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Original empirical article

THE REPRODUCIBILITY OF THE VENTILATORY ANAEROBIC THRESHOLD DETERMINATION METHODS AMONG TWO MAXIMAL TREADMILL EXERCISE PROTOCOLS IN ELITE ORIENTEERS

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Abstract. *The characteristics of the workload in orienteering presumes a specific dynamic of the functional parameters when applying testing protocols with increase of speed and gradient of the treadmill, compared to the sole increase of the running speed. There is a limited number of publications analyzing the influence of the modifications in the speed and gradient (without changes in the stage length) on the ventilatory threshold (VT) in orienteers. We compared the reproducibility of the most widely used methods for VT determination using protocols with an increase of the running speed alone and with modifications of the speed and the gradient. The results demonstrated that the VT_{EqO2} and $VT_{V\text{-}slope}$ – methods were more reproducible for the determination of VT than the $VT_{RER=1.0}$. Probably, the differing characteristics of the motor activity and direct dependence of the determined $VT_{RER=1.0}$ on RER = 1.0 – values have an impact on the lower reproducibility of the $VT_{RER=1.0}$ – method.*

Key words: *exercise testing protocol, orienteering, ventilatory threshold*

INTRODUCTION

The maximal oxygen consumption (VO_{2max}) is a key parameter for maximal functional capacity (MFC) assessment of athletes (Ostojić, 2000). However, an extremely high VO_{2max} is not the only determinant of athletic performance and success (Mészáros et al., 1998). Furthermore, high level athletes very often show a smaller increase in VO_{2max} than those who are beginners (Kostić & Zagorc, 2005). Researchers have also found wide variations in improvement of VO_{2max} with specific aerobic training. Bouchard et al.

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(1999) established that the increase in VO_{2max} under a particular training program is influenced by genetic factors and each individual responds differently to the same exercise stimulus. On the basis of these studies, it seems that the anaerobic threshold (AT) is the main scientific criterion, which corresponds acceptably to the race performance (Robinson et al., 1991) and can be used for prescribing intensity during a competition (Billat, 1996). The invasive character of the procedures for lactate AT assessment motivates many researchers to use the respiratory gas exchange and/or the heart rate deflection point for non-invasive AT determination (Ignjatović et al., 2008). The contemporary methods for ventilatory threshold (VT) assessment include analysis of the respiratory exchange ratio (RER) at the level of RER=1.0 ($VT_{RER=1.0}$), the breakpoint of the nonlinear increase of the ventilatory equivalent for oxygen (VT_{EqO2}) and the VCO_2 versus VO_2 curve ($VT_{V\text{-}slope}$) (Solberg et al., 2005; Wasserman et al., 2005).

The motor characteristics of the workload in orienteering (which includes cross-country running) presumes specific changes of the assessed MFC when applying the incremental exercise testing protocol (IETP), which includes an increase in the speed and the gradient of the treadmill, compared with the sole increase of the running speed (Smekal et al., 2003). The influence of the test protocol on the estimation of MFC and VT has been addressed in a number of studies. Researchers reported that there was no effect on the VT_{EqO2} – method in trained subjects following manipulation of the stage length in IETP (Amann et al., 2004). According to Weston et al. (2002), an incremental ramp test comparing "fast" or "slow" increments resulted in a significant difference in VO_{2max} and unchanged VT, which was defined by the VT_{EqO2} – method. On the other hand, in another study on trained athletes, no VO_{2max} changes were observed when the stage duration during the IETP was influenced (Bentley et al., 2007). Despite the cited studies, there are limited data analyzing the influence of the modifications in the speed and gradient of the treadmill band (without changes in the stage length) on functional parameters. Sloniger et al., (1997) reported that the muscle volume activated is greater for uphill maximal treadmill running than for horizontal running. He concluded that the increase in active muscle volume is associated with an increase in peak oxygen deficit which may be due to decreased running efficiency and/or more anaerobic energy release. Based on the above mentioned, we agreed that it may be of interest to investigate the application of the methods for VT assessment and the difference in MFC in orienteers, using two different exercise testing protocols, which did not include changes in stage duration.

The main purpose of the study was to test the reproducibility of the described methods for VT determination in elite orienteers using two IETPs: Protocol I (PI) – with incremental increase of the running speed alone and protocol II (PII) – with modifications of the speed and the gradient. In addition, we compared the difference in some key maximal functional capacity parameters resulting from these protocols.

METHOD

Participants

Ten high-level national male orienteers (age – 24.46 (± 3.72) years; height – 180.91 (± 6.75) cm; weight – 69.25 (± 5.35) kg; BMI – 21.16 (± 2.04)) volunteered to take part in the study. All of the subjects were healthy, injury-free, non-smokers and were not taking any medications. They gave written, informed consent before participation and were fully familiar with the laboratory testing procedure. All of the athletes were instructed to avoid strenuous exercise 2 days prior to testing. Each subject took part in the two tests at the same time of day (± 1.0 h) to minimize the effects of diurnal biological variation on the results. There was a 48-hour of recovery period between the applications of the two IETPs. Before the first exercise test, blood samples were taken from an arm vein and analyzed (RAL, Spain) for the following parameters: hemoglobin (HB); red blood cell count (RBC); hematocrit (HTC) and mean corpuscular hemoglobin (MCH). For all of the subjects the registered hematological characteristics were within the normal reference value range, so we concluded that the arterialized blood had an effective oxygen-carrying capacity; these parameters were not expected to limit the MFC (HB = 146.14 (± 9.89) (g l^{-1}); RBC = 5.31 (± 0.24) (10E 12 l^{-1}); HTC = 47.76 (± 2.89) (%); MCH = 27.44 (± 0.98) (g l^{-1})).

Experimental design

After a 5-minute warm-up walking at low speed, which allowed the subjects to adapt to the exercise, they did a maximal exercise treadmill test (Quasar – 4.0, Med; HP Cosmos, Germany). In the first part of the experiment, each subject performed PI as a maximal test; 48 hours later, PII was applied. PI started at an at initial speed of 6 km h^{-1} with incremental increase of the running speed alone with 1.2 km h^{-1} every 90 sec. until objective exhaustion was reached (Tzvetkov et al., 2008). In PII the workload was realized through increase of both the speed and gradient: the stage length was again 90 sec., the initial speed was 5 km h^{-1} and the initial gradient – 0 %; only speed was increased by 2 km h^{-1} at every stage up to 7.5 min, afterward – only the gradient was increased by 2% at a constant speed of 13 km h^{-1} until exhaustion was achieved. The following criteria were used to verify maximal exhaustion: a plateau in VO_2 dynamics; maximal respiratory exchange ratio (RER_{max}) > 1.10; real maximal heart rate close to the HR_{max} predicted based on the subject's age ($HR_{max(real)} = HR_{max(theory)} \pm 5$ beats min^{-1})); physical inability to continue the test (Wasserman et al., 2005). We registered the following parameters: maximal duration of the test (T_{max}); maximal work capacity (WC_{max}); VO_{2max}; VO_{2max} kg^{-1}, maximal pulmonary ventilation (VE_{max}); maximal tidal volume (Vt_{max}); maximal breathing frequency (BF_{max}); maximal VE/VCO_2 ratio (VE/VCO_{2max}); HR_{max}; RER_{max}. The values of VO_2 corresponding to the determined VTs (VO_{2VT}) were also analyzed.

Gas exchange measurements and Ventilatory threshold assessment

During the exercise test, pulmonary gas-exchange was registered continuously with a metabolic system (Erich Jaeger GmBH & Co Wuerzburg, Germany), using the breath-by-breath method. Subjects breathed through a low-dead space mouthpiece (110 ml) and turbine assembly. The calibration of the turbine flow-meter of the volume sensor was performed with a standard 3l syringe. Before each test, the gas-analyzer was calibrated using ambient air (20.9 % O_2 and 0.04 % CO_2) and calibration gas (0.00 % O_2 and 4.83 % CO_2). Heart rate was recorded by ECG throughout the test. VTs were determined using three different approaches. In the $VT_{RER=1.0}$ – method we accepted that VT_{RER} corresponds to RER = 1.0 ($VT_{RER=1.0}$) (Solberg et al., 2005). VT_{EqO2} and $VT_{V\text{-}slope}$ (Wasserman et al., 2005) were defined through a linear regression analysis using a mathematic software ("MATLAB" – 6.1) (Diniz & Brochi, 2005). Based on the above criteria, two experienced researchers assessed the VTs. When they could not determine a threshold, the opinion of a third researcher was sought.

Procedure

A paired sample T – test was performed for analysis of the maximal parameters and VO_{2VT} – s values, registered during the PI and PII protocols. The obtained values for VO_2 at VT_{EqO2}, $VT_{V\text{-slope}}$ and $VT_{RER=1.0}$ were statistically processed with a pair-wise comparison analysis and Bland – Altman method (Stuart et al., 1999). It was preceded by a distribution normality check of the samples with the standard Kolmogorov – Smirnov and Shapiro – Wilk tests. The reproducibility of the methods for VT determination was assessed by 95 % confidence intervals of the differences between the respective VTs values during the two tests. For the same purpose, the coefficients of variations (CV) of the VTs values, determined with the three methods for each protocol were also calculated. Pearson's correlation coefficient and Student's T – criterion were used for evaluation of the statistical results with a standard level of significance ($p < 0.05$) /SPSS 14/.

RESULTS

High values for MFC were registered during both studied IETPs (Table 1). The conducted paired sample T – test between the maximal functional parameters registered during PI and PII revealed that there is a significant difference in the maximal duration of the tests. The average total time for PI was longer than the performance for PII (T_{max} (PI) – T_{max} (PII) = 0.89 min (p* < 0.05)). The peak values (PI vs. PII) of most maximal respiratory parameters were not significantly different. Maximal Vt was statistically higher in PII compared to PI (Vt_{max} (PI) - Vt_{max} (PII) = - 0.88 l (p* < 0.05)). A higher statistically significant value was also found in RER_{max} (PII) compared to the RER_{max} (PI) (RER_{max} (PI) - RER_{max} (PII) = - 0.05 (p* < 0.05)).

Table 1. Maximal functional parameters during the two studied protocols (PI, PII) ($p < 0.05$; * - statistically significant differences)

Parameters	PI		PII		$\overline{X}_1 - \overline{X}_2$	t	p
	$\overline{X}_1$ ±	Sx_1	$\overline{X}_2$ ±	Sx_2			
T_{max} (min)	16.85±	(1.23)	15.96±	(1.13)	0.89	3.32*	< 0.05
WC_{max} (MET)	18.89±	(1.14)	19.36±	(1.83)	−0.47	0.96	0.389
VO_{2max} (ml min^{-1})	4560.50±	(499.23)	4667.90±	(506.19)	−107.4	0.81	0.440
VO_{2max} kg^{-1} (ml kg^{-1}min^{-1})	65.86±	(3.94)	66.70±	(5.56)	−0.84	0.97	0.356
VE_{max} (l min^{-1})	159.7±	(21.52)	169.3±	(19.36)	−9.60	2.11	0.064
Vt_{max} (l)	2.92±	(0.49)	3.11±	(0.47)	−0.88	4.21*	< 0.05
BF_{max} (min^{-1})	58.0±	(6.31)	55.0±	(9.98)	3.00	1.02	0.333
VE/VCO_{2max}	29.79±	(3.01)	31.06±	(3.07)	−1.27	1.75	0.115
HR_{max} (beat min^{-1})	195±	(7.64)	194±	(8.69)	1.00	1.23	0.250
RER_{max}	1.11±	(0.04)	1.16±	(0.05)	−0.05	3.80*	< 0.05

For all of the subjects participating in the study, VTs were determined; none were dropped from the statistical analysis. A paired T – test between the obtained mean VO_{2VT} - s values calculated with the three methods of assessment during PI and PII ($VO_{2VTEqO2}$ (PI) = 3757.64 ml min^{-1}; $VO_{2VTEqO2}$ (PII) = 3732.36 ml min^{-1}; $VO_{2VTV\text{-slope}}$ (PI) = 3446.41 ml min^{-1}; $VO_{2VTV\text{-slope}}$ (PII) = 3398.17 ml min^{-1}; $VO_{2VTRER=1.0}$ (PI) = 4160.92 ml min^{-1}; $VO_{2VTRER=1.0}$ (PII) = 3955.14 ml min^{-1}) indicated no significant difference (p_{VTEqO2} = 0.76;

$p_{VTV\text{-}slope} = 0.23$; $p_{VTRER=1.0} = 0.07$). The multiple pair-wise comparison analysis for VO_{2VT} - s values revealed a strong correlation between VT_{EqO2} (PI) – VT_{EqO2} (PII) and $VT_{V\text{-}slope}$ (PI) – $VT_{V\text{-}slope}$ (PII) pairs ($r = 0.891$, $t = 0.26$, $p = 0.80$; $r = 0.837$, $t = 0.63$, $p = 0.54$). In the $VT_{RER=1.0}$ (PI) – $VT_{RER=1.0}$ (PII) pair a significantly lower correlation coefficient was observed ($r = 0.474$, $t = 1.98$, $p = 0.36$) with higher standard deviation ($S_{(VTRER=1.0\ (PI)\ -\ VTRER=1.0\ (PII))} = 706.26$ ml min^{-1}; $S_{(VTEqO2\ (PI)\ -\ VTEqO2\ (PII))} = 117.59$ ml min^{-1}; $S_{(VTV\text{-}slope\ (PI)\ -\ VTV\text{-}slope\ (PII))} = 195.96$ ml min^{-1}). There was a tendency for higher values of $VT_{RER=1.0}$ than VT_{EqO2} and $VT_{V\text{-}slope}$ in both IETPs (Figure 1 – a), b), c)).

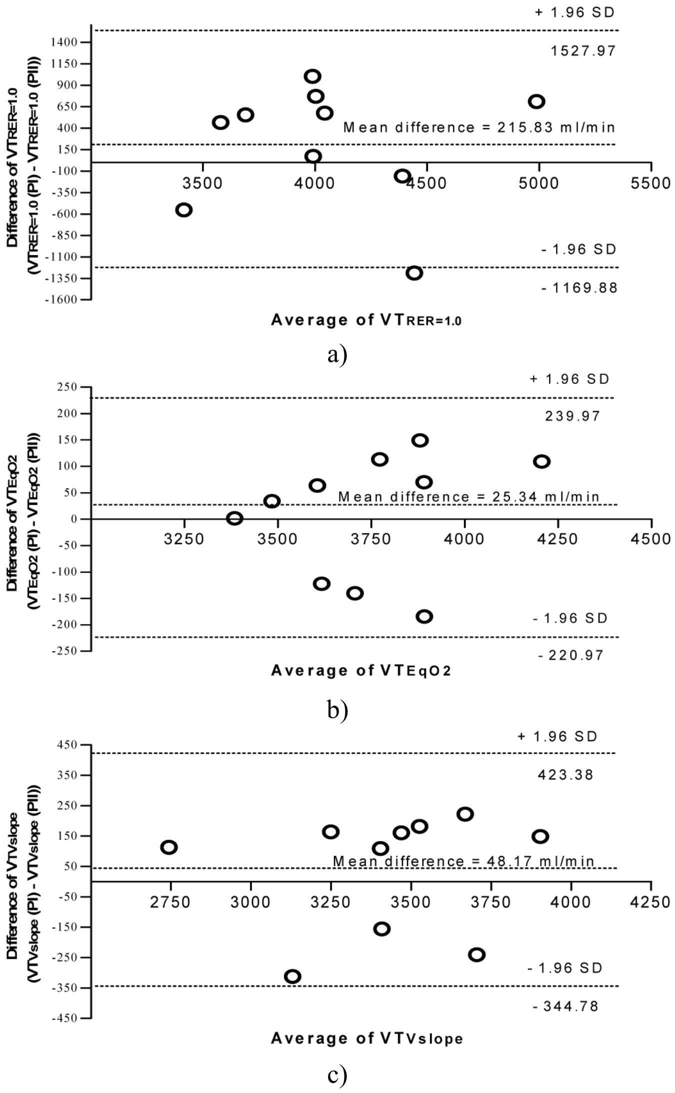

Fig. 1. Bland - Altman plots showing differences between ventilatory thresholds detected by the $VT_{RER=1.0}$, VT_{EqO2} and $VT_{V\text{-}slope}$ – methods during both testing protocols (a) $VT_{RER=1.0}$ – values; b) VT_{EqO2} – values and c) $VT_{V\text{-}slope}$ – values). Values of VO_2 in ml min^{-1}.

The higher variability of the $VT_{RER=1.0}$ (PI) – $VT_{RER=1.0}$ (PII) values is demonstrated by the higher 95% confidence interval for the limit of agreement (- 1169.88 ÷ 1527.97 ml min^{-1}) compared to the confidence intervals for VT_{EqO2} (PI) – VT_{EqO2} (PII) (- 220.97 ÷ 239.97 ml min^{-1}) and $VT_{V\text{-}slope}$ (PI) – $VT_{V\text{-}slope}$ (PII) (- 344.78 ÷ 423.38 ml min^{-1}) values. This higher variability differences in the $VT_{RER=1.0}$ – method was further evident by the analyses of the coefficient of variation of the VO_{2VT} - s values for both IETPs. There were significantly greater CV of $VO_{2VTRER=1.0}$ values (CV ($VT_{RER=1.0}$ (PI) = 12.2 %; CV ($VT_{RER=1.0}$ (PII) = 17.8 %) among the two protocols than CV of $VO_{2VTEqO2}$ (CV (VT_{EqO2} (PI) = 6.2 %; CV (VT_{EqO2} (PII) = 7.9 %) and CV of $VO_{2VTV\text{-}slope}$ values (CV ($VT_{V\text{-}slope}$ (PI) = 9.6 %; CV ($VT_{V\text{-}slope}$ (PII) = 11.8 %). These results suggested that VT assessment by the $VT_{RER=1.0}$ method in PI and PII has a lower reproducibility than the VT_{EqO2} and $VT_{V\text{-}slope}$ – methods.

DISCUSSION

A lot of studies have been conducted to examine the validity of measuring MFC and AT using incremental protocols with different stage duration. Many investigations considered that using IETPs with stages ≥ 3 minutes in duration may induce more valid lactate AT (Bentley et al., 2007). This is mainly due to the delay in the changes in lactate blood concentration after an increase in workload at each stage. However, using a test with longer stages may compromise the VO_{2max} and WC_{max} measurements. Furthermore, the respiratory gas exchange has enhanced sensitivity for detection of the VT while using shorter stage duration (Wasserman et al., 2005). Thus, it is not necessary to have longer stage duration during VT tests. Based on these findings, in the present study we considered it appropriate to apply a compromise stage duration of 90 sec. for both IETPs without changes in stage length. This shorter stage duration guarantees acceptable reliability of both MFC measurement and VTs assessment. The values of the maximal functional parameters of the subjects participating in the study obtained during PI and PII were comparable to data published in scientific literature on elite orienteers (Creagh & Reilly, 1997). Thus, their fitness level as well as their successful performance in international competitions allowed us to qualify them as high-level athletes with high MFC.

The PII protocol combined both horizontal and uphill running, which corresponds better with the motor characteristics of the workload in orienteering. Researchers have suggested that the IETP for highly trained athletes should involve a speed/gradient combination that is similar to the subjects' regular training (Bosquet et al., 2002). Creagh & Reilly (1997) reported that greater energy and oxygen cost of the running on rough terrain (horizontal and uphill running) is expected compared with horizontal running. In this context we assumed that PII would yield higher VO_{2max} in testing orienteers. Our results confirmed this expectation, although the difference between the obtained values was very small, statistically insignificant (Table 1). Since it is acknowledged that the biomechanical differences are associated with changes in energy demand, we accepted that these findings are in connection with the differing characteristics of motor activity during the PI vs. PII protocol. From this point of view, it is possible that the lack of a significant difference between the VO_{2max} (PI) and VO_{2max} (PII) was due to the greater involvement of anaerobic metabolism during the PII. This position is consistent with

Sloniger et al., (1997), who found a more active participation of the anaerobic energy supply during maximal uphill rather than horizontal running. According to him, the activated muscle volume for uphill vs. horizontal running is higher and he concluded that this finding was associated with more anaerobic energy release. In our study, this hypothesis was supported by slightly higher VE_{max} (PII), lower maximal respiratory efficiency (VE/VCO_{2max}) during PII and particularly, significantly higher values of RER_{max} (PII) (RER_{max} (PI) – RER_{max} (PII) = - 0.05; $p^* < 0.05$), which are indirect indexes for greater anaerobic metabolism at the end of PII (Wilmore & Costill, 2006). Generally, it should be stressed that there were no significant evidences for obtaining higher values of the maximal functional parameters, when applying PII. Therefore, we can summarize that both IETPs are interchangeable for testing the MFC. On the other hand, the testing with PII was associated with significantly lower maximal duration of the test (T_{max} (PI) – T_{max} (PII) = 0.89 min; $p^* < 0.05$). This result suggests that using PII vs. PI in practice could be more time–efficient alternative in testing MFC.

The validity of the methods for VT assessment is essential when assessing changes in functional condition. The paired T – test analysis between the produced VO_{2VT} - s values (PI vs. PII) for all tree methods of VT assessment demonstrated a lack of significant difference. According to the Bland – Altman method, the T – test of significance may show that the examined methods are significantly related, but this test is irrelevant to the question of agreement and reproducibility. For this reason, the fact that the pairs $VO_{2VTEqO2}$ (PI) – $VO_{2VTEqO2}$ (PII); $VO_{2VTV\text{-}slope}$ (PI) – $VO_{2VTV\text{-}slope}$ (PII) and $VO_{2VTRER=1.0}$ (PI) –$VO_{2VTRER=1.0}$ (PII) were related, did not allow us to make a reasoned conclusion on the reproducibility of the VT_{EqO2}, $VT_{V\text{-}slope}$ and $VT_{RER=1.0}$ – methods. Based on the abovementioned study, we conducted the Bland – Altman analysis, which produced a very high, 95%, confidence interval for the limit of agreement for $VT_{RER=1.0}$ vs. VT_{EqO2} and $VT_{V\text{-}slope}$ values (Fig.1: a, b, c). The 95% confidence interval for $VT_{RER=1.0}$ (PI) – $VT_{RER=1.0}$ (PII) differences can be found in the broader range vs. VT_{EqO2} (PI) – VT_{EqO2} (PII)) and $VT_{V\text{-}slope}$ (PI) – $VT_{V\text{-}slope}$ (PII) values, which could be a problem when applying this method for practical purposes. The obtained lower correlation coefficient in the $VT_{RER=1.0}$ (PI) – $VT_{RER=1.0}$ (PII) pair (r = 0.474) than in the VT_{EqO2} (PI) – VT_{EqO2} (PII) (r = 0.891) and $VT_{V\text{-}slope}$ (PI) – $VT_{V\text{-}slope}$ (PII) pairs (r = 0.837) again confirmed the very large variations in the determined $VT_{RER=1.0}$ values when applying the two IETPs. This lower reproducibility of the $VT_{RER=1.0}$ – method was also confirmed by the significantly greater CV ($VT_{RER=1.0}$) than CV (VT_{EqO2}) and CV ($VT_{V\text{-}slope}$).

There are probably multiple factors influencing the observed problem with the application of $VT_{RER=1.0}$ during PI and PII. There are contradictory opinions on the use of this method in scientific literature. The study of Beaver et al. (1986) supported the opinion on the questionable efficacy of the VT_{RER} method for VT determination. Furthermore, despite the widespread application of the $VT_{RER=1.0}$ – method, some researches assume that VT corresponds to the level of RER = 0.95 (Amann et al. 2004). Another factor which can determine the lower reproducibility of $VT_{RER=1.0}$ is the possibly higher involvement of anaerobic metabolism at the end of the PII vs. PI protocol. This suggestion is in concordance with the obtained higher $VO_{2VTRER=1.0}$ (PII) values (compared to $VO_{2VTEqO2}$ (PII) and $VO_{2VTV\text{-}slope}$ (PII) values), which corresponded to a higher gradient of the workload (higher degree of acidosis) and considerably greater variations of the $VT_{RER=1.0}$ (PII) values related to the more pronounced hyperventilation. This hypothesis is consistent with Meyer et al. (2004), who reported that exercise-induced metabolic acidosis stimulated hyperventilation during an intensive workload in the IETP. Moreover, according to Deruelle et al. (2006), highly trained athletes demonstrated varying respiratory responses to the induced acidosis. He accepted that the experience of elite athletes ensured advantage in the voluntary control of the respiratory function and due to this, they were able to modify their breathing strategy in accordance to the design of the used protocol. It should also be noted that RER values might be affected by a number of environmental factors (the size of glycogen stores, the amount of adipose tissue, the fat/carbohydrate mix in the regular diet and genetic factors) (Kempen et al., 1998). In this context, we can conclude that the application of $VT_{RER=1.0}$ in different IETPs is associated with a risk of obtaining results with very low reproducibility, compromising their practical value. This problem requires further detailed investigations on the criteria for VT_{RER} assessment, depending on the design of the exercise testing protocol.

CONCLUSION

In conclusion, we can summarize that the differences in the main maximal functional parameters obtained during PII compared to PI were insignificant and the examined IETPs are relatively interchangeable for testing MFC. $VT_{RER=1.0}$ compared to VT_{EqO2} and $VT_{V\text{-}slope}$ – methods tended to show a less reliable performance, with a lower correlation coefficient between the respective values and higher, 95%, confidence interval for the limit of agreement. Possibly, the differing characteristic of the motor activity during PII and PI and the multiple factors affecting RER dynamics during exercise may compromise the practical application of $VT_{RER=1.0}$.

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MOĆ REPRODUKCIJE ANAEROBIČNOG PRAGA DISANJA METODA DETERMINACIJE IZMEĐU DVA MAKSIMALNA PROTOKOLA TREDMIL VEŽBANJA KOD ELITNIH UČESNIKA U ORIJENTIRINGU

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Karakteristike opterećenja u orijentiringu predviđaju specifičnu dinamiku funkcionalnih parametara kada se primenjuju protokoli testiranja sa povećanjem brzine i uspona na tredmilu u poređenju sa povećanjem samo brzine trčanja. Postoji ograničavajući broj publikacija koja analiziraju uticaj modifikacija u brzini i usponu, (bez promena u dužini etape) na prag disanja (VT) u orijentiringu. Mi smo upoređivali ponavljanje najčešće korišćenih metoda za određivanje praga disanja korišćenjem protokola sa povećanjem samo brzine trčanja i sa modifikacijom brzine i uspona. Rezultati su pokazali da su VT_{EqO2} i $VT_{V\text{-}Slope}$ - metodi bili više reproduktivni za određivanje praga disanja nego za $VT_{RER=1.0}$. Verovatno, različite karakteristike motorne aktivnosti i direktne zavisnosti određenih $VT_{RER=1.0}$ na RER =1.0- vrednosti imaju uticaj na manje ponavljanje $VT_{RER=1.0}$- metoda.

Ključne reči: *protokoli tredmil vežbanja, orijentiring, prag disanja*