RESEARCH ARTICLE

Validation of a 5 Minute 5 Hz protocol for Muscle Specific Endurance

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Abstract

Muscle endurance has been measured using an electrical stimulation twitch protocol and an accelerometer. This protocol used stimulation frequencies of 2, 4, and 6 Hz over nine minutes. This study compared endurance index values using a shorter protocol of 5 Hz for 5 Minutes to the original nine minute protocol. Fifteen healthy subjects (19-22yrs) were electrically stimulated on the forearm muscles twice: one day for 5 minutes at 5 Hz and on another day for 3 minutes at each at 2, 4 and 6 Hz. A triaxial accelerometer measured the resultant vector of the movements and the decrease in acceleration was used to indicate fatigue. There was no difference in Endurance Index (EI) between the 5 and 9 minute protocols (63.2±18.7%, 64.1±19.8%, respectively, p=0.532). The correlation between the two EI measurements was y= 0.91x+4.99%, r2=0.93. Equivalency testing found the mean difference between EI values for the two methods was 0.88% with a 95% confidence interval of the difference in EI of -2.09 to 3.81%. In conclusion, the shorter 5 Hz five minute endurance index protocol provided similar results to the longer 2 ,4, 6 Hz nine minute protocol. These results support the use of the shorter protocol when measuring muscle specific endurance.

Keywords: 1; Muscle fatigue, 2; fatigability, 3; accelerometer, 4; electrical stimulation

1. Introduction

Muscle fatigue or a lack of muscle endurance is present in clinical diseases or injuries including the muscular dystrophies, after spinal cord injury, and neurological disorders[1, 2]. Related to muscle fatigue, reduced mitochondrial energy capacity is associated with muscle dysfunction[3-5]. A number of different methods have been developed to evaluate skeletal muscle endurance[6-8].

An endurance test that utilizes twitch contractions has been previously developed to properly evaluate the fatigue of specific muscles in clinical populations[9]. The twitch endurance test uses neuromuscular electrical stimulation frequencies that vary from 2 to 6 Hz, and measures muscle activation as the acceleration associated with each contraction. The amount of acceleration remaining as a percentage of the initial highest acceleration has been called the endurance index. This approach has subsequently been used to evaluate skeletal muscle in a number of clinical populations[2, 10-12]. In addition, the twitch endurance test has been applied to a number of different muscle groups[13, 14]. A key advantage of this approach is that it is noninvasive and requires only relatively inexpensive equipment: a neuromuscular stimulator capable of performing repeated twitch contractions and a tri-axial accelerometer with storage or Bluetooth capability.

While the twitch endurance index test is relatively simple, the current protocol requires nine minutes of stimulation and the ability to change stimulation rates multiple times[2]. The purpose of this study was to test whether using a shorter and simpler protocol that is five minutes long and uses one stimulation frequency provides comparable results to the nine minute variable frequency test. We hypothesized that the Endurance Index for the five minute 5Hz test would have a high degree of correlation (R2 > 0.90) with the Endurance Index at 6Hz for the nine minute test.

2. Materials and Methods

Participants Fifteen healthy individuals without known neurological or physical conditions and between the ages of 19 to 22 years old participated in this study (Table 1). This study was approved by the Institutional Review Board at the University of Georgia (VERSION00000437). All subjects were given written, informed consent prior to any testing.

Procedures All subjects were tested on two occasions, with the one of the two EI protocols being performed on each day.

Experimental setup The participant was placed in a supine position. Two gel electrodes (5 x 10 cm) were placed on the right or left forearm muscles approximately 5 cm apart. The twitch electrical stimulator (Theratouch 4.7, Rich-Mar, USA) was used. A submaximal current sufficient to produce a visible high intensity muscle contraction without causing discomfort was used (25.2 ± 4.77mA). Previous studies have shown that the endurance index is not influenced by the amount of current applied as long as a visible contraction occurs[2].

The triaxial accelerometer was set at a frequency of 400Hz and measurement range of 2Gs. The device (WAX3, Axivity, UK) was placed equidistant between the two electrodes. The 5-minute muscle endurance test protocol was performed with continuous muscle stimulation set at the same current and frequency throughout the test. The 2, 4, 6Hz protocol used 3 minutes of stimulation at each frequency separated by 10 seconds[9]. The 10 second delay allowed switching the stimulation frequency with the stimulation device.

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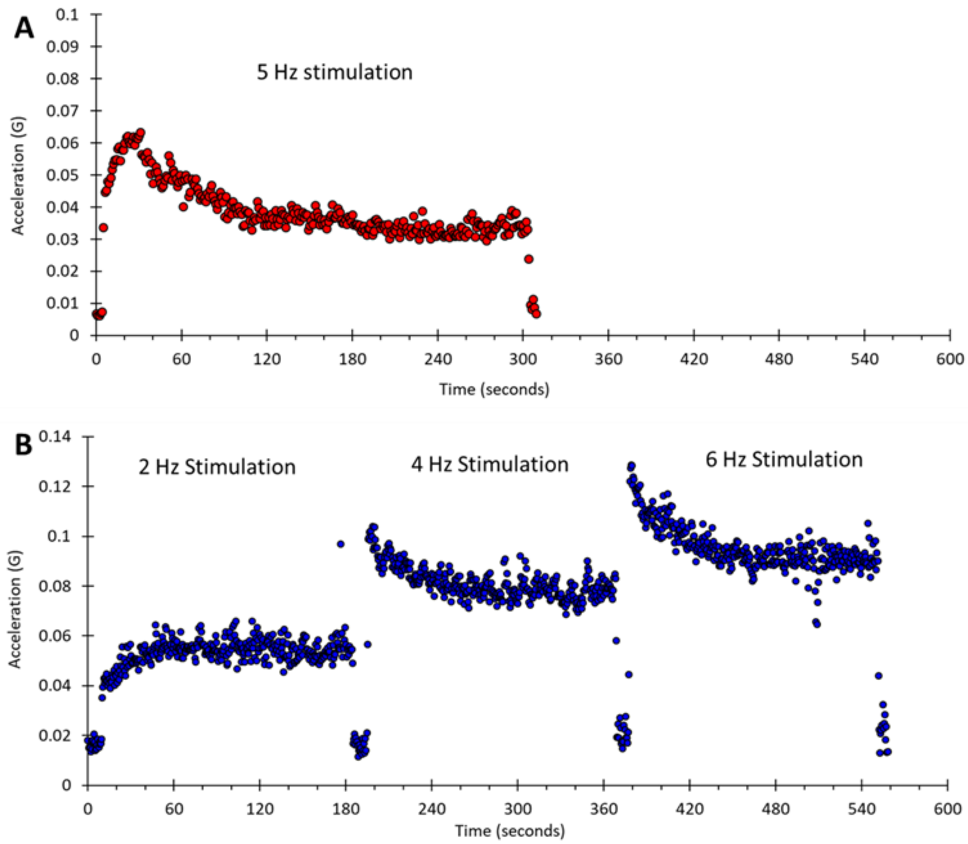

Figure 1. a) Representative example of the accelerometer data from the 5 Hz protocol. b) Representative data from the same subject from the 2 ,4, 6 Hz protocol. Data are the resultant vector of acceleration in the x, y, and z directions.

Data analysis

The data from the three axes were used to calculate a resultant vector (Figure 1). The resultant vector was analyzed using a Matlab routine to determine the maximal changes in acceleration at the beginning of the test and at the end of the test. For the single frequency 5 Hz protocol, the endurance index was calculated as the ending acceleration over the maximal acceleration times 100%. For the 2, 4, 6 Hz protocol, the ending acceleration was divided by the maximal acceleration times 100 for each frequency. In addition, the maximal acceleration at 4 Hz was normalized to the ending acceleration for 2 Hz. The maximal acceleration at 6 Hz was normalized to the ending acceleration for 4 Hz. In this approach, the EI for 4 and 6 Hz included the cumulative reductions in acceleration from the previous stimulation frequencies [9].

Comparisons between the two EI protocols were made using paired student t-tests for the mean values across subjects (two tailed, equal variance). Equivalency testing was also performed for the two methods of determining the endurance index [15]. Data are reported as means ± standard deviations. The accepted significance level was determined to be 0.05.

Results

Subject Demographics Participant demographics are shown in Table 1. The average time between the two testing days was 3.7 days with all tests performed within 14 days. The 5 Hz protocol was performed first five times, and the 2,4, 6 Hz protocol performed first 10 times.

Table 1. Subject demographics.

	Total	**Male**	**Female**
Sample size	15	7	8
Age (years)	20.6(1.0)	21.0 (1.5)	20.6 (1.0)
Height (m)	1.74 (0.11)	1.82 (4.07)	1.66 (0.09)
Weight (kg)	76.3 (18.8)	87.5 (37.9)	66.5 (9.2)

Values are means (standard deviations)

The forearm muscle showed evidence of fatigue in both protocols (Figure 1). The endurance index for the 2, 4, 6 Hz protocol was 90.2 ±11.7%, 77.2 ±15.2%, and 64.1 ±19.8% for 6 Hz. The EI value was 63.2 ± 18.7% for the 5 Hz protocol. There was no difference between the EI value for 6% and the EI value for the 5 Hz protocol, p = 0.532. The EI values for 2 Hz and 4 Hz were lower than the EI value for 5 Hz ($p < 0.001$). There was also no difference in the endurance index values between men and women for either 6 Hz (61.3± 26.5% for men and 66.5 ± 20.8% for women, p = 0.634) or 5 Hz protocols (62.2 ± 26.5% for men and 64.1 ± 19.6% for women, p = 0.854).

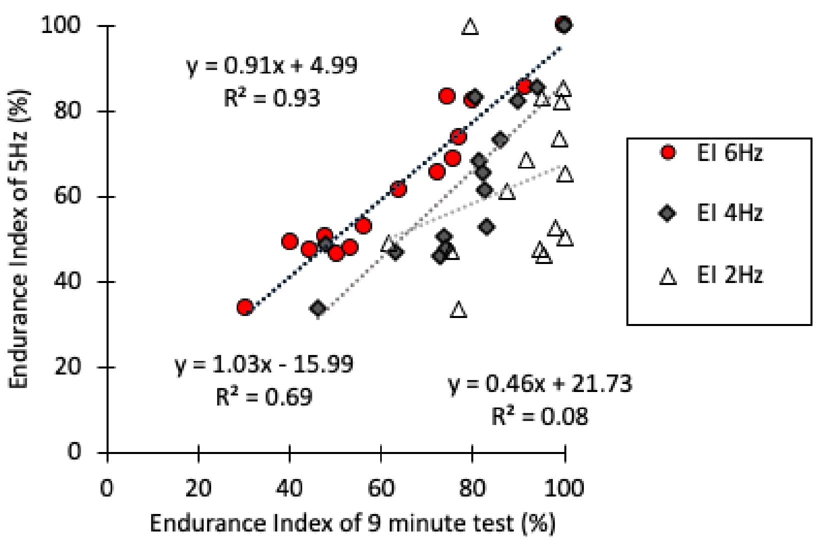

Figure 2. The correlations between the 2, 4 and 6 Hz EI valum/es and the 5 Hz EI values. The R^2 values for correlations with 2, 4, and 6 Hz with the 5 Hz EI values were 0.08, 0.69, and 0.93, respectively.

There was a good correlation between the EI value for 5 Hz and the EI value for 6% ($R^2 > 0.9$ (Figure 2). The correlation between the EI value for 5 Hz was lower for the 2 Hz and 4 Hz EI values from the nine minute test ($R^2 < 0.9$). Equivalency testing for the two endurance index tests are shown in Figure 3. The difference in the mean values for the 6 Hz and 5 Hz EI protocols was not significant (p = 0.532). The 95% confidence interval for individual data was within 5% of the average.

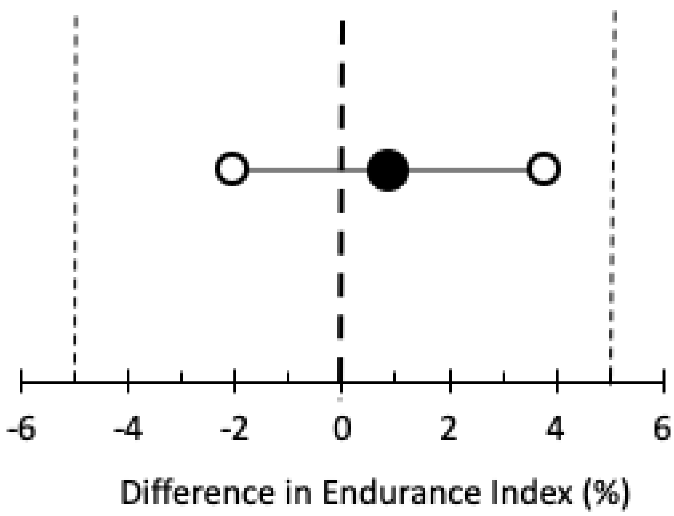

Figure 3. The large solid symbols are the mean difference between EI values for the 2, 4, 6Hz protocol at 6 Hz and the 5 Hz protocol. The open symbols are the 95% confidence intervals of the difference between the accelerometer and the video analyses. The thin vertical dotted lines are the plus and minus 5% values.

4. Discussion

This study found that the 5 Hz five minute protocol produced similar EI values to the 2, 4, 6 Hz nine minute protocol. Previous studies have used the nine minute protocol to measure muscle specific endurance[2, 10]. These studies have shown that the 6 Hz EI correlated with measurements of muscle mitochondrial capacity in clinical populations[2, 10]. In addition, the 6 Hz EI value improved with body weight support treadmill training in patients with multiple sclerosis[16]. The advantage of the 2, 4, 6 Hz nine minute protocol is that it is a progressive test with increasing stimulation protocols. The metabolic rate of the muscle increases with stimulation frequency[17]. However there can be advantages to using a shorter protocol. The 5 Hz for 5 minutes protocol is shorter in duration, which can speed up data collection and reduce the burden on the research participant. It also reduces the chance that the research subject might move or spasm during data collection. The purpose of this study was to test whether the 5 Hz protocol produced consistent EI values to the longer 2, 4, 6 Hz protocol. This was found, but in addition the actual values for the EI was not different. This suggests that there can be direct comparisons between different studies that use either protocol.

The EI values found in this paper were similar to previous studies. The endurance index found in this study (64%) was similar to that seen in previous studies of control subjects[12]. Previous studies have shown that EI values are greater in the leg muscles (vastus lateralis and medial gastrocnemius muscles)[13, 14], but lower in trunk muscles like the lower back and shoulder muscles[13, 14]. No difference in the EI was seen between males and females. However, the sample size for the comparison between males and females was small. Thus small differences between males and females may still be possible, as some studies report sex differences in fatigue [18, 19].

The endurance protocols used in this study used stimulation frequencies varying from 2-6 Hz. At these stimulation frequencies, the muscle contractions can be considered individual twitch contractions[9]. The muscle contraction can be considered 'isotonic' as the contraction does not last long enough to take up the compliance in the muscle and generate significant force levels across the wrist joint. The movement of the muscle in these contractions can be measured as acceleration with an accelerometer. Decreases in contractile activity due to fatigue will reduce the magnitude of acceleration of the twitch contractions. The advantage of using an accelerometer to measure muscle contraction level rather than force, is that accelerometers are inexpensive compared to force transducers. In addition, accelerometers can be used to evaluate the movement of muscles such as those in the lower back and neck, which are difficult to measure with a force transducer[13, 14]. Because twitch contractions are low force levels, they can be considered safer for patients at risk for musculo-skeletal injury that fatigue/endurance protocols that use isometric or tetanic contractions. A limitation to using twitch contractions is that the energy demand of the 2-6 Hz contractions may not be enough to produce muscle fatigue. This supports the use of the twitch contraction endurance protocol for subject populations with low levels of muscle endurance. Twitch endurance protocols may not be suitable for subjects with high endurance, such as endurance trained muscles in athletes.

The use of low frequency twitch electrical stimulation and accelerometry has a number of significant advantages when studying muscle endurance/fatigability. The low force levels that result from submaximal stimulation and twitch contractions makes the test easy to tolerate and low risk of physical damage. The use of electrical stimulation overcomes potential problems in fatigue tests that use voluntary contractions. Voluntary contractions require motivation as well as adequate motor control, something that many clinical populations lack [10, 16]. Instead of measuring force development, this test measures changes in muscle movement with contraction. Previous studies have shown that accelerometer based movements correlate with torque measurements [20 21], and thus they provide similar information. An advantage of using an accelerometer is that muscles can be tested with an accelerometer that would be difficult to study with a force measuring device [13, 14]. Another strength of the study is that previous studies have shown that measurements of muscle endurance with the twitch acceleration test correlated with independently measured muscle mitochondrial capacity [2, 10]. This suggests that the physiological basic for the endurance test is related to aerobic energy capacity of the muscle.

A potential limitation to this study was the use of a single muscle group. In is not clear if similar results would be seen in other muscle groups. This is particularly true for the absolute value for the EI. For example, little fatigue has been seen in the quadriceps muscles of healthy subjects[13], and it is not clear if there would be consistent agreements. Or in patients with MS who have very low EI values, it might be more useful to use the 2, 4, 6 Hz protocol so that the 2, or 4Hz EI values could be used in the 6 Hz EI values are too low [16]. However, we feel that for most skeletal muscle, the relationship between the 5 Hz five minute protocol to the 2, 4, 6 Hz nine minute protocol should be consistent.

5. Conclusions

The results from this study suggest that a shorter 5 Hz five minute electrical stimulation protocol can capture endurance index values from the forearms of healthy subjects. The advantage of this protocol is that it is shorter and simpler than the previously used 2, 4, 6 Hz nine minute endurance protocol.

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Conflicts of Interest: One of the authors; Kevin McCully is the President and Chief Science Officer of Infrared Rx, Inc, a company that develops analysis software related to Endurance Index measurements.

References

1. Reetz K, Dogan I, Hilgers RD, et al. Progression characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS): a 2 year cohort study. *Lancet Neurol*. Dec 2016;15(13):1346-1354. doi:10.1016/S1474-4422(16)30287-3

2. Willingham TB, Backus D, McCully KK. Muscle Dysfunction and Walking Impairment in Women with Multiple Sclerosis. *Int J MS Care*. Nov-Dec 2019;21(6):249-256. doi:10.7224/1537-2073.2018-020

3. Mitchell T, Chacko B, Ballinger SW, Bailey SM, Zhang J, Darley-Usmar V. Convergent mechanisms for dysregulation of mitochondrial quality control in metabolic disease: implications for mitochondrial therapeutics. *Biochem Soc Trans*. Feb 1 2013;41(1):127-33. doi:10.1042/BST20120231

4. Mitchell T, Darley-Usmar V. Metabolic syndrome and mitochondrial dysfunction: insights from preclinical studies with a mitochondrially targeted antioxidant. *Free radical biology & medicine*. Mar 1 2012;52(5):838-40. doi:10.1016/j.freeradbiomed.2011.12.014

5. Kendall FD. Mitochondrial disorders: Overview of diagnostic tools and new diagnostic trends. *Journal of Pediatric Biochemistry*. 2012 2012;2(4):193-203.

6. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. *Neurology*. Jan 22 2013;80(4):409-16. doi:10.1212/WNL.0b013e31827f07be

7. Al-Mulla MR, Sepulveda F, Colley M. A review of non-invasive techniques to detect and predict localised muscle fatigue. *Sensors (Basel)*. 2011;11(4):3545-94. doi:10.3390/s110403545

8. Taylor JL, Amann M, Duchateau J, Meeusen R, Rice CL. Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again. *Med Sci Sports Exerc*. Mar 22 2016;doi:10.1249/MSS.0000000000000923

9. Willingham TB, McCully KK. Assessment of Muscle Fatigue during Twitch Electrical Stimulation using Accelerometer-based Mechanomyography. *Advances in Skeletal Muscle Function Assessment*. August, 2017 2017;1(2):14-20.

10. Bossie HM, Willingham TB, Schoick RAV, O'Connor PJ, McCully KK. Mitochondrial capacity, muscle endurance, and low energy in friedreich ataxia. *Muscle Nerve*. Oct 2017;56(4):773-779. doi:10.1002/mus.25524

11. Faxon JL, Sanni AA, McCully KK. Hamstrings muscle endurance in subjects with prior knee injuries. *J Functional Morphology and Kinesiology*. 2018;3(56):1-10. doi:10.3390/jfmk3040056

12. Hewgley RA, Moore BT, Willingham TB, Jenkins NT, McCully KK. Muscle mitochondrial capacity and endurance in adults with type 1 diabetes. *Medical Research*

Archives. 2020;8(2):1-13. doi:https://doi.org/10.18103/mra.v8i2.2049

13. McCully KK, Moraes C, Patel SV, M. G, Willingham TB. Muscle-specific endurance of the lower back erectors using electrical twitch mechanomyography. *J Functional Morphology and Kinesiology*. 2019;4(12):1-7. doi:10:3390/jfmk4010012
14. McCully KK, Prins P, Mistry K, Willingham TB. Muscle-specific endurance of the trapezius muscles using electrical twitch mechanomyography. *Shoulder Elbow*. Apr 2018;10(2):136-143. doi:10.1177/1758573217726269
15. Lakens D. Equivalence Tests: A Practical Primer for t Tests, Correlations, and Meta-Analyses. *Soc Psychol Pers Sci*. May 2017;8(4):355-362. doi:10.1177/1948550617697177
16. Willingham TB, Melbourn J, Moldavskiy M, McCully KK, Backus D. Effects of Treadmill Training on Muscle Oxidative Capacity and Endurance in People with Multiple Sclerosis with Significant Walking Limitations. *Int J MS Care*. Jul-Aug 2019;21(4):166-172. doi:10.7224/1537-2073.2018-021
17. Ryan TE, Brizendine JT, McCully KK. A comparison of exercise type and intensity on the noninvasive assessment of skeletal muscle mitochondrial function using near-infrared spectroscopy. *J Appl Physiol (1985)*. Jan 15 2013;114(2):230-7. doi:10.1152/japplphysiol.01043.2012
18. Hicks AL, Kent-Braun J, Ditor DS. Sex differences in human skeletal muscle fatigue. *Exerc Sport Sci Rev*. Jul 2001;29(3):109-12. doi:10.1097/00003677-200107000-00004
19. Hunter SK. Sex differences and mechanisms of task-specific muscle fatigue. *Exerc Sport Sci Rev*. Jul 2009;37(3):113-22. doi:10.1097/JES.0b013e3181aa63e2
20. Gobbo M, Ce E, Diemont B, Esposito F, Orizio C. Torque and surface mechanomyogram parallel reduction during fatiguing stimulation in human muscles. *Eur J Appl Physiol*. May 2006;97(1):9-15. doi:10.1007/s00421-006-0134-8
21. Orizio C, Diemont B, Esposito F, et al. Surface mechanomyogram reflects the changes in the mechanical properties of muscle at fatigue. *Eur J Appl Physiol Occup Physiol*. Sep 1999;80(4):276-84. doi:10.1007/s004210050593