Original article

Non-exhaustive double effort test is reliable and estimates the first ventilatory threshold intensity in running exercise

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Abstract

Purpose: The present study aimed to investigate the reliability of the non-exhaustive double effort (NEDE) test in running exercise and its associations with the ventilatory thresholds (VT1 and VT2) and the maximal lactate steady state (MLSS).

Methods: Ten healthy male adults (age: 23 ± 4 years, height: 176.6 ± 6.4 cm, body mass: 76.6 ± 10.7 kg) performed 4 procedures: (1) a ramp test for VT1 and VT2 determinations measured by ratio of expired ventilation to O2 uptake (VE/VO2) and expired ventilation to CO2 output (VE/VCO2) equivalents, respectively; (2) the NEDE test measured by blood lactate concentration (NEDELAC) and heart rate responses (NEDEHR); (3) a retest of NEDE for reliability analysis; and (4) continuous efforts to determine the MLSS intensity. The NEDE test consisted of 4 sessions at different running intensities. Each session was characterized by double efforts at the same running velocity (E1 and E2, 180 s), separated by a passive recovery period (90 s rest). LAC and HR values after E1 and E2 (in 4 sessions) were used to estimate the intensity equivalent to “null delta” by linear fit. This parameter represents, theoretically, the intensity equivalent to maximal aerobic capacity.

Results: The intraclass correlation coefficient indicated significant reliability for NEDELAC (0.93) and NEDEHR (0.79) (both p < 0.05). There were significant correlations, no differences, and strong agreement with the intensities predicted by NEDELAC (10.1 ± 1.9 km/h) and NEDEHR (9.8 ± 2.0 km/h) to VT1 (10.2 ± 1.1 km/h). In addition, despite significantly lower MLSS intensity (12.2 ± 1.2 km/h), NEDELAC and NEDEHR intensities were highly correlated with this parameter (0.90 and 0.88, respectively).

Conclusion: The NEDE test applied to running exercise is reliable and estimates the VT1 intensity. Additionally, NEDE intensities were lower but still correlated with VT2 and MLSS.

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Keywords: Aerobic exercise; Anaerobic threshold; Maximal lactate steady state; Non-exhaustive protocol; Training prescription; Ventilatory threshold

1. Introduction

Aerobic exercise (long duration at low-to-moderate intensity) promotes several morphologic and physiological adaptations, such as improved oxidative metabolism, higher fat mobilization, weight loss, and optimization of cardiorespiratory capacities.1 Individual detection of maximal aerobic intensity is the first step for an optimal training prescription and monitoring. In this regard, detection of maximal oxygen consumption (VO2max) and aerobic and anaerobic thresholds are the main determinants of aerobic fitness in athletes,2,3 subjects with impaired health,4,5 and healthy individuals.6,7

Initially, the first studies that identified the metabolic thresholds used gas analyzers during incremental efforts to determine anaerobic threshold (first increase in ratio of expired ventilation to O2 uptake (VE/VO2)) and the respiratory compensation point (first increase in ratio of expired ventilation to CO2 output (VE/VCO2)), currently also known as ventilatory thresholds 1 and 2 (VT1 and VT2).8 Later, another research group determined these phenomena by measuring blood lactate (LAC) concentration and conceptualizing them as aerobic (~2 mmol/L lactate) and anaerobic thresholds (~4 mmol/L lactate).9 Despite being determined by different physiological measures, the metabolic thresholds represent
and can estimate MLSS, at least in a swimming exercise. Recently, our research group conducted an investigation with elite swimmers and confirmed that the NEDE test is reliable for anaerobic threshold determination and aerobic training prescription. Among these efforts is one proposed by Chassain, which is based on non-exhaustive double efforts (NEDE). This procedure is characterized by low cost, short duration, and low-to-moderate intensity of effort.

The NEDE test uses non-exhaustive loads to determine the highest intensity at which the physiological variables (LAC and heart rate (HR)) do not increase beyond the initial transient level after 2 rounds of exercise. In summary, the NEDE test consists of 4 sessions at different intensities. Each session is characterized by double efforts at the same intensity (E1 and E2, 180 s), separated by a passive recovery period (90 s rest). Physiological variables are measured at the ends of E1 and E2 (in each of 4 sessions), and the physiological deltas at each different intensity are used to estimate the “null delta intensity” by linear fit. This parameter represents, theoretically, the intensity equivalent to maximal aerobic capacity (i.e., the highest intensity in which changes of LAC and HR are equal to 0).

In the original study, Chassain did not compare the NEDE test with other methods used to determine the aerobic and anaerobic thresholds. Despite making some assumptions regarding the relation of the NEDE test and a protocol similar to MLSS, it was not tested with the same subjects. Other studies have investigated the relationship between the “null delta intensity” based on the original NEDE test and other aerobic parameters (i.e., critical velocity, lactate threshold, and MLSS). However, despite the use of the double effort test, the protocols had longer time durations than were used in the original study (14 min and 20 min) and only tested the correlation between protocols, not their similarity in mean values.

In an experimental study using swimming and running rats, the “null delta intensity” obtained using the NEDE test showed an association with MLSS. However, in a study involving menopausal women, these results were not confirmed. Recently, our research group conducted an investigation with elite swimmers and confirmed that the NEDE test is reliable and can estimate MLSS, at least in a swimming exercise.

Considering that the intensity predicted by the NEDE test indicates a work rate of physiological equilibrium, we hypothesized that this intensity is related to other aerobic physiological parameters, such as the VTs and the MLSS, in running exercise. Thus, the present study aimed to investigate the reliability of the NEDE test for LAC responses (NEDE_LAC) and HR measures (NEDE_HR) in running exercise. Also, we investigated the association of the null delta intensity obtained in the NEDE test with the VTs (VT1 and VT2) and MLSS in order to determine whether the NEDE test can be an effective alternative method for aerobic evaluation in running exercise.

2. Methods

2.1. Volunteers

Ten healthy young male adults (age: 23 ± 4 years, height: 176.6 ± 6.4 cm, and body mass: 76.6 ± 10.7 kg; mean ± SD) who were moderately active participated in the present study. All volunteers filled out the International Physical Activity Questionnaire and met one of the following 3 scores for the “moderately active” classification: (1) 3 or more days of vigorous-intensity activity lasting at least 20 min per day, (2) 5 or more days of moderate-intensity activity or walking lasting at least 30 min per day, or (3) 5 or more days of any combination of activities (including walking) at moderate or vigorous intensity to achieve a minimum total physical activity level of at least 600 MET-min per week (where MET is metabolic equivalent value). All participants were informed of the risks and benefits of participating in the research and, in accordance with the Declaration of Helsinki and approved by the Institutional Research Ethics Committee of Human Research of University of Campinas (protocol number: 12-03-184), signed an informed consent form prior to the tests.

2.2. Experimental design

During the study, volunteers were instructed to maintain the same nutritional habits, avoid high-intensity exercise, and avoid caffeine or alcohol ingestion for 24 h before each test. All procedures were performed on a motorized treadmill (Super ATL; Inbrasport, Porto Alegre, Brazil) at a laboratory in a controlled environment (22˚C ± 1˚C temperature and 50% ± 2% relative humidity). After anthropometric measurements, the volunteers performed an individualized ramp protocol to determine VT1, VT2, and VO2max. Thereafter the MLSS and NEDE test and retest (NEDE-1 and NEDE-2) were carried out in random order separated by a minimal period of 24 h between each test. All volunteers completed all procedures within 2 weeks.

2.3. Ramp protocol and respiratory parameters

Immediately after a 5 min warm-up at 7 km/h, the individualized ramp protocol was carried out on a treadmill without inclination, at 8.0 km/h initial velocity and then constantly increased at a rate of 0.7 to 1.0 km/h (depending on the predicted VO2max by individuals’ age) as previously suggested. During the whole test, the HR, ventilation, and expired gas responses were continuously measured by an integrated, computerized, breath-by-breath gas analyzer system (K4b2; COSMED, Rome, Italy) that was calibrated before each test according to manufacturer’s instructions. The end of the test was determined by the following criteria: (1) achievement of...
VO2 plateau (an increase less than 1.5 mL/kg/min), (2) attain-ment of the predicted maximal HR (220−age), or (3) voluntary desistence. The data of VO2, VCO2, VE, VE/VO2, and VE/VCO2 were processed with a moving average of 15 breaths, with the breath-average aligned to the center of the time interval (8th breath). This procedure aimed to reduce approximately 90% of variability error inherent to any breath-by-breath gas analyzer system.24

As previously suggested,24 the VO2max was determined as the highest 15-breath average value for VO2 during the ramp protocol and only after the attainment of the first criterion to end the test (achievement of plateau in VO2 kinetics). The VT1 was characterized by the first rise in the ventilatory equivalent VE/VO2 without a concomitant increase in the VE/VCO2.25 The VT2 was determined by the initial increase in the VE/VCO2.2 The HR (in beats per minute, bpm) at VT1, VT2, NEDELAC, NEDEHR, and MLSS were determined by interpolation of a linear regression between HR and the intensity (km/h) during the ramp protocol as previously described.26 A similar procedure was carried out to determine the VO2 (mL/kg/min) relative to VT1, VT2, NEDELAC, and NEDEHR.

2.4. MLSS

To evaluate the MLSS, participants ran for 30 min at the intensity of VT2 (km/h). Blood samples were collected from the ear lobe for blood lactate determination at rest and each 5 min until the end of the test. If a difference higher than 1 mmol/L was found between the blood lactate at the 10th and the 30th min, another test was carried 24 h later at a lower intensity. On the other hand, if a difference smaller than 1 mmol/L was obtained, a higher intensity was applied. The higher intensity in which the LAC does not increase more than 1 mmol/L between the 15th and the 30th min was considered MLSS intensity. Visits ranging from a minimum of 2 days to a maximum of 5 days were necessary to determine MLSS for all subjects. The maximal difference between the MLSS and the intensity at which LAC accumulation surpassed 1 mmol/L was set to 0.5 km/h allowing for a high precision of the MLSS intensity determination. The oxygen consumption at the MLSS intensity. The following describes a single double effort.

2.6. LAC determination

After local asepsis, a 25 μL blood sample was collected from the ear lobe with a previously calibrated and heparinized
capillary tube. The blood was transferred to a 1.5 mL capacity microtube containing 400 µL of trichloroacetic acid (4%) and immediately stored at a temperature between 2°C and 8°C. After centrifugation at 3000 rpm for 3 min, 50 µL of supernatant was homogenized with 250 µL of reagent based on hydrazine hydrate (88%; pH 9.45), ethylenediaminetetraacetic acid (EDTA), glycine, β-nicotinamide adenine dinucleotide (NAD), and lactate dehydrogenase (LDH). Analysis was carried out on a microplate reader (Asys Expert Plus UV; Biochrom, Cambridge, UK) at 340 nm using a spectrophotometric method against a calibration curve.27

2.7. Statistical analyses

All analyses were conducted with a statistical software package (Statistica Version 7.0; StatSoft, Inc., Tulsa, OK, USA), and data are expressed in terms of mean ± SD. After data normality was attested to by the Shapiro-Wilk test, one-way ANOVA was used for mean comparison of the intensities parameters (VT1, VT2, MLSS, NEDELAC, and NEDEHR). When necessary, the differences among variables were indicated by Newmann-Keuls post hoc test. In addition, to attest to the concordance between the NEDE test and the other physiological parameters, the bias and limits of agreement analysis was employed as previously described.28 To assess the reliability of the NEDE test—retest, an analysis was conducted using the intraclass correlation coefficient (ICC; 2,1 fixed model for absolute agreement) and the typical error to determine the coefficient of variation (CV) relative to the mean, as suggested elsewhere.29 The Pearson product moment was used to verify correlations among NEDELAC or NEDEHR and VT1, VT2, and MLSS. The effect size and power of ANOVA and the correlation analysis were conducted using G*Power Version 3.1.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). For all analyses, the significance level was set to 5% (p < 0.05).

3. Results

3.1. NEDE reliability

Intensities for test (NEDELAC-1) and retest (NEDELAC-2) were 10.1 ± 1.9 km/h and 10.6 ± 1.6 km/h (mean ± SD), respectively, and for test (NEDEHR-1) and retest (NEDEHR-2) were 9.8 ± 2.0 km/h and 9.7 ± 2.1 km/h, respectively (Table 1). Average linear coefficients of determination (R²) for NEDELAC-1 and NEDELAC-2 were 0.92 ± 0.06 and 0.89 ± 0.09, and for NEDEHR-1 and NEDEHR-2 were 0.90 ± 0.08 and 0.89 ± 0.10, respectively. ICC and CV for NEDELAC intensities in the test and retest were 0.93% (p < 0.05) and 5.1%, respectively, and for NEDEHR they were 0.79% (p < 0.05) and 11.7%, respectively.

3.2. NEDE vs. VTs and MLSS

Data regarding intensity, HR, and VO2 related to the physiological parameters of NEDE, VT1, VT2, MLSS, and VO2max are presented in Table 1. In short, NEDELAC and NEDEHR intensities presented statistically lower values than VT2 and MLSS intensities (p < 0.001; effect size = 0.74; power = 0.99); however, test and retest NEDELAC and NEDEHR intensities presented no differences from VT1 intensity. The same results were observed when the HR related to each physiological parameter (p < 0.001; effect size = 0.70; power = 0.97) were compared. No differences were observed between VT2 and MLSS intensities. The intensity, HR, and VO2 relative to VO2max were significantly higher than all other physiological parameters (p < 0.001; effect size = 0.64; power = 0.92).

The Bland and Altman analysis was also employed to evaluate the agreement between intensity determined by the NEDE test and the other physiological parameters (VT1, VT2, and MLSS). NEDE intensity presented considerable agreement to VT1, with the mean difference (bias) between NEDE and VT1 varying from −0.05 to 0.52 for NEDELAC and NEDEHR, respectively. Additionally, all data were within the limits of agreement (Fig. 2A and 2B). The NEDE intensity underestimated the VT2 and MLSS intensities, with bias ranging from 1.23 to 2.53 for NEDELAC and NEDEHR vs. VT2 and from 2.07 to 2.55 for NEDELAC and NEDEHR vs. MLSS (Fig. 2C–F).

In addition, NEDELAC and NEDEHR intensities were statistically correlated with VT1 and MLSS intensities, and only NEDEHR was significantly correlated with VT2. Fig. 3 illustrates correlations between the NEDE test and the other protocols.

4. Discussion

The present study hypothesized that NEDE is a reliable protocol for aerobic evaluation and training prescription. This hypothesis was confirmed by the finding that the “delta null intensities” obtained by the NEDE test (from LAC and HR) were reproducible and presented significant associations with VT1 and MLSS.

4.1. NEDE test reliability

To assess NEDE reliability, volunteers were asked to complete a retest procedure. The significant ICC observed for
NEDE intensities, determined by the physiological variables (LAC and HR), pointed to statistical agreement between the test–retest results, indicating a temporal stability of these measures. Additionally, the CV ranged from 5.1% to 11.7% (depending on the physiological variable utilized). These results are partially in accordance with the CV found by incremental protocols for anaerobic threshold determination (1.2% to 6.4%),\textsuperscript{29} for critical power models (2.3% to 7.6%),\textsuperscript{29} and for MLSS (3%).\textsuperscript{30} Despite statistically significant ICC indexes for NEDELAC and NEDEHR, these results should be carefully interpreted because only NEDELAC presented a considerably low CV (5.1%). However, the higher variation in NEDEHR was an expected result since HR response is highly susceptible to multiple variables, including metabolic, emotional, nutritional, and training-state variables.\textsuperscript{31,32}

The NEDE test measurements were based on the accumulation of different physiological variables during 2 efforts at the same intensity to calculate the delta values for each intensity. These calculations are used in a linear regression to determine the intensity of null delta, which means the intensity of physiological homeostasis. Besides the expected positive deltas for high intensities (above MLSS or VT\textsubscript{2}), we found some negative deltas in low intensities (Fig. 1), as have been reported in several other studies with procedures based on the NEDE test.\textsuperscript{15,18} In the study by Billat et al.,\textsuperscript{18} they argued that at low intensities (close to 63%VO\textsubscript{2max}), the early lactate produced is reused in gluconeogenesis or oxidized, which explains its subsequent reduction during low-intensity efforts. Another possible explanation for negative deltas is the lack of a warm-up session previous to the double effort. Without a warm-up session, the anaerobic metabolism must compensate for the initial energy demand until the necessary O\textsubscript{2} is captured, transported, and used in active muscles, which leads to an exaggerated increase in lactate production even in low intensities. When reporting on his original study, Chassain\textsuperscript{16} did not mention or show any negative deltas in his results, which suggests that he used only higher intensities and extrapolated the linear regression for the NEDE intensity determination. Despite the unknown relation between the negative and positive deltas, we found a large coefficient of determination ($R^2$) for the NEDE tests, suggesting that both deltas can be used for NEDE intensity determination by linear interpolation rather than extrapolation.

### 4.2. NEDE test vs. MLSS

The MLSS indicates the highest intensity at which LAC concentration reaches stabilization, thus allowing for exercise continuity for long periods and estimation of maximal aerobic capacity.\textsuperscript{20,34} In the present study, NEDELAC and NEDEHR underestimated the MLSS intensity by 15% and 20%, respectively, and the MLSS relative HR by 10% and 13%, respectively (Table 1). In 2 different studies, Manchado-Gobatto
et al.\textsuperscript{19,20} compared the MLSS intensity to the NEDE intensity determined for swimming and running rats. No difference was found between the protocols for the swimming exercise,\textsuperscript{19} but the NEDE test underestimated the MLSS by 20\% for running rats.\textsuperscript{20} Interestingly, a recent study conducted by our group found that NEDE precisely estimated the MLSS in humans during swimming exercise.\textsuperscript{21} Thus, it seems that prediction of MLSS by NEDE is ergometer-dependent for humans and rodents, given its underestimation during running exercise. In the 1990s, Billat et al.\textsuperscript{18} presented a protocol for the estimation of MLSS in humans from 2 levels of submaximal cycling exercise based on the null delta foundation of the NEDE test. In 2005, this method was compared with the classical MLSS\textsuperscript{33} and also presented significantly lower values for the MLSS (~18.2\%). According to Kilding and Jones,\textsuperscript{33} the proposed protocol indicates the intensity in which the difference between the LAC is equal to 0, while the MLSS admits an accumulation of 1 mmol/L during the last 20 min effort, which perhaps could be achieved at higher intensities, as observed in the present study.

Recently, Rossi et al.\textsuperscript{15} compared the NEDE test to the MLSS in menopausal women during running exercise. Their results suggested that the NEDE test underestimated MLSS intensity in the same proportions observed in the present study. One of their hypotheses was that the linear regression would fit better if the predictive intensities of the test were relativized according to individual physical fitness. In reporting on his original study, Chassain\textsuperscript{16,19} also suggested that workloads should be selected to vary near the anaerobic threshold. Following these precedents, the selection of NEDE workloads in the present study was based on parameters obtained in a previous ramp protocol. However, this previous procedure aimed only to standardize the selection of individualized workloads for each volunteer and to determine the VTs and VO\textsubscript{2max} for further comparisons. In practical situations, other indicators (i.e., targets of predicted maximal HR or rating of perceived exertion) could easily substitute for the workloads selection, which would dispense with a previous incremental protocol.

Another possible explanation for the higher intensities of MLSS compared to the NEDE test is the use of intervals of 5 min each for blood samples. Despite having no influence on MLSS concentration, the recovery period may contribute to an overestimation of MLSS intensity.\textsuperscript{11} Also, it is noteworthy that unlike the study by Rossi et al.\textsuperscript{15} the NEDE test in the present study presented strong correlations to MLSS (Fig. 3E and 3F). Despite an elevated bias from the Bland and Altman analysis (~2.0 km/h), the difference between NEDE and MLSS intensities were all inside the limits of agreement. The lower values observed in NEDE compared to MLSS are most likely due the fact that NEDE predicts the aerobic threshold (VT\textsubscript{1}) while MLSS is equivalent to the anaerobic threshold (VT\textsubscript{2}) during running exercise.\textsuperscript{10,16}

4.3. NEDE vs. VT\textsubscript{1} and VT\textsubscript{2}

In the present study, there were statistical correlations and no differences between NEDE intensities (determined in the test and retest by LAC and HR) and VT\textsubscript{1} (Fig. 2). Despite being determined by completely different methods and physiological variables, it is possible that NEDE and VT\textsubscript{1} intensities are associated with similar physiological phenomenon. It is known that VT\textsubscript{1} represents the first increase in ventilation followed by an increase in CO\textsubscript{2} production from anaerobic metabolism.\textsuperscript{12} In support of this conjecture, VT\textsubscript{1} has been associated with the first lactate increase during incremental protocol.\textsuperscript{17} Therefore, considering that NEDE predicts an intensity of physiological balance between production and clearance of LAC, it is plausible that NEDE intensity indicates the beginning of anaerobic participation in energy production. This hypothesis is corroborated by the study by Sid-Ali et al.\textsuperscript{17} who found significant correlations between an adaptation of the NEDE test (double efforts of 7 min and 14 min) with the first increase in LAC in incremental exercise. In addition, all data analysis suggests that the NEDE test indicates the VT\textsubscript{1} intensity because (1) no differences were found between NEDE and VT\textsubscript{1} intensities, HR, or VO\textsubscript{2} (Table 1); (2) NEDE and VT\textsubscript{1} intensities presented strong agreement in the Bland and Altman analysis (Fig. 2A and 2B); and (3) NEDE and VT\textsubscript{1} intensities presented significant correlation coefficients (Fig. 3A and 3B).

Hence, our results suggest that the NEDE test estimates the intensity correspondent to VT\textsubscript{1}. This is an important finding considering that VT\textsubscript{1} is an important evaluative and prescriptive parameter of aerobic training for athletes,\textsuperscript{3} especially for individuals with impaired exercise capacity, such as patients with cardiovascular disease\textsuperscript{1,4} and other endocrine metabolic comorbidities.\textsuperscript{5} Thus, the NEDE test appears to be an easier and more practical method for conducting aerobic evaluation and developing training prescriptions because it does not require incremental protocols or high-cost equipment such as gas analyzers. In addition, the short duration of each double effort implies a lower total workload of exercise that could be applied as a warm-up before training sessions.\textsuperscript{15}

5. Conclusion

Our results suggest that the NEDE test applied to running exercise is reliable and can estimate VT\textsubscript{1} intensity. Moreover, despite being conducted at lower running velocities, the intensity obtained by the NEDE test showed significant correlations with VT\textsubscript{2} and MLSS.

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Authors’ contributions

LDMF performed data collection, statistical analysis and manuscript writing; FBMG contributed to data interpretation and revision of the paper; RCMR designed and reviewed the study; MCG designed and reviewed the study; CAG designed and coordinated the study, interpreted data and reviewed the paper. All authors have read and approved the final version of the paper, and agree with the order in which the authors are presented.
Competing interests

The authors declare that they have no competing interests.

References

1. Tamburis NY, Kunz VC, Salviati MR, Castello Simoes V, Catai AM, Da Silva E. Interval training based on ventilatory anaerobic threshold improves aerobic functional capacity and metabolic profile: a randomized controlled trial in coronary artery disease patients. *Eur J Phys Rehabil Med* 2016;52:1–11.

2. Rabadan M, Diaz V, Calderon FJ, Benito PJ, Peinado AB, Maffulli N. Physiological determinants of speciality of elite middle- and long-distance runners. *J Sports Sci* 2011;29:975–82.

3. Pedro RE, Milanez VF, Boullosa DA, Nakamura FY. Running speeds at ventilatory threshold and maximal oxygen consumption discriminate futsal competitive level. *J Strength Cond Res* 2013;27:514–8.

4. Mourot L, Tordi N, Bouhaddi M, Teffaha D, Monpere C, Regnard J. Heart-rate variability threshold as an alternative for spiro-ergometry tests in professional basketball players. *Int J Sports Physiol Perform* 2015;10:92–100.

5. Castro EA, Peinado AB, Benito PJ, Galindo M, González-Gross M, Cupeiro R; the PRONAF Study Group. What is the most effective exercise protocol to improve cardiovascular fitness in overweight and obese subjects? *J Sport Health Sci* 2017;6:45–61.

6. Neves CD, Lacerda AC, Lage VK, Lima LP, Fonseca SF, de Avelar NC, et al. Cardiorespiratory responses and prediction of peak oxygen uptake during the shuttle walking test in healthy sedentary adult men. *PLoS One* 2015;10:e0117563. doi: 10.1371/journal.pone.0117563

7. Novais LD, Silva E, Simoes RP, Sakabe DI, Martins LE, Oliveira L, et al. Anaerobic threshold by mathematical model in healthy and post-myocardial infarction men. *Int J Sports Med* 2016;37:112–8.

8. Wasserman K, Whipp BJ, Koyl SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 1973;35:236–43.

9. Kindermann W, Simon G, Keul J. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Appl Physiol Occup Physiol* 1979;42:25–34.

10. Dekker J, Baron B, Dupont L, Vanvelcenaere J, Pelayo P. Maximal lactate steady state, respiratory compensation threshold and critical power. *Eur J Appl Physiol* 2003;89:281–8.

11. Beneke R, Hutter M, Von Duvillard SP, Sellens M, Leithauser RM. Effect of test interruptions on blood lactate during constant workload testing. *Med Sci Sports Exerc* 2003;35:1626–30.

12. Ramos-Campo DJ, Rubio-Arias JA, Avila-Ganá V, Marín-Pagán C, Luque A, Alcañiz PE. Heart rate variability to assess ventilatory thresholds in professional basketball players. *J Sport Health Sci* 2017;6:468–73.

13. Llodio I, Gorostiaga EM, García-Tabar I, Granados C, Sanchez-Medina L. Estimation of the maximal steady state in endurance runners. *Int J Sports Med* 2016;37:539–46.

14. Mankowski RT, Michael S, Rozenberg R, Stokla S, Stam HJ, Preat SF. Heart-rate variability threshold as an alternative for spiro-ergometry testing: a validation study. *J Strength Cond Res* 2017;31:474–9.

15. Rossi FE, Kalva-Filho CA, Araújo RG, Neto JG, Campos EZ, Pastre CM, et al. Critical velocity determined by a non-exhaustive method in menopausal women. *Sci Sports* 2015;30:17–22.

16. Chassain AP. A method for objective evaluation of body tolerance to effort applied to measurement of critical peaks in heart rate and lactate-mia. *Sci Sports* 1986;1:41–8.

17. Sid-Ali B, Vandewalle H, Chair K, Moreaux A, Monod H. Lactate steady state velocity and distance-exhaustion time relationship in running. *Arch Int Physiol Biochim Biophys* 1991;99:297–301.

18. Billat V, Dalmas F, Antonini MT, Chassain AP. A method for determining the maximal steady state of blood lactate concentration from two levels of submaximal exercise. *Eur J Appl Physiol Occup Physiol* 1994;69:196–202.

19. Manchado FB, Gobatto CA, Voltarelli FA, de Mello MAR. Non-exhaustive test for aerobic capacity determination in swimming rats. *Appl Physiol Nutr Metab* 2006;31:731–6.

20. Manchado-Gobatto FB, Gobatto CA, Contarteze RV, Mello MA. Non-exhaustive test for aerobic capacity determination in running rats. *Indian J Exp Biol* 2011;49:781–5.

21. Gobatto CA, De Araujo GG, Santiago V, Papoti M, Manchado-Gobatto FB. Validation of non-exhaustive test to determine the aerobic capacity in swimming. *J Sports Med Phys Fitness* 2018;58:407–13.

22. Hallal PC, Victora CG. Reliability and validity of the International Physical Activity Questionnaire (IPAQ). *Med Sci Sports Exerc* 2004;36:556.

23. Myers I, Buchanan N, Smith D, Neutel J, Bowes E, Walsh D, et al. Individualized ramp treadmill. Observations on a new protocol. *Chest* 1992;101(Suppl. 5):S236–41.

24. Robergs RA, Dwyer D, Asttorino T. Recommendations for improved data processing from expired gas analysis indirect calorimetry. *Sports Med* 2010;40:95–111.

25. Smith TB, Stonell C, Purkayastha S, Parasekvas P. Cardiopulmonary exercise testing as a risk assessment method in non cardio-pulmonary surgery: a systematic review. *Anaesthesia* 2009;64:883–93.

26. Azevedo LF, Perlingeiro PS, Braga AM, Negrao CE, de Matos LD. Exercise intensity optimization for men with high cardiorespiratory fitness. *J Sports Sci* 2011;29:555–61.

27. Engel PC, Jones JB. Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of NAD+ in alkaline hydrazine buffers: improved conditions for the assay of L-glutamate, L-lactate, and other metabolites. *Anal Biochem* 1978;88:475–84.

28. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1986;1:307–10.

29. Hopkins WG, Schabort EJ, Hawley JA. Reliability of power in physical performance tests. *Sports Med* 2001;31:211–34.

30. Hauser T, Bartsch D, Baumgartel L, Schulz H. Reliability of maximal lactate-steady-state. *Int J Sports Med* 2013;34:196–9.

31. Jeukendrup A, VanDieren A. Heart rate monitoring during training and competition in cyclists. *J Sports Sci* 1998;16(Suppl. 1):S91–9.

32. Plews DJ, Laursen PB, Le Meur Y, Hausswirth C, Kilding AE, Buchheit M. Monitoring training with heart rate-variability: how much compliance is needed for valid assessment? *Int J Sports Physiol Perform* 2014;9:783–90.

33. Kilding AE, Jones AM. Validity of a single-visit protocol to estimate the maximum lactate steady state. *Med Sci Sports Exerc* 2005;37:1734–40.

34. Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. *Comp Biochem Physiol A Mol Integr Physiol* 2001;130:21–7.