standard deviation.

Figure 3.7: Relationship between EIVL and EI2. A Pearson Correlation was run and there was a statistically significant correlation of 0.568 (p=0.05).

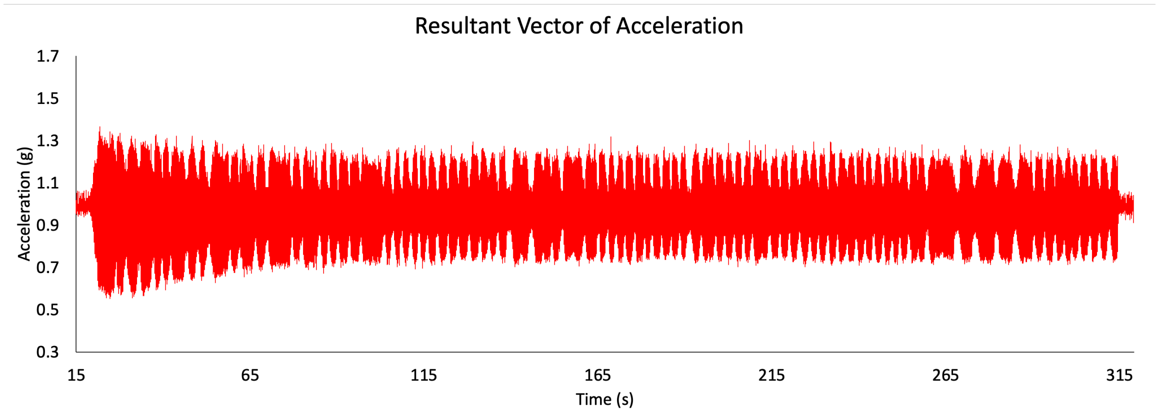
Figure 3.8: Time to fatigue in seconds of the inactive and highly active groups. There was not statistically significant difference between groups. Data shown are individual data points with mean and standard deviation.

Table 3.1

Activity Category	Males	Females		Height (cm)	Weight (kg)	Age (years)
Inactive	5	5	Mean STDEV	171.7 11.87	68.73 15.27	24.1 3.43
Highly Active	8	2	Mean STDEV	176.02 8.13	76.45 11.29	24.6 3.95

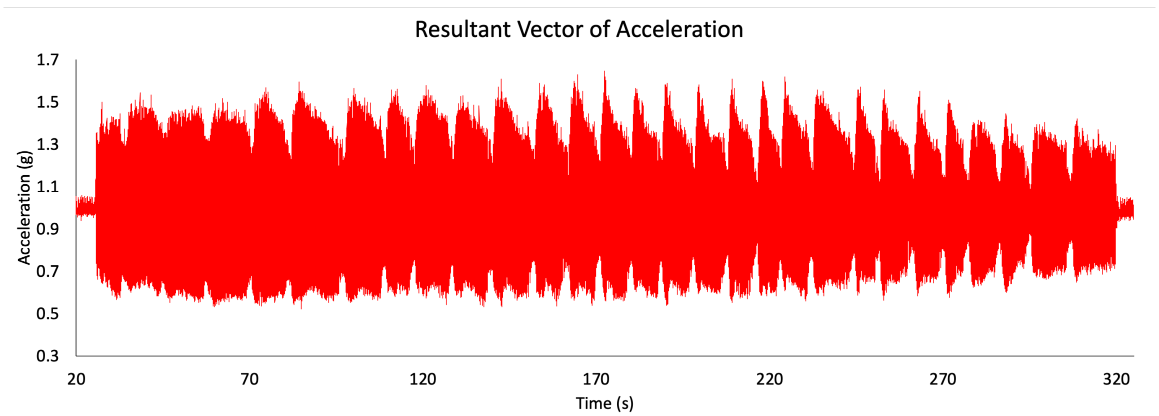
Figure 3.1

A)



Resultant Vector of Acceleration
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
Acceleration (g)
15
65
115
165
215
265
315
Time (s)

B)



Resultant Vector of Acceleration
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
Acceleration (g)
20
70
120
170
220
270
320
Time (s)

Figure 3.2

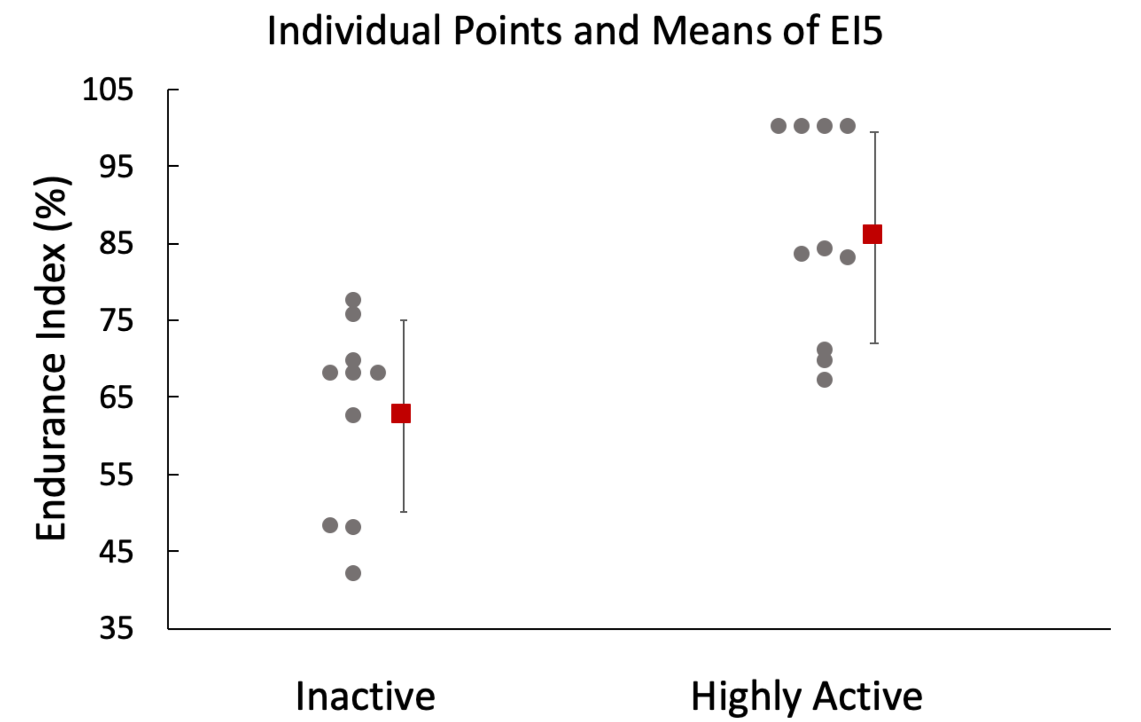
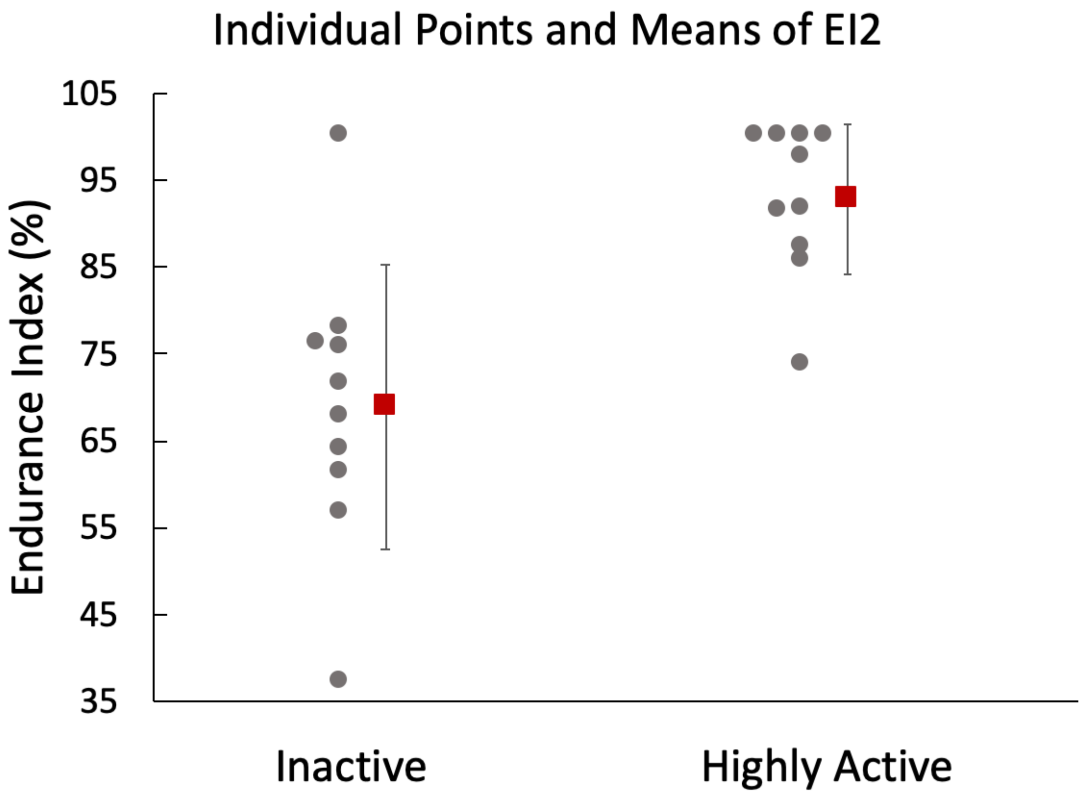

Individual Points and Means of EI5
Endurance Index (%)
105
95
85
75
65
55
45
35
Inactive
Highly Active

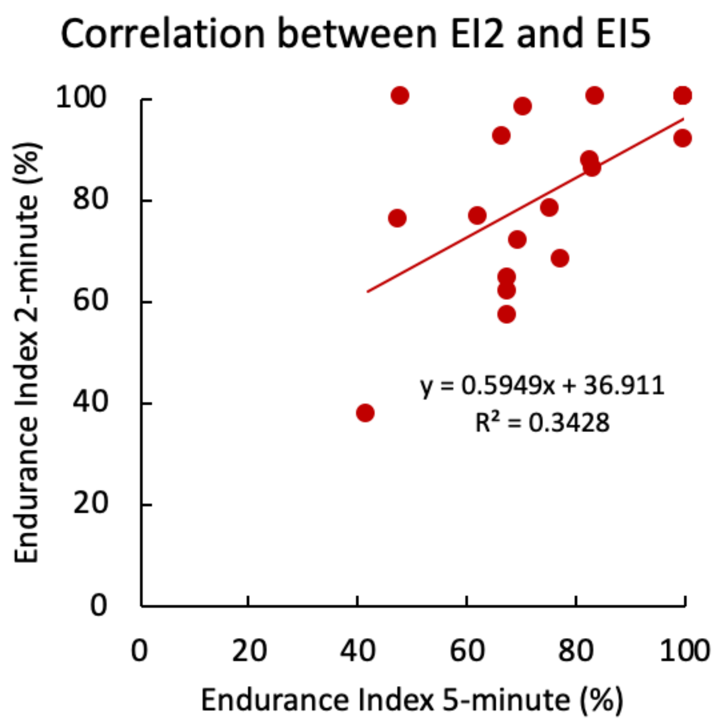
Figure 3.3



Individual Points and Means of EI2
105
95
85
75
65
55
45
35
Endurance Index (%)
Inactive
Highly Active

Figure 3.4

A)


Correlation between EI2 and EI5
Endurance Index 2-minute (%)
Endurance Index 5-minute (%)
y = 0.5949x + 36.911
R² = 0.3428
0
20
40
60
80
100

B)

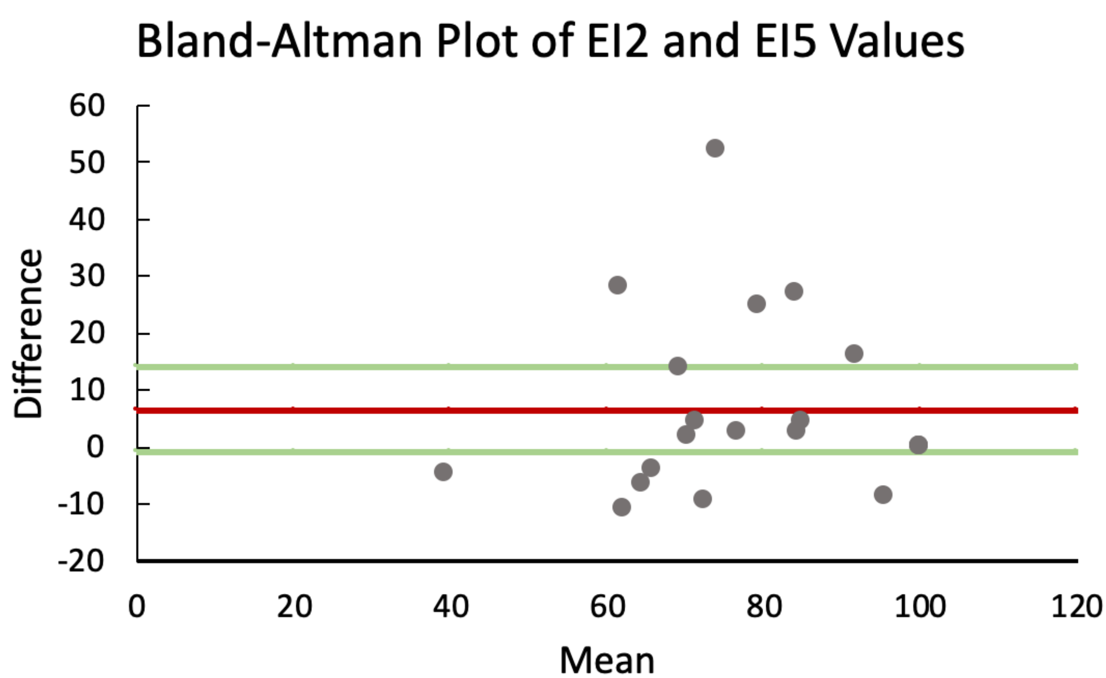

Bland-Altman Plot of EI2 and EI5 Values
Difference
Mean
60
50
40
30
20
10
0
-10
-20
0
20
40
60
80
100
120

Figure 3.5

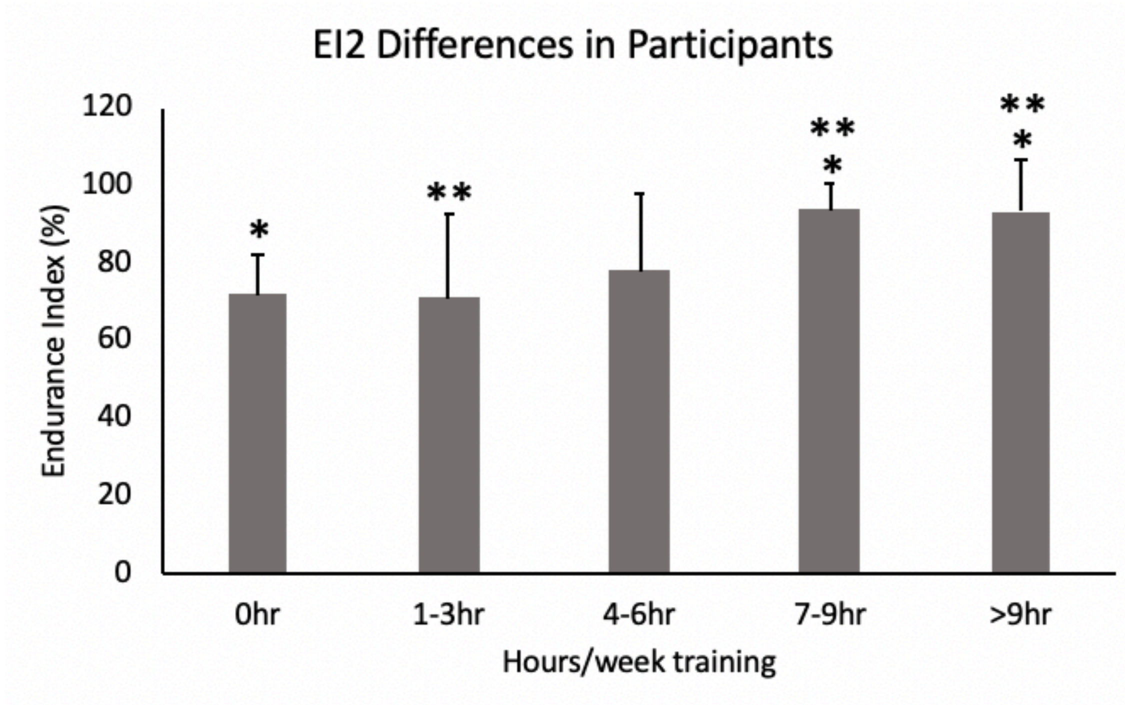

EI2 Differences in Participants
120
100
80
60
40
20
0
Endurance Index (%)
*
**
**
*
**
*
0hr
1-3hr
4-6hr
7-9hr
>9hr
Hours/week training

Figure 3.6

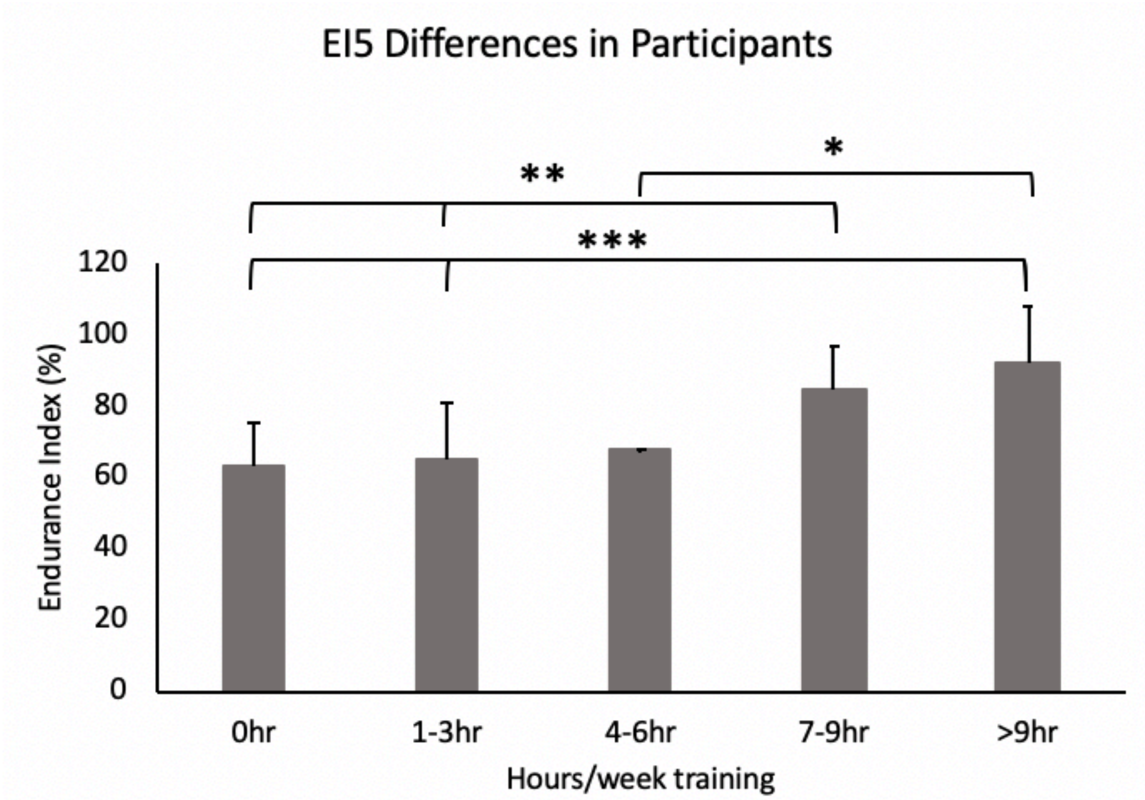

EI5 Differences in Participants
**
*
***
120
100
80
60
40
20
0
Endurance Index (%)
0hr
1-3hr
4-6hr
7-9hr
>9hr
Hours/week training

Figure 3.7

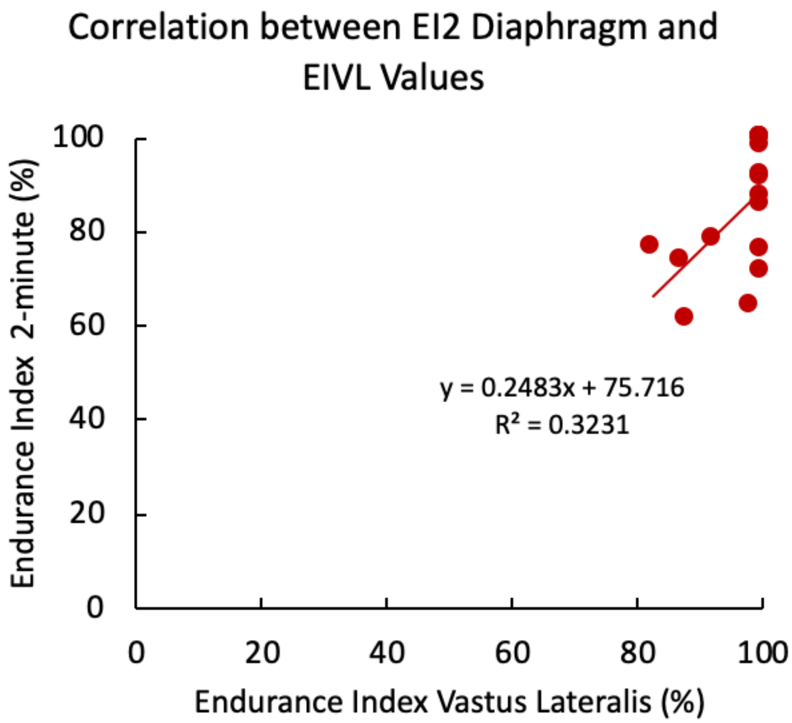
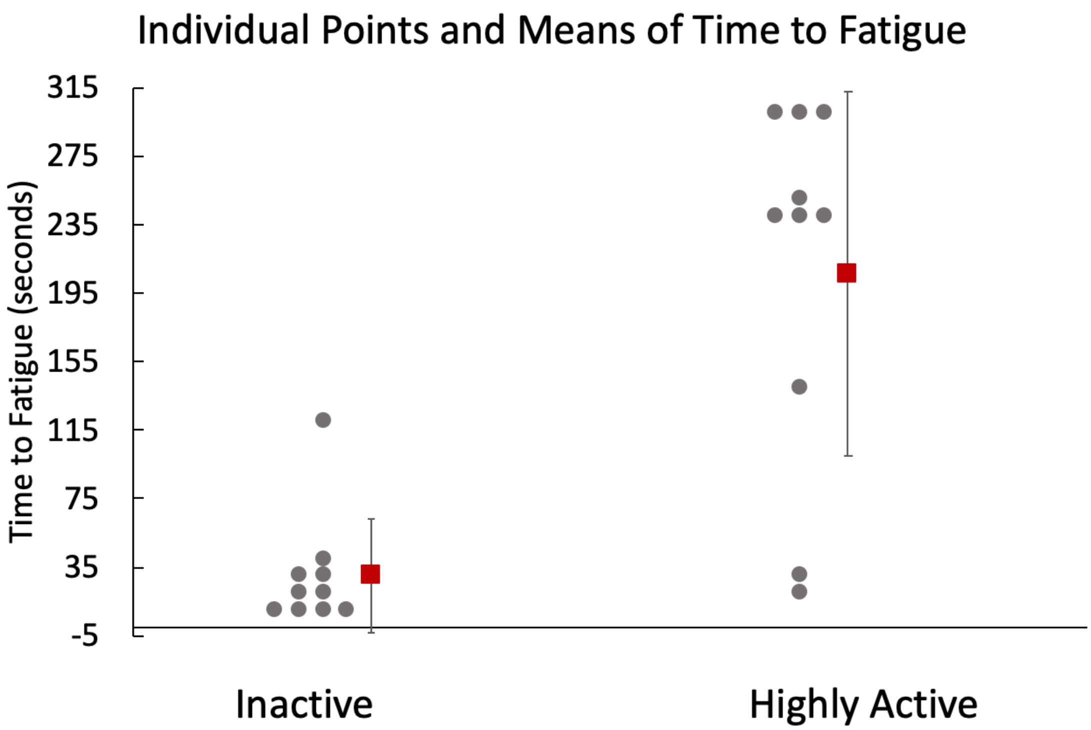

Correlation between EI2 Diaphragm and EIVL Values
Endurance Index 2-minute (%)
100
80
60
40
20
0
0
20
40
60
80
100
Endurance Index Vastus Lateralis (%)
y = 0.2483x + 75.716
R² = 0.3231

Figure 3.8


Individual Points and Means of Time to Fatigue
Time to Fatigue (seconds)
315
275
235
195
155
115
75
35
-5
Inactive
Highly Active

REFERENCES

1. Carson SS, Cox CE, Holmes GM, Howard A and Carey TS. The Changing Epidemiology of Mechanical Ventilation: A Population-Based Study. Journal of Intensive Care Medicine. 2006;21:173-182.

2. Stefan MS, Shieh MS, Pekow PS, Rothberg MB, Steingrub JS, Lagu T and Lindenauer PK. Epidemiology and outcomes of acute respiratory failure in the United States, 2001 to 2009: a national survey. Journal of hospital medicine. 2013;8:76-82.

3. Sieck GC, Ferreira LF, Reid MB and Mantilla CB. Mechanical properties of respiratory muscles. Compr Physiol. 2013;3:1553-1567.

4. Tokizane T, Kawamata K and Tokizane H. Electromyographic studies on the human respiratory muscles; studies on the activity pattern of neuromuscular units. Jpn J Physiol. 1952;2:232-47.

5. De Troyer A, Sampson M, Sigrist S and Macklem PT. The diaphragm: two muscles. Science. 1981;213:237-8.

6. Drake R, Vogl AW and Mitchell AWM. Gray's Anatomy for Students E-Book: Elsevier Health Sciences; 2009.

7. Pickering M and Jones JFX. The diaphragm: two physiological muscles in one. J Anat. 2002;201:305-312.

8. Bordoni B and Zanier E. Anatomic connections of the diaphragm: influence of respiration on the body system. J Multidiscip Healthc. 2013;6:281-291.

9. Nicaise C, Hala TJ, Frank DM, Parker JL, Authelet M, Leroy K, Brion J-P, Wright MC and Lepore AC. Phrenic motor neuron degeneration compromises phrenic axonal circuitry and diaphragm activity in a unilateral cervical contusion model of spinal cord injury. Experimental Neurology. 2012;235:539-552.

10. Lieberman DA, Faulkner JA, Craig AB, Jr. and Maxwell LC. Performance and histochemical composition of guinea pig and human diaphragm. J Appl Physiol. 1973;34:233-7.

11. Mizuno M. Human respiratory muscles: fibre morphology and capillary supply. European Respiratory Journal. 1991;4:587.

12. Syabbalo N. Assessment of respiratory muscle function and strength. Postgraduate medical journal. 1998;74:208-15.

13. Bossie HM, Willingham TB, Schoick RAV, O'Connor PJ and McCully KK. Mitochondrial capacity, muscle endurance, and low energy in friedreich ataxia. Muscle & Nerve. 2017;56:773-779.

14. McCully KK, Moraes C, Patel VS, Green M and Willingham BT. Muscle-Specific Endurance of the Lower Back Erectors Using Electrical Twitch Mechanomyography. Journal of Functional Morphology and Kinesiology. 2019;4.

15. McCully KK, Prins P, Mistry K and Willingham TB. Muscle-specific endurance of the trapezius muscles using electrical twitch mechanomyography. Shoulder & Elbow. 2017;10:136-143.

16. Willingham T and McCully K. Assessment of skeletal muscle endurance using twitch electrical stimulation and accelerometer-based mechanomyography. Advances in Skeletal Muscle Function Assessment. 2017;1:9.

17. Willingham TB, Backus D and McCully KK. Muscle Dysfunction and Walking Impairment in Women with Multiple Sclerosis. International Journal of MS Care. 2019;21:249-256.
18. Smith JM. Reproducibility and the Effects of Exercise on the Endurance Index. 2019.
19. Pryor EK. WH, McCully KK. Evaluation of a 5-Minute Endurance Test of Human Diaphragm Muscle. 2019.
20. Brizendine JT, Ryan TE, Larson RD, McCully KK. Skeletal muscle metabolism in endurance athletes with near-infrared spectroscopy. Medicine and Science in Sports and Exercise. 2013; 45:869-75.
21. Polkey MI, Kyroussis D, Hamnegard CH, Hughes PD, Rafferty GF, Moxham J, Green M. Paired phrenic nerve stimuli for the detection of diaphragm fatigue in humans. European Respiratory Journal. 1997;10:1859-1864.
22. Esteban A, Frutos-Vivar F, Muriel A, Ferguson ND, Penuelas O, Abraira V, Raymondos K, Rios F, Nin N, Apezteguia C, Violi DA, Thille AW, Brochard L, Gonzalez M, Villagomez AJ, Hurtado J, Davies AR, Du B, Maggiore SM, Pelosi P, Soto L, Tomicic V, D'Empaire G, Matamis D, Abroug F, Moreno RP, Soares MA, Arabi Y, Sandi F, Jibaja M, Amin P, Koh Y, Kuiper MA, Bulow HH, Zeggwagh AA and Anzueto A. Evolution of mortality over time in patients receiving mechanical ventilation. American journal of respiratory and critical care medicine. 2013;188:220-30.
23. Jaber S, Petrof BJ, Jung B, Chanques G, Berthet JP, Rabuel C, Bouyabrine H, Courouble P, Koechlin-Ramonatxo C, Sebbane M, Similowski T, Scheuermann V, Mebazaa A, Capdevila X, Mornet D, Mercier J, Lacampagne A, Philips A and Matecki S. Rapidly progressive diaphragmatic weakness and injury during mechanical ventilation in humans. American journal of respiratory and critical care medicine. 2011;183:364-71.
24. Searle JF. The outcome of mechanical ventilation: report of a five year study. Ann R Coll Surg Engl. 1985;67:187-189.

CHAPTER 4

CONCLUSIONS

This study evaluated the endurance index for the diaphragm muscle. As part of the process for validating the method, this study evaluated trained and untrained subjects. Much like the paper by Brizendine et al, this study showed that trained subjects had higher diaphragm endurance index values that untrained subjects. Because previous studies have showed trained subjects to have higher respiratory endurance than untrained subjects, our study could be considered a validation study. This is just the first step, future studies need to do other validation tests such as testing the effects of the breathing patterns, current level, and phrenic nerve side on the endurance index values.

A potential limitation of this study was the technical expertise needed to perform the EI measurements through the phrenic nerve. The test required the researcher to find the phrenic nerve with little to no stimulation of the brachial plexus; on some participants this was a small stimulation area and thus difficult to find and maintain. Holding the electrodes in the same position for 5 minutes was difficult for the tester. Any movement of the electrodes could result in a lost contraction or a diminished contraction. This method should be improved upon in subsequent studies to allow for greater success rates of testing. The two major factors of if a test would be successful were the number of prior tests and how recently the researcher had completed a test. As testing increased there was an increase in the testers success rates and with lulls in testing there was a decrease in success rate. One approach to make the diaphragm endurance test easier to perform would be to shorten the test. This study evaluated a two-minute version of the five-minute endurance test. These values were seen to be only weakly associated with each other. This indicates that further testing of the 2-minute modified version should be done prior to using it in place of the current 5-minute test.

While analyzing the data from this study, the investigator noticed that the time when most of the loss of acceleration occurred was different between the two groups. In the inactive groups there appeared to be a quicker loss of acceleration in comparison to the highly active groups. This could be another indicator of respiratory fitness and should be further investigated to see if there is an implication of respiratory health dependent on the rate at which fatigue occurs.

While analyzing data in this study the investigator noticed that there seemed to be two different breathing patterns by the research subjects. When the diaphragm is contracting during inspiration, the diaphragm muscle maybe stiffer and thus the acceleration is decreased. This could have had an impact on endurance index values. In studies of respiration or heart rate variability, there is a debate as to whether to use a forced breathing pattern or let the subject breathe spontaneously. In this study the subjects were told to breathe in a way that was comfortable to them. The impact on breathing pattern should be studied to determine if in subsequent studies there should be a standardization of breathing patterns.

One of the long-term goals for the diaphragm endurance test is to study patients with suspected diaphragm impairments. They should have much lower endurance index values. This study was to study older adults, but they were not tested due to limitations in recruitment. We believed that older adults would have a decreased diaphragm endurance compared to the younger populations. The major population that should be tested to improve and create clinical significance of this test is older inactive adults. It would allow us to determine what an ‘at risk’ diaphragm endurance profile would look like. This could then be utilized to evaluate patients determine what preventative steps should be taken to ensure acute respiratory failure does not occur.

In conclusion, this test, while difficult to conduct in its current methodology, appears to be sensitive to differences in respiratory muscle training and could be a promising clinical test used to prevent serious respiratory problems. This is a novel, inexpensive protocol that includes submaximal contractions, is non-invasive, and still diaphragm specific. There are different experiments that should be completed to further ensure the reliability and sensitivity of this test that were previously stated. Some of these conditions are currently being tested and will provide further insight into the usefulness of this test in a clinical setting.

REFERENCES

1. Stefan MS, Shieh MS, Pekow PS, Rothberg MB, Steingrub JS, Lagu T and Lindenauer PK. Epidemiology and outcomes of acute respiratory failure in the United States, 2001 to 2009: a national survey. *Journal of hospital medicine*. 2013;8:76-82.
2. Esteban A, Frutos-Vivar F, Muriel A, Ferguson ND, Penuelas O, Abraira V, Raymondos K, Rios F, Nin N, Apezteguia C, Violi DA, Thille AW, Brochard L, Gonzalez M, Villagomez AJ, Hurtado J, Davies AR, Du B, Maggiore SM, Pelosi P, Soto L, Tomicic V, D'Empaire G, Matamis D, Abroug F, Moreno RP, Soares MA, Arabi Y, Sandi F, Jibaja M, Amin P, Koh Y, Kuiper MA, Bulow HH, Zeggwagh AA and Anzueto A. Evolution of mortality over time in patients receiving mechanical ventilation. *American journal of respiratory and critical care medicine*. 2013;188:220-30.
3. Jaber S, Petrof BJ, Jung B, Chanques G, Berthet JP, Rabuel C, Bouyabrine H, Courouble P, Koechlin-Ramonatxo C, Sebbane M, Similowski T, Scheuermann V, Mebazaa A, Capdevila X, Mornet D, Mercier J, Lacampagne A, Philips A and Matecki S. Rapidly progressive diaphragmatic weakness and injury during mechanical ventilation in humans. *American journal of respiratory and critical care medicine*. 2011;183:364-71.
4. Mansel JK and Norman JR. Respiratory Complications and Management of Spinal Cord Injuries. *CHEST*. 1990;97:1446-1452.
5. Mehta S. Neuromuscular disease causing acute respiratory failure. *Respiratory care*. 2006;51:1016-21; discussion 1021-3.
6. Syabbalo N. Assessment of respiratory muscle function and strength. *Postgraduate medical journal*. 1998;74:208-15.
7. Aliverti A, Cala SJ, Duranti R, Ferrigno G, Kenyon CM, Pedotti A, Scano G, Sliwinski P, Macklem PT and Yan S. Human respiratory muscle actions and control during exercise. *Journal of applied physiology (Bethesda, Md : 1985)*. 1997;83:1256-69.
8. Fuglevand AJ. Neural Aspects of Fatigue. *The Neuroscientist*. 1996;2:203-206.
9. Allen DG, Lamb GD and Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiological reviews*. 2008;88:287-332.
10. Potvin JR and Fuglevand AJ. A motor unit-based model of muscle fatigue. *PLoS computational biology*. 2017;13:e1005581.
11. Willingham T and McCully K. Assessment of skeletal muscle endurance using twitch electrical stimulation and accelerometer-based mechanomyography. *Advances in Skeletal Muscle Function Assessment*. 2017;1:9.
12. McCully KK, Moraes C, Patel VS, Green M and Willingham BT. Muscle-Specific Endurance of the Lower Back Erectors Using Electrical Twitch Mechanomyography. *Journal of Functional Morphology and Kinesiology*. 2019;4.
13. McCully KK, Prins P, Mistry K and Willingham TB. Muscle-specific endurance of the trapezius muscles using electrical twitch mechanomyography. *Shoulder & Elbow*. 2017;10:136-143.
14. Smith JM. Reproducibility and the Effects of Exercise on the Endurance Index. 2019.
15. Bossie HM, Willingham TB, Schoick RAV, O'Connor PJ and McCully KK. Mitochondrial capacity, muscle endurance, and low energy in friedreich ataxia. *Muscle & Nerve*. 2017;56:773-779.

16. Willingham TB, Backus D and McCully KK. Muscle Dysfunction and Walking Impairment in Women with Multiple Sclerosis. *International Journal of MS Care*. 2019;21:249-256.
17. Shebl E and Burns B. Respiratory Failure *StatPearls* Treasure Island (FL): StatPearls Publishing
StatPearls Publishing LLC.; 2020.
18. Carson SS, Cox CE, Holmes GM, Howard A and Carey TS. The Changing Epidemiology of Mechanical Ventilation: A Population-Based Study. *Journal of Intensive Care Medicine*. 2006;21:173-182.
19. Vincent JL, Akca S, De Mendonca A, Haji-Michael P, Sprung C, Moreno R, Antonelli M and Suter PM. The epidemiology of acute respiratory failure in critically ill patients(*). *Chest*. 2002;121:1602-9.
20. Elliott MW, Steven MH, Phillips GD and Branthwaite MA. Non-invasive mechanical ventilation for acute respiratory failure. *BMJ*. 1990;300:358-360.
21. Searle JF. The outcome of mechanical ventilation: report of a five year study. *Ann R Coll Surg Engl*. 1985;67:187-189.
22. Meduri GU, Abou-Shala N, Fox RC, Jones CB, Leeper KV and Wunderink RG. Noninvasive face mask mechanical ventilation in patients with acute hypercapnic respiratory failure. *Chest*. 1991;100:445-54.
23. Wunsch H, Wagner J, Herlim M, Chong DH, Kramer AA and Halpern SD. ICU occupancy and mechanical ventilator use in the United States. *Critical care medicine*. 2013;41:2712-2719.
24. Munoz Bonet JI. [Definitions in mechanical ventilation]. *An Pediatr (Barc)*. 2003;59:60-6.
25. Powers SK, Wiggs MP, Sollanek KJ and Smuder AJ. Ventilator-induced diaphragm dysfunction: cause and effect. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2013;305:R464-R477.
26. Jubran A. Critical illness and mechanical ventilation: effects on the diaphragm. *Respiratory care*. 2006;51:1054-61; discussion 1062-4.
27. Picard M, Jung B, Liang F, Azuelos I, Hussain S, Goldberg P, Godin R, Danialou G, Chaturvedi R, Rygiel K, Matecki S, Jaber S, Des Rosiers C, Karpati G, Ferri L, Burelle Y, Turnbull DM, Taivassalo T and Petrof BJ. Mitochondrial dysfunction and lipid accumulation in the human diaphragm during mechanical ventilation. *American journal of respiratory and critical care medicine*. 2012;186:1140-9.
28. Tams C, Stephan P, Euliano N, Gabrielli A, Martin AD, Efron P and Patel R. Clinical decision support recommending ventilator settings during noninvasive ventilation. *J Clin Monit Comput*. 2019.
29. Alía I and Esteban A. Weaning from mechanical ventilation. *Crit Care*. 2000;4:72-80.
30. Murray CJ and Lopez AD. Evidence-based health policy--lessons from the Global Burden of Disease Study. *Science*. 1996;274:740-3.
31. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ and Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. 1998;157:1418-22.

32. Chronic obstructive pulmonary disease among adults--United States, 2011. *MMWR Morb Mortal Wkly Rep*. 2012;61:938-43.
33. Lopez-Campos JL, Tan W and Soriano JB. Global burden of COPD. *Respirology*. 2016;21:14-23.
34. Guarascio AJ, Ray SM, Finch CK and Self TH. The clinical and economic burden of chronic obstructive pulmonary disease in the USA. *Clinicoecon Outcomes Res*. 2013;5:235-45.
35. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;385:117-71.
36. Kraus KH. The pathophysiology of spinal cord injury and its clinical implications. *Semin Vet Med Surg (Small Anim)*. 1996;11:201-7.
37. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A and Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol*. 2014;6:309-331.
38. Yavagal DR and Mayer SA. Respiratory complications of rapidly progressive neuromuscular syndromes: Guillain-Barre syndrome and myasthenia gravis. *Semin Respir Crit Care Med*. 2002;23:221-9.
39. Rezania K, Goldenberg FD and White S. Neuromuscular Disorders and Acute Respiratory Failure: Diagnosis and Management. *Neurologic Clinics*. 2012;30:161-185.
40. Bella I and Chad DA. Neuromuscular disorders and acute respiratory failure. *Neurol Clin*. 1998;16:391-417.
41. Sieck GC, Ferreira LF, Reid MB and Mantilla CB. Mechanical properties of respiratory muscles. *Compr Physiol*. 2013;3:1553-1567.
42. Tokizane T, Kawamata K and Tokizane H. Electromyographic studies on the human respiratory muscles; studies on the activity pattern of neuromuscular units. *Jpn J Physiol*. 1952;2:232-47.
43. De Troyer A, Sampson M, Sigrist S and Macklem PT. The diaphragm: two muscles. *Science*. 1981;213:237-8.
44. Drake R, Vogl AW and Mitchell AWM. *Gray's Anatomy for Students E-Book*: Elsevier Health Sciences; 2009.
45. Pickering M and Jones JFX. The diaphragm: two physiological muscles in one. *J Anat*. 2002;201:305-312.
46. Bordoni B and Zanier E. Anatomic connections of the diaphragm: influence of respiration on the body system. *J Multidiscip Healthc*. 2013;6:281-291.
47. Nicaise C, Hala TJ, Frank DM, Parker JL, Authelet M, Leroy K, Brion J-P, Wright MC and Lepore AC. Phrenic motor neuron degeneration compromises phrenic axonal circuitry and diaphragm activity in a unilateral cervical contusion model of spinal cord injury. *Experimental Neurology*. 2012;235:539-552.
48. Lieberman DA, Faulkner JA, Craig AB, Jr. and Maxwell LC. Performance and histochemical composition of guinea pig and human diaphragm. *J Appl Physiol*. 1973;34:233-7.
49. Mizuno M. Human respiratory muscles: fibre morphology and capillary supply. *European Respiratory Journal*. 1991;4:587.
50. de Troyer A and Loring SH. Action of the Respiratory Muscles. *Compr Physiol*. 2011:443-461.

51. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiological reviews*. 2001;81:1725-89.
52. Edwards RH. Human muscle function and fatigue. *Ciba Found Symp*. 1981;82:1-18.
53. Allen DG, Lannergren J and Westerblad H. Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol*. 1995;80:497-527.
54. Hermansen L and Stensvold I. Production and Removal of Lactate during Exercise in Man. *Acta Physiologica Scandinavica*. 1972;86:191-201.
55. Knuttgen HG and Saltin B. Muscle metabolites and oxygen uptake in short-term submaximal exercise in man. *Journal of Applied Physiology*. 1972;32:690-694.
56. Fiedler GB, Schmid AI, Goluch S, Schewzow K, Laistler E, Niess F, Unger E, Wolzt M, Mirzahosseini A, Kemp GJ, Moser E and Meyerspeer M. Skeletal muscle ATP synthesis and cellular H+ handling measured by localized 31P-MRS during exercise and recovery. *Scientific Reports*. 2016;6:32037.
57. Cooke R and Pate E. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys J*. 1985;48:789-798.
58. Hibberd MG, Dantzig JA, Trentham DR and Goldman YE. Phosphate release and force generation in skeletal muscle fibers. *Science*. 1985;228:1317.
59. Pate E and Cooke R. A model of crossbridge action: the effects of ATP, ADP and Pi. *Journal of Muscle Research & Cell Motility*. 1989;10:181-196.
60. Webb MR, Hibberd MG, Goldman YE and Trentham DR. Oxygen exchange between Pi in the medium and water during ATP hydrolysis mediated by skinned fibers from rabbit skeletal muscle. Evidence for Pi binding to a force-generating state. *J Biol Chem*. 1986;261:15557-64.
61. Gibson H and Edwards RH. Muscular exercise and fatigue. *Sports Med*. 1985;2:120-32.
62. Dubowitz V and Pearse AGE. A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. *Zeitschrift für Zellforschung und mikroskopische Anatomie Abt Histochemie*. 1960;2:105-117.
63. Edstrom L and Nystrom B. Histochemical types and sizes of fibres in normal human muscles. A biopsy study. *Acta Neurol Scand*. 1969;45:257-69.
64. Stienen GJ, Kiers JL, Bottinelli R and Reggiani C. Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. *J Physiol*. 1996;493 (Pt 2):299-307.
65. Essen B, Jansson E, Henriksson J, Taylor AW and Saltin B. Metabolic characteristics of fibre types in human skeletal muscle. *Acta Physiol Scand*. 1975;95:153-65.
66. Sahlin K, Tonkonogi M and Soderlund K. Energy supply and muscle fatigue in humans. *Acta Physiol Scand*. 1998;162:261-6.
67. Kemp GJ, Ahmad RE, Nicolay K and Prompers JJ. Quantification of skeletal muscle mitochondrial function by 31P magnetic resonance spectroscopy techniques: a quantitative review. *Acta Physiologica*. 2015;213:107-144.
68. Holloszy JO and Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *Journal of Applied Physiology*. 1984;56:831-838.
69. Henriksson J and Reitman JS. Quantitative measures of enzyme activities in type I and type II muscle fibres of man after training. *Acta Physiol Scand*. 1976;97:392-7.

70. Riek S and Bawa P. Recruitment of motor units in human forearm extensors. *Journal of Neurophysiology*. 1992;68:100-108.
71. Morgan TE, Cobb LA, Short FA, Ross R and Gunn DR. Effects of Long-Term Exercise on Human Muscle Mitochondria. In: B. Pernow and B. Saltin, eds. *Muscle Metabolism During Exercise: Proceedings of a Karolinska Institutet Symposium held in Stockholm, Sweden, September 6–9, 1970 Honorary guest: E Hohwü Christensen* Boston, MA: Springer US; 1971: 87-95.
72. Powers SK, Criswell D, Lieu F-K, Dodd S and Silverman H. Diaphragmatic fiber type specific adaptation to endurance exercise. *Respiration Physiology*. 1992;89:195-207.
73. Powers SK, Criswell D, Lieu FK, Dodd S and Silverman H. Exercise-induced cellular alterations in the diaphragm. *Am J Physiol*. 1992;263:R1093-8.
74. Pryor EK. WH, McCully KK. Evaluation of a 5-Minute Endurance Test of Human Diaphragm Muscle. 2019.
75. Aubier M, Murciano D, Lecocguic Y, Viires N and Pariente R. Bilateral phrenic stimulation: a simple technique to assess diaphragmatic fatigue in humans. *Journal of applied physiology (Bethesda, Md : 1985)*. 1985;58:58-64.
76. Bellemare F and Bigland-Ritchie B. Assessment of human diaphragm strength and activation using phrenic nerve stimulation. *Respir Physiol*. 1984;58:263-77.
77. Laroche CM, Mier AK, Moxham J and Green M. Diaphragm strength in patients with recent hemidiaphragm paralysis. *Thorax*. 1988;43:170-174.
78. Whittenberger JL, Sarnoff SJ and Hardenbergh E. Electrophrenic respiration; its use in man. *J Clin Invest*. 1949;28:124-8.
79. Mier A, Brophy C, Moxham J and Green M. Twitch pressures in the assessment of diaphragm weakness. *Thorax*. 1989;44:990-996.
80. Polkey MI, Green M and Moxham J. Measurement of respiratory muscle strength. *Thorax*. 1995;50:1131-1135.
81. Mier-Jedrzejowicz A, Brophy C, Moxham J and Green M. Assessment of diaphragm weakness. *Am Rev Respir Dis*. 1988;137:877-83.
82. Caruso P, Albuquerque ALPd, Santana PV, Cardenas LZ, Ferreira JG, Prina E, Trevizan PF, Pereira MC, Iamonti V, Pletsch R, Macchione MC and Carvalho CRR. Diagnostic methods to assess inspiratory and expiratory muscle strength. *J Bras Pneumol*. 2015;41:110-123.
83. Hart N, Hawkins P, Hamnegård CH, Green M, Moxham J and Polkey MI. A novel clinical test of respiratory muscle endurance. *European Respiratory Journal*. 2002;19:232.
84. Fiz JA, Romero P, Gomez R, Hernandez MC, Ruiz J, Izquierdo J, Coll R and Morera J. Indices of respiratory muscle endurance in healthy subjects. *Respiration*. 1998;65:21-7.
85. Woszezenki CT, Heinzmann-Filho JP, Vendrusculo FM, Piva TC, Levices I and Donadio MVF. Reference Values for Inspiratory Muscle Endurance in Healthy Children and Adolescents. *PLoS One*. 2017;12:e0170696-e0170696.
86. Johnson PH, Cowley AJ and Kinnear WJ. Incremental threshold loading: a standard protocol and establishment of a reference range in naive normal subjects. *Eur Respir J*. 1997;10:2868-71.