RESEARCH ARTICLE

Evaluation of a 5-Minute Endurance Test of Human Diaphragm Muscle

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Abstract

Diaphragmatic function is important in treating respiratory failure. Muscle endurance has been measured using twitch electrical stimulation and accelerometer-based mechanomyography. The purpose of this study was to evaluate the reproducibility and the time to perform an endurance test on the diaphragm muscle using electrical stimulation of the phrenic nerve. Twenty healthy subjects (21.6±1.6yrs) were tested in the supine position on two separate occasions. Stimulation electrodes were placed on the left (n=18) or right (n=2) phrenic nerve underneath the sternocleidomastoid muscle. A tri-axial accelerometer was placed on the abdomen and 5 minutes of submaximal electrical stimulation at 5Hz was performed. The resultant vector of acceleration at peak and end acceleration was analyzed: endurance index = end/peak*100). The test was successfully completed 41/47 times. The endurance index for the two days was 73.8±9.4% and 72.6±11.1%, respectively. The time to find the phrenic nerve was 34.8±13.1s for the first 10 subjects and 13.0±2.8s for the second 10 subjects. For the second group, the mean difference in endurance between days was 0.03% with a 95% confidence interval of -1.6% to 1.5%. In conclusion, the diaphragm endurance test was reproducible with practice.

Keywords: Respiratory fatigue; fatigability; electrical stimulation; phrenic nerve

Introduction

The diaphragm is a primary inspiratory muscle in ventilation and plays an essential role in normal respiratory function [1, 2]. The diaphragm muscle is normally considered to a highly aerobic muscle that is fatigue resistant during normal respiratory activities. Although it does contain fatigable muscle fibers, and diaphragmatic fatigue is normally thought to occur with activities that recruit these fatigable fibers, such as high intensity exercise or conditions that increase airway resistance[1, 3]. In addition, patients who have been placed on a ventilator are at greater risk of respiratory failure (diaphragmatic fatigue) [4]. Diaphragmatic fatigue is defined as the loss of capacity to develop force in response to a load [5-7] and is related to respiratory failure [2, 4, 7-13].

Many aspects of the respiratory system can be studied, including overall respiratory function, respiratory muscle strength and endurance, as well as respiratory muscle fatigue [14]. Current approaches to studying diaphragm muscle endurance typically involves using voluntary efforts and measure pressure changes with balloons placed above and below the diaphragm[15-17]. These measurements often do not specifically address diaphragmatic function as they require difficult to perform breathing techniques and therefore depend on an individual's ability to perform the task. Previous studies explored non-invasive techniques of studying diaphragm function but have mainly focused on the strength of the diaphragm [17]. It is critical to develop a non-invasive, clinically applicable test to assess diaphragm muscle endurance.

Previous studies demonstrated the effectiveness of using electrical stimulation and accelerometry mechanomyography (aMMG) to measure limb muscle fatigue[17-19]. Twitch accelerometry has been shown to reflect isometric twitch force [20]. An endurance index (EI) has been determined using twitch accelerometry and applied to superficial skeletal muscles in the limbs and trunk [21-24]. Activation of the diaphragm via phrenic nerve stimulation has been used in the past [6, 16, 25, 26]. This study seeks to evaluate the validity and reproducibility of an endurance test of the diaphragm muscle using phrenic nerve stimulation. It was hypothesized that these measurements are possible and will be reproducible, and that the results of this study will provide the foundation for future studies to explore diaphragmatic endurance in other populations and in clinical settings.

2. Materials and Methods

2.1 Participants

Twenty-four young, healthy participants (13 female, 11 male) between ages 18-35 years were recruited to participate in this study. Exclusion criteria included anyone with respiratory disease or musculoskeletal weakness such as asthma or diaphragmatic weakness. All volunteers provided written formal consent before participation. The study was approved by the Institutional Review Board at the University of Georgia (Athens, GA). Twenty participants completed both test sessions (Table 1).

2.2 Experimental Design

Subjects were tested on two separate occasions by the same tester. The tests were completed within two weeks, and the same protocol to determine the EI of the diaphragm was used on both days. Subjects laid on a table in the supine position, with the tester on their left side for left phrenic nerve access. An arm rest for the tester was placed over the participant's left arm. Two custom surface point stimulation electrodes (2.5 cm diameter) were placed on the left (n = 18) or right (n = 2) phrenic nerve, which lies underneath the sternocleidomastoid (SCM) muscle. The right phrenic nerve was only used if the left was not accessible or able to be found. One electrode was placed in between the two origins of the SCM, the manubrium of the sternum, and the medial clavicle, while the other electrode moved in the posterior triangle until the nerve was located (Figure 1A). A small amount of pressure was applied to the electrodes in the area to improve activation of the phrenic nerve.

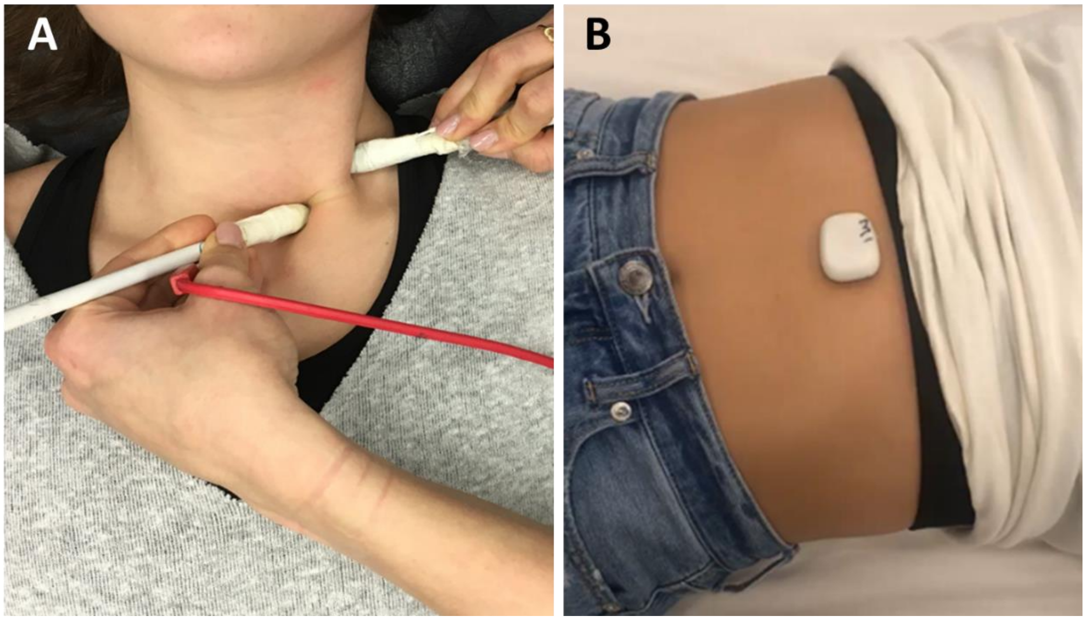

Figure 1. A) Electrode placement on the left phrenic nerve. The phrenic nerve lies underneath the sternocleidomastoid (SCM) muscle. One electrode was placed in between the two origins of the SCM, the manubrium of the sternum and the medial clavicle, while the other electrode moved in the posterior triangle until the nerve was located. B) Location of the accelerometer on the abdomen. The accelerometer was placed below the ribcage.

The stimulation intensity varied among participants (mean 49.5, range 25-70 mA) and was chosen based on the production of a visual vigorous contraction of the diaphragm. The stimulation levels were considered tolerable by the subjects, and produced submaximal activation [27]. Because maximal or supramaximal activation of the phrenic nerve are poorly tolerated and not practical, submaximal currents must be used. A previous study done in limb muscles showed that submaximal electrical stimulation produced the same endurance index values with different amounts of current[27]. The exact amount of current needed to produce a vigorous contraction will vary depending on the placement of the electrodes and the skin electrical resistance, as well as the amount of other tissue in the neck above the phrenic nerve. An accelerometer (WAX3, Axivity, UK) was placed on the left upper abdomen, approximately 1 inch medial and distal to the costal cartilage of the 11th and 12th ribs (Figure 1B). Movement of the upper abdomen is solely due to diaphragm contraction and has no contribution from the abdominal muscles, as they are not stimulated during this procedure. The endurance test protocol that was used in this study was modified from previously published literature, which was 9 minutes long using frequencies of 2, 4, and 6 Hz [27].

The current endurance test consisted of 5 minutes of electrical stimulation of 5 Hz stimulation frequency, with a sampling rate of 400 Hz. A shorter protocol was used to make the study easier to perform for both the investigator and the participant. Previous unpublished results showed a good agreement between the endurance index for 6 Hz in the previous protocol with the endurance index for 5 Hz in this protocol. The time from placing the electrodes to the start of the endurance test was recorded. All participants were required to follow up with the tester to identify any adverse effects from the test.

2.3 Data Analysis

Acceleration values were converted to absolute values by subtracting the devices value for earth's gravity from each point and getting the absolute value. The sum of the vibrations averaged over each second (five twitches) was obtained and the values during expiration when the diaphragm was relaxed were identified and used for analysis. No filtering or smoothing was performed. The EI, which equals the ending value/peak value*100, was calculated. Data are presented as means ± SD. The average EI values for the first 10 participants was compared to the last 10 participants to evaluate potential differences in the time to achieve successful stimulation over the course of data collection for this study.

Statistical Analysis included reliability testing using an intraclass correlation coefficient (ICC) and a coefficient of variation (CV). Two- tailed paired t-test was used to compare values for the two days. Alpha was set to 0.05. These were analyzed using Microsoft Excel software (IBM Corp., Armonk, NY). Equivalency testing was also performed for the two methods of determining the endurance index [28].

Table 1.

	#		Age (years)	Height (M)	Weight (kgs)	BMI kg/m^2
Females	13	Mean	22.0	1.65	60.7	22.1
		SD	1.8	0.06	6.8	6.8
Males	7	Mean	20.9	1.87	87.4	24.9
		SD	0.4	0.07	8.6	2.0

3. Results

Participant characteristics are listed in Table 1. Twenty out of the twenty-four participants completed both tests, and the test was successfully completed 41/47 times. Four participants did not complete both tests. Two participants did not complete their second test. The left phrenic nerve could not be located on two participants, in which their right phrenic nerve was used. The right phrenic nerve was successfully located for these participants in the tests following. No participants reported any major adverse effects from testing. All participants reported the test feeling "unusual" but not painful. One participant reported lightheadedness immediately post testing, and five participants reported labored or fatigued breathing only during endurance exercise later in the day after the test. None of the subjects considered the symptoms to be significant enough to limit their daily activities or to require medical treatment.

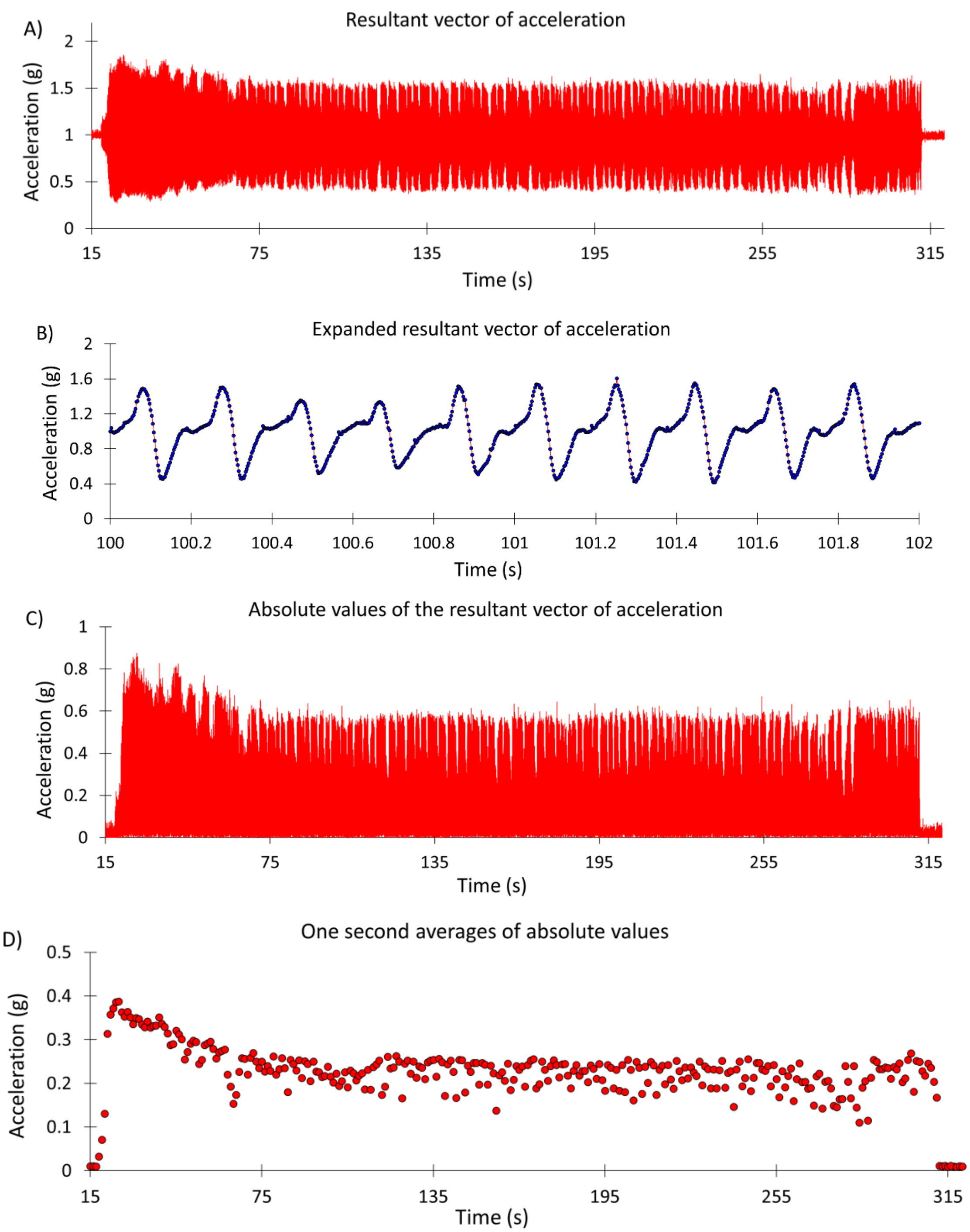

Figure 2. Representative example of diaphragm acceleration from electrical stimulation. A) The resultant vector from the three axes (x,y,z) collected by the accelerometer. The acceleration of contraction decreased over time. Breathing cycles were demonstrated by the decrease in acceleration with each breath. B) Two seconds of individual twitches showing data collection at 400 Hz (approximately 80 data points per twitch) and stimulation at 5 Hz. C) Absolute values of the raw 3-axis data corrected for gravity. D) One second averages (400 points) showing the decline in acceleration over the five minutes of stimulation.

A representative example of diaphragm acceleration is shown in Figure 2. The average EI was not significantly different between days (73.8 ± 9.4% vs 72.6 ± 11.1%, between days (p = 0.52). There was no significant differences between males and females (71.2 ± 8.7 vs 74.3 ± 10.1, p = 0.29). The time to find the phrenic nerve was lower for the second 10 participants and compared to the first 10 participants, although the results were not statistically different (p = 0.07) (Figure 3a). For all tests combined, the CV was 7.9% and the ICC was 0.73. When comparing the last 10 subjects to the first 10 subjects, the ICC improved from 0.57 to 0.97. The CV also improved in the last 10 subjects compared to the first 10 subjects from 13% to 2.8% (P = 0.006) (Figure 3b). There was no difference in the EI between the first 10 and last 10 subjects (EI = 70.3 ± 9.9 and EI = 76.1 ± 8.7, p = 0.19)

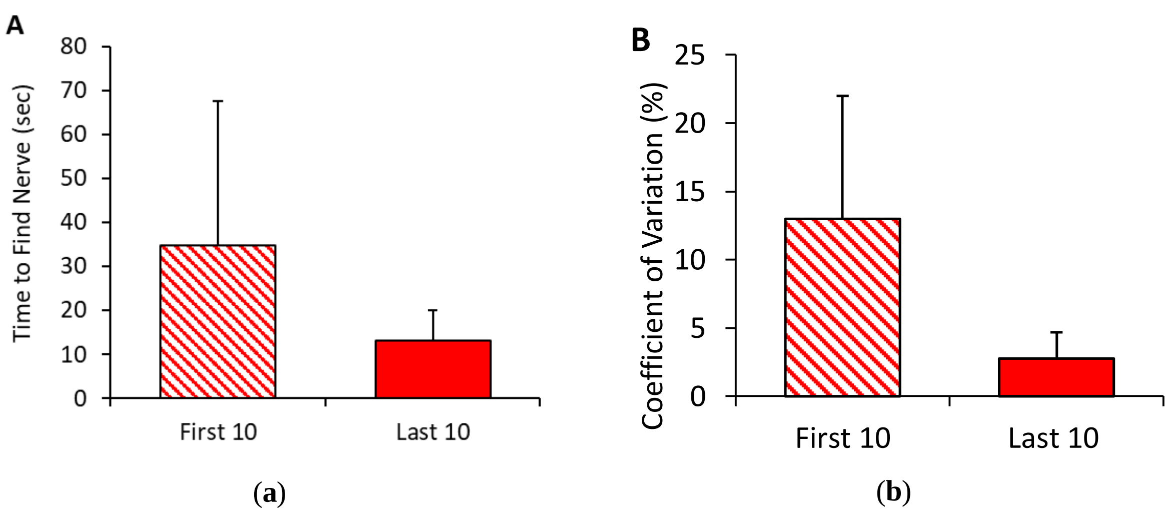

Figure 3. A) Comparison of the time to find the phrenic nerve in the first 10 subjects and the last 10 subjects (P = 0.07). B) Average Coefficient of Variations between the first 10 subjects and the last 10 subjects (P = 0.006).

Equivalency testing for repeated testing of the endurance index is shown in Figure 4. The difference in the mean values between for the first and second groups was not significant (p = 0.187). The differences between the EI on the two testing days was not different for the First (p = 0.656) or second groups (p = 0.994). The 95% confidence interval for individual data was within 5% of the average value for the second group, while it was outside 5% for the first group.

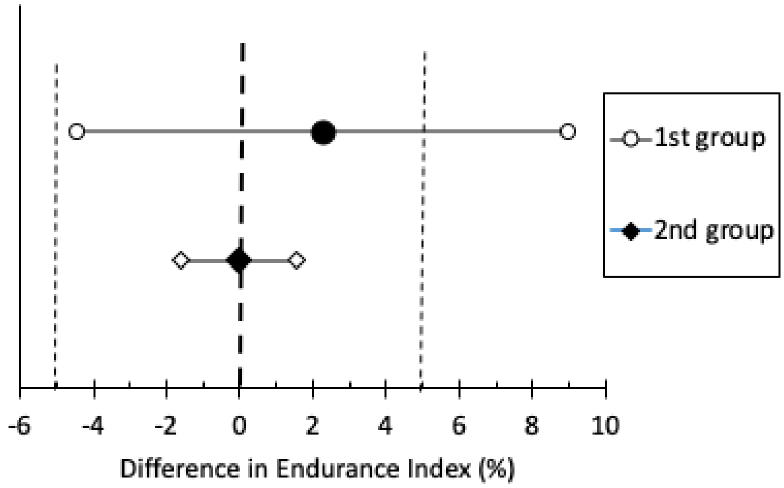

Figure 4. The large marks are the mean difference in endurance index between groups. The open symbols are the 95% confidence intervals of the difference in endurance index between groups. The thin vertical dotted lines are the plus and minus 5% values.

4. Discussion

The present study demonstrated that phrenic nerve stimulation can be used to perform an accelerometry-based endurance test successfully on a population of young, healthy subjects. The test was successfully completed 88% of the time. This success rate is not far from previous studies which reported success rates 90-100% with healthy subjects [15, 29]. A primary finding in this study was that the time to achieve successful stimulation of the phrenic nerve and the reproducibility of the EI values both markedly improved from the first 10 subjects to the last 10 subjects. This did not include 10-15 practice tests on different participants prior to initiation of data collection. Practice with evaluation methodology is routinely recommended, but evaluation of the actual number of tests needed to perform accurate measurements is not usually presented. The amount of training in the studies of phrenic nerve stimulation were not reported [6, 15-17, 29-31]. The location of the phrenic nerve varies across subjects [32], which sometimes makes it difficult to locate and isolate from the brachial plexus [15, 31, 32]. This problem was often solved by adjusting the stimulation electrodes until the diaphragm produced a visibly controlled, large contraction without significant arm movement. The vagus and phrenic nerve lie in close proximity to each other in the anterior cervical region, and therefore will both most likely be stimulated when an electrode is placed in the area. Significant stimulation of the vagus nerve most likely did not occur in this study. Subjects did not show or report obvious signs of vasovagal syncope. Vagus nerve stimulation is a common treatment for epilepsy and depression; it has been shown to be safe and with no identifiable risks[33].

This study resulted in good reproducibility of the EI values for the diaphragm. Previous studies reported CV for the EI of the forearm muscles of 7-12% [27], the lower back muscle of 10-20% [21], and shoulder muscles of 7-24% [24]. These values are consistent with our study, especially if the CV for the last 10 subjects is used for comparison.

The actual EI values from this study are hard to compare to previous studies of the diaphragm. Twitch stimulation of phrenic nerve (1-5 Hz) has been performed to evaluate patients with myasthenia gravis, however the duration of stimulation was only a few seconds and EMG signal intensities were used to evaluate fatigue [16]. The range of EI values in this study (58-92%) is consistent with the human diaphragm having some proportion of fatigable muscle fibers. The human diaphragm is reported to have fast twitch fibers percentages of 19-67% based on *in vitro* histochemical analyses [8]. Because the diaphragm performs regular breathing with a small percentage of its muscle fibers and also performs high force contractions for other reasons [34], some fatigability might be expected.

The endurance test protocol used was based on previous literature [27]. Because the test did not result in large amounts of fatigue, and only involved one costal hemidiaphragm, we did not expect the fatigue that occurred in our test to influence respiratory function. Respiratory gases were not measured during our study, however we did not expect them to change. This can be considered one advantage of the 5 minute endurance test used in this study. Performing a test that minimized respiratory deficits would be useful in clinical populations that have respiratory symptoms. The 5 minute 5 Hz endurance test was adapted from a 9 minute long protocol (3 minutes each at 2, 4 and 6 Hz) to be shorter and more comfortable for this study (5 minutes at 5 Hz). Limiting the test to 5 minutes reduces the potential respiratory deficit that might occur to the subject, and a shorter protocol is easier for the tester. Strong correlations above $R^2 > 0.9$ have been seen between the 6 Hz EI of the 9 minute protocol and the 5 Hz EI of the five minute protocol (unpublished observations).

There were some limitations to the study. One was the use of submaximal stimulation currents. High currents producing maximal activation would have been more uncomfortable and difficult for subjects to tolerate. A previous study showed that different stimulation currents did not affect endurance test measurements in the arm when using submaximal stimulation [27]. However, this should be determined stimulation of the diaphragm. Another limitation to the study was the stimulation of a hemidiaphragm instead of the entire muscle; this may not give a complete, accurate representation of diaphragm endurance. Furthermore, the technique itself; there was difficulty in maintaining solid, consistent contact on the nerve with the hand held electrodes [35, 36]. Movements of other body parts may also skew accelerometer data [32]. Finally, subjects were allowed to breathe freely during the procedure. A deep breath overrode the movement of the diaphragm caused by the stimulation and resulted in no movement of the accelerometer. These lags in the data collection had to be accounted for in the data analysis. Breathing could potentially impact measurements and the acceleration produced by the diaphragm and therefore the use of spontaneous breathing is considered a limitation.

5. Conclusions

The diaphragm endurance test was reproducible and did not demonstrate an order effect with testing on different days. The coefficient of variation of the measurement improved with practice, as did the time to find the phrenic nerve. This strongly suggests that practice is necessary to adequately perform these measurements. The diaphragm endurance test is non-invasive, uses inexpensive equipment, and doesn’t require voluntary hyperventilation. With further testing, the diaphragm endurance test may become an important method of assessing respiratory endurance capacity.

6. Patents

A provisional patent has been applied for.

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Conflicts of Interest: Kevin McCully is the President of Infrared Rx, Inc,

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