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Original article

Application of the neuromuscular fatigue threshold treadmill test to muscles of the quadriceps and hamstrings

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Abstract

Purpose: The purposes of the present study were: (1) to determine whether the physical working capacity at the fatigue threshold (PWC_{FT}) model that has been used for estimating the onset of neuromuscular fatigue in the vastus lateralis (VL) during incremental treadmill running could also be applied to the vastus medialis (VM), biceps femoris (BF), and semitendinosus (ST) muscles; and (2) if applicable, to compare the running velocities associated with the PWC_{FT} among these muscles.

Methods: Eleven subjects (age 21.7 ± 1.8 years) performed an incremental treadmill test to exhaustion with electromyographic signals recorded from the VL, VM, BF, and ST.

Results: The results indicated there were no significant (p > 0.05) mean differences in the running velocities associated with the PWC_{FT} for the VL (14.4 ± 2.0 km/h), VM (14.3 ± 1.9 km/h), BF (13.8 ± 1.8 km/h), and ST (14.7 ± 2.3 km/h). In addition, there were significant inter-correlations (r = 0.68-0.88) among running velocities associated with the PWC_{FT} of each muscle. Individual results also indicated that 9 of the 11 subjects exhibited identical PWC_{FT} values for at least 3 of the 4 muscles, but there were no uniform patterns for any intra-individual differences. *Conclusion*: The findings of the present study suggested that the PWC_{FT} test is a viable method to identify neuromuscular fatigue in the quadri-

ceps and hamstrings during incremental treadmill exercise and results in consistent PWCFT values among these muscles.

Keywords: EMG amplitude; Muscle activation; PWC_{FT}

1. Introduction

The physical working capacity at the fatigue threshold (PWC_{FT}) test of deVries et al.¹ estimates the maximal power output that can be sustained for an extended period of time without evidence of neuromuscular fatigue during cycle ergometry. Specifically, the PWC_{FT} test is based on within-stage increases in electromyographic (EMG) amplitude that reflect fatigue-induced increases in muscle activation (i.e., motor unit recruitment and firing rates) required to maintain the desired power output. It has been demonstrated that the PWC_{FT} provides an accurate measurement of the highest non-fatiguing

workload² and results in consistent values among superficial muscles of the quadriceps (i.e., vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM).³ Previous studies have used the PWC_{FT} test to examine physiological factors associated with neuromuscular fatigue,⁴⁻⁶ to assess physical fitness⁷ and factors related to the degeneration of neuromuscular function^{8,9} in the elderly, to prescribe exercise training intensities,¹⁰ as well as to determine the effectiveness of exercise training programs¹¹ and various nutritional supplements as ergogenic aids.¹²⁻¹⁶ Collectively, these findings illustrate that the PWC_{FT} serves as a valid and reliable tool for estimating the onset of neuromuscular fatigue during cycle ergometry with athletic performance and clinical applications.

Recently, Camic et al.¹⁷ applied the PWC_{FT} model used for cycle ergometry to incremental treadmill exercise to derive a new neuromuscular fatigue threshold test for running.

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Utilizing the method of deVries et al.¹ and recording the EMG signal from the VL, Camic et al.¹⁷ reported that the PWC_{FT} model was able to identify fatiguing from non-fatiguing running velocities by examining the slope coefficients for the EMG amplitude vs. time relationships during each 2-min stage of an incremental treadmill test to exhaustion. Theoretically, the PWC_{FT} treadmill test estimates the fastest running velocity that can be sustained without progressive increases in muscle activation to compensate for the development of fatigue. It was also reported that the running velocity associated with the PWC_{FT} from the VL was significantly correlated (r = 0.70) and occurred at the same running velocity (14.0 \pm 2.3 km/h, mean \pm SD) as the respiratory compensation point (RCP) $(14.0 \pm 1.8 \text{ km/h})$.¹⁷ These findings suggested that the PWC_{FT} determined from the VL during incremental treadmill exercise, like the RCP, (1) can be used to identify the boundary between the heavy and severe domains of exercise intensity, and (2) represents the maximal exercise intensity that can be achieved with oxygen uptake (VO₂) and lactate still maintaining a steady state.¹⁸ It has recently been demonstrated, however, that the patterns (linear, quadratic, cubic) of responses for muscle activation (i.e., EMG amplitude) across exercise intensity (i.e., VO₂) are unique among muscles of the thigh and may be due to variations in muscle architecture, fiber type, or biomechanical differences.¹⁹ A number of other studies^{20–23} have also reported differences in muscle-activation patterns between the quadriceps and hamstring groups with increases in running velocity or the development of fatigue. For example, Kyröläinen et al.²¹ showed that biarticular muscles (e.g., biceps femoris) exhibited changes in muscle activity across running phases with increases in velocities from 4.0 m/s to 8.5 m/s that were distinct from the activation patterns of monoarticular muscles (e.g., VL). Thus, it is currently unknown whether the PWC_{FT} model is applicable to other muscles of the quadriceps as well as the hamstrings while running due to these variations in activation. Based on the anatomical and biomechanical differences that exist among these muscles, an assessment of their fatigue-induced activation strategies is warranted. Therefore, the purposes of the present study were: (1) to determine whether the PWC_{FT} model that has been used for estimating the onset of neuromuscular fatigue in the VL during incremental treadmill running could also be applied to the VM, biceps femoris (BF), and semitendinosus (ST) muscles; and (2) if applicable, to compare the running velocities associated with the PWC_{FT} among these muscles.

2. Methods

2.1. Subjects

Nine college-aged males (age = 22.0 ± 1.7 years, body mass = 75.5 ± 9.0 kg, height = 178.2 ± 5.8 cm) and 2 females (20.5 ± 2.1 years, 58.7 ± 3.1 kg, 165.8 ± 9.8 cm) volunteered to participate in this investigation. These subjects were selected based on their diverse running backgrounds, which included regular participation in recreational races (i.e., 5 km, 10 km) (n = 3), marathons (n = 4), ultramarathons (n = 1), triathlons (n = 1),

collegiate track (n = 1), and collegiate soccer (n = 1). Each subject visited the laboratory on 2 occasions (separated by at least 48 h) and was instructed to: (1) maintain normal dietary habits and sleep patterns during the course of the study and (2) avoid exercise for 48 h, caffeine and alcohol for 24 h, and food intake for 3 h prior to each visit. The study was approved by the University of Wisconsin-La Crosse Institutional Review Board for Human Subjects, and all subjects completed a health history questionnaire and signed a written informed consent prior to testing.

2.2. Incremental Treadmill Tests

The first laboratory visit was structured as an orientation session to familiarize the subjects with the testing procedures (i.e., measurement of gas exchange and EMG while running on a treadmill). During the second laboratory visit, each subject performed an incremental test to exhaustion for the determination of PWC_{FT}, RCP, and peak oxygen uptake (VO_{2peak}). The incremental treadmill test involved a standard warm-up of walking at 4.8-6.4 km/h for 4 min. Immediately following the warm-up, the test began at 9.7 km/h and increased 1.6 km/h every 2 min until volitional fatigue. This increment of 1.6 km/h was consistent with the original protocol¹⁷ and was selected for the practical purpose of estimating the PWC_{FT} across a wide range of running velocities in the diverse sample. The grade remained constant at 1.0% during the test and was selected to represent the energy cost that is typically experienced by running outdoors.²⁴

2.3. EMG Measurements and Signal Processing

During the second laboratory visit, bipolar (10 mm centerto-center) wireless surface electrode sensors (Tringo Lab Wireless EMG System; Delsys, Natick, MA, USA) were placed on the right thigh over the VL, VM, BF, and ST muscles according to the recommendations of the SENIAM Project.²⁵ Because of the lack of reliability of the EMG signal for the RF.^{26,27} this muscle was not examined. Prior to electrode sensor placement. the skin at each electrode site was dry-shaved, lightly abraded with gauze, and cleaned with alcohol. The EMG signals were amplified (gain: × 1000) (Tringo Lab Wireless EMG System, bandwidth = 20-450 Hz), sampled at 2000 Hz, recorded continuously throughout the incremental test, and stored in a personal computer (Latitude E6540; Dell Inc., Round Rock, TX, USA) for subsequent analyses. All signal processing was performed using custom programs, which were written with MATLAB programming software (Version 8.2; Mathworks, Natick, MA, USA). The EMG signals were digitally bandpass filtered (fourth-order Butterworth) at 20-450 Hz.

2.4. Determination of PWC_{FT}

The PWC_{FT} values were determined using the model of deVries et al.¹ During each 2-min stage of the incremental treadmill test, six 10-s EMG samples were selected from the signal (10–20 s, 30–40 s, 50–60 s, 70–80 s, 90–100 s, and 110–120 s). The EMG amplitude (microvolts root mean



Fig. 1. Illustration of the method used to determine the physical working capacity at the fatigue threshold (PWC_{FT}) during treadmill running. The PWC_{FT} in this example (15.3 km/h) was calculated by averaging the highest running velocity (14.5 km/h) that resulted in a non-significant (p > 0.05) slope coefficient for the EMG amplitude *vs.* time relationship and the lowest running velocity (16.1 km/h) that resulted in a significant (p < 0.05) slope coefficient. * Slope significantly (p < 0.05) greater than 0.

square, μ Vrms) values were calculated for each of the 10-s epochs (MATLAB) and separately plotted across time for each stage (i.e., running velocity) of the test (Fig. 1). The PWC_{FT} for each muscle was defined as the average of the highest running velocity that resulted in a nonsignificant (p > 0.05, single-tailed *t* test) slope coefficient for the EMG amplitude *vs.* time relationship and the lowest running velocity that resulted in a significant (p < 0.05) positive slope coefficient (Fig. 1).

2.5. Measurements of Gas Exchange

All subjects wore a nose clip and breathed through a 2-way valve (2700, Hans Rudolph, Kansas City, MO, USA) during the incremental tests. Expired gas samples were collected and analyzed using a calibrated metabolic cart AEI Moxus (AEI Technologies, Pittsburgh, PA, USA) with O2, CO2, and ventilatory parameters expressed as 30-s averages. Each subject was also fitted with a Polar Heart Watch system (Polar Electro, Lake Success, NY, USA) to monitor heart rate throughout the test. VO_{2peak} was defined as the highest VO₂value in the last 30 s of the exercise test that met the criteria of Day et al.²⁸ The test-retest reliability for VO_{2peak} testing from our laboratory indicated that the intraclass correlation coefficient was R = 0.95, and the standard error of measurement²⁹ (SE_m) = 97 mL/min, with no significant (p > 0.05) mean difference between test and retest values. The RCP was determined by noninvasive gas-exchange measurements using the method of Beaver et al.³⁰ For each subject, running velocities from the incremental treadmill test attained during the second laboratory visit were plotted against VO₂values, and the regression equation derived was used to determine the running velocity that corresponded to their RCP. The test-retest reliability for RCP testing from our laboratory indicated that the intraclass correlation coefficient was R = 0.93, and $SE_m = 103$ mL/min, with no significant mean difference between test and retest values.

2.6. Statistical Analyses

Mean \pm SD values were calculated for the PWC_{FT} from the VL, VM, BF, and ST as well as RCP and VO_{2peak}. For determination of PWC_{FT} values, the relationships for EMG amplitude *vs*. time for each individual muscle and running velocity were examined using linear regression (IMB SPSS Statistics, Version 24; IMB Corp., Armonk, NY, USA). A 1-way repeated-measures ANOVA was also used to determine whether there were significant mean differences in running velocities among the PWC_{FT} from each muscle and the RCP. Follow-up *post hoc* analyses included paired *t* tests with Bonferroni correction. A 0-order correlation matrix was used to determine the relationships among the PWC_{FT} from each muscle and the RCP. An $\alpha < 0.05$ was considered statistically significant for the 1-way repeated-measures ANOVA and all 0-order correlations.

3. Results

The results of the 1-way repeated-measures ANOVA and *post hoc* analyses indicated there were no significant (p > 0.05) differences among the running velocities associated with the VL PWC_{FT}, VM PWC_{FT}, BF PWC_{FT}, ST PWC_{FT}, and RCP (Table 1). In addition, the individual running velocities associated with the PWC_{FT} were identical for all 4 muscles in 2 subjects, 3 of the 4 muscles in 7 subjects, and 2 of the 4 muscles in 2 subjects (Table 2). There were no consistent intra-subject patterns for the PWC_{FT} values that distinguished the VL, VM, BF, and ST muscles (Table 2). Furthermore, there were significant (p < 0.05) 0-order correlations (r = 0.60-0.88) among the running velocities associated with the VL PWC_{FT}, ST PWC_{FT}, and RCP, except for the VM *vs*. RCP (r = 0.52) (Table 3).

Table 1

Physical characteristics, running velocities, and metabolic parameters of the subjects (n = 11).

$Mean \pm SD$	Range
21.7 ± 1.8	19.0-25.0
73.9 ± 9.8	56.5-86.9
176.8 ± 7.7	158.8-185.4
45.2 ± 39.9	6.4-160.9
14.4 ± 2.0	10.5-16.9
14.3 ± 1.9	10.5-16.9
13.8 ± 1.8	10.5-16.9
14.7 ± 2.3	10.5-18.5
14.5 ± 1.7	12.4-17.5
4.08 ± 0.77	2.68 - 4.98
4.78 ± 0.84	3.11-5.53
64.4 ± 9.4	51.1-78.1
	$\begin{array}{c} \mbox{Mean} \pm \mbox{SD} \\ 21.7 \pm 1.8 \\ 73.9 \pm 9.8 \\ 176.8 \pm 7.7 \\ 45.2 \pm 39.9 \\ 14.4 \pm 2.0 \\ 14.3 \pm 1.9 \\ 13.8 \pm 1.8 \\ 14.7 \pm 2.3 \\ 14.5 \pm 1.7 \\ 4.08 \pm 0.77 \\ 4.78 \pm 0.84 \\ 64.4 \pm 9.4 \\ \end{array}$

Notes: No significant differences (p > 0.05) were found among the running velocities associated with the PWC_{FT} values for the vastus lateralis, vastus medialis, biceps femoris, or semitendinosus muscles as well as the RCP.

Abbreviations: BF = biceps femoris; PWC_{FT} = physical working capacity at the fatigue threshold; RCP = respiratory compensation point; ST = semitendinosus; VL = vastus lateralis; VM = vastus medialis; VO_{2peak} = peak oxygen uptake.

Table 2 Individual PWC_{FT} values (km/h) for each muscle and subject.

Subject code	VL	VM	BF	ST
1	16.9	16.9	16.9	16.9
2	15.3	15.3	13.7	15.3
3	12.1	10.5	10.5	10.5
4	15.3	15.3	13.7	15.3
5	15.3	12.1	13.7	13.7
6	15.3	15.3	13.7	15.3
7	10.5	13.7	13.7	13.7
8	13.7	13.7	13.7	13.7
9	13.7	13.7	12.1	12.1
10	16.9	16.9	16.9	18.5
11	13.7	13.7	13.7	16.9

Abbreviations: BF = biceps femoris; $PWC_{FT} = physical$ working capacity at the fatigue threshold; ST = semitendinosus; VL = vastus lateralis; VM = vastus medialis.

Table 3

Correlations among running velocities associated with the $\rm PWC_{FT}$ of each muscle and respiratory compensation point.

	RCP	VL	VM	BF	ST
RCP	_				
VL	0.60*	_			
VM	0.52	0.70*	_		
BF	0.80*	0.69*	0.85*	_	
ST	0.85*	0.68*	0.84*	0.88*	_

* *p* < 0.05.

Abbreviations: BF = biceps femoris; $PWC_{FT} = physical$ working capacity at the fatigue threshold; RCP = respiratory compensation point; ST = semitendinosus; VL = vastus lateralis; VM = vastus medialis.

4. Discussion

One of the main findings of the present study was that the PWC_{FT} model that has been used to estimate neuromuscular fatigue in the VL¹⁷ during incremental treadmill running was also applicable to other muscles of the quadriceps femoris (VM) and hamstring (BF, ST) groups. That is, the PWC_{FT} method of deVries et al.¹ was able to identify fatiguing from non-fatiguing running velocities during the incremental treadmill test by statistically examining the slope coefficient of the EMG amplitude vs. running velocity relationship of each stage for all muscles. Specifically, the EMG amplitude values at the velocities associated with fatigue increased across time for the VL (r = 0.74 - 0.99), VM (r = 0.78 - 0.99), BF (r = 0.76 - 0.98), and ST (r=0.77-0.99) for all subjects, whereas the nonfatiguing running velocities resulted in non-significant relationships. These findings illustrated that the PWC_{FT} test is a viable tool for estimating the running velocities associated with neuromuscular fatigue in individual muscles of the thigh. Previous studies^{20,23,31} using other methods have also identified neuromuscular fatigue in various lower-limb muscles based on increases in EMG amplitude during running. For example, Hanon et al.^{20,23} examined the difference in EMG amplitude values associated with 5-10 running bursts of activation at 0:45 (min:s) and 3:40 (min:s) of each 4-min stage for the VL, BF, gluteus maximus, RF, tibialis anterior, and

gastrocnemius muscles during discontinuous, incremental treadmill running to exhaustion. In these studies,^{20,23} running velocities were defined as "fatiguing" if they exhibited significant increases in EMG amplitude at the end compared to the beginning of a stage. One of the major advantages of the PWC_{FT} model^{1,17} used in the present study, however, is the evaluation of change in EMG amplitude across the entire stage, compared to only 5–10 running bursts of activation at 2 different time points. Thus, the use of the PWC_{FT} model^{1,17} may provide greater insight into the evolution of fatigue during incremental treadmill running and may be less susceptible to outliers that exist among EMG activation bursts.

The findings of the present investigation also indicated that there were no significant mean differences in running velocities associated with the VL PWC_{FT} (14.4 \pm 2.0 km/h), VM PWC_{FT} (14.3 ± 1.9 km/h), BF PWC_{FT} (13.8 ± 1.8 km/h), and ST PWC_{FT} (14.7 \pm 2.3 km/h) (Table 1). In addition, there were significant inter-correlations for the PWC_{FT} values that existed among all muscles (r=0.68-0.88) (Table 3). Therefore, the identification of neuromuscular fatigue for the VL, VM, BF, and ST was associated with the same running velocity and was consistent among all muscles. These findings were similar with those of Housh et al.,³ which indicated that the PWC_{FT} occurred at the same power output for the superficial muscles of the quadriceps during incremental cycle ergometry. Using the PWC_{FT} model and 30-W incremental stages, the authors³ reported: (1) no significant differences in the power output associated with neuromuscular fatigue for the VL (226 \pm 58 W), VM (223 \pm 58 W), and RF (203 \pm 54 W), and (2) significant inter-correlations (r=0.78-0.92) among the muscles. In conjunction, the findings of the present study and those of Housh et al.³ suggested that the PWC_{FT} serves as a reliable tool to identify neuromuscular fatigue during incremental treadmill running and cycle ergometry. It is important to note, however, that the running velocities associated with the PWC_{FT} in the current investigation were identical for: (1) all 4 muscles in 2 subjects, (2) 3 muscles for 7 subjects, and (3) 2 muscles for 2 subjects (Table 2). As proposed by Housh et al.,³ it is possible that these intra-subject differences in the PWC_{FT} among muscles may be due to variations in fiber-type distribution, training status or protocols, and biomechanical differences. Thus, the current findings suggested that an examination into intra-subject variability in the PWC_{FT} can be used to identify muscle imbalances among the quadriceps and hamstring groups during running as well as to determine individual training strategies for athletes. For example, the potential uses for PWC_{FT} identification in various muscles of interest include: (1) to determine the effectiveness of training programs through pre- and post-assessments, (2) to prescribe exercise training intensities (i.e., running velocities) based on %PWC_{FT}, (3) to assess the impact of running-form adjustments on fatigue-related aspects of muscle activation, and (4) to assess the rehabilitative progress in recovering from injury. It is also possible that the PWC_{FT} protocol could be modified to identify muscle fatigue across differing velocities and grades, depending on performance requirements. In particular, deVries et al.¹ suggested that the PWC_{FT} treadmill test could be customized for the elderly by using slower velocities to assess fatigue-related aspects of neuromuscular function during walking. Therefore, the PWC_{FT} treadmill test has practical applications that can be potentially useful in both athletic and special populations.

The PWC_{FT} values for each muscle (VL = 14.4 ± 2.0 km/h, $VM = 14.3 \pm 1.9 \text{ km/h}$, $BF = 13.8 \pm 1.8 \text{ km/h}$, and ST = 14.7 \pm 2.3 km/h) also occurred at the same running velocity as the RCP (14.5 \pm 1.7 km/h) (Table 1). In addition, there were significant inter-correlations among the PWC_{FT} values for each muscle vs. the RCP (r = 0.60 - 0.88), except the VM (r = 0.52) (Table 3). As described previously,¹⁷ the relationships among neuromuscular (PWCFT) and ventilatory-based thresholds (ventilatory threshold and RCP) provide information related to the practical applications and validity of the PWC_{FT} treadmill test. That is, the running velocity associated with neuromuscular fatigue in muscles of the thigh, like the RCP, demarcates the border between the heavy and severe domains of exercise intensity and, thus, represents the maximal running velocity that can be maintained for an extended period of time with VO₂ and lactate still reaching a steady state.¹⁸ In particular, it has been established that continuous exercise above the heavyintensity domain results in VO2 and lactate values that do not stabilize, and VO₂ reaches its maximum.¹⁸ These findings offer physiological validation that the PWC_{FT} is an accurate estimate of the highest non-fatiguing running velocity. Practical validation of the PWC_{FT} model during treadmill exercise through constant runs to exhaustion at, below, and above the estimated PWC_{FT}, however, has not been examined.

5. Conclusions

In summary, the findings of the present investigation illustrated that the PWC_{FT} that has previously been used to identify the onset of neuromuscular fatigue in the VL is also applicable to other muscles of the thigh (i.e., VM, BF, and ST) during incremental treadmill running. The non-significant mean differences and significant correlations among the running velocities of the VL PWCFT, VM PWCFT, BF PWC_{FT}, and ST PWC_{FT} suggested that this neuromuscular fatigue threshold results in consistent estimates across muscles of the quadriceps femoris and hamstring groups and may be used to identify muscle imbalances as well as to determine individual training strategies for athletes. In addition, the development of fatigue in these muscles as indicated by the PWC_{FT} coincided with the running velocity associated with the RCP. These findings provide physiological validation for the PWC_{FT} model during incremental treadmill running.

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Authors' Contributions

CLC conceived the study design, data collection, and analysis and drafted the manuscript; AJK assisted with study design and carried out the electromyographic analyses; TAV, ECH, and EAE were involved with study design and coordination, data collection and analysis. All authors have read and approved the final manuscript, and agreed with the order of presentation of the authors.

Competing Interest

The authors declare that they have no competing interests.

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